The Relation between Preharvest Sprouting and Embryonic Sugars Levels in Two-rowed Malting Barleys.

by

Angela B.M. Tessier

A Thesis

submitted to the Faculty of Graduate Studies in partial fulfillment of the requirements for the degree of

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ABSTRACT

Angela B. M. Tessier, M.Sc. The University of Manitoba.
The Relation between Preharvest Sprouting and Embryonic Sugar levels in Two-rowed Malting Barleys.
Major professor: Dr. G. M. Ballance.

Malting barleys that germinate quickly are termed "rapidly modifying" and these barleys have a propensity to sprout in the field when poor harvesting conditions prevail. The purpose of this work was to evaluate if the mobilization of raffinose and sucrose, (sugars located in the embryo of the kernel) that occurs in the early phase of germination might serve as indicators of premature sprouting.

The susceptibility of ten two-rowed malting varieties to sprouting was determined using a rain simulator. Results demonstrated that eight of the ten examined varieties were susceptible to sprouting. Standard germination tests were conducted on all varieties to evaluate the levels of germination in the post-harvest phase. The rates of germination in this phase revealed a parallel between those varieties exhibiting low levels of germination and little or no sprouting.

The levels of sucrose and raffinose in the embryos of varieties susceptible and resistant to sprouting were determined by High Performance Liquid Chromatography

(H.P.L.C). In Hannchen, a variety resistant to sprouting, raffinose constituted on average 50% of the total embryo sugars of all kernels on the spike. In Ellice, a variety susceptible to sprouting, raffinose constituted on average 36% of the total embryo sugars for all kernels on the spike.

Heads of Ellice were subjected to conditions that would induce sprouting, and the levels of sucrose and raffinose in the embryos of kernels at all positions on the spike were analysed by H.P.L.C. Raffinose was mobilized most rapidly and essentially depleted from the topmost sector of the head before sprouting was visible.

There was a depletion of raffinose when threshed seed was subjected to cycles of steeping and drying. Reduced germination rates occurred and a decrease in vigour was also observed.

The mobilization of raffinose and sucrose has been demonstrated prior to visible sprouting occurring. More research must be conducted to evaluate different varieties in different environments. This study may be of benefit to the malting industry as it is now possible to identify grain which appears to be of good quality, but as a result of poor harvesting conditions has sprouted prematurely.

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To Dad, Mom, Liz, Stef, John, Gran and Poppa.."Cé go rabhamar thar lear, bhí a fhios againn go rabhamar le chéile" and to the T's for a home away from home...Thank you.

This thesis is dedicated to my husband Tom..Thanks for being there.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BET	Betzes
ELL	Ellice
HAN	Hannchen
HAR	Harrington
NOR	Norbert
201	TR201
219	TR219M
479	TR479
490	TR490
LAM	Lamont

These abbreviations are only used in Figures and Tables.

FOREWARD

This thesis is written in a classical form. The Results and Discussion is divided into two sections. Section I discusses the susceptibility of the examined varieties to sprouting. Section II discusses the mobilization of the sugars in the embryo and how it might serve as an indicator of premature sprouting.

INTRODUCTION

Barley is a grain frequently used in the production of alcoholic beverages. In the malting industry, the quality of barley used is the highest grade possible. Any deviation from high quality has a negative impact on the quality of products manufactured in breweries and distilleries.

Malt is derived from the controlled steeping, germination and kilning of malting barley. The quality parameters determining high grade malting barley include varietal purity (>95%) of an acceptable malting variety, a low percentage in screenings and low moisture content (11-12%). There are also a number of factors that reduce the suitability of barley for malting. These factors are the presence of diseased, broken, peeled, weathered and frost damaged kernels. Protein content is a major factor in determining the quality of malting barley. Desirable protein levels in two-rowed barleys are 10.5 to 12.5% on a dry weight basis. Barley, which is high in protein, produces a malt with a low level of extract. Also, protein precipitation may occur in the resulting alcoholic products particularly when they are stored at lower temperatures.

The most important criteria are that the barley is uniformly plump and has vigorous germination. This ensures that a rapid and homogenous malt occurs. Formerly, malt was used as the source of simple sugars, which when fermented resulted in the production of alcohol. In current North

American brewing, when malt is used, it is primarily used as a source of enzymes to degrade starch from an unmalted source referred to as an adjunct.

Adjunct brewing has been the standard in North America for many years, and the more enzymic 6-rowed barleys were favoured by the malting industry as a source of malt. With the introduction of Klages and the realization that enzymic 2-rowed barleys could be produced, the demand for such barleys by the industry increased as did production. The development of rapidly modifying 2-rowed barleys has been reported to be accompanied by the increased incidence of preharvest sprouting.

Preharvest sprouting has been defined as the initiation of germination in physiologically mature grain in response to poor harvesting conditions (e.g. rain and high humidity) (Derera, 1988). The level of sprouting damage can vary widely, depending on temperature and the intensity of rainfall experienced at harvest time (Mares, 1984). The effect of 5 centimetres of rain over three days will be more detrimental than the same quantity of rainfall over three hours. The stage of ripeness, and the degree of lodging present in the crop are also important factors (Mares, 1984).

Sprouted grain is not acceptable for malting. The embryo contains primordia, (growth initiative tissue) that instigate root and shootlet development. If the primordia begin to develop, and this growth is interrupted, the

process may not be initiated again. In sprouted grain, there is a loss in germination uniformity and a reduction in germination vigour. In sprouted grain, the embryo is slightly exposed, and therefore susceptible to physical damage and microbial infection. The kernel density is reduced and a non-homogenous malt with a lower extract than expected occurs (Burger and LaBerge, 1986). Problems of poor lautering, low extract yield and fermentation difficulties arise in the brewery (Burger and LaBerge, 1986). The beer tends to have a poor flavour and a shortened shelf life. Schildbach (1987) described how malt prepared from barley subjected to differing quantities of rainfall had produced increased levels of gushing in bottled beer as a result of sprouted barley. Thus, sprouted barley affects both malting and brewing procedures.

In Western Canada, in the past decade there has been an increase of 15% in the area seeded to two-rowed malting barley*. This is a result of a greater demand on the international market for barley of this nature (LaBerge, 1988). Current 2-rowed malting barleys modify rapidly and are reported to have a propensity to sprout in the field when poor harvesting conditions prevail.

When barley is being purchased, a standard germination test is conducted on what is termed a 'pre-load sample'.

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*An historical background detailing the development of two-rowed malting barley in Canada is presented in Appendix I.

The rate of germination must be 95% or higher and all other standards must be met in order that the barley is of acceptable malting quality. Prior to shipping, which could be many months later, a 'loading sample' is taken; the standard germination test is repeated and all other criteria are re-examined.

Problems have recently occurred in the malting industry. On occasion, grain has been purchased which has met all the factors determining quality and which appears on a visual basis to be acceptable. However, when placed in the conditions of malting, the barley would not germinate, or germinated poorly. Sprouting was not visible. A loss in germination vigour may be attributed to poor storage conditions or preharvest factors.

Therefore, the purpose of this experimental work was to (i) to determine the degree of varietal susceptibility to sprouting in ten two-rowed malting barleys, and (ii) to ascertain if the mobilization of raffinose and sucrose that occurs during the initial stages of germination could be used as an indicator of the early stages of sprouting that precede any visible changes.

LITERATURE REVIEW

2.1 Physical characteristics influencing susceptibility to preharvest sprouting

The physical characteristics of the cereal plant may influence the degree of resistance the plant has to preharvest sprouting. The presence of the pendant spike and tightly closed glumes has been demonstrated to be important. Derera (1988) found that pendant spikes shed the water of a quick shower very readily, but there is no protection against soaking rain. The same situation arises in the case of tightly closed glumes. Gaping glumes, as found in rye and male-sterile wheats, absorb water quickly and this stimulates the seed to sprout readily if dormancy is not present. The club head character has also been shown to increase water uptake (King, 1984).

King and Richards (1984) found that the variation in sprouting among cultivars of wheat differed significantly after 30 hours of showering with water. The differences were partially explained by the presence of awns. Awnless lines of wheat took up significantly less water and were less susceptible to sprouting. Isogenic lines with and without awns demonstrated similar results, even when higher rainfall intensities were employed. However, the removal of awns did not increase or decrease sprouting susceptibility, as awned and deawned heads of the same cultivar absorbed water at the

same rate. King (1988) suggested that the husk structure associated with awns, in addition to the degree of gaping in the bracts may be responsible for a greater degree of water uptake by awned cultivars.

King (1984) reported that isogenic lines of wheat differing in grain hardness and seed coat colour did not demonstrate differences in water uptake and rates of germination. Other ear characteristics suggested by King warranting further investigation to decrease the rate of ear wetting include the effect of glabrousness (smoothness), glaucousness (waxiness), and the ear nodding angle. The latter suggestion requires further research as the optimum ear nodding angle(s) have yet to be determined. Work conducted by Miller and Brinkman (1983), demonstrated that the nodded spike characteristic is sufficiently heritable to manipulate in a barley breeding program, provided that evaluation and selection is made on the basis of F3 or F4 head or plant rows, rather than individual F2 plants.

2.2 Mobilization of endosperm reserves

2.2.1 Structure of barley

The barley seed is composed of two distinctive regions, the endosperm and the embryo. The non-living starchy endosperm contains both carbohydrate and protein reserves, and constitutes the bulk of the cereal grain. It is surrounded by the living tissues of the aleurone layers and is separated from the embryo by the scutellum. The embryo,

scutellum, and aleurone tissues (all living) have major roles to play in the mobilization of the reserves in the endosperm. The two maternal tissues (integuments), the inner (testa) and the outer (pericarp) envelop the whole caryopsis. These integuments are non-living at maturity. In the mature grain of barley, the dead glumes (hull) adhere tightly to the grain.

2.2.2 Initial events in the mobilization of reserves

In the mature, air-dry cereal grain the water potential is very low and therefore, initially the rate of water uptake is high, with physical imbibition processes dominating (King, 1988). Living and heat-killed caryopses show the same initial rate of water uptake. This confirms that, initially, only physical processes are involved. Temperature affects the initial rate of water uptake (5% increase per 10^0C) through physical, not biochemical changes (King, 1988).

Hallam *et al.*, (1972) examined the biochemical and fine structural changes during the germination of rye (*Secale cereale*). The imbibition of water measured as an increase in fresh weight in embryos occurred in three phases. The first phase of ten minutes was accounted for by a rapid uptake in water into the outer layers of the seed. In the next stage (50 minutes duration) there was a slight increase in the fresh weight of the embryos. The third phase from one hour onwards was one of continuous water uptake. The levels of

CO_2 in the seed increased in a linear fashion after one hour of imbibition. The latter coincides with an increase in both the number of mitochondria and the number of cristae in this organelle.

2.2.3. Pathway of Water Permeability

Work conducted by Briggs and MacDonald (1983a) examined the permeability of the surface layers of cereal grains using aqueous solutions of salts or eosin. In naked grains, the pericarps and in husked grains the husk and the pericarp were stained with eosin to varying extents. This indicates that regions of differing permeability are found in these tissues. The dyes did not permeate the testa or pigment strand or gain access to the interior unless the surface layers were perforated, cut or otherwise damaged. The structure of the micropyle was also implicated in regulating the entry of aqueous solutions. While the availability of water within the grain is essential for the initiation of germination, Briggs and MacDonald also demonstrated that the level of humidity within the seed is also important. Stripped grains held for 80 hours in a water-saturated atmosphere did not germinate unless they were placed in wet sand. In a dry atmosphere grains with their apices in wet sand failed to grow. The authors concluded that in the absence of evaporation from the grain surface, insufficient moisture was conducted from the apex to the embryo to permit germination.

Briggs and MacDonald (1983a) demonstrated that the testa limited the penetration of iodine into barley. Iodine penetrated irregularly over the whole surface of decorticated barley, causing blotches of blue-black staining in the starchy endosperm. When potassium iodide was used (iodine was present as the tri-iodide ion) iodine penetrated at the embryo end of undamaged, decorticated barley. The presence of iodine was indicated by the formation of a blue-black collar in the starchy endosperm around the scutellum.

Based on this information the authors concluded that the main path of embryonic water uptake is via the pericarp to the embryo, and not by means of the endosperm. The form of the compound (ionized or unionized) in the case of iodine determines whether or not it gains access into the grain.

2.2.4 Mobilization of protein reserves

Germinating barley is reported to contain at least eight different peptidases: three carboxypeptidases, three aminopeptidases which act on the *B*-naphthylamides of various amino acids, and two peptidases characterized by their action on the peptides Leu-Gly-Gly and Ala-Gly (Mikola and Kolehmainen, 1972). The hydrolysis of reserve proteins in the germinating barley grain proceeds in two distinct phases. First, the reserve proteins in the cells of the aleurone layer are hydrolysed to provide amino acids for the synthesis of various hydrolytic enzymes, including

proteinases. When germination occurred, the aleurone layer contained high activities of all three groups of peptidases. There were no changes in the activities of the five aminopeptidases, while the carboxypeptidases exhibited a small increase in activity. Work performed in other systems (Chrispeels and Boulter, 1975) for example, mung bean suggest that protein bodies contain acid proteinases and carboxypeptidases. The combined action of both of these enzymes results in the degradation of protein. Free amino acids and small peptides pass into the cytosol where the derived amino acids are subsequently used in the production of hydrolytic enzymes (Chrispeels and Boulter, 1975).

In the second stage of mobilization the proteins of the starchy endosperm are hydrolysed. Proteolysis is initiated by acid proteinases which degrade the water insoluble storage proteins (hordeins and glutelins) into soluble peptides. As germination progresses, gibberellins induce further synthesis of proteinases in the aleurone layer. These enzymes are then secreted into the starchy endosperm (Jacobsen and Varner, 1967). The soluble peptides serve as substrates for carboxypeptidases. The amino acids and peptides derived as a consequence of the action of the carboxypeptidases are absorbed by the scutellum. Work performed by Salmenkallio and Sopanen (1989), demonstrated that the uptake of amino acids by barley scutella was similar to that of wheat and was by means of four known systems: two nonspecific amino acid systems, one system

specific for proline and another specific for basic amino acids. Peptides not completely degraded in the endosperm are hydrolysed in the scutellum (Mikola and Kolehmainen, 1972). The amino acids are transported to the embryo and are in turn, used in growing tissues.

At the end of germination as the amino acid concentration in the endosperm decreases, there appears to be de novo synthesis of new carrier proteins (permeases or translocases) (Nyman et al., 1983). This synthesis does not appear to be regulated by gibberellins, but is slowed down or stopped by glutamine which appears to regulate the flow of amino acids from the endosperm to the seedling.

2.2.5 Carbohydrate Reserves in Barley

Carbohydrates constitute between 78 - 83.9% of the dry weight of barley grains (Henry, 1988). Starch composes approximately 62% of the barley grain (Henry, 1988) and it consists of a mixture of amylose, an essentially linear (1-4)-alpha-glucan, and amylopectin, a branched polymer with (1-6)-alpha-linkages at the branch points of an otherwise (1-4)-alpha glucan (Henry, 1988). Barley starch consists of two populations of granules, large A type (10 - 25 um) and small B type (1 - 5um). The small granules account for approximately 90% of the total number of granules, but only 10% of the total starch weight (Bathgate and Palmer, 1972). The fine structure of the two populations of starch granules appears to be similar but not identical (MacGregor et al.,

1971 and Rahman et al., 1982). Small granules tend to have lower amylose content, higher protein and lipid contents and higher temperatures of gelatinisation. Starch granules are usually compact bodies containing crystalline and amorphous regions, which give starch granules their characteristic lamella structure (Palmer, 1989).

The structural polysaccharides of the barley grain comprise the cellulose microfibrils and the matrix polysaccharides, mainly the beta-glucans and the arabinoxylans. Found mainly in the endosperm, the beta-glucan content varies with variety and environment, but is usually 3 to 6% (Henry, 1988). The arabinoxylans (composed of beta-xylan chains with arabinose residues attached) make up 25% of the matrix polysaccharides in the cell walls of the barley endosperm (Henry, 1988). Although these polysaccharides are structural in the mature seed, specific polysaccharides, e.g. beta-glucan, which is rapidly degraded may also serve as a reserve polysaccharide.

LaBerge et al., (1973) studied the changes in the sugar content of developing barley grains. The concentration of free sugars decreased from 6.5% of grain dry weight immediately following anthesis to 2% of dry weight at maturity. Initially, reducing sugars constituted 80% of the total sugars present. Fructose and glucose were present in large quantities: lower levels of galactose, mannose and maltose were also present. In the mature grain the proportion of reducing sugars had declined to 10% of the

total sugars present. As kernel dry matter increased, the level of the trisaccharide, raffinose increased, from trace amounts 20 days after anthesis to 0.7% of kernel dry weight (i.e. 250 ug/kernel) 36 days after anthesis, and declined to 0.4% in mature kernels. Sucrose decreased slightly from 1.8% to 1.2% of total kernel dry matter, but actually increased from 90 ug/kernel in immature kernels to 500 ug/kernel in mature kernels.

O'Sullivan first reported raffinose in barley in 1886 (Henry, 1988). The presence of other oligosaccharides structurally related to raffinose namely, stachyose and verbascose has not been established, although trace amounts of stachyose have been reported in barley (Kuo et al., 1988). The average amount of raffinose in intact barley grains is approximately 0.5% of the total dry weight, although figures ranging from 0.14 - 0.83% have been recorded (MacLeod, 1956).

The distribution of raffinose and sucrose within the grain is not uniform. MacLeod (1956) examining differentially milled fractions of barley reported 3.6% raffinose in the fraction containing the embryo but only 0.008% in endosperm material. She therefore concluded that raffinose was primarily associated with the 'living meristematic tissues of the embryo'.

