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PHYSIOLOGICAL CONTROL OF HAGBERG FALLING NUMBER AND SPROUTING IN WINTER WHEAT AND DEVELOPMENT OF A PREDICTION SCHEME

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PHYSIOLOGICAL CONTROL OF HAGBERG FALLING NUMBER AND SPROUTING IN WINTER WHEAT AND DEVELOPMENT OF A PREDICTION SCHEME

by

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ABSTRACT

The aim of this research was to understand the causes of low Hagberg falling number in the UK and formulate a prediction scheme. Literature review revealed three recognised routes of *alpha*-amylase production, causing low HFN, with the possibility of a fourth. Visits to researchers operating differing types of HFN prediction schemes in Finland and France allowed identification of the components of those schemes appropriate for use in the UK. Factors influencing the four routes of *alpha*-amylase synthesis in the UK were therefore investigated in multi-site field, controlled environment and laboratory studies.

Lowering of HFN by pericarp amylase (α-AMY-2) isozyme activity, was demonstrated by reconstitution of flours with immature grains. This highlighted the possibility of retention of pericarp *alpha*-amylase activity (RPAA) as a route to low HFN in the UK. Experiments with air-drying and desiccant spraying indicated these as possible control measures for RPAA.

A comparison of crops at two sites in two years did not show a relationship between pre-maturity *alpha*-amylase accumulation in the absence of sprouting (PMAA) and grain drying rate, reported by other workers. Transfers of developing wheat plants between warm and cool controlled environment cabinets during grain development showed induction of PMAA by both cold and heat shock, even in cultivars such as Pastiche which have never shown PMAA in the field. The factors inducing PMAA were too complex to allow modelling with meteorological data.

Pre-maturity sprouting (PrMS) during the grain dough stage was demonstrated, by isolated grain germination and grain cutting experiments to be caused by severe cold, wet weather, by pericarp damage or interaction of the two factors. Increased susceptibility to PrMS was found to be associated with infestation of wheat grain by the orange wheat blossom midge (*Sitodiplosis mosellana*) during grain development. Midge-damaged grains showed only the characteristic pattern of plant germination amylases, with PrMS enhanced by splitting of the pericarp caused by larval feeding, leading to dormancy break and germination.

Multi-site field studies at four sites over four years were used for the investigation of the relationship between dormancy and temperature with the aim of predicting post-maturity sprouting (PoMS). The relationship found between dormancy index of harvested grain and temperature in France was not seen with UK data. A relationship was found between dormancy duration and temperature accumulated during grain development, for sprouting resistant cultivars, although it was too weak to allow modelling of dormancy.

Consideration of the visits abroad and the progress in defining the complex routes of *alpha*-amylase synthesis in the UK allowed development of a prototype scheme for HFN forecasting. During the field studies, a good relationship between pre-harvest HFN and combine harvest HFN, in the absence of subsequent rain, was found. Therefore, combine harvest HFN potential can be assessed at about 35% grain moisture (Stage 1). Crops with low HFN due to PMAA, PrMS or RPAA can be given low harvest priority. Improvement of crops with RPAA may be possible, but not of crops with PMAA or PrMS. Assessment of the level of dormancy (or germinability) in crops in Stage 2 (using the sample as Stage 1) with a germination test allows identification of crops with a high HFN potential but at high risk of HFN loss (due to PoMS) in poor weather. These crops would benefit from an early, high moisture harvest. Trial operation of the scheme in 1996 and 1997 allowed assessment of the logistics of operating such a scheme and allowed predictions of combine harvest HFN to be made with a success rate of 75-85%.

ABBREVIATIONS

α-AMY-1	Malt or high-pI *alpha*-amylase isozymes
α-AMY-2	Pericarp, green or low-pI *alpha*-amylase isozymes
AB	ADAS Bridgets
ADAS	Agricultural Development and Advisory Service
ANOVA	Analysis of variance
ARC	Agricultural Research Centre of Finland
CCFRA	Campden & Chorleywood Food Research Association
°Cd	Degree (Celsius)-days
CV	Coefficient of variation (%)
DM	Dry mass
DPA	Days post-anthesis
FDB	Fluorescein dibutyrate (test)
FM	Fresh mass
HAAC	Harper Adams Agricultural College (Crop & Environment Research Centre)
HFN	Hagberg falling number
ICC	International cereals committee
IEF	Iso-electric focusing
IGG	Isolated grain germination
ITCF	Institut Technique des Céréales et des Fourrages
IVED	*In vitro* ear dormancy
JIC	John Innes Centre, Norwich
LSD	Least significant difference
$mEUgdw^{-1}$	Milli-enzyme units per gramme dry weight
OWBM	Orange wheat blossom midge (*Sitodiplosis mosellana,* Géhin)
p	Probability
pI	Iso-electric point
PMAA	Pre-maturity *alpha*-amylase activity in the absence of sprouting
PoMS	Post-maturity sprouting
PrMS	Pre-maturity sprouting
RPAA	Retained pericarp *alpha*-amylase activity
SB	Sutton Bonington Campus (University of Nottingham, School of Biological Sciences, Division of Agriculture and Horticulture)
SED	Standard error of the differences between means
UA	University of Aberdeen, Department of Agriculture
ZGS	Zadoks' growth stage

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TABLES AND FIGURES

1. OBJECTIVES.

As stated in the initial research proposal, the project objectives were:

1. To become familiar with two HFN/sprouting prediction schemes already in operation, one in France and one in Finland.

2. To differentiate clearly the modes and periods of formation of *alpha*-amylase in wheat grain, both with (a) a desk study, and in

(b) field experiments with a range of cultivars chosen for their known susceptibility to sprouting and/or low Hagberg and using irrigation.

3. To describe the whole *alpha*-amylase/sprouting phenomenon in a model/conceptual framework.

4. (a) With (1) and (3) as background, to assess the appropriateness for UK conditions of existing overseas prediction schemes.

(b) These will be modified to take account of the conceptual framework.

(c) A desk study will use historic data sets over several years of Hagberg falling number and weather from various sites, *e.g.* Harper Adams, ADAS farms.

5. To carry out experiments in the field and controlled environments to:

(a) characterise the dormancy/environment relationships for UK cultivars;

(b) clarify the environmental triggers for pre-dormancy (pre-maturity) sprouting, pre-maturity *alpha*-amylase formation and other routes of Hagberg falling number reduction.

6. To produce a scheme for the prediction of grain *alpha*-amylase activity and sprouting in the UK (and France in collaboration with ITCF).

Progress on all of the objectives was achieved, apart from 4 (c), the desk study of historic HFN and weather data. The impetus for this objective changed in the light of the field study results and the nature of the prediction scheme, as described in the text.

2. INTRODUCTION.

Low Hagberg falling number (high *alpha*-amylase activity) in wheat is an intermittent problem which has serious financial consequences for farmers, the milling and baking industry and exporters. Over the last two decades, there has been a UK wheat crop with an average Hagberg falling number below 200 s about one year in seven, and below 250 s about one year in three (*Figure 1*, HGCA data). In 1997, low HFN was again a problem for many crops in the North and West of the UK due largely to a wet harvest.

Some aspects of grain quality, *e.g.* specific weight, can be manipulated to a certain extent by husbandry or post-harvest treatments, but there is very limited scope for manipulation of Hagberg falling number before harvest. Post-harvest improvement of low Hagberg wheat is possible to a limited extent by blending with lots of higher HFN (Vaidyananthan, 1987). Gravity separation may also improve Hagberg (Hook *et al.*, 1988), but this is not effective for all batches of low Hagberg wheat and may not lead to concomitant improvement in baking quality.

Therefore, in the absence of effective methods for manipulation of Hagberg falling number before harvest, the probable value to farmers of pre-harvest Hagberg prediction has been identified (Kettlewell, 1993; Kettlewell *et al.*, 1996). The virtues of pre-harvest prediction of grain quality include the allocation of harvesting priority, allowance for separate storage, more informed decision-making on the timing of harvest as well as planning for marketing of the grain. The ability to sometimes successfully manipulate Hagberg after harvest does not necessarily negate these benefits to the farmer, since the cost of prediction and subsequent decisions may be less than the cost of post-harvest manipulation. One of the benefits of pre-harvest prediction may be to help decide which lots of grain may benefit from blending or gravity separation and allow preparation for the necessary processing.

Therefore, HGCA project 0056/01/93 was initiated in April 1994 to undertake the research required to design an appropriate HFN prediction scheme for the UK. An operable scheme will provide UK farmers with the intelligence to support the decision making necessary to optimise the HFN of the national crop.

3. SUMMARY OF PROGRESS WITH NON-EXPERIMENTAL OBJECTIVES

3.1. Objective 1: Investigation of schemes already in operation in other EU countries

In some European Union countries prediction schemes to warn farmers of sprouting risk by predicting the end of dormancy from weather data have already been developed or tested (Netherlands, Germany, France). There are also forecasting schemes in existence in Scandinavia which rely on regular sampling of regional reference crops to monitor changes in Hagberg ('prognosis'-type schemes). The first aim of this project was to transfer as much information as possible from existing prediction schemes overseas to a scheme in the UK.

3.1.1. ***Dormancy prediction in France*** *(Philippe Gate of ITCF Service Innovations Techniques visited 23-26 May 1994. Funded by ITCF)*

The scheme currently used in France is based largely on the work of Belderok (1961, 1965, 1968, 1973) completed at the TNO (Netherlands Cereal, Flour and Bread Institute) in Wageningen. A prediction service ran at least from 1964-1973 in the Netherlands, although it appears no longer to operate. Using plants transferred at various times between different constant temperature glasshouses, Belderok reported an inverse curvilinear relationship between the length of dormancy (defined as the time from harvest ripeness to germination of 50% of grains in 3 days at 18°C) and the temperature accumulated above 12.5°C during the dough stage of development (*Figure 2*). The scheme was also tested in Northern Germany (Grahl and Schrödter, 1975) although it was found to be unsuitable for the conditions in the then Federal Republic.

Dormancy prediction in France was instigated after severe sprouting affected the value of the crop in the North and West of France in 1987. The work of Belderok (1973, in French) was re-evaluated (Beaux and Labonne, 1990) and a French version of the scheme initiated.

French cultivars are tested for their genetic susceptibility to sprouting which is assessed by the *in vitro* ear dormancy test (IVED) required for official registration of commercial cultivars (Gate, personal communication). This information allowed cultivars to be put into five groups, from

very sprouting-sensitive to very sprouting-resistant (*Table 1*), as previously classified by Belderok.

***Table 1**: Classification of Classes of Sprouting Susceptibility in France*

1	Très résistantes	Very sprouting-resistant
2	Résistantes	Sprouting-resistant
3	Assez résistantes	Reasonably sprouting-resistant
4	Sensibles	Sprouting-susceptible
5	Très sensibles	Very sprouting-susceptible

With monitoring of crop development and weather conditions, the observed accumulated temperature (above 12.5°C) in the dough stage is used to calculate the likely duration of dormancy from the temperature-dormancy curves presented by Belderok in his original work (*Figure 2*). If a duration of dormancy of less than 10 days is predicted, then, in the case of a forecast of rain, warnings are disseminated on the radio and in newspapers and farmers' bulletins.

The rapid implementation of the prediction scheme did not allow validation. The scheme only forecasts a potential *risk* of pre-harvest field-sprouting and work is ongoing to predict more precisely the *expression* of PoMS. The recent, ongoing work by ITCF, has focused on the investigation of the relationship between a dormancy index and temperature parameters in an attempt to improve the accuracy of the prediction system. Investigation of this approach with UK field sites and cultivars is reported in *Sections 4.4.2.3-4.4.2.6.*

At the start of the project, the dormancy break prediction schemes, apart from cultivar-specific information, were thought largely transferable to the UK and for this reason collaboration with the Institut Techniques des Céréales et des Fourrages was initiated. Originally it was anticipated that successful dormancy prediction might be possible using only meteorological data. However,

low Hagberg falling number appears to be a more complex phenomenon in the UK than is recognised in France, and pre-harvest dormancy prediction from weather data more difficult than anticipated. Testing of Belderok-type systems by other authors (Grahl and Schrödter, 1975; Olsson and Mattisson, 1976; Reiner and Loch, 1976; Strand, 1989) has cast doubt on the validity of the Belderok temperature-dormancy relationship, especially in maritime climates, in a number of countries.

3.1.2. ***Pre-harvest HFN monitoring in Finland*** *(Markku Kontturi and Tuula Suvanto, Agricultural Research Centre of Finland, visited 19-24 August 1995. Part-funded by a grant from the Anglo-Finnish British Council)*

The forecasting schemes used in Scandinavia appear to be simple since they require little or no understanding of the modes of formation of *alpha*-amylase. On the other hand they are logistically complex, involving up to one reference crop for every 5,000 ha land area and are thus relatively expensive to operate.

Farming conditions in Finland verge on the marginal, with the weather a major limiting factor for cereal production. Specifically, low Hagberg falling number due to sprouting is a recurrent problem because of the pattern of rainfall (increasing in August and September during crop maturation and harvest). Although low HFN is less of a problem for the type of breads preferred by Finnish consumers (including acid-dough rye-breads) compared to the Anglo-American style soft, sliced loaves, minimum HFN requirements of 190 s are often unattainable.

The HFNs of cultivars of spring wheat, winter wheat and rye are monitored in field trials at the Agricultural Research Centre (Jokioinen) and at eight ARC field stations in the major wheat-growing regions of Finland (Mietoinen, Kokemäki, Vihti, Pälkäne, Mikkeli, Laukaa, Ylistaro and Tohmajärvi). Data is collected over a 6-8 week period, depending on the season. Grab samples of ears are collected by hand, or by combining small areas of the plot, from a maximum of 15 representative commercial cultivars. Sampling occurs from approximately 40% moisture content, the yellow ripe stage, on Mondays and Wednesdays in the early morning. Grab samples are threshed before drying overnight in large drying ovens at < 60°C. HFN determinations are

performed at each site rather than centrally.

Early the next morning, dried samples are cleaned and milled with a falling number mill and the HFN determined using the standard ICC method and falling number apparatus. The results must be ready by 12.00 noon on the Tuesday or Friday for publication, which places the upper limit on the possible number of samples that can be analysed (particularly when HFNs are in the 300-400 s region). The data is placed on an answering phone system, along with advice about the trend and weather effects, as well as on the ARC Agronet computer database. The information is also published in the twice-weekly agricultural newspaper, '*Maasedun Tulevaissus*', the following Monday or Thursday, with simple weather forecasts. The process is repeated on Thursday/Friday for the Monday newspaper.

The answerphone service operates on one telephone number, with access to weather forecasts and agricultural bulletins for 21 individual districts. The computer system can detect the district the call is received from to direct it to the relevant announcement. The farmer can select the weather forecast (2 and 5 days) and agricultural bulletins (cereals, or the other major crops of the area). Call costs were 4.65 Finnmarks (approx. 66 pence)/min in 1995, with a standard call lasting two minutes. At the time of the visit, government subsidies and support for agricultural research was being cut back due to membership of the European Union, with the resulting loss of some field sites from the scheme. With declining use of the information system by farmers due to the economic climate, the future of the scheme was in some doubt.

No theoretical weather-based models are in operation in Scandinavia, despite some research into this (Karvonen and Peltonen, 1991). A similar scheme to that described above also operated for cereal crops (rye and wheat) in Scania in Sweden (Larsson, 1987).

3.2. Objective 2 (a): Differentiation of the modes of *alpha*-amylase accumulation of importance in the UK, by desk study (literature review).

At the start of the project, three modes of grain *alpha*-amylase formation in wheat grain were thought to be important in the UK. These modes needed to be clearly separated if a UK

prediction scheme was to be successful. In some previous years low Hagberg falling number was associated with post-maturity sprouting in wet weather after grain matured and dormancy was broken, *e.g.* 1977. In other years there has been little evidence of sprouting as pre-maturity *alpha*-amylase formed in the grain in the absence of sprouting, *e.g.* 1985. High grain *alpha*-amylase at maturity has also been associated with pre-maturity sprouting, *e.g.* 1987. During the course of the research, experiments also indicated that a fourth possible mode, due to pericarp or green *alpha*-amylase, could not be ignored.

3.2.1. Retained pericarp alpha-amylase activity (RPAA).

In the initial development stages, grain *alpha*-amylase activity is very high (> 1000 $mEUgdw^{-1}$), but this activity is located in the pericarp tissues and consists of separate families of amylase isozymes (α-AMY-2 and α-AMY-3; Olered and Jonsson, 1970; Daussant and Renard, 1987) to the major form expressed in the other syndromes (α-AMY-1). The activity usually largely disappears during grain development and drying, leading to high Hagberg falling number in seasons of good weather, *e.g.* 1990 (with low *alpha*-amylase activity, 10-100 $mEUgdw^{-1}$). However, if for some reason a sub-population of green grains remains in the crop at harvest, this may affect the Hagberg falling number, as shown by the research described in *Section 4.1.* Aside from vague anecdotal evidence, this route was not previously recognised in the UK, but our research has identified its potential importance. The heterogeneity in ear development necessary to cause RPAA problems may arise by a number of different mechanisms discussed in more detail in *Section 4.1.*

3.2.2. Pre-maturity alpha-amylase accumulation in the absence of sprouting (PMAA).

The appearance of a high *alpha*-amylase activity in grains showing no visible signs of germination has been long recognised in some UK cultivars (Bingham and Whitmore, 1966). The phenomenon has also been recorded in cultivars from as far afield as Mexico, Australia (Mares and Gale, 1990) and Japan (Mrva and Mares, 1994). The syndrome is also sometimes known as late maturity *alpha*-amylase accumulation (LMAA). In this phenomenon, mainly the α-AMY-1 (malt or germination) amylase isozymes are expressed, although the α-AMY-2 family may also

be observed in severe cases. The enzymes are expressed by the aleurone cells in the crease region of the grain rather than by the embryo (as is seen in the first stages of germination). The final *alpha*-amylase activity in affected grains is usually in the range 100-300 $mEUgdw^{-1}$. Slow grain drying in cool, damp weather between 40-20% grain moisture (Gale *et al.*, 1983) has been proposed as a factor leading to *alpha*-amylase formation in the absence of sprouting, *e.g.* 1985. However, there appears to be some form of pre-conditioning or initial trigger stimulus which may start the syndrome (Gold *et al.*, 1990, Gold, 1992). The exact nature of the stimulus was unknown at the start of the research, although induction of PMAA by glasshouse conditions implicated high humidity and diurnal temperature variation. In addition, some research has related the appearance of PMAA to large grain size (Evers *et al.* 1995; Alberti *et al.*, 1996). Our research has shown the importance of periods of temperature shock in early grain development in triggering PMAA.

3.2.3. Pre-maturity sprouting (PrMS).

This phenomenon has been reported less often than PMAA or PoMS (Mitchell *et al.*, 1980; Flintham and Gale, 1988). In PrMS, the grain becomes capable of germinating whilst immature which may lead to premature sprouting in poor weather, *e.g.* 1987, with expression of the germination pattern of *alpha*-amylase isozymes (α-AMY-1 and α-AMY-2) by the embryo and aleurone layers. The *alpha*-amylase activity in individual pre-mature sprouted grains can be very high (> 1000 $mEUgdw^{-1}$). The physiology of, and environmental triggers for, this process in the field are hardly known. The research has concentrated on assessing the relative importance of severe cold, wet weather conditions and damage by infestation with the orange wheat blossom midge (*Sitodiplosis mosellana*, Géhin), the two factors implicated on initial review of the literature and anecdotal evidence.

3.2.4. Post-maturity sprouting (PoMS).

Post-maturity sprouting, the classic 'pre-harvest sprouting' is the most clearly recognised, frequently occurring and intensively studied route of *alpha*-amylase accumulation leading to low HFN. Most UK wheat cultivars experience a period of dormancy after harvest ripeness where

germination can only occur under very specific conditions (low temperature) despite competence of the isolated embryo to germinate under a much wider set of conditions and availability of water and oxygen. In the period of after-ripening following harvest maturity (nominally defined in all our work as 15% moisture content), the range of conditions under which germination is possible gradually widens. Once dormancy has broken, germination is possible over a wide temperature range (*e.g.* 4-25°C) with the presence of exogenous moisture and oxygen. When grains become germinable *alpha*-amylase accumulates in the endosperm as the grain begins to sprout in wet weather, *e.g.* 1977, 1997. The germination pattern of enzymes (α-AMY-1 and α-AMY-2) is expressed, eventually leading to very high *alpha*-amylase activities (> 1000 mEUgdw^{-1}). Activity is first observed adjacent to the embryo, but is also later released from the aleurone layer. Dormancy is a very heterogeneous property and the germination of only a very few grains can degrade the HFN of a much larger lot of grain. Despite much research, and some theories (Gate, 1996), there is still no firm consensus or complete understanding of the exact mechanism of dormancy or its breakage.

At a biochemical level, much is understood of the process of *alpha*-amylase synthesis and its control by gibberellins, although the exact way in which the controls operate in the different modes of formation is not clear. The emphasis in this project, however, was to establish detailed inter-relationships between the field environment, formation of *alpha*-amylase and germination rather than to take biochemical investigations further

3.3. Objective 3: Conceptual framework of *alpha*-amylase accumulation in the UK

The separate modes and periods of the four possible routes of grain *alpha*-amylase accumulation in the UK, described above, are shown in the final version of the conceptual framework (Version 4, Parts 1 and 2, *Figures 3.1* and *3.2).* Part 1 of the conceptual framework schematically shows the relative phasing of the four different modes of enzyme accumulation, in comparison with the simplest yardsticks of grain development, grain moisture content and Zadoks growth stage (Tottman and Broad, 1987). Also shown in Part 1 are the major factors which *promote* the individual routes.

In Part 2 of the Framework, the effect of each of the syndromes on *alpha*-amylase activity is shown graphically, in comparison with grain development measures (fresh/dry mass accumulation and percentage moisture content) and dormancy/embryo germination levels.

Due to the complexity of the four processes, their interaction with each other and with environmental triggers, mathematical modelling of the processes from field and controlled environment data has not been possible. However, such models are not necessary for operation of the envisaged Prediction Scheme described in *Section 4.5.*, as opposed to the meteorologically-based scheme originally visualised.

3.4. Objective 4 (a): Assessment of the appropriateness of existing prediction schemes for use in the UK

3.4.1. Discussion of European schemes in relation to the UK situation

From consideration of the two schemes already in operation, it appears that neither of the Continental prediction systems studied are completely appropriate for operation in the UK. The scheme developed by the ITCF in France predicts a risk of sprouting rather than Hagberg falling number, which is the critical commercial criterion in the UK. The effects of a given degree of sprouting on HFN may vary widely with cultivar and the interaction of PoMS with the three early routes of *alpha*-amylase formation, which are not considered in the French system. Also, a relationship between the *in vitro* dormancy index measurement and sprouting in the field has not been clearly demonstrated. The early routes of *alpha*-amylase accumulation (RPAA, PMAA and PrMS) appear to be of less significance in France, possibly due to the drier and hotter climate. Also, due to the larger market for non-sliced loaves, French HFN requirements are slightly less stringent than in the UK.

The prediction scheme operating in France has used the relationships developed by Belderok, over which much doubt has been cast by workers in other countries (Grahl and Schrödter, 1975; Strand, 1989). The Belderok relationship could not be used without validation for UK cultivars and conditions. Despite the good relationships developed between accumulated temperature and

dormancy index in France, there is little evidence of this relationship in the UK or that this prediction of risk correlates well with field observations of the expression of sprouting and Hagberg falling number reduction in poor weather.

The Finnish scheme is initially attractive as a fall-back. However, as it effectively shows the 'live' HFN of reference crops, by the time sprouting commences and reports of falling Hagberg are disseminated it is often too late for farmers to take remedial action. Also, wide variations in response to particular conditions may result from crops of widely differing sowing date or micro-environment. Due to the logistics of operating many reference sites, this sort of scheme might prove prohibitively expensive in the UK. A very small scale scheme with widely dispersed sites, few cultivars and restricted sowing dates would probably prove too inaccurate to be of general use.

