PROJECT REPORT No. 298

CAUSES AND CONTROL OF GAPE, SPLITTING AND SKINNING IN GRAINS OF MALTING SPRING BARLEY

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by

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PART A: ABSTRACT

Malting barley grain that is damaged by gape, splitting or skinning presents product and processing problems and puts a grower's malting quality premium at risk. Split grain may be rejected for use in both the UK and for export. Gape, splitting and skinning were defined to assist in the development of assessment procedures within the malting industry and in variety testing.

Significant regional differences in splitting and skinning have been noted in the UK indicating that environmental factors, probably due to certain weather patterns, are triggers for splitting and skinning. At locations where there are likely to be a number of high-risk factors, e.g. weather or other seasonal conditions, it is desirable to choose low risk varieties. There is also a significant genetic component to gape, splitting and skinning as varieties and breeding lines vary in their susceptibility to these conditions. Surveys of National List and Recommended List trials would allow the industry to rank varieties according to their risk.

The influence of agronomic practice is not the same in varieties of high or low susceptibility. In susceptible varieties, treatments that enhance excessive grain-filling or prolong canopy greenness have the disadvantageous side effects in that they may increase the risk of splitting or skinning. Crop management in terms of fungicide and nitrogen fertiliser usage must be considered in relation to the relative risks predisposing the crop to grain damage or loss of yield and quality due to disease. In varieties that are predisposed to skinning it is best to avoid the more abrasive combine settings and mechanical damage associated with some post-harvest processing.

Growth and physiological changes during husk and grain development were associated with incidence of gape, splitting and skinning. However, there was no strong evidence to suggest that gape *per se* leads to splitting. Splitting and skinning are examples of traits that are determined by a number of genes whose expression is under considerable environmental influence but associations have been identified between genetic markers and a number of loci affecting the grain traits; gape, splitting and skinning.

This project offers the prospect of developing molecular markers of real value in marker-assisted selection. Variety improvement depends on successful selection that is best attained in traits with a high proportion of genetic variation and low environment or genetic *x* environment influences. In respect to splitting there would appear to be sufficient genetic variability to permit progress using a combination of marker-assisted selection and phenotypic screening.

PART B: SUMMARY REPORT

1. Introduction

Use of split or skinned grain for malting presents both product and processing problems putting maltsters' sales and accreditation at risk. The main concern for growers is the loss of malting premium. Bulks with split or skinned grain may be rejected for use both in the UK and abroad. Maltsters reject a sample if it contains more than a few percent of split, skinned or damaged grains. Splitting results in irregular germination and starch modification during the malting process. 'Splits' and 'gapes' also affect drainage of water during malting and provide entry points for micro-organisms which may affect malt production and value. Splitting in the field causes pre-harvest conversion of starch to sugar; thus reducing potential levels of malt extract and spirit yield. Micro-organisms in split grains may produce mycotoxins that reduce the quality of grain, both for malting and feed. If, in a batch of barley, there are grains without husks (i.e. skinned), then these grains will germinate more rapidly than those with firmly adhering husks, thus giving rise to uneven malting. However, if the embryo is damaged, then grains without husks may not germinate or be at risk from mould growth. In grains with a loosely adhering husk, the growth of the plumule (acrospire) tends to be more vigorous than in grains with tightly adhering husks and this leads to handling problems and to greater malting losses.

Levels of splitting or gape vary considerably between years and between regions, and splitting tends to be more prevalent in Scotland, whilst skinning is more common in England. An SAC survey of maltsters' intake between 1992-94 indicated wide variation in levels of split grain. At some locations a significant proportion of bulks were rejected for being above threshold values. Assessments by SAC on HGCA-funded Recommended List trials in Scotland between 1992-1998 revealed that the content of split grains was up to 20% in susceptible varieties such as Chariot. High levels of gape were recorded in 1996 and 2001 and skinning was particularly severe across the UK in grain harvested in 1997 and 2001.

2. Defining and assessing gape, splitting and skinning

The evaluation and acceptance of grain is based on many criteria. The definitions developed in this project for gape, splitting and skinning were designed to complement assessment procedures that might be used within the malting industry and in variety testing. It is important that farmers, maltsters and grain merchants are able to work according to the same definitions for gape, splitting and skinning: this will enable better quantification of these characteristics across the industry. Each character is described in terms of a standard definition and a range of categories or variations from the standard. The descriptions below also provide a basis from which industry procedures could be standardised.

2.1 The intact barley grain

Barley grains have an adherent husk which is composed of two parts from the flower, the palea and the lemma (Fig. 1a). The palea covers the ventral side of the grain which is characterised by a central crease and the lemma covers the dorsal side of the grain. In most grains, the lemma overlaps the palea along the sides of the grain. Several layers of tissues separate the husk from the endosperm, which comprises about 80% of the mature grain (Fig. 1b). Immediately beneath the husk lies the pericarp or ovary wall, which protects and supports the growing endosperm and embryo. The caryopsis (also referred to as a kernel) is the term used to describe all the tissues beneath the husk, including the endosperm. As the grain matures, the palea and lemma become cemented to the pericarp by "glue" that is secreted from the pericarp. From about two weeks after anthesis, the husk becomes very difficult to remove from the caryopsis.

2.2 Gape

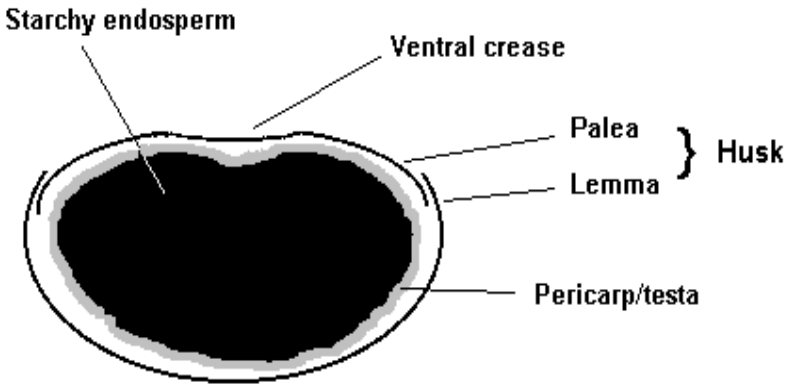
In a normal grain the lemma overlaps the palea (Fig. 1a). If a gap is present between the lemma and palea but the pericarp remains intact and there is no exposure of the endosperm, the condition is known as gape. In assessments gape is defined as a gap of 0.5 mm or more between the palea and lemma in the middle third of the grain. When describing and measuring gape there are two other categories to consider: 'overlapping' is used to describe a grain in which the palea and lemma overlap along its entire length and 'abutting' occurs when the palea and lemma meet without overlapping or leaving a gap. Scores for gape will vary widely if different sizes of gap are used (e.g. 0.5, 1.0 or 2.0 mm). Therefore, for clarity of assessments within the industry it is recommended that a standard gap between the palea and lemma is measured.

2.3 Splitting

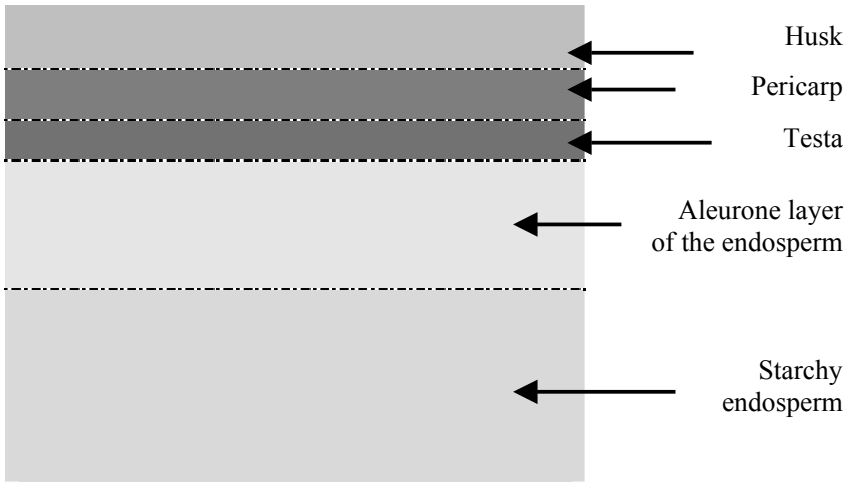
Splitting is a crack through the pericarp/testa/aleurone tissues that exposes the starchy endosperm. There are three types of splitting – each can be regarded as causing the same degree of damage. Lateral (side) splitting occurs along the side of the grain and is most often associated with gape that exposes a crack or opening in the pericarp/testa/aleurone which encloses the endosperm. In ventral (front) and dorsal (back) splitting the husk adheres to the pericarp and lesions in both the husk and the pericarp/testa expose the starchy endosperm. For assessment of splitting, all types of cavity or exposure of the endosperm can be scored equally, though in some cases in may be appropriate to categorise the condition into lateral, ventral and dorsal. Splitting can be assessed with or without the use of an iodine-based dye to stain the areas of exposed endosperm blue/black. Although the use of a dye is a more time-consuming task than an assessment on unstained grains, the dye makes the identification of split grains easier.

Figure 1. (a) Cross-section of a grain showing how the husk (palea and lemma) covers the grain and the starchy endosperm within. (b) Schematic diagram of the main grain tissues (not to scale). The husk overlays the pericarp/testa which surrounds the caryopsis (or kernel) which is comprised of an outer aleurone layer and the starchy endosperm.

(a)



(b)



2.4 Skinning

Skinning occurs when there is a loss of grip between the husk and the pericarp (Fig. 1a). Skinned grains are defined as those in which 25% or more of the husk (palea and/or lemma) has failed to adhere to the caryopsis. Skinning can be further defined as dorsal (i.e. removal of the lemma), ventral (i.e. removal of the palea) or lateral (i.e. removal of a longitudinal strip of palea and/or lemma). A pearled grain is one in which the entire husk, pericarp/testa have been removed. Skinning can also occur at the ends of the grain, especially at the distal end when there has been damage to, or removal of, the awn resulting in a loss of husk from the end towards the mid-grain. A 5% level of skinning is common in barley because of this type of damage to the awn. In assessments of skinning, the threshold (e.g. 25%) will comprise a sum of the types described above.

3. What are the risk factors?

3.1 Environment

Figure 2 summarises the main environmental and crop warning signs of gape, splitting of skinning. Some weather patterns appear to be important triggers of splitting. In 2001, which was a bad year for splitting in Scotland compared to 2000 and 1999, there was low spring rainfall combined with high rainfall in July-August. It is possible that crops were stressed during husk development in spring and that the high rainfall in summer created good conditions for grain filling and also gave rise to repeated wetting and drying, which caused tensions in the outer layers of the grains during grain development and maturation.

In Scotland, growing conditions are generally wetter, cooler and longer than those in England giving rise to high TGWs which may explain why the incidence of splitting is usually higher in grain grown in Scotland than in grain grown in England. The incidence of skinning tends to increase when the crop is exposed to wetting and drying cycles.

3.2 Variety

In recent SAC surveys, the varieties Chalice and Cellar were identified as having a relatively low risk of gape or splitting, whilst Chariot was high risk and Decanter, Optic and Prisma were intermediate (Table 1). For skinning, Chalice, Cellar and Decanter appear to have low risk, whilst Prisma is high risk. Limited evidence suggests that variability in skinning, across a range of treatments, is greater in Chariot than in Optic. Other SAC surveys of Recommended List (RL) trials suggest that there is a degree of consistency in ranking for each of gape, splitting and skinning over seasons. However, in bad splitting or skinning years (e.g. 1997 and 2001) we have to conclude that most current varieties will display some degree of these undesirable conditions. The results in Table 1 are based on surveys in Scotland only and the ranking order of

variety susceptibility for gape and splitting was similar. Annual surveys of National List and RL trials would allow the industry to rank varieties according to their susceptibilities, as well as regional variation in these conditions.

Table 1. The relative risk of gape and splitting or skinning

Risk of condition	Gape or splitting	Skinning
Low	Chalice, Cellar	Chalice, Cellar, Decanter
Moderate	Decanter, Optic, Prisma	Chariot, Optic
High	Chariot	Prisma

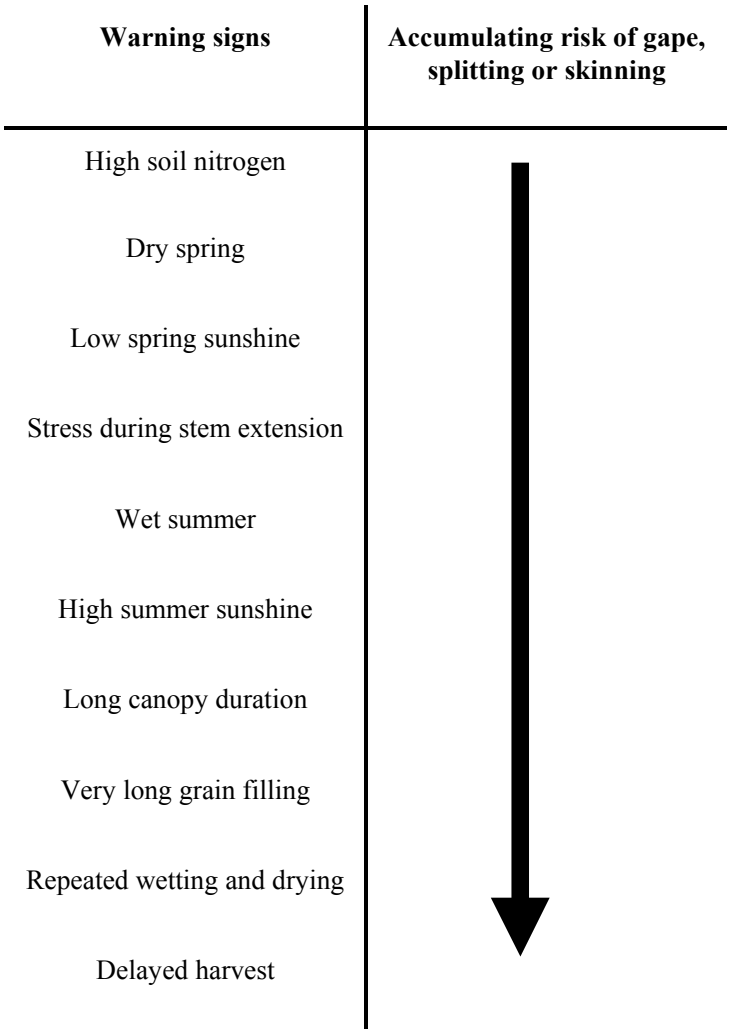
3.3 Agronomy

The influence of agronomic practice is clearly not the same in varieties of high susceptibility (e.g. Chariot) as it is in varieties of low susceptibility (e.g. Landlord). In susceptible varieties, treatments that prolong canopy greenness or create excessive grain-filling are likely to increase the risk of splitting or skinning. However, regional differences are important in that splitting and skinning do not appear to be equally affected across different parts of the UK.

In Scottish trials, increasing the rate of application of N fertilisers and the number of fungicide applications increased splitting, but not necessarily gape or skinning. However, in English trials, late fungicide applications increased the incidence of skinning. Although there was no consistent effect of the rate of application of N fertiliser or the number of fungicide applications on TGW, there was a clear relationship between TGW and splitting in Chariot, across trials and treatments. By contrast, in Landlord there was only a weak relationship between TGW and splitting.

Although variety differences in mean TGW is not itself a good indicator of gape, splitting or skinning risk, changes in TGW within a variety appear to be important (see section 4). There appears to be a greater risk of splitting (in Scotland) and skinning (in England) under situations where TGW is likely to be high.

Figure 2. (a) The environmental factors or warning signs that may indicate accumulating risk of gape, splitting of skinning.



3.4 Mechanical damage during harvesting or post-harvest

Skinning can be worsened by physical damage that occurs during harvest and post-harvest processing. When the grain is threshed the awn, which is tapered from the lemma, can have sufficient strength to strip part of the lemma at the tip of the grain. ADAS trials compared combined and hand-harvested grain samples and indicated that abrasion during harvesting increased the risk of skinning. In vulnerable varieties, it is desirable to avoid highly abrasive combine settings. For example, a combination of a low concave setting (e.g. 7-9 mm, the lowest for barley) and high drum speed (1200-1250 rpm, the maximum for barley) is likely to place

the crop at risk of excessive abrasion and skinning. Further grain cleaning or transfer operations can result in a more widespread abrasion of the husk from the grain and modification of malting performance.

There is no evidence to suggest that gape or splitting are strongly influenced by combine settings. However, other types of damage such as chipped grain or removal of the embryo can occur if combining is too abrasive.

4. Effects of the environment on grain physiology and growth

Alterations in growth conditions and plant structure resulted in physiological changes during husk and grain development that were associated with incidence of gape and splitting, and an increased predisposition of the grain to skin during harvesting. There are a number of possible explanations and differential growth of husk and caryopsis could be the key. Excessive expansion during grain filling could predispose grains to gape, splitting or skinning. This mismatch between the size of the husk and the caryopsis is most likely to occur if the husk is poorly developed because of stress or less than optimal growing conditions during stem extension, i.e. dull weather or low rainfall or low temperature.

Poor contact between a large husk and a poorly filled grain, or variable grip between husk and grain, could lead to skinning. This could be a result of a modification of the 'glue' that binds the lemma and palea to the pericarp.

If grain-filling stresses the structure of the grain such that the mechanical strength of the grain is reduced then splitting becomes more likely. This appears to be particularly important when the grain fills to excess. In favourable situations, excessive grain-filling at the positions of the largest grains on the main-stem ear may increase the risk i.e. increases the proportion retained over a larger sieve (e.g. 2.8 mm). By contrast, grain in the lower sieve-size fraction is likely to be derived from the distal positions on the main-stem ear and from any position on tiller ears. Grains in these positions will be shorter as well as thinner due to competition from larger grains for carbohydrates. Splitting is probably less likely to occur in these grains than in the large grains from the middle of the main stem.

There is a possibility that increases in the observed levels of splitting are a result of reduction in the mechanical strength of the endosperm cell walls because of changes in the partitioning of carbohydrate in the developing grain brought about by selection for improved malting quality, particularly selection for rapid modification and low beta-glucans.

Where skinning is a risk, physiological changes that give rise to excessively large grain size can increase the proportion of skinned grain in the harvested crop. This may be due to the use of combine settings inappropriate for the largest grains in the population.

Stress (e.g. lack of sunlight) before anthesis was shown to affect grain development by a direct effect on reducing husk growth and also by slowing down caryopsis dehydration, presumably by disrupting the formation during early development of adequate pathways for grain dehydration. However, slow dehydration *per se* does not explain how grains split because shading after anthesis reduced the rate of grain maturation and was also associated with low levels of gape and splitting.

Gape, splitting and skinning can occur together or independently of each other. This is partly because some types of splitting, e.g. lateral splitting are likely to be preceded by gape, but dorsal and ventral splitting can occur in grains in which there is no gape. Splitting and skinning, as described in sections 2.3 and 2.4, are also associated with different physiological or developmental processes and differ in their predisposition to mechanical damage, as described above. Furthermore, gape is difficult to assess in samples of grain in which the incidence of splitting or skinning are high.

5. Reducing the risk of gape, splitting and skinning in the field

5.1 Environment and variety choice

At locations where there are likely to be a number of high-risk factors, e.g. weather or seasonal conditions then it is best to choose varieties with a low risk of the undesirable conditions. Likewise, if there is history of gape, splitting or skinning at a particular location, which cannot be directly related to either environmental or agronomic factors, then growers are likely to be benefit from selecting a variety with low or medium risk rather that one with high risk. Surveys of gape, splitting and skinning across the UK, as suggested above, are required to establish the extent to which the ranking order of variety susceptibility to each condition changes across regions.

5.2. Agronomy and crop handling

Although agronomic treatments that prolong canopy greenness or create excessive grain-filling are likely to increase the risk of splitting or skinning, the grower faces the dilemma of how best to manage a crop to reduce the undesirable grain conditions, whilst at the same time protect the crop from disease. Fungicides applied late (i.e. after flag leaf stage) can result in the prolonging of green leaf area and high TGW in some grains, which increases the risk of all three conditions. However, growers need to be careful because late-season diseases such as *Ramularia* or leaf-spotting complexes can reduce yield severely if not adequately

controlled with fungicides applied between flag leaf to booting stage (or flag leaf to ear emergence in feed crops). Therefore, crop management in terms of fungicide and nitrogen fertiliser usage must be considered in relation to both the risks predisposing the crop to the undesirable grain conditions and to the loss of yield and quality due to disease.

In varieties that are predisposed to skinning it is best to avoid highly abrasive combine settings. This means adjusting the combine settings so that the concave setting is not too low and the drum speed is not too fast.

6. Genetic control of gape, splitting and skinning

The existence of definite varietal differences for splitting and gape indicate a high level of genetic control over these undesirable traits. Associations have been identified between genetic markers and the grain traits, gape, splitting and skinning. Splitting and skinning are examples of traits that are determined by a number of genes whose expression is under considerable environmental influence. The grain dimensions show an interesting contrast because while genetic control accounts for more than 60% of the phenotypic variation in grain length the corresponding figure for grain breadth is about 10%. This is an obvious consequence of the differences in development and growth of grain components before and after anthesis. The analyses suggest that alterations in grain length or width that affect the width to length ratio may reflect a disruption of the appropriate grain dimensions to retain the integrity of the pericarp and/or testa and these can lead to grain splitting.

The genetic location of characters or traits (i.e. Quantitative Trait Loci, QTLs) were detected for grain traits in the populations from Tankard x Livet and Derkado x B83-12/21/5. In most cases these QTLs detected over 50% of the estimated genetic variation for each of the grain traits. In fact, QTLs were detected that accounted for over 60% of the genetic variation in splitting and nearly 60% in skinning. This offers the prospect of developing molecular markers of real value in marker-assisted selection for reducing in these undesirable traits.

There is evidence of some independent genetic control of TGW and splitting (i.e. in the Tankard x Livet cross). For example, QTL alleles from Tankard at one locus that increase TGW, width to length ratio, gape and yield are not co-located with a QTL for splitting. Conversely, three QTLs from Tankard decreasing splitting are located in regions of chromosomes (Bmag353 on 4H and Bmag323 and HvLOX2 on 5H) that are not co-located with any other QTLs. Therefore, selection of Tankard alleles at these regions of the genome could reduce overall splitting and boost TGW and yield.

Some comparison of QTL locations across the two populations can be made as many of the molecular markers on the Derkado x B83-12/21/5 map are also represented on the Tankard x Livet map. For example, the major locus for gape in Derkado x B83-12/21/5 was in the same region of chromosome 6H as a locus of large effect for the same character in the Tankard x Livet population. The latter was part of a QTL cluster with effects upon TGW, grain length and grain width, though no effects on these characters were detected in the Derkado x B83-12/21/5 population. A QTL for grain width to grain length ratio was detected in the same region of chromosome 7H in both populations.

Genetic markers correctly identified eight of the worst 11 lines for splitting and could therefore be used in direct selection with a good level of confidence. This is not an independent test, however, and further work is required to assess the value of these markers in selection. Derivation of more closely linked, and even direct, gene markers would vastly improve the potential to use molecular markers for selection.

7. The role of plant breeding in controlling gape, splitting and skinning

7.1 Genetic approaches

Variety improvement depends on successful selection and thus is best attained in traits with a high proportion of genetic variation and low environment or genetic *x* environment influences. Successful selection also depends on how easy it is to recognise traits, either in the phenotype or genome. In respect to splitting there would appear to be sufficient genetic variability to permit progress, given an efficient selection process.

Selection of lines with low expression of undesirable characters is an obvious means of avoiding the problems but care must to taken to limit the expression of a desirable yield or malting quality component as well as a possible increase in screenings if TGW is reduced too much.

The possibility of using marker-assisted selection offers obvious advantages in allowing the breeder to take a broad view of the genetic control of plant traits. In a large-scale breeding programme, where F_2 populations could far exceed 100,000 plants, even weak correlations between traits may have considerable effects on the outcome of selection.

Given the problem of environmental variation for splitting, the ability to use molecular markers as a means of selecting lines resistant to split that is environmentally independent would be of great advantage to plant breeders and/or official testing authorities. There remains a requirement to demonstrate that the markers

identified as being associated with splitting would provide such a method of identifying lines resistant to the character. This would best be achieved by testing a range of lines genotypically and phenotypically to determine if alleles at the key loci are associated with resistance over a broad genetic background.

7.2 Other screening tests

Other phenotypic screening tests developed in this project can be used to complement the use of markers. Glasshouse tests, such those used in the shading experiments at SAC, could be used to screen out vulnerable cultivars. In addition, the 'half-ear' test, described by SAC and ADAS, which encourages grains to fill to excess and thus predispose them to gape or splitting in the field or skinning during combining, can be used in field plots. Whichever method is used, genetic or phenotype, the elimination of vulnerable varieties from the National or Recommended Lists would provide greater security to growers.

8. Introduction to Part C: Technical Papers

A series of five Technical Papers (listed on pages i and ii) present different aspects of this investigation. A combination of agronomic, physiological and genetic studies were undertaken to give a new insight into the characters gape, splitting and skinning and to provide an understanding of the principal risk factors and clearer guidelines for control measures. This facilitated the collection of new developmental and genetic information to reduce these undesirable characteristics in new varieties in future. Environmental and agronomic data should lead to guidelines designed to minimise damage to vulnerable crops before and at harvest. Four main approaches were used to offer the most suitable approach to addressing the problems of gape, splitting and skinning and thus protect grain quality:

(1) Identifying the main agronomic and environmental risk factors (Technical Papers 1, 2 & 3).

(2) Gaining an understanding of the developmental morphology and physiology associated with gape, splitting and skinning (Technical Papers 1 & 4).

(3) Determination of the genetic architecture and identification of genetic markers to assist in selection against splitting, gape and skinning (Technical Paper 2).

(4) Providing better guidelines for defining and measuring splitting, gape and skinning (Technical Paper 5).

PART C: TECHNICAL PAPERS

TECHNICAL PAPER 1

GAPE, SPLITTING AND SKINNING IN GRAINS OF MALTING BARLEY: (1) CONTROLLED ENVIRONMENT STUDIES ON GRAIN DEVELOPMENT AS INFLUENCED BY ENVIRONMENTAL FACTORS AND (2) FIELD STUDIES OF THE INFLUENCE OF AGRONOMIC FACTORS AND WEATHER ON THE INCIDENCE OF GAPE, SPLITTING AND SKINNING

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Introduction

Results from an SAC survey carried out in 1994 indicated that some degree of gape or splitting was present in up to 30 % of samples of malting barley harvested in Scotland between 1992-1994, and this may result in up to 10% of samples being rejected by maltsters (personal communication with maltsters). SAC has carried out assessments of splitting on varieties in HGCA funded RL trials. Table 1 presents mean splitting scores between 1992 to 1998 for selected varieties. In those trials where splitting was detected, varieties such as Chariot and Delibes showed values significantly above threshold levels (i.e. 3-4 % of split grain in a sample), whereas varieties such as Landlord and Derkado had low values. As well as variation between years in the amount of splitting, there may be high levels of variation between sites in any particular year. For example, Table 2 indicates mean scores for malting varieties across several sites in 1996.

Although there is very little scientific literature investigating the causes of grain splitting in barley, field observations in the UK suggest that some weather patterns may increase the risk of splitting and skinning. Two reports form Germany (Zimmerman, 1998; Muller and Schildbach, 1998) suggest that both husk and kernel (caryopsis) splitting present at high levels in the barley harvest were the result of repeated exposure to heavy rain followed immediately by hot dry weather. Furthermore, repeated periods of wetting were implicated in major occurrences of skinning in southern England in 1979

Table 1. Mean splitting scores (%) for malting varieties 1992-1998.

	Entry to RL	1992 (4 sites)	1993 (1 site)	1994 (3 sites)	1995 (3 sites)	1996 (5 sites)	1997 (4 sites)	1998 (4 sites)
Chariot	1992	19.2	9	11	3.3	10	6	4.5
Chalice	1998	*	*	*	*	*	2.5	2.25
Delibes	1994	*	*	12.7	*	10.8	6.25	13.5
Derkado	1992	4.7	4	1.3	2	2.2	2.0	*
Landlord	1997	*	*	*	*	0	0	0.25
Optic	1995	*	*	2.7	*	3.6	1.0	2.25
Prisma	1989	1.0	0	*	*	3.0	0.3	4.0
Tankard	1996	*	*	*	3.7	13.8	4.0	*

* no data collected

Table 2. Mean splitting scores (%) for malting varieties at 5 sites in 1996.

	Inverness	Udny, Aberdeen	Rennyhill, Fife	Hoprig, East Lothian	Spotsmains, Borders	Variety Mean
Chariot	12	27	8	2	1	10.0
Delibes	14	22	7	7	4	10.8
Derkado	3	5	1	1	2	2.2
Landlord	0	0	0	0	0	0.0
Optic	6	4	0	6	2	3.6
Prisma	2	7	2	2	2	3.0
Tankard	9	21	16	13	10	13.8
Site Mean	6.6	12.3	4.9	4.4	3.0	6.2

and 1997; both years had high June rainfall. Work in Japan by Tsuyuzaki and Tekeda (1989) reported that low light levels before anthesis followed by high light levels after anthesis caused an increase in the proportion of split grains.

There is little understanding of the anatomical and morphological features during grain development which determine gape and splitting, though there are clear differences between varieties in degree of gape and splitting (and of skinning). There may be a greater tendency for grains to split when there is little or no overlap of the lemma and palea and it is conceivable that excessive expansion during grain filling results in gape which in turn makes the grain more likely to split. Unfavourable conditions prior to anthesis could result in poor husk development. If this is followed by good grain fill then the palea and lemma may not overlap, resulting in gape.

Hamachi, Yoshino, Furusho and Yoshida in Japan (1990) showed that the growth and development the lemma and palea between flag leaf appearance to heading was strongly affected by environmental conditions. There appeared to be interactions between different factors and poor husk development was linked to shading or low temperature combined with excess soil moisture. Tsuyuzaki and Tekeda (1989) showed that leaf blade removal (i.e. reducing the source of assimilates) reduced splitting. Thus, it appears that splitting can be induced by environmental conditions and a crop's physiological condition.

Agronomic factors such as the timing of fungicide applications, plant growth regulators (PGRs) and the rate and timing of nitrogen fertiliser applications have also been implicated in increasing gape and splitting. High N and fungicides applications applied late in crop development were associated with splitting in trials in Berwickshire, Borders and Morayshire (unpublished data), however, no precise cause was identified. One hypothesis to test is that applications of nitrogen and fungicides (made to increase canopy size and or duration) may inadvertently create physiological changes, including excessive grain filling that lead to gape and splitting in vulnerable varieties such as Chariot.

The Materials & Methods and Results sections in this report are arranged into two parts:
(1) glasshouse/controlled environment studies on grain development as influenced by environmental factors and (2) field studies of the influence of agronomic factors and weather gape, splitting and skinning.