2.2.6 Mobilization of Carbohydrate Reserves

In the first stage of carbohydrate mobilization, the reserves in the embryo namely, sucrose and raffinose are degraded. Investigations by Palmer (1969) revealed that the ungerminated grain contains large quantities of sucrose in the embryonic axis and the scutellum. Raffinose was also found in these tissues in smaller amounts. There was a rapid decline in sucrose content in both embryonic organs during the first twelve hours of germination. At 12 hours the decline in sucrose content in the scutellum stopped, but 4 hours later an increase was evident as hexose sugars from the modifying endosperm passed into the scutellum. In the embryonic axis the decrease in sucrose continued until 18 hours of germination had elapsed. This was followed by an increase in sucrose levels. As development of the seedling progressed, sucrose was transported to the main growing areas where it was utilized for development and growth.

In contrast to the mobilization of sucrose, the raffinose content of the embryo declined 73% over 24 hours and did not increase during germination (Palmer, 1969). Chromatographic studies based on ethanolic sugar extracts supplemented with galactose support the concept that raffinose may be degraded to galactose and sucrose in the embryo. The galactose fraction may, in turn be epimerized enzymatically to glucose. Glucose, in turn enters the pathway of general metabolism. Further evidence for this suggestion in the barley system is lacking. Main (1983)

demonstrated in the early stages of germination of soybean (Glycine max) increases in enzyme activities correlating with a decrease in galactosyl sugars. Differences were demonstrated between cultivars in the levels of UDP-glc-4-epimerase (EC 5.1.3.2) in addition to other germinative enzymes. This is the scientific basis of this study i.e. to evaluate if the mobilization of the sugars in the embryo that occurs in the early phase of germination might serve as indicators of premature sprouting.

The mobilization of starch present in the endosperm occurs after the initiation of changes in the embryo and involves the enzymes alpha-amylase, limit dextrinase, alpha-glucosidase and beta-amylase. The first three are known to be synthesized de novo in the aleurone layer in response to germination onset (Hardie, 1975).

A minor point of controversy has been whether the major source of alpha-amylase is the scutellar region of the embryo or the adjacent aleurone layer. A proposal by Gibbons (1981), Briggs and MacDonald (1983b) and Briggs (1987) that the scutellum was also involved in alpha-amylase synthesis was disputed by Palmer (1988) on the basis that the excised scutella used in the experiments were likely contaminated with aleurone cells. The scutellum accounted for the small amount of alpha-amylase first detected in the germinating grain (MacGregor et al., 1984). A large proportion of scutellar alpha-amylase was excreted into the endosperm compared to aleurone synthesized alpha-amylase. However, the

aleurone layer synthesized more alpha-amylase compared to scutellar tissue (MacGregor et al., 1984).

Large dextrins are released into solution by alpha-amylase which acts by hydrolysing alpha-1,4-glucosidic linkages randomly in the chains of amylose and semi-randomly in amylopectin. The dextrins are further degraded partly, by this enzyme and partly by beta-amylase. The latter is an exo-enzyme and attacks the amylose chain from the non-reducing end, liberating beta-maltose. The mode of action of limit dextrinase involves hydrolysis of the alpha-1,6-linkages in amylopectin. This enzyme liberates substrates for alpha and beta amylases. The maltose derived as a consequence of the action of amylases is hydrolysed to glucose by alpha-glucosidase. This enzyme which was found in small quantities in the ungerminated grain appears to be secreted by the aleurone layer (Hardie, 1975). Glucose is taken up by the scutellum, converted to sucrose and translocated to the developing seedling (Enari and Sopanen, 1986).

2.3 Gibberellins in Germination and the Sprouting Response

There are at least 72 known gibberellins (GAs), 61 of which have been identified in higher plants (Mayer and Poljakoff-Mayber, 1989). The major bioactive GA in barley is 18-OH-GA_4. Grain GAs are distinctive from those in seedlings. Lenton and Gale (1987) found in immature grains less than 0.003ng GAs/g fresh weight, but in excess of

0.2ng/g fresh weight in 7 day old seedlings. Atzorn and Weiler (1983) using immunological techniques examined gibberellins in germinating barley. In dry seeds GA_1 was the dominant gibberellin, but GA_3 and GA_4 were also present in small quantities. Neither GA_1 nor GA_3 increased alpha amylase production. There was however, a very high degree of correlation between the level of GA_4 and alpha-amylase production. The time lag between the changes in this gibberellin and the corresponding changes in the rates of synthesis of the enzyme was approximately 2 hours. The authors demonstrated that the level of GA_4 in the endosperm rose earlier than in the embryo. The aleurone layer contained the major fraction of the GA_4 extracted from the grain. This suggests that the aleurone layer rather than the embryo is the source of GA_4 accumulating in the endosperm. Using inhibitors of GA synthesis and exogenous applications of GA_1 and GA_4, the authors showed that the effect of GA_1 on the induction of alpha-amylase is indirect and that the process is dependant on the synthesis of an aleurone gibberellin GA_4. There has been, to date, no confirmation of this report. Mayer and Poljakoff-Mayber (1989) commented.."The GA produced by barley embryos is probably GA_1, but its identity is still in dispute. In many, if not all seeds, GA_{4+7} are far more effective than GA_3. Since GA_3 cannot be converted to GA_4 or GA_7 one must assume that it is acting at the same site, but less effectively and therefore much higher concentrations are required.."

GA_3 enhanced the synthesis of alpha-amylase in isolated aleurone layers (Chrispeels and Varner, 1967). The removal of gibberellic acid in mid-course of alpha-amylase production resulted in a slowing down in the synthesis of the enzyme. The authors suggested that there was a continued requirement of GA for alpha-amylase production. These events were paralled by a continuous requirement for RNA synthesis. The addition of 6-methylpurine in mid-course resulted in an inhibition of alpha-amylase synthesis within 3 to 4 hours. The newly formed isozymes of alpha-amylase hydrolyse the starch in the endosperm (Cornford et al., 1987).

There appears to be a link between GA and sprouting responses. Work performed by Gale et al., (1987) on wheat described a peak in GA activity prior to maturity and the authors suggested that this could be the embryonic stimulus required for germinative alpha-amylase production. In addition, other environmental and edaphic factors may dictate the capacity to produce alpha-amylase. For example, differences in nitrogen nutrition may not only alter grain protein, but may also increase amylase synthesis (King, 1988). Morris and Paulsen (1985) demonstrated a positive correlation between protein content and the degree of sprouting in winter wheat.

2.4 Abscisic Acid (ABA) and sprouting responses

Meredith and Pomeranz (1985) suggested that ABA in wheat has no part in grain set or subsequent grain growth, but has a role in grain maturation. The pattern of ABA change in developing grains follows that of dry matter accumulation (Lenton and Gale, 1987). A decrease in ABA content occurs at the time of grain dessication near maturity. There is an accumulation of metabolites of ABA including phaseic acid, dihydrophaseic acid, bound forms of ABA and an unknown highly polar metabolite (Lenton and Gale, 1987). An accumulation of ABA in the later stages of grain growth may prevent precocious germination and therefore, may be associated with dormancy. Schopfer and Plachy (1985) postulated that abscisic acid inhibits germination by means of inhibiting cell wall plasticity.

ABA blocks GA-induced production of alpha-amylase (Chrispeels and Varner, 1967) while, simultaneously inducing production of specific proteins, one of which is a protein inhibitor of alpha-amylase (Weselake *et al.*, 1983). Atzorn and Weiler (1983) suggested a dual action for ABA in the course of their studies namely, a reduction in the level of GA_4 in the aleurone tissue and by additional interference in alpha-amylase formation at a later stage. This latter suggestion implies perhaps the formation of an inhibitor of alpha-amylase.

2.5 Dormancy and the sprouting response

A state of dormancy exists in seeds when healthy physiologically ripe seeds do not germinate when placed under optimal conditions of moisture, temperature, and light (Salisbury and Ross, 1985). A period of after-ripening is necessary before dormancy breaks down.

The mechanism(s) of barley dormancy is not understood. Palmer (1989) suggested that failure of the pentose phosphate pathway to operate in freshly harvested grains, is the primary cause of dormancy. In this theory, treatments such as high oxygen levels, chilling and oxidising agents will reduce dormancy by inducing the development of the pentose phosphate pathway. Since the normal respiratory pathway operates in dormant grains, it has been suggested that 'extra' oxygen is required to oxidize 'inhibitors' or to activate enzymes or co-factors of the germination process. Using this concept, Palmer (1989) suggested that the thickness of the pericarp may regulate the permeability of oxygen to the seed. He reported that a dormant variety of barley had a thick pericarp when compared to a variety that had a higher level of germination.

Work performed by Hagemann and Ciha (1987) on wheat demonstrated that seed dormancy at harvest ripeness was a function of the cultivar and the environment during grain development. Grain that developed in cool environments generally lost their dormancy more rapidly than grain that grew in a warm climate. Loss of seed dormancy during the

after-ripening period is a function of the cultivar, the environment in which the seed was grown and the after-ripening environment. High temperature during the after-ripening period accelerates the loss of seed dormancy.

Work conducted by Harland and Madson (1989) investigated the influence of kernel surface lipids on barley dormancy. Kernels with surface lipids extracted did not differ significantly in the rates of germination when compared to the control material. Differences in fatty acid composition occurred in both surface and total kernel lipids when the grain was held in storage. These changes, however could not be used to determine the levels of germination in the post-harvest phase.

Researchers can only speculate on what is the cause of dormancy in the seed and how it might be incorporated into breeding programmes to reduce preharvest sprouting problems. There are however, a number of practical suggestions to avoid sprouting in the field. Ringlund (1987) suggests using early maturing varieties, particularly in areas where there is increased rainfall towards the end of the season. Sprouting occurs most readily when the crop is lodged particularly, in cool, wet harvests as water remains and tends to seep into the kernels. A reduction in lodging and potential sprouting damage may be achieved as a result of a minimal application of nitrogen, as high nitrogen fertilization frequently leads to grain lodging.

MATERIALS AND METHODS

3.1 Selection of varieties

Varieties selected for this study were Betzes, Ellice, Hannchen, Harrington, Norbert and the breeders' lines TR201, TR219M, TR479 and TR490 (obtained from Agriculture Canada, Winnipeg, Manitoba) and Lamont (obtained from Dr. D. Wesenberg, Aberdeen, Idaho, U.S.A.). All are 2-rowed malting barleys, seven of which originate in Canada. Seed used for the study was pedigree or breeders' seed. The breeders' lines TR479 and TR490 are now licensed varieties named Stein and Manley respectively.

Betzes was introduced into North America from Krakow, Poland in 1938 by the United States Department of Agriculture. The origin of the variety is unknown. It was licensed for sale in Canada in 1960 (Metcalfe, 1987).

Hannchen originated in Svalof, Sweden and was licensed before 1923 in Canada (Metcalfe, 1987).

Lamont is an American 2-rowed malting barley derived from 'Zephyr' * '61Ab4965' cross. Zephyr originated in the Netherlands; 61Ab4965 in Aberdeen, Idaho, U.S.A. It is a variety that has demonstrated good malting potential (Wesenberg and Robbins, 1988). The background of the other varieties used is summarized in Table 1.

TABLE 1: THE BACKGROUND OF SEVEN OF THE CHOSEN VARIETIES

	SEVEN CHOSEN VARIETIES						
PARENTAL SOURCE	ELL	HAR^	NOR	201	219	479^	490^
KLAGES	*	*	*		*	*	*
GAZELLE		*			*		
BETZES	*	*	*	*	*	*	*
CENTENNIAL	*	*	*	*	*	*	*
CI5791	*		*	*	*	*	*
PARKLAND	*		*	*	*	*	*
PIROLINE	*		*	*	*		
AKKA	*		*	*	*		
CAMBRINUS	*						
TERN	*						
FIRBECKS III					*		
HECTOR						*	*

Source: Metcalfe, 1987; ^Harvey, 1987.

3.2 Growing and harvesting of barley samples

The varieties were grown in the summer of 1988 at the University of Manitoba Research Plots, Winnipeg, Manitoba. Each variety was planted in a varietal 'strip' and there were five 'blocks' within each strip. Each block contained 4 rows, each row being 5 m in length and 15 cm apart.

With the exception of the variety Lamont (seeded at a rate of 200 g per strip), the remaining varieties were planted at a rate of 250 g per strip, 3 to 5 cm deep. Seed was pretreated with Benlate T (DuPont Corporation, Mississauga, Ontario) to protect against smut infection. Data pertaining to this field experiment are presented in Table 2.

TABLE 2: DATA PERTAINING TO THE FIELD EXPERIMENT CONDUCTED DURING THE SUMMER OF 1988 AT THE UNIVERSITY OF MANITOBA.

	Mean Temp. (^{0}C)	Normal Temp. (^{0}C)
May	15.0	11.3
June	22.9	16.8
July	22.2	19.6
August	20.8	18.3
	Precipitation (mm)	Normal Precipitation (mm)
May	34.9	65.7
June	44.3	80.1
July	69.4	75.9
August	15.0	75.2

Spike samples were taken for three weeks prior to attainment of maturity. Twenty-five primary stem samples, chosen on a random basis were removed every second day. To minimize differences that may have arisen due to circadian

rhythms, samples were taken at the same time in the early morning. The samples were air-dried at ambient temperature for two days, and stored at -19°C.

Ninety-one days after seeding, the material remaining in the field for each variety was bulk harvested. Maturity was determined by a complete loss of green colour at the peduncle (Copeland and Crookston, 1985). Precautions were taken to ensure that no mixing of the varieties occurred. The threshed seed was dried for two days at ambient temperature, cleaned and stored at 4°C.

3.2.1. Moisture determination in samples

The level of moisture was determined in accordance with procedure as sanctioned by the American Association of Agricultural Engineers (1987). Three replicates (15g per replicate) were examined for each variety.

3.2.2. Postharvest germinability

The levels of germination for all varieties were determined at weekly intervals for 8 weeks after harvesting. Two varieties demonstrated low levels of germination, and for these cultivars germination testing continued periodically until 22 weeks post-harvest had elapsed. In all cases the following procedure was employed:-

Two layers of Whatmans #1 filter paper were placed in 9 cm petri dishes. Four mL of distilled water were added. One hundred seeds of each variety, chosen on a random basis were

placed in separate dishes. The dishes were sealed with 'Parafilm M' laboratory film, and placed in a growth cabinet in the dark at 22°C and examined after 72 hours. Germination was determined by the presence of visible rootlets 2 mm or greater in length.

The varieties that demonstrated low levels of germination at 22 weeks post harvest, were also subjected to the following conditions. The germination test was conducted as described except that 4 mL of gibberellic acid (10^{-6} M) were used, rather than water. At 26 weeks post harvest material was prepared as described with 4 mL of water, placed overnight in a refrigerator (4°C), and then placed in a growth cabinet at 22°C.

3.3 Assessment of sprouting susceptibility

The spikes which had been stored frozen, were removed from the freezer and allowed to thaw at ambient temperature. Sixteen spike samples chosen on a random basis were put into a rain simulation cabinet (Coldstream Plant Growth Cabinet, Coldstream Refrigerator Ltd., Winnipeg, Manitoba) on slotted trays. The samples were initially subjected to 3 hours of showering with water. The experiment was conducted in an atmosphere of 100% relative humidity and a temperature of 15.5°C. Rating was done every 12 hours for 4 days. Each kernel position from the bottom to the top of the spike was rated visually on rootlet development based on a scale of one to four. The rating of one indicated that no sprouting

was visible; the rating of two indicated that sprouting was just visible; three indicated that sprouting was visible and four implied extensive rootlet proliferation (Figure 1). Following each examination, the samples were returned to the rain simulation cabinet, showered with water for 30 minutes and maintained at the temperature and relative humidity described.

Samples at designated physiological maturity and one week prior to this event were examined by this procedure. Material representing these two stages is illustrated in Figure 2.

3.4 Isolation of embryonic tissue

The dehusked kernel was held between the thumb and forefinger and the embryo was removed manually using a metal probe. Precautions were taken to ensure that there was minimal contamination with endosperm.

3.5 Extraction of embryonic raffinose and sucrose

Each embryo was put into a glass homogenizing tube and 500 uL of 80% ethanol was added. The embryo was ground using a motor-driven glass pestle (Caframo, Ontario) until a very fine suspension was produced. The tube was covered with 'Parafilm M' laboratory film to minimize evaporation and vortexed for 1.5 minutes. The extract was subjected to centrifugation (1350g for 5 minutes) and the supernatant was removed. The pellet was reground in 100 uL of 80% ethanol,

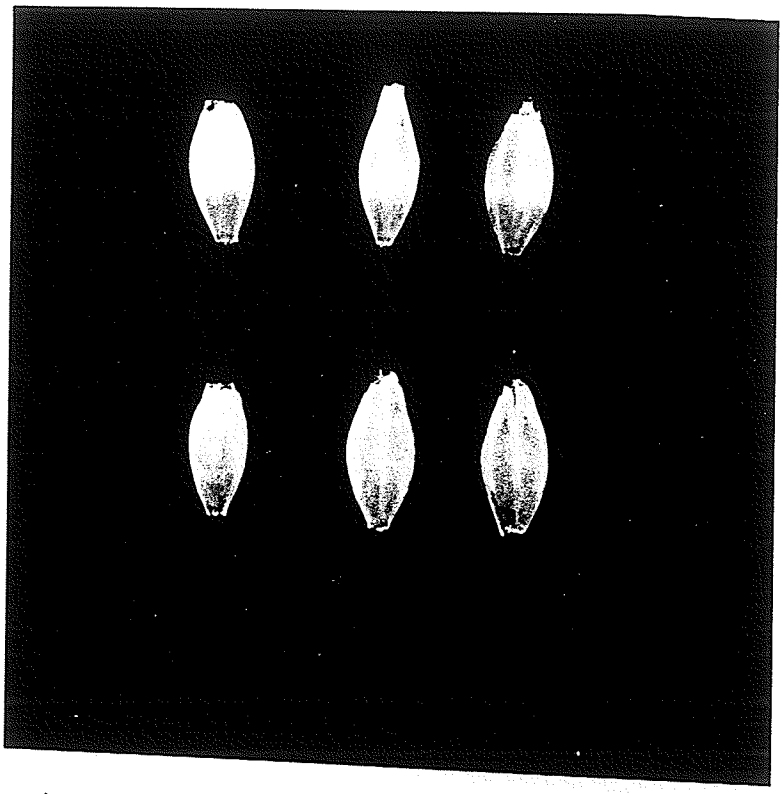


Figure 1a: Sprouting rating 1.