Therefore, it seems that a hybrid scheme incorporating the two diverse approaches might be the most successful and economical option for the UK, with the possibility of operation on an individual field basis. A completely meteorologically based HFN prediction model was initially envisaged as a possible solution for the UK. Substantial progress has been made to further our understanding of the critical factors affecting UK HFN, which will be an important foundation for any future modelling exercise. However, the experimental results presented in the ensuing sections show that the complex interactions of the routes of *alpha*–amylase accumulation and environmental conditions have precluded this option at the moment.

Due to the incomplete definition of discrete environmental triggers for RPAA, PMAA, PrMS and PoMS, a meteorological model was not developed. With the practical nature of the envisaged prediction scheme, completion of Objective 4 (b), which would have tested any meteorologically-based prediction methods, was deemed unnecessary.

3.4.2. Identification of the gaps in knowledge requiring further research (Objectives 2 (b), 5 (a) and 5 (b))

The gaps in knowledge, identified at the beginning of project 0056/1/93, concerned elucidation of the environmental factors promoting each of the *alpha*-amylase accumulation routes, which might allow the production of a prediction scheme based on meteorological data. On review of the potential prediction scheme at the mid-point of the project, a further set of research requirements was identified for the successful operation of the pragmatic two-stage prediction scheme which replaced the initially planned meteorological model.

3.4.2.1. Initial research areas (potential meteorologically-based scheme).

A.1) Testing of the hypothesis that a temperature stimulus (high temperature heat shock) can induce PMAA (revised to include tests of high and low temperature shock on intelligence from Australian workers at the Seventh International Symposium on Pre-harvest Sprouting in Cereals, Mrva and Mares, 1996).
A.2) Testing of the hypothesis that slow drying exacerbates PMAA.
A.3) Investigation of the weather factors which can induce PrMS (in the absence of other factors)
A.4) Investigation of the involvement of *S. mosellana* infestation with PrMS induction.
A.5) Testing of the hypothesis that dormancy duration or dormancy index is related to temperature/accumulated temperature experienced during grain development.
A.6) Investigation of the potential use of the dormancy/temperature relationship for prediction of sprouting and Hagberg falling number, specifically in the UK.

3.4.2.2. Revised research areas (prototype two-stage prediction scheme research)

B.1) Testing the hypothesis that α-AMY-2 is active in HFN test and is therefore of consequence to pre-harvest HFN measurement, or as a route to low combine harvest HFN.
B.2) Quantification of the relationship of pre-harvest to combine harvest HFN.
B.3) Determination of the appropriate time for sampling to measure pre-harvest HFN for combine harvest HFN prediction.

B.4) Quantification of the relationship between HFN of hand/combine harvest samples.

B.5) Determination of the appropriate sample size for pre-harvest HFN measurement.

B.6) Investigation of appropriate transport methods for pre-harvest HFN samples.

B.7) Testing of the hypothesis that an early dormancy test can be used to predict later germinability.

B.8) Investigation of the logistics of operating an HFN prediction scheme

3.4.3. Initial outline of experimental work (Objectives 2 (b), 5 (a) and 5 (b))

In 1994, outline experimental methods were tested at Sutton Bonington and Harper Adams on eight cultivars, chosen for their known propensities to different routes of *alpha*-amylase accumulation and including four French cultivars grown in a reciprocal agreement with ITCF. French methods of *in vitro* dormancy assessment were assessed. Methods for the various diagnostic techniques (iso-electric focusing, FDB staining, *alpha*-amylase assay) were also developed.

Experience from 1994 was used to design an appropriate protocol for operation in 1995-1994 using the same cultivars grown at the University of Aberdeen, Harper Adams, Sutton Bonington and ADAS Bridgets with some additional intelligence from ITCF sites in France. Specific modifications to the protocol were made in the light of experience gained in each year, noted in each section below.

The multi-site field protocol was designed to study the relationship between dormancy and temperature and the operation of the proposed two-stage scheme (tested in outline in 1996 and 1997). Grain growth and development were monitored and serial samples were taken to allow identification of the early routes of *alpha*-amylase accumulation. Meteorological data was taken from the wide stretch of sites to allow testing of the various potential factors inducing each *alpha*-amylase route described in the previous section. In particular, crop dormancy was assessed to allow testing of the potential dormancy-temperature relationship across the wide range of sites. Serial HFN samples were taken, with irrigation of plots to assess the effects of sprouting and the relationship between dormancy and HFN.

In addition to the multi-site field study, controlled environment, laboratory experiments and small-scale field trials were undertaken to study PMAA at Harper Adams and RPAA and PrMS at Sutton Bonington during 1994-1997. Specific samples and plots from the multi-site field trial were investigated further for these routes where appropriate. In 1996 and 1997 a pilot HFN prediction scheme was operated using intelligence from the first half of the project, and specific sub-experiments were completed to provide the data needed to fine-tune the protocol of the prototype scheme.

4. OBJECTIVES 2 (b), 5 (a), 5(b) and 6: RESEARCH NEEDED TO INVESTIGATE THE FOUR ROUTES OF *ALPHA*-AMYLASE ACCUMULATION AND IMPLEMENT AN HFN PREDICTION SCHEME FOR THE UK: EXPERIMENTAL DESIGN AND RESULTS.

4.1. Investigation of the effects of pericarp *alpha*-amylase (RPAA) on HFN.

4.1.1. Materials and Methods - RPAA Studies

4.1.1.1. Methods for determination of the route of alpha- amylase accumulation

Samples with significant *alpha*–amylase accumulation (after HFN was measured at 800-850 °Cd post-heading as part of the investigations of the prototype scheme) were identified due to their low HFN, < 250 s (see *Section 4.4.1.5.* for HFN method). Further testing allowed identification of the route of *alpha*-amylase accumulation.

4.1.1.1.1. Visual observation

Observation of subsamples of grain often allowed a preliminary diagnosis of the probable cause of low HFN. Immature grains, with the possibility of RPAA activity, could be identified by their obvious green colour. Orange wheat blossom midge damage (see *Section 4.3.*) was obvious due to shrivelling of the grain, bleaching and indentation of the pericarp, secondary fungal infection, or characteristic splitting of the pericarp with concomitant plumule growth and presence of the small orange midge larvae. PrMS/PoMS could be identified by visible radicles and/or plumules, although these could be knocked off by grain processing (combine harvesting/threshing). PMAA could be intimated in samples of low HFN with no visual signs of germination or the other phenomena.

4.1.1.1.2. Fluorescein-dibutyrate staining

Samples of 50-300 grains per replicate were examined for lipase activity associated with the

embryo which is an indicator of sprouting. Grains were mounted crease upper-most and were fixed on a cernit clay block (Danbrew Ltd, Frederiksberg, Denmark) using a seed fixation system. After dorsoventral longitudinal rotary sanding, to reveal the embryo, the remaining half-seeds obtained were stained for 10 minutes with fluorescein dibutyrate (ICN Biochemicals, Cleveland, Ohio, USA) solution (236 mg in 90% aqueous ethanol). Plates were then soaked in water at 60°C for 2 minutes. Excess water was dabbed off before the plates were viewed under ultraviolet light in a Malt Modification ultra-violet analyser (Carlsberg system; Danbrew Ltd, Frederiksberg, Denmark). The appearance of yellow fluorescence near the embryo indicated the presence of a sprouted grain. The degree of sprouting in individual grains was assessed subjectively according to the method of Jensen *et al.* (1984).

4.1.1.1.3. Iso-electric focusing

Alpha-amylase was extracted from 0.15 g - 0.50 g milled flour samples (or an equivalent mass of single grains crushed with a pestle and mortar) in eppendorf tubes with 1 ml of standard extraction medium (20 gl^{-1} sodium chloride, 0.2 gl^{-1} calcium acetate). After a 1 hour incubation in a 30°C waterbath (with agitation each 15 minutes), the tubes were centrifuged at 2700 rpm for 10 minutes. The supernatant was carefully removed and heated at 70°C in a water bath for 15 minutes to inactivate *beta*-amylase activity. The sample was finally centrifuged to remove any remaining solid material. These procedures were modified from the method of Sargeant and Walker (1978).

Extracts were separated according to their iso-electric point (pI) by IEF using Ampholine PAGplate pre-cast pH 3.5-9.5 polyacrylamide IEF gels and a Multiphor II electrophoresis tank and power pack (Pharmacia Biotech, Uppsala, Sweden). The anode was soaked in 0.1 M glutamic acid/0.5 M phosphoric acid and the cathode was 0.1 M β-alanine. 20µl aliquots were placed in wells or on paper applicator strips on gels cooled to 10°C. The gel was prefocused and samples run in before iso-electric focusing at 200 V/25 mA for 2.5 h.

After IEF, the gel was soaked in pre-warmed 1% Fluka potato starch (Fluka Chemie AG, Buchs, Switzerland) solution at 30°C for 10 minutes. Distilled water was used to wash the gel twice

to remove excess starch. The gel was then stained by gently rocking for 5 minutes in 0.26 gl^{-1} iodine/0.78 gl^{-1} potassium iodide solution, with two washes in distilled water to remove excess iodine. *Alpha*-amylase activity was then clearly visualised as transparent bands against a purple-brown background. Gel photographs were taken with a Nikon SLR camera with transillumination as soon as possible after iodine staining.

4.1.1.1.4. Location of *alpha*-amylase activity with the *beta*-limit dextrin gel test.

A solution of 1% (w/v) agarose (BDH, Poole, UK) was prepared by heating 0.5 g agarose in 50 ml 20 gl^{-1} sodium chloride/0.2 gl^{-1} calcium chloride buffer (standard *alpha*–amylase autoanalysis extraction medium). A 10% (w/v) stock *beta*-limit dextrin solution was made using the same stock buffer. After the agarose dissolved, 10 ml of *beta*-limit dextrin solution was added and mixed, before gels were poured out in standard petri dishes and allowed to set at room temperature. Grains, dissected transversely or longitudinally, were placed cut-side down on the set gel and were kept for 25 minutes at 30°C in an incubator. The gel was developed with 0.13 gl^{-1} iodine/0.039 gl^{-1} potassium iodide solution made from diluted standard 6.5 gl^{-1} iodine/19.5 gl^{-1} potassium iodide stock. The gel test could identify three general locations for *alpha*-amylase activity: a faint peripheral halo associated with the pericarp (RPAA), stronger staining associated with the crease region but not the embryo (PMAA) and very strong staining (often forming an amorphous blob covering the position of the whole grain), associated with sprouting (PrMS/PoMS).

4.1.1.1.5. Location of *alpha*-amylase activity with the Phadebas gel test.

The Phadebas method used was developed at CCFRA (A.D. Evers and D.G. Bhandari, personal communication - manuscript in preparation). A solution of 1.3% (w/v) agarose (BDH, Poole, UK) was prepared by heating 0.033 g agarose in 2.5 ml distilled water at 50°C with a magnetic stirrer. 0.066 g Phadebas powder (Pharmacia Biochem, Uppsala, Sweden) was washed out with 2.5 ml distilled water into the agarose solution to make to make a final 0.65% agarose, 1% Phadebas solution. Aliquots of 2 ml were pipetted onto clean petri dishes and left to set at 10°C fro 24 hours. The gels were used within 7 days. Grains (25 per plate) were cut transversely and

longitudinally and were carefully placed on the set gel plates, avoiding pressure. Plates were incubated at 10°C showing *alpha*-amylase activity by digestion of the blue Phadebas substrate to leave a clear area of gel on transillumination. Sprouted grains were detectable in 30 minutes, whereas detection of PMAA took four hours. After incubation, grains were carefully removed from the gels which were cooled in an icebox and washed carefully with distilled water before photography.

The different routes of *alpha*-amylase accumulation could be identified as described in *Section 4.1.1.1.4.* However, the Phadebas gel test was more rapid due to the lack of a staining stage.

4.1.1.2. Reconstitution of high-HFN flours with pericarp alpha-amylase (α-AMY-2) activity

A dilution series of model flours was made from high-HFN flours (*Pastiche* and *Hornet*, Sutton Bonington, 1995) and samples with pericarp *alpha*-amylase activity. Increasing percentages (1-25%) of hammer-milled pre-500°Cd post-heading freeze-dried grains (*Pastiche* and *Hornet*, 1995) were added, with thorough mixing, before HFN determination (as described in *Section 4.4.1.5.*) by the standard method (Anon., 1982). The identity of the *alpha*-amylase isozyme present was checked by IEF and the *alpha*-amylase activity in the freeze-dried green flour was assayed as described in *Section 4.2.1.4.*

A second dilution series of high HFN flours (*Pastiche* and *Hornet*, Sutton Bonington 1996) containing increasing quantities (1-25%) of freshly defrosted non-freeze-dried pre-500°Cd green grains (*Pastiche* and *Hornet,* SB 1996) was also made. Parent flours consisting of a 25% green-grain/75% control flour mixture were milled in a Retsch ZM 1000 cyclone mill (Retsch GmbH, Haan, Germany) since the moisture content was too high for hammer-milling. The rest of the dilution series was made by thoroughly mixing the appropriate amounts of '25% green' and control (high HFN) flours produced from the falling number mill (Falling Number AB, Sweden). HFN values, with correction for moisture content (determined by oven-drying for 90 minutes at 130°C), were then recorded following the usual practice (*Section 4.4.1.5.*).

A final series of flours (*Pastiche* and *Hornet*, Harper Adams 1996) containing 1-25% fresh,

unfrozen green grains (pre-500°Cd *Pastiche* and *Hornet,* HAAC 1997) was made by diluting 25% green parent flours with control flours made by following the same procedure as above in 1997. HFN determinations were made according to the standard method.

4.1.1.3. Induction of late tillering and investigation of the effects of glyphosate

In 1997, late tillering was induced in triplicate non-irrigated plots of the French cultivars *Scipion, Thesée* and *Récital* at Sutton Bonington. Alternate groups of two rows of developing ears were mowed out of 6 m lengths of the plots with a garden strimmer, starting from a randomly chosen end of the plot, on 2nd June 1997, to allow late tillering to progress. Control, 4 m - unmown areas were left at random ends of the plots. On 23rd July 1997, randomly selected halves of the mown areas of the *Scipion* plots were sprayed with 1 lha^{-1} *Roundup Biactive* containing the active ingredient *glyphosate.* Sprayed and unsprayed areas of the *Scipion* plots were harvested by hand on 14th August 1997. Unmown areas of *Scipion* and unmown and mown areas of *Récital* and *Thesée* were combine harvested on 19th August 1997. Green and mature grain numbers in ~ 50 g subsamples were analysed with a Decca Mastercount seed counter. HFN values were determined according to the usual method, although not all samples were directly comparable due to the logistics of timing harvesting.

4.1.1.4. Effects of air-drying on pericarp alpha-amylase activity

Grain samples with various drying treatments were produced by air- or forced-air oven-drying hand-threshed freshly defrosted grains (*Hornet* and *Pastiche* SB 1996). Subsamples were dried at various temperatures between 15-55°C for various times between 2-14 days. The affect of this process on *alpha*-amylase enzyme activity was assessed by iso-electric focusing (as in *Section 4.1.1.1.3*) and semi-automated *alpha*-amylase analysis (as described in *Section 4.2.1.4.*)

Reconstituted flours were made by mixing high HFN control (*Hornet* and *Pastiche,* SB 1996) flours and hammer-milled flours from freshly defrosted grains (*Hornet* and *Pastiche* 1996) air-dried at 25°C or oven-dried at 55°C. The HFN was determined as previously described.

Replicate mixtures of high-HFN grain (*Pastiche* and *Hornet*, Harper Adams 1996) containing 10% freshly isolated grains (*Pastiche* and *Hornet*, Harper Adams 1997, or *Hornet*, Sutton Bonington, 1997) were made. The samples were left to air-dry in the laboratory, with individual replicates removed for milling and HFN determination during a 0-20 day period to assess the effects of air-drying in whole grain mixtures.

4.1.1.5. Markers for pericarp alpha-amylase activity.

In 1997 triplicate plots of *Pastiche* at Harper Adams and Sutton Bonington were intensively sampled during the dough stage. Grains were isolated and freeze-dried for assessment of moisture content (*Section 4.4.2.1.*) and *alpha*-amylase activity (*Section 4.2.1.4.*). Grain colour was determined on replicate samples of 100 grains by reference to Royal Horticultural Society colour charts, apportioning grains to the different colour classes in any one sample and recording the proportion in each. The accumulated temperature (°Cd) for each sample was determined from the sum of the mean diurnal temperature from heading (Zadoks growth stage 55, Tottman and Broad, 1987) to the date of the sample.

4.1.2. RPAA Study Results

4.1.2.1. Identification of the route of alpha-amylase accumulation, especially RPAA

Fluorescein dibutyrate testing was not appropriate for demonstrating incipient sprouting as identification of lipase fluorescence lagged behind visual observation of sprouting, *i.e.* emergence of the plumule and radicle from the embryo. It could however show the lipases associated with more advanced sprouting and was useful for combine samples when visible signs of sprouting were removed (*i.e.* small roots and shoots knocked off during harvesting).

Iso-electric focusing identified the different isozyme groups due to their different isoelectric points (α-AMY-1 high pI, α-AMY-2 low pI, α-AMY-3 very high pI: *Figure 4*). This allowed identification of RPAA if only low pI α-AMY-2 enzymes were present. In the other syndromes the expression of both isozyme families, in different concentrations and at different times, was

possible. In these cases further testing was required for diagnosis. There were some problems with the IEF technique due to temperature-sensitivity of the enzymes which could lead to some *alpha*-amylase denaturation during the 15 minute 70°C heat treatment used to inactivate *beta*-amylase enzymes. In particular, work in collaboration with CCFRA showing differences in the temperature sensitivity of various isozyme families indicates that the preparations for IEF may destroy some of the evidence required to distinguish the PMAA/PrMS/PoMS syndromes.

Beta-limit or Phadebas gel testing was useful to show incipient sprouting before FDB or visual germination and was also good for discrimination of the routes due to the different location of the *alpha*-amylase in each syndrome (RPAA peripheral, PMAA in the crease, PrMS/PoMS the embryo; *Figure 5* showing a *beta*-limit dextrin gel). Phadebas gel testing eventually proved the more useful and rapid test as it had no requirement for staining. This test may eventually prove useful in any further development of the HFN prediction scheme to speed up the assessment of dormancy from germination testing, or for use in the field. However, since a combination of the various *alpha*-amylase locations was often seen in particular samples, it was difficult to ascribe low HFN to one major cause in some situations.

The methods described can therefore be used in concert to identify the various routes of accumulation (Lunn *et al.*, 1996). Consideration of the methods available for identification of *alpha*-amylase location showed that the possibility of RPAA could be considered in samples with low HFN, without visible signs of sprouting (no FDB-positive staining) and possibly with the presence of green grains. In the case of RPAA, iso-electric focusing shows the presence of α-AMY-2 isozymes (*Figure 4*) of low pI *in the absence of high-pI α-AMY-1* and Phadebas gel testing shows a halo of starch digestion around the periphery of the grain (*Figure 5*).

4.1.2.2. Activity of a-AMY-2 in reconstitution experiments

Iso-electric focusing of the milled flours reconstituted with freeze-dried, freshly defrosted and native green grains showed only low pI α-AMY-2 activity diagnostic of pericarp amylase (*Figure 4*), with no α-AMY-1 activity. Therefore, all the effects in the reconstitution experiments were due to pericarp amylase. Reconstitution with freeze-dried, freshly defrosted and native green

grains containing α-AMY-2 showed conclusively that α-AMY-2 isozymes were active in the HFN test, which had been previously discounted due to a reported thermal instability of the enzyme, since HFN was reduced compared to the control in all the reconstitution experiments.

All the reconstitutions fitted the same general negative exponential relationship, with increasing incorporation of green flour/green grains (corresponding to increasing α-AMY-2 activity) causing reduction in HFN. *Figure 6* shows the raw curves for the cultivar *Pastiche,* showing HFN against w/w percentage green grain incorporation. The cultivar *Hornet* showed the same general trend. For *Pastiche*, it was possible to reduce a very high HFN (475 s) to below commercially acceptable criteria by addition as little as 5% green grains (75-100 $mEUgdw^{-1}$) α-AMY-2 activity. The curves differ in shape due to differences in moisture content and *alpha*-amylase activity in the raw materials.

4.1.2.3. Late tillering and effects of glyphosate

Late tillering was effectively induced by mowing out regions of the developing plot. Assessment of late tiller numbers showed about 20% green ears were induced in mown, non-*glyphosate*-sprayed areas of the plots, between mowing on 2nd June 1997 and harvesting on 14th - 19th August 1997 (*Table 2*). Spraying with 1 lha^{-1} *Roundup Biactive* killed all of the late-developing green ears in the sprayed areas of the *Scipion* plots within two weeks of spraying.

Analysis of combined and hand-threshed samples showed there were significantly more ($p<0.05$) green grains in samples from mown areas than non-mown areas (ANOVA of a standard angular transformation of the percentage green grain data). Due to the logistics of sample harvesting, processing and transport, direct comparison of HFNs was not possible. Comparison of grain samples from hand-threshed mown-sprayed and mown-unsprayed areas of the *Scipion* plots showed that the *glyphosate*-containing herbicide *Roundup Biactive* significantly reduced ($p<0.05$) numbers of green grains in sprayed plots by killing late tillers. A significantly improved HFN ($p<0.05$) was found in some mown-*glyphosate*-sprayed samples compared to mown-nonsprayed samples (*Table 2*). More detailed direct comparison of HFN was not possible due to complications caused by differences in the timing of sample processing procedures. Further work

is needed to fully investigate the effect of *glyphosate* on HFN.

***Table 2**: Effects of mowing and glyphosate-spraying on green grains and Hagberg falling number in combined samples.*

		Récital	*Thesée*	*Scipion*
% Green ears before harvest (18.8.97)	Mown	35.70	28.7	40.11
	Mown, Sprayed	-	-	0.00
	Unmown	0.00	0.00	0.00
	SED	4.64		
	LSD	10.53		
% Green grains[1] in combine (or hand-threshed) sample (19.8.97)	Mown	9.13 (2.58)	11.68 (4.58)	13.61 (5.59)
	Mown, Sprayed	-	-	2.78 (0.45)
	Unmown	0.00 (0.00)	0.00 (0.00)	2.36 (0.29)
[1]Angularly transformed data, original data in brackets	SED	1.87		
	LSD	4.21		
Hagberg falling number (s)	Mown	268.00	330.00	348.30
	Mown, Sprayed	-	-	413.30
	Unmown	266.00	342.00	333.00
	SED	26.60		
	LSD	67.50		

4.1.2.4. Air-drying experiments

Analysis of *alpha*-amylase activity in air- and oven-dried immature grain samples (containing only pericarp amylase activity) showed that α-AMY-2 activity was reduced by about 70% by air-drying, but that it was not completely abolished (*Table 3*). Both air- and oven-drying over the range 15-55°C reduced α-AMY-2 activity by similar amounts.

***Table 3**: Effects of air and oven-drying on α-AMY-2 activity in green grains*

Treatment	Fresh	15°C Air-dry	20° Air-dry	27° Oven-dry	55°C Oven-dry
Amylase activity (mEUgdw^{-1})	1555	380	350	432	444

Air-dried α-AMY-2 was still effective in reducing HFN, as shown by reconstitution of sound, high HFN control flours (*Hornet* and *Pastiche*, SB 1995) with increasing amounts of air- or oven-dried flours (*Figure 7*). The HFN was reduced to a much lesser extent than with addition of similar amounts of freeze-dried α-AMY-2, although there appeared to be no difference between the effects of air-drying at 25°C and oven-drying at 55°C on residual α-AMY-2 activity.

The high α-AMY-2 activity in freshly defrosted grains was rapidly reduced (from about 750 mEU gdw^{-1} to about 400 mEU gdw^{-1} in 10 days, *Figure 8*) by air-drying, showing the potential for rapid change in HFN of RPAA-affected grain lots due to reduction in α-AMY-2 activity during storage. However, air-drying of bulk grain reconstituted with 10% fresh green grains showed that the effects of pericarp amylase could not be negated by drying in all situations (*Figure 9*). Although one cultivar, *Pastiche*, showed rapid inactivation of α-AMY-2 activity, which initially depressed HFN, in *Hornet* the reduction caused by α-AMY-2 activity was observed even after 20 days air-drying, by which time the HFN in the *Pastiche* was the same as the control.