In the glasshouse/controlled environment studies, a series of four experiments (I, II, III and IV) was carried out to establish how environmental and developmental factors influenced gape and splitting. The varieties Landlord and Chariot were used as examples of varieties that have a history of very low and very high risks of splitting, respectively. Shade treatments (pre-anthesis) were used to test the hypothesis that husk condition, measured as dimension and weight, could be environmentally controlled and consequently influence gape and splitting. A range of shading and temperature treatments (post-anthesis) were used to test the hypothesis that gape and splitting were influenced by the rate at which biomass and volume accumulated in the developing grains, and the rate at which grains dehydrated during ripening.

Experiments I and II investigated husk and caryopsis growth and development, as influenced by post- and/or pre-anthesis shading. Experiment III examined light and warm and cool temperature effects on gape and splitting. Experiment IV compared gape and splitting in Landlord and Chariot with that in four inbred lines selected to have different susceptibilities to gape and splitting. The latter were selected on 1999 field scores from a population of random inbred lines derived from the Tankard *x* Livet cross by the Scottish Crop Research Institute (SCRI). Further details of the Tankard *x* Livet population are presented by Rajasekaran, Thomas, Wilson, Lawrence, Young and Ellis (2002) in Technical Paper 2, in this Report.

In the field studies, four experiments (A, B, C and D) examined the influence of the timing of fungicide applications and the rate of which nitrogen was supplied to the crop on the occurrence of gape, splitting and skinning in Landlord and Chariot. Two trials were carried out in 2000 (A and B) and two in 2001 (C and D). Monthly rainfall, sunshine hours and temperature were also recorded. Two further trials carried out in 2000 (E and F) examined the effects of excessive grain filling on splitting in Landlord and Chariot. A complementary study on the influence of agronomic treatments, environmental conditions and excessive grain filling on skinning was carried out at ADAS by Froment and South (Technical Paper 3, in this Report).

Materials and Methods – Glasshouse/Controlled Environment Studies (Experiments I, II, III, and IV)

Plant material

Ten seeds of varieties Chariot and Landlord were sown into a peat based rooting medium in 4.3 l pots. At growth stage (GS) leaf 3 (GS13) plants were thinned to 6 per pot. Pots were irrigated daily and supplied with N, P and K (at the ratio 5.2 : 5.2 : 6.0) twice weekly from leaf 4 (GS14) to anthesis (GS61). Plants were treated with Pirimor (50% w/w Pirimicarb) for aphid control.

Growing conditions

Plants were grown in a glasshouse in which day/night temperatures were maintained at a minimum of $15^{o}C/10^{o}C$ for an 18 h day (details of each experiment are given below). Natural daylight was supplemented with mercury vapour lamps so that the minimum photosynthetically active radiation (PAR) at ear level was 150 μmol m^{-2} s^{-1} for 18 h each day. Between late tillering (GS25) to anthesis plants were either shaded (to reduce PAR by 30% or 70%) or kept unshaded. Anthesis was determined by visual assessment of dissected flowers. After anthesis, plants were either: (1) maintained in the glasshouse with or without further shading treatments or (2) transferred to growth rooms in which temperature was maintained at either $13^{o}C$ or $18^{o}C$ and PAR was 160 μmol m^{-2} s^{-1} at ear level. Ears on main stems and tillers were tagged at anthesis. Table 3 provides a schedule and summary of treatments in Experiments I, II, III and IV. Further details of pre- and post-anthesis treatments are provided in the individual experimental sections below.

Measurements of grain growth and development (Experiments I and II only)

Ears (four) were sampled from each treatment during the second week after anthesis (GS75) and twice a week thereafter until harvest ripeness. The time course was measured as days after anthesis (daa). Five grains were removed from the middle of one side of each ear. The palea and lemma of each were removed and their length and width measured using a micrometer. Dry weight on the pooled paleas and lemmas from each ear was determined after drying at $70^{o}C$ for 48 h. Fresh and dry weight were measured on the pooled caryopses. Five additional grains were removed from the middle of the other side of each ear. The length and width of each caryopsis was measured after removing the palea and lemma. The caryopses from each ear were pooled and measured for volume by displacement of water (by weight) using a 5 cm^{-3} graduated flask. In

Experiment III, measurements of caryopsis fresh and dry weight and volume were made at maximum grain size i.e. GS77.

Light microscopy

Examination of husk and endosperm in Chariot as influenced by shade pre- or post-anthesis was made by light microscopy in grain sampled from Experiment II. Preparation details are given in Cochrane and Hoad (Technical Paper 4, in this Report).

Assessments of gape, splitting and skinning and Thousand Grain Weight (TGW) (Experiments I, II, III and IV)

At harvest-ripeness all ears were harvested, pooled and hand-threshed. Grains were sieved over a 2.5 mm mesh; those falling through were discarded. One hundred grains selected at random were counted onto a white background. Gape was scored as the percentage of grains with a gap of 0.5 mm or more between the palea and lemma in the middle third of the grain. Splitting was scored after staining grains with a solution of iodine (I) in potassium iodide (KI). Two g of KI was dissolved in 100 cm^3 water and 0.2 g of I was dissolved into the KI solution. One hundred grains were placed into a 50 cm^3 beaker and immersed in approximately 20 cm^3 of KI/I solution. The dish was shaken gently to ensure that all grains were thoroughly soaked by the solution. After 10 min the solution was poured off and the grains rinsed with water. Each grain was examined against a white background using magnification (x 6 to x 10). Splitting was scored as the percentage of grains stained black or blue-black. Each assessment used either 3 or 6 replicates of 100 grains. Skinning was scored as the percentage of grains with more than 25% of the entire husk missing. Thousand grain weight was determined from the 100-grain samples retained over a 2.5 mm sieve. Grains were dried at 80^{o}C for 24 h, weighed and TGW was recalculated to 15 % moisture content.

Statistical analysis

In Experiments I and II, measurements of grain growth are shown as mean values with standard error at days after anthesis. In each experiment, data for gape, splitting and TGW were analysed by two or three factor analysis of variance with standard errors for the difference between means (SEDs) for two or three means using Genstat Release 4.22.

Table 3. Timetable of glasshouse/controlled environment experiments I, II, III, and IV.

	Experiment I	Experiment II	Experiment III	Experiment IV
Sown	14/02/99	04/06/99	17/04/00	12/01/01
Pre-anthesis treatments	Shade imposed on half of plants 26/03/99 (GS25-30)	Shade imposed on half of plants 30/06/99 (GS25-31)	Shade imposed on half of plants 22/05/00 (GS25-31)	Shade imposed on all plants 05/03/01 (GS25-31)
Anthesis	20 to 30/04/99	16 to 26/07/99	17 to 26/06/00	26/03/01 to 03/05/01
Post-anthesis treatments	Shade removed 02/05/99 (GS71)	Either, shade and unshaded treatments reversed 30/07/99 (GS71) or, shade removed and cool grain- filling temperature imposed 30/07/99 (GS71)	Shade removed 20/06/00 (GS59-71) and, warm or cool grain-filling temperature imposed 30/06/00 (GS69-73)	Shade removed 06/04/01 (GS59-71) and, warm or cool grain-filling temperature imposed 12/04/01 or 24/04/01 (GS59-73)
Harvested	01/07/99	08/09/99 or 25/10/99	15/08/00 or 04/10/00	11/07/01 or 28/08/01

Experiment I. Grain development, gape and splitting as influenced by shading during husk development

The aim of Experiment I was to examine how changes in husk and caryopsis development, as influenced by shading before anthesis, affected the incidence of gape and splitting at harvest. The two experimental factors examined were variety susceptibility to splitting (Landlord = low risk and Chariot = high risk) and shade or no shade before anthesis. The latter was used as a means to induce poor or adequate husk development (shade = poor husk and no shade = adequate husk).

Sixty pots of Chariot and Landlord were divided into two equal groups and arranged in alternate rows of five pots Landlord and 5 pots Chariot along both sides of an east-west aligned glasshouse. At the end of tillering (GS25), all plants on the north-facing side were shaded to reduce ambient light by 30%. At early stem extension (GS31), the shading was increased to reduce ambient light by 70%. Plants on the south-facing side were left unshaded. After anthesis (end of anthesis to early milk development (GS69-71) shading was removed. Grain development was measured as described above. After harvest, gape, splitting and TGW were measured at harvest ripeness on 6 replicate samples of 100 grains from bulked samples of ears from main stems and main tillers.

Experiment II. Grain development, gape and splitting as influenced by shading during husk development or grain filling/maturation

The design of Experiment II was similar to that in Experiment I. The main aim was to examine the effects of pre- or post-anthesis shading on husk and caryopsis development, and gape/splitting in the varieties Landlord and Chariot. This procedure was designed to exaggerate the shading effects observed in Experiment I by (i) providing poor conditions for husk development before anthesis and promoting gape and splitting by high light and good grain filling after anthesis and (ii) providing good conditions for husk development before anthesis and reducing the risk of gape or splitting by avoiding excessive grain fill after anthesis.

Sixty pots of Chariot and Landlord were divided into two equal groups and arranged in alternate rows of five pots of Landlord and 5 pots of Chariot along both sides of an east-west aligned facing glasshouse. At late tillering (GS25), all plants on the north-facing side were shaded to reduce ambient light by 70%. After anthesis (GS60-71), half of the plants were retained in the glassshouse and the shade/unshaded treatments were reversed so that the previously shaded plants were left unshaded (denoted as S/US) whilst the previously unshaded plants were moved into the shade (denoted as US/S). These plants were used for measurements of grain growth/development, gape, splitting and TGW as described in Experiment I. The remaining plants were placed in a growth room at a constant temperature of 13^{o}C. This additional treatment was designed to simulate a longer, cooler, grain filling/maturation period after shade or no shade pre-anthesis. These plants were used for measurements of gape, splitting and TGW only.

After harvest, gape, splitting and TGW were measured on 6 replicate samples of 100 grains from bulked samples of ears from main stems and main tillers. An examination of husk and endosperm development by light microscopy was made in grains of Chariot sampled at 24 and 45 days after anthesis (daa) from plants under the glasshouse treatments S/US and US/S.

Experiment III. Gape and splitting as influenced by: shade during husk development, warm or cool temperatures during grain filling and maturation, and by anthesis date

The aim of Experiment III was to examine how temperature during grain filling and maturation influenced gape and splitting in grains of plants that had been previously exposed to either shade or no shade before anthesis (as in Experiments I and II). The additional factor of anthesis date was examined to establish if there were differences in gape and splitting between a population of predominantly main stem ears that had anthesed relatively early and a population of tiller ears that had anthesed relatively late.

Sixty pots of Chariot and Landlord were divided into two equal groups and arranged in alternate rows of five pots Landlord and 5 pots Chariot along both sides of an east-west aligned glasshouse. At late tillering (GS25), all plants on the north-facing side were shaded to reduce ambient light by 70%. After anthesis (GS69-71), half of the pots (i.e. 15 from each pre-anthesis treatment) were transferred to a growth room to simulate a long, cool, (13^{o}C) grain filling period, whilst the remainder were kept unshaded in the glasshouse to provide a shorter grain filling period with higher light and temperature. The change over was carried out over a three-day period according to anthesis date. Caryopsis fresh and dry weight and volume were measured at maximum grain volume i.e. late milk stage (GS77). At harvest, ears were divided into two groups, those that had anthesed in days 1 to 4 (early anthesis) and those that had anthesed 7 to 10 (late anthesis) after the first ear had anthesed. Gape and splitting were assessed on 3 replicate samples of 100 grains from bulked samples of ears from main stems and main tillers.

Experiment IV. Gape and splitting in genotypes (varieties and selected lines) as influenced by warm or cool temperatures during grain filling and maturation, and by anthesis date

The aim of Experiment IV was to examine how temperature during grain filling and maturation influenced gape and splitting in grains of varieties (Landlord and Chariot) and four selected lines. The selected lines (provided by SCRI) were from a cross between Tankard *x* Livet. The lines B96-76/24 and B96-76/179, had previously been identified as having a low splitting risk under 1999 field conditions whereas B96-76/96 and B96-76/193 had a higher risk of splitting. Twenty pots of the two varieties, Landlord and Chariot, and the four lines were divided into two equal groups and arranged in alternate rows of five pots of each genotype along both sides of an east-west aligned glasshouse. At late tillering (GS25), all plants were shaded to reduce ambient light by 70%.

After heading, plants were transferred to growth rooms set at a temperature of either 13^{o}C or 18^{o}C. There was a wide range of anthesis dates, both between and within genotypes so plants within each genotype were put into two groups (early or late anthesis) according to anthesis date. Transfer to the growth rooms was carried out on 2 dates (12 or 24 April) (Table 4). The variation was such that in some ears anthesis took place in the glasshouse whilst in other ears anthesis took place in the growth rooms. Gape and splitting were assessed on 3 replicate samples of 100 grains from bulked samples of ears from main stems and main tillers.

Table 4. Experiment IV: range of anthesis dates for genotypes (varieties and selected lines) in each grain filling/maturation temperature. Plants were grouped into early or late anthesis according into the range of anthesis dates.

	Grain filling/maturation temperature 13°C		Grain filling/maturation temperature 18°C	
	Early anthesis	Late anthesis	Early anthesis	Late anthesis
Landlord	05/04 to 07/04	26/04 to 27/04	03/04 to 10/04	28/04 to 30/04
Chariot	07/04 to 19/04	26/04 only	16/04 to 19/04	27/04 to 29/04
B96-76/24	29/03 to 02/04	26/04 to 03/05	01/04 to 04/04	27/04 to 30/04
B96-76/179	29/03 to 06/04	26/04 to 30/04	09/04 to 11/04	25/04 to 03/05
B96-76/96	31/03 to 03/04	17/04 to 19/04	29/03 to 01/04	20/04 to 24/04
B96-76/193	26/03 to 04/04	16/04 to 19/04	26/03 to 31/03	17/04 to 21/04

Materials and Methods – Field Studies (Experiments A, B, C, D, E and F)

Agronomy trials using variety, rate of nitrogen fertiliser application and number of fungicide applications as main factors were carried in both 2000 and 2001 (Experiments A, B, C and D). In 2000, two additional studies were carried out using ear (sink) manipulation and nitrogen fertiliser rate as main factors (Experiments E and F).

General agronomy

The varieties Landlord and Chariot were sown at SAC trials locations in Midlothian, East Lothian and Berwickshire. The seed rate was 360 seeds m^{-2} and the plot size was 2 x 18 m. Treatments are indicated in the description of each experiment below.

Harvesting and grain samples

In Experiments A to D, each plot was harvested using a plot combine and grain yield was calculated at 15% moisture content. A 150 g sample of grain was taken during combining and used for assessments of gape, splitting, skinning and TGW. Leaf canopy duration was assessed by a visual percentage score of total green leaf area (GLA) at GS73. In Experiments E and F, ears were harvested from the treated areas (described below) and hand-threshed for assessment of splitting and TGW.

Assessments of gape, splitting, skinning and TGW

Gape, splitting and skinning were measured as in the glasshouse/controlled environment experiments described above.

Statistical analysis

Data for gape, skinning, splitting, TGW and % GLA were analysed by three factor analysis of variance with 2 or 3 replications using Genstat Release 4.22. That is, variety *x* nitrogen *x* fungicide treatments in Experiments A, B, C and D and variety *x* nitrogen *x* ear manipulation in Experiments E and F. SEDs were calculated for comparing any two treatment means.

Experiments A and B. Gape, splitting and skinning in Landlord and Chariot at harvest 2000 as influenced by the rate of N fertiliser application and the number of fungicide applications at two sites (Boghall Farm, Midlothian & Hutton Hall Barns, Berwickshire)

The varieties Landlord and Chariot were sown at Boghall Farm, Midlothian (Experiment A) and Hutton Hall Barns, Berwickshire (Experiment B). Site details are indicated in Tables 5 and 6. In both experiments, each variety, was grown at 2 levels of N fertiliser and two levels of fungicide giving the following treatments:

1. Control: 120 kg N ha^{-1} and a 2-spray fungicide programme
2. Extra fungicide: 120 kg N ha^{-1} and a 3-spray fungicide programme
3. Extra N: 170 kg N ha^{-1} and a 2-spray fungicide programme
4. Extra N and fungicide: 170 kg N ha^{-1} and a 3-spray fungicide programme

Full details of treatments are given in Tables 5 and 6. Treatments were applied to each plot by hand. Routine applications of herbicide and manganese were applied to all plots as required.

Table 5. Experiment A: Site details and treatments applied to varieties Landlord and Chariot at Boghall Farm, Midlothian, in 2000.

Grid reference:	NT 241 656
Soil type:	Loam
Previous crop:	Spring barley

Nitrogen	Level 1	Level 2
Seedbed	60 kg ha^{-1}	60 kg ha^{-1}
GS12	60 kg ha^{-1}	110 kg ha^{-1}

Fungicides	Level 1	Level 2
GS30	Amistar Pro 2 litre ha^{-1} + Unix 0.67 kg ha^{-1}	Amistar Pro 2 litre ha^{-1} + Unix 0.67 kg ha^{-1}
GS45-49	Amistar Pro 2 litre ha^{-1}	Amistar Pro 2 litre ha^{-1}
GS59		Atlas Cropguard 2 litre ha^{-1} + Opus 0.5 litre ha^{-1}

Table 6. Experiment B: Site details and treatments applied to varieties Landlord and Chariot at Hutton Hall Barns, Chirnside, Berwickshire, in 2000.

Grid reference:	NT 888 544
Soil type:	Sandy loam
Previous crop:	Winter wheat

Nitrogen	Level 1	Level 2
Seedbed	60 kg ha^{-1}	60 kg ha^{-1}
GS12	65 kg ha^{-1}	115 kg ha^{-1}

Fungicides	Level 1	Level 2
GS30-31	Amistar Pro 2 litre ha^{-1} + Unix 0.67 kg ha^{-1}	Amistar Pro 2 litre ha^{-1} + Unix 0.67 kg ha^{-1}
GS45-50	Amistar Pro 2 litre ha^{-1}	Amistar Pro 2 litre ha^{-1}
GS59-60		Atlas Cropguard 2 litre ha^{-1} + Opus 0.5 litre ha^{-1}

Experiments C and D. Gape, splitting and skinning in different varieties as influenced by the rate of N fertiliser application and the number of fungicide applications at Boghall Farm, Midlothian and Seton West Mains, East Lothian, at harvest 2001

Varieties Landlord and Chariot were sown at Boghall Farm, Midlothian (Experiment C) and varieties Landlord, Chariot, Chalice and Optic were sown at Seton West Mains, East Lothian (Experiment D). Site details are indicated in Tables 7 and 8.

In Experiment C, each variety was grown at 2 levels of N fertiliser and three levels of fungicide giving the following treatments:

1. No fungicide: 110 kg N ha^{-1} and no fungicide applications
2. Control: 110 kg N ha^{-1} and a 2-spray fungicide programme
3. Extra fungicide: 110 kg N ha^{-1} and a 3-spray fungicide programme
4. Extra N, no fungicide: 160 kg N ha^{-1} and no fungicide applications
5. Extra N, normal fungicide: 160 kg N ha^{-1} and a 2-spray fungicide programme
6. Extra N and fungicide: 160 kg N ha^{-1} and a 3-spray fungicide programme

In Experiment D, each variety was grown at two levels of nitrogen and two levels of fungicide giving the following treatments:

1. Control: 120 kg N ha^{-1} and a 2-spray fungicide programme
2. Extra fungicide: 120 kg N ha^{-1} and a 3-spray fungicide programme
3. Extra N: 170 kg N ha^{-1} and a 2-spray fungicide programme
4. Extra N and fungicide: 170 kg N ha^{-1} and a 3-spray fungicide programme

Full details of N treatments and fungicide programmes are given in Tables 7 and 8. Treatments were applied to each plot by hand. Routine applications of herbicide and manganese were applied to all plots as required.

Table 7. Experiment C: Site details and treatments applied to the varieties Landlord and Chariot at Boghall Farm, Midlothian, in 2001.

Grid reference:	NT 246 648
Soil type:	Alluvial fan
Previous crop:	Winter oilseed rape

Nitrogen	Level 1	Level 2
Seedbed	60 kg ha^{-1}	60 kg ha^{-1}
GS12	60 kg ha^{-1}	110 kg ha^{-1}

Fungicides	Level 1	Level 2	Level 3
GS30-31	None	Amistar Pro 2 litre ha^{-1} + Unix 0.67 kg ha^{-1}	Amistar Pro 2 litre ha^{-1} + Unix 0.67 kg ha^{-1}
GS37-39	None	Amistar Pro 2 litre ha^{-1}	Amistar Pro 2 litre ha^{-1}
GS59	None	None	Atlas Cropguard 2 litre ha^{-1} + Opus 0.5 litre ha^{-1}

Table 8. Experiment D: Site details and treatments applied to the varieties Landlord, Chariot, Optic and Chalice at Seton West Mains, East Lothian, in 2001.

Grid reference:	NT 409 746
Soil type:	Sandy loam
Previous crop:	Spring barley

Nitrogen	Level 1	Level 2
Seedbed	60 kg ha^{-1}	60 kg ha^{-1}
GS12	65 kg ha^{-1}	115 kg ha^{-1}

Fungicides	Level 1	Level 2
GS30-31	Amistar Pro 2 litre ha^{-1} + Unix 0.67 kg ha^{-1}	Amistar Pro 2 litre ha^{-1} + Unix 0.67 kg ha^{-1}
GS37-39	Amistar Pro 2 litre ha^{-1}	Amistar Pro 2 litre ha^{-1}
GS59	None	Atlas Cropguard 2 litre ha^{-1} + Opus 0.5 litre ha^{-1}

Experiments E and F. TGW and the incidence of splitting as influenced by the rate of N fertiliser application and treatments affecting ear (sink) size in Landlord and Chariot at Boghall Farm, Midlothian and Hutton Hall Barns, Berwickshire, at harvest 2000

Additional plots of Landlord and Chariot were sown adjacent to Experiments A and B. Each variety, was grown at two levels of N fertiliser with a single fungicide programme (as in level I in Table 6) and replicated twice. Sink size was modified using three ear-manipulation treatments in each plot at anthesis (GS69). The following treatments were set up in 1 m diameter circles within each plot and marked by a central cane: (1) no ear manipulation (control), (2) top half of each ear cut off to reduce the sink size of each ear by 50% and (3) half of ear population removed to reduce ear number by 50%. Details of nitrogen and ear treatments are given in Table 9. Other routine applications of herbicide and manganese were applied to all plots as required.

Table 9. Experiments E and F: Treatments applied to varieties Landlord and Chariot at Boghall Farm, Midlothian, and Seton West Mains, East Lothian, in 2001. Site details are the same as shown in Tables 7 and 8.

Nitrogen	Level 1	Level 2
Seedbed	60 kg ha^{-1}	60 kg ha^{-1}
GS12	60 kg ha^{-1}	110 kg ha^{-1}

Ear treatment	Level 1	Level 2	Level 3
GS69	**None (control)**	Top half of each ear removed by cutting the rachis across central spikelets	Ear population reduced to 50% by cutting at base of collar

Results – Glasshouse/Controlled Environment Studies (Experiments I, II, III, and IV)

Experiment I. Grain development, gape and splitting as influenced by shading during husk development

In this experiment, a comparison between shading and no shading before anthesis was made in the varieties Landlord and Chariot.

Lemma and palea (husk)

In all treatments lemma width increased after anthesis (Fig. 1) whilst lemma length decreased slightly, though generally this was not significant (Fig. 2). Initially, the lemma of Chariot was significantly wider than that of Landlord at 4 days after anthesis (4 daa). Thereafter, there was no significant difference between varieties or the shade treatments. There were no significant differences in lemma length between varieties or shade treatments. Generally, palea width (Fig. 3) increased after anthesis but not significantly so and there were no significant differences between varieties or shade treatments. Initially, palea length of Chariot was longer than that of Landlord, but not significantly so (Fig. 4). There was no significant change in lemma dry weight between 4 and 25 daa (Fig. 5). Lemma dry weight was significantly lower in shaded Landlord than in other treatments. Palea dry weight increased significantly between 4 to 11 daa (Fig. 6). Thereafter, there were no significant differences between treatments.

Caryopsis

In all treatments, the width of the caryopsis was at its maximum value of between 4.5-4.7 mm at approximately 25 daa (Fig. 7). By contrast, caryopsis length was at its maximum between 11 to 17 daa (Fig. 8.). Generally, caryopsis width and length had decreased to approximately 80% of their maximum values at 45 to 60 daa. The widest and longest caryopsis was in shaded Chariot, but generally the differences between this and other treatments were not significant.

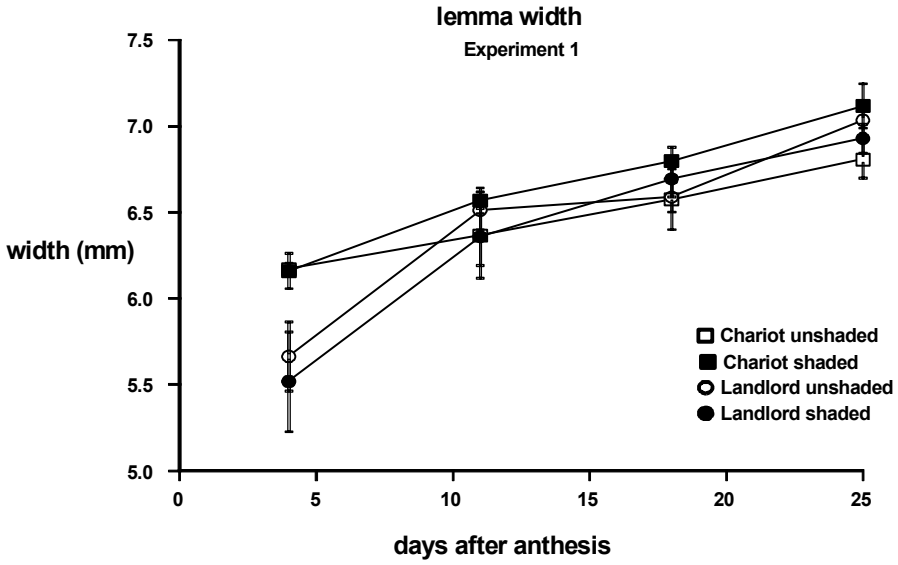
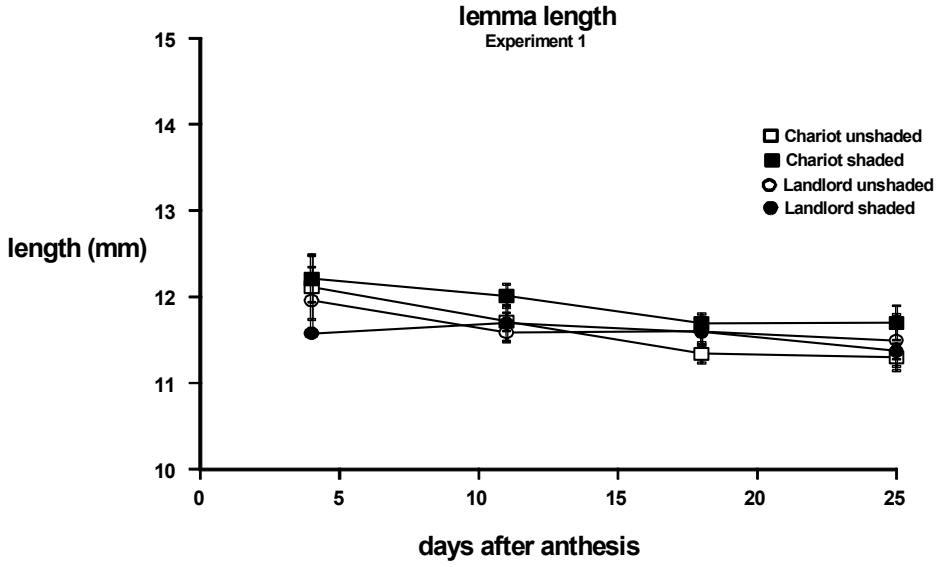


Figure 1



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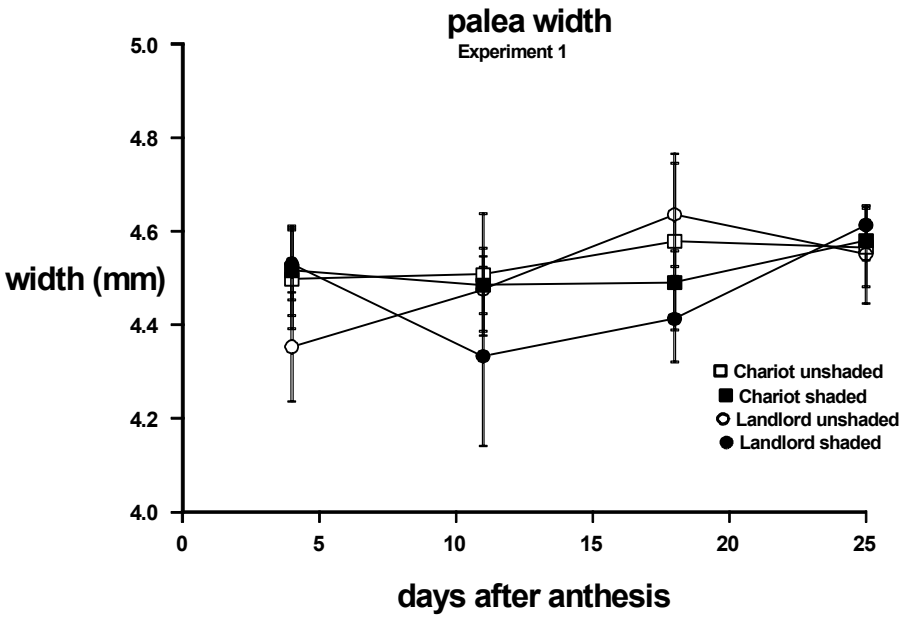

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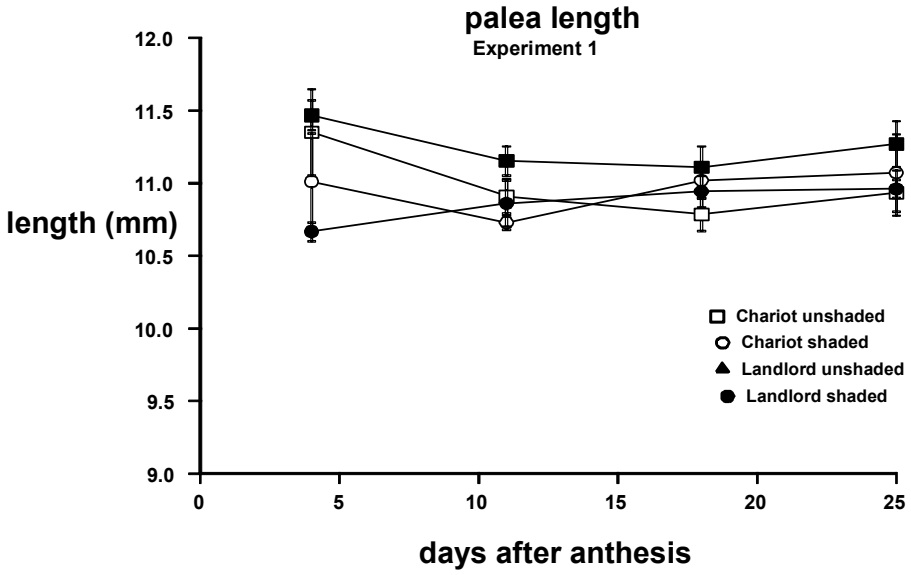