Radicle not visible.

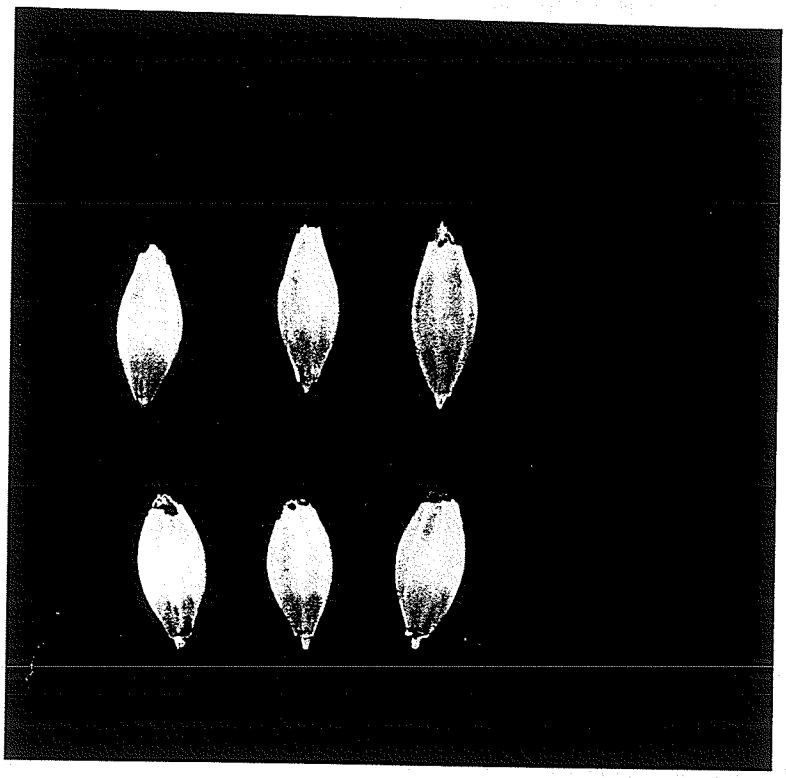


Figure 1b: Sprouting rating 2.

Radicle development just visible.

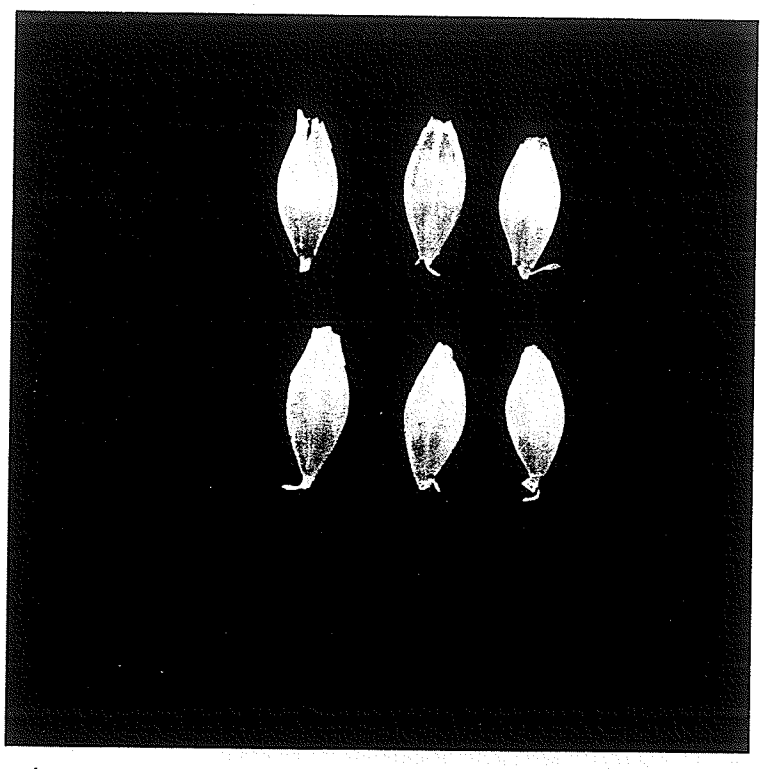


Figure 1c: Sprouting rating 3.

Radicle development visible.

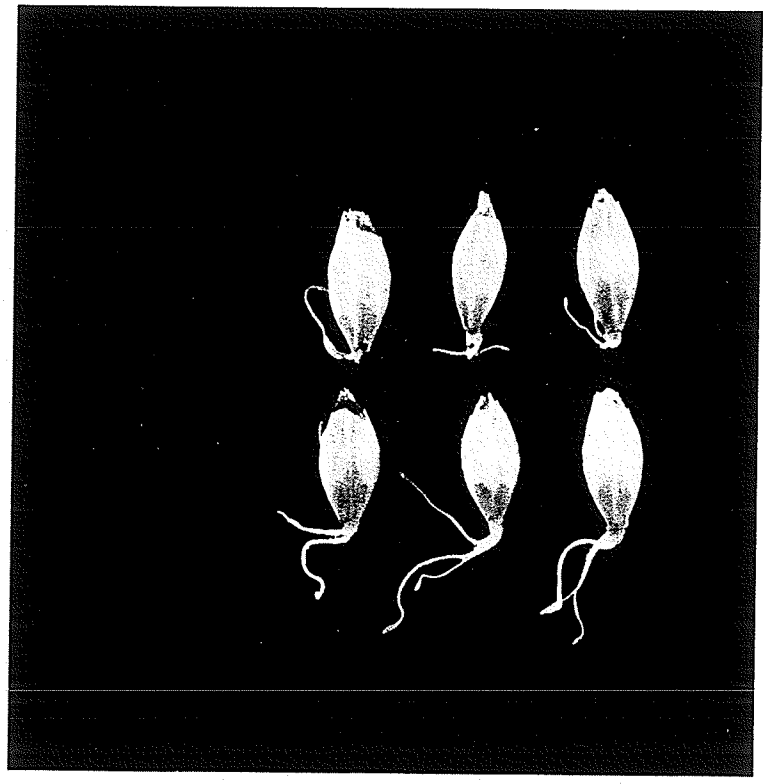


Figure 1d: Sprouting rating 4.

Radicle development clearly visible.

Figure 2:(a) Material at physiological maturity and(b)material one week prior to physiological maturity subjected to sprouting tests.

re-extracted and the two supernatants were combined. The 80% ethanol was evaporated under a stream of air while being maintained in a water bath at 40^{0}C. The residue was dissolved in 500 uL of water. Samples were frozen until examined by High Performance Liquid Chromatography (H.P.L.C.).

3.6 Separation and quantification of embryonic sugars

A Beckman, model 322 H.P.L.C. equipped with an Aminex HPX-87P (BioRad Laboratories, California, U.S.A) carbohydrate column (300 x 7.8mm), was maintained at a running temperature of 84.4^{0}C. A solvent system of distilled, filtered and degassed water operating at a flow-rate of 0.6 mL per minute was used. A refractive index detector and automatic computing integrator were used for quantification.

Raffinose and sucrose standards were prepared in distilled water from vacuum oven dried samples. Calibration solutions of raffinose and sucrose were prepared by dilution from the standard solutions. Samples were injected manually into the system using a 20 uL injection loop. Prior to analysis of embryonic extracts, samples were filtered through a 0.45 micron filter. Quantification was based on the comparsion of the integration values of peaks for raffinose and sucrose from test samples with standard curves. To ensure reproducibility, two injections per sample were examined. Since this analysis gave the quantity of each

sugar present in 20 uL, results were then multiplied by a factor of 25 to determine the quantity of each sugar in each embryo.

3.7 Subjection of Ellice barley to repeated cycles of wetting and drying

Plump Ellice seed from the bulk harvest was selected by collecting the grain retained on a 6/64 inch slotted sieve. Approximately 40 g of this Ellice barley was weighed into perforated bottles. The material was steeped for 4 hours in water at 15^{0}C, before it was removed and allowed to drain. Surface water was removed by centrifugation (1350g for 5 minutes). The moisture was determined in accordance with A.S.A.E. regulations as described in section 3.2.1.

Material subjected to the described steeping treatment was dried at 22^{0}C, in an atmosphere of 40% relative humidity. The grain was spread over a perforated tray and an air-flow of 100 cubic feet per minute was maintained through the grain for 8 hours. The moisture content was determined as described in section 3.2.1.

Material was subjected to 4 cycles of wetting and drying. After 12, 24 and 36 hours the seed was rated for the levels of visible sprouting present as described in section 3.3. To ensure reproducibility the grain was thoroughly mixed at each stage, and three replicates of randomly chosen samples were analysed for moisture content as described in section 3.2.1 in two separate runs of the experiment.

3.7.1. Determination of raffinose and sucrose levels in the embryos of Ellice barley after wetting and drying cycles

Plump seed of the variety Ellice was subjected to 3 cycles of steeping and drying as described. When each steeping cycle was completed and the surface water was removed, the material was thoroughly mixed and the embryos of 15 seeds were removed and frozen. Raffinose and sucrose were extracted and determined as described in sections 3.5 and 3.6 respectively. In accordance with Statistical Analysis System (S.A.S.) guidelines (1982), results were subjected to an analysis of variance; cycle, time and injection were considered as treatments.

3.7.2. Germination capability of Ellice barley following repeated cycles of wetting and drying

At the end of each cycle of wetting and drying, samples were removed and dried as described in section 3.7 to a final moisture of 16.3%. A portion of each grain sample (40g) was subjected to an accelerated aging test (44^0 C, 100% relative humidity for 48 hours) (A.S.O.C. 1983).

Three replicates of 100 seeds from each cycle, both before and after the accelerated aging test were tested to determine if the rates of germination had changed. The material was subjected to the germination conditions as described in section 3.2.2. Results were analysed in accordance with S.A.S. regulations. Replicates and cycles were considered as treatments.

RESULTS AND DISCUSSION

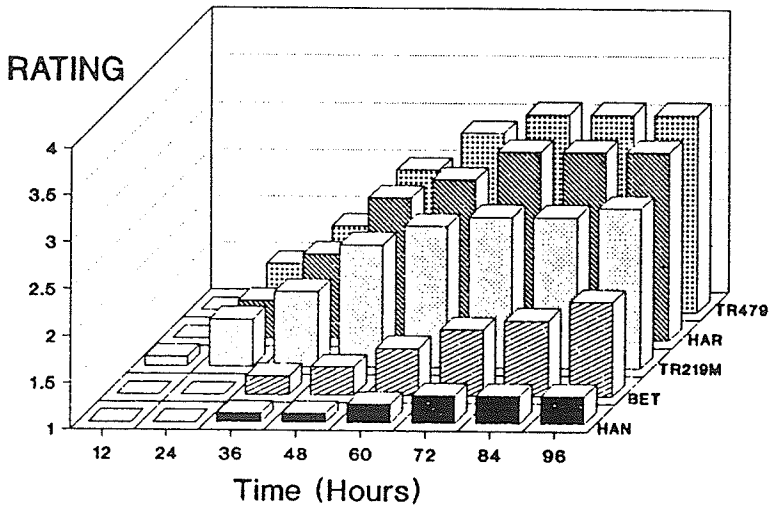
4.1 Sprouting Susceptibility

4.1.1 Mean sprouting susceptibility of ten two-rowed barleys

Seven of the ten varieties chosen for this study were 2-rowed Canadian barleys, and Lamont is an American variety. These varieties are known to be rapidly modifying barleys. The other two barleys Hannchen and Betzes are older barleys which do not have this trait. Some of the varieties studied are known to exhibit some degree of susceptibility to preharvest sprouting, but the variation in susceptibility among the varieties has not been previously demonstrated. Hence, the purpose of this work was to determine if different levels of sprouting susceptibility existed in the selected 2-rowed malting barleys.

To evaluate the relative sprouting susceptibility of the varieties, 16 heads of each variety were subjected to rain simulation and rated as detailed in Materials and Methods. At physiological maturity (Figure 3a, Appendix II), there were three patterns clearly visible. The varieties Ellice, Norbert, TR201, Lamont and TR490 demonstrated high levels of sprouting from the onset of the rain simulation experiment. Over the course of the experiment, the

Figure 3a: Sprouting over 96 hours in ten varieties at physiological maturity



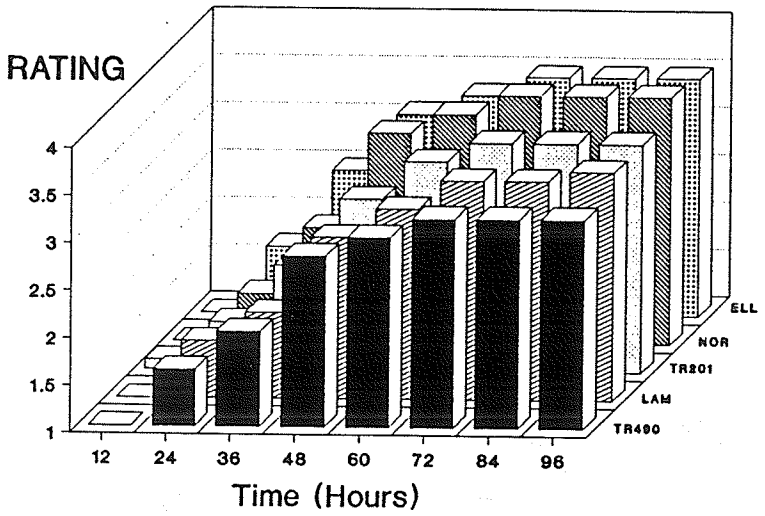
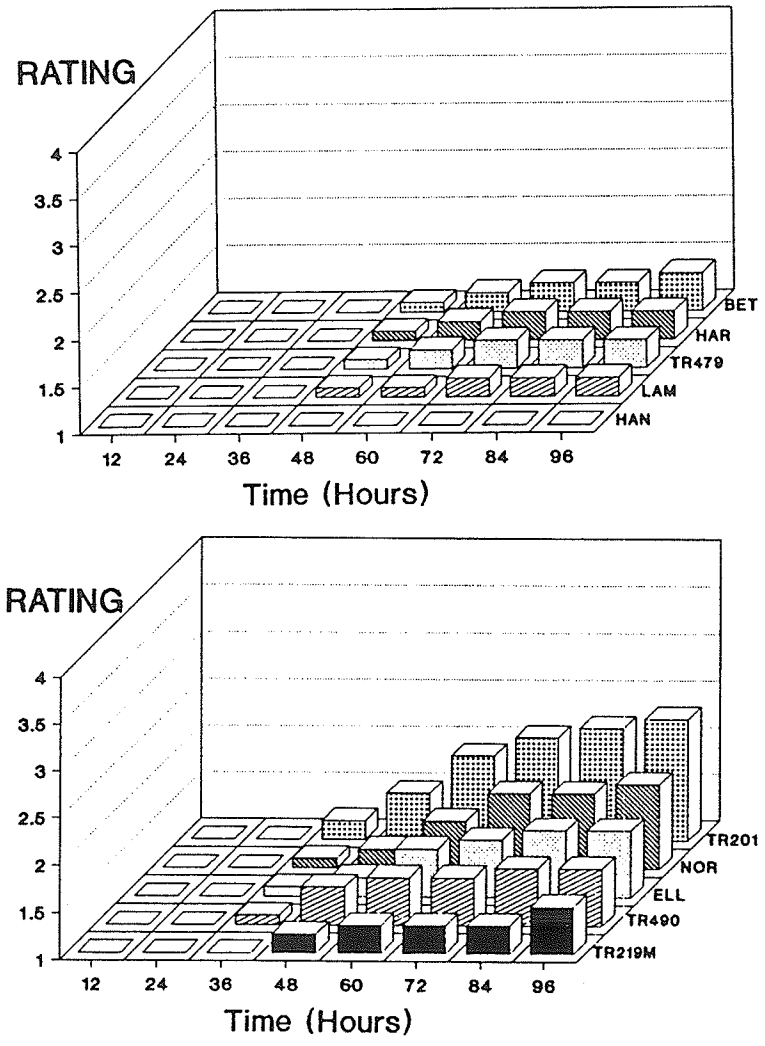


Figure 3b: Sprouting over 96 hours in ten varieties one week prior to physiological maturity



varieties TR479, Harrington and TR219M had increased levels of sprouting, but not to the levels observed in the former varieties. The varieties Betzes and Hannchen had low levels of sprouting throughout the experimental period.

One week prior to physiological maturity (Figure 3b, Appendix III), the levels of sprouting observed were much less when compared to the levels seen at physiological maturity. The breeders' line TR201 demonstrated the highest level of sprouting. Norbert, Ellice and the breeders' line TR490 demonstrated intermediate-to-high levels of sprouting. TR219M, Betzes, Harrington, TR479 and Lamont demonstrated low-to-intermediate levels of sprouting at one week prior to physiological maturity. The variety Hannchen did not sprout over the duration of the experimental period. With the exception of TR201, the varieties demonstrated a very gradual increase in sprouting levels. In the case of the variety TR201 the pattern demonstrated a slow increase followed by noticeably higher levels of visible sprouting towards the end of the experimental period.