4.1.2.5. Markers of amylase activity

The crop moisture content at the 825°Cd sample point (*Figure 10*) specified for pre-harvest HFN measurement (*Section 4.4.2.2.*) shows a great degree of variation between sites and seasons, indicating that accumulated temperature is a poor marker for this developmental stage in grain development. The *alpha*-amylase activity in the cultivar *Hornet* at various sites and seasons also showed considerable variation in the end of α-AMY-2 degradation when plotted on an accumulated temperature scale (data not shown). Crop colour was also a poor marker of *alpha*-amylase activity due to a large amount of heterogeneity between grains. Significant *alpha*-amylase activity persisted in serially sampled grains even after the disappearance of the green colour traditionally associated with pericarp *alpha*-amylase (data not shown).

4.2. Investigation of the factors inducing pre-maturity amylase accumulation in the absence of sprouting (PMAA)

4.2.1. Materials and Methods -PMAA Studies

4.2.1.1. Effect of drying rate on alpha-amylase activity

The grain moisture content of crops grown in the multi-site field study was determined at approximately 100°Cd intervals throughout grain development, commencing at 300°Cd post-heading (Gate, personal communication) using the method described in *Section 4.4.1.3.* The *alpha*-amylase activity in combine harvested grains was measured according to the method described in *Section 4.2.1.4.* The moisture content data was used to calculate drying rates (against time or accumulated temperature) which were then related to *alpha*-amylase activity.

4.2.1.2. Effect of a short period of high-temperature on PMAA (Experiments 1 and 2)

Dressed grains of the cultivars *Pastiche* and *Riband* were sown in John Innes No. 2 compost in a glasshouse and were watered daily until ZGS 10-11 (7 days). The seeds were then vernalised for 70 days (4°C, 8 h day). The seedlings were potted in John Innes No. 2 compost in 4" pots and

allowed to develop to anthesis (ZGS 61) when tillers were individually tagged. Plants were watered daily and received two applications of fungicide (Corbel-Fenpropimorph) and 1 application of aphicide (Aphox-Pirimicarb).

20 days post-anthesis (dpa), 30 plants of each variety were placed in a completely randomised layout in a Conviron (Controlled Environments Ltd., Winnipeg, Canada) controlled environment cabinet (A), 16 h light/8h dark (0.204 $mmolm^{-2}s^{-1}$ light intensity) and 16 (±2)/10 (±2)°C cycle. Relative humidity was maintained at 85 (±15)% and plants were watered daily.

After periods of 32, 38, 43 and 50 days post-anthesis, six randomly-chosen plants from each variety were transferred to Conviron cabinet B, which had a light-dark temperature cycle of 26 (±2)/20 (±2)°C, with other conditions the same as cabinet A except for watering twice-daily to reduce water stress. After 72 hours in cabinet B, the plants were transferred back to cabinet A until harvest ripeness at 63 dpa (*Experiment 1*) . The transfers are summarised in *Table 4.*

At 63 dpa, six grains from the central spikelets of the main stem of each plant (36 in total) were removed from each treatment and freeze-dried for determination of moisture content (Edwards 4k Modulyo Freeze Drier). *Alpha*-amylase activity was then measured in each single grain using a Skalar (UK) *alpha*-amylase autoanalyser based on a system described by Smith (1974), using the Farrand (1964) technique to measure enzyme activity calibrated in Phadebas units (Barnes and Blakeney, 1974) as described in detail in *Section 4.2.1.4.*

A subsequent replication of this experiment was also completed.

4.2.1.3. Effect of reciprocal transfer between hot and cold temperatures on PMAA (Experiments 3 and 4).

This experiment was undertaken to identify whether it was a temperature change *per se* or a specific transient change to a high temperature that was responsible for causing changes in *alpha*-amylase activity. Reciprocal transfers of plants for three days between 16/10 °C and 26/20 °C environments were undertaken at three different stages during grain development. In *Experiment*

3, transfers were for 3 days. In *Experiment 4* transfer time between environments was increased to five and ten days, with transfers being undertaken at two stages during grain development.

The experimental treatments are summarised in *Table 4*

4.2.1.4. Semi-automatic alpha-amylase assay

Thirty single grains from each treatment were assayed for *alpha*-amylase activity using an air-segmented flow autoanalyser (Skalar (UK) Ltd, York, UK) based on a system described by Smith (1974). Sample extracts were compared by grinding single grains in a pestle and mortar. The ground material was then carefully transferred to a 1.5 ml centrifuge tube and 1 ml extracting solution (85.5 mM sodium chloride, 1.8 mM calcium chloride) was added. The tubes were then shaken for 5 minutes, centrifuged at 2700 rpm and the supernatant assayed. The system was calibrated in Phadebas units using flours of known *alpha*-amylase activity.

IEF, FDB and gel test methods were used as described elsewhere to confirm that the *alpha*-amylase activity recorded was due to PMAA and no to RPAA, or PrMS/PoMS sprouting.

Table 4: *Treatments used in Controlled-Environment Experiments Investigating PMAA*

No.	Cultivar	*Length of Transfer Time	Treatment Growth Stage (°C days after anthesis)	Treatment Growth Stage ZGS	*Alpha*-Amylase Activity Assayed on	Light Intensity ($\mu mol\ s^{-1}$)
1	Pastiche Riband	3 day Hot C→H→C	560	75-77	Single grains	204
			630	77-83		
			700	85-87		
			798	87		
2	Pastiche Riband	3 day Hot C→H→C	324	73	Single grains	200
			390	73-75		
			488	75		
			739	83		
3	Hornet Pastiche Riband	3 day Hot C→H→C 3 day Cool H→C→H	518	73	Whole ear	350
			645	77		
			811	83		
4	Hornet Riband	5-day Hot C→H→C 10-day Hot C→H→C 5-day Cool H→C→H 10-day Cool H→C→H	600	77	Whole ear	250
			700	83		

*Hot = H = 26 /20 °C, Cool = C = 16/10 °C

4.2.2. PMAA Study Results

4.2.2.1. Effects of drying rate on alpha-amylase activity

Linear regression analysis (*Figure 11*) of the drying rate of the cultivar *Hornet* (the cultivar most susceptible to PMAA in our trials) between 50 and 20% moisture content from 3 separate trials (Harper Adams 1994 and 1995, Sutton Bonington 1994) revealed significant effects of site and time ($p < 0.005$, $R^2 = 94.5$), but identified no significant site.time interaction. This illustrates that the moisture loss of *Hornet* (between 50 and 20%) could not be used to explain the differences in HFN (thus *alpha*-amylase) of the samples and the data cannot be used to support the hypothesis of Gale *et al.* (1983).

4.2.2.2. Effects of a short period of high temperature on alpha-amylase activity

A short period of high-temperature shock in cool-grown plants increased *alpha*-amylase activity in harvest mature grains, even in cultivars (*e.g. Pastiche*) that never show PMAA in the field.The effect was more marked the earlier in development the transfer occurred.

Freeze-dried weights of harvested grain varied from 10 mg - 47.5 mg, the low weight attributable to the relatively low light intensity available in the controlled environment cabinets. There was large variation in the *alpha*-amylase activity of individual grains, although the effect of intra-ear variation in grain weight and *alpha*-amylase (Evers and Ferguson, 1980), was minimised by sampling consistently from central spikelets. No relationship between grain weight and *alpha*-amylase activity was observed, although this does not rule out the hypotheses of Evers *et al.* (1995) concerning grain size.

For data analysis, specific *alpha*-amylase activities were subjected to a natural log transformation. Analysis of variance revealed there was a significant effect ($p<0.05$) of time of transfer between cabinets A and B on *alpha*-amylase activity in the grain, although there was no significant cultivar effect. *Figure 12* illustrates the mean log *alpha*-amylase activity ($mEUgdw^{-1}$) in grains at each transfer date. Transfer to high temperatures for 3 days after 32 dpa significantly

increased ($p<0.05$) *alpha*-amylase activity in the grains compared to control grains. IEF showed the source of the activity was high pI α-AMY-1. The later transfers did not vary significantly in activity from the control grains. There were no visible signs of sprouting in any grains at harvest. As shown in *Figure 12*, the fitting of polynomial functions to *alpha*-amylase activity with time revealed a clear linear relationship between accumulated temperature (°Cd) from anthesis to transfer and ln *alpha*-amylase activity. The replication of this experiment showed broadly similar results. The untransformed data for the two experiments are shown in *Figure 13*.

4.2.2.3. Effects of reciprocal transfer between hot-cold and cold-hot conditions on alpha-amylase activity

Transfers from hot/cold/hot environments at early milk (511°Cd), late milk (631°Cd) and the soft dough stage (823°Cd) increased *alpha*-amylase activity in all three cultivars studied (*Riband, Hornet* and *Pastiche*), compared to control plants kept solely in the hot environment (*Figure 14*). The effects of cold/hot/cold transfers on *alpha*-amylase activities in this experiment were more variable, with only *Hornet* showing a clear increase in *alpha*-amylase activity at all developmental stages (*Figure 14*). Both *Riband* and *Pastiche* showed increase in PMAA activity at 631°Cd only, The lack of response to temperature change in the other two developmental stages reflects the results seen in the previous experiments.

Hornet appeared to be stimulated to produce PMAA by transient hot and cold changes in temperature at the three developmental stages investigated. *Riband* and *Pastiche* appeared to be less susceptible to this phenomenon with transient changes to cold temperature stimulating PMAA at the three developmental stages investigated, but transient changes to high temperature being less effective. These results support the hypothesis that transient change to cold temperature can be effective in stimulating PMAA formation (Mrva and Mares, 1994).

The results from these experiments have confirmed that the stimulation of PMAA formation is a complex phenomenon governed by both the genetic basis of the cultivar and its interaction with environmental temperature. Therefore, it appears that it is temperature changes during early grain development which are important stimuli for PMAA formation in certain wheat cultivars. These

effects will need to be quantified by further experiments to vary the length of exposure to temperature change in an effort to develop a simple robust model for predicting PMAA formation in the field. The inconsistent effects of transfer time may indicate an effect of an even earlier conditioning period (since plants for the separate experiments were grown in glasshouse conditions at different times of the year) which also requires further investigation.

4.3. Investigation of the factors inducing pre-maturity sprouting

4.3.1. Materials and Methods - PrMS Studies

4.3.1.1. Investiagtion of weather factors affecting pre-maturity sprouting

In 1995, wheat plants (cultivar *Hornet* and *Riband*) were grown to anthesis in a glasshouse as described in *Section 4.2.1.2.* After ZGS 83 (early dough stage), experimental plots were irrigated manually three times a day (9 am, 12 am, 5 pm) until harvest ripeness, with the addition of a minimum of 3 $lday^{-1}$ of water to each plant, completely drenching the ears and leaves. Pre-maturity sprouting was assessed by dissection of ear samples and assessment of moisture content in comparison with non-irrigated control plots, grown in an adjacent bay of the glasshouse. Glasshouse temperatures varied greatly in the range 10-45°C.

In 1996, 100 pots of wheat (cultivars *Hornet, Riband, Pastiche* and *Récital*) were grown in a randomised block design to ear emergence as described previously. Potting on of vernalised plants was staggered on a sequential fortnightly basis to produce material at different accumulated temperatures post-heading at the time of irrigation. At ZGS 55, main tillers were tagged and a running total of accumulated temperature (sum of mean daily temperature > 0°C) was calculated. Ten pots of experimental plants were transferred to beneath an overhead irrigation system (Access Irrigation Ltd), in a separate bay of the glasshouse, for 5 days at approximately 500, 700, 800, 900 and 1000°Cd post-heading and were subsequently allowed to dry down in the control bay of the glasshouse. The irrigation operated on a standardised daily cycle (2 minutes irrigation each half hour between 0800-2000) with the amount of irrigation (mm) measured using a plastic raingauge. Corresponding control plants were kept in an adjacent,

dry bay of the glasshouse and were sampled on the same date as the experimental plants. Ears from the main and secondary tiller were collected and pooled from each pot of each experimental treatment and were then hand-threshed, winnowed and assessed for PrMS by visual observation of the percentage of sprouted grains. Temperatures in the glasshouse were in the range 10-45°C.

4.3.1.2. Relationship of wheat orange blossom midge infection to pre-maturity sprouting

Samples from ADAS trials investigating insecticidal efficiency at midge control at ADAS Boxworth in 1994 and ADAS Bridgets in 1995 were tested for pre-maturity sprouting using the *in vitro* ear dormancy test described in *Section 4.4.1.6.2*. Data concerning the degree of midge infestation in plots was provided by ADAS, to allow sampling from plots with a range of midge infestation.

In 1995, orange wheat blossom midge pupae were collected in April from a field (Place Farm, Bury St. Edmunds, Suffolk) with a high inoculum (up to 65 pupae kg^{-1} recorded in April). Topsoil samples up to 5 cm deep were collected with a trowel and were pooled into ~ 3 kg samples. The orange pupae were removed from sifted soil by hand. Pupae were then incubated at 17-19 °C in the laboratory, in fine (sifted) soil samples in polythene bags, kept moist by spraying every other day with a domestic hand-trigger sprayer. As adult midges started to emerge, these samples were placed in 5 replicate plots of the cultivars *Hornet* and *Riband* at Sutton Bonington, in small (1 m^2) enclosures made of Agrifleece 25 (Bourne Supplies Ltd, Bourne, UK) polyester material, to attempt to produce a high degree of midge infestation by preventing midge flight. The mesh size was small enough to restrict midge flight, whilst allowing entry of diffuse sunlight without excessive temperature increase. Samples of 30 ears were assessed for increased susceptibility to PrMS by IVED and IGG testing at various stages in the dough stage of development. Midge activity was analysed by dissection of ears to record the number of infesting larvae.

In 1997, samples from a sub-population of late tillers in plots of *Pastiche* were dissected and analysed for midge infestation, when high numbers of larvae were noted during other routine analyses.

4.3.1.3. Iso-electric focusing of midge amylase activity

Samples of frozen larvae and midge-infected grains from 1994 ear dissections at Boxworth and samples of severely midge-infected grains from 1987-1993 (various sites) were provided by ADAS. These samples were ground in a pestle and mortar and were extracted for *alpha*-amylase activity using the standard extraction medium as described in *Section 4.1.1.1.3.* IEF was performed as described previously.

4.3.1.4. Simulation of midge damage

Grains isolated from developing ears (as described in *Sections 4.4.1.2. - 4.4.1.3.)* in various years were tested for germination (as described in *Section 4.4.1.6.2.*) after simulated midge damage. Simulated damage was caused by an ~ 4 mm long acropetal dorsal cut through the pericarp with a razor blade. Some of the treatments were incubated (10-25°C) without wetting to test damage-induced sprouting using endogenous moisture, others were incubated on wet filter papers, simulating wet weather conditions. Control grains were not cut but also had wet and dry treatments.

In 1997, plants were grown for a cabinet experiment as described in *Section 4.2.1.2.* Replicate plants, 32 per cultivar, of *Riband* and *Hornet* were grown in cool (16/10°C ± 2°C) and warm (26/20 ± 2°C) cabinets with a 16/8 h light/dark cycle and humidity maintained at 85 ± 15%. The plant pots were watered daily to prevent drying. After 7th July 1997, temperatures were reduced to 20/16°C and 14/10°C (± 2°C) in the warm and cold cabinets respectively, to slow drying. On 27th June 1997 (warm cabinet) and 7th July 1997 (cool cabinet) 6 grains in florets 1 and 2 of the central 6 spikelets were cut acropetally through the glumes with a razor blade to simulate midge damage whilst *in situ* on the plant. Sixteen plants per cultivar were cut. Each day the same eight cut and eight uncut plants per cultivar (originally randomly chosen) were removed from the cabinets to have their ears thoroughly irrigated manually with a domestic hand trigger sprayer on three occasions. The ears were harvested at growth stage 92 (8th July and 8th August 1997 for warm and cool cabinets respectively) and the cut grains were recovered after hand-threshing and were analysed for the percentage sprouted grains.

4.3.2. Results - PrMS Studies

4.3.2.1. Effects of irrigation in the absence of midge infection.

The effect of intense irrigation at relatively high temperature during the late dough stage of grain development was first investigated with a glasshouse experiment in 1995. Experimental plants were subjected to intense irrigation three-times daily during the dough stage of development, commencing on 15th August 1995 when the moisture content of the grains was approximately 45%. During each irrigation event, the whole plant was completely drenched, receiving at least 1 litre per irrigation event. However, the temperatures in the glasshouse were very high (on some days the maximum recorded temperature was in excess of 45°C) during the very hot summer so the ears often soon dried out. Ears were sampled and dissected to analyse degrees of sprouting at 7-14 d intervals, with sampling on the 5th and 22nd of September and the final harvest on 29 September. *Table 5* shows that pre-maturity sprouting was possible at about 35 % moisture in *Hornet* due to the application of exogenous moisture, despite the very high daytime ambient temperatures. In *Riband*, the start of sprouting was not observed until approximately 20% moisture, although this was still before nominal ripeness (defined as 15% moisture).

***Table 5**: Pre-maturity sprouting induction by intense irrigation at high grain moisture content*

Date	Zadoks Growth Stage	Moisture Content (approx.)	Percentage Sprouting			
			Hornet		*Riband*	
			Control	Wet	Control	Wet
15/8/95	79	45%	0.0	0.0	0.0	0.0
5/9/95	82-5	35%	0.0	0.0	0.0	1.5
22/9/95	85-90	25%	0.0	1.3	0.0	3.6
29/9/95	90-92	20%	0.0	2.9	0.0	7.5

In 1996 a similar experiment was carried out, except that replicate groups of plants of *Haven, Riband, Pastiche* and *Récital* were placed under an irrigation system for a fixed number of days at various accumulated temperatures after ear emergence, throughout development. The results appeared to show a phase of increased sensitivity to irrigation at 800°Cd for the cultivar *Haven*, although not for the other cultivars (*Figure 15*). Although the amounts of irrigation received during the cycle varied due to the design of the irrigation system, this alone could not account for the variation in sprouting seen in *Haven*. The results shown were normalised for 100 mm of irrigation, although actual irrigation levels varied from c. 70-160 mm.

4.3.2.2. Relationship of pre-maturity sprouting to midge damage

Samples from ADAS Boxworth investigated in 1994 had no significant difference in midge infestation ($p > 0.1$) due to generally unsuitable conditions for oviposition (*i.e.* cold, windy June conditions), so no significant relationship ($p > 0.1$) of degree of midge infestation to PrMS could be determined. In 1995, the samples from ADAS Bridgets did have significantly different levels of midge attack and midge damage ($p < 0.05$). However, differences in PrMS-susceptibility were not observed ($p > 0.1$), possibly due to general insensitivity of the IVED test. In 1995, high levels of artificial infection of enclosed areas at Sutton Bonington did not arise due to the difficulty in synchronising midge emergence and crop susceptibility and unsuitable conditions for oviposition (again, cold June temperatures)

A sub-population of heavily midge-infected ears of the *Pastiche* from Sutton Bonington in 1997 was found in the pursuit of other observations. These samples showed a high degree of midge damage (about 30%), much greater than that seen in the more mature ear population. Coincident with the high degree of midge damage was a high level of pre-mature sprouting in grains of high moisture content, with severe pericarp damage (*Table 6*)

Table 6: *Degree of midge damage in a sub-population of green* Pastiche *ears, Sutton Bonington 1997*

	Green grains (40% moisture)	*Mature grains (15% moisture)*
% midge-damaged grains	33.30	0.32
% pre-mature sprouted grains	3.20	0.00

4.3.2.3. Midge damage iso-electric focusing

Iso-electric focusing studies (*Figure 16*) using material sourced from minor outbreaks at ADAS Boxworth and ADAS Bridgets in 1994 demonstrated that endogenous midge amylolytic activity was very weak and transient compared to standards of the plant *alpha*-amylase isozymes α-AMY-1 and α-AMY-2. The clear band, at high pI (~ 3.5) rapidly faded after staining of the gel with iodine, possibly due to high acid lability (Kruger, 1989). This activity could only be isolated from immature, feeding larvae or immature (green-yellow ripe) parasitised grains. The midge activity could not be isolated from mature, resting larvae or mature, parasitised (obviously 'midge-damaged') grains, indicating that midge amylase *per se* does not affect HFN.

Analysis of mature midge-damaged grains provided by ADAS showed that the reduction in HFN could be explained by presence of the characteristic sprouting or germination pattern of plant enzymes, α-AMY-1 and α-AMY-2. This pattern was not seen in 'Type I' (shrivelled) or minor 'Type II' or 'III' damage (indentation or bleaching of the pericarp on dorsal or ventral sides respectively). The pattern was observed only in severe Type II or III damage where the pericarp was split to reveal the endosperm. The pattern was observed in such cases both with and without visible sprouting. The effect of midge-damage on HFN is therefore manifested by damage to the pericarp, abolishing dormancy as well as allowing easier ingress of water.

4.3.2.4. Midge damage simulation (cutting) and irrigation experiments

During dissection of developing midge-infected samples from Sutton Bonington and ADAS Bridgets during the very hot, dry summer of 1995, a some dough-stage grains demonstrating severe Type II damage (split pericarp) and visible sprouting were observed. It was postulated that midge-damage to the pericarp could break dormancy and allow germination of the embryo of dough-stage grain due to its high endogenous moisture content. The affect of artificial damage to the seed coverings in promoting dormancy breakage was demonstrated by Belderok in 1960. In a small experiment, the positive effects on sprouting of an incision in the pericarp, with incubation at low temperature conducive to germination, was demonstrated for this kind of 'viviparous' sprouting in dough-stage grain (*Figure 17*). Germination was present in the absence of exogenously applied moisture, at low temperature, but was much more apparent with addition of exogenous moisture, at higher temperatures. The effect of the cutting treatment was to increase the temperature range over which germination was possible, compared to uncut controls.

In 1997, an analogous experiment was carried out on intact plants grown in controlled environment cabinets. Only treatments involving pericarp cutting and irrigation showed any signs of sprouting, with the cold cabinet condition showing then greatest degree of germination (up to 50%, *Table 7*). These experiments showed that midge damage and water availability interacted to increase the likelihood of pre-maturity sprouting. Either could promote the phenomenon on their own, although the syndrome was most marked when both factors interacted. The removal or dormancy caused by midge damage to the pericarp allows PrMS at less extreme conditions than would be required in the presence of PrMS.

Table 7: Degree of sprouting in midge damage simulation/irrigation interaction controlled environment experiment

	Cultivar and wetting treatment			
Treatment	*Hornet*		*Riband*	
	Wet	*Dry*	*Wet*	*Dry*
Hot, cut	2.0	0.0	0.0	0.0
Hot, uncut	0.0	0.0	0.0	0.0
Cool, cut	26.8	0.0	51.0	0.0
Cool, uncut	0.0	0.0	0.0	0.0

4.4. Multi-Site Field Trial and Investigation of PoMS

4.4.1. Materials and Methods - Multi-Site Field Trial/PoMS

4.4.1.1. Field Sites, Plot Design and Husbandry.

In 1994 field sites were at Sutton Bonington and Harper Adams. In 1995-1997, field sites were set up at Aberdeen University (Tilliecorthie Farm), Harper Adams Agricultural College, ADAS Bridgets and Sutton Bonington. Intelligence from sites at ITCF St. Aubin (near Paris) and ITCF Baziège (near Toulouse) was also available.

The cultivars used were *Récital, Thesée, Soissons and Scipion* (French) and *Pastiche, Riband, Hornet* and *Haven* (UK). The cultivars were grown in triplicate in a randomised split-split plot design, with the main plot treatment as irrigation/non-irrigation and the sub-plot as cultivar.

Husbandry and cultivation practices followed standard agronomic procedures in order to keep pests, diseases and lodging to a minimum.

4.4.1.2. Sampling.

Samples were initially scheduled following French protocols using accumulated temperature. Samples were taken at 100°Cd intervals from 300°Cd post ear-emergence (ZGS 55, Tottman and Broad, 1987). Accumulated temperature was calculated on a daily basis as the sum of the daily mean temperature from the date of ear emergence, using meteorological data recorded at the nearest local weather station (< 1 km from the field site). Samples of approximately 40-450 ears, according to the tests required at each sample point, were randomly selected from around the position of pre-marked ears. In 1994 and 1995, individual 60-grain samples were isolated and freeze-dried for *alpha*-amylase assay, identification of enzyme accumulation route, *etc.* From 1996, contingency samples were frozen at -20°C or dried at 50°C (after about 30% moisture content in the field) and examined only if a problem with *alpha*-amylase accumulation was identified from Hagberg samples. From 1996, sampling after irrigation moved onto a once weekly schedule due to logistical problems with continued use of 100°Cd intervals.