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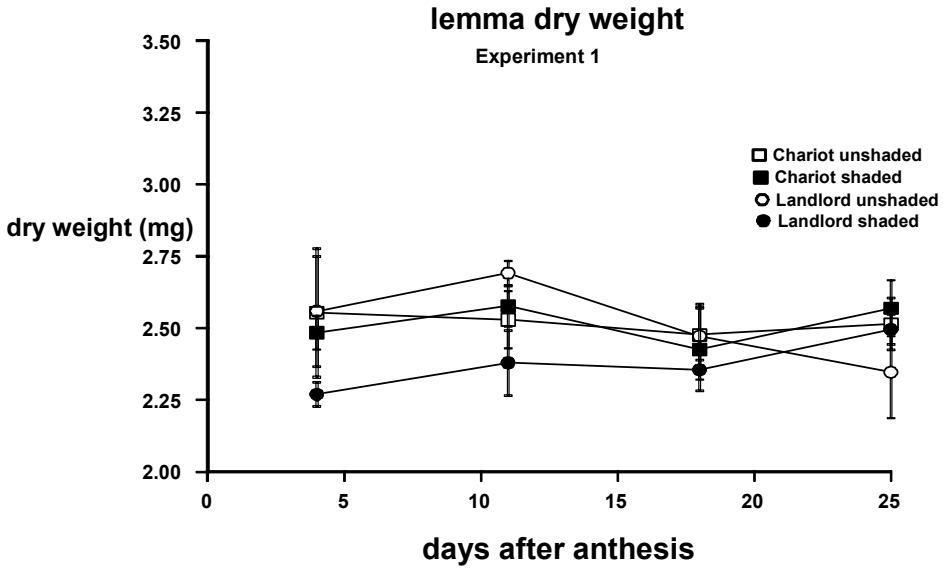
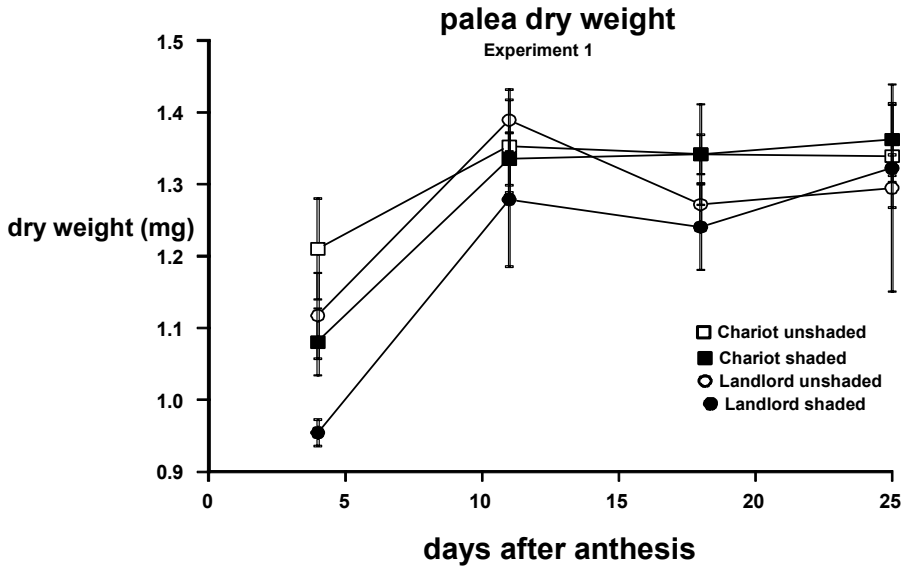


Figure 5



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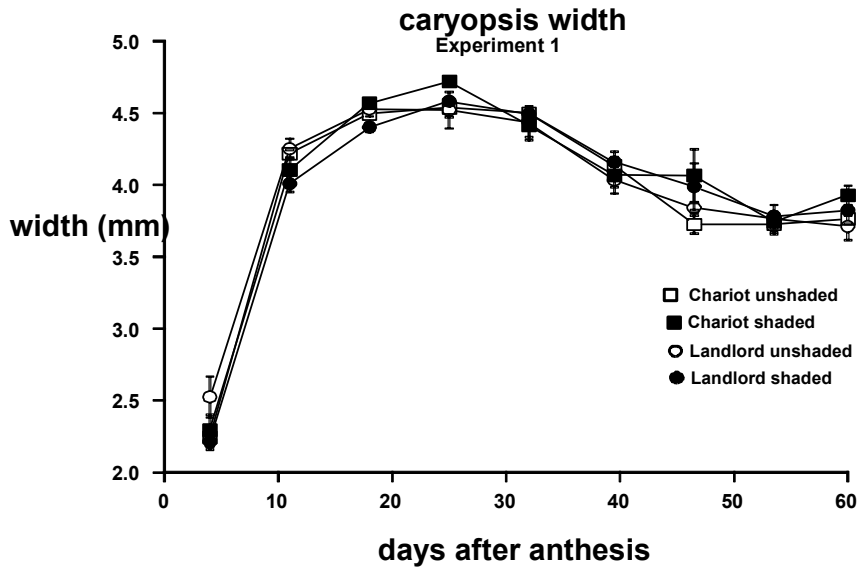


Figure 7

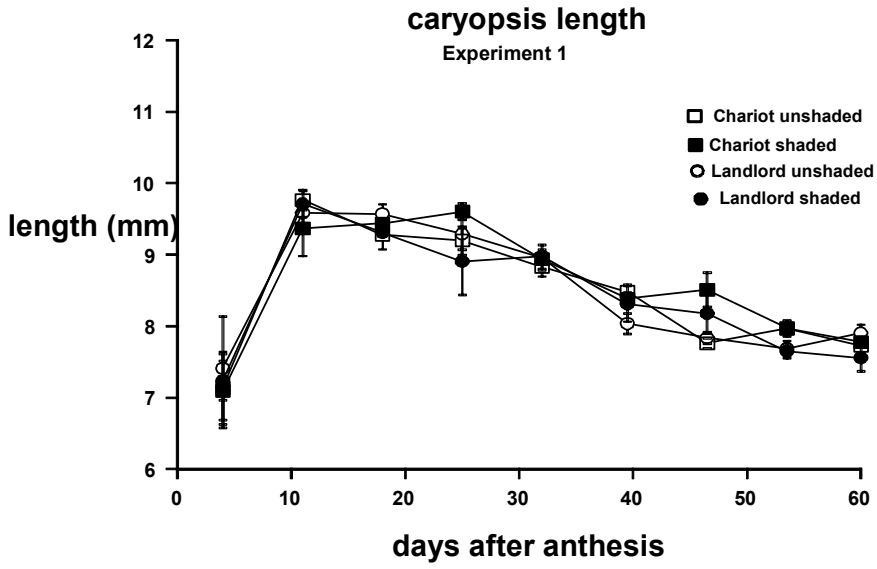


Figure 8

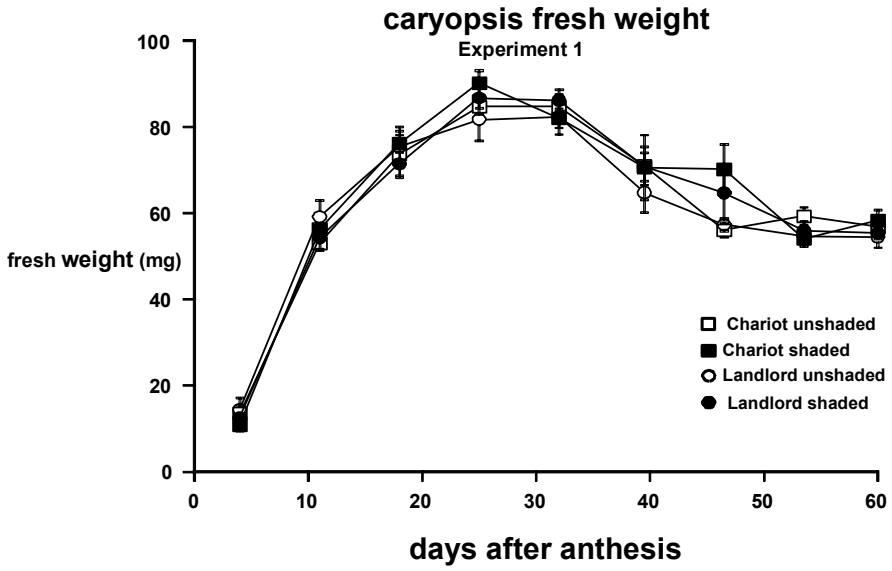


Figure 9

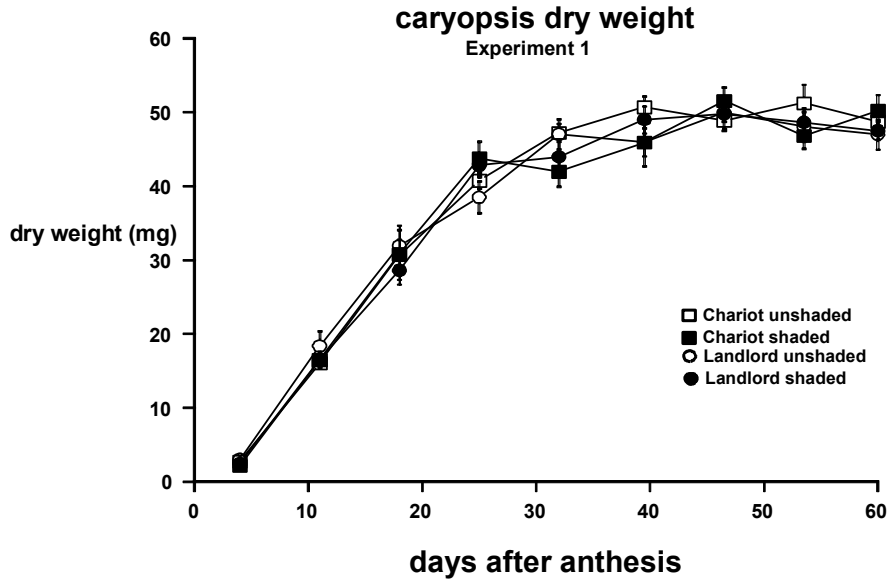


Figure 10

In all treatments, the fresh weights of caryopses were at their maximum (80 to 90 mg) at between 25 to 35 daa (Fig. 9). Caryopsis dry weight was between 48-50 mg by 45 daa (Fig. 10). There were no significant differences in fresh weight or dry weight between treatments. Caryopsis volume increased to its maximum value between 25 to 31 daa (Fig. 11). Chariot that had been shaded before anthesis had a significantly larger caryopsis volume than other treatments. The rate at which caryopsis volume declined between 35 to 60 daa was slowest in Chariot and Landlord that had been shaded before anthesis. Caryopsis water content was highest in Chariot that had been shaded before anthesis (Fig. 12). The rate at which water content and % moisture content decreased during grain maturation was slower in plants that had been shaded before anthesis, especially in Chariot (Figs. 12, 13).

Relationships between caryopsis and husk dimensions

Before 5 daa, the lemma width to caryopsis width ratio was higher in Landlord that had been shaded before anthesis than in other treatments (Fig. 14). Before 5 daa Landlord that had not been shaded before anthesis had the lowest values for the palea width to caryopsis width ratio (Fig. 15) and for the combined lemma + palea width to 2x caryopsis width ratio (Fig. 16). Thereafter, there were no differences in husk and caryopsis dimensions between treatments. Before 5 daa the caryopsis volume to dry weight ratio was significantly higher in plants that had been shaded before anthesis than in those that had not been shaded (Fig. 17). Thereafter, the difference between shaded and unshaded plants remained, but the differences were not significant.

Gape, splitting and TGW

There were significantly higher levels of splitting in Chariot than in Landlord (Table 10). Shading plants before anthesis had significantly increased splitting compared to unshaded plants in Chariot, but not in Landlord. Gape was higher in Chariot than in Landlord, but was not significantly affected by shading. In both varieties, TGW was significantly higher in plants that had not been shaded before anthesis than in plants that had been shaded.

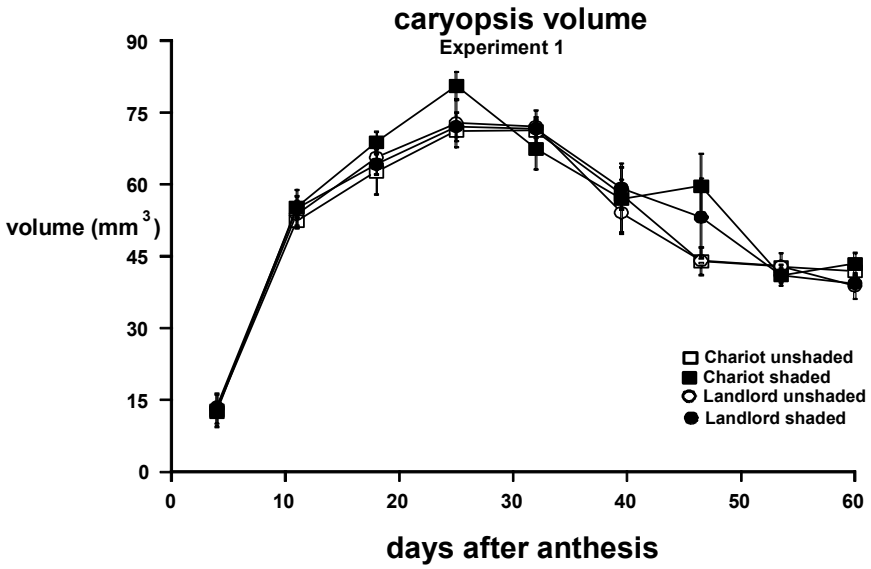


Figure 11

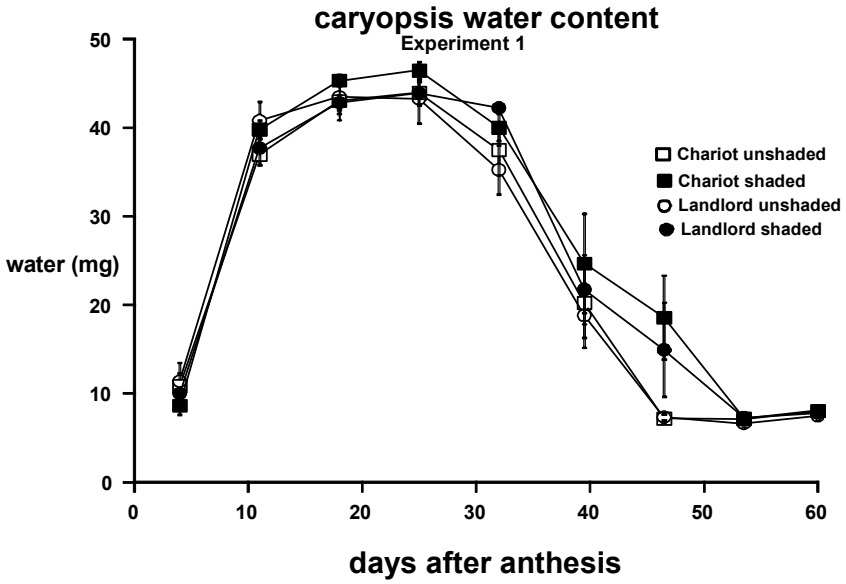


Figure 12

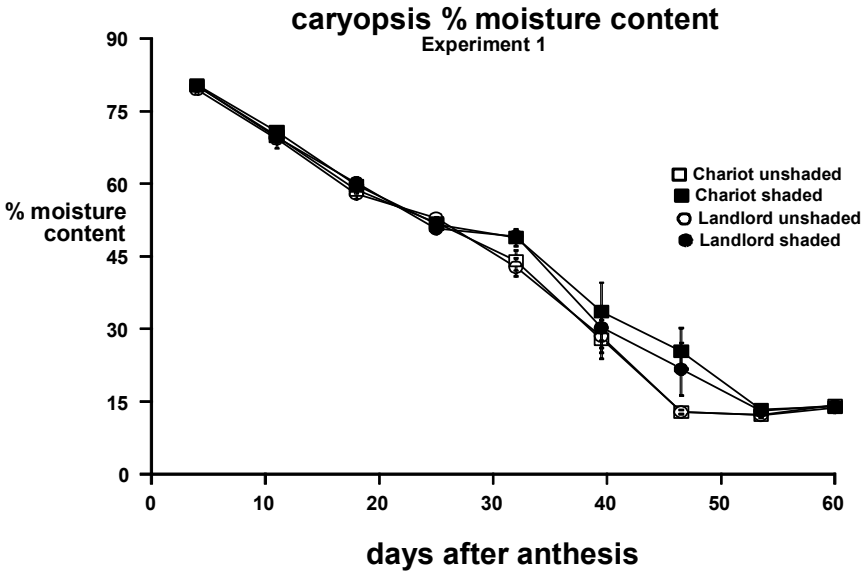


Figure 13

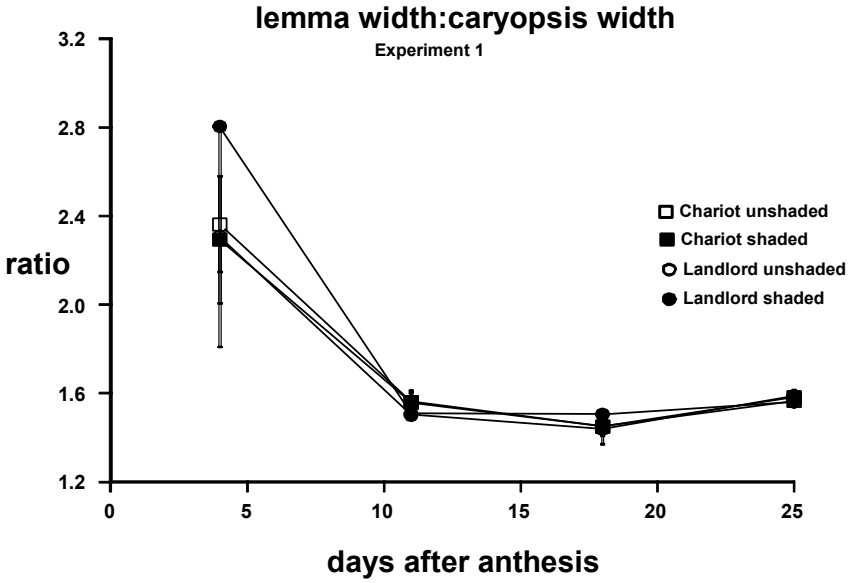


Figure 14

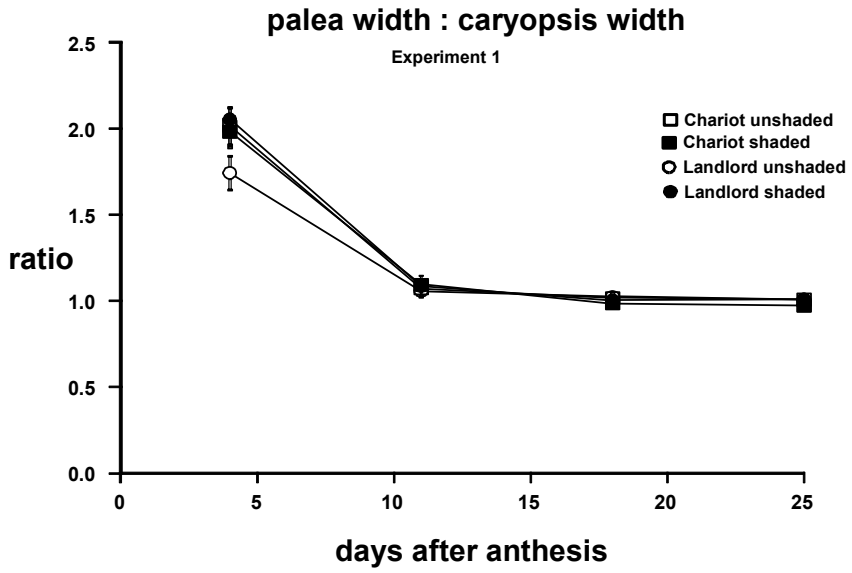


Figure 15

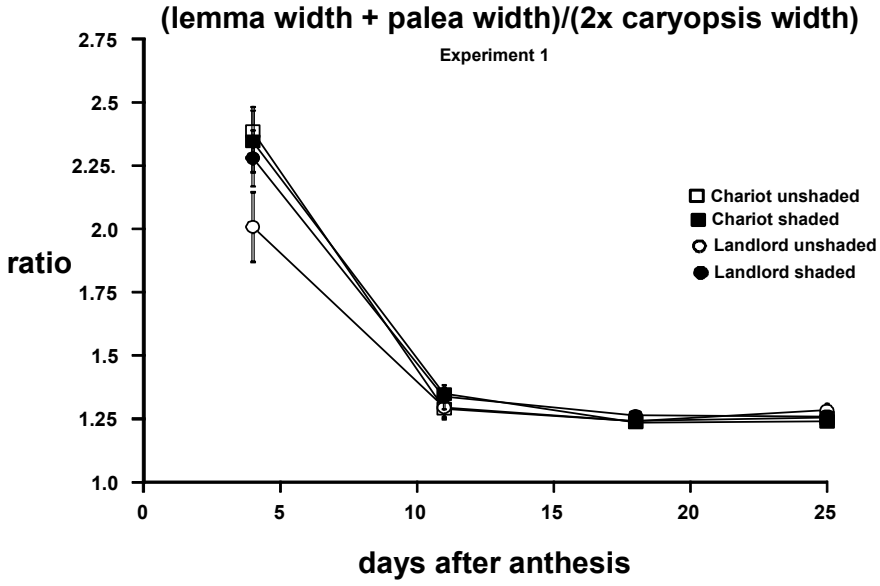


Figure 16

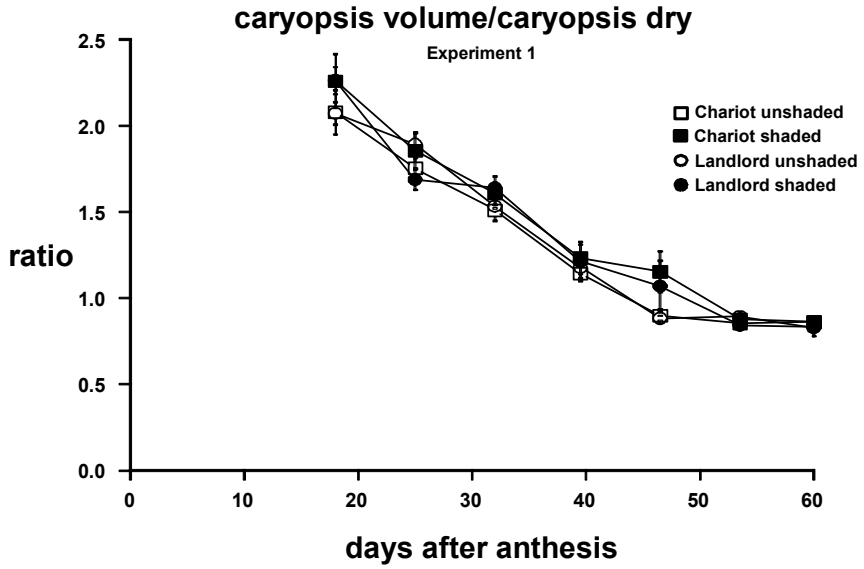

caryopsis volume/caryopsis dry
Experiment 1
Chariot unshaded
Chariot shaded
Landlord unshaded
Landlord shaded
ratio
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
days after anthesis

Figure 17

Table 10. Experiment I: Effect of pre-anthesis shading on TGW and the incidence of gape and splitting in Landlord and Chariot. Measurements were made on bulked samples ears from main stems and main tillers.

	Landlord			Chariot		
Pre-anthesis Treatment	Splitting (%)	Gape (%)	TGW (g)	Splitting (%)	Gape (%)	TGW (g)
No shade	0.8	13.8	54.3	2.7	27.0	54.0
Shaded	1.2	7.0	51.8	6.8	31.3	51.5

SED for comparing % splitting in any variety *x* shade combination = 1.19

SED for comparing % gape in any variety *x* shade combination = 9.97

SED for comparing TGW in any variety *x* shade combination = 0.89

Temperature and sunshine hours in the glasshouse

Approximately 1850 day ^{o}C had been accumulated between sowing and harvest. Anthesis occurred between 20 to 30 April and there were 930 to 1050 day ^{o}C from anthesis to grain harvest-ripeness. The sunshine hours were estimates from a local meteorological station.

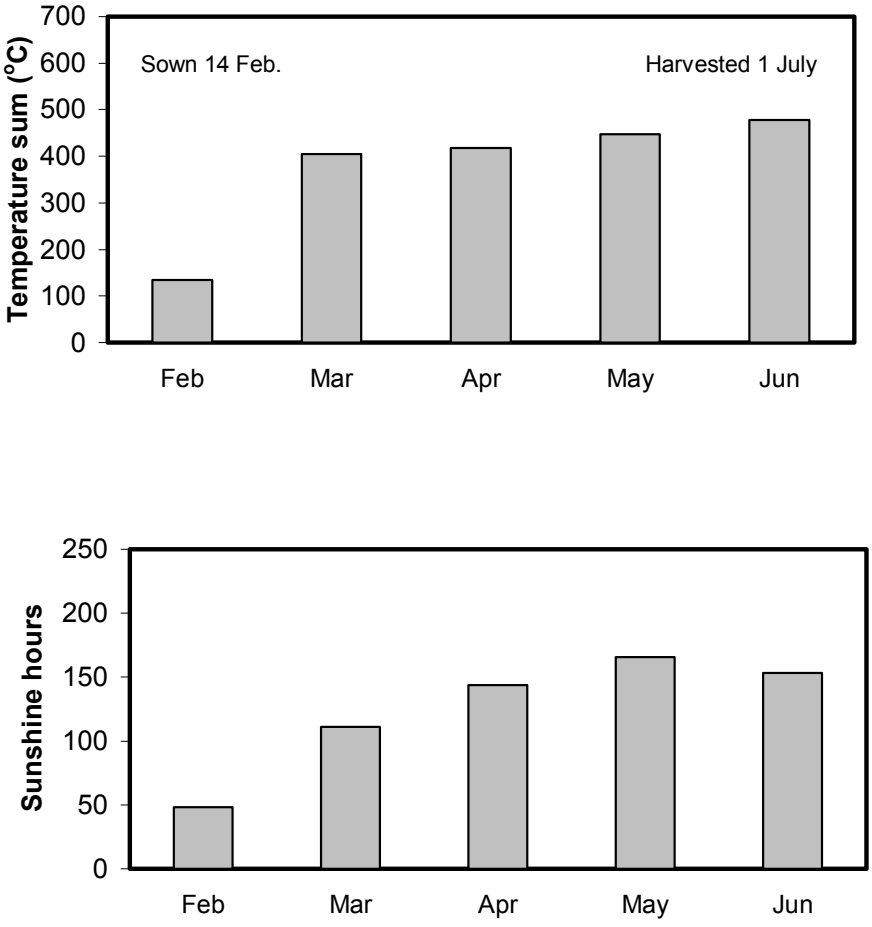


Figure 18. Experiment I: Monthly temperature sums and sunshine hours in the glasshouse.

Experiment II. Grain development, gape and splitting as influenced by shading during husk or grain filling/maturation

In this experiment, plants of the varieties Landlord and Chariot were shaded either only before anthesis or only after anthesis. Otherwise, the plants were maintained under ambient light levels. The shaded to unshaded and unshaded to shaded treatments are denoted by S/US and US/S, respectively.

Lemma and palea

In both varieties, lemma width and length, palea width and length and lemma and palea dry weight were significantly lower in S/US plants than in US/S plants (Figs. 19, 20, 21, 22, 23, 24). Landlord from US/S had the widest, longest and largest (by weight) lemma and palea, whereas Chariot from S/US had the narrowest, shortest and smallest (by weight) lemma and palea.

Caryopsis

In all treatments, the width of the caryopsis was at its maximum value of between 3.9-4.3 mm at approximately 19-26 daa (Fig. 25). By contrast, caryopsis length was at its maximum between 10 to 14 daa (Fig. 26). Generally, caryopsis width and length had decreased to approximately 80-85% of their maximum values at 40 to 45 daa

The caryopsis was significantly narrower in S/US Chariot than in other treatments. In both Landlord and Chariot, caryopsis length was significantly greater in US/S plants than in S/US plants.

In all treatments, the fresh weights of caryopses were at their maximum (65 to 72 mg) at approximately 26 daa (Fig. 27). Caryopsis dry weight was between 35 to 40 mg by 40 daa (Fig. 28). Caryopsis fresh weight tended to accumulate more rapidly and later decrease more slowly in US/S plants that in S/US plants. The accumulation of caryopsis dry weight was more rapid in those S/US plants than in US/S plants. Thus, maximum dry weight was achieved earliest in those plants that been shaded before anthesis and unshaded after anthesis.