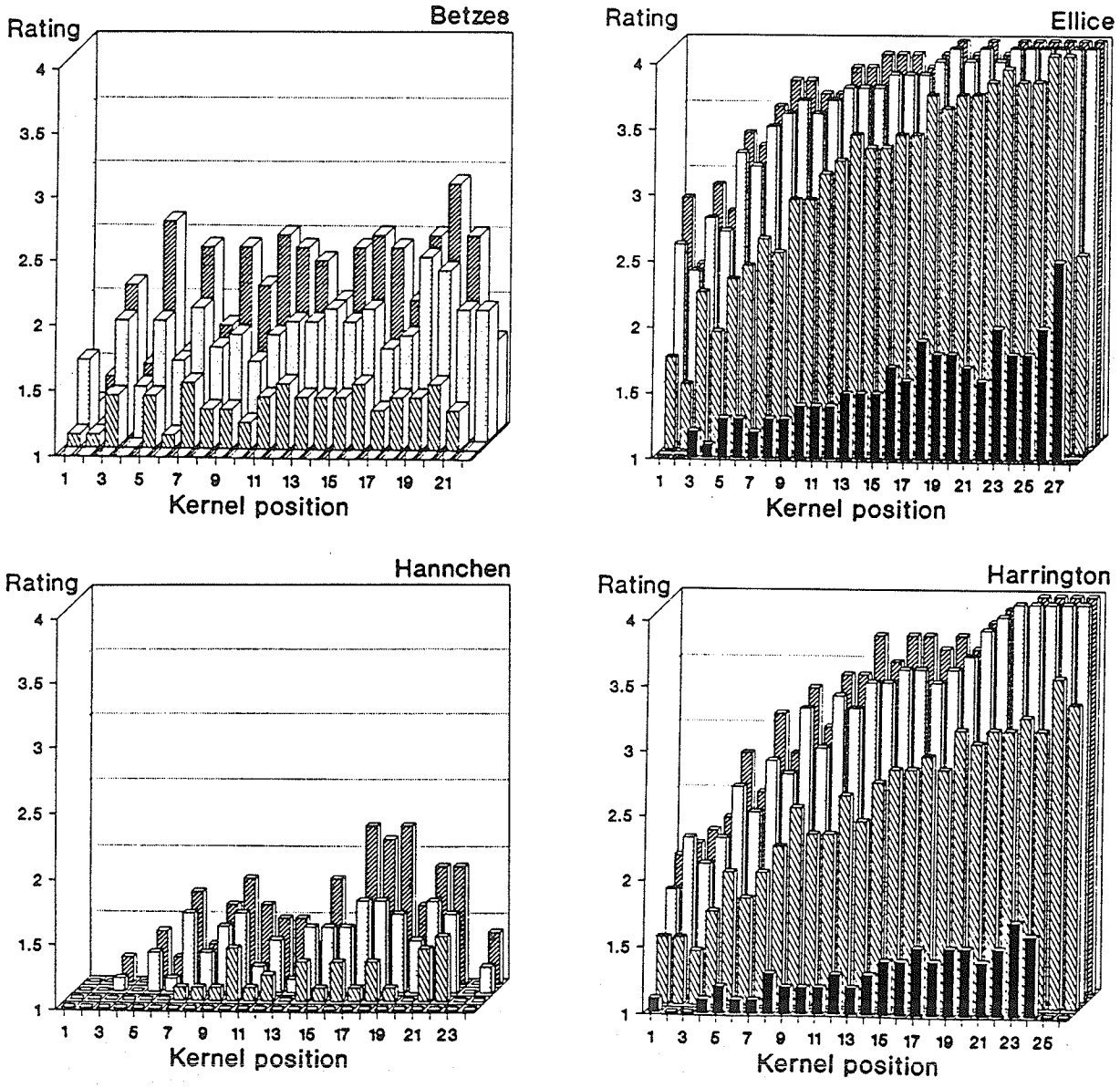
Two varieties, namely, Lamont and TR479 demonstrated an increased susceptibility to sprouting, relative to the other varieties as dry-down occurred to physiological maturity. The variety Lamont had little or no visible sprouting one week before maturity, but demonstrated intermediate-to-high level of sprouting at physiological maturity. The same effect, though not as dramatic, was observed in the breeders' line TR479.

4.1.2 Effect of kernel position on sprouting susceptibility

The above data summarizes the situation averaged across all positions. However, positional differences were noted. Sixteen spikes were examined in each variety; the number of kernel positions varied, depending on the variety. The mean sprouting value at each kernel position was determined from the 16 spikes as follows. Twenty-four hours refers to the mean sprouting value observed at each kernel position for twelve and twenty-four hours. Forty-eight hours refers to the mean sprouting value observed at each kernel position for thirty six and forty eight hours. Seventy-two hours refers to the mean sprouting value observed at each kernel position for sixty and seventy-two hours. Ninety-six hours refers to the mean sprouting value observed at each kernel position for eighty-four and ninety-six hours.

On examining the effect of kernel position on sprouting susceptibility the following trends emerged in eight of the ten varieties examined at physiological maturity (Figure 4). For the varieties Ellice, Harrington, Norbert, TR201, TR219M, TR479, TR490 and Lamont in the first twenty-four hours, the lower kernel positions of the spike had a lower level of visible sprouting than the upper kernel positions. As the level of visible sprouting throughout the head continued to increase, the patterns established in the first twenty-four hours were maintained. The patterns described were also present in the varieties Betzes and Hannchen. However, the levels of visible sprouting were greatly

Figure 4: The sprouting value at each kernel position of physiological mature barley rated after 24 (▬), 48 (▨), 72 (▭) and 96 (▨) hours. The rating system used is presented in figures 1a, 1b, 1c and 1d.



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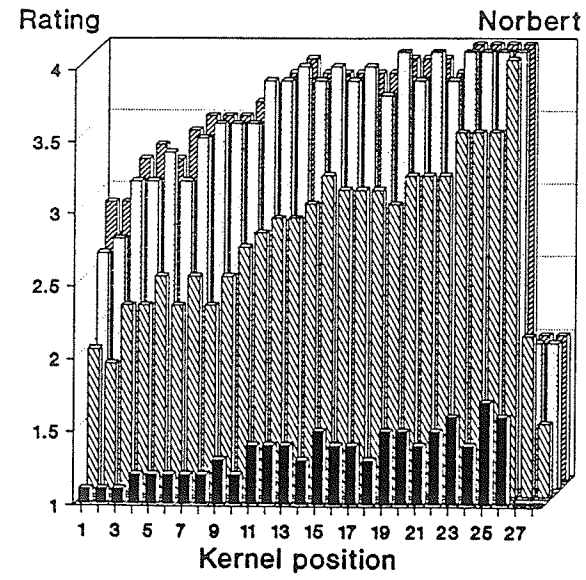
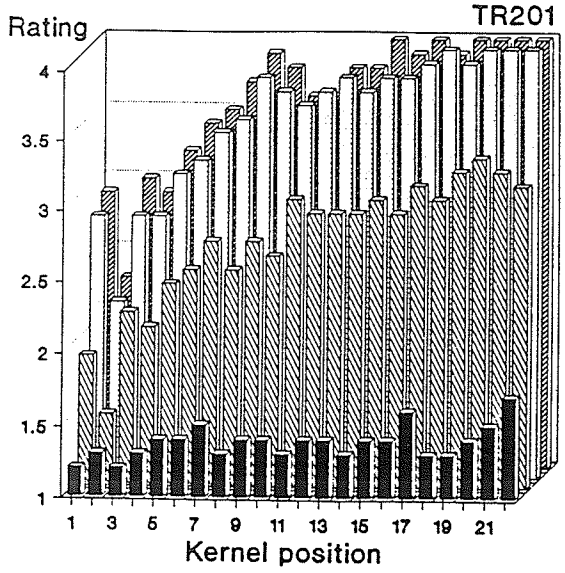
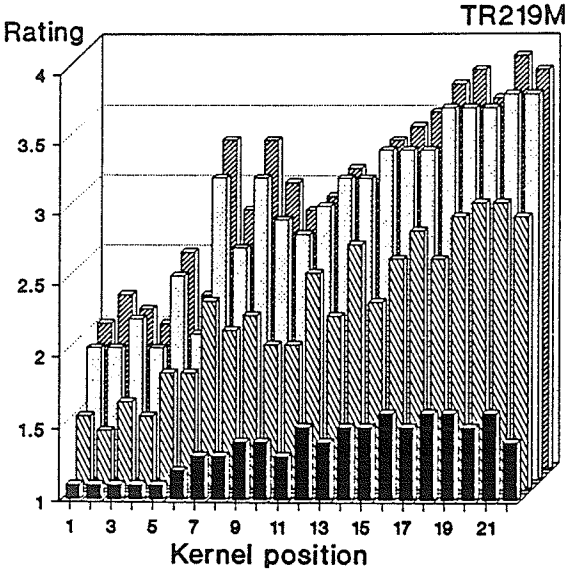
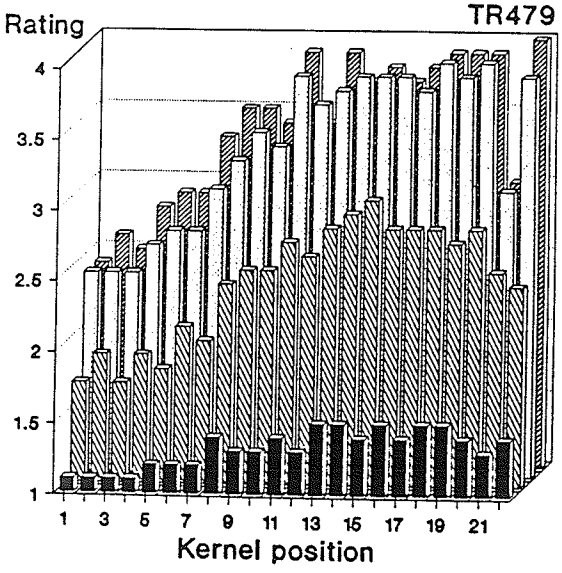
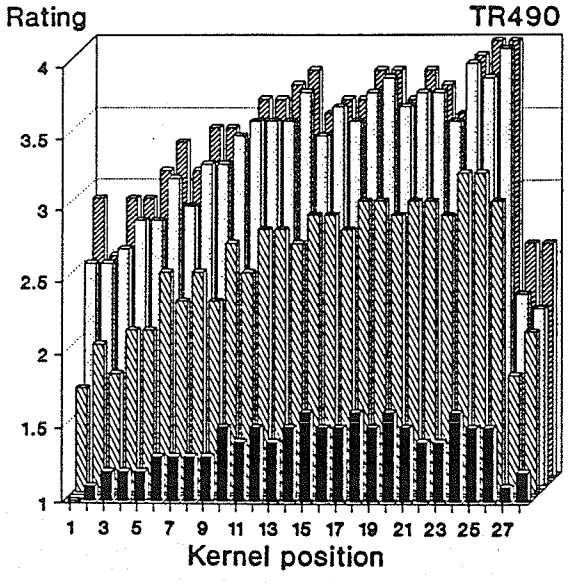
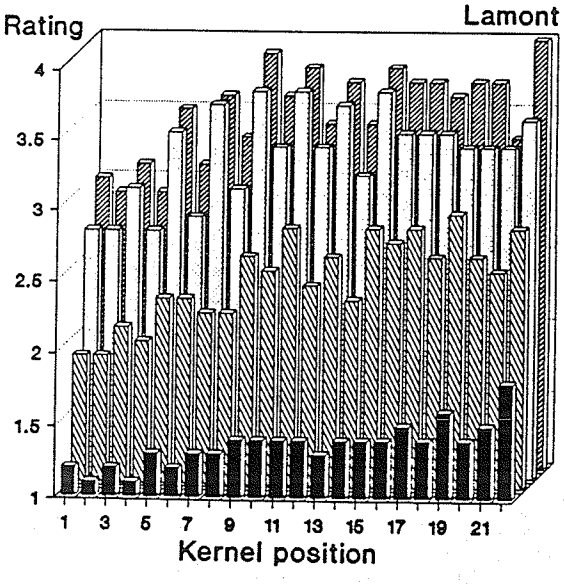

Rating
Norbert
4
3.5
3
2.5
2
1.5
1
1 3 5 7 9 11 13 15 17 19 21 23 25 27
Kernel position


Rating
TR201
4
3.5
3
2.5
2
1.5
1
1 3 5 7 9 11 13 15 17 19 21
Kernel position


Rating
TR219M
4
3.5
3
2.5
2
1.5
1
1 3 5 7 9 11 13 15 17 19 21
Kernel position


Rating
TR479
4
3.5
3
2.5
2
1.5
1
1 3 5 7 9 11 13 15 17 19 21
Kernel position


Rating
TR490
4
3.5
3
2.5
2
1.5
1
1 3 5 7 9 11 13 15 17 19 21 23 25 27
Kernel position

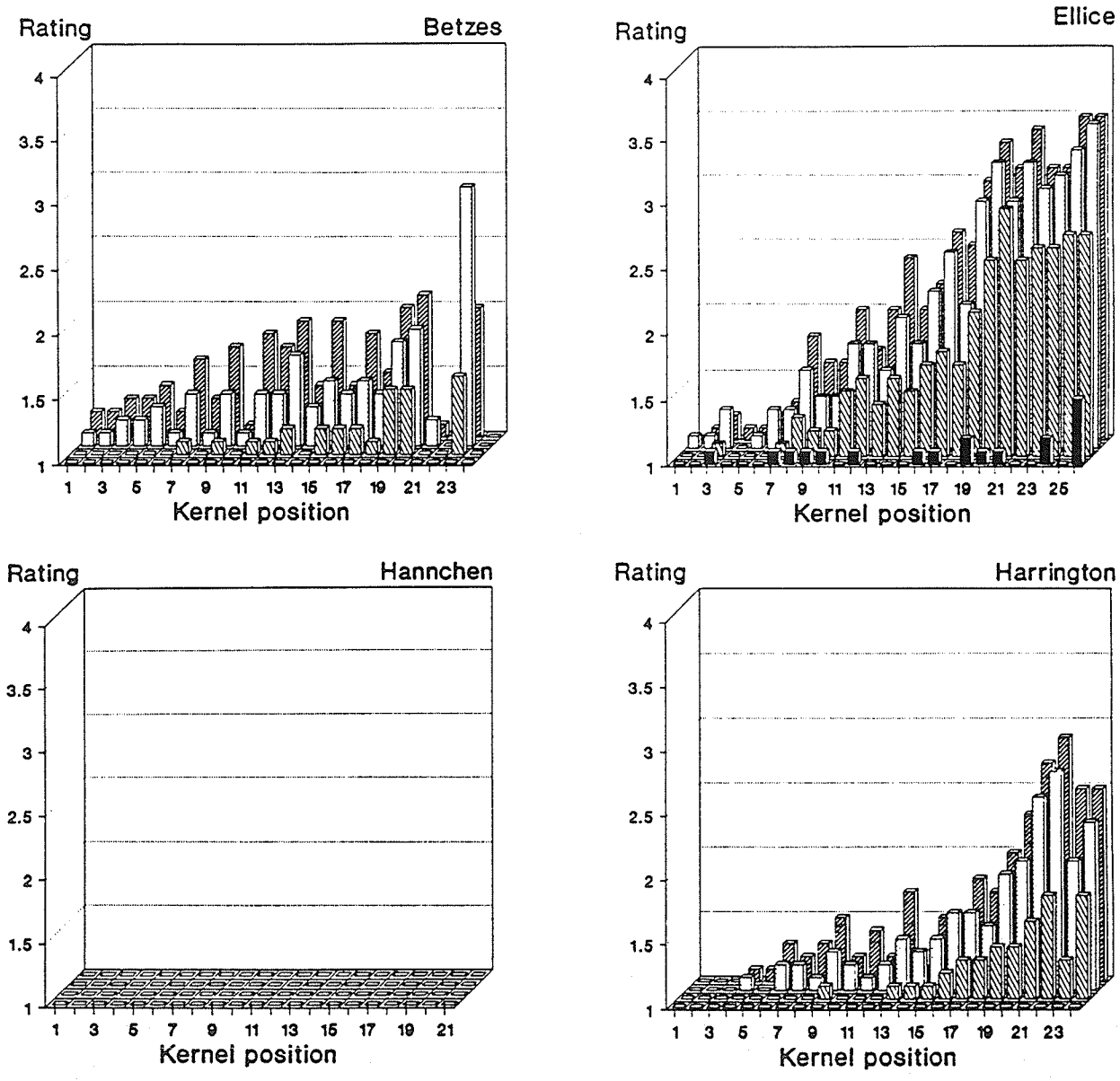

Rating
Lamont
4
3.5
3
2.5
2
1.5
1
1 3 5 7 9 11 13 15 17 19 21
Kernel position

reduced in comparsion to the previously mentioned varieties. In most instances the level of sprouting increased from one time period to the next. However, in a few cases e.g. kernel position 22 in Betzes (Figure 4) and kernel position 23 in Betzes (Figure 5) the rating decreased between 72 and 96 hours. This is attributed to the subjective nature of the rating system.

At one week prior to physiological maturity (Figure 5), the sprouting patterns observed in the varieties were similar to that at physiological maturity in that the kernels in the upper part of the head had a greater propensity to sprout than those in the lower positions. As already shown, the levels of visible sprouting in all varieties were at that time noticeably less than levels observed at physiological maturity. There was no sprouting visible at any kernel position on the variety Hannchen.

Barley fills from the bottom to the top of the head, but matures from the top of the head downwards (Wych et al., 1985). The results presented here suggest that the kernels situated near the top of the head exhibit a greater susceptibility to sprouting. Hence, seed found in the upper sectors of the head are more susceptible to sprouting when placed in conditions that would induce sprouting. This is further supported by the differences in sprouting between mature barley and one week prior to physiological maturity.

Figure 5: The sprouting value at each kernel position of barleys one week prior to physiological maturity rated after 24 (■), 48 (▨), 72 (▭)and 96 (▨) hours. The rating system used is presented in figures 1a, 1b, 1c and 1d.