4.4.1.3. Fresh and Dry Mass, Moisture Content and Growth Stage

The fresh mass of duplicate or triplicate samples of 60 grains isolated from florets 1 and 2 of the central three spikelets of ten randomly selected ears was recorded. The dry mass of cooled samples was recorded after 48 h in a forced air oven at 80°C or 2 h at 130°C. Dry mass of samples for *alpha*-amylase assay or iso-electric focusing was recorded after freeze-drying in an Evans Modulyo 4k freeze-drier. Moisture content was determined as (FM-DM)*100/FM. Zadoks' growth stages (ZGS) were recorded subjectively by reference to published descriptions (Tottman and Broad, 1987).

4.4.1.4. Hagberg falling number sampling, irrigation and sprouting

In 1994, pre-harvest HFN samples were taken at ~ 900°Cd (> 0°C) post ear-emergence and in 1995 the target was ~ 800°Cd (> 0°C) post ear-emergence. In 1996 and 1997, pre-harvest HFN samples were taken on a Monday offering the closest compromise accumulated temperature to 800-850°Cd (> 0°C) post ear-emergence for all cultivars, with staggering of the sampling date between French and UK cultivars at some sites. Ear samples were gently dried overnight (to < 15% moisture content) in a forced air oven at < 50°C to preserve *alpha*-amylase activity. Dried ears were then threshed as soon as possible; the cleaned grain was despatched to Harper Adams Agricultural College for central HFN analysis. Fresh, undried samples of the cultivars *Riband* and *Hornet* were also despatched, by overnight courier immediately after sampling, to allow central germination testing from live material and investigation of the reliability of drying off-site.

After pre-harvest HFN assessment (Monday), the field irrigation systems were used in 3-4 day bursts (during Tuesday-Friday) to simulate a period of wet weather. The irrigation was then stopped for at least two days (Saturday and Sunday) allowing drying before re-sampling on the following Monday at ~ 850°Cd + 7d. The process was repeated at ~ 850°Cd +14d, ~ 850°Cd + 21d, *etc.*, to provide an irrigation series. HFNs of dried, threshed grain from the irrigation series was determined at the end of the experiment as described in *Section 4.4.1.5*. Subsamples of 2 x 100 grains were taken from bulk threshed samples for analysis of the percentage sprouting.

4.4.1.5. Hagberg Falling Number.

Samples were collected at <18% moisture content from the field, or if sampled at higher moisture content dried to <18% overnight in a 50°C forced-air oven. Approximately 300 ears were threshed (Walter and Wintersteiger, Maschinenbau Riedinnkr, Austria) and cleaned with an aspirated sample cleaner (A/S Rationel Kornservice, Esbjerg, Denmark). Grain samples of 50-300 g were milled with a hammer mill (Falling Number AB, Sweden). Flour moisture content was measured as described previously after drying duplicate 5 g samples in metal moisture cups for 2 h at 130°C in a forced-air oven. HFN was recorded on duplicate samples with flour mass

adjusted for moisture content, using the British Standard method (Anon., 1987). Duplicate values with > 5% difference were repeated until the difference was < 5%.

4.4.1.6. Dormancy Measurement.

4.4.1.6.1. *In vitro* ear dormancy tests

Twelve ears from each sub-plot were pooled for each cultivar and immersed for 6 h in water containing 2% sodium hypochlorite or 6% copper oxyquinoleate fungicide for surface sterilisation. The ears were then placed in sealed centrifuge tubes with approximately 4 cm of the culm attached and standing in 2 cm of distilled water. The tubes were left at ambient temperature in the laboratory for 14 days with the number of sprouted ears and visible sprouts per ear assessed at 7 and 14 days and the total number of sprouts per ear assessed at 14 days by dissection. The test was carried out from approximately 700°Cd post-heading to harvest, and on material weathered in the field after harvest. In 1995, the tests were dissected after 7 days incubation. The IVED test was not used in 1996 or 1997.

4.4.1.6.2. Isolated grain germination tests

Dormancy duration was assessed using isolated grain germination tests carried out for 7-14 days on damp filter paper in petri dishes at 20 and 25°C. In various years and at various sites, additional incubation temperatures of 10 and 15°C were included. Grains were isolated from florets 1 and 2 of spikelets from the central half of 40 randomly chosen ears. The tests required (n x 50) grains per replicate, where n = the number of different temperatures for incubation of the tests. The grains were incubated in 2% sodium hypochlorite solution for one hour before plating out, for surface sterilisation. In each test, 50 grains were spread out on four layers of 9 cm diameter Whatman No. 1 filter paper in Petri dishes, with 5 ml distilled water added. Dormancy was calculated as the duration in days from the acquisition of maturity (defined here as a combine harvestable moisture content of about 15%) to the point when 50% of grains germinated in 3 days at 20°C (modified after Belderok, 1968, who used a temperature of 18°C). The 25°C test was completed to allow the calculation of dormancy index (Gate, 1995) to compare with

relationships developed in France. Dormancy index was calculated as $(G_{20}-G_{25})/G_{20}$ after 7 days, where G_{20} = germination at 20°C (number or percentage of sprouted grains), G_{25} = germination at 25°C.

4.4.1.7. Meteorological Recordings

Meteorological recordings were taken from the nearest local weather station to the field site (usually < 1 km) providing Meteorological Office-approved manually or automatically-recorded data. Records of maximum and minimum temperature, rainfall, relative humidity, sunshine hours and radiation were taken.

4.4.1.8. Statistical Analysis

All statistical analyses were completed using Genstat 5 for DOS or Release 3.33 for Windows 3.1.

4.4.2. Results - Multi-site Field Trial/PoMS

4.4.2.1. General trial husbandry, moisture content, growth stage and accumulated temperature

Generally, there were no significant amounts of disease, pest attack, lodging, *etc.* affecting the trial. Analysis of the development of crop moisture content and growth stage with accumulated temperature showed very poor correlation. Grain moisture content was the best indicator of development stage, although further investigation is required to allow easier and more rapid estimation of this quantity.

4.4.2.2. Hagberg falling number

A wide variation in combine harvest Hagberg falling number was found across the site-season-cultivar matrix, from 62-475 s, as shown in *Table 8* (1994-1995). Data for 1996-1997 is shown in *Tables 10* and *12*, showing the accuracy of the prototype scheme.

Table 8: Hagberg falling number, 1994-1995

Site	*Year*	*Cultivar*	*Pre-harvest HFN*	*Combine HFN*	*Cause of low HFN*
SB	1994	Récital	386	417	-
		Thesée	392	406	-
		Soissons	420	429	-
		Scipion	397	407	-
		Pastiche	449	447	-
		Riband	360	322	-
		Hornet	308	361	-
		Haven	376	327	-
HA	1994	Récital	162	201	PoMS
		Thesée	297	234	PoMS
		Soissons	362	352	-
		Scipion	322	302	-
		Pastiche	445	427	-
		Riband	268	236	PMAA/PoMS
		Hornet	154	152	PMAA/PoMS
		Haven	173	178	PMAA/PoMS
SB	1995	Récital	416	416	-
		Thesée	401	368	-
		Soissons	421	411	-
		Scipion	418	397	-
		Pastiche	424	401	-
		Riband	369	365	-
		Hornet	359	336	-
		Haven	380	340	-
HA	1995	Récital	414	398	-
		Thesée	398	399	-
		Soissons	420	421	-
		Scipion	408	395	-
		Pastiche	434	413	-
		Riband	374	376	-
		Hornet	368	390	-
		Haven	364	378	-
AB	1995	Récital	365	355	-
		Thesée	366	341	-
		Soissons	361	362	-
		Scipion	336	302	-
		Pastiche	350	345	-
		Riband	247	245	PrMS/PoMS
		Hornet	234	227	PrMS/PoMS
		Haven	238	227	PrMS/PoMS
UA	1995	Récital	351	62	PoMS
		Thesée	376	62	PoMS
		Soissons	374	62	PoMS
		Scipion	346	62	PoMS
		Pastiche	374	62	PoMS
		Riband	326	62	PoMS
		Hornet	297	62	PoMS
		Haven	322	62	PoMS

4.4.2.2.1 Causes of low combine harvest HFN

Most of the low combine HFN values were due to post-maturity sprouting. However, in some cases, low HFN before harvest was identified, caused by the other routes as shown in *Tables 8, 10* and *12*.

4.4.2.2.2. Relationship between percentage sprouting and HFN

The relationships between percentage sprouting and HFN vary between cultivars (*Figure 18*) with a larger drop in HFN for a given percentage of sprouting for occurring for some cultivars than others. If the prediction of sprouting risk in the Prototype Prediction Scheme is to be developed into a prediction of HFN loss, the drop in HFN for given levels of dormancy and particular environmental conditions, for commercial cultivars, would need to be probed more deeply.

4.4.2.3. In vitro ear dormancy test

The *in vitro* ear dormancy test was found to be an inappropriate research test for UK conditions. The test results were very variable, sample analysis was time-consuming and simple statistical analysis was not possible due to the lack of replication. As an exponential relationship (*Figure 19*) was demonstrated between the percentage sprouted ears (which would be recorded in a rapid test) and percentage sprouted grain (which would relate to Hagberg falling number and combine harvested samples), linear predictions would not be simple. Therefore, use of the IVED test was discontinued after 1995.

4.4.2.4. Dormancy index

French work (*Figure 20*) has, over a number of years, demonstrated good relationships (for the cultivars *Soissons* and *Thesée*) for dormancy index (defined in *Section 4.4.1.6.2.*) taken at harvest and accumulated temperature (> 12.5°C) between ear emergence and physiological maturity +250°Cd. Here, 'physiological maturity' is defined as 40% moisture content. The UK dormancy

index results for *Soissons* and *Thesée* did not fit the developed relationship (*Figure 20*), possibly due to more variable environmental conditions in the UK. Investigation of UK cultivars to identify any similar tends showed no consistent relationship between dormancy index and the accumulated temperature parameter used by the French (data not shown). The accumulated temperature parameter would be difficult to use in a predictive scheme since it cannot be calculated until after the appropriate decision-making period for high-moisture harvest. In the UK tests the dormancy index measured at harvest showed no relationship to the level of germination in 20°C germination tests or the sprouting induced by overhead irrigation (data not shown).

4.4.2.5. Dormancy duration

Due to the inapplicability of dormancy index in the UK, return to first principles of Belderok (1952, 1965, 1968, 1973) was used to investigate dormancy duration and its possible relationship to temperature. No relationship was found for sprouting-susceptible cultivars (*i.e. Haven, Hornet, Récital, Thesée*). The more sprouting-resistant cultivars (*i.e. Pastiche, Riband, Soissons, Scipion*) showed a relationship, between dormancy duration (as defined in *Section 4.4.1.6.2.*) and temperature accumulated in excess of 12.5°C between heading and harvest maturity (15% moisture), analogous to that reported by Belderok (*Figure 21,* data for *Riband*). However, as reported in *Section 4.4.2.6.*, the predictive use of this relationship was limited by its restriction to sprouting-resistant cultivars and the inappropriate timing of the accumulated temperature parameter for decision- making.

4.4.2.6. Pre-harvest germination testing.

Due to the difficulty in developing meteorological relationships for dormancy, a pre-harvest germination test was considered the best option to give a 'live' record of germinability for making predictions in the prototype two-stage HFN prediction scheme.

Figure 22 shows the regression relationship between germination in the 15°C IGG test for the UK cultivar *Haven* and the subsequent field sprouting induced by artificial irrigation., which gave the highest R^2 value. However, the 20°C germination test also gave a high R^2 value.

4.5. The proposed prototype UK HFN prediction scheme (Objective 6)

4.5.1. Description of the proposed two-stage prediction scheme

The Prediction Scheme currently envisaged was first tentatively proposed in the Second Annual Interim report to HGCA in April 1996 (Lunn *et al.*, 1996). The prototype consists of a sampling and measurement-based scheme which will provide a pre-harvest measurement of the HFN status of crops followed by a prediction of the subsequent risk of reduction in HFN due to the dormancy status of the crop. Expression of the risk of sprouting would depend on the weather experienced after the pre-harvest HFN determination and germination test.

At the start of project 0056/01/93 it was hoped that it would be possible to predict PMAA and PrMS if the environmental triggers were sufficiently understood. It was anticipated that the later risk of PoMS could then be predicted by adopting the ITCF accumulated temperature scheme for prediction of dormancy. This scheme could then be used by any farmer able to monitor the appropriate measures of weather.

Although substantial progress has been made in discovering the factors stimulating PMAA there is not yet sufficient knowledge to incorporate this effectively in a prediction scheme. For PrMS, intelligence concerning the incidence of midge pupae and likelihood of emergence and egg-laying is available from ADAS entomologists and could be incorporated into the prediction scheme. Knowledge of the rainfall and temperature needed to stimulate PrMS in the field in the absence of blossom midge is, however, scant. The predictions of crop dormancy status from accumulated temperature data developed in France have been shown to be insufficiently accurate for application in the UK (*Section 4.4.2.3.-6.).* Also, a fourth mode of *alpha*-amylase accumulation has been identified which is of particular significance to the pre-harvest HFN measurement. The timescale of degradation of pericarp amylase varies according to the environmental conditions. Thus prediction of combine harvest Hagberg falling number from weather data is not possible yet.

Therefore, we have evolved a prototype scheme which has two stages, and draws on intelligence

gathered from the Finnish and French (ITCF) visits, together with the advances made within project 0056/01/93. The operation of the scheme is outlined in flow-chart format in *Figure 23.* Taking all current knowledge into account, Hagberg measurement (Stage 1) on a sample taken at about 35% moisture (during the second half of July in the Midlands) can reliably indicate the net effect of the three early routes of *alpha*-amylase formation: PMAA, PrMS, RPA). This only leaves the risk of accumulation of late *alpha*-amylase from PoMS to be assessed by a germination test and prediction (Stage 2).

From Stage 1, farmers and their advisors will be able to decide whether their crops, or crops in the same region as a reference site, are still of satisfactory Hagberg, or not, due to *alpha*-amylase accumulation from RPAA, PrMS or PMAA. If they are considered to be of satisfactory Hagberg then a forecast from Stage 2 of a low risk of PoMS in wet weather reduces the pressure for an early, high moisture harvest. Conversely, forecast of a high risk of PoMS in wet weather increases the urgency of harvest and the need for extra drying costs to be incurred. The decision on harvest date must, of course, take into account many other factors such as the weather outlook, the premium likely to be obtained and rate of dryer throughput.

The prototype scheme needs validating over a wider range of sites than the four UK sites used in project 0056/01/93 and the operational details need to be refined.

4.5.2. Materials and Methods for Tests of the Prototype Prediction Scheme

Specific aspects designed to optimise the operation of the envisaged scheme were tested in sub-experiments at HAAC and SB in various years. A prototype scheme was then tested during the second half of the project in 1996-1997, involving all four field sites and France in 1997.

4.5.2.1. Sample size experiment

In 1996, three replicate plots of *Haven* and *Riband* at Harper Adams were sampled at the pre-harvest sample point (moisture content (%)/°Cd post-heading 36/797 and 33/815 respectively). Nine samples (300 ears each) per replicate plot were taken and left to air-dry in the laboratory

before determination of Hagberg falling number by the standard methods. Results were investigated by analysis of variance.

4.5.2.2. Transportation and drying experiments

In 1996, three replicate plots of *Hornet* and *Riband* at Sutton Bonington were sampled at the pre-harvest sample point (800-850°Cd post-heading). Five samples of 300 ears were taken from each replicate plot. Samples was despatched to Harper Adams overnight, one set stored only in plastic bags, another set in plastic bags inside an insulated cooler bag, and a final set in plastic bags in a cooler bag with frozen gel packs to reduce the temperature. Another two sets of samples were left to stand in the laboratory for 7 days, stored in plastic bags or in plastic bags in insulated cooler packs, to simulate delayed delivery. They were then transported by overnight courier to Harper Adams for determination of HFN. At Harper Adams, samples were dried overnight at < 50°C before threshing, cleaning and determination of HFN by the usual methods.

4.5.2.3. Pre-harvest/combine harvest HFN relationship

Data (collected as described previously) from the pre-harvest/combine harvest HFN assessments from 1994 and 1995 at Harper Adams and Sutton Bonington were used for regression analysis to determine an equation for prediction of HFN, with 75% confidence limits, for each cultivar. This equation was used for prediction of likely combine HFN (in the absence of sprouting) during trial operation of the prototype prediction scheme.

In 1995 at HAAC, the correspondence of HFN values taken by hand-sampling (300 ears) from 3 replicate plots and combine harvesting on the same day was investigated.

4.5.2.4. Appropriate temperature of the germination test.

In various years and at various sites, the isolated grain germination tests described in *Section 4.4.1.6.2.* were expanded to include tests at 10°C and 15°C as well as at 20 and 25°C. This was to determine the best test for the prediction of germination in the field. In 1997, tests at all four

temperatures were carried out at all sites.

4.5.2.5. Trial operation of the prototype scheme.

In 1996 and 1997, the prediction scheme was tested in outline in a 'live' format on the cultivars *Riband* and *Hornet* from all sites and on all cultivars from Harper Adams, and those from ITCF La Minière in 1997. In addition in 1997, early and late-sown crops from the Cereals 97 site at Alconbury and a crop of Hereward from NEAC near Newcastle were tested. Predictions were also made for the remaining samples dried on-site and despatched with a slight delay compared to *Riband* and *Hornet*.

In the trial of the scheme, ~ 300 ears (sampled from four randomly-chosen positions in a plot) from triplicate plots between ~ 800 and 850°Cd post ear emergence were posted in plastic bags without refrigeration, to Harper Adams overnight. Grains were isolated for determination of moisture content and isolated grain germination tests at 20°C according to standard protocols. Germination was recorded after 3 days at 20°C as described previously and the samples frozen for future Phadebas gel testing of *alpha*-amylase location.

Fresh samples were dried overnight at 50°C in a forced air oven before threshing, cleaning, milling of grain and determination of HFN by the standard methods. Using the confidence limits for the pre-harvest-combine harvest HFN relationship developed in 1994-1995 (*Section 4.5.2.3.*), HFN values were placed in classes corresponding to their expected HFN. The percentage germination in 3 days at 20°C was used to quantify the risk of a further change in that HFN. In 1996, > 10% germination in the pre-harvest germination test was classified as a high risk of sprouting, < 10% low risk. In 1997, > 2% was classified as a high risk of subsequent sprouting. The prediction classes used (in 1997) are shown in *Section 4.5.3.5.*

Ear samples from other cultivars were dried on-site at 50°C before subsequent despatch to Harper Adams for determination of HFN. The IGG test from the relevant sample period at the originating site was used to assess the risk of HFN reduction in poor weather and assign a prediction class to these samples.

4.5.3. Results of testing the prototype scheme in 1996 and 1997.

4.5.3.1. Sample size experiment.

After the experiment to determine the appropriate number of subsamples to bulk for the greatest accuracy in pre-harvest HFN at HAAC, initial analysis of variance was performed on the whole data set of nine HFN samples per replicate. Then, samples were randomly removed to generate data sets of eight, seven, six, five, four, three, two and one sample(s). The procedure was repeated four times, generating 40 different data sets which were analysed by ANOVA. The coefficient of variation was recorded for each data set and a mean percentage coefficient of variation calculated for each set of samples (*Figure 24*). The results demonstrate that four subsamples are optimal for determination of an accurate pre-harvest HFN measurement. With less than four samples, percentage coefficient of variation increased leading to greater uncertainty in the accuracy of the pre-harvest value. However, taking more than four samples did not decrease the percentage coefficient of variation dramatically and therefore would not be expected to improve the accuracy of the measurement.

4.5.3.2. Transportation and drying

The pre-harvest HFN measurements recorded from fresh material despatched to Harper Adams where it was dried at 50°C before HFN analysis, and from material dried at a nominal 50°C at each experimental site before transfer in 1996 are shown in *Table 9*. The correspondence of values from ADAS Bridgets was excellent (values showed no significant difference), although the HFN values from samples dried at Aberdeen were significantly greater ($p< 0.05$) than those recorded from fresh samples dried at HAAC. This could be due to problems associated with on-site drying, with temperatures in excess of 50°C inactivating some *alpha*-amylase activity. This illustrates that transport of fresh samples and centralised drying would be the preferred method during operation of a full-scale prediction scheme. Excessive drying temperatures (> 50°C) may have inactivated some amylase activity present in fresh material (particularly pericarp or α-AMY-2 which may have been present if the 800-850°Cd sample point was too early). Alternatively, transportation of fresh material may have resulted in accumulation of *alpha*-

amylase not present at the time of sampling.

The transportation experiments in 1997 showed no benefit in insulating or cooling HFN samples during overnight transfer, since there was no significant difference ($p > 0.1$) in the HFN of these differently-treated samples, for the cultivar *Hornet* or *Riband* (*Table 9*). The HFN of delayed samples of *Riband* was significantly greater ($p < 0.05$) than that of overnight-delivered samples probably due to inactivation of α-AMY-2 activity. For *Hornet*, only the HFN of the sample stored in a cool bag was significantly greater. This indicates that delayed delivery of HFN samples would not be a problem causing erroneously low predictions of HFN. However, it also demonstrates how residual α-AMY-2 activity may lower estimates of combine HFN in an inappropriately-timed, early sample.

***Table 9**: Effects of drying and transportation methods on HFN of samples in the test of the prototype scheme*

Drying		*Hagberg falling number (s)*			
		Bridgets		*Aberdeen*	
Method		*Hornet*	*Riband*	*Hornet*	*Riband*
Dried		185.0	224.0	254.0	338.0
Fresh		185.0	224.0	115.0	225.0
SED		7.6		26.8	
LSD		18.5		65.6	
CV (%)		5.1		8.6	
Transportation					
Method		*Riband*		*Hornet*	
Overnight	Bag only	260.0		234.0	
	Cool bag	272.0		255.0	
	Cool bag + gel pack	260.0		244.0	
Delayed 7 days	Bag only	350.0		271.0	
	Cool bag	337.0		311.0	
SED		11.4		21.6	
LSD		26.3		49.8	
CV (%)		8.6		0.9	

4.5.3.3. Pre-harvest/combine harvest HFN relationship

Regression analysis of the Hagberg of hand-samples (taken on the day of combine harvest) and combined samples at HAAC showed a significant ($p < 0.05$) relationship between hand and combine HFN with an R^2 value of 63.3. The relatively low R^2 value could be due to the loss of small grains in combining which would be preserved by hand-threshing. This may also indicate that a larger hand sample would be desirable for improved accuracy of the pre-harvest HFN measurement, although sampling time and logistics precluded this during these experiments.

In the absence of bad weather and RPAA, pre-harvest HFN was a good predictor of likely combine and regression analysis of individual cultivars (*Figure 25* shows *Hornet* as an example) allowed development of equations for the prediction of combine harvest HFN values from pre-harvest data (with 75 % confidence limits).

4.5.3.4. Appropriate temperature of the germination test

The results reported in *Section 4.4.2.6.* and in the description of the trial predictions in *Section 4.5.3.5.* show that the germination test at 20°C gave a quite good indication of the potential for PoMS in the field. During the trial of the scheme, attempts to reduce the time of the pre-harvest germination test were made in order to speed up the turnover of intelligence, by using Phadebas gel testing to give the earliest possible indication of the relapse of *alpha*-amylase enzymes. For operation of the scheme on a commercial scale it may be desirable to tailor the temperature of the germination test to the specific region and season rather than stick to a blanket 20°C test. There are some indications from analysis of the IGG test data that the 15°C test shows optimum expression of pre-harvest germinability and this temperature is more characteristic of the conditions experienced in wet summer weather in the UK than 20°C.

Testing of the Phadebas test showed that it could give an early indication of the potential sprouting and could also distinguish the presence of RPAA or PMAA distinctly from PrMS/PoMS. This could be valuable in enhancing the predictions in the case of PMAA.