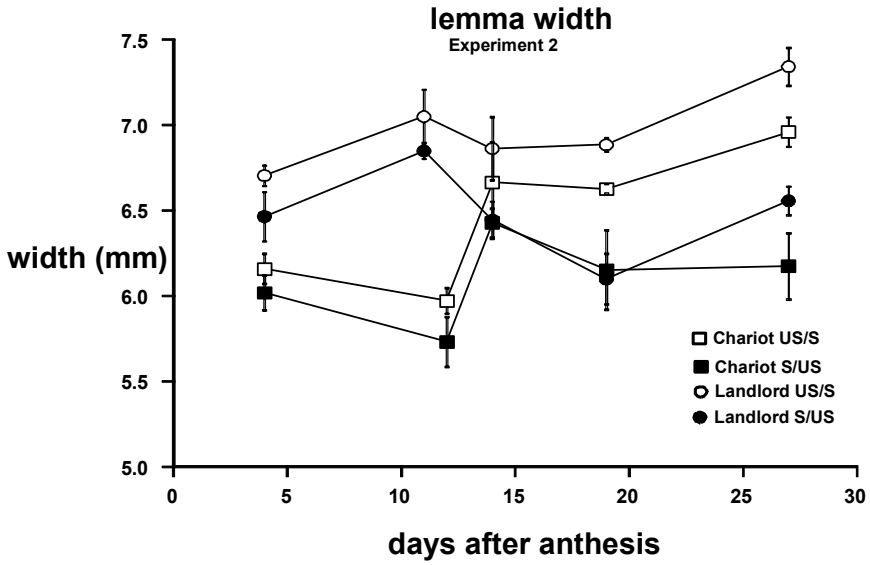


Figure 19

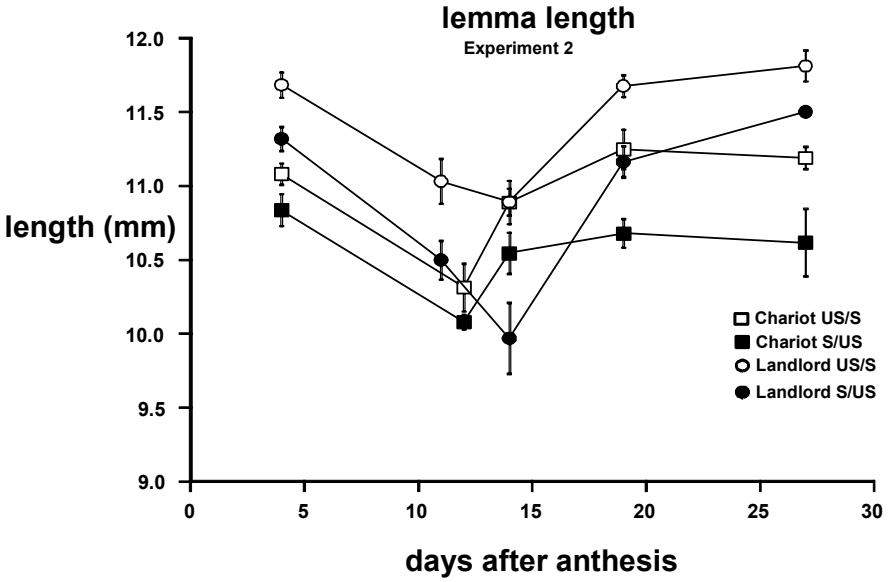


Figure 20

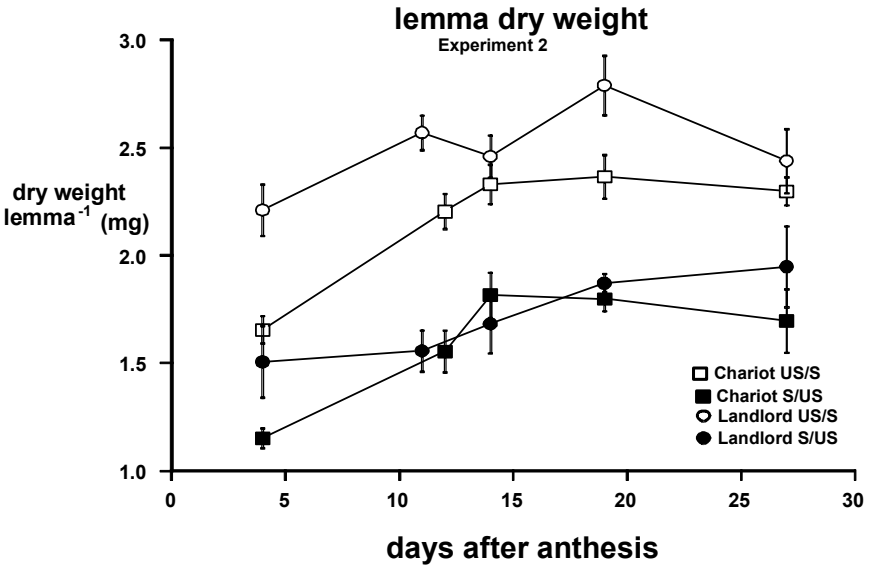


Figure 21

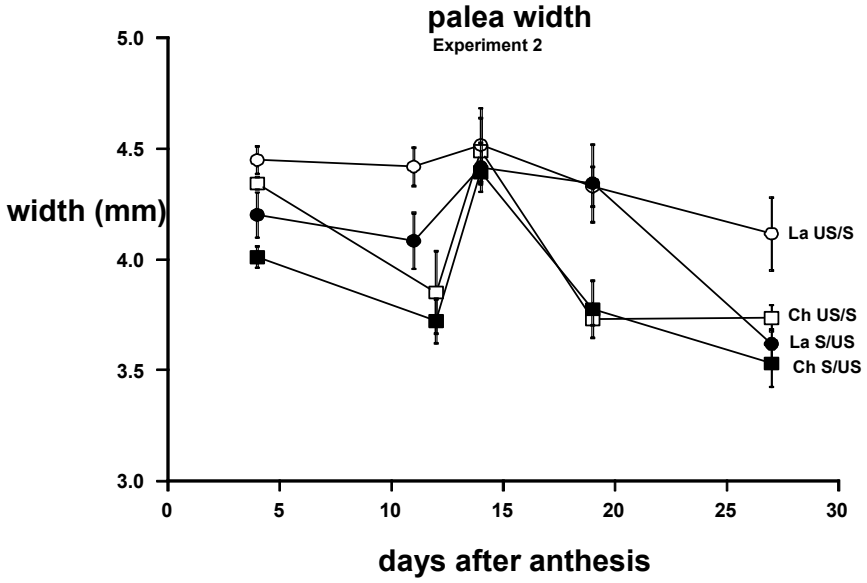


Figure 22

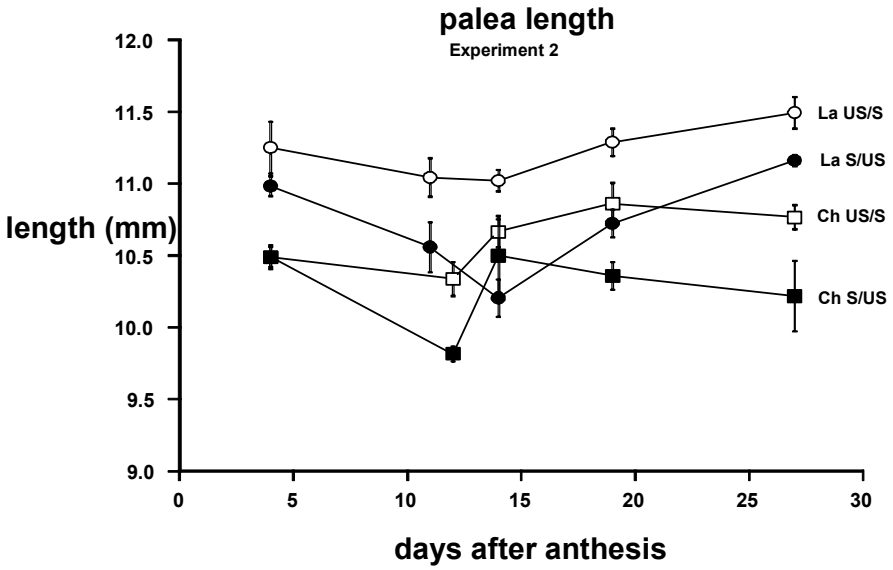


Figure 23

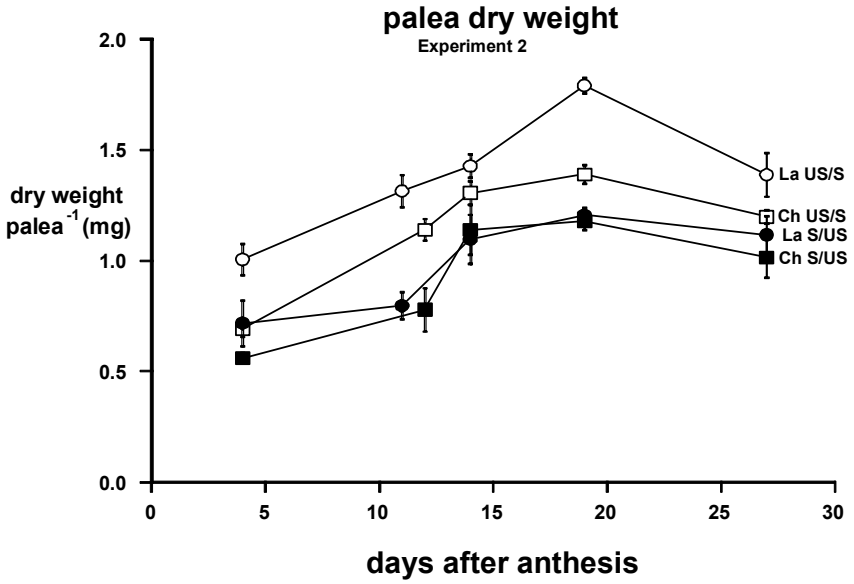


Figure 24

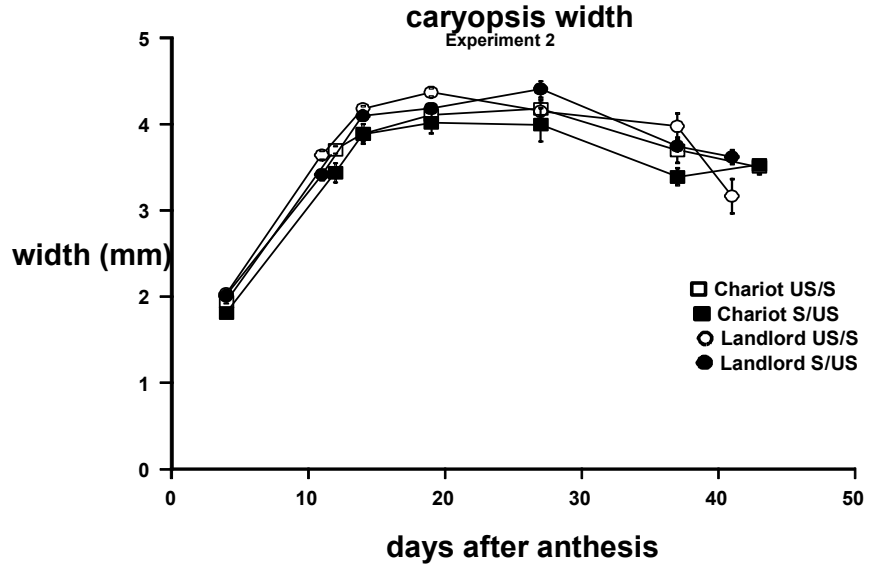


Figure 25

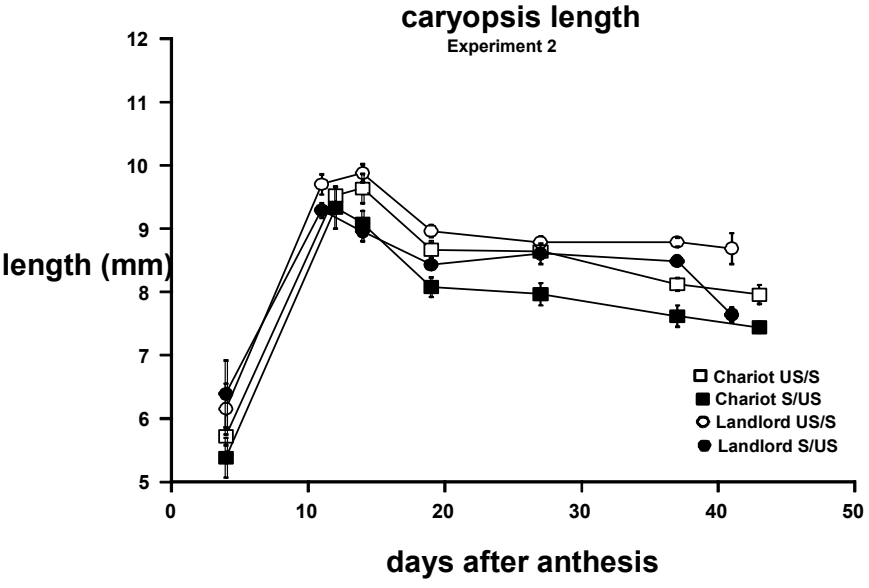


Figure 26

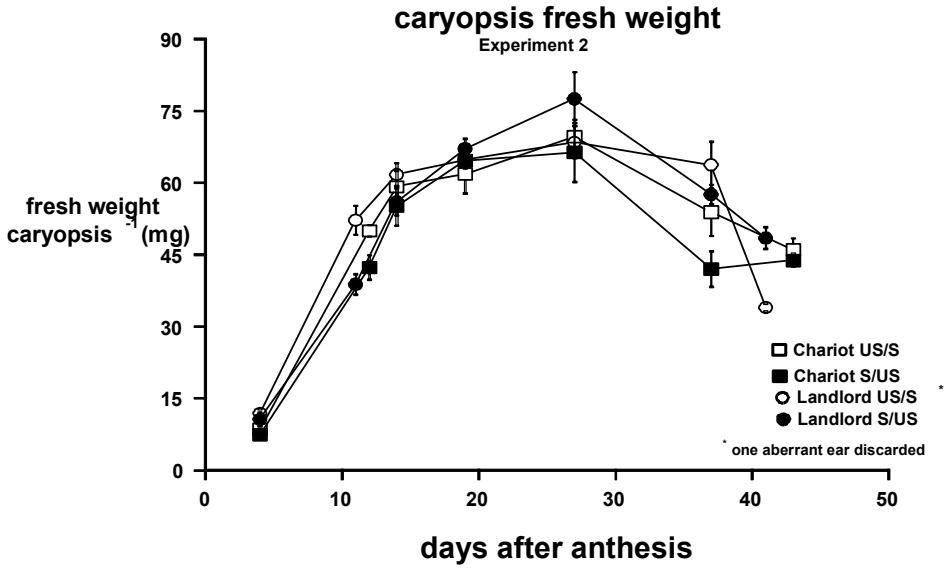


Figure 27

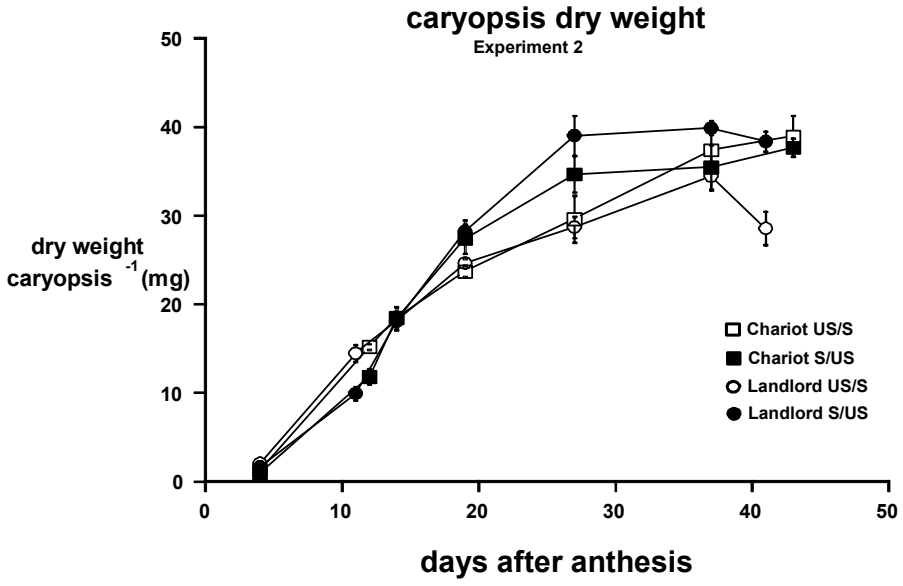


Figure 28

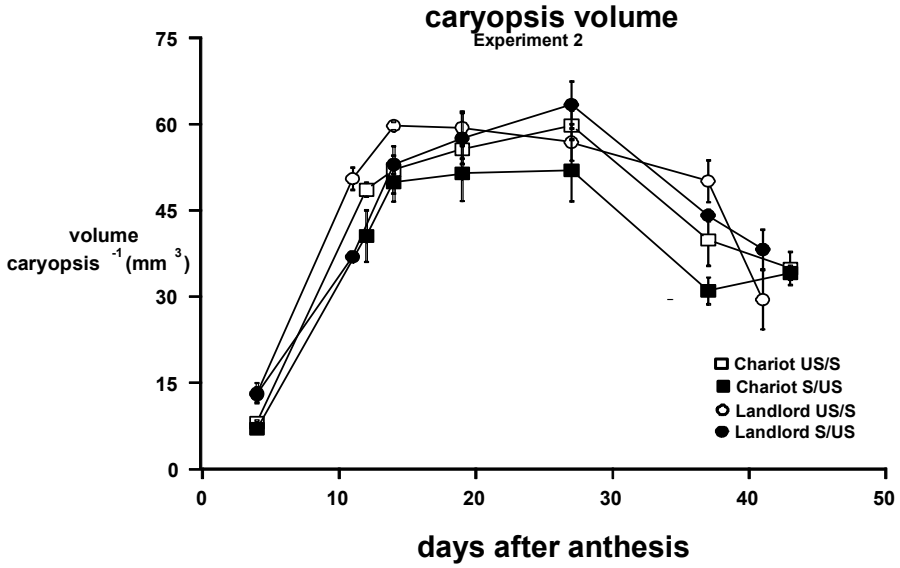


Figure 29

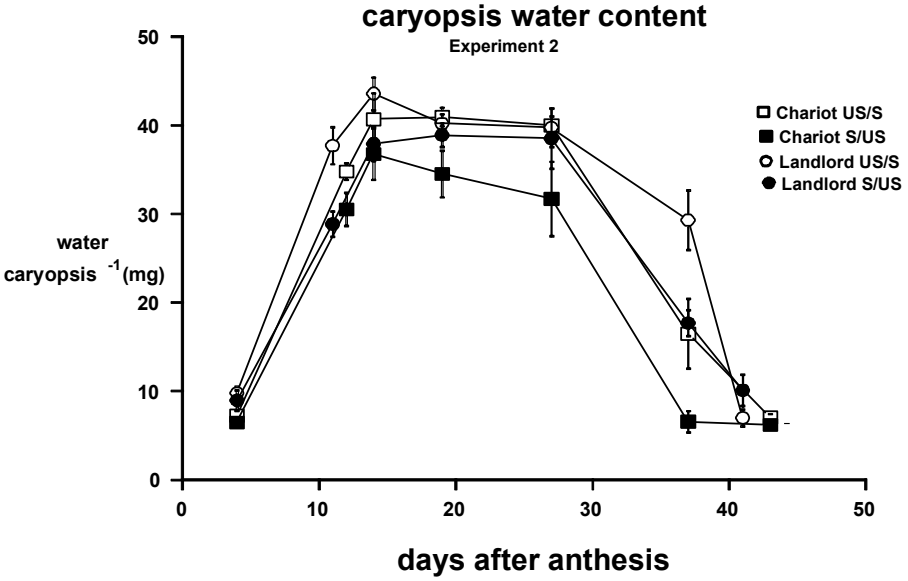


Figure 30

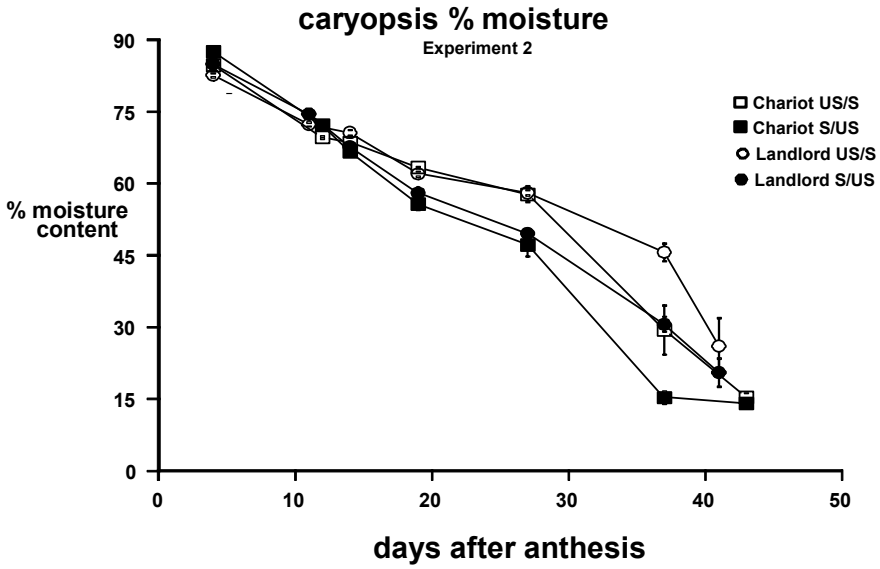


Figure 31

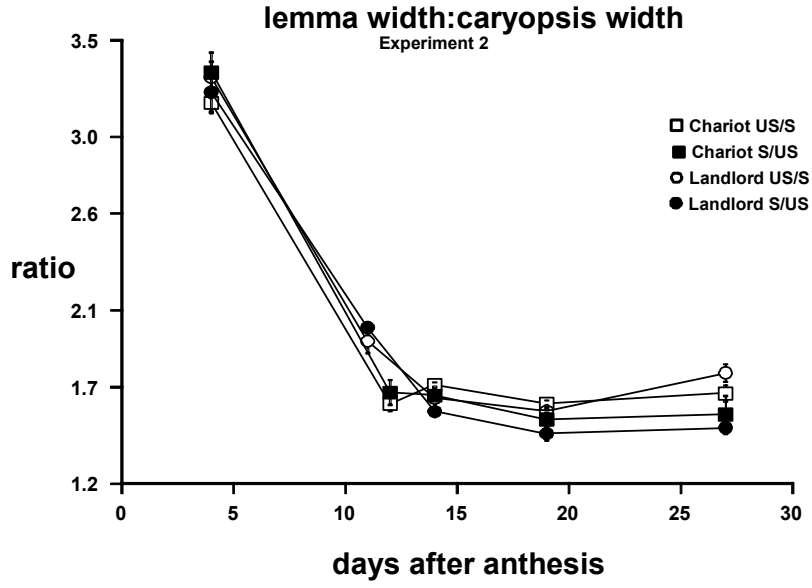


Figure 32

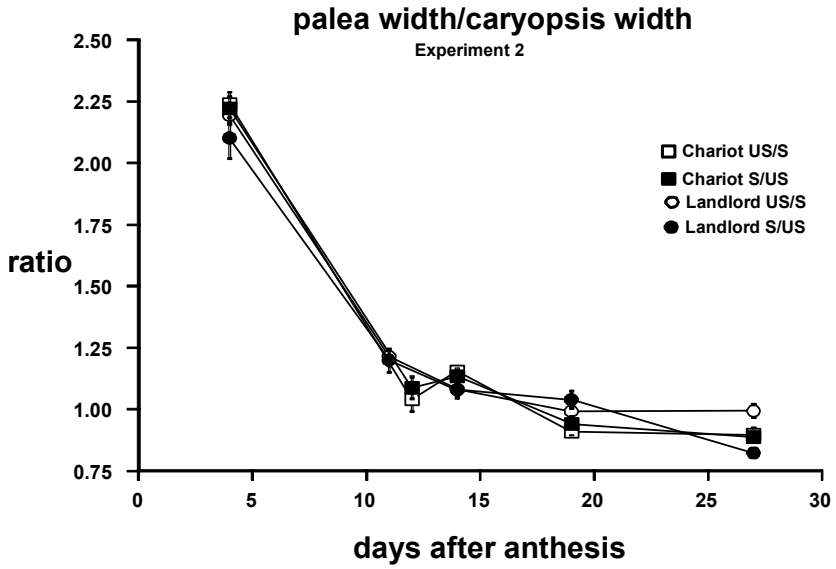


Figure 33

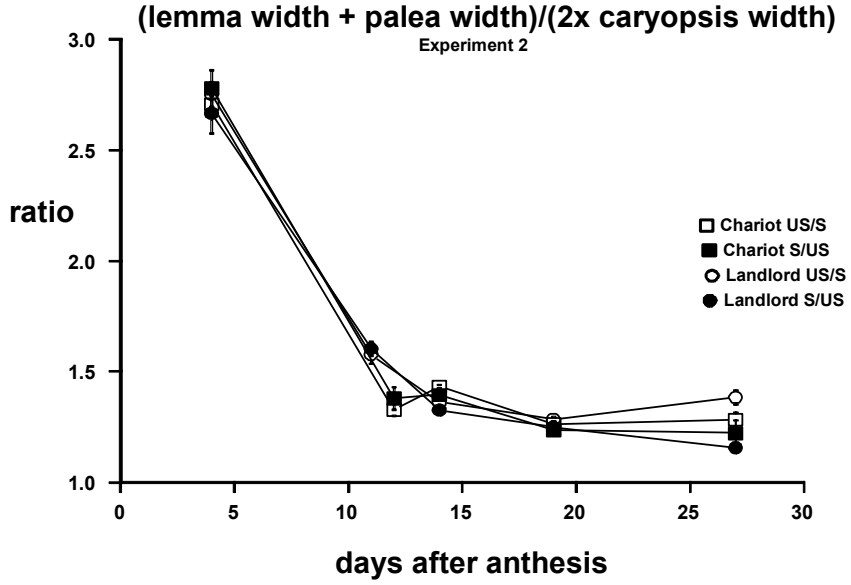


Figure 34

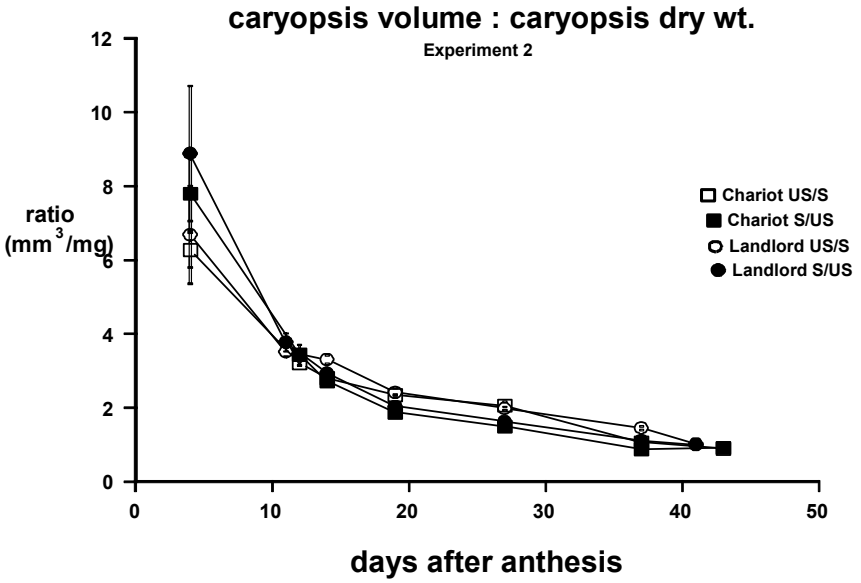


Figure 35

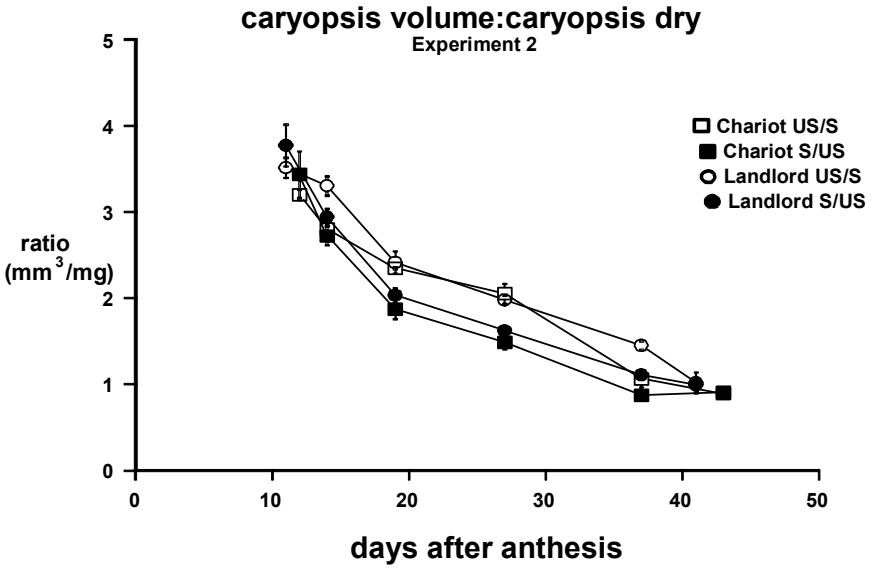


Figure 35a (re-drawn on a different scale from Figure above)

Caryopsis volume and water content increased most rapidly in US/S plants of Landlord (Figs. 29, 30). Caryopsis volume and water content were lowest in S/US plants of Chariot During grain maturation, the decrease in caryopsis water content was slowest in plants of US/S Landlord, but most rapid in S/US plants of Chariot. The % moisture content of caryopses decreased more slowly in Landlord than in Chariot (Fig. 31) and in plants that had been shaded after anthesis than in plants that had not been shaded after anthesis.

Relationships between caryopsis and husk dimensions

Towards the end of grain maturation, the ratios for lemma width to caryopsis width, palea width to caryopsis width and the combined lemma + palea width to caryopsis width were higher in US/S plants than in S/US plants (Figs. 32, 33, 34). Before 5 daa, the caryopsis volume to dry weight ratio was highest in plants that had been shaded before anthesis (S/US) (Fig. 35). However, from the middle of grain filling/maturation the caryopsis volume to dry weight ratio was lowest in S/US plants. The volume to dry weight ratio was higher in Landlord than in Chariot.

Husk and endosperm development – light microscopy

Husk and endosperm development in Chariot at 24 and 45 daa as influenced by shade pre- or post-anthesis was observed by light microscopy. (Plates 1-6). Gape between the palea and lemma overlying the pericarp and the starchy endosperm is shown at 24 daa in a S/US plant (Plate 1). Detail at the edge of the lemma in a gaping grain at 45 daa is shown in Plate 2. Close contact between the husk (lemma) and the underlying pericarp at the dorsal area of a grain is shown in Plate 3. This grain was at 24 daa in a S/US plant. Detail of a husk (lemma) losing contact with the underlying pericarp is shown in Plate 4.

Plates 5 and 6 indicate how shade has affected the relative numbers of large (type A) starch granules and small (type B) starch granules. Both Plates show grains at 24 daa. Large starch granules predominated in a US/S plant (Plate 5). By contrast, when in a S/US plant, there was a large number of small starch granules present among the large starch granules.