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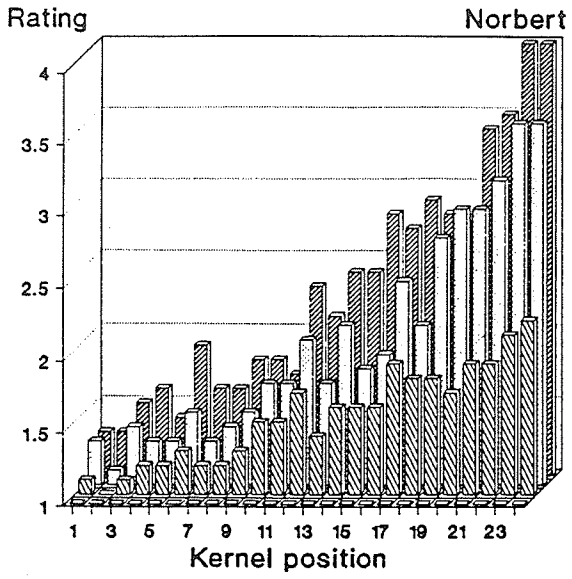
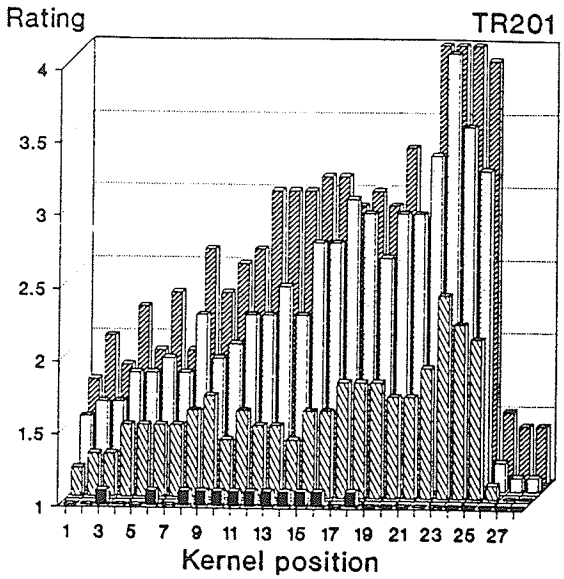
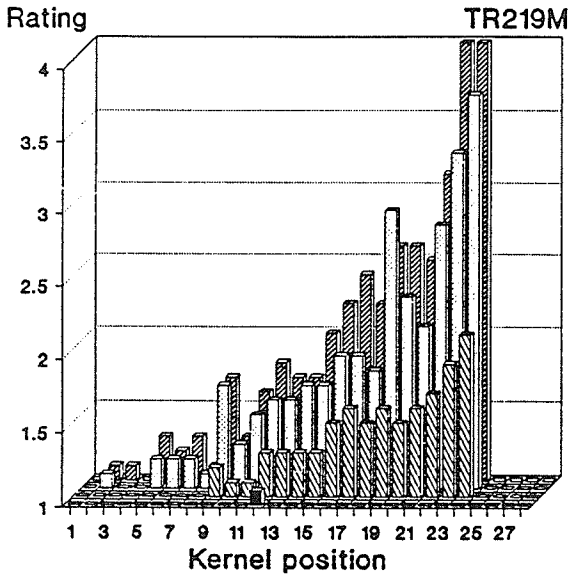
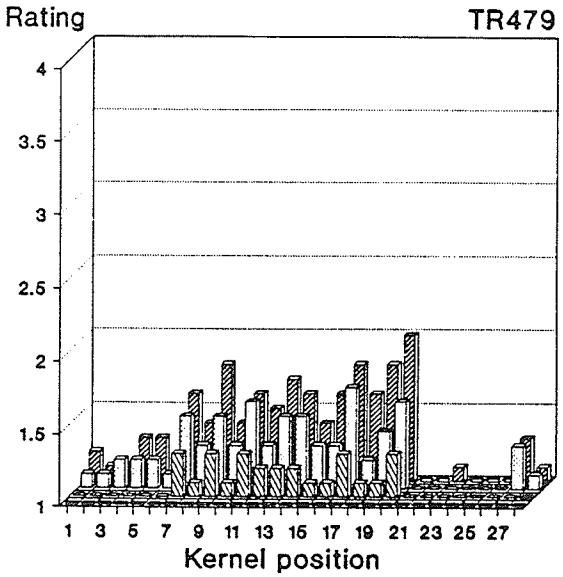
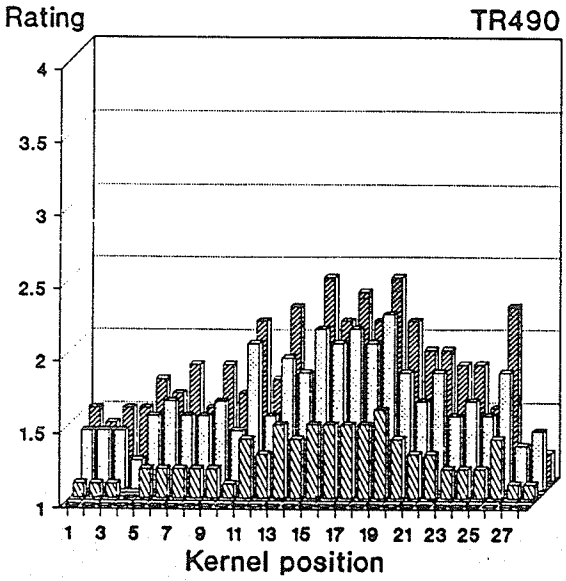
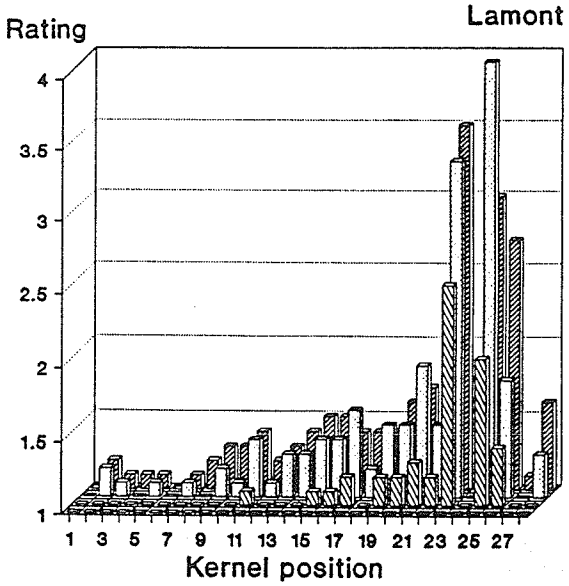

Rating
Norbert
4
3.5
3
2.5
2
1.5
1
1 3 5 7 9 11 13 15 17 19 21 23
Kernel position


Rating
TR201
4
3.5
3
2.5
2
1.5
1
1 3 5 7 9 11 13 15 17 19 21 23 25 27
Kernel position


Rating
TR219M
4
3.5
3
2.5
2
1.5
1
1 3 5 7 9 11 13 15 17 19 21 23 25 27
Kernel position


Rating
TR479
4
3.5
3
2.5
2
1.5
1
1 3 5 7 9 11 13 15 17 19 21 23 25 27
Kernel position


Rating
TR490
4
3.5
3
2.5
2
1.5
1
1 3 5 7 9 11 13 15 17 19 21 23 25 27
Kernel position


Rating
Lamont
4
3.5
3
2.5
2
1.5
1
1 3 5 7 9 11 13 15 17 19 21 23 25 27
Kernel position

4.1.3 Relative dormancy of varieties in the post harvest phase

The germination tests commenced two weeks after material was harvested from the field and ran in parallel with the previously described experiment.

The results given in Table 3, based on an analysis of variance demonstrate high levels of germination at two weeks post-harvest in the varieties TR479, Norbert, Lamont, Harrington, TR490, Ellice, TR201, TR219M. While minor significant differences in the percentage germination were found among these varieties at two and four weeks post-harvest, there was no significant difference among these varieties at eight weeks post harvest. Betzes and Hannchen demonstrated significantly lower levels of germination relative to the other varieties at 8 weeks post harvest. At 22 weeks this grain was subjected to gibberellic acid and at 26 weeks cold treatment to ascertain whether the grain was non-viable or simply dormant. Germination rates rose after both treatments, indicating that these two varieties were exhibiting varying levels of dormancy in the post-harvest phase. Ringlund (1987) described the effect of temperature on the breakdown of dormancy as studied by Strand (1965). If barley grains were stored at very low temperatures (3^{0}C), the dormancy lasted for a few months. On the other hand, if the storage temperature is 20 to 30^{0}C, the dormancy disappeared in a few weeks. As described in Section 3.2 of Materials and Methods, the threshed seed was

TABLE 3: PERCENTAGE GERMINATION* OBSERVED IN VARIETIES IN THE POST-HARVEST PHASE.

	TIME POST-HARVEST (WEEKS)				
VAR	2	4	6	8	
479	92.5 A de	98.5 B f	96.5 AB cd	94.5 AB c	
NOR	95.0 A e	95.0 A e	94.5 A cd	95.5 A c	
LAM	96.0 A e	96.0 A e	92.5 A c	95.5 A c	
HAR	90.0 A d	98.5 B f	94.5 B cd	97.0 B c	
490	93.0 A de	95.0 A e	94.0 A cd	95.0 A c	
ELL	86.5 A cd	93.5 BC de	92.5 B c	97.5 C c	
201	88.0 A d	90.5 AB cd	93.5 BC cd	96.5 C c	
219	83.5 A c	87.0 A c	97.0 B d	96.0 B c	
BET	34.0 A b	59.0 B b	58.0 B b	69.5 C b	98.0$
					98.0#
					98.0@
HAN	20.0 A a	27.5 B a	39.5 C a	39.5 C a	85.0$
					90.0#
					95.0@

*Average percentage germination determined in 2 replicates. $=No treatment at 22 weeks post harvest. #=GA3 treatment at 22 weeks post harvest. @= Cold treatment of grain at 4^0C. at 26

[footnotes to Table 3 contd.]

weeks post harvest. Upper case letters going horizontally describe the L.S.D. analysis ($p<0.05$) determining the differences in percentage germination within each variety over 8 weeks. Lower case letters going vertically describe the L.S.D. analysis ($p<0.05$) within each time period. Varieties are ranked from the lowest to the highest percent germination. Values followed by the same letter are not significantly different from one another.

stored at 4^0C during the experiment.

It has been demonstrated that the levels of germination in the post-harvest period differed significantly among the examined varieties. Different trends emerged among the varieties that exhibited the highest level of germination in the post-harvest period. The varieties Ellice and TR201 demonstrated a very high susceptibility to sprouting. In the post-harvest phase, the initial levels of germination were 87% and 88% respectively. Over the course of eight weeks, the levels of germination increased to 97% and 96% respectively for the varieties Ellice and TR201. Conversely, the varieties Harrington and TR479 demonstrated intermediate levels of sprouting and germination levels at the onset of the post-harvest phase were 90% and 93% respectively. Germination in these varieties had increased after eight weeks to the same levels demonstrated by Ellice and TR201. The varieties, Betzes and Hannchen had the lowest levels of sprouting and the lowest levels of germination in the post-harvest phase. The low level of preharvest sprouting is likely due to high levels of dormancy in the grain.

4.1.4 General Considerations on Section 1

In Western Canada, farmers utilize the practice of swathing as a means of decreasing the moisture content in the kernel. The crop may be swathed a number of days prior, to or just at full maturity. If the swath is subjected to heavy rainfall during this time period, it may be

susceptible to sprouting as suggested by results.

The factors influencing sprouting susceptibility are determined on three levels:- Genetic, environmental and physical characteristics. Genetic and environmental aspects will be discussed. Physical characteristics were considered in section 2.2 of the literature review and were not examined in this study.

In section 3.1 of materials and methods the breeding background of the selected varieties is presented. TR201 and Norbert have a similar background, except that Klages is not in the breeder's line TR201. Norbert has the same genetic diversity as Ellice; traits from the varieties Cambrinus and Tern have been incorporated into the latter variety. The breeder's lines TR479 and TR490 (sister lines) are similar to Norbert except that the varieties Piroline and Akka are not present: Hector has been bred into these breeder's lines. One might suggest that the introduction of Hector has given these lines a greater ability to resist sprouting. Harrington has the unusual combination of Klages, Gazelle, Betzes and Centennial. This breeding has given this variety good resistance to sprouting, relative to the other examined varieties.

Wych and Rasmusson (1983) evaluated six-rowed malting barleys grown in the U.S. mid-west to determine the amount of genetic gain which had been made in 40 years in yield, agronomic and quality traits. The susceptibility to preharvest sprouting was not examined. However, there was a

significant increase in the levels of alpha-amylase in the newer cultivars. In North America, the genetic diversity in malting cultivars is very narrow (Wych and Rasmusson, 1983). One might suggest that the introduction of different varieties into breeding programmes may help alleviate the problem of preharvest sprouting. The desire of breeders to produce two generations of seed within one year, coupled with pressure from maltsters for rapidly modifying barleys may have unintentionally contributed to the breeding of varieties that are susceptible to sprouting. The varieties which will germinate very readily after harvesting when the levels of post-harvest dormancy are minimal are those which may demonstrate the greatest susceptibility to preharvest sprouting. A limited period of post-harvest dormancy should not normally be a problem to the maltster and could be an advantage in reducing preharvest sprouting.

The environment plays a role in determining susceptibility to preharvest sprouting. Strand (1983) examined the effects of temperature and rainfall on dormancy and concluded that low temperatures and high levels of rainfall during the growing season caused high dormancy in grain. In contrast, the growing season of 1988 was very hot and very dry (Table 2) and the varieties had very low levels of dormancy. Hence, "it was an ideal year to determine the susceptibility of varieties to preharvest sprouting" (Czarnecki, 1988).

In conclusion, it has been demonstrated that 2-rowed

Canadian malting barleys differ significantly in their susceptibility to preharvest sprouting. The varieties that have shown the greatest level of sprouting namely, Ellice and Norbert, are grown on a commerical basis on the Canadian Prairies. Lamont is a variety from the United States that has demonstrated good potential for developing into a good malting barley. From a maltsters' point of view rapidly modifying barleys are ideal. Obviously, it is not desirable if the varieties are bred to the extent that they will sprout readily in the field when poor harvesting conditions prevail. Future work must focus on the malting attributes of Harrington and the new line TR479.

SECTION II

4.2: Embryonic Sugars in Mature Barley

4.2.1 Raffinose and sucrose content in embryos of mature barley

Based on the results obtained in sections 4.1 and 4.2, the varieties Ellice and Hannchen were chosen as examples demonstrating extremes in susceptibility to preharvest sprouting.

Three replicate samples (3 spikes) from each variety at physiological maturity were examined. Embryos were removed, extracted and analysed as described in sections 3.4, 3.5 and 3.6, respectively. H.P.L.C. analysis of the sugars present in the 80% aqueous ethanol extract from embryos showed that sucrose and raffinose were the major sugars present. Occasionally, one or two other components of unknown identity were observed (Figure 6). Because of the erratic appearance of these constituents and their low levels when detected, work focused on sucrose, raffinose and the levels of these sugars present in the embryo. Subsequent reference to total sugar per embryo refers to the sum of raffinose and sucrose per embryo.

The heads used in this experiment differed in the number of kernel positions present e.g. In Ellice, replicate 1 had 23 kernel positions and replicate 2 had 21 kernel positions (Appendix IV). For comparsion purposes, the number

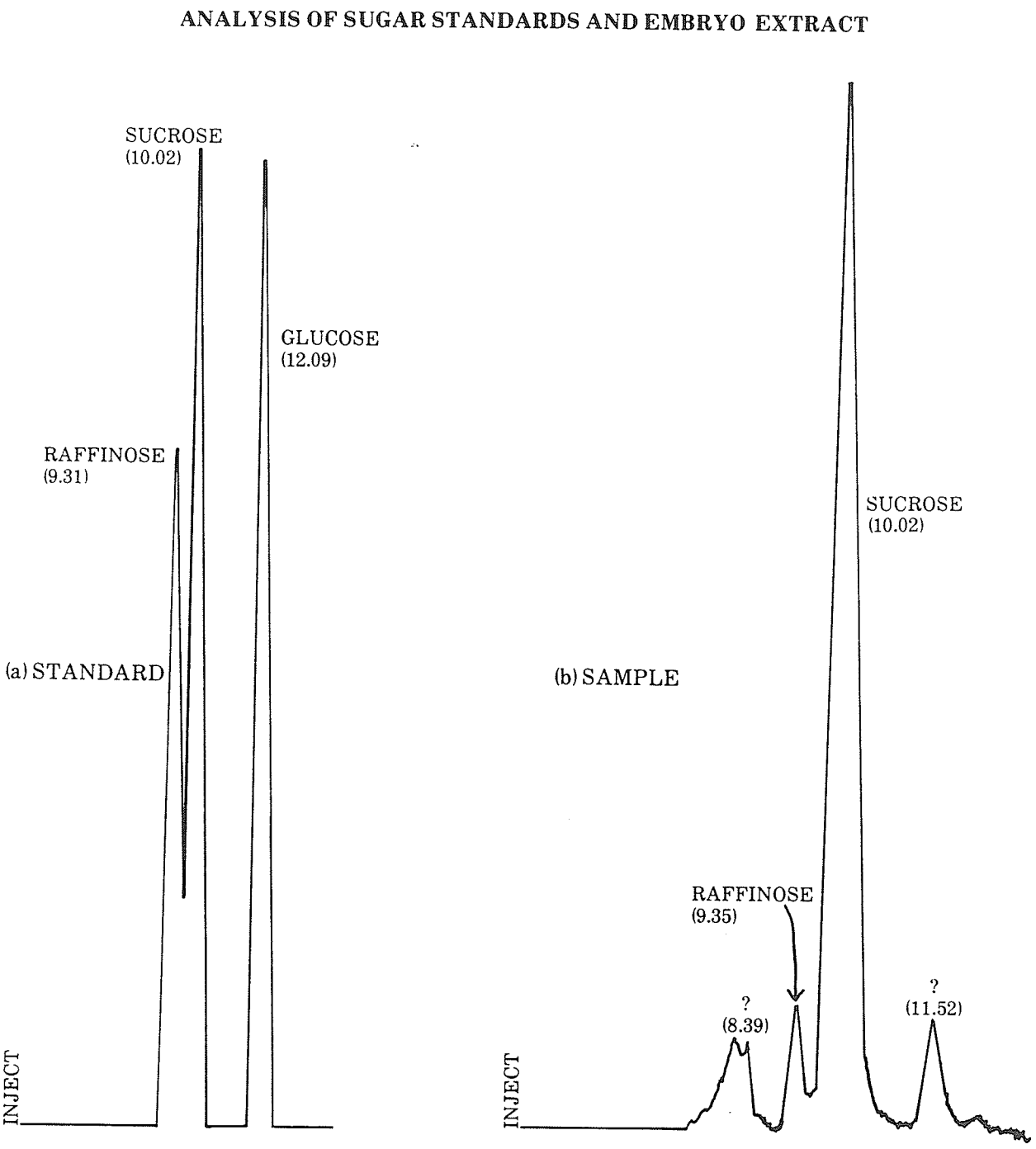


Figure 6: The H.P.L.C. elution profile of (a)standard and (b)sample. The elution time of each sugar is given in parentheses under the name of the sugar.

of kernel positions were divided into 5 sectors. Based on the example given the first 5 kernels (23/5 = 4.6) (from the base of the spike) of replicate 1 were compared with the first 4 kernels (21/5 = 4.2) of replicate 2. This was designated as Sector 1. Sector 2 compared the next four kernels (4.6 + 4.6 = 9.2) (up to kernel position 9) on replicate 1 with the next four kernels (4.2 + 4.2 = 8.4) (up to kernel position 8) on replicate 2. The top sector compared the top five kernels on replicate 1 with the last four kernels on replicate 2.