4.5.3.5. Trial predictions and accuracy

In 1996, a very simple prediction of whether Hagberg falling number of combine samples was expected to be above or below 250 s was made. The prediction used the pre-harvest HFN value (as an indicator of RPAA, PMAA and PrMS) and the percentage germination after 3 days at 20°C (as an indicator of the likely sprouting in any wet, cold weather following the pre-harvest sample, > 10% high risk of germination, <10% low risk). The predictions made and combine harvest results are shown in *Table 10* (with predictions for comparison with combine HFN shown in bold). Out of 30 predictions, 22 were correct, giving an accuracy level of 73.3 %. The causes of low pre-harvest and combine harvest HFN in 1996 and 1997 are annotated on *Table 10* and *12* as follows:

a Combine harvest HFN low due to post-maturity sprouting only

b Pre-harvest HFN low due to retained pericarp *alpha*-amylase

c Pre-harvest HFN low due to pre-maturity *alpha*-amylase accumulation

d Combine harvest HFN low due to the combined effects of PMAA and PoMS

In 1996, the 8 inaccurate predictions fell into two error types. The cultivars *Riband, Hornet* and *Haven* had lower HFNs than expected at Sutton Bonington and Aberdeen, since low (unexpected) levels of germination were recorded pre-harvest. At Harper Adams and Aberdeen, the HFN of the cultivar *Thesée* remained high despite high recorded pre-harvest germination levels, whereas the HFNs of cultivars with lower levels of germination fell. The relevant predictions (on consideration of the weather conditions) are highlighted in bold on *Table 10.*

Table 10: Hagberg falling number predictions from the prototype scheme in 1996

Site	*Cultivar*	*Pre-harvest HFN*	*Pre-harvest Percentage Germination*	*Predicted HFN if hot, dry*	*Predicted HFN if cool, wet*	*Combine HFN*	*Correct Prediction?*
SB	Récital	468	53.0	> 250	**< 250**	62[a]	Y
	Thesée	417	48.0	> 250	**< 250**	78[a]	Y
	Soissons	427	2.6	> 250	**> 250**	267	Y
	Scipion	452	0.0	> 250	**> 250**	346	Y
	Pastiche	449	0.6	> 250	**> 250**	357	Y
	Riband	351	0.3	> 250	**> 250**	150[a]	N
	Hornet	348	0.0	> 250	**> 250**	211[a]	N
	Haven	392	2.0	> 250	**> 250**	239[a]	N
HA	Récital	332	66.0	> 250	**< 250**	164[a]	Y
	Thesée	266[b]	16.4	> 250	**< 250**	302	N
	Soissons	265[b]	4.0	> 250	**> 250**	316	Y
	Scipion	257[b]	1.4	> 250	**> 250**	338	Y
	Pastiche	340	3.3	**> 250**	> 250	356	Y
	Riband	272	0.7	**> 250**	> 250	288	Y
	Hornet	259	2.0	**> 250**	> 250	273	Y
	Haven	258	8.0	**> 250**	< 250	253	Y
AB	Récital	-	-	-	-	-	-
	Thesée	309	4.7	**> 250**	> 250	295	Y
	Soissons	324	0.3	**> 250**	> 250	355	Y
	Scipion	294	0.0	**> 250**	> 250	310	Y
	Pastiche	354	0.0	**> 250**	> 250	418	Y
	Riband	225[c]	1.3	**< 250**	< 250	245[d]	Y
	Hornet	185[c]	6.0	**< 250**	< 250	207[d]	Y
	Haven	163[c]	0.0	**< 250**	< 250	203[d]	Y
UA	Récital	-	-				
	Thesée	282	42.0	> 250	**< 250**	323	N
	Soissons	360	9.4	> 250	**> 250**	352	Y
	Scipion	329	0.0	> 250	**> 250**	306	Y
	Pastiche	391	3.4	> 250	**> 250**	328	Y
	Riband	338	0.0	> 250	**> 250**	236[a]	N
	Hornet	254	2.7	> 250	**> 250**	181[a]	N
	Haven	278	0.6	> 250	**> 250**	117[a]	N

In 1997, samples were placed in one of five prediction classes as shown in *Table 11*. Pre-harvest/combine harvest HFN prediction equations were used to predict combine harvest HFN, with confidence limits, in the absence of rainfall. This allowed classification of likely HFN as much greater than 250 s, about 250 s or much less than 250 s. The percentage germination in 3 days at 20°C was used to classify the risk of loss in predicted HFN in wet weather. Due to the experience of running the scheme in 1996 and knowledge of the low level of sprouted grains required to reduce HFN (*Figure 19*), the criterion for determining high risk was revised (> 2% high risk, < 2% low risk).

***Table 11**: Prediction classes for Prototype HFN prediction scheme used in 1997.*

Prediction class	**Potential Hagberg**	**Risk of drop in Hagberg if weather wet before harvest**
1	well below 250 s	not relevant
2	about 250 s	low
3	about 250 s	high
4	well above 250 s	high
5	well above 250 s	low

The predictions made in 1997 are shown in *Table 12,* with annotations giving the identity of low HFN as in *Table 10,* described on page 66. Out of 40 predictions, five were incorrect and three were partially accurate, giving an accuracy level for the 1997 scheme of 83.8%. Three of the erroneous predictions were due to RPAA in French cultivars sampled at Sutton Bonington and ITCF, which was identified before harvest due to the presence of green grains. If these samples had been re-tested to give more accurate pre-harvest HFN values, the accuracy of the scheme would have risen to 88.6%. Two other errors were due to a greater than expected loss in HFN due to sprouting in samples of *Soissons* and *Scipion* at Aberdeen. If the rules of the scheme had been rigorously applied to the *Soissons* sample, it would have been placed in prediction class 4, raising

the accuracy level of the scheme to 91.1%. However, due to consideration of the experience with this relatively sprouting-resistant cultivar in previous experiments it was placed in class 5. The mis-classification of *Scipion* is a true error.

Of the samples given a Y/N 'qualified correct' classification, *Hornet* at Sutton Bonington was identified as 'at risk' of sprouting. However, due to the relatively light rainfall after pre-harvest HFN measurement and the maintenance of the HFN of other class 3 cultivars > 250 s, the drop in *Hornet* HFN is thought to be due to PMAA. The other two errors of this type were at Aberdeen. For *Pastiche*, placed in group 5, the pre-harvest germination level really warranted a classification of class 4. For *Riband*, the germination recorded pre-harvest would have given a strict classification of group 5 (which would have been erroneous). These particular predictions were made with reference to sprouting-susceptibility of the cultivars in previous experiments rather than germination test data (which was completed on-site and was unavailable at the time of the prediction).The strict classifications for the erroneous samples, made with hindsight with germination data not available at the time of the prediction are shown in bold in brackets on *Table 12*, with the accuracy of the revised prediction also shown in bold in brackets. Considering strict application of the rules of the scheme, including re-testing samples with PMAA identified gives a theoretical maximum accuracy of 93.8% for 1997, using the reclassifications shown in bold on *Table 12*.

4.5.3.6. Logistics of operating the scheme.

The scheme could be operated by many co-operatives or grain merchants with access to oven-drying, threshing, milling, Hagberg and controlled temperature incubator facilities. Any farmer could sample appropriate fields according to a recognised protocol with knowledge of the appropriate sampling time, by reference to growth stage or crop moisture content, possibly by microwave-drying. Submission of samples for testing would require a next-day delivery service (although first class post may prove sufficient) but requires no special storage or cooling facilities provided guaranteed delivery is rapid. During trial operation of the scheme it has proved possible to return predictions to participating sites within 3-5 days of sample arrival by fax, telephone or electronic mail.

***Table 12:** Hagberg falling number predictions using the prototype scheme, 1997*

Site	*Cultivar*	*Pre-harvest HFN*	*% Germination*	*HFN prediction class (see Table 11)*	*Combine HFN*	*Correct Prediction?*
SB	Récital	293	54.0	3	291	Y
	Thesée	302	4.0	4	369	Y
	Soissons	255[b]	0.0	2 (**5**)	387	N (**Y**)
	Scipion	270	0.0	5	371	Y
	Pastiche	391	0.0	5	411	Y
	Riband	260	0.0	2	287	Y
	Hornet	234[c]	1.0	3	212[c]	Y/N
	Haven	294	12.0	3	278	Y
HA	Récital	376	72.0	4	62[a]	Y
	Thesée	390	26.0	4	219[a]	Y
	Soissons	365	0.0	5	296	Y
	Scipion	385	0.6	5	367	Y
	Pastiche	410	3.7	5	324	Y
	Riband	319	4.0	4	257	Y
	Hornet	293	2.3	3	241[a]	Y
	Haven	300	18.0	4	163[a]	Y
AB	Pastiche	335	0.0	5	424	Y
	Riband	298	1.3	3	219[d]	Y
	Hornet	187[b]	1.7	1	241[d]	Y
	Haven	184[b]	2.0	1	163[d]	Y
UA	Récital	235[a]	7.3	1	62[a]	Y
	Thesée	288	4.0	3	99[a]	Y
	Soissons	372	2.7	5 (**4**)	160[a]	N (**Y**)
	Scipion	320	0.7	5	228[a]	N
	Pastiche	300	3.3	5 (**4**)	270	Y/N (**Y**)
	Riband	331	1.0	4 (**5**)	153[a]	Y/N (**N**)
	Hornet	127[c]	2.0	1	86[a]	Y
	Haven	259	2.7	3	158[a]	Y
ITCF	Récital	183[b]	22.0	1 (**3**)	263	N (**Y**)
	Thesée	226[b]	13.3	1 (**3**)	257	N (**Y**)
	Soissons	236[b]	0.0	2	315	Y
	Scipion	262[b]	0.3	2	319	Y
	Pastiche	321	0.0	5	430	Y
	Riband	212[c]	0.0	2	245[d]	Y
	Haven	157[c]	2.8	1	194[d]	Y
Velcourt	RibE	265	0.0	3	261	Y
	RibL	236	3.0	3	270	Y
	HerE	322	3.0	4	305	Y
	HerL	337	38.0	4	392	Y
NEAC	Her	224[a]	5.0	1	159[a]	Y

4.5.4. Further development of the prototype scheme

Although the proposed scheme could be set up in outline format at any number of sites, or operated by any commercial company or grain merchants, additional research would be needed to develop an optimised, more broadly operable scheme because:

- A demonstration of the prototype scheme needs to be operated using current commercial cultivars, whereas project 0056/01/93 used model cultivars as experimental tools, chosen for their specific propensities to different modes of *alpha*-amylase formation. It will be essential to test the scheme on current cultivars and evaluate on these cultivars the validity of the time of pre-harvest sampling chosen using model cultivars. Additionally, regression equations and confidence limits for the pre-harvest/combine harvest HFN relationship need to be worked out for current commercial cultivars.

- Further evidence that the time of sampling is appropriate to avoid the normal period of pericarp *alpha*-amylase activity over a wider range of sites and seasons is needed. There is an indication that a slightly later sampling may be needed further north because of normally greater persistence of pericarp amylase. The French method of accumulated temperature has proven an inaccurate predictor of grain moisture content and potential evapotranspiration, rapid assessment of moisture content by microwave drying, or modelling moisture content may be better measures. Although sample size and number of sampling positions have been optimised for small trial plots, assessment of the correct sample size and pattern for large commercial fields needs to be undertaken.

- The details of the germination test applicable to UK conditions need to be elucidated in more detail. In particular, the appropriate temperature for the test requires detailed investigation since dormancy is normally less marked at cooler temperatures. The appropriate temperature for the germination test may vary regionally in the UK. In addition, the speed of prediction may be increased by the use of various tests (*e.g.* Phadebas gel test) allowing more rapid quantification of germination than visual observation.

- It is not yet sufficiently clear how great a drop in Hagberg occurs for given amounts of rain and different depths of dormancy. This information is would allow refinements in the predictions according to the weather conditions after pre-harvest sampling. The current data set is far too small to give adequate guidance, and particularly needs to be generated with a range of commercial cultivars.

- Instigation of a weather-based prediction scheme would require more research into the weather factors that stimulate and moderate each of the four routes of *alpha*-amylase accumulation. The appropriate areas for future research into the four routes are outlined in the results and discussion sections appropriate to each route.

5. DISCUSSION AND CONCLUSIONS

The experimental work has underlined the complexity and interaction of the different routes of *alpha*-amylase accumulation which can lead to low Hagberg falling number in the UK. Due to the very complex physiology of these routes and the various antagonistic environmental factors which trigger and promote them, the aim of producing an HFN prediction scheme based solely on meteorological data has proved over-ambitious. However, this project has laid an important foundation for further work towards this goal and has designed a more pragmatic HFN prediction scheme suitable for use in UK conditions.

5.1. Retention of pericarp *alpha*-amylase activity.

The work has shown a fourth, initially overlooked, route of *alpha*-amylase accumulation to be important to combine harvest HFN and HFN prediction, namely RPAA. This route was initially discounted due to the reported temperature lability and normal degradation of α-AMY-2 isozymes (Gale, personal communication; Kruger, 1989). However, our research has conclusively demonstrated the activity of α-AMY-2 in the HFN test, by showing the reduction in HFN caused by reconstituting flours with freeze-dried, freshly defrosted or native forms of the enzyme.

The presence of α-AMY-2 could cause errors in the envisaged pre-harvest HFN prediction

scheme and was indeed responsible for some of the inaccuracies during the trial operation of the scheme in 1996-1997. If not positively identified, RPAA causes an underestimate of combine HFN. Unlike the other two early routes of low HFN, RPAA allows scope for improvement by α-AMY-2 inactivation, which the work presented above demonstrated could occur during air-drying.

Several routes for production of non-uniform crops containing green grains with α-AMY-2 exist, including pest grazing, lodging, rainfall after drought, tramlines, *etc.* Our research has demonstrated that a large proportion of the green grains in non-homogeneous crops easily persists through to combined samples. Therefore any of the aforementioned phenomena could affect HFN. Anecdotal evidence of improvement in crop HFN during the first few weeks of storage (also observed in data from Zimbabwe) could be explained by α-AMY-2/RPAA inactivation on drying.

Appropriate drying management and use of desiccants provide options for addressing the problem of RPAA in farm crops. Since the mechanisms leading to non-uniform crops are so diverse, modelling of the RPAA syndrome from meteorological data would be very difficult. The advantage of the proposed HFN prediction scheme is that crops suffering from RPAA could be identified at the pre-harvest sample point (by low HFN, observation of green grains or further testing by IEF or the Phadebas gel test) allowing remedial action to be taken, for example use of glyphosate-containing herbicides which can kill the green tillers in non-uniform crops.

For timing of the pre-harvest HFN sample, a marker for the normal degradation of α-AMY-2 activity would be advantageous. In the multi-site field studies, development rate varied across sites and calendar date, and accumulated temperature from heading and grain colour proved to be poor markers of α-AMY-2 degradation. It is suspected that moisture content, possibly estimated by accumulated potential evapotranspiration or tested by rapid microwave drying, might prove to be the best marker, although this requires further research. Other appropriate avenues for research include the manipulation of crop uniformity by mowing, to simulate the result of the diverse routes described previously and allow further investigation of the effects of RPAA on HFN and amelioration of the syndrome by use of desiccants and/or air-drying.

5.2. Pre-maturity *alpha*-amylase activity in the absence of sprouting

The environmental triggers of pre-maturity *alpha*-amylase activity proved more complex than originally anticipated. Investigation of field grain drying rates showed no relationship to *alpha*-amylase activity, despite other work supporting slow grain drying as a PMAA stimulus in the scientific literature (Gale *et al.*, 1983). The short high-temperature shock experienced in Cabinet Experiment 1 did promote PMAA, even in *Pastiche,* a cultivar not susceptible to the syndrome in the field. In the first experiments, the extent of PMAA was related to the time of transfer, with earlier transfers in the milky stage increasing PMAA. The mechanism of action of this stimulus is unknown, although review of the scientific literature indicates the possibility that it may be associated with changes in membrane permeability to gibberellins which may be induced by temperature shock.

After consideration of Australian work which showed that the PMAA syndrome in Australia could be induced by a cold-temperature shock, the emphasis of the work was changed to look at reciprocal transfers between hot and cold environments to investigate the hypothesis that a temperature shock of any sign away from the average growing conditions could induce PMAA. The experiments showed that this was indeed the case, although due to differences in control *alpha*-amylase activity and in responses to time of transfer, interpretation of the results is difficult. Many more experiments investigating further stimuli and susceptible periods and cultivars would be necessary before any kind of accurate meteorological model could be produced, since the condition in the field is very different than transfer between two constantly maintained temperature régimes.

The prediction scheme proposed for the UK can assess crops which have lost commercial value , due to severe PMAA, by pre-harvest HFN measurement. For crops with an HFN in the region of 250 s on pre-harvest measurement, augmentation of the scheme with tests to demonstrate PMAA activity (such as the Phadebas gel test) would be advantageous in order to improve the accuracy of the predictions.

5.3. Pre-maturity sprouting.

Research into pre-maturity sprouting has shown that the phenomenon may be induced by very intense irrigation, simulating wet weather, during the dough stage of grain development, even at high temperatures in the glasshouse. Subsequent experiments gave limited evidence that a window of increased sensitivity to pre-maturity sprouting may exist during the dough stage for certain cultivars. This possibility needs further research, at lower ambient temperatures, with more rigorous control of irrigation amounts, but could form the basis of a test to attribute varietal sensitivity to PrMS. Review of isolated grain germination data gave indications that induction of PrMS by severe rainfall would be most likely at low temperatures, < 15°C, as shown for 'hidden germination' by Lalluka (1976). Since these conditions could not be simulated in the available glasshouse facilities, more research to delineate the exact weather stimuli responsible for PrMS in the field is required.

Direct demonstration of a causal relationship between *in vivo* orange wheat blossom midge infection and pre-maturity sprouting was difficult, due to diminishing midge pupal inoculum after the 1993 epidemic. Weather conditions in June 1994 and 1995 were generally unsuitable for midge oviposition, so there was little material available for investigation. However, a small sub-population of late green tillers at Sutton Bonington in 1997 provided positive evidence for the correlation between *S. mosellana* infection and PrMS. These ears were heavily infested with orange blossom midge larvae and showed a high degree of PrMS in grains with split pericarps, at high grain moisture content.

Reports in the literature have demonstrated a negative correlation between Hagberg falling number and the percentage of midge-damaged grains (Helenius and Kurrpa, 1987; Oakley, 1993). However, the exact cause of the reduction in HFN has not been conclusively demonstrated. Iso-electric focusing studies showed that midge amylase enzymes were not sufficient to account for the low HFN observed in some midge-damaged crops, since they showed transient activity and were only present in low concentrations. Only plant *alpha*-amylase enzymes were found expressed in mature midge-damaged grains, specifically in severely damaged grains with split pericarps, some showing obvious signs of germination.

A combination of cutting (simulating midge damage) and wetting/irrigation provided indirect evidence to support the relationship between midge damage and PrMS. In isolated grain experiments, cutting the pericarp of immature grains increased the temperature range in which gains could germinate, with exogenously applied moisture. Germination of the uncut immature grains was only possible at low temperature. Additionally, cut grains incubated without excess exogenous moisture were able to germinate using endogenous moisture at low temperatures, which was not possible with uncut grains.

A forecasting system exists to predict the likelihood of severe blossom midge outbreaks, although some infestation is possible in all fields in all seasons. This system requires soil monitoring rather than just meteorological observation, but in years of high midge infestation risk data concerning the insect activity may be available from organisations such as ADAS. This information could be used to augment the proposed prediction scheme by more thorough observation of pre-harvest grain samples. The classification of sprouting risk in pre-harvest samples could be adjusted according to the level of blossom midge damage observed during sample preparation. More study of the relationship between PrMS in midge-damaged grains and weather factors is also required before this could be modelled using meteorological data.

5.4. Post-maturity sprouting.

Study of dormancy index-temperature relationships in France has shown some fairly robust relationships with French cultivars and weather conditions, with high accumulated temperature values correlated with low dormancy index. The relationship between dormancy index and accumulated temperature was however not very good when investigated with UK data and was not suitable for incorporation in the prediction scheme.

Analysis of dormancy duration data (analogous to the original Belderok work) showed some relationships similar to those demonstrated in the initial research. The inverse relationship between dormancy duration and accumulated temperature was only found for sprouting-resistant cultivars, not sprouting-sensitive cultivars. Accumulated temperature from heading to 15% moisture gave a better best-fit curve than accumulated dough stage temperature, although this

is of limited use for the prediction scheme.

Due to the change of emphasis of the scheme from meteorological prediction to crop monitoring, pre-harvest germination testing has proved the best method of assessing the likelihood of post maturity sprouting. Isolated grain germination tests in the multi-site study show that germination levels at 15-20°C correlate well with artificially induced sprouting using the overhead irrigation system. The percentage germination in isolated grain germination test at 15-20°C should therefore be a good discriminating quantity to place crops tested in the prediction scheme in the risk categories shown in *Table 11*. However, there are indications that a variation in the germination test may be required to enhance predictions of the risk of HFN loss for certain cultivars and in certain regions, which requires further research.

5.5. Prediction of HFN and sprouting.

Consideration of the advances in understanding of the four routes of *alpha*-amylase accumulation allows assessment of the most appropriate route towards HFN prediction in the United Kingdom. There is still insufficient understanding of the effects of environment on dormancy for this to be assessed from weather records following the French example. Similarly, much further research into the environmental factors responsible for the three early pathways of *alpha*-amylase accumulation is required to enable modelling of these processes.

Although possible, constant live HFN monitoring would probably be too expensive for the UK, and discussion with farmers shows a general preference for intelligence concerning their own, rather than reference, crops. The results of our studies show that serial HFN sampling may reasonably be reduced to a single appropriately-timed pre-harvest sample point to assess early modes of *alpha*-amylase accumulation (RPAA, PMAA and PrMS). Combined with a pre-harvest germination test to assess the likelihood of PoMS, this gives a hybrid scheme combining aspects of the two European models studied.

A prototype of the scheme has been successfully tested and demonstrated in 1996-1997 with success rates of 75 and 85% accuracy in those respective years, based on classification of crops

into broad expected combine HFN categories. This initial testing allowed consideration of the logistics of the scheme with elucidation of appropriate sample size and transportation method. As the scheme has been developed using model cultivars with a known range of responses to Hagberg, and only over a limited two-year period, further research is required to set up a commercially operable scheme.

In particular, the scheme needs to be assessed for current and upcoming commercial cultivars. Appropriate timing of the pre-harvest HFN sample, which might vary with location in the UK, along with a marker for this time point, needs to be determined. Assessment of an appropriate sample pattern and number for commercial fields is required since this has been investigated only for small experimental plots. Further consolidation of the pre-harvest germination test is also required to improve its accuracy. Delineation of the relationship between temperature, rainfall and given levels of germination/germinability would allow the prediction scheme to be more quantitative, refining the broad classifications currently envisaged.

In conclusion, project 0056/1/93 has successfully completed its stated objectives of assessing the routes of *alpha*-amylase accumulation of importance to the UK. This has demonstrated four separate possible methods of *alpha*-amylase accumulation as shown in the conceptual framework (*Figure 3.1-3.2*). Significant steps have been made towards improving the understanding of the physiology of these four routes and their interaction with the environment. Consideration of the available evidence from the literature, visits to institutions abroad and our own research has demonstrated how a prediction scheme, taking account of all the routes of *alpha*-amylase accumulation, could be instigated. Since solely meteorological predictions are not possible from the current knowledge base, the most appropriate and cost-effective scheme would be a synthesis of the two Continental schemes studied, using HFN and germination tests on farmer-supplied samples (*Figure 23*). Trial operation of the scheme has shown a good success rate for the prototype giving information which could enhance farmer profitability by increasing the chance of winning quality premia. It is now up to the industry to support implementation of any practical prediction scheme.

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We extend our thanks to our collaborators at various institutions, particularly Campden and Chorleywood Food Research Association and the John Innes Centre. We would also like to thank all the technical staff at the University of Nottingham, Harper Adams Agricultural College, University of Aberdeen and ADAS Bridgets.

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APPENDIX: PUBLICATIONS

1: Lunn, G. D., Kettlewell, P. S., Major, B. J. and Scott, R.K. (1995): Effects of orange wheat blossom midge (*Sitodiplosis mosellana*) infection on pre-maturity sprouting and Hagberg falling number of wheat. *Aspects of Applied Biology,* ***42****: Physiological responses of Plants to Pathogens:* 355-358.