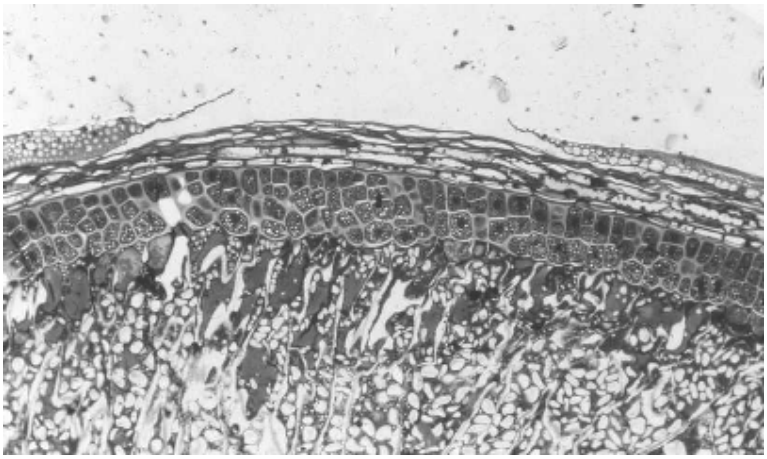


Plate 1. Gape between the lemma (left) and palea (right) overlying the pericarp that encloses the aleurone layer (block-like cells) and the starchy endosperm within. The grain was at 24 daa in a S/US plant of Chariot (Experiment II).

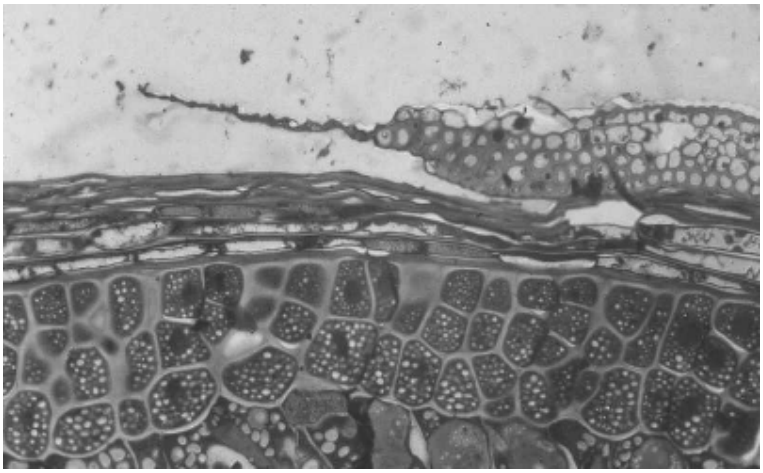


Plate 2. Detail at the edge of the lemma in a gaping grain. The lemma overlies the pericarp that encloses the aleurone layer (block-like cells). The grain was at 45 daa in US/S plant of Chariot (Experiment II).

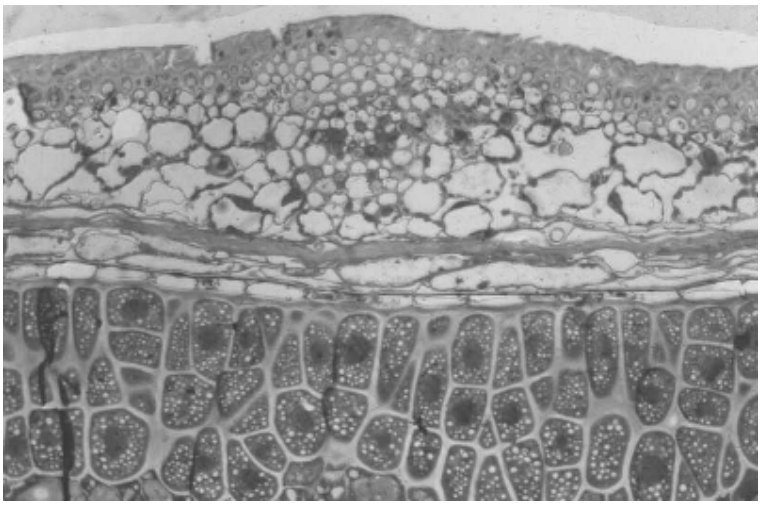


Plate 3. Detail of the contact between the husk (lemma) and the underlying pericarp at the dorsal area of a grain. The husk has not been separated from the pericarp (i.e. the grain has not skinned). A vascular bundle is present within the husk, in mid-picture. The grain was at 24 daa in a S/US plant of Chariot (Experiment II).

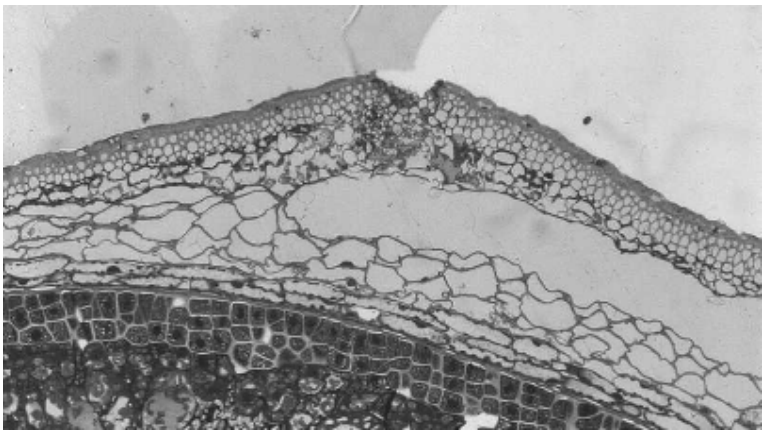


Plate 4. Detail of the husk (lemma) losing contact with the underlying pericarp at the dorsal area of a grain (i.e. skinning). The grain was at 24 daa in a US/S plant of Chariot (Experiment II).

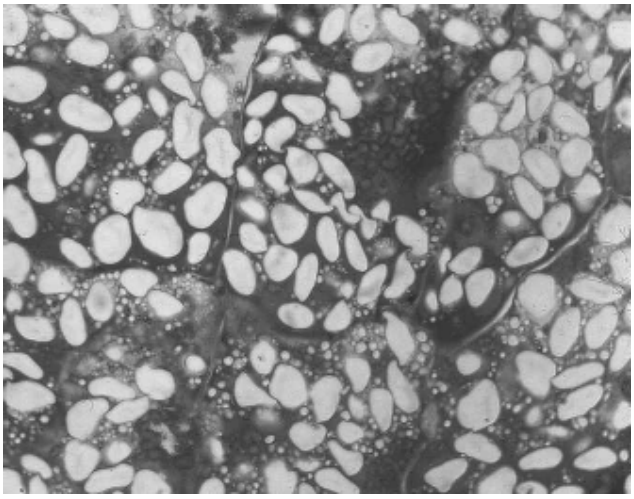


Plate 5. Detail of the starchy endosperm in which large (type A) starch granules predominate. The grain was at 24 daa in a US/S plant of Chariot (Experiment II).

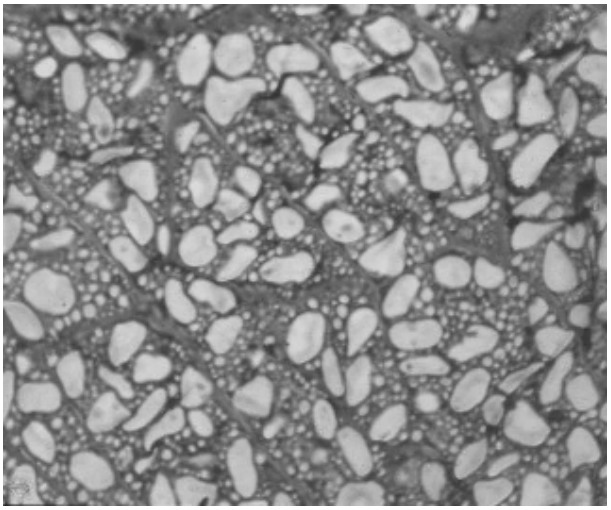


Plate 6. Detail of the starchy endosperm in which a large number of small (type B) starch granules are present among the large (type A) starch granules. The grain was at 24 daa in a S/US plant of Chariot (Experiment II).

Gape, splitting and TGW

There was a significantly higher level of splitting in S/US plants of Chariot than in US/S plants of Chariot or in either S/US or US/S plants of Landlord (Table 11a). There were no significant differences in splitting between US/S plants of Chariot and either Landlord treatment. Shading after anthesis resulted in lower levels of gape than did shading before anthesis, though, in Chariot, the scores for gape were not significantly different between S/US and US/S. In both varieties, TGW was significantly higher in S/US plants than in US/S plants. Overall, levels of splitting were similar to those in Experiment I, but levels of gape and TGW were considerably lower than those in Experiment I.

At the grain filling temperature of 13^{o}C, Chariot had significantly higher levels of splitting than Landlord (Table 11b). TGWs in plants of both Landlord and Chariot that had not been shaded before anthesis were significantly higher than those in plants that had been shaded before anthesis. Levels of splitting in plants of Chariot at a grain filling/maturation temperature of 13^{o}C (either shaded or unshaded before anthesis) were similar to those recorded for plants at the S/US treatment (compare Table 11a and 11b). Thus, where cooler conditions followed anthesis the effect of the pre-anthesis treatment was either unaffected or increased.

Table 11. Experiment II: (a) Effect of S/US and US/S on TGW and the incidence of gape and splitting in Landlord and Chariot. (b) Effect of shade (S) or no shade (US) pre-anthesis in plants placed in a constant temperature (13°C) growth room post-anthesis. Measurements were made on bulked samples ears from main stems and main tillers.

(a)	Landlord			Chariot		
Pre- / post-anthesis treatment	Splitting (%)	Gape (%)	TGW (g)	Splitting (%)	Gape (%)	TGW (g)
S / US	0.4	2.5	48.3	5.4	3.5	45.4
US / S	0	0	37.4	0.8	2.5	39.4

SED for comparing % splitting in any variety *x* shade combination = 0.76

SED for comparing % gape in any variety *x* shade combination = 1.19

SED for comparing TGW in any variety *x* shade combination = 3.41

(b)	Landlord			Chariot		
Pre / post- anthesis treatment	Splitting (%)	Gape (%)	TGW (g)	Splitting (%)	Gape (%)	TGW (g)
S / 13°C	1.1	0.6	46.7	3.5	0.0	42.2
US / 13°C	0.2	0.4	57.0	4.7	3.8	53.9

SED for comparing % splitting in any variety *x* shade combination = 1.51

SED for comparing % gape in any variety *x* shade combination = 1.24

SED for comparing TGW in any variety *x* shade combination = 2.87

Relationship between splitting and TGW in Experiments I and II

The data below are from splitting (%) scores and TGWs in grains from main stems and main tillers of plants in Experiments I and II. In Chariot there was a significant, positive, relationship between TGW and the % split grains. By contrast, there was only a weakly positive relationship between TGW and % of split grains in Landlord.

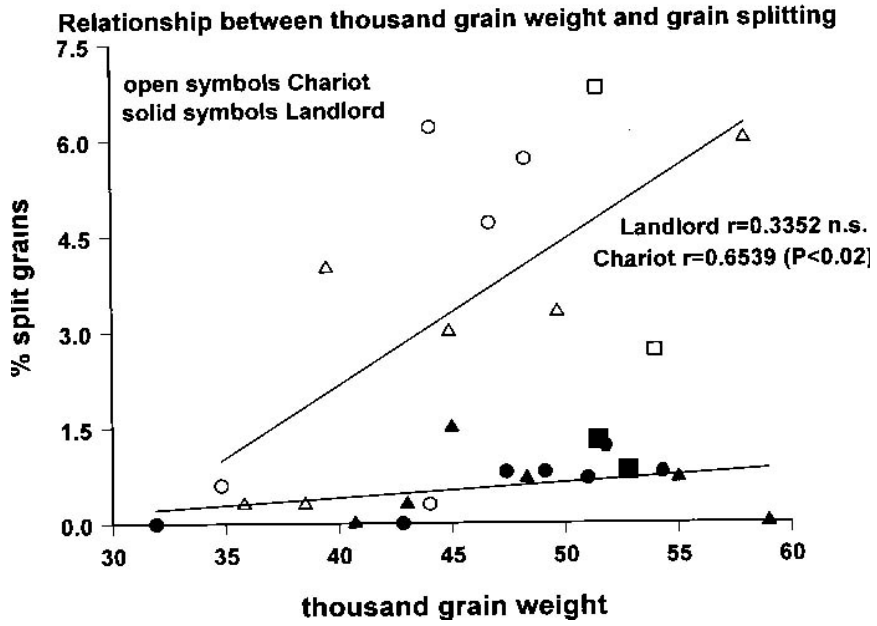


Figure 36. Relationship between splitting (%) and TGW in grains from Experiments I and II. Data points are: Landlord (■, ●, ▲) and Chariot (□, ○, △) in Experiment I (■, □) and Experiment II S/US or US/S main stems or main tillers (●,○) and Experiment II S/13^{0}C or US/13^{o}C main stems or main tillers (▲, △).

Relationship between lemma and palea width and caryopsis width

The combined maximum lemma and palea width relative to 2x caryopsis width at harvest-ripeness was less in Chariot than in Landlord and less in plants that had been shaded before anthesis than in plants unshaded before anthesis. (Table 12). There was no evidence of gape which would need [A] / 2x [B] to be less than 1.

Table 12. Relationship between maximum lemma + palea width and caryopsis width in Chariot and Landlord, Experiments I and II.

Experiment	Variety and treatment*	Maximum lemma width + palea width (27 daa (mm) ± sd [A]	Caryopsis width at harvest-ripeness** (mm) ± sd [B]	[A] / 2x [B]
I	**Landlord U**	**11.58 ± 0.26**	**3.71 ± 0.19**	**1.56**
I	**Landlord S**	**11.54 ± 0.24**	**3.82 ± 0.75**	**1.51**
I	**Chariot U**	**11.37 ± 0.30**	**3.77 ± 0.07**	**1.51**
I	**Chariot S**	**11.70 ± 0.21**	**3.93 ± 0.13**	**1.49**
II	**Landlord US/S**	**11.45 ± 0.54**	**3.16 ± 0.40**	**1.81**
II	**Landlord S/US**	**10.17 ± 0.18**	**3.62 ± 0.16**	**1.41**
II	**Chariot US/S**	**10.69 ± 0.19**	**3.50 ± 0.16**	**1.52**
II	**Chariot S/US**	**9.70 ± 0.48**	**3.53 ± 0.49**	**1.37**

*U = no shade; S = shade before anthesis; US/S = unshaded before anthesis and shaded after anthesis; S/US = shaded before anthesis and unshaded after anthesis.

**60 daa in Experiment I; 43 daa in Experiment II

Temperature and sunshine hours in the glasshouse

Approximately 1700 day ^{o}C had been accumulated between sowing and harvest. Anthesis occurred between 16 to 26 July and there were between 840 to 1020 day ^{o}C from anthesis to grain harvest-ripeness filling. The sunshine hours were estimates from a local meteorological station.

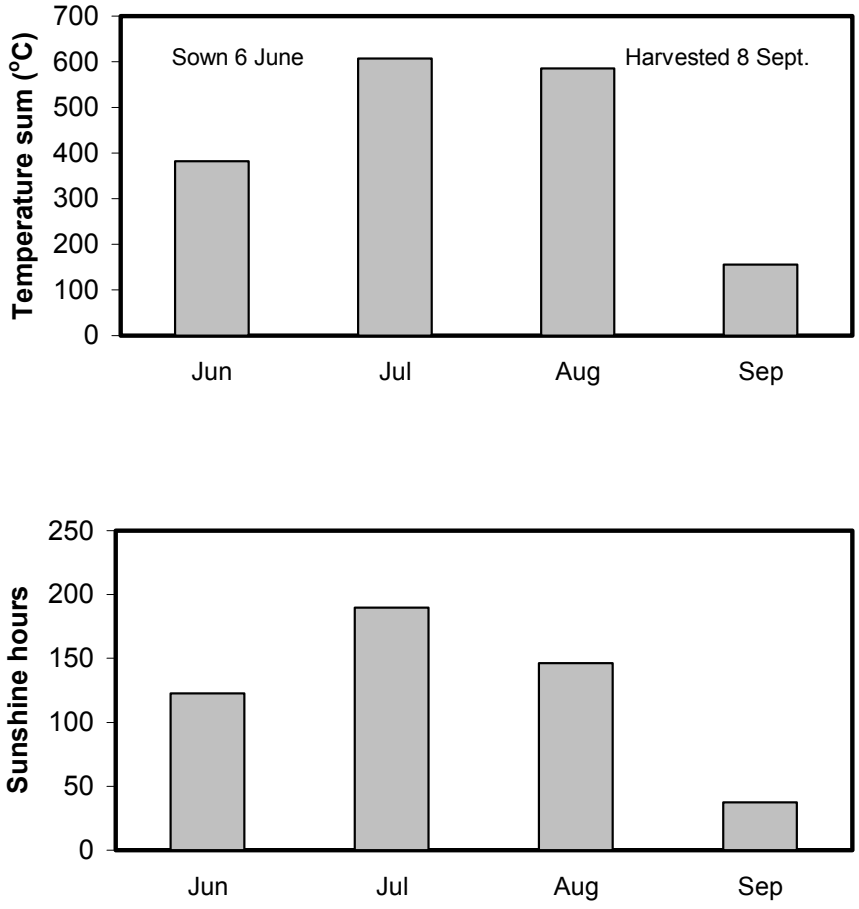


Figure 37. Experiment II: Monthly temperature sums and sunshine hours in the glasshouse.

Experiment III. Gape and splitting as influenced by: shade during husk development, warm or cool temperature during grain filling/maturation, and by anthesis date

In this experiment, the varieties Landlord and Chariot were either shaded or not shaded before anthesis. After anthesis, all plants were kept unshaded and either remained in the glasshouse or were transferred to a cool growth room (13 ^{o}C). Differences in gape and splitting between a population of predominantly main stem ears that had anthesed relatively early and a population of tiller ears that had anthesed relatively late were also considered.

Gape, splitting and TGW

Gape in Chariot was markedly higher in grains from plants that had been shaded before anthesis than in those from plants in full light prior to anthesis (Table 13a,b). There were significantly higher levels of splitting in Chariot than in Landlord and significantly more splitting in grains of Chariot that had anthesed late (i.e. predominantly tillers) than in grains that had anthesed early. The TGW from ears that had anthesed early (i.e. main stem ears) was markedly higher than those from ears that had anthesed late, in plants that had not been shaded before anthesis. The TGW was the same in main stems and tillers in plants that had been shaded before anthesis.

Relationships between gape or splitting and TGW

Figure 38 combines data from early and late anthesed ears (as shown in Table 13). In Chariot there was a significant negative correlation between the % of split grains and TGW. Likewise, % gape was negatively correlated with TGW. However, there was no significant relationship between gape or splitting and TGW in Landlord.

Table 13. Experiment III: Effect of pre- and post-anthesis treatments on TGW and the incidence of gape and splitting in ears of Landlord and Chariot from (a) early anthesis and (b) late anthesis.

(a) Early anthesis

	Landlord			Chariot		
Pre- / post-anthesis treatment	**Splitting** (%)	Gape (%)	TGW (g)	Splitting (%)	Gape (%)	**TGW** (g)
No shade / warm	0.7	5.7	58.1	2.0	4.7	55.1
Shade / warm	0.0	4.3	52.3	5.3	12.3	48.1
No shade / cool	0.0	1.0	59.0	1.3	2.0	55.7
Shade / cool	0.3	7.0	50.8	3.0	8.3	50.9

(b) Late anthesis

	Landlord			Chariot		
Pre- / post-anthesis treatment	**Splitting** (%)	Gape (%)	TGW (g)	Splitting (%)	Gape (%)	**TGW** (g)
No shade / warm	1.3	4.7	53.9	11.3	6.7	50.4
Shade / warm	1.7	13.3	51.5	12.3	21.3	48.3
No shade / cool	0.0	0.0	45.4	8.0	10.0	49.9
Shade / cool	0.7	11.3	51.2	10.0	16.0	49.3

SED for comparing % splitting in any variety *x* shade/temperature *x* anthesis date combination = 3.57

SED for comparing % gape in any variety *x* shade/temperature *x* anthesis date combination = 3.29

SED for comparing TGW in any variety *x* shade/temperature *x* anthesis date combination = 2.47

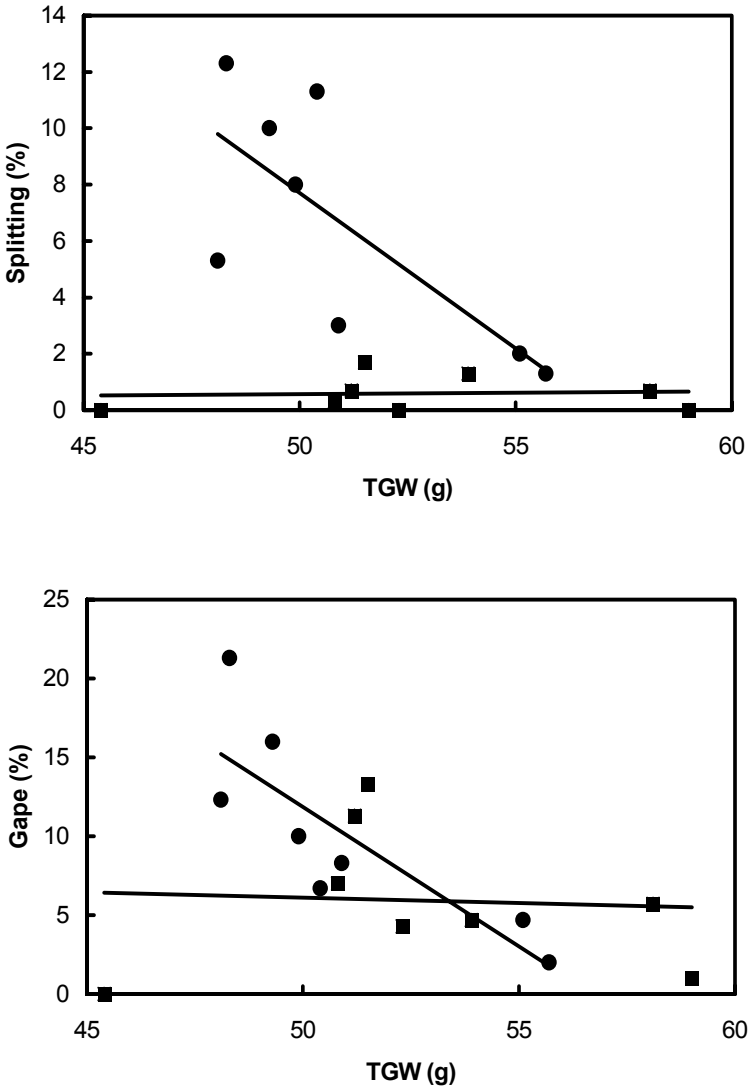


Figure 38. Relationship between (a) splitting and TGW and (b) gape and TGW in Landlord (■) and Chariot (●). Data are from the combined early and late anthesed ears in Experiments III.

Grain volume and dry weight (at maximum grain volume, GS77)

In both Landlord and Chariot, grain volume and dry weight were higher (though not always significantly so) in grains of plants that had not been shaded before anthesis than in grains of plants that had been shaded (Table 14). The differences were much greater in plants kept in warm conditions after anthesis than in plants kept in cool conditions after anthesis. The volume to dry weight ratio was higher in Landlord than in Chariot and higher under the cool grain filling/maturation temperature that under the warm grain filling temperature.

Table 14. Experiment III: Effect of pre- and post-anthesis treatments on grain volume and dry weight (at maximum grain volume, GS77) in Chariot and Landlord. Data for early anthesis (i.e. main stems) only.

	Landlord			Chariot		
Pre- / post-anthesis treatment	**Volume** (mm^3)	Dry weight (mg)	Vol : dry wt	**Volume** (mm^3)	Dry weight (mg)	Vol : dry wt
No shade / warm	78.8	54.3	1.45	53.7	44.0	1.22
Shade / warm	66.4	47.6	1.40	44.7	42.9	1.04
No shade / cool	63.2	40.9	1.55	62.2	40.9	1.52
Shade / cool	61.5	38.5	1.60	59.2	39.2	1.51

SED for volume at any shade/temperature combination = 2.71

SED for dry weight at any shade/temperature combination = 1.85

SED for vol : dry wt at any shade/temperature combination = 0.23

Measurements of grain sterility

Higher than expected levels of grain sterility were observed in Experiment III. Consequently, numbers of sterile grain sites were counted in main stem ears of both varieties. The percentage of sterile grain sites was higher in ears of plants that had been shaded (Table 15).

Table 15. Experiment III: Percentage of sterile grain sites per ear (n= 8) in main stem ears.

	Sterility (%)	
	No shade before anthesis	Shaded before anthesis
Landlord	8.2	12.6
Chariot	7.9	16.4

Temperature and sunshine hours in the glasshouse/growth room

Approximately 2000 day ^{o}C had been accumulated in the glasshouse between sowing and grain harvest-ripeness: between 950 to 1150 day ^{o}C were accumulated between anthesis and grain harvest-ripeness. By contrast, approximately 2400 ^{o}C days had been accumulated in the combination of glasshouse and growth room. Plants were transferred to the growth room at 4-10 daa and between 1300 and 1450 day ^{o}C were accumulated between anthesis and grain harvest-ripeness in the growth room.

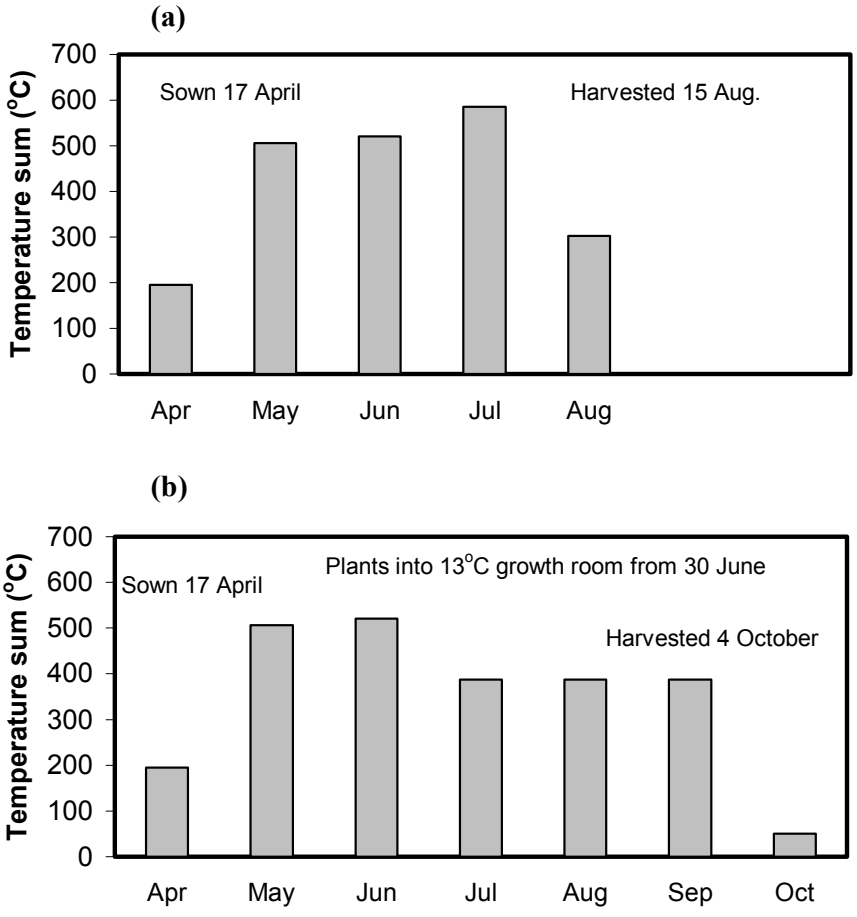


Figure 39. Experiment III: Monthly temperature sums for plants in (a) the glasshouse only and (b) glasshouse before anthesis and growth after anthesis.

Experiment IV. Gape and splitting in genotypes (varieties and selected lines) as influenced by warm or cool temperatures during grain filling and maturation, and by anthesis date

Data presented in Table 16 shows that although no consistency in the rank order of selected lines was observed, B96-76/96 and B96/76-193 had, on average (combined early and late anthesed ears), a higher level of splitting than B96-76/24 and B96-76/179. This is consistent with previous scores in field trials in 1999 by SCRI i.e. B96-76/96 and B96/76-193 were scored as high for splitting whilst B96-76/24 and B96-76/179 were scored as low for splitting. The incidence of splitting in Chariot was lower than that observed in previous experiments, but was in all cases equal to or greater than the incidence of splitting in Landlord. The highest incidence of splitting occurred in grains from ears that had anthesed late (i.e. predominantly tillers) and the lines B96-76/96 and B96/76-193 had higher splitting scores than Chariot (Table 16b). Generally, the level of splitting at a temperature of 13 ^{o}C from anthesis to harvest-ripeness was equal to or greater than that at a temperature of 18 ^{o}C from anthesis to harvest-ripeness. TGW was higher in plants grown from anthesis onwards at 13 ^{o}C than in those grown from anthesis onwards at 18 ^{o}C. TGWs for the selected lines were comparable to those for the two varieties and similar to those recorded in the previous three experiments.

The percentage of sterile grain sites in the ears of shaded plants was greater in the four selected lines than in the 2 varieties (Table 17).

Table 16. Experiment IV: Effect of pre- and post-anthesis treatments on TGW and the incidence of gape and splitting in Landlord, Chariot and four lines. (a) early anthesis and (b) late anthesis.