The quantification results were subjected to an analysis of variance in accordance with S.A.S. regulations: replicate, sector position and injection were considered as treatments.

Raffinose

The levels of raffinose per embryo at sequential sectors on the spike of Ellice barley are presented in Table 4. Some kernels were missing and the number of kernel positions analysed at each sector is presented in Appendix IV. Raffinose content per embryo was highest in the first three sectors (not significantly different) and lowest in the top sector. The fifth (top) sector had significantly lower levels of raffinose when compared with sectors 1 to 4.

The levels of raffinose in embryos at sequential sectors on the spike of Hannchen barley are presented in Table 4. Some kernels were missing and the number of kernel

TABLE 4: RANGE AND MEAN QUANTITIES* OF RAFFINOSE PER EMBRYO WITHIN THE DESIGNATED SECTORS OF ELLICE AND HANNCHEN BARLEY.

	SECTOR	RAFFINOSE
ELLICE	1	45-63ug 52ug abc
	2	55-64ug 60ug ab
	3	32-62ug 48ug bc
	4	41-53ug 47ug c
	5	26-40ug 31ug d
HANNCHEN	1	59-61ug 61ug a
	2	63-63ug 63ug a
	3	62-63ug 63ug a
	4	43-58ug 53ug abc
	5	40-48ug 45ug c

*L.S.D. analysis ($p<0.05$). Means followed by the same letter are not significantly different.

positions analysed at each sector is presented in Appendix VI. The first four sectors were not significantly different in the levels of raffinose per embryo. The fifth sector had significantly lower levels of raffinose when compared to the first three sectors. With the exception of the third and fifth sectors in Ellice, there was no significant difference between the two varieties in the levels of raffinose per embryo within the designated sectors.

Sucrose

The levels of sucrose per embryo at sequential sectors on the spike of Ellice barley are presented in Table 5. Some kernels were missing and the number of kernel positions analysed at each sector is shown in Appendix IV. The sucrose content per embryo was not significantly different among the five sectors.

The levels of sucrose per embryo at sequential sectors on the spike of Hannchen barley are presented in Table 5. Some kernels were missing and the number of kernel positions analysed at each sector is shown in Appendix VI. Between sectors the sucrose content did not differ significantly. When Ellice and Hannchen were compared, all sectors of Ellice contained significantly higher sucrose levels than the corresponding sectors of Hannchen.

TABLE 5: RANGE AND MEAN QUANTITIES* OF SUCROSE PER EMBRYO WITHIN THE DESIGNATED SECTORS OF ELLICE AND HANNCHEN BARLEY.

	SECTOR	SUCROSE	
ELLICE	1	71-92ug 80ug	a
	2	74-94ug 83ug	a
	3	82-93ug 87ug	a
	4	72-102ug 89ug	a
	5	66-88ug 79ug	ab
HANNCHEN	1	51-66ug 59ug	c
	2	52-73ug 64ug	bc
	3	49-65ug 58ug	c
	4	50-63ug 56ug	c
	5	46-51ug 49ug	c

*L.S.D. analysis ($p<0.05$). Means followed by the same letter are not significantly different.

Total

The average levels of total sugar per embryo determined within the designated sectors of Ellice barley are presented in Table 6-Appendix V. The levels which occurred within individual embryos ranged from 65 ug to 203 ug per embryo. The lowest quantities were found in the topmost sector of the spike. The slightly lower levels in total sugar content near the spike apex are due to lower raffinose content. Only the highest (sector 2) and lowest (sector 5) values were significantly different in the levels of total sugar per embryo.

The levels of total sugar expressed on a per embryo basis within each designated sector of Hannchen barley were determined (Table 6-Appendix VII). The levels which occurred within individual embryos ranged from 76 ug to 144 ug per embryo. As was the situation in Ellice, the lowest quantities of sugar were found at the top of the spike. As with Ellice, only the highest (sector 2) and lowest (sector 5) values were significantly different. There was no significant difference between the two varieties in the levels of total sugar per embryo within the designated sectors.

Percent

The level of raffinose expressed as a percentage of total sugar (raffinose plus sucrose) at each sector in Ellice barley is presented in Table 7-Appendix VIII. In the

TABLE 6: RANGE AND MEAN QUANTITIES* OF TOTAL SUGARS PER EMBRYO WITHIN THE DESIGNATED SECTORS OF ELLICE AND HANNCHEN BARLEY.

	SECTOR	TOTAL
ELLICE	1	118-155ug 131ug ab
	2	129-157ug 139ug a
	3	117-151ug 134ug ab
	4	109-149ug 130ug ab
	5	92-128ug 110ug bc
HANNCHEN	1	114-123ug 120ug abc
	2	108-136ug 124ug ab
	3	93-128ug 114ug abc
	4	106-120ug 113ug bc
	5	97-99ug 98ug c

*L.S.D. analysis ($p<0.05$). Means followed by the same letter are not significantly different.

TABLE 7: RANGE AND MEAN* PERCENT RAFFINOSE OF TOTAL SUGAR PER EMBRYO WITHIN THE DESIGNATED SECTORS OF ELLICE AND HANNCHEN BARLEY.

	SECTOR	PERCENT		
ELLICE	1	37-42%	40%	b
	2	39-43%	40%	b
	3	27-40%	35%	bc
	4	31-34%	32%	cd
	5	24-32%	28%	d
HANNCHEN	1	46-55%	51%	a
	2	46-52%	49%	a
	3	49-56%	52%	a
	4	47-53%	50%	a
	5	47-53%	49%	a

*L.S.D. analysis ($p<0.05$). Means followed by the same letter are not significantly different.

lower two sectors of the spike, raffinose constituted 40% of the total sugar content per embryo, while in the upper two sectors it averaged 30%. These differences were significant as illustrated in Table 7. When raffinose was averaged across all head positions, it represented 35% of the total sugar in the embryos of mature seed. There were no significant differences within the designated sectors in the levels of raffinose expressed as a percent of total sugar.

The level of raffinose expressed as a percentage of total sugar content within each sector of Hannchen barely is presented in Table 7-Appendix VIII. The percent raffinose remained on average, at 50% from the bottom to the top of the spike. There were no significant differences between sectors. The percent raffinose of total sugar per embryo was significantly higher in all five sectors of Hannchen when compared with Ellice.

Some of the variation in replicates may be accounted for by differences in the actual size of the embryos. Large embryos may contain more sugar overall or, more of a particular sugar. This study focused on the levels of raffinose and sucrose on a per embryo basis because of the difficulties in obtaining reproducible constant moisture weights for the dissected embryos. Care was taken to ensure that there was minimal contamination of the embryo with the endosperm, but such contamination may also contribute to some of the observed variation. The percentage variation due to differences in the injection of the samples was minimal in all cases. This infers that the H.P.L.C. system used was reproducible in its sample loading, separation and quantification of raffinose and sucrose.

The general trend observed for embryo sugars levels along the spike was higher levels in the middle of the spike and lower levels at the extremes. The observed variation may be due to several factors:-

(a) developmental or morphological differences during seed filling yielding a higher concentration of sugars in some embryos, or (b) larger embryos may result in a larger sink for sugar storage, or (c) differences in the germination state of the embryos along the spike may lead to partial depletion of the sugar resources in embryos.

Of these factors embryo size appeared initially to be the easiest to examine. However, attempts to weigh the embryo after removal from the seed yielded highly variable

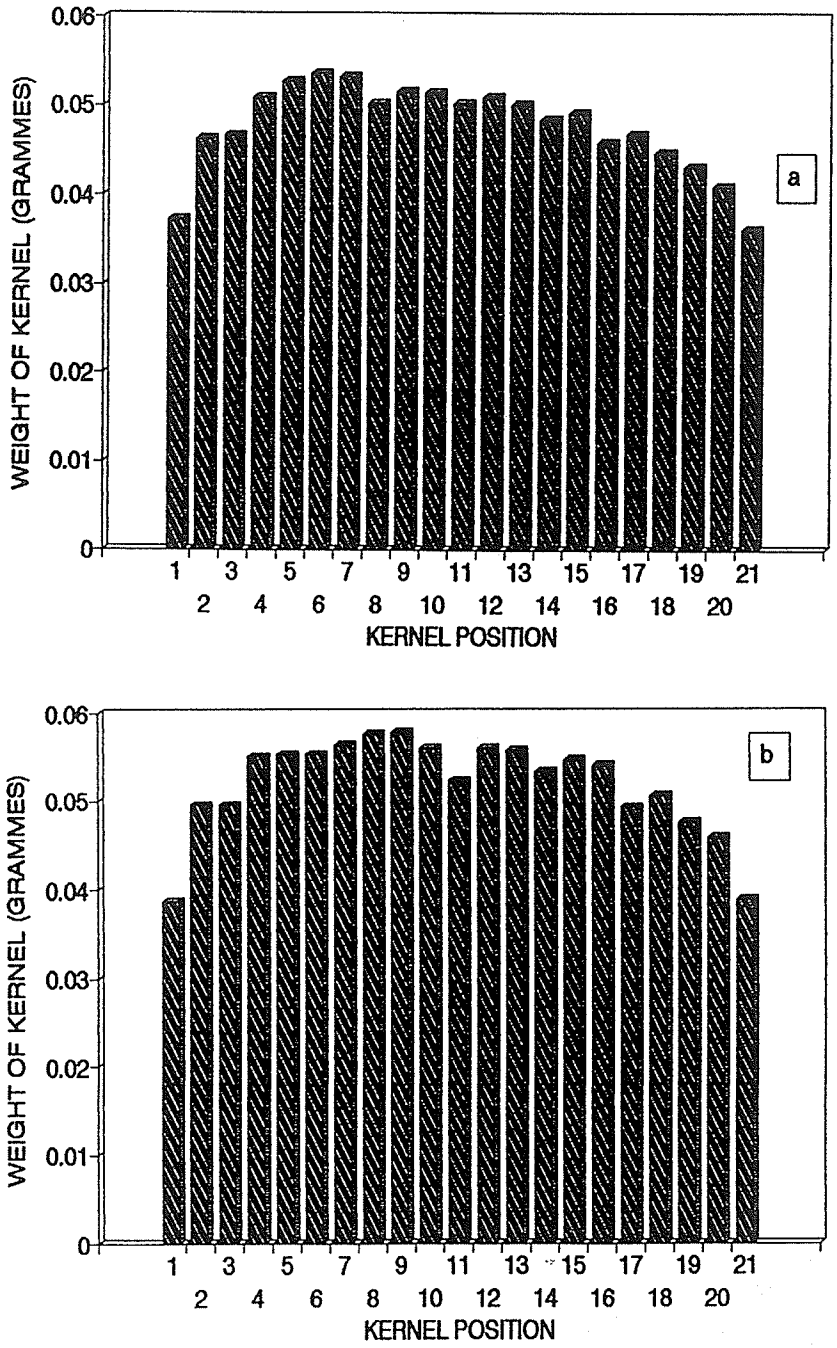
results due to the small weight being determined and moisture changes. Embryo size however, appeared to vary with kernel size. Because of this analysis of kernel weight was carried out to look at the effect of kernel position on kernel weight in the spike. Six spikes from the two cultivars, Harrington and Norbert were examined because the remaining spikes of Hannchen and Ellice were committed to other studies.

As presented in Figure 7 (Appendix IX) the trend in kernel weight with kernel position is similar for both Harrington and Norbert. It is also very similar to the general trends observed for the total sugars suggesting that much of the variability observed might be accounted for by variation in the size of the embryos along the spike.

Dubois et al., (1960) reported on results obtained by hand dissection and analysis of wheat. The total embryo comprised 2.64% by weight of the kernel. Sucrose and raffinose were the main sugars detected by paper chromatography. Trace amounts of glucose were detected but, no fructose was found. The determined sugars constituted 20.1% of the total weight of the defatted embryo, of which 58.5% was sucrose, 41.5% was raffinose.

The varieties chosen represent the extremes in sprouting behaviour and they had different levels of basal embryo sugar levels. The levels of raffinose in the upper sector of Ellice were lower when compared to the corresponding sector in Hannchen, which had higher levels of

Figure 7: Kernel weight in relation to kernel position in (a) Harrington and (b) Norbert barley.



raffinose. Since the sugar levels were determined in physiologically mature embryos, the next stage of the study involved examining the effect of sprouting conditions on the levels of these sugars.

4.2.2 Raffinose and sucrose content in embryos from seeds on spikes exposed to sprouting conditions

Using the variety Ellice the objectives of the work were (i) to investigate the effect of simulated sprouting conditions on the levels of raffinose and/or sucrose in the embryo and (ii) to determine where on the spike raffinose and sucrose are initially mobilized

Six heads of the variety Ellice were placed in a rain simulation cabinet and subjected to the conditions that induced sprouting as described in section 3.3. Two heads were removed every 3 hours until 9 hours of these conditions had elapsed.

At each interval the sprouting value for each kernel position on each head was noted. The embryo was removed and the sugars extracted. The levels of raffinose and sucrose were determined as described in section 2.6. The heads were sectored as described in Section 4.2.1. Results, including the analyses from mature, dry seed (hereafter referred to as "physiological maturity") based on these adjustments were subjected to an analysis of variance in accordance with S.A.S. regulations: time and sector were considered as treatments.

The effect of simulated sprouting conditions on the levels of raffinose in the embryo is presented in Table 8. Nine hours of simulated sprouting conditions significantly reduced the level of raffinose in the bottom four sectors of the head with an apparent but non-significant reduction in the fifth sector.

The effect of simulated sprouting conditions on the levels of sucrose in the spike is presented in Table 9. In the case of sucrose levels there was an apparent decline in the upper sectors of the spike, but the change was not significant. Sprouting was not visible over the course of this experiment.

The implication of the results in this study is that a rapid mobilization of raffinose occurs, particularly in the upper regions of the spike prior to actual sprouting being visible. In the case of sucrose there is a decline in the levels in the spike. The decline was not significant. Results demonstrating a significant decrease in the level of sucrose may have occurred if a larger sample were examined.

4.2.3 Wetting and drying cycles of threshed seed

The previously described experimental work was conducted on whole heads of the variety Ellice. Over the time course of the experiment, the three lower sectors tended to have higher levels of total sugars (raffinose and sucrose) (Sector 1: 114.5ug, sector 2: 127.1ug and sector 3: 116.2 ug) when compared with the other sectors (sector 4:

TABLE 8: EFFECT OF SIMULATED SPROUTING CONDITIONS APPLIED TO THE SPIKE OF MATURE ELLICE BARLEY ON THE LEVELS OF RAFFINOSE* IN THE EMBRYO.

	RAFFINOSE LEVEL (ug)/embryo			
SECTORS	0 HRS	3 HRS	6 HRS	9 HRS
FIRST	52.0 B ab	51.5 B a	31.5 A a	26.0 A a
SECOND	60.0 B b	51.5 B a	35.5 AB a	27.0 A a
THIRD	48.0 B a	45.5 B a	35.0 AB a	21.0 A a
FOURTH	47.0 B a	46.0 B a	31.0 AB a	12.5 A a
FIFTH	31.0 A a	33.5 A a	17.5 A a	22.0 A a

*Upper case letters going horizontially describe the L.S.D. analysis ($p<0.05$) within each sector over the duration of the experiment. Lower case letters going vertically describe the L.S.D. analysis ($p<0.05$) within each time period. Values followed by the same letter are not significantly different from one another.

TABLE 9: EFFECT OF SIMULATED SPROUTING CONDITIONS APPLIED TO SPIKES OF MATURE ELLICE BARLEY ON THE LEVELS OF SUCROSE* IN THE EMBRYO.

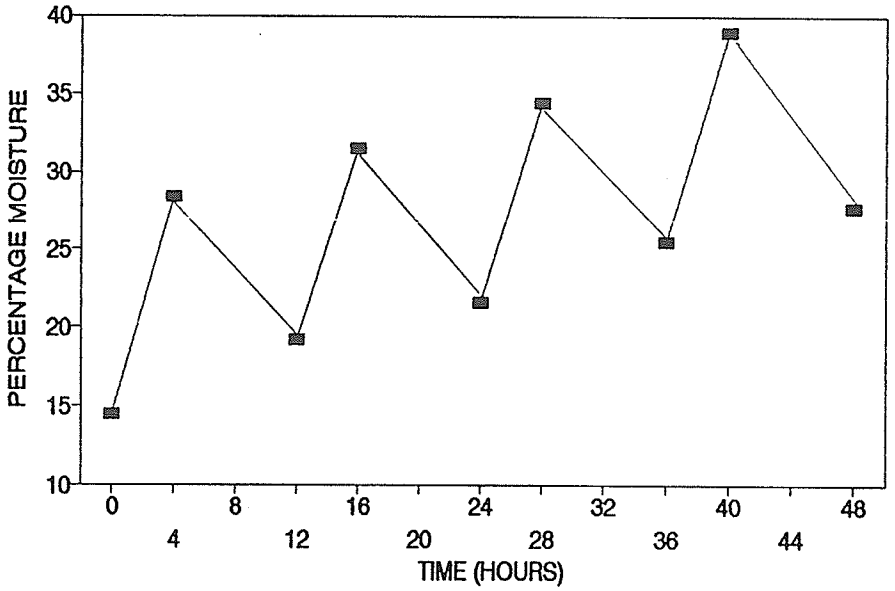
	SUCROSE LEVEL (ug)/embryo			
SECTORS	0 HRS	3 HRS	6 HRS	9 HRS
FIRST	80.0 A a	77.0 A a	62.0 A a	78.0 A ab
SECOND	83.0 A a	81.5 A a	87.5 A b	82.5 A ab
THIRD	87.0 A a	80.5 A a	87.0 A b	61.0 A a
FOURTH	89.0 B a	83.0 B a	69.0 AB ab	47.5 A a
FIFTH	79.0 A a	83.0 A a	57.0 A a	60.0 A a

*Upper case letters going horizontially describe the L.S.D. analysis ($p<0.05$) within each sector over the duration of the experiment. Lower case letters going vertically describe the L.S.D. analysis ($p<0.05$) within each time period. Values followed by the same letter are not significantly different from one another.