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Figure 1: Graph of percentage imports and percentage crops with high *alpha*-amylase against harvest year, 1977-1993. Shows high imports in years of high *alpha*-amylase activity (low Hagberg). HGCA data (after Flintham and Gale).

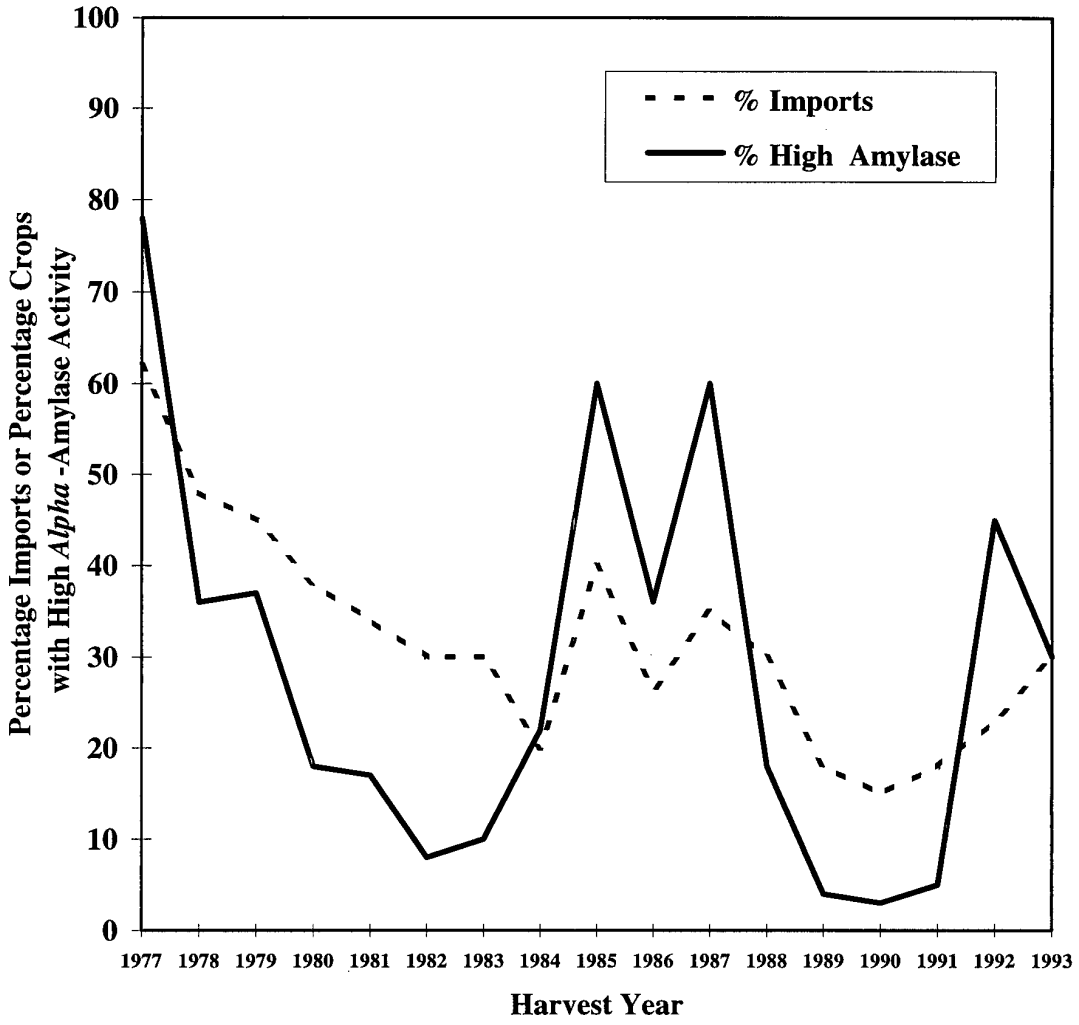
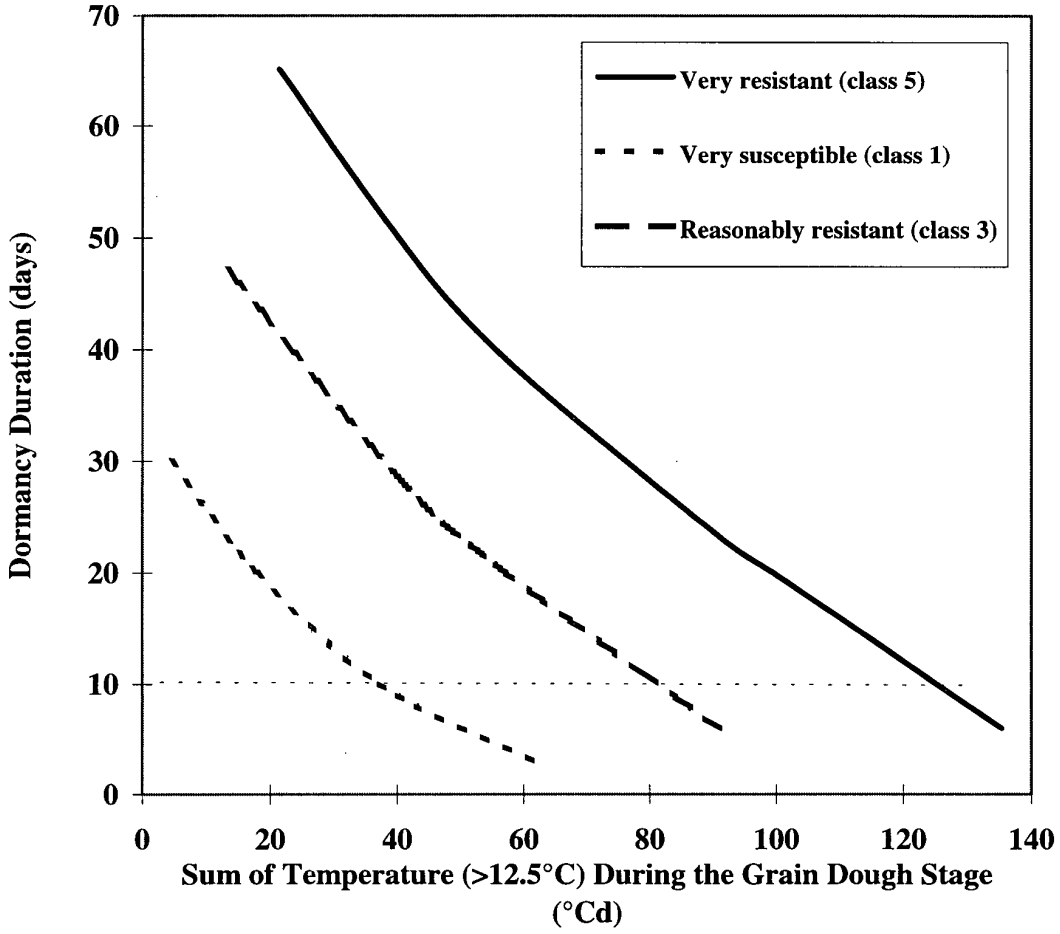


Figure 2: Graph of the relationship between dormancy duration[1] and accumulated temperature during the dough stage[2] for sprouting-susceptible and resistant cultivars. After Belderok (1973)

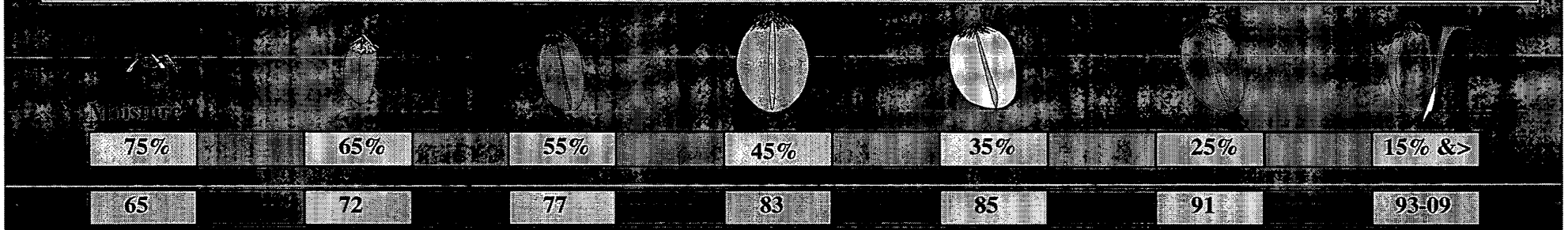


[1] Defined as the time between the date of harvest ripeness (~ 15% moisture) to the date 50% of grains in isolated grain germination tests germinated in 3 days at 18°C

[2] Defined as the time between 40-20% grain moisture

The fine dotted horizontal line shows the cut-off point of dormancy duration of 10 days, with temperature sums in excess of 37, 80 and 125°Cd indicative of sprouting risk for susceptible, reasonable resistant and very resistant varieties respectively.

Figure 3.1: University of Nottingham/Harper Adams Conceptual Framework of *Alpha*-Amylase Accumulation in Developing Wheat Grains: Version 4, Part 1



Late tillering, non-uniform crops.....

* Drought then rain	* Temperature shock + or -	* Severe cold/ wet	* Temperature during grain development
* Pest Grazing	* Slow drying ?	* Blossom midge	* Cold, wet harvest weather
* Lodging	* Cultivar (single gene?)	* Cultivar ?	* Cutivar (red colour, *Phs* gene *etc.*)
* Tramlines			

Figure 3.2: University of Nottingham/Harper Adams Conceptual Framework of Alpha-Amylase Accumulation in Developing Wheat Grains: Version 4, Part 2

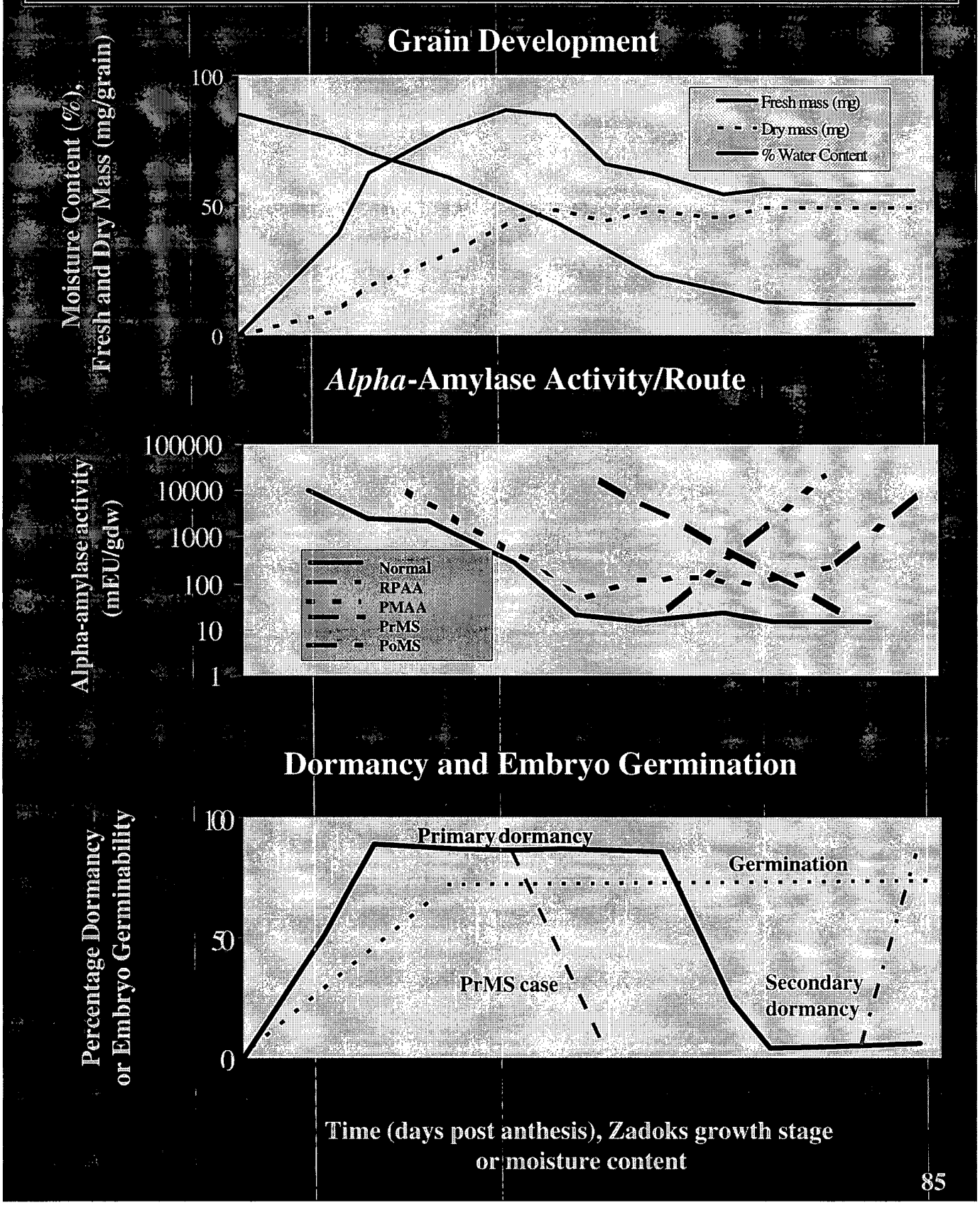


Figure 4: Photograph of an iso-electric focusing gel (pH 9-3.5 range) showing standard positions of α-AMY-1 (high pI, malt, track 2) and α-AMY-2 (low pI, pericarp, tracks 3, 4-10) isozymes. IEF standards (only amyloglucosidase activity visible) were run in track 1. α-AMY-3 isozymes can only be observed by non-equilibrium polyacrylamide gel electrophoresis. Additional tracks show the presence of only α-AMY-2 enzymes in some of the samples used for pericarp amylase studies (reconstitution experiments/air-drying, *etc.*)

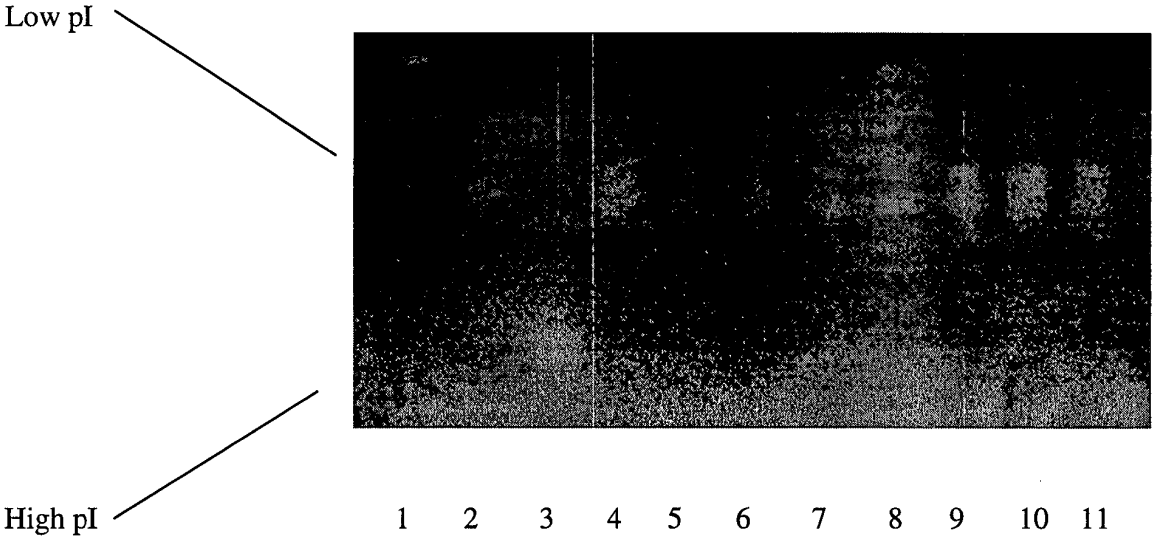


Figure 5: Photograph of a *beta*-limit dextrin gel showing location of *alpha*-amylase activity from various syndromes (i) embryo-associated activity from PrMS/PoMS, (ii) crease-associated activity from PMAA, (iii) peripheral pericarp activity from RPAA.

(i) (ii) (iii)

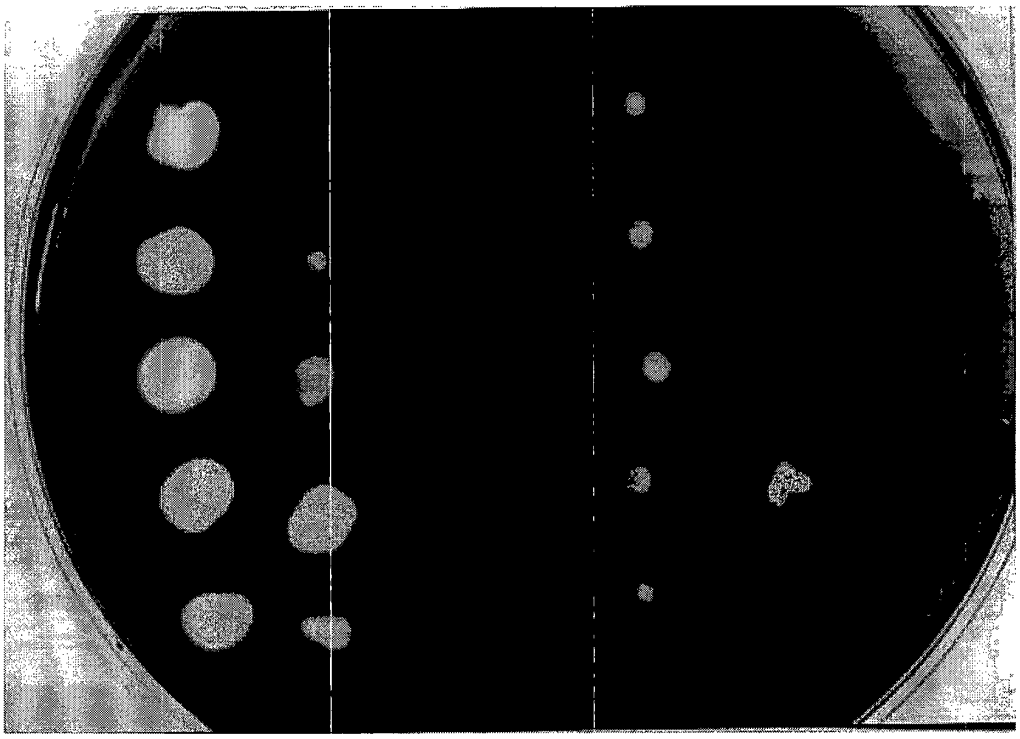


Figure 6: Graph showing effects of α-AMY-2 (green or pericarp) *alpha*-amylase activity on Hagberg falling number of the cultivar *Pastiche* (control HFN 475 s). (i) Freeze-dried α-AMY-2 activity (ii) freshly defrosted frozen α-AMY-2 activity, (iii) fresh, native α-AMY-2 activity.

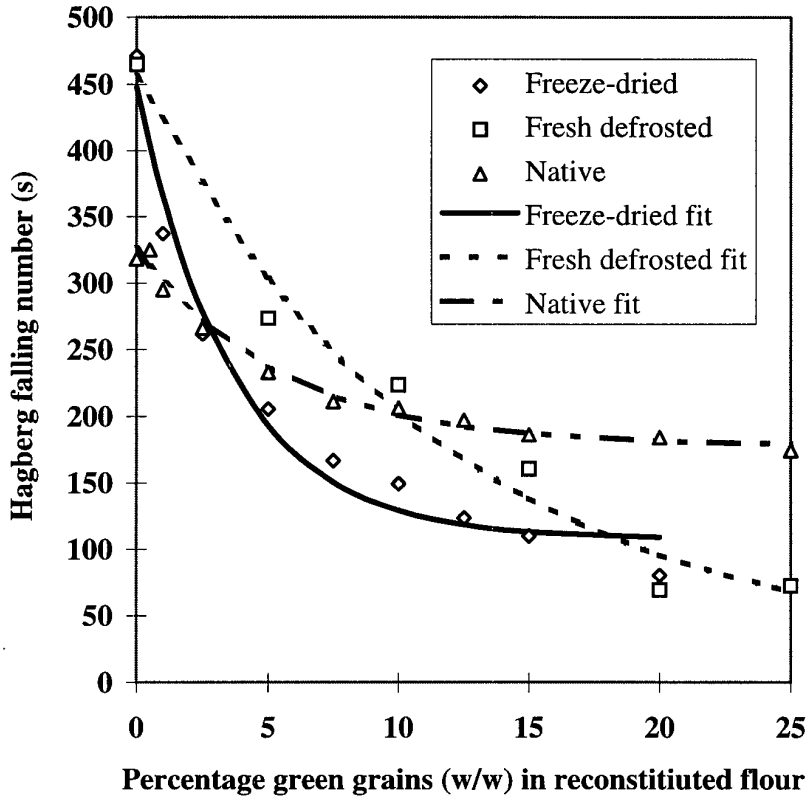


Figure 7: Graph showing effects of reconstitution with air-dried green grains (with α-AMY-2 *alpha*-amylase activity) on Hagberg falling number of the cultivar *Pastiche* (control HFN 475 s) in comparison with the effect of reconstition with freeze-dried *alpha*-amylase activity.

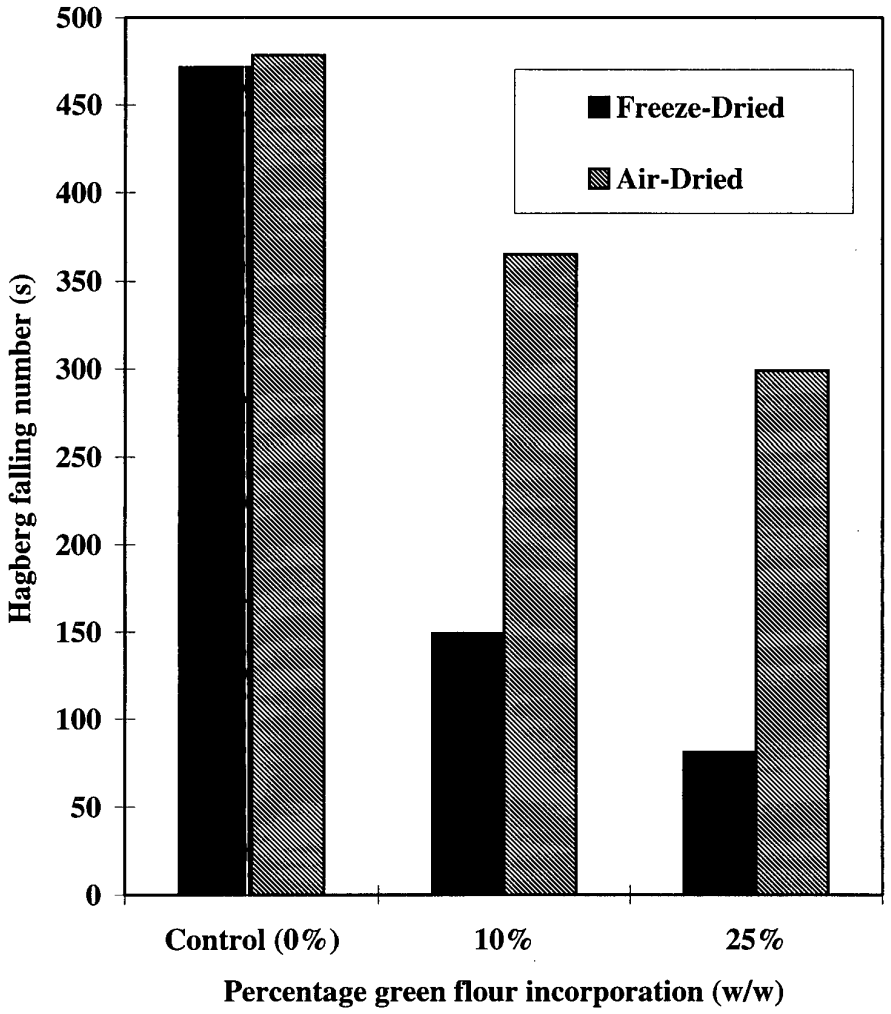


Figure 8: Graph of change in *alpha*-amylase activity and percentage moisture content in threshed green grains of the cultivar *Hornet* left to air-dry at approximately 18-21°C in the laboratory.