(a) Early anthesis — Post-anthesis grain filling/maturation temperature

	13°C			18°C		
	Splitting (%)	Gape (%)	TGW (g)	**Splitting** (%)	Gape (%)	TGW (g)
Landlord	0	1.0	53.3	1.3	2.0	51.9
Chariot	5.0	6.7	53.9	1.3	13.7	53.9
B96-76/24	3.0	20.0	53.8	3.7	15.0	51.5
B96-76/179	3.3	2.0	52.7	1.3	2.7	43.9
B96-76/96	1.7	8.7	50.8	2	12.3	47.7
B96-76/193	5.3	3.3	49.8	1.3	4.7	45.3

(b) Late anthesis — Post-anthesis grain filling/maturation temperature

	13°C			18°C		
	Splitting (%)	Gape (%)	TGW (g)	Splitting (%)	Gape (%)	TGW (g)
Landlord	0	0	51.9	0	2.7	45.1
Chariot	0.3	3.7	48.5	3.3	7.0	46.1
B96-76/24	4.7	1.0	51.7	0.3	1.7	42.9
B96-76/179	3.0	1.0	48.6	3.0	0.3	44.0
B96-76/96	10.0	2.7	49.0	6.3	2.0	43.0
B96-76/193	5.0	7.3	51.9	5.7	6.0	45.7

SED for % splitting at any genotype *x* temperate *x* anthesis date combination = 1.29

SED for % gape at any genotype *x* temperate *x* anthesis date combination = 1.53

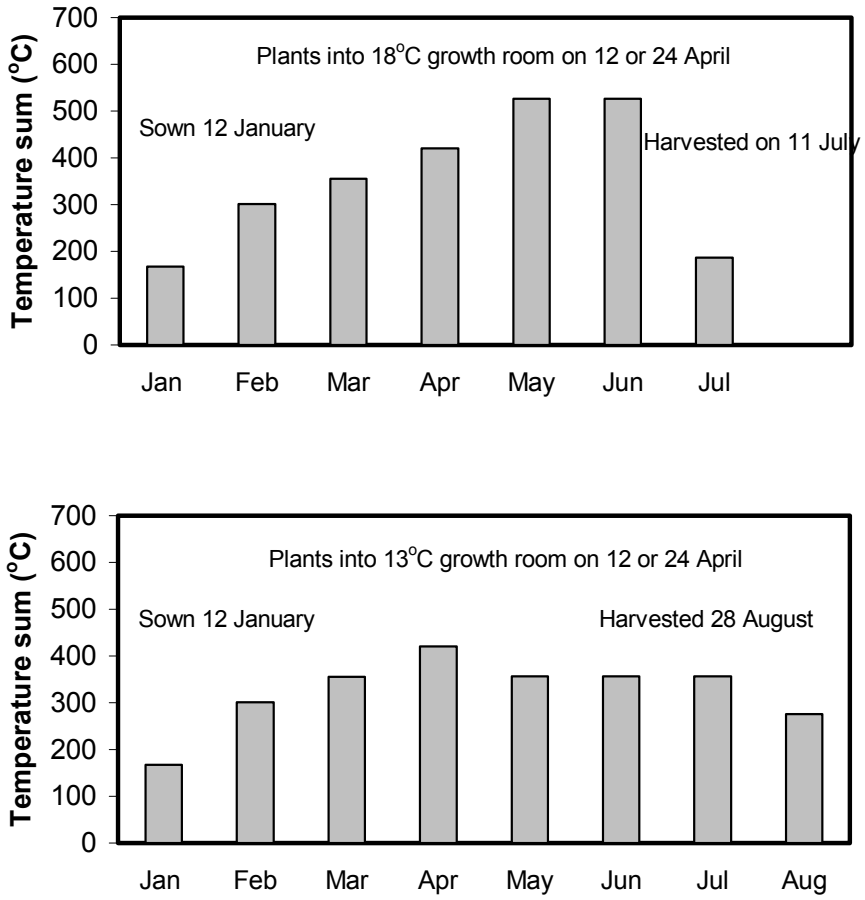
Table 17. Percentage of sterile grain sites per ear in genotypes (varieties and selected lines). All plants were shaded before anthesis. (n= 8).

	Sterility (%)
Landlord	10.8
Chariot	10.4
B96-76/24	26.9
B96-76/179	22.8
B96-76/96	24.2
B96-76/193	19.9

Temperature accumulation in Experiment IV

Approximately 2,500 day °C were accumulated from sowing to grain harvest-ripeness in the glasshouse and 18 °C growth room. By contrast, approximately 2,600 day were accumulated from sowing to grain harvest-ripeness in the glasshouse and 13 °C growth room. A very wide range of anthesis dates, most likely as a consequence of early sowing (12 January), resulted in a much wider range of day °C accumulated between anthesis to harvest than those reported in Experiments I, II or III.

Figure 40. Experiment IV: Monthly (2000) temperature sums in the glasshouse and growth rooms (a)



glasshouse and 18°C growth room after anthesis and (b) glasshouse and 13°C growth after anthesis.

Results – Field Studies (Experiments A, B, C, D, E and F)

Experiments A and B. Gape, splitting and skinning in Landlord and Chariot at harvest 2000 as influenced by the rate of N fertiliser application and the number of fungicide applications at two sites (Boghall Farm, Midlothian and Hutton Hall Barns, Berwickshire)

There were no significant effects of the rate of N fertiliser or of the number of fungicide applications on the levels of gape, splitting and skinning in Landlord (Tables 18 and 19). However, in all treatments, the levels of splitting were significantly higher in Chariot than in Landlord.

In Chariot (Experiment A), there were significantly higher levels of splitting following an increase in the rate of N fertiliser application and with an increase in the number of fungicide applications (Table 18). In Chariot (Experiment B) there was a increase in splitting following an increase in the number fungicide applications, but this was only significant at the lower rate of N fertiliser i.e. 125 kg N ha^{-1} (Table 19).

There were no significant effects of variety, rate of N fertiliser application or the number of fungicide applications on levels of gape or skinning (Tables 18 and 19). Generally, yields increased with an increase in the rate of N fertiliser application and in the number of fungicide applications (Tables 18 and 19). Landlord out-yielded Chariot across all treatments. TGW was consistently higher in Landlord than in Chariot. An increase in the rate of N fertiliser application resulted in a lower TGW at Boghall Farm, Midlothian (Table 18) and a higher TGW at Hutton Hall Barns, Berwickshire (Table 19).

In both varieties, the % of green leaf area (GLA) tended to be higher with an increase in the rate of N fertiliser application or in the number of fungicide applications (Tables 18 and 19).

Table 18. Experiment A: Effects of the rate of N fertiliser and the number of fungicide applications on gape, splitting and skinning in Landlord and Chariot at Boghall Farm, Midlothian , in 2000. SEDs are for comparing any variety x N x fungicide combination.

(a) Landlord

Treatment		Split (%)	Gape (%)	Skin (%)	Yield (t/ha)	TGW (g)	%GLA (GS73)
Control	120 kg N/ha, 2 fungicide applications	2.5	0	0	5.87	48.1	70
Extra fungicide	120 kg N/ha, 3 fungicide applications	3.2	0	1	6.21	48.6	72
Extra N	170 kg N/ha, 2 fungicide applications	1.3	0.3	0.7	6.58	46.9	78
Extra N and fungicide	170 kg N/ha, 3 fungicide applications	2.3	0	2.7	6.89	46.6	84

(b) Chariot

Treatment		Split (%)	Gape (%)	Skin (%)	Yield (t/ha)	TGW (g)	%GLA (GS73)
Control	120 kg N/ha, 2 fungicide applications	7.3	0.2	1.2	5.60	46.0	67
Extra fungicide	120 kg N/ha, 3 fungicide applications	8.3	0.2	1.0	5.86	46.2	67
Extra N	170 kg N/ha, 2 fungicide applications	10.0	0.2	0.8	6.32	45.6	80
Extra N and fungicide	170 kg N/ha, 3 fungicide applications	16.8	0.2	2.0	6.42	45.9	78
	SED	1.91	0.24	0.65	0.42	0.57	2.7

Table 19. Experiment B: Effects of the rate of N fertiliser application and the number of fungicides applications on gape, splitting and skinning in Landlord and Chariot at Hutton Hall Barns, Chirnside, Berwickshire, in 2000. SEDs are for comparing any variety x N x fungicide combination.

(a) Landlord

Treatment		Split (%)	Gape (%)	Skin (%)	Yield (t/ha)	TGW (g)	%GLA (GS73)
Control	125 kg N/ha, 2 fungicide applications	3.7	0.2	1.3	7.15	46.3	60
Extra fungicide	125 kg N/ha, 3 fungicide applications	3.2	0.2	1.2	7.11	46.4	65
Extra N	175 kg N/ha, 2 fungicide applications	1.8	0.3	0.7	7.73	47.1	64
Extra N and fungicide	175 kg N/ha, 3 fungicide applications	3.0	0.2	1.0	8.07	47.5	68

(b) Chariot

Treatment		Split (%)	Gape (%)	Skin (%)	Yield (t/ha)	TGW (g)	%GLA (GS73)
Control	125 kg N/ha, 2 fungicide applications	8.8	0.3	2.0	6.85	44.2	56
Extra fungicide	125 kg N/ha, 3 fungicide applications	12.5	0.4	3.0	6.76	44.5	61
Extra N	175 kg N/ha, 2 fungicide applications	10.2	0.3	1.3	7.60	45.5	67
Extra N and fungicide	175 kg N/ha, 3 fungicide applications	12.0	0.3	2.2	7.41	45.7	68
	SED	1.41	0.29	0.83	0.26	0.95	3.1

Experiment C. Gape, splitting and skinning in Landlord and Chariot as influenced by the rate of N fertiliser application and the number of fungicide applications at Boghall Farm, Midlothian, at harvest 2001

The levels of splitting were 2-3 fold higher at Boghall Farm 2001 than in 2000 (compare Tables 18 and 20). The levels of splitting were significantly higher in Chariot than in Landlord (Table 20). There were no significant effects of the rate of N fertiliser application or of the number of fungicide applications on splitting or gape in Landlord or Chariot. However, in Chariot, levels of splitting were greater at the higher number of fungicide applications or at the higher rate of N application with fungicides applied. There was a tendency for the higher rate of N fertiliser application or the number of fungicide applications to reduce skinning in both varieties, though this was not was not significant across in all treatment combinations.

Yields were higher in Landlord than in Chariot. Yields, in both varieties, were significantly higher at the higher rate of N fertiliser application, with two or three fungicide applications, compared to the control or zero fungicide application. TGWs were significantly higher in Landlord than in Chariot, but were not significantly affected by the rate of N fertiliser application or the number of fungicide applications. In both varieties, the % of green leaf area (GLA) at GS73 tended to be higher with an increase in the rate of N fertiliser application or in the number of fungicide applications (Table 20).

Table 20. Experiment C: Effects of rate of N fertiliser application and number of fungicides applications on gape, splitting and skinning in Landlord and Chariot at Boghall Farm, Midlothian, in 2001. SEDs are for comparing any variety x N x fungicide combination.

(a) Landlord

Treatment		Split (%)	Gape (%)	Skin (%)	Yield (t/ha)	TGW (g)	%GLA (GS73)
Zero fungicide	110 kg N ha^{-1}	4.4	0.9	10.2	6.41	47.0	55
Control	110 kg N/ha, 2 fungicides	6.0	1.5	9.7	6.71	45.3	64
Extra fungicide	110 kg N/ha, 3 fungicides	7.3	1.5	7.5	7.14	47.2	70
Extra N and zero fungicide	160 kg N ha^{-1}	5.9	0.7	9.9	6.88	46.6	65
Extra N	160 kg N/ha, 2 fungicides	9.2	1.0	7.8	7.03	45.9	72
Extra N and fungicide	160 kg N/ha, 3 fungicides	5.7	1.2	6.0	7.40	47.0	78

(b) Chariot

Treatment		Split (%)	Gape (%)	Skin (%)	Yield (t/ha)	TGW (g)	%GLA (GS73)
Zero fungicide	110 kg N ha^{-1}	34.7	3.0	16.0	5.89	45.6	45
Control	110 kg N/ha, 2 fungicides	37.4	3.0	14.5	6.05	44.1	62
Extra fungicide	110 kg N/ha, 3 fungicides	46.9	2.0	9.9	6.76	45.0	66
Extra N and zero fungicide	160 kg N ha^{-1}	39.5	2.2	10.0	6.62	44.0	55
Extra N	160 kg N/ha, 2 fungicides	41.4	2.2	13.3	6.90	43.3	75
Extra N and fungicide	160 kg N/ha, 3 fungicides	43.0	2.4	9.0	7.05	44.5	73
	SED	4.70	1.03	1.58	0.19	0.82	3.9

Experiment D. Gape, splitting and skinning in Landlord, Chariot, Optic and Cellar as influenced by the rate of N fertiliser application and the number of fungicide applications at Seton West Mains, East Lothian, at harvest 2001

Chariot had significantly higher levels of splitting across all treatments compared to the other varieties, whilst Landlord had significantly lower levels of splitting across all treatment compared to other varieties (Table 21). Optic and Chalice had levels of splitting intermediate to those of Landlord and Chariot; Chalice tended to have higher levels of splitting than Optic.

Gape was significantly lower in Landlord than in other varieties. Skinning was highest in Chalice.

There were no significant effects of the rate of N fertiliser or of the number of fungicide applications on splitting, gape or skinning in Landlord. There was no consistency in the effects of rate of N fertiliser or of the number of fungicide applications on the levels of gape, splitting or skinning in Chariot, Optic or Chalice.

TGW was highest in Chalice and least in Chariot. There was no consistency in the effects of the rate of N fertiliser of the number of fungicide applications on TGW across varieties. Across all varieties, the % of GLA at GS73 tended to be higher with an increase in the rate of N fertiliser or in the number of fungicide applications (Table 21).

Table 21. Experiment D: Effects of N fertiliser and number of fungicides applications on gape, splitting and skinning in Landlord, Chariot, Optic and Chalice at Seton West Mains, East Lothian, in 2001. SEDs are for comparing any variety x N x fungicide combination.

(a) Landlord

Treatment		Split (%)	Gape (%)	Skin (%)	Yield (t/ha)	TGW (g)	%GLA (GS73)
Control	120 kg N/ha, 2 fungicides	7.7	1.0	8.0	5.40	50.4	66
Extra fungicide	120 kg N/ha, 3 fungicides	7.0	0	4.0	4.45	50.1	74
Extra N	170 kg N/ha, 2 fungicides	8.3	0	4.7	7.20	49.5	73
Extra N and fungicide	170 kg N/ha, 3 fungicides	6.7	0	2.7	7.12	50.6	77

(b) Chariot

Treatment		Split (%)	Gape (%)	Skin (%)	Yield (t/ha)	TGW (g)	%GLA (GS73)
Control	120 kg N/ha, 2 fungicides	29.7	2.0	6.0	6.44	46.8	60
Extra fungicide	120 kg N/ha, 3 fungicides	36.0	1.7	4.0	6.02	46.0	62
Extra N	170 kg N/ha, 2 fungicides	27.7	2.0	6.3	6.67	48.1	72
Extra N and fungicide	170 kg N/ha, 3 fungicides	28.0	0.7	5.0	7.31	48.1	73

(c) Optic

Treatment		Split (%)	Gape (%)	Skin (%)	Yield (t/ha)	TGW (g)	%GLA (GS73)
Control	120 kg N/ha, 2 fungicides	12.0	1.3	6.7	7.37	52.2	72
Extra fungicide	120 kg N/ha, 3 fungicides	7.3	1.3	4.0	8.17	52.2	76
Extra N	170 kg N/ha, 2 fungicides	17.0	1.3	5.0	9.05	50.7	81
Extra N and fungicide	170 kg N/ha, 3 fungicides	16.0	2.0	6.0	8.58	52.8	81

(b) Chalice

Treatment		Split (%)	Gape (%)	Skin (%)	Yield (t/ha)	TGW (g)	%GLA (GS73)
Control	120 kg N/ha, 2 fungicides	17.3	2.7	9.7	6.92	54.8	73
Extra fungicide	120 kg N/ha, 3 fungicides	11.3	3.3	6.7	7.21	54.0	77
Extra N	170 kg N/ha, 2 fungicides	19.3	1.7	8.7	8.03	54.0	76
Extra N and fungicide	170 kg N/ha, 3 fungicides	13.0	3.3	8.7	8.12	54.8	77
	SED	1.40	0.34	0.46	0.38	0.21	2.4

Experiments E and F. TGW and the incidence of splitting as influenced by the rate of N fertiliser application and treatments affecting ear (sink) size in Landlord and Chariot at Boghall Farm, Midlothian and Hutton Hall Barns, Berwickshire, at harvest 2000

At both sites, the levels of splitting were significantly higher in Chariot than in Landlord (Tables 22 and 23). In the control plants of Landlord (i.e. no ear manipulation treatment) the levels of splitting were 1% or less, but in the control plants of Chariot splitting ranged from 3.8% to 15%. Splitting in Chariot was higher, in all treatments, at Boghall Farm than at Hutton Hall Barns (Tables 22 and 23).

Reducing the ears (sink) size by 50% (i.e. cutting the ears in half) resulted in a significant increase in splitting compared to the control. In both varieties at both sites the TGW of grain from ears cut in half was significantly higher than the TGW in control ears. At Boghall Farm, the effect of reducing ear size on TGW was significantly more pronounced in Landlord than in Chariot. Removal of 50% of the ears increased levels of splitting relative to the control in Chariot, but not in Landlord. Although removal of 50% of the ears had less affect on TGW than did cutting ears in half it tended to have a negative effect on TGW at Boghall Farm.

Table 22. Experiment E: TGW and the incidence of splitting as influenced by the of rate N fertiliser application and treatments affecting ear (sink) size in Landlord and Chariot at Boghall Farm, Midlothian in 2000. SEDs are for comparing any treatment combination.

(a) Landlord

Ear treatment	**Nitrogen** (kg/ha)	Split (%)	TGW (g)
Control	120	0.2	47.0
Control	170	1.0	52.6
All ears cut in half	120	4.0	54.6
All ears cut in half	170	5.7	57.0
50% of ears removed	120	0.8	51.0
50% ears removed	170	0.8	49.3

(b) Chariot

	Nitrogen (kg/ha)	Split (%)	TGW (g)
Control	120	10.8	50.0
Control	170	15.0	50.5
All ears cut in half	120	28.2	53.5
All ears cut in half	170	29.5	53.2
50% of ears removed	120	14.8	47.3
50% of ears removed	170	18.8	48.9
	SED	3.83	1.56

Table 23. Experiment F: TGW and the incidence of splitting as influenced by the of rate N fertiliser application and treatments affecting ear (sink) size in Landlord and Chariot at Hutton Hall Barns, Berwickshire, in 2000. SEDs are for comparing any treatment combination.

(a) Landlord

Ear treatment	**Nitrogen** (kg/ha)	Split (%)	TGW (g)
Control	125	0.7	44.6
Control	175	0.2	44.5
All ears cut in half	125	7.3	51.1
All ears cut in half	175	5.0	51.1
50% of ears removed	125	1.5	45.1
50% of ears removed	175	3.3	46.7

(b) Chariot

Ear treatment	**Nitrogen** (kg/ha)	Split (%)	TGW (g)
Control	125	3.8	41.7
Control	175	10.2	42.3
All ears cut in half	125	12.5	48.0
All ears cut in half	175	17.2	47.5
50% of ears removed	125	14.2	42.2
50% of ears removed	175	11.5	43.3
	SED	2.64	1.07

Relationship between splitting and TGW in Experiments E and F

In Chariot there were significant, positive, relationships between TGW and the percentage of split grains in both Chariot ($P < 0.01$) and Landlord ($P < 0.05$).

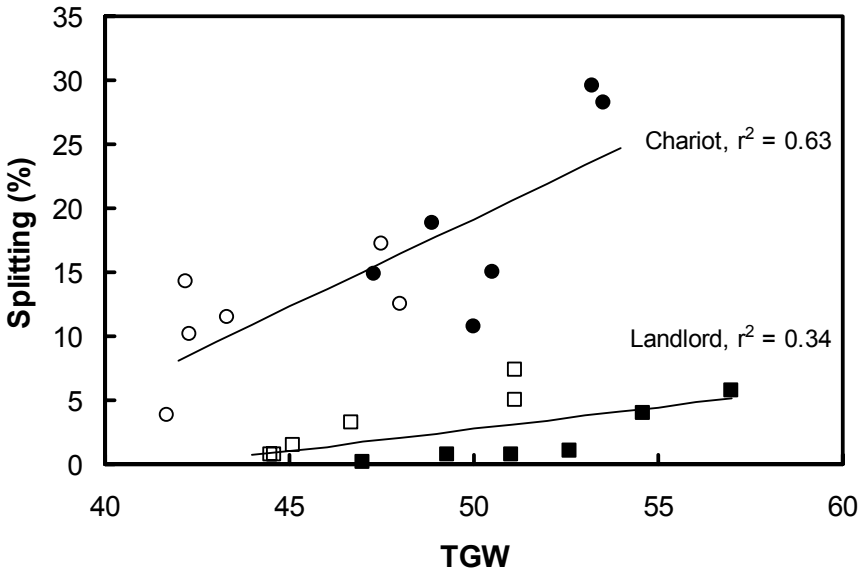


Figure 41. Relationship between splitting and TGW in Landlord (■, □) and Chariot (●,○). Data are from Experiments E (Boghall Farm: ■, ●) and F (Seton West Mains: □, ○).

Weather conditions in 2000 and 2001

In 2000, there were higher monthly sums for temperature at Hutton Hall Barns, Berwickshire than at Boghall Farm, Midlothian (Fig. 42). Likewise, in 2001, there were higher monthly sums for temperature at Seton West Mains, East Lothian than at Boghall Farm, Midlothian (Fig 43). There was less rainfall in March, April and May at the two sites in 2001 than at the two sites in 2000. By contrast, there was more rainfall in July and August at the two sites in 2001 than at the two sites in 2000.

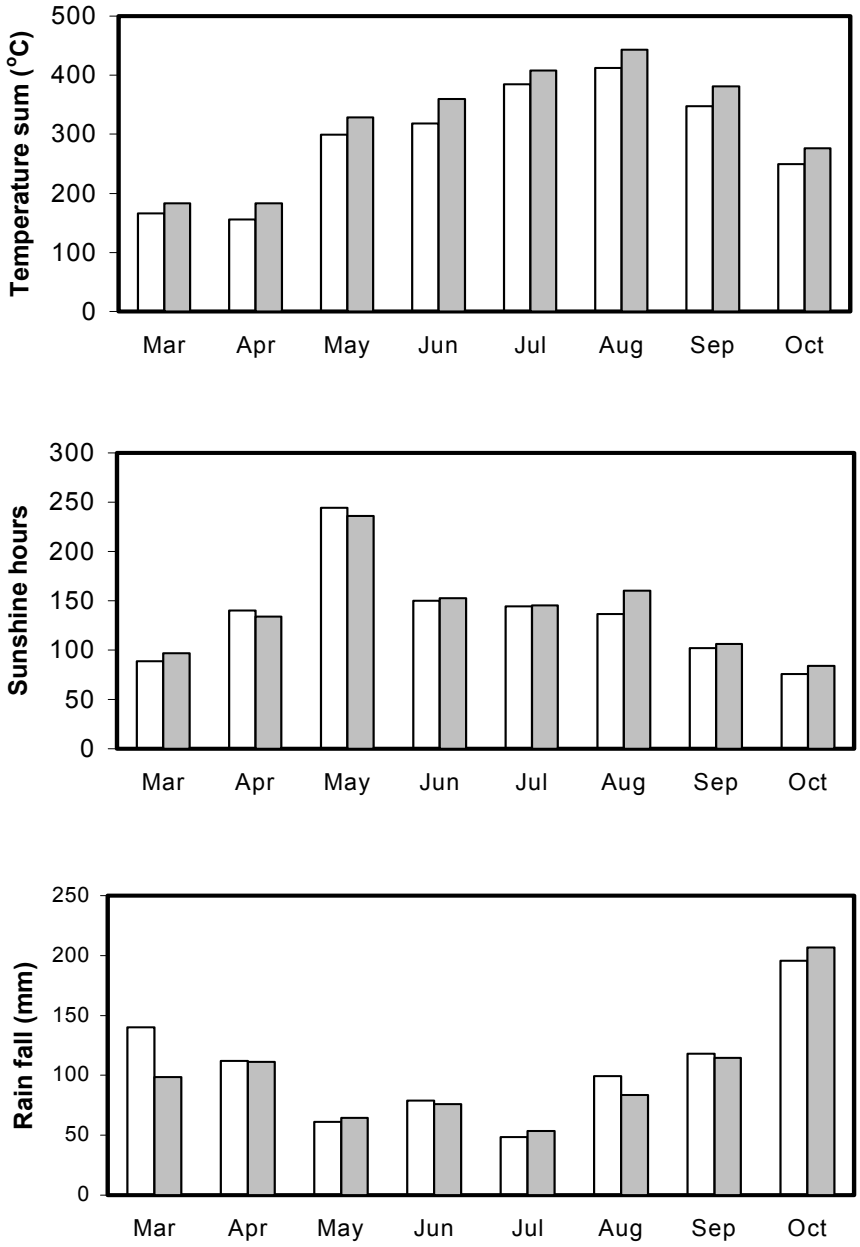


Figure 42. Weather data for Boghall Farm, Midlothian ( ) and Hutton Hall Barns, Berwickshire (■), in 2000

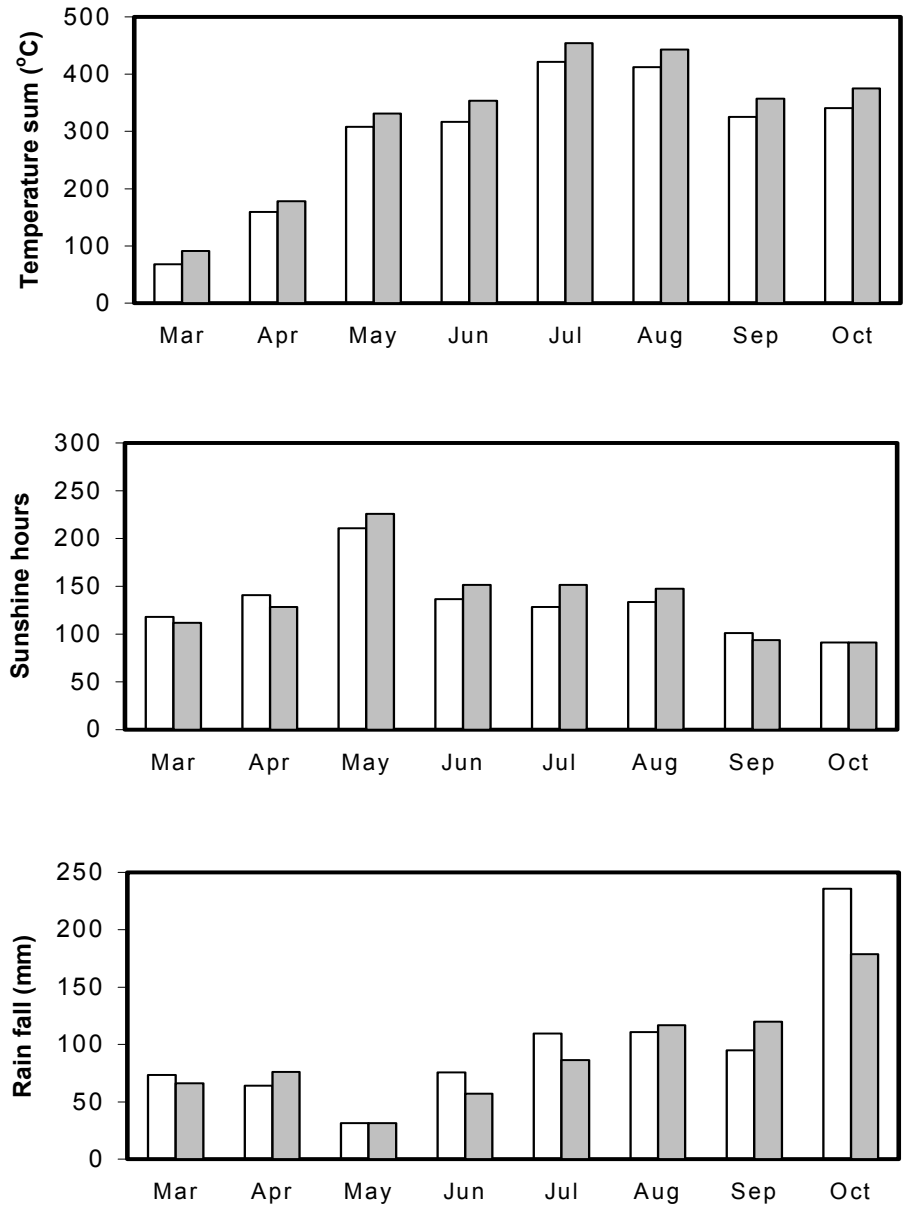


Figure 43. Weather data for Boghall Farm, Midlothian ( ) and Seton West Mains, East Lothian (■), in 2001

Discussion

The results presented above confirm that levels of splitting and gape in malting barley are influenced by environmentally induced changes in a crop's growth patterns during critical phases of development: before anthesis as the husk develops and after anthesis as the grain fills and matures. Although some of the general growth responses e.g. increasing TGW are correlated with splitting, the precise physiological mechanisms of husk and caryopsis development that give rise to these undesirable characteristics are less clear.

The low (or zero) scores for gape and splitting in Landlord grown across a wide range of environmental conditions confirm the very low susceptibility towards splitting of this variety. However, the term resistant to splitting should be avoided, because in some circumstances e.g. as in field Experiments C, D, E and F, splitting did occur in Landlord. Chariot, as expected, was much more prone to splitting than was Landlord. This contrast between varieties is consistent with earlier work and clearly demonstrates the influence of genotype on the incidence of splitting, but to a lesser extent gape.

The effect of pre- and post anthesis treatments on husk dimensions and caryopsis growth was as great in Landlord as in Chariot (Experiments I and II), but splitting was induced only in Chariot. This suggests that Landlord is better able than Chariot to cope with conditions that cause variation in the patterns of husk and caryopsis development.

In a survey of Japanese malting barley, Hamachi, Furusho and Yoshida (1989) reported high levels of husk under-development resulting in exposure of the caryopsis between the palea and lemma (i.e. gape). The length, width and dry weight of palea and lemma were less in cultivars that had a high incidence of gape. Furthermore, plants grown under shade or at low temperatures and subjected to excess moisture from flag leaf to ear emergence had less developed paleas and lemmas and a high incidence of gape. In Experiment II of our investigation, shading before anthesis markedly decreased the length, width and dry weight of both the lemma and the palea in both Landlord and Chariot. This was associated with the occurrence of splitting in Chariot (but not in Landlord) and a small but statistically non-significant increase in the incidence of gape in both varieties.

Contrary to our earlier thoughts, the ratio of grain volume to dry weight does not appear to be a good indicator of splitting risk. A high volume to dry weight ratio was associated with both a high incidence of splitting (Experiment I) and a low incidence of splitting (Experiments II and III). By contrast, there was some evidence to suggest that husk and grain dimensions may be more useful in indicating splitting risk. The maximum combined lemma and palea width relative to caryopsis width ratio at harvest-ripeness was less in

Chariot than in Landlord and less in plants shaded before anthesis than in plants not shaded before anthesis (Experiments I and II) (Table 12). This supports the view that a smaller husk makes grains more vulnerable to splitting. The difference in this ratio between shading treatments was greater in Landlord than in Chariot. However, even when husk development was relatively poor, Landlord grains did not split. The fact that these values were all above 1.00 reflects the fact that little if any gape was found in these samples.

Hamachi and Yoshida (1990) showed that husk thickness varied across Japanese varieties. Low husk thickness and weight were associated with high malt extract and the amount of wrinkling across the husk. They found no difference in husk thickness between grains that exhibited gape and those that did not. However, there is sufficient evidence for the barley industry to consider that there is a link between husk quality and susceptibility to gape and splitting (or skinning) in modern varieties, and that husk quality has been compromised in the improvement of grain malting quality.

Plants that were stressed before anthesis (i.e. shade in Experiments I and II) had increased levels of splitting. This finding is consistent with the results of work on malting barley carried out in Japan by Tsuyuzaki and Tekeda (1989) who reported that low light levels before anthesis followed by high light levels after anthesis caused an increase in the proportion of split grains. Shade before anthesis appeared either to reduce husk growth (Experiment II) or slow down caryopsis dehydration during grain maturation (Experiment I). If shade before anthesis affect endosperm or pericarp cell wall development (early in grain development) such that pathways to grain dehydration have increased resistance, then it is feasible that increased tensions during maturation could make grains more prone to splitting.