106.4ug and sector 5: 95.8ug). These changes in sugar content at different sectors within the spike is of little benefit to maltsters because they utilize threshed seed in their operations. The work reported here has shown that kernels at the extremes of the head tend to be smaller (Fig. 7). Lower levels of measurable sugar were found in embryos of the upper sectors of the head. To reduce the kernel to kernel variability, threshed barley was size fractionated to select only plump kernels. A further justification for doing this is that the maltster also size fractionates barley before malting. The purpose of this work was to evaluate if repeated cycles of wetting and drying would result in changes in embryo sugars.

The wetting and drying cycles (hereafter referred to as "weathering") were conducted by soaking threshed seed for four hours, and allowing the seed to dry under the conditions described in Section 3.7. The moisture content was determined at four and twelve hours of each cycle. The fluctuations in moisture content due to weathering cycles are presented in Figure 8. The initial level of moisture in the kernels was 14.5%. After 4 hours of steeping, the level of moisture had increased to 28.4%. After 8 hours of drying at the described conditions the level of moisture was reduced to 19.2% in the kernels. The repeated cycles of wetting and drying caused the moisture content to fluctuate as shown. Sprouting was not visible after three cycles of weathering but could be seen after the fourth cycle. A

Figure 8: Grain moisture content during repeated wetting and drying cycles.



Ellice barley was steeped for 4 hours at 15°C, then dried for 8 hours. This cycle was repeated four times with grain moisture determined at the fourth and twelfth hour of each cycle.

sprouting value of two was seen uniformly throughout the grain subjected to four cycles of weathering.

4.2.4 The raffinose and sucrose content of threshed seed subjected to cycles of weathering

The sugar content of embryos removed from kernels after each cycle of weathering was determined. The average level of raffinose in physiologically mature grain, regardless of kernel position was 45ug per embryo (Table 10). After the first cycle of weathering the level of raffinose per embryo had declined to 35ug per embryo. The second cycle and third cycles of weathering further reduced the levels of raffinose to 14ug and 4ug per embryo respectively. Results serve to demonstrate that three cycles of weathering resulted in the mobilization of the raffinose present in the embryo. In Ellice, at physiological maturity raffinose on average constituted 35% of the total extracted sugars in the embryo. When three cycles of weathering had elapsed, it constituted only 5% of the total weight of the total sugars indicating the rapid and selective depletion of raffinose in the embryo.

Changes in sucrose levels as presented in Table 11 yield a different pattern to that of raffinose. The mean quantity of sucrose was 84ug per embryo at physiological maturity. The level of sucrose oscillated around this value with repeated cycles of wetting and drying.

Kuo et al., (1988) reported that during seed

TABLE 10: RAFFINOSE CONTENT OF EMBRYOS OF ELLICE BARLEY AFTER REPEATED CYCLES OF WETTING AND DRYING.

NO.OF EMBRYOS	CYCLE	RANGE*	MEAN*
	0$	18 - 78	45
15	1	4 - 68	35 a
15	2	4 - 33	14 b
15	3	1 - 7	4 c

*Units micrograms. $ All positions on 3 heads of Ellice barley at physiological maturity. L.S.D. analysis ($p<0.05$). Actual values are presented in Appendix X.

TABLE 11: SUCROSE CONTENT OF EMBRYOS OF ELLICE BARLEY AFTER REPEATED CYCLES OF WETTING AND DRYING.

NO.OF EMBRYOS	CYCLE	RANGE*	MEAN*
	0$	58 - 103	84
15	1	50 - 111	78 a
15	2	56 - 115	94 a
15	3	27 - 135	78 a

*Units microgrammes. $ All positions on 3 heads of Ellice barley at physiological maturity. L.S.D. analysis ($p<0.05$). Means followed by the same letter are not significantly different. Actual values are presented in Appendix XI.

germination, the levels of raffinose saccharides (stachyose, raffinose and verbascose) declined rapidly in the embryonic axes of mung bean, soybean and cotton. The monosaccharides glucose and fructose accumulated in the embryonic axes of soybeans and mung beans. The decrease in these monosaccharides was not so apparent in cotton. These results concur with the results described by Palmer (1969), who suggested that the raffinose saccharides were directly hydrolysed during germination to produce sucrose, which was then transported to the embryonic axes for further metabolism.

The implication of the results in this study is that a dramatic decline in raffinose can occur without visible sprouting occurring. The potential exists to use raffinose levels as an indicator that the grain may have already been exposed to germination conditions. One must then ask if these conditions altered the germinability of the grain?

To examine this question, the next stage of the study involved examining the effect of weathering on the germination capacity of grain in the short and long term.

4.2.5 The germination of threshed seed subjected to weathering

Samples of Ellice barley from each cycle of controlled weathering were prepared and germinated as described in Section 3.7.2. Repeated cycles of weathering caused no significant decline in overall germination (Table 12)

TABLE 12: EFFECT OF REPEATED CYCLES OF WETTING AND DRYING ON THE GERMINATION OF ELLICE BARLEY*

TREATMENT	GERMINATION (%)
NO CYCLES	99 a
ONE CYCLE	94 a
TWO CYCLES	96 a
THREE CYCLES	95 a

*Three replicates per cycle were examined.

following short term storage (weeks after weathering treatment).

The accelerated aging test was used in conjunction with the standard germination test as a predictive test to determine within a short period of time how well the grain might germinate in the future i.e. following long term storage. The accelerated aging test was run on samples subjected to the three cycles of weathering treatment as well as samples of the original, unweathered Ellice. Examination at 24 hour intervals of the 3 day germination test (Table 13) revealed that the levels of germination in material subjected both to the accelerated aging test and three cycles of weathering were significantly reduced when compared to the untreated material. The differences between the percent germination after three cycles of weathering as presented in Tables 12 and 13 is assumed to reflect the negative effect of aging on the weathered samples.

Vigour was also affected as germination of treated samples tended to be erratic and the seedlings were spindly in nature compared to untreated material (Figures 9 - 13). The grain was shrivelled and tended to be paler in appearance once subjected to weathering cycles.

The differences described in germination may be attributed to the fact that raffinose provides part of the essential energy to initiate germination. The depletion of this sugar removes that energy source and as a consequence of this may decrease the rate and/or vigour of germination.

TABLE 13: PERCENTAGE GERMINATION IN ELLICE BARLEY* SUBJECTED TO THREE CYCLES OF WEATHERING AND AN ACCELERATED AGING TEST.

	GERMINATION (%)	
GERMINATION TIME	TREATED	UNTREATED#
24 HOURS	85b	90a
48 HOURS	89b	94a
72 HOURS	90b	97a

*Two replicates per 24 hours were examined. L.S.D. analysis ($p<0.05$) within time periods between treated and untreated material. Means followed by the same letter are not significantly different. #=Non-weathered and non-aged.

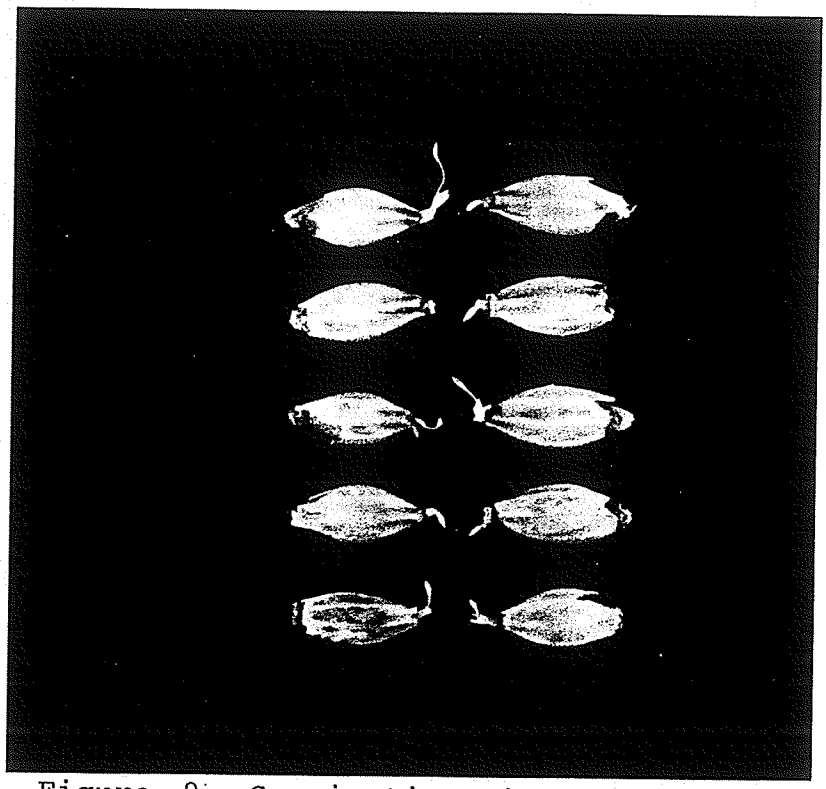


Figure 9 : Germination vigour observed at 24 hours in material not subjected to wetting and drying cycles.

Figure 10 : Germination vigour observed at 48 hours in material not subjected to wetting and drying cycles.

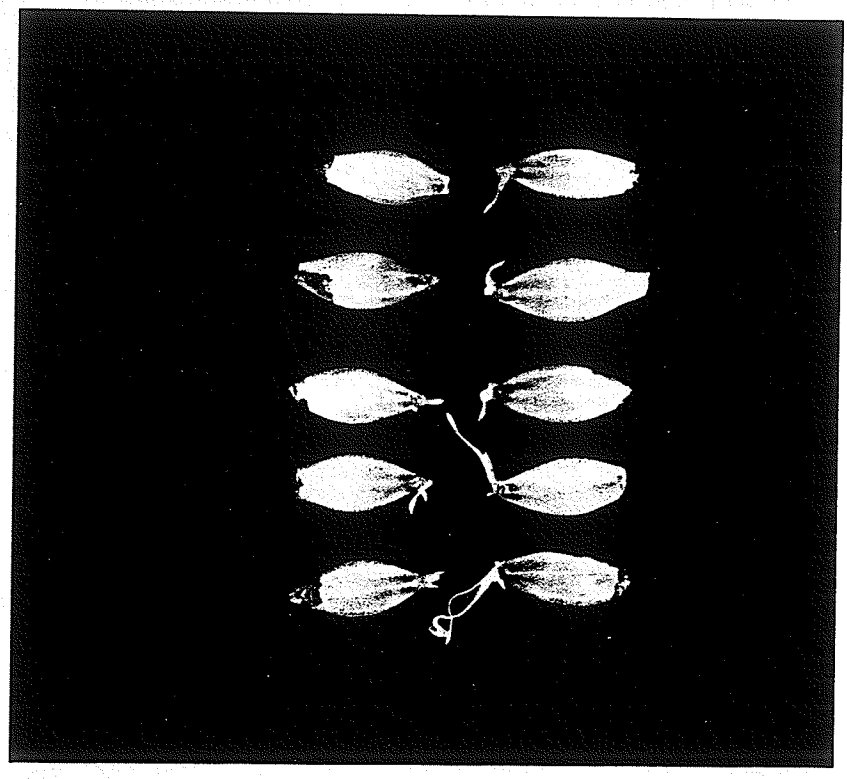


Figure 11: Different rates of germination vigour observed at 24 hours in material subjected to three cycles of wetting and drying.

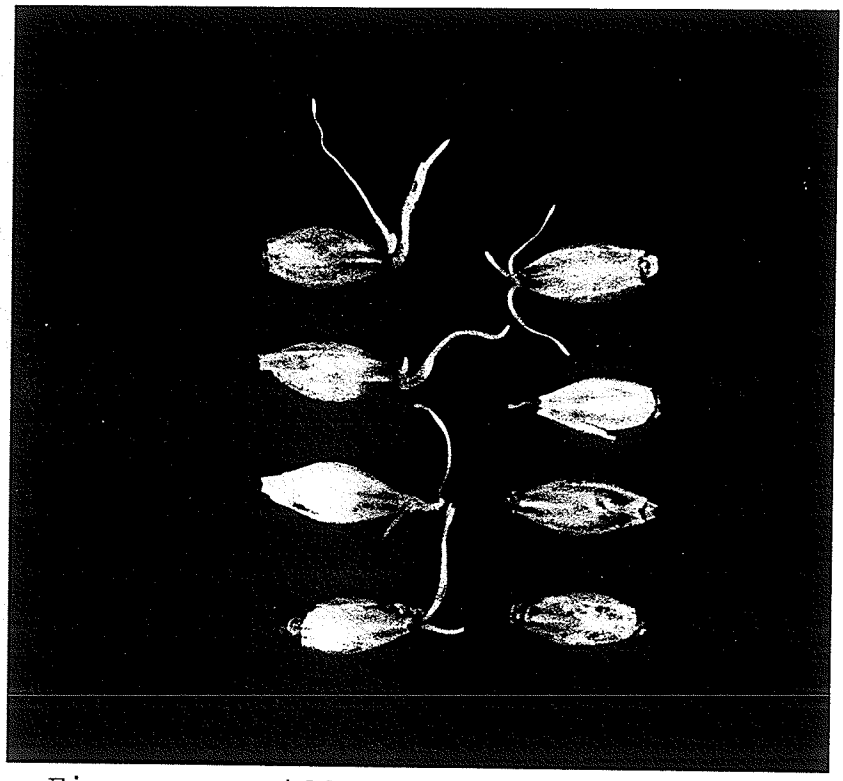


Figure 12 : Different rates of germination vigour observed at 48 hours in material subjected to three cycles of wetting and drying.

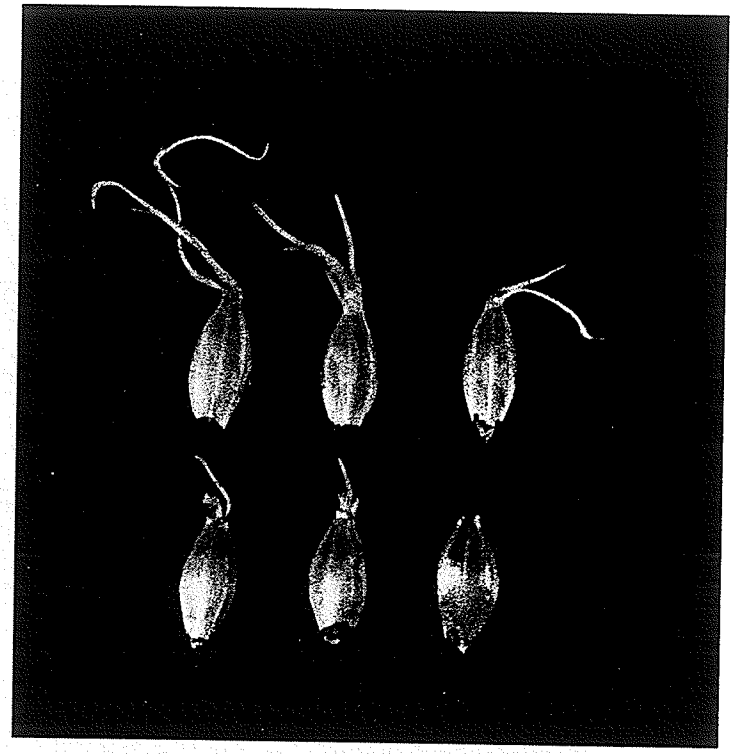


Figure 13: Different rates of germination vigour observed at 72 hours in material subjected to three cycles of wetting and drying.

Ovcharov and Koshelev (1974) reported that corn with a high moisture content had reduced sugar content after prolonged storage. The decrease in sugars was especially large in seeds which had lost their viability. Sucrose and raffinose were almost totally absent in the non-viable seeds, which contained fructose and glucose instead. The evidence suggested a parallel between the presence of sucrose in the seed and germination capacity of that seed. Main et al., (1983) demonstrated in soybean that an accelerated aging treatment reduced the rate of germination. Reduced development may be related to an inability to mobilize stored carbon sources. However, it has not been demonstrated that a decline in enzyme activities is responsible for the effects of accelerated aging on growth and sugar metabolism.

The results of this study may be important for maltsters and farmers. The wetting and drying of physiologically mature grain in a swath because of dew or heavy rainfall, followed by good drying conditions may result in a decline in the levels of raffinose and sucrose. It has been demonstrated that these fluctuations in seed moisture content can result in a decline in the level of germination and vigour following aging. The repeated cycles of weathering were a crude simulation of the potential seed moisture fluctuation which might occur during showery conditions while the grain is in the swath.

4.2.6 General Considerations on Section II

When one examines the trends that emerge from this work the following is seen:- Based on Section I it was clearly evident that Ellice was susceptible to preharvest sprouting, whereas Hannchen exhibited very low levels of preharvest sprouting. In these two varieties, basal levels of raffinose and sucrose in the embryo were determined. On examining the levels of sucrose plus raffinose per embryo, at sequential sectors on the spike it was clear that in Ellice, raffinose constituted 36% of the total sucrose and raffinose content per embryo. This percentage was significantly different than the corresponding percentage in Hannchen. In Hannchen, raffinose composed 50% of the weight of raffinose and sucrose. Obviously, further examination of other cultivars, in other environments is necessary but perhaps, the higher the percent raffinose of the total weight of sucrose plus raffinose in the embryo the less susceptible the cultivar is to preharvest sprouting.