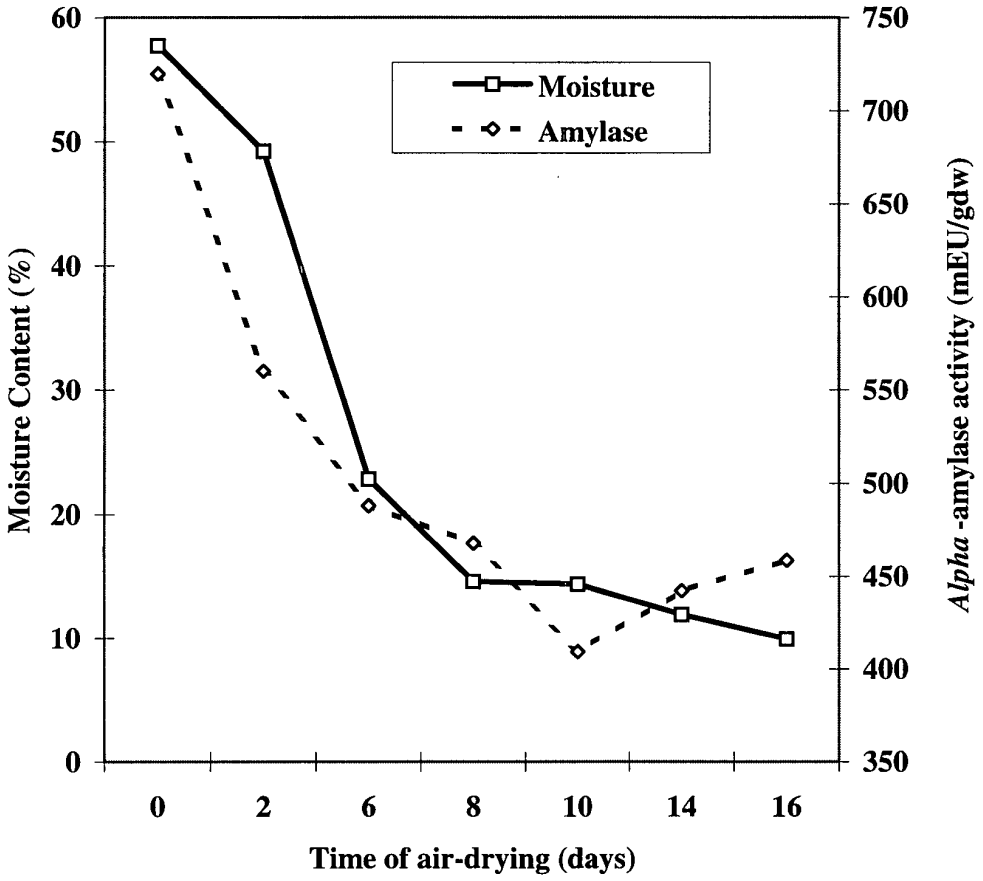


Figure 9: Effect of time of air-drying of whole grain samples (originally reconstituted with 10% green grains containing α-AMY-2) on Hagberg falling number. Control values for each experiment shown by the identified dotted lines.

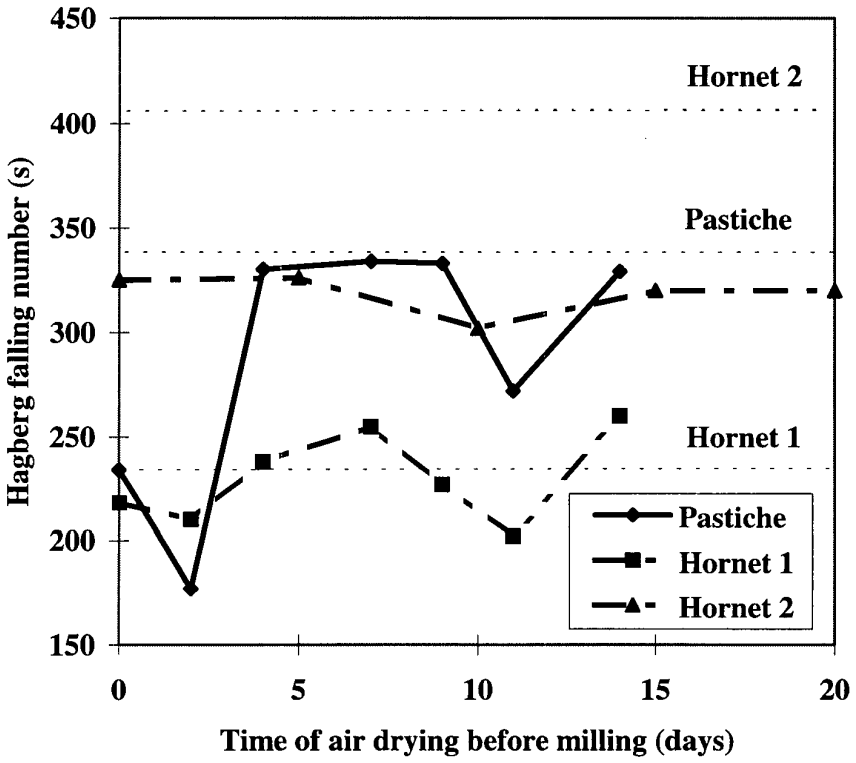


Figure 10: Graph of variation in grain percentage moisture content determined between 800-850°Cd post-heading in samples of *Riband* from the multi-site field trial.

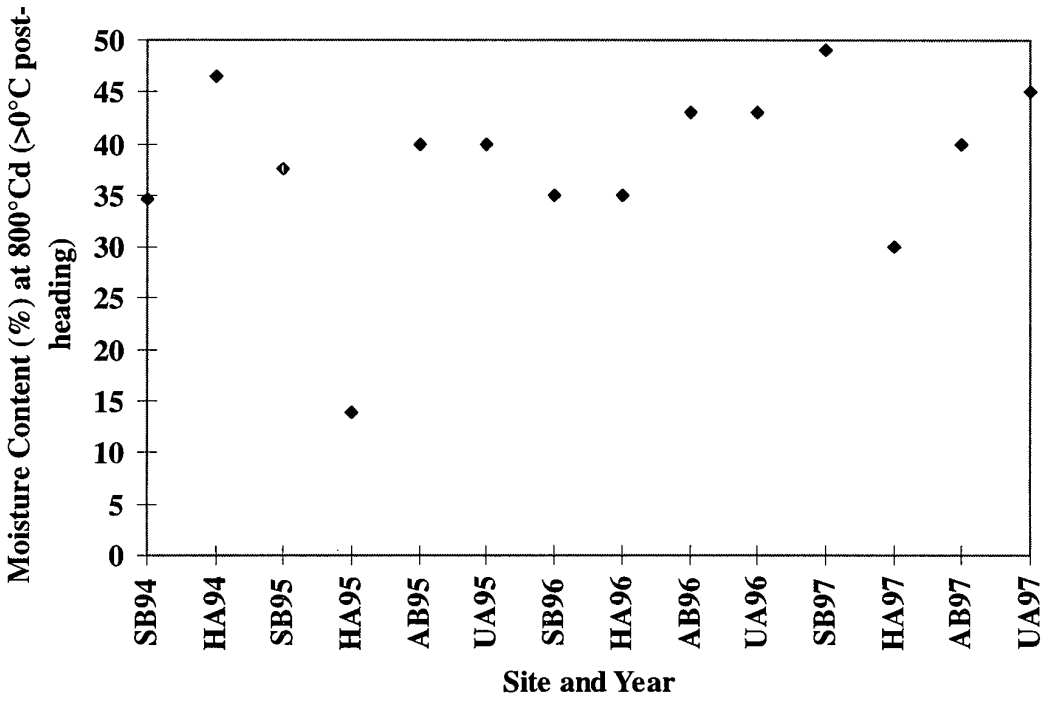


Figure 11: Graph showing linear regression analysis of *Hornet* drying rate between 50-20% moisture content in 1994-1995 field experiments.

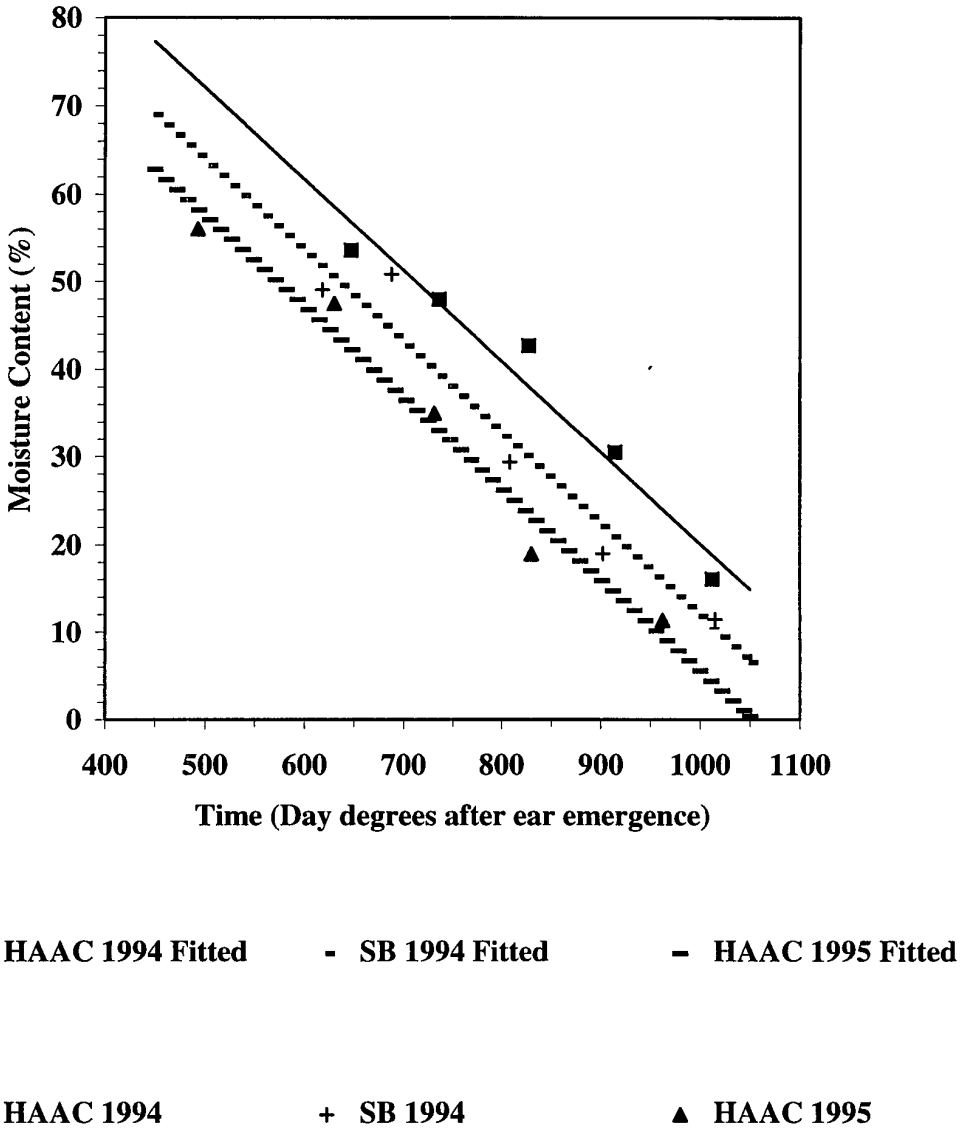
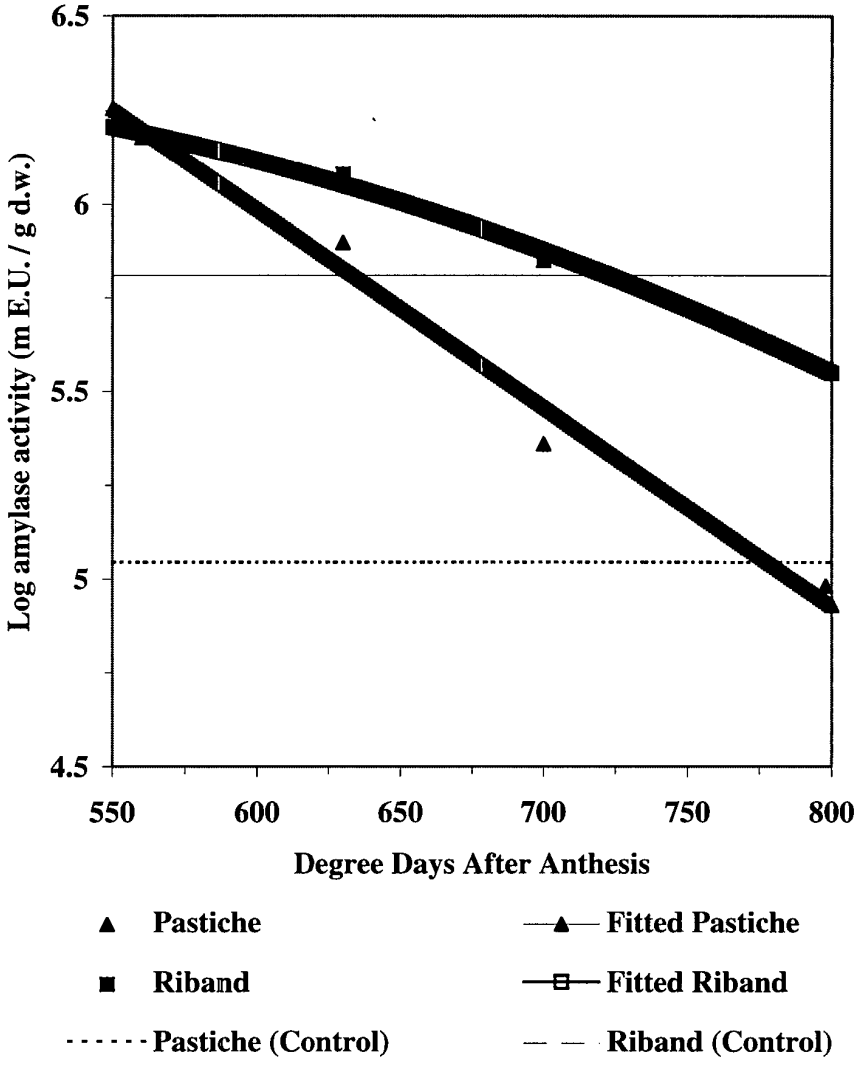


Figure 12: **:** Linear fit of *alpha*-amylase activity at harvest (log mEUgdw^{-1}) with time of transfer to transient high-temperature shock in Experiments 1 and 2.



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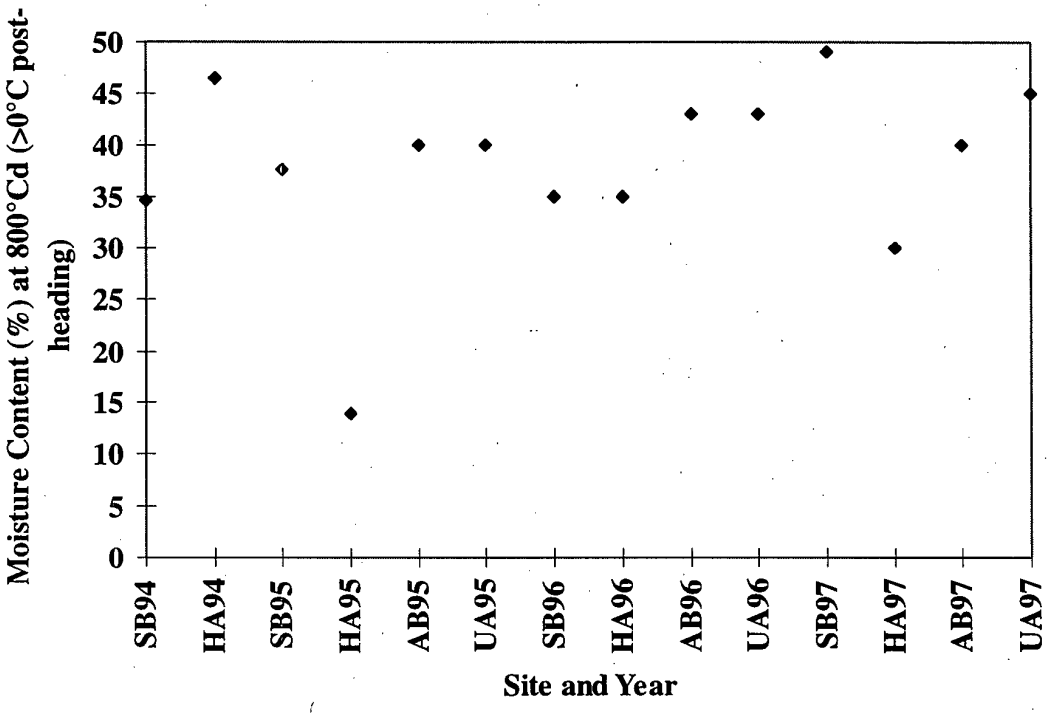
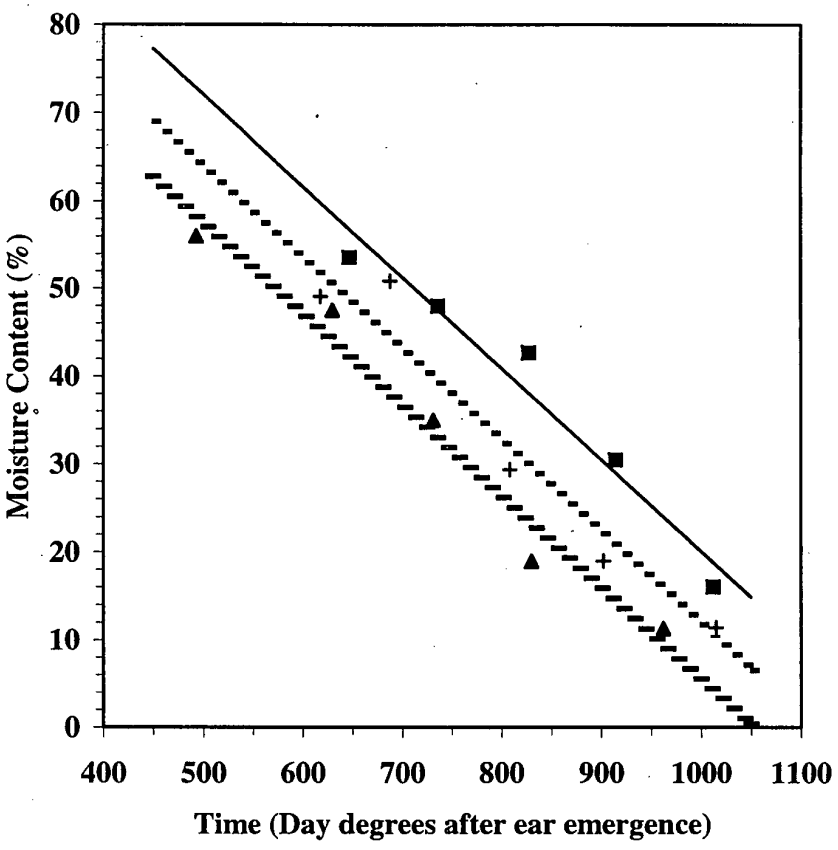


Figure 11: Graph showing linear regression analysis of *Hornet* drying rate between 50-20% moisture content in 1994-1995 field experiments.



HAAC 1994 Fitted SB 1994 Fitted HAAC 1995 Fitted

HAAC 1994 SB 1994 HAAC 1995

Figure 15: Pre-maturity sprouting induction in glasshouse plants irrigated intensively for five days at different stages of development.

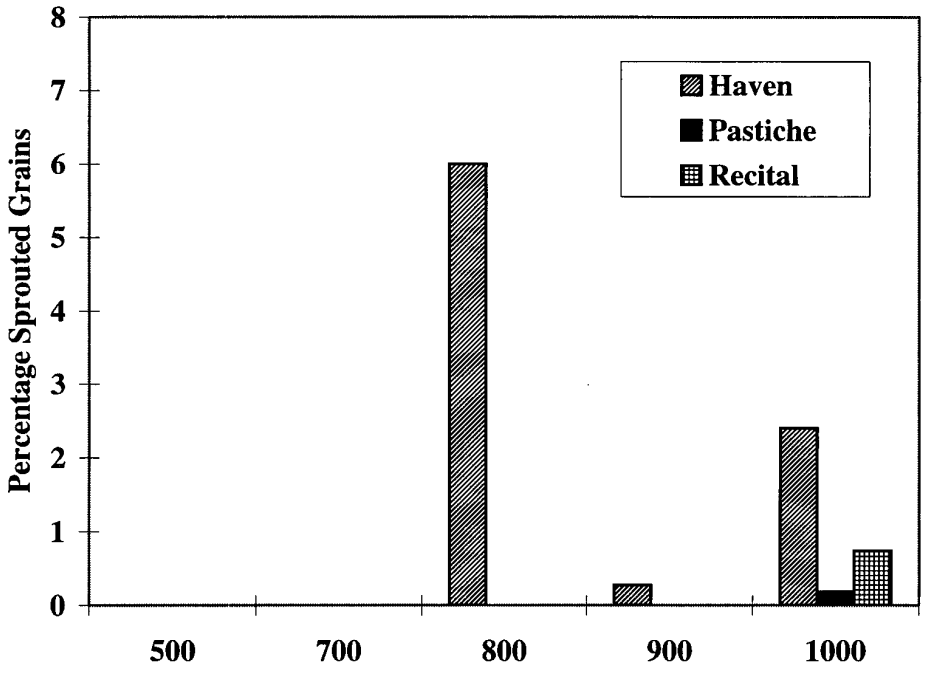
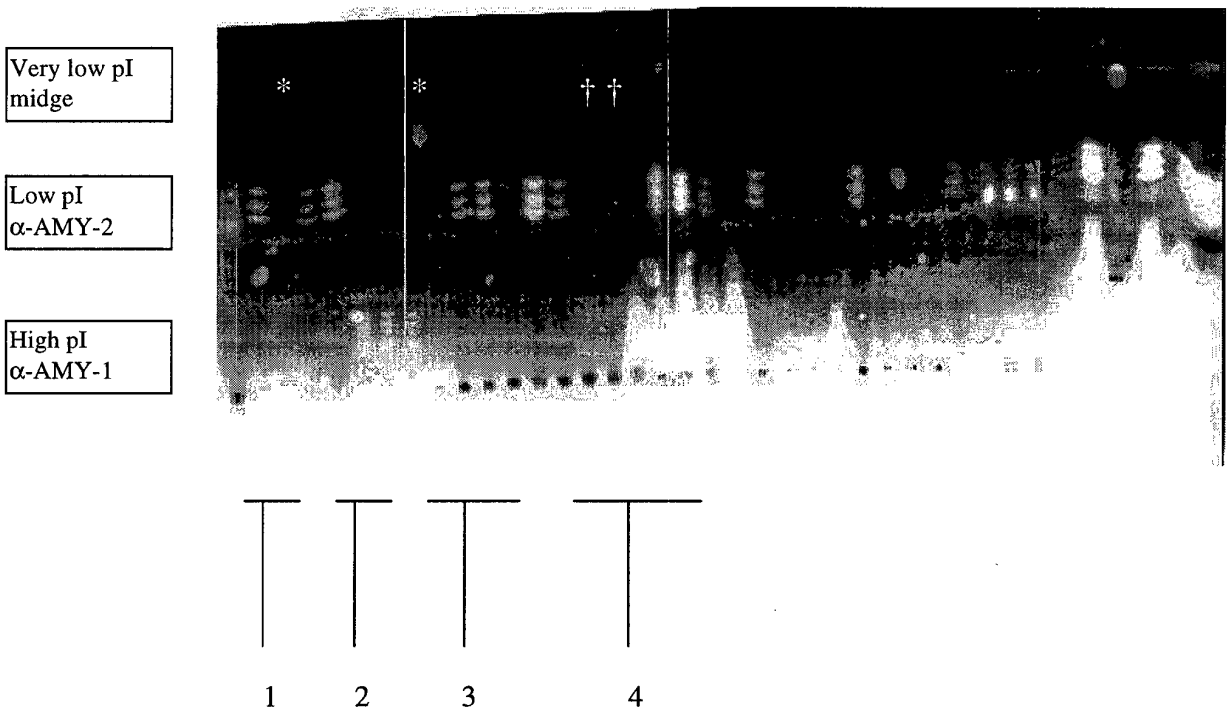