The consequences of shade after anthesis may be very different to that of shade before anthesis. Slower caryopsis dehydration, under low-light conditions, in grains with adequately formed dehydration pathways, could result in very low splitting (e.g. Experiment II). Whereas, fast dehydration in a vulnerable variety could make grains more prone to splitting.

An exception to low splitting under low-light conditions would be if the grain-filling period was too long, and grains split because they had filled to excess. This was observed under the cooler grain filling period in Experiment II. However, unless grain size is significantly changed, there would appear to little effect of grain-filling temperature on the incidence of splitting or gape (Experiment III, Table 13).

Another indication of the ways in which shading may affect grain filling can been observed in the transverse sections of grains of Chariot harvested 24 daa (Plates 5 and 6). These show that endosperm cells in grains from the S/US treatment had numerous small (B-type) starch granules whereas endosperm cells in grains

from the US/S treatment had very few small starch granules. The small starch granules start to develop later and continue developing for longer than the larger (A-Type) granules (Palmer, 1989). In wheat, starch composition has an influence on grain cracking i.e. hard wheats crack more cleanly than soft wheats. Consequences of environmentally-induced changes in the proportions of starch granules for splitting in barley are not clear, and further investigations are required to understand if starch-granule type or the overall rate of grain development have a direct link to splitting.

The TGW in the bulked samples used for splitting assessments was affected by shade both before and after anthesis. This is consistent with dry weight accumulation in the grain being affected by assimilate supply both before and after anthesis. In Experiment I, all grains filled under the same conditions i.e. no shade. However, the relatively low TGW in plants that had been shaded before anthesis, may indicate an effect of shade on the amount of assimilate available for grain filling presumably because pre-anthesis shading causes a reduction in the amount of carbohydrate stored in stems. It is also possible that if the development of the vascular tissues of the pericarp were affected by shade, sugar transport to the endosperm through the chalazal cells in the ventral crease would be disrupted. The discrepancy between the dry weight values of individual grains and the TGW values of bulked grain is attributable to the different sampling methods used for the two determinations and to the moisture content of the samples used for the TGW determinations.

Evidence is provided for a relationship between TGW or excessive grain filling and splitting. Glasshouse Experiments I and II showed there was a positive correlation between splitting and TGW in Chariot, but not in Landlord (Fig. 36) and the field Experiments E and F, showed that excessive grain filling was associated with increased levels of splitting in both Chariot and Landlord (Fig, 41). This is supported by Rajasekaran *et al.* (Technical Paper II, in this Report) who report that gape and splitting were associated with TGW, lines of low TGW were less prone to splitting and that larger mid-point grains tend to split.

However, other data suggests that caution must be made before accepting the hypothesis that high TGW alone is associated with splitting. TGW is an average value and a sample with a relatively low average grain weight could contain a small proportion of very large grains, and these grains could be the ones at risk of splitting. This could occur in a sample that includes a high proportion of late tillers or ears have anthesed late. This would appear to be a likely explanation for the negative relationship TGW and splitting in Chariot in Experiment III (Fig. 38). Likewise, data from field Experiments A, B, C and D indicated no positive correlation between TGW and splitting: this could be a consequence of weather conditions or grain weathering (see discussion, page 90).

Therefore, grain size, the distribution of grain sizes within a sample and the relationship between husk development and size of the caryopsis may be equally important in the occurrence of splitting.

Data from Experiment III indicates that plants shaded before anthesis had higher levels of sterile grain sites in each ear than plants that had not been shaded (Table 15). Sterility was also present in Experiments I and II, though to a lesser extent than in Experiments III and IV. Grain sterility would have reduced the number of potential sinks and resulted in assimilate being shared by fewer grains on each ear, possibly leading to an increase in grain size. However, shading before anthesis reduces stem sugars and so grains on ears of shaded plants with high sterility did not necessarily become large. Nevertheless, the possible influence of sterility has to be borne in mind when interpreting the effect of pre-anthesis shade in terms of the relationship between grain weight and splitting.

The results from field experiments indicate a strong seasonal influence on splitting. Although site may influence the risk of splitting, the most likely direct effect is that of weather, but indirect effects on grain size, as influenced by agronomy and crop structure are also important.

The incidence of both splitting and skinning may be affected by the seasonal distribution and frequency of rainfall or by changes in atmospheric humidity. Two reports from Germany (Zimmerman, 1998; Muller and Schildbach, 1998) suggest that both husk and kernel splitting were present at high levels in the barley harvest and were the result of the repeated exposure to heavy rain followed immediately by hot dry weather. Although frequent spells of hot dry weather are not likely in northern Britain, it may be that quite a short spell of hot weather is all that is required to dry grain after a heavy shower. Other factors such as the seasonal distribution of rainfall or the frequency of wet and drying weather (i.e. warmer temperatures combined with wind) are likely to be important.

In our study, the main weather differences between the low splitting year in 2000 and the high splitting year in 2001 were: half the normal rainfall in May followed by double the normal rainfall in July in 2001 than in the same months in 2000 (Figs. 42 and 43).

Hamachi, Yoshino, Furusho and Yoshida (1990) showed that the lemma and palea of malting barley (which grows rapidly between flag leaf appearance to heading) was strongly affected by environmental conditions. Their results suggested that there were interactions between different factors and poor husk development was linked to shading or low temperature combined with excess soil moisture. However, excess moisture is seldom a problem in typical spring barley soils i.e. sandy loams. Our field experiments examined rainfall

only, and results suggest that if splitting is a consequence of poor husk development, then it is more likely to be associated with low, rather than high, water availability during the period when the husk is being formed.

The significance of the effect of spring and summer weather patterns on gape and splitting is confirmed by observations from Rajasekaran *et al*. (Technical Paper 2, in this Report). These authors indicate that in the three seasons 1999, 2000 and 2001 levels of splitting increased tenfold in cultivars when particularly low early summer (May) rainfall was followed by higher rainfall in mid to late summer. Furthermore, low levels of solar radiation in May and June doubled the level of grain splitting and skinning in the same genotypes.

The incidence of skinning in England has also been linked to the seasonal distribution of rainfall and to repeated periods of wetting and drying. For example, skinning was a major problem in southern England in 1979 and 1997, both years with high June rainfall. Froment and South (Technical Paper 3, in this Report) demonstrated that skinning in Chariot was significantly increased by periodic misting of plots in 1999, though misting of plots of Chariot and Optic in 2000 did not have a significant effect on the level of skinning in either variety. Furthermore, the data of Froment and South (Technical Paper III, in this Report) suggest that excessive grain filling increased the risk of skinning.

The hypothesis that gape might lead to splitting was not supported by our data. Gape was recorded at levels of up to 21% in the glasshouse/controlled environment experiments, but not above 4% in the field experiments. In the field, levels of splitting were two to four times higher than those recorded in the glasshouse/controlled environments, and there was little or no correlation between gape and splitting in the field (Fig. 44a, page 91). Although poor correlation between gape and splitting can be partly explained by the high levels of splitting, which would have precluded the measurement of gape in grains that had split badly, it should be concluded that these two characters are often independent of each other, but may share common associations.

Both field experiments and glasshouse-based shading experiments provided strong evidence of the importance of assimilate supply and sink size in producing the physiological conditions that give rise to splitting or gape. In field-grown plots in which grain size was influenced by sink size manipulation (Experiments E and F) there was a strong positive correlation between percentage splitting and TGW in both Landlord and Chariot (Fig. 41).

Supplying crops with high rates of N fertiliser or an increased number of fungicide applications may delay the loss of green leaf area (GLA) or result in other physiological changes that induce splitting, possibly by allowing the excessive filling of some grains to occur. Therefore, protection of leaf canopies from late season diseases such as *Ramularia* or leaf spotting complexes may inadvertently increase the risk of splitting. However, the influence of increasing the rate of N was not always clear in respect of splitting. There could be differential effects of N on tiller production or survival between varieties, with consequences for individual grain weight and splitting as described above. The retention of GLA was not always positively correlated with TGW, (Fig. 44b) and unless TGW of the bulk is excessive it is not necessarily an indicator of splitting risk.

When the results of Experiments A, B, C and D were combined no positive correlation was found between the TGW and the incidence of splitting (Fig. 44c). Moreover, the data for Chariot grown at two sites in 2001 (a year with high levels of splitting) were plotted, a highly significant negative correlation was found (Fig. 44c). One explanation is that growing conditions that led to high levels of splitting also had a negative effect on grain size. An alternative, and perhaps more likely explanation of this negative correlation may be that the split grains had had a substantial proportion of their endosperm lost by weathering or digested away by micro-organisms. Though large and heavy when intact, if they had split some days before harvest they would have been relatively light when the TGW determinations were carried out.

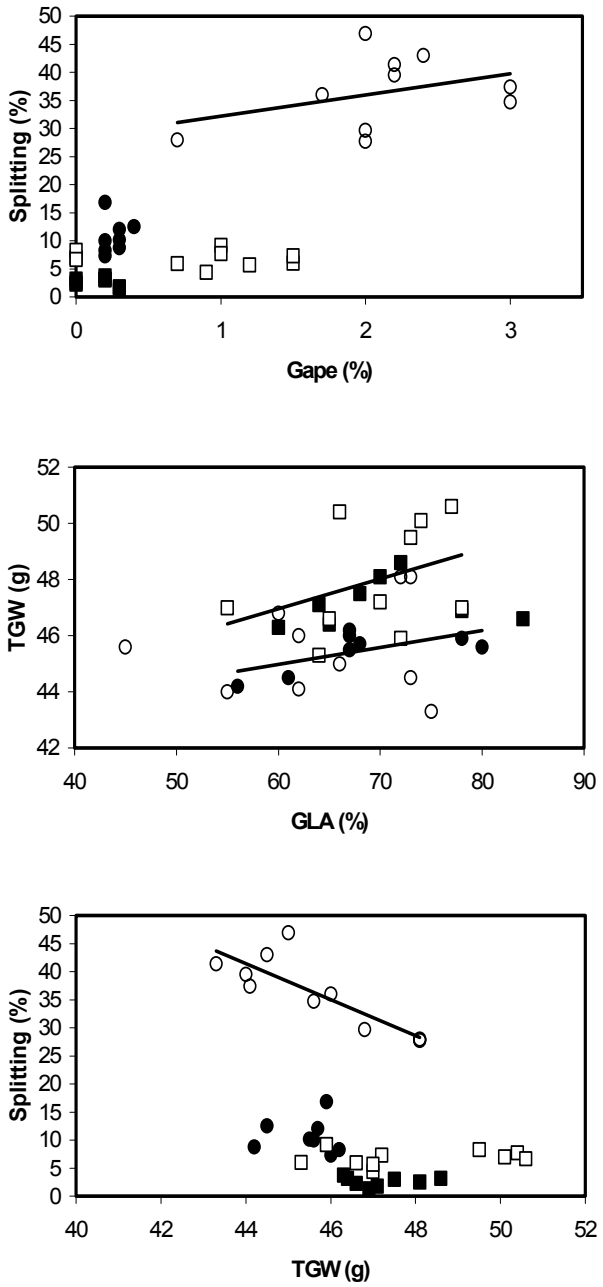


Figure 44. Relationships between (a) splitting and gape, (b) GLA and TGW and (c) splitting and TGW in Landlord (■, □) and Chariot (●, ❍) from years 2000 (■,●) and 2001 (□,❍) in Experiments A, B C and D. Significant correlations are shown for (a) Chariot in 2001 (b) Landlord and Chariot in 2000 and (c) Chariot in 2001.

Data from the glasshouse studies suggested that grain shape may also have a role in grain susceptibility to splitting. The extent to which changes in grain shape and husk/caryopsis proceed together under field conditions remains to be established, though this could be an important step forward in associating splitting (or skinning) with grain development and environmental conditions pre- or post-anthesis.

The use of breeding lines provides the potential to study different genotypes and phenotypes having a wide range of physiological and grain characteristics that may be linked to splitting. A detailed analysis of the genetic controls of gape, splitting and skinning is given in Rajasekaran *et al.* (Technical Paper 2, in this Report). In the current Paper, a comparison was made between two varieties (Landlord and Chariot) and four inbred lines provided by SCRI and thought to have either low or high susceptibility to gape, splitting and skinning (Experiment VI). All plants were shaded before anthesis and grown at either 13^{o}C or 18^{o}C during grain filling in attempt to induce a wide range of scores for gape, splitting and skinning. However, the range of dates for anthesis across the breeding lines and varieties was wider than in previous experiments and to a large extent confounded some of the effects of the treatments. The resultant relatively low incidence of splitting across all breeding lines and varieties made it impossible to draw statistically-based conclusions from the data. For example, atypically low scores for splitting in Chariot were recorded. These may have been a consequence of the fact that anthesis occurred in plants of Chariot two to three weeks after shading was removed. Generally, all lines were as prone as Chariot was to splitting. The lines of low splitting risk (B96-76/24 and B96-76/179) had higher levels of splitting than did Landlord. B96-76/24 and B96-76/179 appeared to have a splitting risk similar to that of Chariot, whereas the lines of high splitting risk (B96-76/96 and B96-76/193) appeared to have a susceptibility to splitting greater than that of Chariot.

The high splitting scores for breeding lines B96-76/96 and B96-76/193 were from ears that anthesed after removal of shade, whereas the lowest scores in lines B96-76/24 and B96-76/179 tended to be from ears that had anthesed in the shade. This could be interpreted as an effect of high light immediately after anthesis inducing a high potential for grain filling. The highest scores for gape occurred in plants with ears that anthesed early, at or before shading was removed. These ears tended to have the highest levels of sterility. This sterility could have induced high levels of gape by increasing the size of remaining grains on the worst affected ears.

Concluding remarks

Some varieties are inherently more vulnerable to splitting than others. Although weather conditions are very important in determining the incidence of splitting, gape and skinning in any one variety. In the field, it is not yet possible to separate out the precise conditions that lead to gape, splitting and skinning: in some circumstances, gape and splitting occur together. Although there is no conclusive evidence to indicate that splitting is consequence of gape, both conditions may share genetic and environmental causes.

In both field and glasshouse conditions, poor conditions for assimilate production and accumulation before anthesis (i.e. when the husk is formed) seems to be a common factor that leads to splitting, especially if conditions favourable to high rates of assimilate production prevail during grain filling and grain maturation. Evidence suggests that a dry May and wet July increase the risk of splitting.

Varieties with large grains do not necessarily gape or split more than varieties with small grains (e.g. Experiments A to F). However, within a variety there is evidence that grain size is an important factor in determining splitting risk. Thus agronomic practices such as the application of extra nitrogen fertiliser or late fungicide treatments may increase the incidence of splitting. It should be remembered that the presence of unusually large grains would not be detected in TGW estimations if the sample contained a larger number of smaller than average grains, or if the large grains had split and had lost some of their starchy endosperm. Grain samples with a high content of split grains might have a wide range of grain sizes. This remains to be investigated.

Husk dimensions were affected by the shading of plants before anthesis. These changes took place in both a resistant variety (Landlord) and a susceptible variety Chariot but were accompanied by splitting only in Chariot. In some conditions however, splitting did occur in Landlord. It may prove very difficult to breed a variety that would not split in any conditions. The relationship between splitting risk and malting quality should be explored further.

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TECHNICAL PAPER 2

CAUSES AND CONTROL OF GAPE, SPLITTING AND SKINNING IN GRAINS OF MALTING BARLEY: GENETIC INVESTIGATIONS

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Introduction

The fact that grain splitting has become a concern in Scotland recently (for example see "The Courier", Harvest Roundup 24/9/01) may reflect an increase in the level of technical specification for malting barley, changes in the cultivars used for malting or because of particular weather patterns. Spring barley is widely grown in Scotland with comparatively little winter barley because the whisky industry uses only the highest quality malting barley. While winter barley out yields spring barley in the field it currently yields less malt and so is seldom used for malt whisky production. In contrast to the area in which the crop is grown, Scotland and Northern England, there are now no breeding programmes for spring barley based in Scotland.

Genetic associations have been identified between markers and the grain traits; gape, splitting and skinning (Rajasekaran et al., 2000). Grain splitting and skinning are examples of traits that are determined by a number of genes whose expression is under considerable environmental influence. A fuller knowledge of the genetic basis of grain gape, splitting and skinning will allow the use of markers to select for new cultivars with the appropriate genotype, and so the desired phenotype, without the requirement for testing over a wide range of environments in unpredictable weather conditions.

Splitting is the visible damage that occurs when the outer tissues of the grain; husk, testa, pericarp and aleurone are ruptured and the starchy endosperm is exposed. Depending on the pre-harvest environment further damage can occur when moisture allows the development of microbial organisms that in turn degrade the starch. Splitting occurs in a range of cultivars but those bred with intensive selection for malting quality and initially evaluated in Southern Britain, where splitting is rarely experienced, are particularly prone to this problem when grown in Scotland. For example, cv Tankard was placed on the UK Recommended List of spring barley in 1996 and was approved by the Institute of Brewing as a malting barley for the North East

region but suffered extensive splitting during the 1997 harvest. This resulted in the cultivar being immediately dropped and therefore wasted money spent by breeders, testing authorities and maltsters as well as loss to growers. Elimination of such cultivars from the Recommended List makes the whole system more efficient as well as providing greater security to growers. There are a number of possible explanations of the phenomenon such as; (1) a reduction of mechanical strength of the grain, (2) a mismatch between the size of the husk (lemma and palea) and the grain (3) a modification of the gum that binds the lemma and palea to the pericarp or (4) poor adaptation to the wetter, cooler growing conditions of Scotland. In practise it may be difficult to completely separate grain responses into these categories.

Skinning is the physical damage that occurs during harvest and post-harvest processing. When the grain is threshed the lemma awn can have sufficient strength to strip part of the lemma at the tip of the grain. This exposes a small part of the testa and may affect the permeability of the grain to water during steeping. Further grain cleaning or transfer operations can result in a more widespread abrasion of the husk from the grain and modification of malting performance (Baxter et al., 1974).

The events in the endosperm following germination support the possibility of splitting occurring as a result of reduction in the mechanical strength of the endosperm cell walls because of selection for improved malting quality. Sectioning of the grain and staining with a fluorescent dye allows the cell walls to be clearly observed with a microscope (Ellis et al., 1992) (Figure 1). As germination proceeds the release of enzymes from the aleurone results in a progressive breakdown of cell walls in the sub-aleurone followed by the inner starchy endosperm. At the end of malting the grain is very fragile and the preparation of sections is difficult mechanically. Selection in the UK for improvement of malting quality by reduction in grain beta-glucan content was started at the Plant Breeding Institute, Cambridge (Barley, 1973). Malting barley has been differentiated from feed barley in studies of endosperm structure as having a characteristic mealy structure as opposed to a steely endosperm (Allison, 1986). In one particular study (Chandra et al., 1999) Derkado, which can show intermediate levels of splitting, was found to have a mealy endosperm while Blenheim, with a lower tendency to split, more frequently had a steely endosperm. The difference in endosperm texture was associated with differences in beta-glucan and protein content although the differences in endosperm composition between genotypes that differ greatly in splitting, e.g. Chariot and Blenheim, were small.

The process of selecting malting barley in breeding programmes started with hand evaluation of grain samples, essentially the process used in the breeding of Proctor (Whitehouse and Whitmore, 1964). Laboratory micro-malting was introduced as a result of research at the Plant Breeding Institute, Cambridge (Whitmore and Sparrow, 1957). The laboratory procedure allowed selection for all the characteristics measured in commercial malting laboratories, principally germination, hot water extract, cold water extract,

soluble nitrogen index, wort viscosity and fermentability. The complexity of genotype with environment interaction (Ellis et al., 1989), the range of testing regimes and genetic variation in malting potential led to the development of sophisticated statistical methods for selection by micromalting (Whitehouse and Whitmore, 1964). The testing of such a wide range of traits was not economically viable in breeding programmes and a number of "small scale tests", so called because they can be applied to the small grain samples, were developed for use in breeding programmes (Ellis et al., 1979). One of the alternative assays of grain structure measured the energy required to mill a grain sample (Allison et al., 1976). The Milling Energy test permits the whole grain structure to be examined and in breeding lines allows the selection of low or high energy requiring lines with great speed. The milling energy of a grain sample depends on the thickness of the husk, the protein composition of the endosperm and the adherence of the protein matrix to the endosperm starch granules (Jagtap et al., 1993).

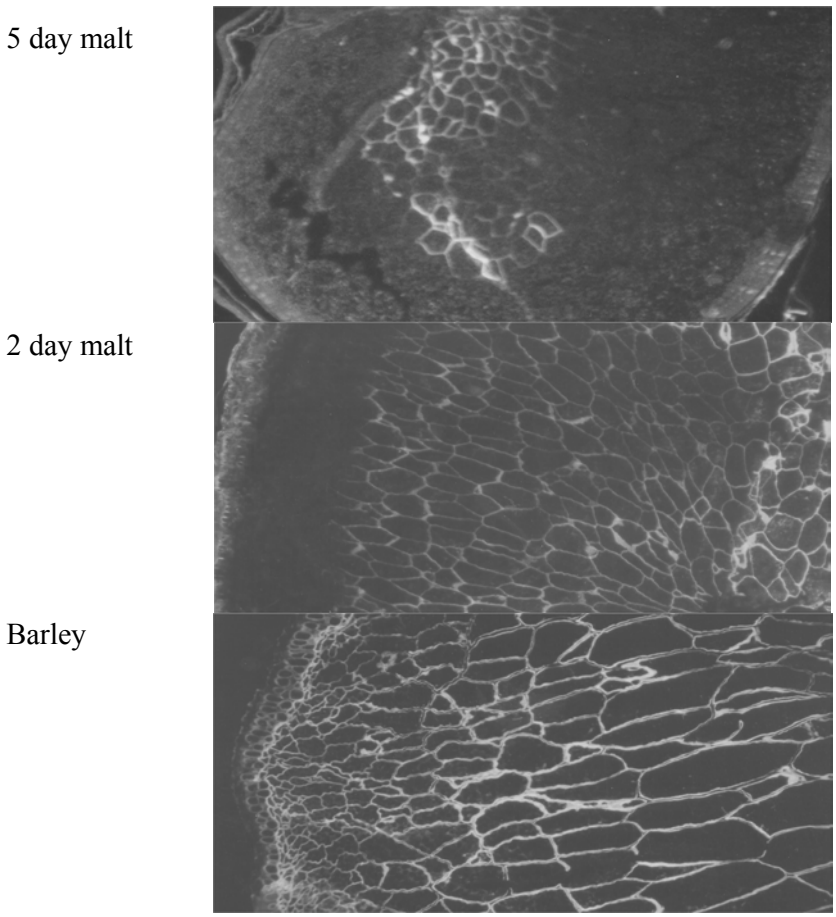


Figure 1. Progressive degradation of cell walls in microscope sections stained with Calcoflor and viewed with UV illumination. From bottom to top, the cell walls of barley are degraded by enzymes from the aleurone so that the wall in the sub-aleurone layer disappear in 2-day malt. In five-day malt all the cell walls have been degraded.

The origin of all mature plant traits in barley lies in the processes of meristem differentiation, development and growth of the plant. When grain is planted the germination of the embryo starts a sequence of events that, through these processes leads to a complete life cycle (Kirby and Appleyard, 1984). Many of the critical stages of grain development and growth occur after anthesis but pre-anthesis events determine yield and grain size. The cooler, moist conditions in Scotland result in barley crops with larger grain size and greater yield by comparison with the same genotypes grown in England (Ellis and Kirby, 1980). If spikelet differentiation and growth occur under markedly different conditions then it is possible that the lemma and palea, the husk, and the ovule may not be matched for size and gaping will occur.

Many "small scale tests" have been developed (Baxter et al., 1990) but they all suffer faults as tools for selection. For example the assay of grain nitrogen content is a, superficially, attractive technique but genetical analysis indicates that at least three loci are involved in determining the trait with the possibility of contrasting alleles in the parents (Ellis et al., 2002). In this situation the possibility of using marker-assisted selection offers obvious advantages in allowing the breeder to take a holistic view of the genetic control of plant traits (Thomas, 2001). Measurement of the target traits, together with other relevant traits, in a population of random inbred lines can reveal much about their genetic control but is of little value in identifying desirable or undesirable lines in the absence of suitable environmental conditions.

Over the past 10 years, a range of molecular marker technologies have been developed that permit the construction of complete genetic maps of single crosses from a species. This provides detailed genotypic information about the individuals within a cross which can be combined with measurements of a trait (phenotyping) to identify genetic regions that are important in the control of the trait. These regions are termed Quantitative Trait Loci (QTL) and can be used to directly select lines to produce a target phenotype. The simplest means of detecting QTL is, for each marker in the genotyping set, to group the data for each character according to their parental genotypes. The means of each marker group can then be compared and tested for statistical significance by analysis of variance or regression. This approach is most useful when markers are very close to QTL and an alternative approach, termed interval mapping, was developed to establish more precise locations of QTL.

We have therefore combined more traditional genetic studies with genetic map construction in a cross between parents that contrast for splitting and related traits to (1) explore the relationship of traits and (2) identify diagnostic markers. We have investigated the genetic control of phenotypic traits in inbred lines from the cross Tankard x Livet by locating genetic markers, such as AFLPs, SSRs, S-SAPs and IRAP on a genetic map and exploring QTL locations.

Materials and Methods - Tankard x Livet Population

One hundred and eighty four random inbred lines (RIL) were developed by multiplying seed from random F_3 plants from a cross between Tankard and Livet in the field in 1998. The F_5 seed from each RIL was then used to generate phenotypic and genotypic data to examine the genetic control of grain splitting and skinning. The 184 RILs and the parents along with controls were grown in replicated plot trials at Dundee in 1999 (F_5 generation) and 2000 (F_6 generation), using a row and column design. Seed was limited in the first year and only sufficient to sow two replicates, each a 3 m^2 plot, at an average seed rate of 180 kg ha^{-1}. In the second year, another two replicate trial was grown but in 7.625 m^2 plots, each containing a constant number of seeds (425 m^2). The trials were sown with a compound fertiliser with a top dressing (Table 1). Each year, the plots were kept free of foliar pathogens by a prophylactic regime as noted in Table 1. The plots were scored for heading date (Head, estimated in days from June 1st when 50% of the plot was at GS 53) and height (Height, measured in cm from the ground to the average position of the collar). When mature, the plots were harvested with a small plot combine, seed dried to a constant moisture, weighed and yield expressed as tonnes ha^{-1} (Yield). The grain was then cleaned and sub-samples measured for the proportions passing over 2.8, 2.5 and 2.2 mm sieves (GT28Sv, GT25Sv and GT22Sv respectively) using a Sortimat (Pfeuffer GmbH, Kitzingen, Germany). All the fractions passing over a 2.5 mm sieve were retained, bulked and mixed and used for all subsequent analyses. The grain fraction greater than 2.5 mm was milled in the Comparamill to determine milling energy, grain dimensions were assessed by image analysis, tested for germination, grain nitrogen assessed by NIR and samples malted according to a standard micro-malting procedure (Swanston et al., 1999).

Extra Fieldwork

The planned fieldwork programme for the project included trials grown in 1999 and 2000. These seasons were not outstanding for the amount of splitting observed in Scotland so a small number of plots were grown, as an ex-project contingency in 2001, according to the protocols of the 2000 trial. The weather experienced in the 2001 season resulted in a very high incidence of splitting. Grain samples were treated and measured as in the previous seasons (see below).

Table 1. Agronomic treatments carried out on the 1999 and 2000 SCRI field trials.

Date	Commodity	Rate/ha	Notes
24-Mar-99	22-4-14-(7.5SO3)	350 kg	N.P.K.fertilizer
09-Apr-99	Stomp 400	5 L	Weed seal
19-May-99	22-4-14-(7.5SO3)	100 kg	N.P.K.top dress
19-May-99	Asset	2 L	Weeds
19-May-99	Amistar Pro	2 L	Fungicide
19-May-99	Unix	0.67 kg	Fungicide
19-May-99	Manganese	3 L	Manganese supplement
23-Jun-99	Amistar Pro	2 L	(Part)Fungicide
20-Mar-00	22-4-14-(7.5SO3)	350 kg	N.P.K.fertilizer
22-Mar-00	Stomp 400	5 L	Weed seal
08-May-00	Gramoxone	5 L	Plot definitions
16-May-00	22-4-14-(7.5SO3)	100 kg	N.P.K.topdress
22-May-00	Amistar Pro	2 L	(Part)Fungicide
22-May-00	Unix	0.67 kg	(Part)Fungicide
22-May-00	Harmony M	60 g	Weeds
24-May-00	Manganese	2.5 L	Mn supplement
30-May-00	Manganese	3 L	Mn supplement
15-Jun-00	Amistar Pro	2 L	Fungicide

N.B. 22-4-14-(7.5SO3) is 22%N, 4%P2O5, 14%K2O, 7.5%SO3

Grain scoring

The completeness of the outer tissues of the grain i.e. the husk, testa/pericarp and aleurone was assessed in scores of splitting, skinning and gape. From each seed lot 100 grains were taken using a seed counter, each seed observed under a 10x binocular microscope and scored for gaping, splitting and skinning. Gape was assessed on a three point scale from (1) lemma and palea overlapping, through (2) lemma and palea abutting to (3) gap between lemma a palea greater than 1 mm (gape). Skinning was scored on a five point scale from (1) complete, (2) 5% skinning, (3) 25% skinning, (4) 50% skinning and (5) 100% skinning. Grain that was classified as gaping was scored for testa/pericarp splitting (see Appendix 1 for detail on scoring these traits). Each grain was placed in a matrix of weighing boats according to the scores for gape and skinning and the classes counted. The grain samples were weighed to permit the calculation of thousand grain weights (TGW).

Seed Size by Image analysis

Cleaned grain samples were assessed with the MARVIN Digital Seed Analyser (GTA Sensorik GmbH, Neubrandenburg, Germany). MARVIN software was used to estimate thousand corn weight (TKW) and average grain length and width (GLength and GWidth respectively) from a 15 cm^{-3} sample of seed i.e. approximately 100 grains. The grain width:length ratio (Wid/Len) was calculated from GWidth and GLength.

Table 2. A complete list of all the traits measured on trials of the Tankard x Livet population grown at SCRI in 1999 – 2001.