Knowing the range of basal levels, one could explore the changes in these levels due to induced weathering. Within the variety Ellice, there were changes in raffinose content in the upper sectors of the head when placed in simulated sprouting conditions. Sprouting, however was not visible. Examination of the data (used in Section I) demonstrated that during the initial stages of sprouting the topmost sector of the head had the greatest susceptibility to sprouting. Therefore, a parallel may be drawn between the

susceptibility of the topmost sector to sprouting and the reduction in the levels of raffinose during the initial stages of germination. The percent raffinose of the total embryo sugar may be used to identify varieties with an increased susceptibility to preharvest sprouting, but also to determine the most susceptible part of the spike to sprouting.

The repeated cycles of weathering demonstrated that there was a significant reduction in the level of raffinose and sucrose. Repeated cycles of weathering also caused a reduction in the germinative capacity of the grain. The effect of simulated weathering on germinative capacity was amplified over time.

CONCLUDING REMARKS

Variation in varietal susceptibility to preharvest sprouting has been demonstrated in two-rowed malting barleys. The differences may be accounted, in part by the levels of post harvest dormancy as the varieties having the lowest level of sprouting also demonstrated the greatest dormancy. The varieties Ellice and Hannchen, chosen as examples demonstrating extremes in sprouting susceptibility, differed significantly in their distribution of embryo raffinose and sucrose on the head.

Under weathering conditions, there was a decline in the raffinose content in the embryo and in the germination capability of the treated grain. This decrease in germination capability may be partially accounted by the depletion of sugars in the embryo. Sprouting was not visible after three cycles of weathering. However, after four cycles of weathering, sprouting was visible.

Dormancy has been cited as the most important criterion in the control of preharvest sprouting (Ringlund, 1988). Breeders will have to consider incorporating a degree of dormancy into new lines of malting barley if the problem of preharvest sprouting is to be resolved. The methodology for determining the susceptibility of varieties to sprouting is also a major consideration. As demonstrated by the variety Harrington at physiological maturity, germination tests conducted in petri dishes have a different microenvironment

for stimulating germination to that of intact heads placed in a rain simulation cabinet. Other factors, for example the head type, the possibility of inhibitors within the intact head, the genetic background of Harrington and the presence of awns on the spike influence this variety's response to sprouting.

It may be possible to use the mobilization of raffinose and sucrose in the early phase of germination as an indicator of premature sprouting. The effect on germination rates and on grain quality has been demonstrated. Further investigations are necessary to examine if similar trends are found in other varieties grown in different environmental and edaphic conditions. The procedure for determining the levels of raffinose and sucrose in the embryo is not difficult to perform and may be completed with good reproducibility in 30 minutes. The economics of such a system are obvious when one considers that the standard germination test currently undertaken by the malting industry takes three days to complete.

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Appendix I: Historical background detailing the development of two-rowed malting barley in Canada.

The earliest evidence available suggests that barley was first imported into this country at the beginning of the seventeenth century to assess if it could be grown and used in the manufacture of beer (LaBerge, 1988). English settlers establishing agricultural production (c.1763) in the area surrounding the Saint Lawrence river brought varieties that were grown in their homeland (LaBerge, 1988). It was used in the production of 'home brew' (Burger and LaBerge, 1986). In 1786, John Molson founded his brewing company in Montreal (Burger and LaBerge, 1986).

Two-rowed barley was grown at this time. 'Thorpe' was English in origin and 'Chevalier' came from Europe (LaBerge, 1988). Maltsters used varieties that were available in the locality for malt production. The advantages of using ripe, sound material that resulted in a rapid, homogenous malt was known by the mid-nineteenth century (Burger and LaBerge, 1986). In North America, there was a period of advancement in malting and brewing, as a result of refrigeration and electricity in the late 1800's (LaBerge, 1988). Refrigeration enabled long term storage of beer to occur (LaBerge, 1988). The introduction of high yielding Manchuria barley by both the Wisconsin Experimental Farm in 1873, and the Ontario Agricultural College in 1889 caused a decline in the use of locally grown barley varieties for malt and beer

production (Burger and LaBerge, 1986).

In the United States, it was not until the arrival of German settlers in the late nineteenth century that beer was produced on a commerical scale (Burger and LaBerge, 1986). Farmers, particularly those in Southern Ontario, were growing two-rowed barley as a market existed in the United States for this product because of increased beer production (Burger and LaBerge, 1986).

The implementation of the McKinley tariff (1890) (Taussig, 1930) imposed a surcharge on Canadian barley being exported to the United States. As quality was no longer important, varieties were mixed and grown as fodder in addition to becoming contaminated with weeds (LaBerge, 1988). As a consequence of increased prices for the American buyer, the market demand for two-row Canadian malting barley declined. Canadian farmers turned to growing 6-rowed types for two reasons: Firstly, the loss of trade between Canada and the United States caused a rapid decline in market demand as a result of the imposition of the McKinley tariff and secondly, the introduction of 6-rowed types from Eastern Russia. This proved to be very successful because the barley was already adapted to the climatic conditions present in Canada. 'Mensury Ottawa 60' was the variety initially grown; this was, replaced by OAC21 (LaBerge, 1988).

Statutory standards of malting quality for barley were included in the Canada Grains Act in 1929. OAC21 was named as the statutory standard of quality for six-rowed barleys

in Canada. Currently, the variety Harrington determines standards for two-rowed types; Bonanza for six-rowed barley (LaBerge, 1988). In Canada, plant breeding developed new and improved existing varieties suitable for malting. It was quickly realized that more refined methods for measuring differences between varieties were required. Anderson and Co-workers (1941) established many of the important relationships between barley and malt properties (LaBerge, 1988).

Despite the fact that 6-row types were grown extensively in Canada, 2-row types continued to be bred and improved on a small scale. The problem with introducing new two-row types into the market is that Canadian brewers use six-row types in their brewing process. However, within the last decade there has been an increased demand for good quality 2-row malting types (Statistics Canada, 1989). The Canadian farmer has responded to this market demand and since 1979, the total area seeded to 2-row types has increased 15% on the Canadian prairie provinces (LaBerge, 1988).

In Canada, barley ranks second to wheat both in terms of the area seeded to the crop and levels of production (Statistics Canada, 1989). On the Prairies, the major producer is Alberta, followed by Saskatchewan and finally, Manitoba (Statistics Canada, 1989).

APPENDIX II: MEAN SPROUTING VALUE* OBSERVED AT 12 HOURLY INTERVALS FOR EACH VARIETY AT PHYSIOLOGICAL MATURITY.

	VARIETIES									
TIME	ELL	NOR	201	LAM	490	479	HAR	219	BET	HAN
12 HOURS	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.1	1.0	1.0
24 HOURS	1.7	1.5	1.5	1.6	1.6	1.5	1.4	1.5	1.0	1.0
36 HOURS	2.5	2.2	2.1	1.9	2.0	1.9	1.9	1.8	1.2	1.1
48 HOURS	3.1	3.2	2.8	2.7	2.8	2.5	2.5	2.3	1.3	1.1
60 HOURS	3.3	3.4	3.2	3.0	3.0	2.9	2.7	2.5	1.5	1.2
72 HOURS	3.5	3.6	3.4	3.3	3.2	3.1	3.0	2.6	1.7	1.3
84 HOURS	3.5	3.6	3.4	3.3	3.2	3.1	3.0	2.6	1.8	1.3
96 HOURS	3.5	3.6	3.4	3.4	3.2	3.1	3.0	2.7	2.0	1.3

* The value was calculated by totalling the sprouting rates observed at each 12 hourly intervals (16 replicates), and dividing this number by the total number of observations made.

APPENDIX III: MEANS SPROUTING VALUES OBSERVED* AT 12 HOURLY INTERVALS FOR EACH VARIETY ONE WEEK PRIOR TO PHYSIOLOGICAL MATURITY.

	VARIETIES									
TIME	201	NOR	ELL	490	219	BET	HAR	479	LAM	HAN
12 HOURS	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
24 HOURS	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
36 HOURS	1.2	1.1	1.1	1.1	1.0	1.0	1.0	1.0	1.0	1.0
48 HOURS	1.5	1.2	1.2	1.4	1.2	1.1	1.1	1.1	1.1	1.0
60 HOURS	1.9	1.5	1.5	1.5	1.3	1.2	1.2	1.2	1.1	1.0
72 HOURS	2.1	1.8	1.6	1.5	1.3	1.3	1.3	1.3	1.2	1.0
84 HOURS	2.2	1.8	1.7	1.6	1.3	1.3	1.3	1.3	1.2	1.0
96 HOURS	2.3	1.9	1.7	1.6	1.5	1.4	1.3	1.3	1.2	1.0

* This value was calculated by totalling the sprouting rates at each 12 hourly interval (16 replicates) and dividing this number by the number of observations made.

APPENDIX IV: WEIGHT OF SUCROSE* AND RAFFINOSE* PER EMBRYO IN ELLICE BARLEY AT PHYSIOLOGICAL MATURITY.

POSITION	REP 1		REP 2		REP 3	
	SUC	RAF	SUC	RAF	SUC	RAF
1	.	.	72	43	44	44
2	79	57	73	48	59	56
3	75	61	90	39	81	56
4	103	65	72	50	.	.
5	111	70	78	78	98	34
6	60	50	81	50	79	67
7	131	71	.	.	64	52
8	72	65	.	.	78	55
9	111	60	97	35	71	50
10	73	65	95	27	79	51
11	98	56	91	33	79	59
12	84	67	81	39	96	36
13	123	71	61	28	66	49
14	87	52	.	.	85	55
15	104	61	69	59	86	57
16	70	53	.	.	83	47
17	137	45	74	46	97	33
18	98	31	.	.	70	36
19	.	.	82	26	113	57
20	102	40	74	30	95	34

21	99	40	42	23	87	28
22	65	41			80	28
23	85	40			80	42
24					81	18
25					82	18

*Units microgrammes. Missing values are indicated by a period. Replicates 1, 2 and 3 had 23, 21 and 25 kernel positions respectively in each complete head. The lines drawn within each replicate indicate how the head was divided into sectors.

APPENDIX V: TOTAL* WEIGHT OF SUCROSE AND RAFFINOSE PER EMBRYO IN ELLICE BARLEY AT PHYSIOLOGICAL MATURITY.

POSITION	REP 1	REP 2	REP 3
1	.	115	87
2	136	121	115
3	135	129	137
4	168	122	.
5	181	129	132
6	118	131	146
7	203	.	116
8	137	.	133
9	170	131	120
10	138	123	130
11	124	124	137
12	160	120	132
13	194	88	115
14	139	.	141
15	165	98	143
16	123	.	130
17	181	120	130
18	128	.	106
19	.	108	170
20	142	103	128
21	139	65	115
22	107		108

23	125	122
24		99
25		100

*Units microgrammes. Missing values are indicated by a period. Replicates 1, 2 and 3 had 23, 21 and 25 kernel positions respectively in each complete head. The lines drawn within each replicate indicate how the head was divided into sectors.

APPENDIX VI: WEIGHT OF SUCROSE* AND RAFFINOSE* PER EMBRYO IN HANNCHEN BARLEY AT PHYSIOLOGICAL MATURITY.

POSITION	REP 1		REP 2		REP 3	
	SUC	RAF	SUC	RAF	SUC	RAF
1	77	48	56	63	54	54
2	62	66	.	.	.	.
3	59	58	45	65	65	63
4	.	.	.	.	61	61
5	57	60	63	60	63	59
6	49	54	69	65	.	.
7	49	55	.	.	80	64
8	50	57	71	65	.	.
9	41	51	59	61	66	62
10	57	38	41	61	.	.
11	.	.	73	60	71	69
12	53	64	57	68	64	61
13	44	53	66	60	69	68
14	53	52	68	60	56	52
15	51	60	69	61	54	68
16	.	.	66	65	57	55
17	47	61	57	52	54	57
18	41	35	54	50	49	52
19			41	44	.	.
20			61	55	63	50

21	.	.	41	56
22	49	44	56	42
23			46	45
24			48	41

* Units microgrammes. Missing values are indicated by a period. Replicates 1, 2 and 3 had 18, 22 and 24 kernel positions respectively in each complete head. The lines drawn within each replicate indicate how the head was divided into sectors.

APPENDIX VII: TOTAL* WEIGHT OF SUCROSE AND RAFFINOSE PER EMBRYO IN HANNCHEN BARLEY AT PHYSIOLOGICAL MATURITY.

POSITION	REP1	REP 2	REP 3
1	124	118	109
2	128	.	.
3	117	110	128
4	.	.	123
5	117	123	132
6	103	134	.
7	104	.	144
8	107	136	.
9	92	120	128
10	95	102	.
11	.	132	140
12	117	125	125
13	97	124	137
14	105	128	108
15	110	130	122
16	.	131	112
17	107	109	111
18	76	104	101
19		84	.
20		115	112
21		.	105
22		93	98

23	91
24	88

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* Units microgrammes. Missing values are indicated by a period. Replicates 1, 2 and 3 had 18, 22 and 24 kernel positions respectively in each complete head. The lines drawn within each replicate indicate how the head was divided into sectors.

APPENDIX VIII: LEVEL OF RAFFINOSE EXPRESSED AS A PERCENTAGE OF TOTAL SUCROSE AND RAFFINOSE CONTENT AT EACH KERNEL POSITION IN THE EMBRYOS OF HANNCHEN AND ELLICE BARLEY AT PHYSIOLOGICAL MATURITY.

	HANNCHEN			ELLICE		
Position	REP1	REP2	REP3	REP1	REP2	REP3
1	38.5	52.9	49.9	.	37.5	50.0
2	51.5	.	.	42.2	39.6	48.7
3	49.9	56.9	49.1	45.0	30.4	41.2
4	.	.	50.0	38.7	41.0	.
5	51.2	49.0	52.0	38.9	39.7	25.5
6	52.2	48.5	.	42.1	38.2	46.0
7	52.7	.	44.6	35.1	.	44.8
8	53.5	47.9	.	47.1	.	41.4
9	55.8	51.0	48.3	35.0	26.4	41.1
10	58.5	59.8	.	46.9	22.4	39.2
11	.	45.3	49.5	36.3	26.8	42.7
12	54.4	54.6	48.7	42.1	32.3	27.3
13	54.3	48.4	49.6	36.4	31.5	42.7
14	49.2	46.7	48.4	37.3	.	39.3
15	54.1	46.8	51.6	36.8	29.4	39.8
16	.	49.7	49.1	43.5	.	36.4
17	56.6	47.9	51.1	24.7	38.1	25.3
18	45.8	48.3	51.3	23.9	.	34.1
19		51.8	.	.	24.0	33.5

20	47.4	44.2	28.1	28.7	26.4
21	.	53.1	29.1	35.0	24.2
22	46.9	42.6	38.6		26.0
23		49.1	32.1		34.6
24		46.1			17.8
25					17.8

APPENDIX IX: WEIGHT* OF KERNELS IN THE HEADS OF HARRINGTON AND NORBERT BARLEY.

HARRINGTON		NORBERT	
POSITION	WT. OF KERNEL	POSITION	WT. OF KERNEL
6	0.0536a	9	0.0577a
7	0.0533a	8	0.0575a
5	0.0528a	7	0.0564a
9	0.0515a	10	0.0560a
10	0.0514a	12	0.0560a
4	0.0510a	13	0.0558a
12	0.0509a	6	0.0553a
8	0.0503a	5	0.0553a
11	0.0502a	4	0.0551a
13	0.0501a	15	0.0546a
15	0.0491a	16	0.0541a
14	0.0484a	14	0.0534a
17	0.0468a	11	0.0523a
3	0.0467a	18	0.0506a
2	0.0462a	2	0.0496a
16	0.0457a	3	0.0496a
18	0.0447a	17	0.0493a
19	0.0431a	19	0.0476a
20	0.0410a	20	0.0459a
1	0.0372a	21	0.0390b

21	0.0362a	1	0.0387b

*Units grammes. L.S.D. analysis ($p<0.05$). Means followed by the same letter are not significantly different. Six replicates of each variety examined were not significantly different. The lowest number indicates the kernel nearest to the peduncle; the highest number is the topmost kernel present on the head.

APPENDIX X: DETERMINATION OF RAFFINOSE* LEVELS IN THE EMBRYOS OF ELLICE BARLEY AFTER THREE CYCLES OF WETTING AND DRYING.

EMBRYO	INJECTION	CYCLE1	CYCLE2	CYCLE3
1	1	35	33	4
	2	31	33	4
2	1	31	16	3
	2	37	18	3
3	1	4	26	5
	2	8	25	6
4	1	11	8	3
	2	14	8	3
5	1	50	15	3
	2	45	15	3
6	1	10	20	2
	2	10	20	2
7	1	68	5	7
	2	66	4	7
8	1	26	16	3
	2	26	17	3
9	1	38	12	2
	2	33	12	4
10	1	60	7	4
	2	61	6	4
11	1	52	5	2
	2	52	5	1

12	1	34	9	5
	2	35	8	5
13	1	40	10	7
	2	39	12	7
14	1	27	20	6
	2	27	20	5
15	1	38	8	6
	2	39	7	6

*Units microgrammes.

APPENDIX XI: DETERMINATION OF SUCROSE* LEVELS IN EMBRYOS OF ELLICE BARLEY AFTER THREE CYCLES OF WETTING AND DRYING.

EMBRYO	INJECTION	CYCLE1	CYCLE2	CYCLE3
1	1	54	114	66
	2	50	115	66
2	1	68	115	104
	2	73	114	103
3	1	83	110	67
	2	89	110	72
4	1	105	83	115
	2	106	81	117
5	1	54	78	87
	2	51	79	91
6	1	105	94	64
	2	106	95	68
7	1	69	95	130
	2	69	93	128
8	1	54	91	66
	2	54	90	69
9	1	87	104	45
	2	85	105	51
10	1	92	112	58
	2	91	113	62
11	1	69	60	31
	2	70	61	27

12	1	82	102	86
	2	83	103	79
13	1	66	58	128
	2	64	56	135
14	1	110	90	61
	2	109	88	65
15	1	67	108	58
	2	68	105	51

*Units microgrammes