Figure 16: Iso-electric focusing of midge larval, midge-damaged- and related grain extracts



* Weak and transient amylolytic activity found in midge larval extracts

† Extracts of mildly midge-damaged grains (indented) showed no *alpha*-amylase activity

1: Extracts of immature grains showed pericarp (α-AMY-2) *alpha*-amylase activity

2: Extracts of immature *Haven* showed PMAA

3: Immature grains (α-AMY-2)

4: Extracts of severely midge-damaged grains (with split pericarps) showed plant germination *alpha*-amylases (α-AMY-1 or α-AMY-1+2)

Figure 17: Effects of pericarp inrtegrity and moisture availability on pre-maturity sprouting of the cultivar *Haven*

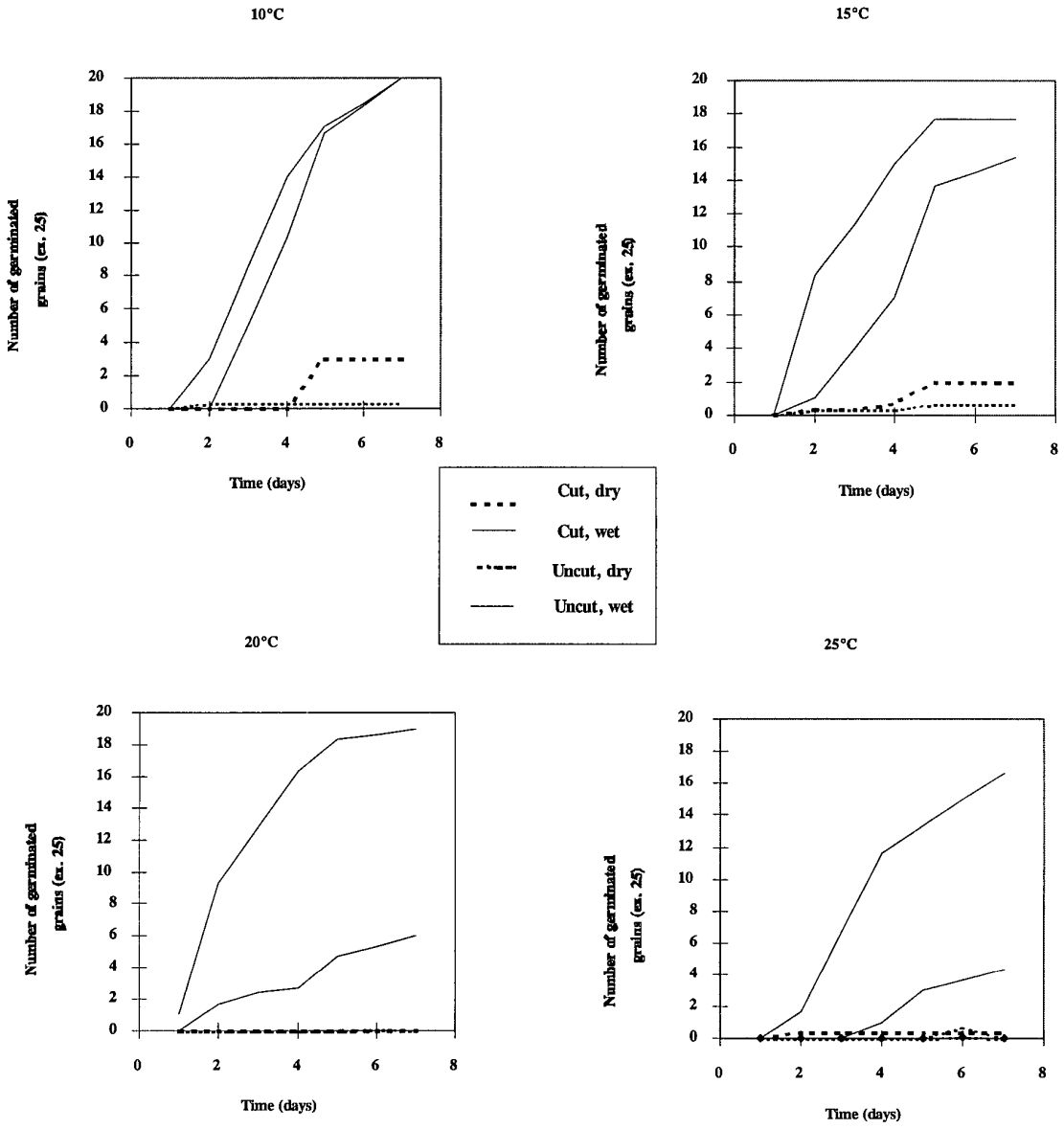
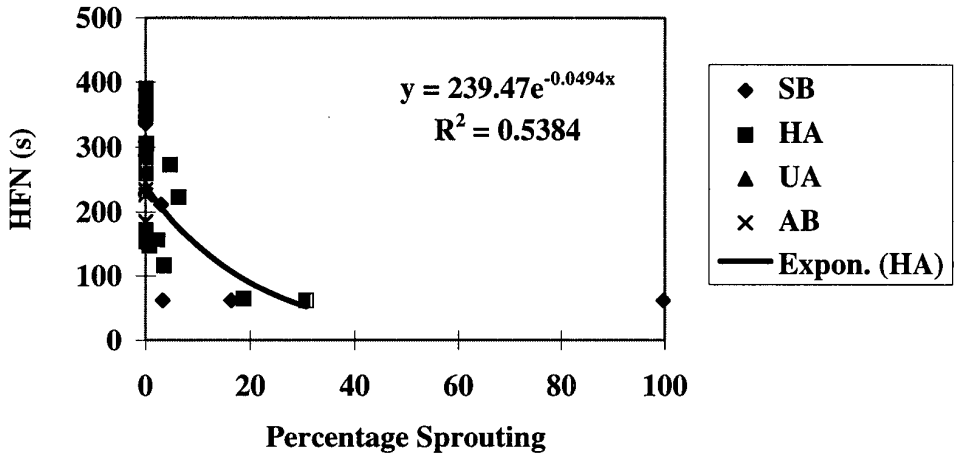


Figure 18: Reduction in HFN caused by sprouting in the cultivars *Hornet* and *Soissons*.

(a) Hornet



(b) Soissons

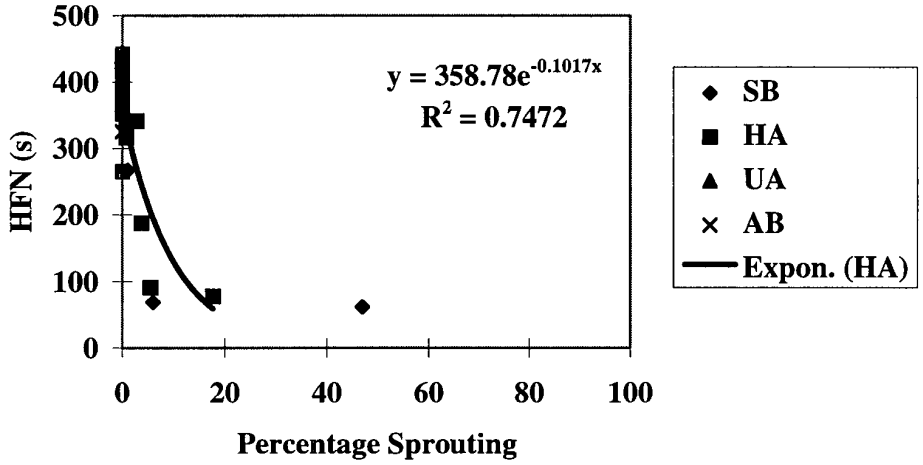


Figure 19: Exponential relationship between percentage sprouted grains and percentage sprouted ears counted in samples analysed by the French *in vitro* ear dormancy test.

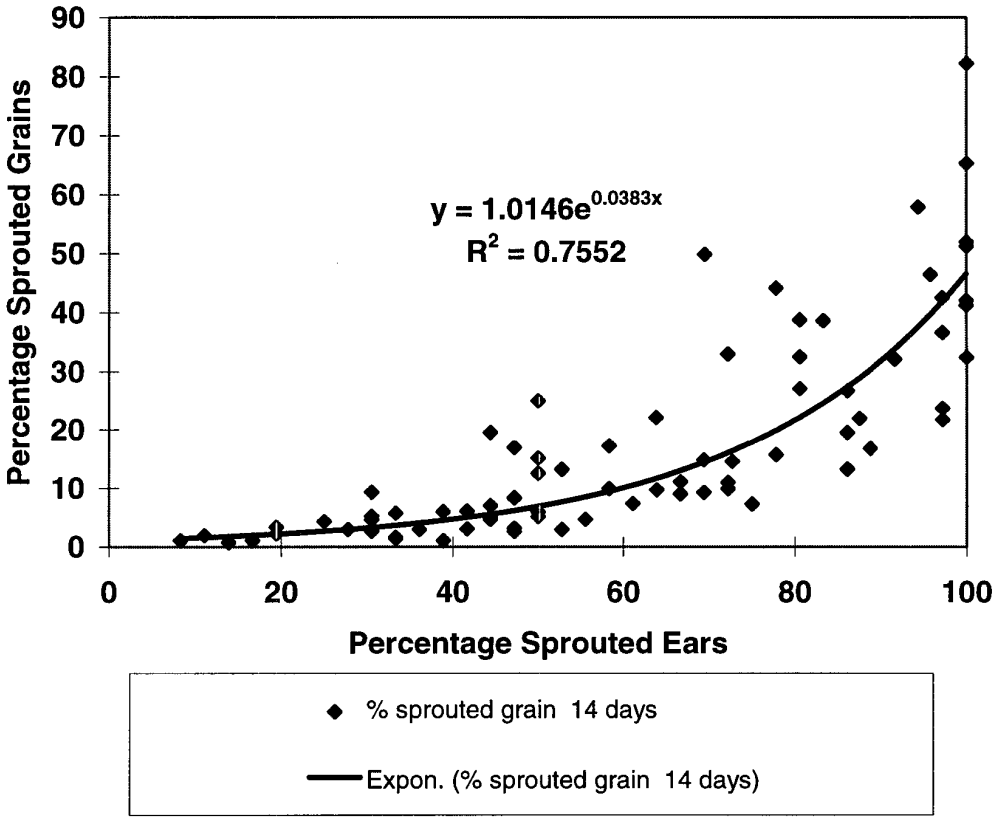


Figure 20: Relationship between dormancy index (G_{20}-G_{25}/G_{20}) for the French cultivar *Soissons*, developed with French data. Data from UK trial sites also shown.

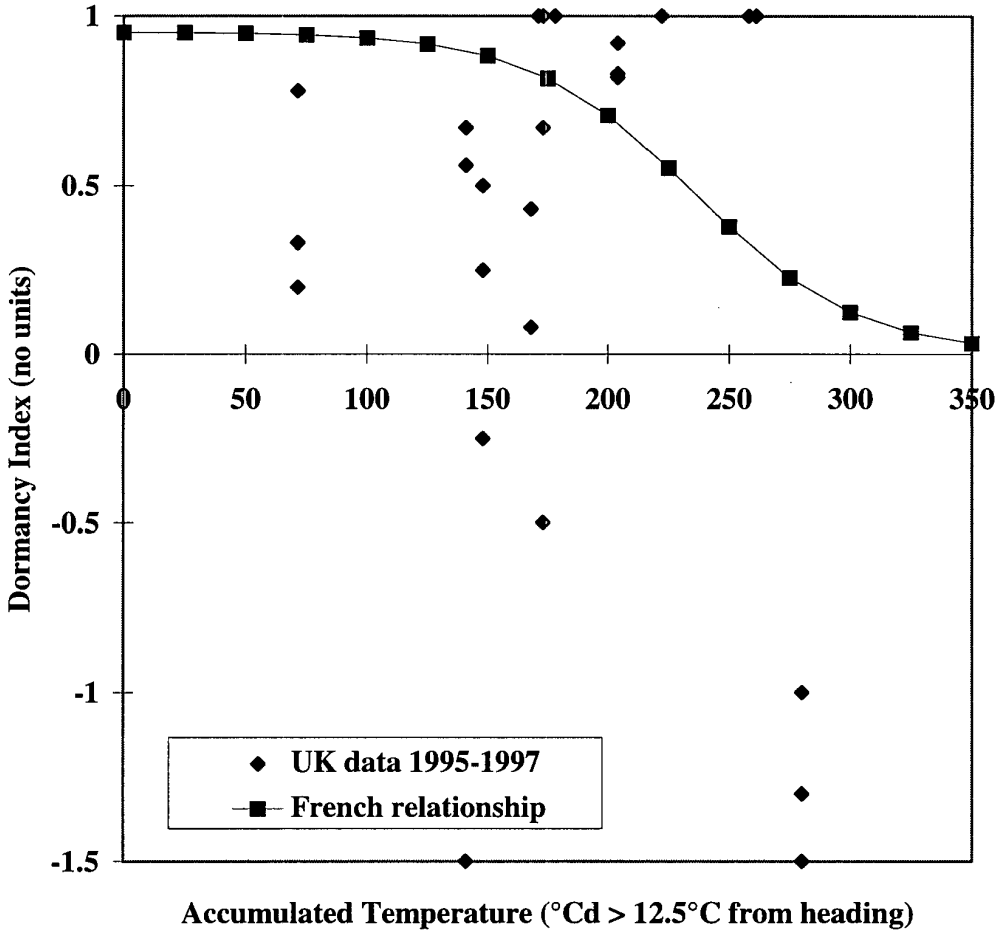


Figure 21: Relationship between dormancy duration in days and temperature accumulated > 12.5°C between heading and 15% moisture. UK data for the cultivar *Riband*.

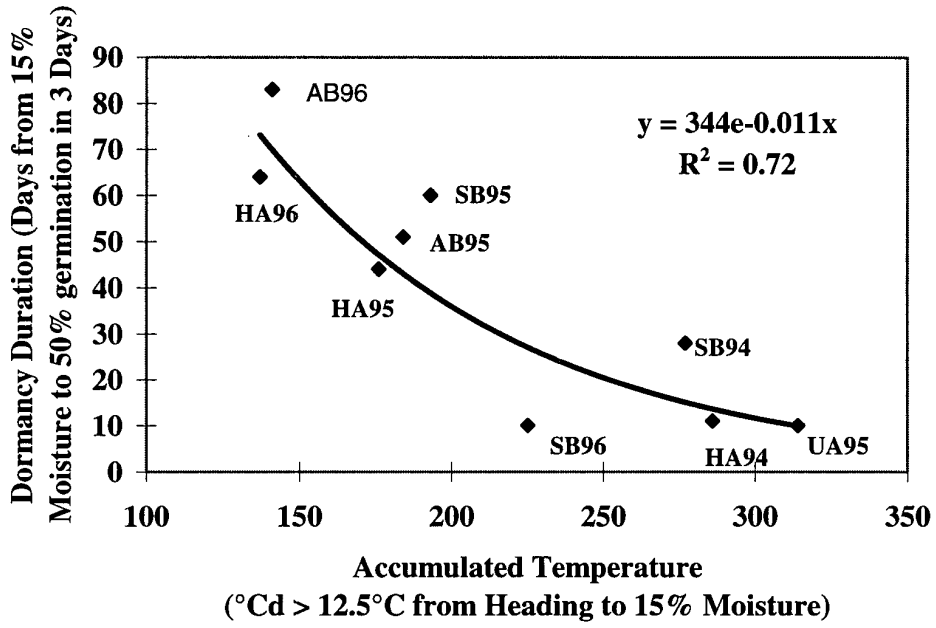


Figure 22: Relationship between the percentage germination recorded in 15°C isolated germination tests and the percentage sprouting recorded in the field after overhead irrigation (cultivar *Haven*, Harper Adams) for four days after sampling for IGG testing.

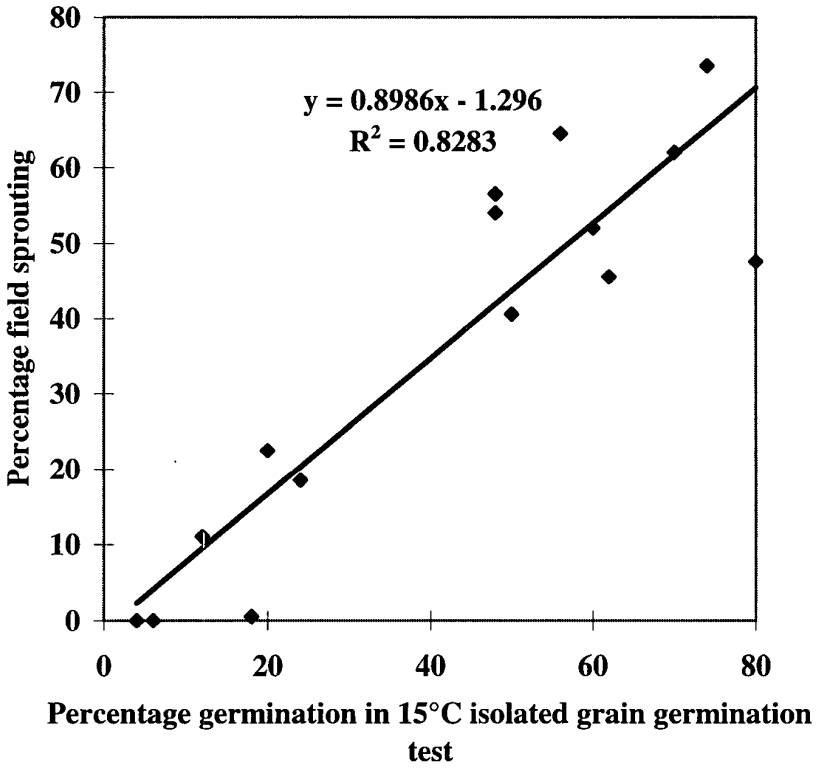


Figure23: Flow Chart Showing Operation of the University of Nottingham/Harper Adams Prototype Hagberg Falling Number Prediction Scheme

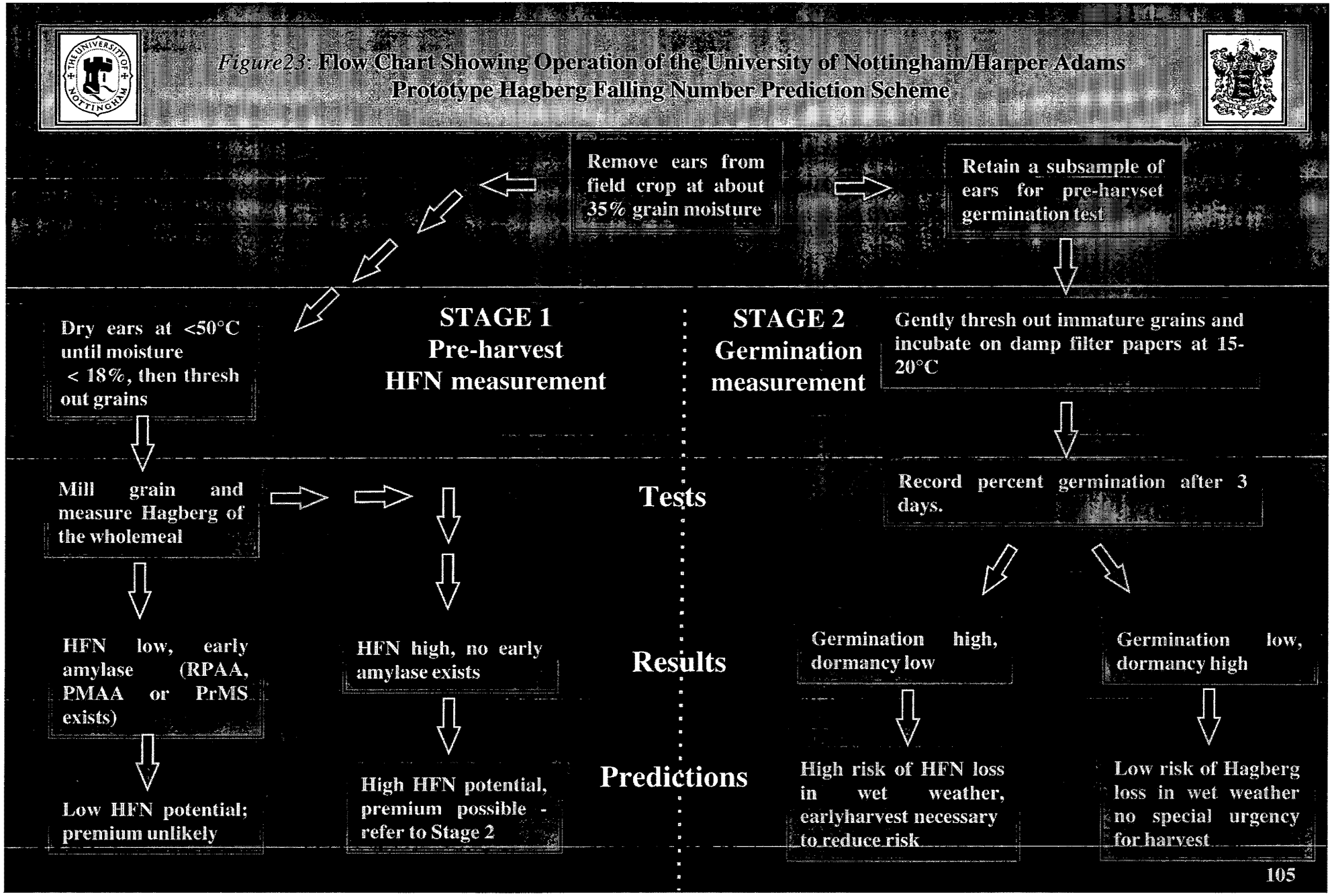


Figure 24: Effect of number of samples on coefficient of variation on ANOVA of Hagberg falling number data.

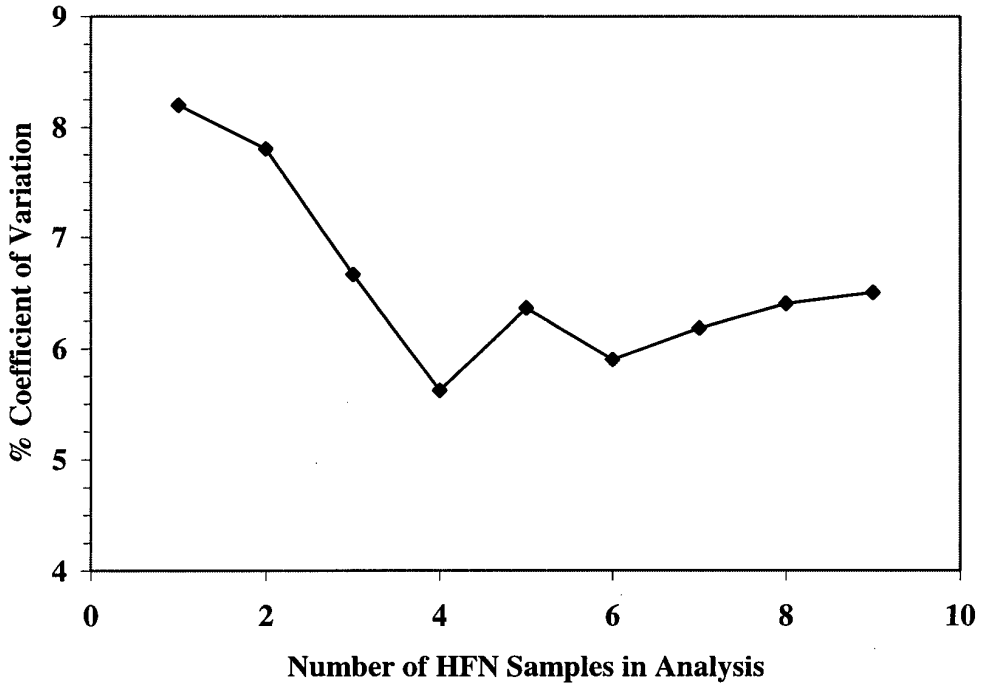


Figure 25: Example regression of pre-harvest HFN to combine harvest HFN, with 75% confidence limits (cultivar *Hornet*)