Abbreviation	Trait description
*Gape1	Gape greater than 1 mm (%)
*GapeAb	Lemma and palea abutting (%)
*GapeOv	Lemma and palea overlapping (%)
*GE3	Germinative energy (IOB 4 ml method) %
*GE8	Germinative energy (8 ml)
GLength	Grain length by image analysis (mm)
GWidth	Grain width by image analysis (mm)
*GT22Sv	Sieve fraction greater than 2.2 mm (%)
*GT25Sv	Sieve fraction greater than 2.5 mm (%)
*GT28Sv	Sieve fraction greater than 2.8 mm (%)
Head	Date of heading (days)
Height	Height (cm)
LT100Sk	Skinning less than 100%
LT50Sk	Skinning less than 50%
*LT25Sk	Skinning less than 25%
LT5Sk	Skinning less than 5%
Complete	No skinning
Moist	Grain moisture before harvest (%dm)
MillEn	Milling energy (J)
*Split	Visible splitting of the testa/pericarp and exposure of starch (%)
TGW	Thousand grain weight (IOB method) (g)
TKW	Thousand grain weight by image analysis (g)
Wid/Len	Ratio grain width to length
Yield	Plot yield (t/ha)

*These traits with the prefix "An" were analysed as angular transformations of the original data

Data Analysis

Data collected from each trial was analysed by REML to correct for any spatial variation due to row and column effects. Data from each trial was also combined in an analysis over years but, as a different design was used each year, each trial was analysed as a Randomised Complete Block by ANOVA. The data for skinning were combined so that the data analysed (LT25Sk and its angular transform) constituted the sum of the scores "complete" and "5% skinning". As many of the values in the data were scored as percentages and were either <30% or >60%, an angular transformation was applied to all the percentage scores in an attempt to normalise the data. From the combined analysis over years, the significance of the influences of genotype, year and their interaction upon each variate were estimated, as well as the magnitude of each effect. In addition, the overall genotypic means were used to calculate pair-wise correlations between the variates and to examine the multi-variate relationships between the variables by using Principle Components Analysis (PCP) of the correlations. All the data analyses were carried out using GENSTAT for Widows software (GenStat Release 4.21 (PC/Windows NT) Copyright 2001, Lawes Agricultural Trust, Rothamsted Experimental Station).

DNA extraction and marker development

Seed harvested from the 1999 trial was used to isolate DNA to ensure the closest possible match between genotype and phenotype. Because each plot was at the F_5 generation but had been derived from a single F_3 plant, it was highly likely that each genotype would have been heterozygous at a number of loci. To capture this potential variation we therefore sampled 10 seeds from each genotype and grew them in the glasshouse. Young fresh leaves from all the 10 seedlings of each genotype were collected and bulked to represent one gram of total leaf material to isolate genomic DNA using the Phytopure plant DNA extraction kit (Nucleon biosciences, SCOTLAB LTD., UK) with RNase treatment. DNA quality and quantity were checked by running it out on an agarose gel with lambda DNA as control. The RIL population was used to map molecular markers comprising Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphism (AFLP), Sequence-Specific Amplified Polymorphisms (S-SAP), Retrotransposon-Microsatellite Amplified Polymorphism (REMAP), Inter-Retrotransposon Amplified Polymorphism (IRAP) and Sequence Tagged Sites (STS) within some Restriction Fragment Length Polymorphism (RFLP) probes.

A range of previously mapped SSRs Ramsay et al. (2000) were chosen to provide a stratified sample of the barley genome and analysis was carried out in two stages. The first stage was a parental screen to identify polymorphic SSRs and the second stage was to screen the polymorphic SSRs on the whole recombinant population. In each case, PCR was performed in a 10 μl reaction mix consisting of approximately 0.2 μg genomic DNA, 10x PCR buffer with Mg, 0.2 U Taq DNA polymerase (Roche Diagnostics, GmbH), 50 pMol

of labelled forward primer, 50 pMol of unlabelled reverse primer and 200 μM dNTPs. Radio-active labelling was done by incorporating 0.5 μCi of gamma ^{32}P-ATP 4000 Ci / μMol. The primers and PCR protocols for each SSR were those given by Ramsay et al. (2000).

AFLP analysis (Vos et al., 1995) proceeded in two stages, similar to that for SSRs, but with the first stage being to identify primer pairs that revealed the greatest number of polymorphic bands between the parents. Approximately 0.5 μg of genomic DNA was digested with PstI and MseI at 37 ^{o}C for 1 h in Reverse Ligation (RL) buffer consisting one-phor-all buffer, 10 mg/ml BSA, 1 M DTT. Adapter ligation was achieved by adding 10 μl of a ligation mixture consisting PstI (5 pmol) and MseI (50 pmol) adapters, RL buffer, 1U T4 DNA ligase and 10 mM ATP to the restriction digest and incubated at 37 ^{o}C for 3 hours. The adapter sequences were:-

Pst I adapter:	5'-CTCGTAGACTGCGTACATGCAG
	3'-TACTCAGGACTCAT
MseI adapter	5'-GACGATGAGTCCTGAG
	3'-TACTCAGGACTCAT

The pre-amplification was carried out using PstI and MseI primers and the primer sequence are as given below:-

PstI	(P00) 5 '-GACTGCGTACATGCAG-3'
MseI	(M00) 5'-GATGAGTCCTGAGTAA-3'

Pre-amplification of prepared template was performed in 25 μl PCR reaction contained 2 μl of 1:10 diluted ligation mixture, 50 ng PstI primer, 50 ng of MseI primer, 10x PCR buffer, 0.2 mM dNTPs, 0.2U of Taq DNA polymerase. The PCR reaction was performed in a Perkin Elmer 9600 or 9700 thermal cycler using the program consisted 25 cycles of 30 s at 94 ^{o}C, 30 s at 56 ^{o}C, and 1 min at 72 ^{o}C. PCR products were diluted in 180 μl sterile distilled water prior to selective amplification. For selective radioactive amplification only PstI primers were labelled with 10 mci/ml r ^{33}P-ATP(Redivue, Amersham); 16 PstI and MseI primer combinations, containing the same sequences as those used in the pre-amplification (P00 M00 and P01 M01) but with two to three selective nucleotides at the 3' end, were employed in this study. The PCR amplifications were carried out using the following cycling parameters: 1 cycle of 30 s at 94 ^{o}C, 30 s at 65 ^{o}C, and 1 min at 72 ^{o}C followed by 8 cycles in which the annealing temperature decreases 1 ^{o}C each cycle followed by 24 cycles 30 s at 56 ^{o}C, and 1 min at 72 ^{o}C. The PCR product was analysed by running the

samples in 6% denaturing polyacrylamide gels. The electrophoresis conducted by constant power setting of 80 W. The gels were then dried and exposed to X-ray films. Only polymorphic bands were scored as present or absent.

The IRAP primers were synthesised according to the information published by (Kalendar et al., 1999). The IRAP PCR was performed in a 20 µl reaction mixture containing 50 ng DNA, 10 x PCR buffer, 5 mM dNTPs, 0.2 U of Taq DNA polymerase and 30 ng of reverse unlabelled primer and 5 ng of labelled primer. Labelling of forward primer was carried out by using 10 mci/ml r ^{33}P-ATP (Redivue, Amersham), 5 ng primer, 10 x T4- buffer and 0.1 unit T4 polynucleotide kinase and the reaction mixture was incubated for one hour at 37 ^{o}C and the reaction was terminated by incubating the reaction mixture at 70 ^{o}C for 10 min. The PCR reaction program consisted of 1cycle at 94 ^{o}C for 2 min, 1 cycle at 94 ^{o}C for 30 s, 60 ^{o}C for 30 s; ramp + 0.5 Cs- 1 to 72 ^{o}C; 30 cycles of 72 ^{o}C for 2 min + 3 s; 1 cycle at 72 ^{o}C for 10 min.
Products were run in 5% polyacrylamide gels for 2 h and exposed to the films and the polymorphic bands were scored as present or absent.

Primers used:	LTR-Forward 6149	CTCGCTCGCCCACTACATCAA CCGCGTTTATT
	LTR Reverse 6150	CTGGTTCGGCCCATGTCTATG TATCCACACATGGTA

The procedure for the REMAP assay is same as above except that the PCR annealing temperature is 58 ^{o}C.

LTR Reverse 7286	GGAATTCATAGCATGGATAA TAAACGATTATC
Forward 8565	GT(CAC)7

We also adapted the S-SAP protocol of Waugh et al. (1997) to assay the Tankard x Livet population for polymorphisms based on an alternative repeat element in the barley genome (L. Ramsay *pers. comm.*).
Preliminary map construction revealed a number of large gaps between different linkage groups upon the same chromosome. We therefore attempted to join such linkage groups by selecting RFLPs from the integrated cytological maps of (Kunzel et al., 2000) that we predicted would fall in the gaps and act as 'bridging' markers. Primers to amplify part of the RFLP probes were available from GrainGenes (http://wheat.pw.usda.gov/ggpages/Barley_physical/STS.html) and were used to amplify products in the parents. A cold PCR, using a program of 5 min denaturing at 95 ^{o}C followed by 30 cycles of 30 s at 94 ^{o}C, 30 s at 58 ^{o}C, and 30 s at 72 ^{o}C, was used to identify primer pairs amplifying a single product. In such cases, the rest of the original reaction mix was purified from primers, nucleotides and polymerase using EXOSAP-IT (USB Corporation,USA). Sequencing reactions were then performed using the BIGDYE terminator kit

(Applied Biosystems-USA) with the 377 DNA sequencer (Applied Biosystems-USA) according to the manufacturer's protocol. The sequences obtained from each parent were then compared to identify any single nucleotide polymorphisms (SNPs) by using SEQUENCE NAVIGATOR software (Applied Biosystems-USA). Of 20 STS primers only two (MWG836 and MWG564) identified SNPs between Tankard and Livet. In addition to two SNPs, the primers for MWG 896 detected a two bp InDel between the parents so it was decided to analyse the RI population by polyacrylamide gel electrophoresis with a radio-active label. The primers for MWG 564 identified one SNP between the parents, which was then assayed on the populations by sequencing all the individuals and comparing their sequences.

Map Construction

A total of 158 markers were available for constructing a genetic map of the Tankard x Livet population. We found 54 SSR primer pairs that detected 66 loci, 8 AFLP primer combinations and four S-SAP primer combinations identified 49 and 41 polymorphic products on the population respectively. The REMAP and IRAP primers identified three and one polymorphic products respectively and with the addition of the two STS markers, there were a total of 158 polymorphic markers scored on the population. The STS and almost all of the SSR markers were co-dominant, i.e. one can detect products from both parents and therefore identify heterozygotes. In such cases, each individual from the RI population could therefore be classified as being homozygous for the Tankard or Livet allele or heterozygous. The remaining markers were dominant in that a product could only be detected from one parent. In these cases, the absence of a product unambiguously identifies the parental allele that does not produce a product. The presence of a product means that an individual could either be homozygous for the parental allele that produces it or heterozygous, i.e. it is definitely not homozygous for the other parental allele. This means that we could place the scores from the dominant markers for individuals into one of four categories: Tankard versus Not Tankard or Livet versus Not Livet depending upon whether Tankard or Livet produced the product.

JOINMAP 2.0 (Stam and van Ooijen, 1995) was used to construct the genetic map. A LOD of 4.0 was used to form 17 linkage groups of three or more markers each from the 158 scored on the population using the JMGRP option. Fourteen of the groups contained at least one previously mapped SSR marker and could therefore be assigned to specific barley chromosomes. The remaining three groups had no obvious relationship to others and could not therefore be assigned to a specific chromosome. For each linkage group, JMMAP was then used to order the markers using a JUMP value between 2 and 5 to exclude markers that did not fit the order after the second round. This produced maps with: chromosome 7H represented by one group covering 186 cM: chromosomes 1H, 2H, 3H, 4H and 6H each represented by two linkage groups covering 183, 201, 226, 130 and 116 cM respectively; chromosome 5H represented by three groups covering

76cM; and the three unassigned groups covered 70 cM. Thus, the genetic map of the population resulted in a genome coverage of 1188 cM and 118 of the markers could be located with confidence.

QTL Mapping

The genotypic data was combined with the overall phenotypic means for each trait and scanned for QTLs using PLABQTL (Utz and Melchinger, 1996). PLABQTL uses a regression approach to carry out Compound Interval Mapping (CIM) with selected markers as co-factors to detect QTL. We used the program default values to select significant co-factors in the initial detection step and then carried out a stepwise elimination of co-factors that were not associated with QTLs that exceeded the default LOD threshold of 2.5. We then carried out permutation of the data with the remaining co-factors to establish the threshold LOD for a genome-wide error rate of 5%. The data were then re-scanned with the new LOD threshold and co-factors associated with QTL that did not exceed this LOD were eliminated in a stepwise manner. At the end of this process, the remaining QTLs were deemed to be significant and included in all subsequent analyses. All the initial QTL detection was carried out assuming that all the genetic effects were additive and, once these were detected, the significance of any epistatic or dominance parameters involving the QTLs was tested by varying the MODEL statement in the input file for PLABQTL. The amount of the phenotypic variation detected by the fitted QTLs was estimated by the CROSS statement in the PLABQTL, which carries out a cross-validation analysis to reduce bias (Melchinger et al., 1998). Finally, the QTLs were also fitted to the means from each trial and their significance in each year tested as well as the interaction of each QTL with environment. As we only had data from two environments and the initial selection of QTL was on the overall mean, this approach will detect QTL x environment magnitude effects but is of limited value. The significance of QTLs in each environment, as well as the overall means, is perhaps more relevant to this study in identifying robust QTL.

Derkado x B83-12/21/5 Population

A large body of data had already been collected on the Derkado x B83-12/21/5 Doubled Haploid (DH) population, including GT25Sv under a previous HGCA project and SEERAD funding (Meyer et al., 2001). Samples had been retained from a number of the trials in which the DH population and controls had been grown and the full grain scoring analysis described above was carried out upon the samples from the 1995 trial. In addition, samples from the 1997 SCRI trial were assessed for Gape1 and samples for both trials measured for TKW, GLength, GWidth and Wid/Len. A genetic map had already been constructed from over 300 molecular markers scored on the population (Meyer et al., 2002). PLABQTL was used to scan this genetic map for QTLs affecting the above phenotypic traits using the same approach as described above for

the Tankard x Livet population, except that no dominance parameters could be fitted as the population was completely inbred.

Results

Phenotype assays

A wide range of measurements was made on the field trials and the grain samples derived from them. After data analysis it was obvious that not all were germane to this report and while they are listed above (Table 2) for completeness they will not be presented in full. For example, the splitting of grain from the 1999 plots was scored as being on the dorsal, lateral or ventral aspects of the grain, but it became obvious from these scores that splitting occurred most commonly on the lateral face of the grain, especially in the gap caused by gape between the palea and lemma. Dorsal and ventral splitting was much less frequent and resulted in data that were more difficult to analyse, so the three categories were combined into a single score of splitting (Split). In addition, notes were taken of the level of heterogeneity in the field plots and plots were rogued, as appropriate, to ensure that only high quality data and grain samples were used in further analyses. After harvest the grain from the 1999 plots was cleaned with records taken of the weight lost in cleaning but, as analysis of these data revealed no genetic differences, this assay was omitted in 2000. The 1999 and 2000 plots were scored for the date of heading (Head), plant height (Height), grain moisture (Moist) before harvest, as a measure of relative maturity, and Yield.

Control varieties were grown in all the trials and their scores for skinning (summarised in Figure 2.) ranged from just under 60 to over 80 with Optic, Decanter and Chalice showing high scores for the 2000 trial which may suggest that these varieties are prone to skinning damage. AnGape1 was more variable with Chime in 2000 showing less than 5 while Cooper and Tankard in 1999 both scored over 45. Splitting scores were more dependent on growing season with 2001 showing higher scores than either 1999 or 2000. The highest splitting scores were 23 (angular transform of the original score), seen in Tankard and Chariot in the 2001 plots.

The results from Analysis of Variance over the 1999 and 2000 trials enable us to visualise the basic population statistics i.e. the parental means and the minima, means and maxima of the RILs. We can use the error variation to establish whether or not the parents were significantly different. We can also compare the range between the RIL minima and maxima to that of the parental range to determine whether or not we observed lines that were better than the higher scoring parent and/or worse than the lower scoring parent

(transgressive segregation). In addition, we expect that the mean of the RIL population would be equal to half the parental difference (mid-parent) if the genetic control of a character is due to simple additive effects of individual genes (Kearsey and Pooni, 1998). All the population statistics are shown in Figures 3a and 3b.

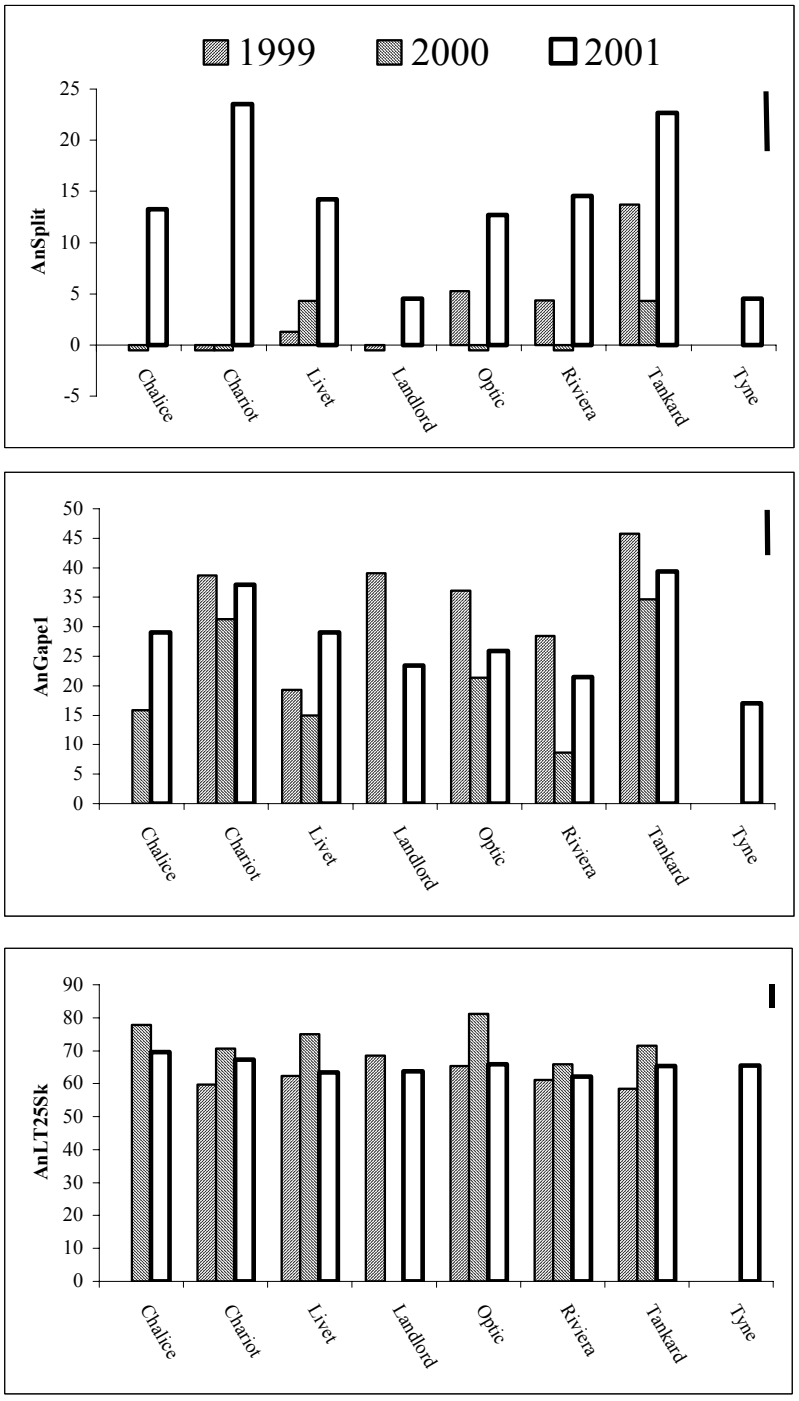


Figure 2. The mean scores for skinning, gape and splitting for control varieties grown in SCRI plots in 1999 – 2001. Some cultivars were omitted from individual trials. LSD for genotypes indicated by the vertical bar on the right of the plot.

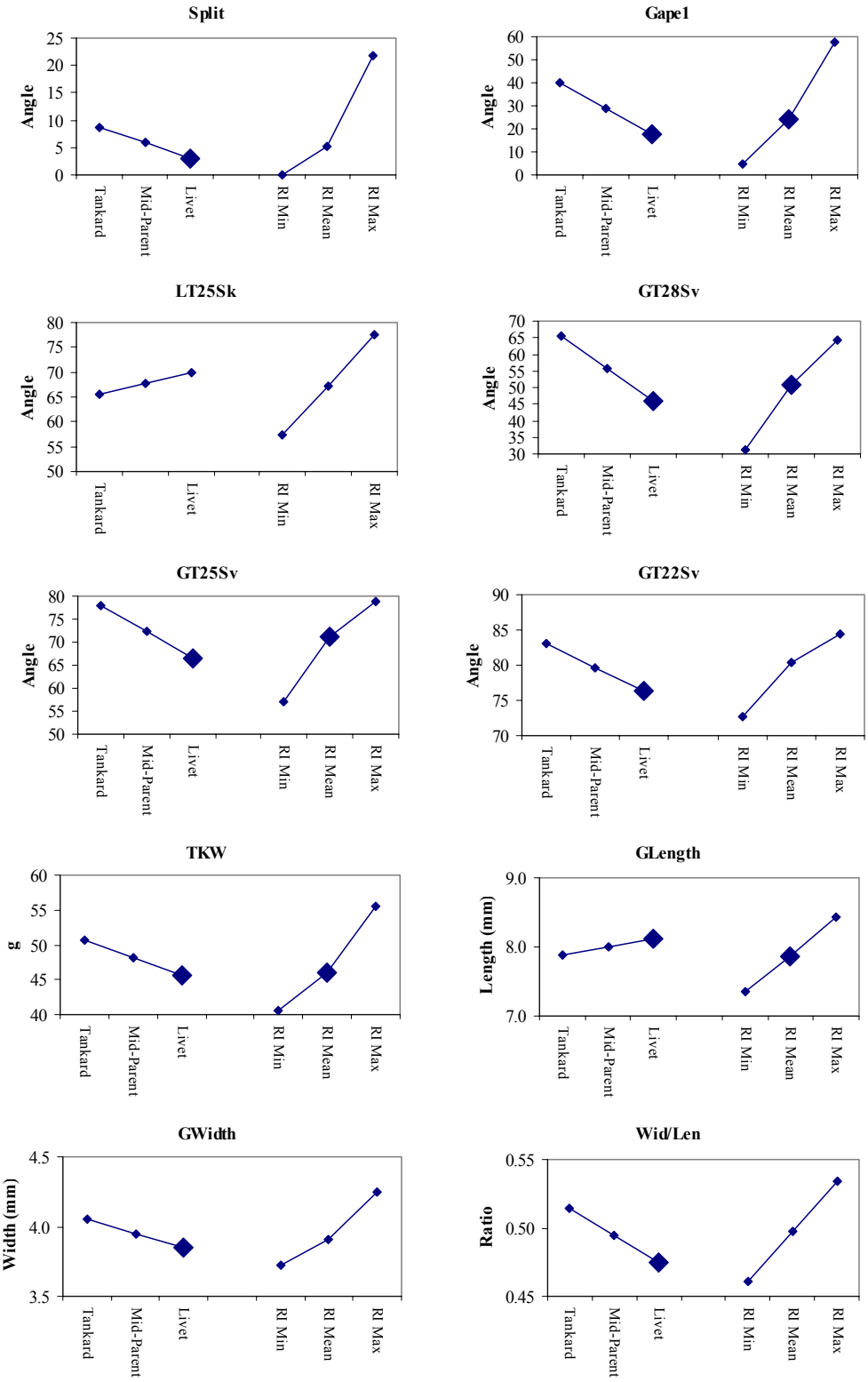


Figure 3. . Overall means of parents and extreme RILs for 10 grain characters measured on trials grown in 1999 and 2000 together with mid-parental and RIL minimum, mean and maximum values. The larger symbols indicate (i) a significant differences between Tankard and Livet or (ii) that the RI mean is significantly different from the Mid-parent. a) Grain traits. Continued below.

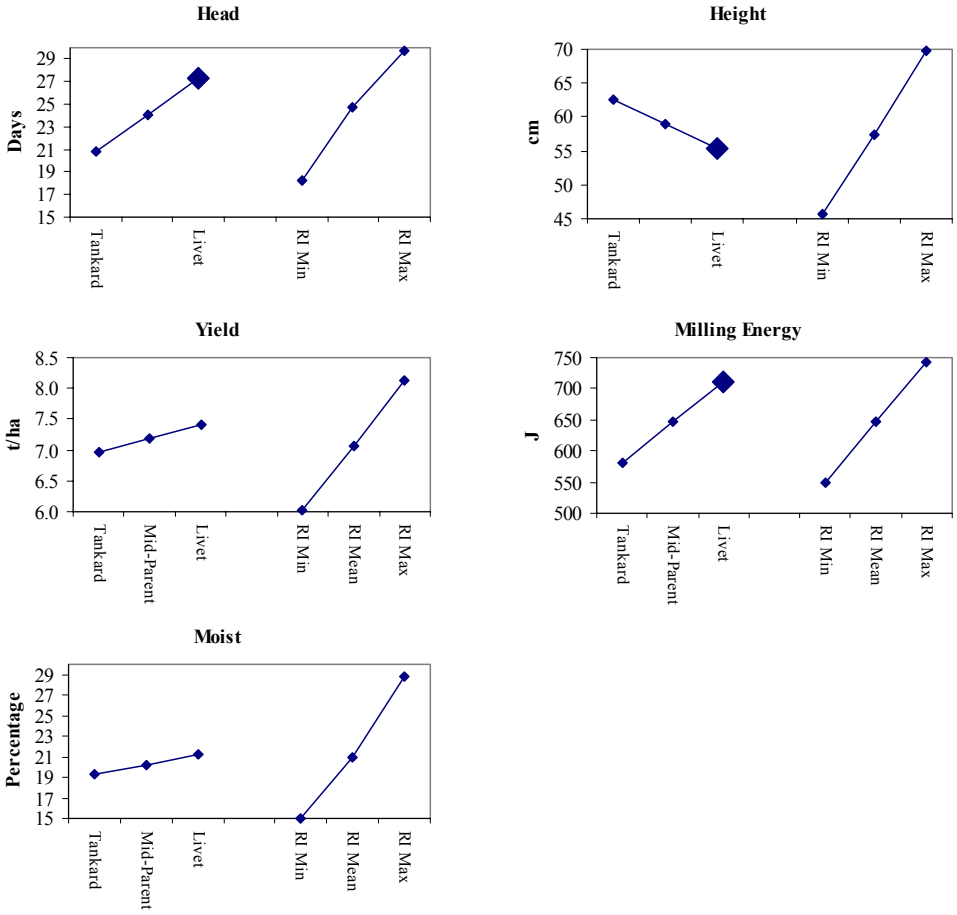


Figure 3b. Agronomic traits and milling energy.

Considering the parental performance, Figure 3a shows that Tankard had a significantly higher level of grain splitting and gape than Livet. The two parents appear to have considerably different grain dimensions with Livet producing grain that is, on average, significantly longer and narrower than that of Tankard, resulting in a significantly lower width to length ratio. The differences in dimensions are reflected in the sieving fractions as a significantly greater proportion of Tankard grain passes over 2.8, 2.5 and 2.2 mm sieves. The difference between the parents for less than 25% grain skinning was not, however, significant. The milling energy test showed that Livet had significantly harder grain than Tankard (Figure 3b). In addition, Livet was significantly later and shorter than Tankard but the observed differences in yield and grain moisture content 10 days prior to harvest were not significant (Figure 3b). The mean of the RIL population was significantly different from the mid-parental value for 4 of the 15 characters presented in Figures 3a and 3b, indicating the

presence of significant non-additive effects in the genetic control of Gape1, GT28Sv, GT25Sv, TKW and GLength. Given that the each line of the population was derived from a single F_3 plant, this could reflect dominance or epistatic effects.

Correlations between the traits

The significant correlations in the grain samples from the 1999 trial between the splitting scores and the other traits varied between r = 0.61 (AnGape1) and r = - 0.41 (AnLT5Sk) (Table 3). This indicates an association between splitting and both gape >1 mm and skinning but does not show a causal relationship between splitting and these traits. Very similar correlations were found between the splitting scores and AnGape1 (r = 0.69) and AnLT5Sk (r = -0.56) in data from the 2000 trial (Table 4) implying consistency over seasons and the extent of genotype with environment interaction for these traits is examined below (Figure 4). In data from the 1999 field trial it was possible to carry out a preliminary examination of the relationships between grain traits and a range of characters that would be subject to selection in a breeding programme. In a large scale breeding programme, where an F_2 population could number in the order of 10^4 plants, even weak correlations between traits may have considerable effects on the outcome. Grain yield showed positive correlations with GapeAb, GE4 and MillEn and negative correlations with Gape1, Moist and Head. Attempts at yield improvement may result in more cultivars that lack overlap between the lemma and palea. Height showed a positive correlation with Gape1, GT25Sv, GT28Sv, GE8 and TGW and a negative correlation with GapeOv, LT5Sk, GT22Sv and Head. Thus if attempts to improve performance result in taller plants there should be synchronized selection for GapeOv, LT5Sk, GT22Sv and Head. Heading date would be expected to have a wide range of effects, because of coincident effects on ear growth and grain growth, and showed the largest correlations with GT28Sv (r = -0.45) and TGW (r = - 0.42).

Among a wide range of other significant correlations the values of r were low but consistency over seasons might highlight interesting relationships. Yield was negatively correlated with heading date in both seasons but the negative correlation between Yield and AnGape1 was not consistent.

Table 3. Correlations between the traits measured in the 1999 SCRI trial. Significant correlations are given in bold.

	AnSplit	*AnDoSp*	*AnLaSp*	*AnVeSp*	*AnGape1*	*AnGapeOv*	*AnGapeAb*	*AnLT5Sk*	*AnLT25Sk*	*AnLT50Sk*	*AnLT100Sk*	*GT28Sv*	*GT25Sv*	*GT22Sv*	*TGW*	*TKW*	*GLength*	*GWidth*	*Wid/Len*	*Head*	*Height*	*Yield*	*MillEn*	*Moist*	*AnGE4*
AnSplit																									
AnDoSp	**0.42**																								
AnLaSp	**0.30**	**0.33**																							
AnVeSp	**0.98**	**0.29**	**0.20**																						
AnGape1	**0.61**	0.16	**0.25**	**0.62**																					
AnGapeOv	**-0.56**	-0.16	**-0.19**	**-0.57**	**-0.93**																				
AnGapeAb	-0.09	-0.01	-0.12	-0.08	0.00	**-0.32**																			
AnLT5Sk	**-0.41**	-0.14	-0.10	**-0.40**	**-0.69**	**0.69**	-0.15																		
AnLT25Sk	-0.05	-0.14	-0.03	-0.02	**0.20**	**-0.23**	0.09	0.07																	
AnLT50Sk	-0.04	-0.18	-0.15	0.00	0.12	-0.16	0.10	0.00	**0.70**																
AnLT100Sk	-0.04	-0.18	-0.05	-0.01	0.05	-0.06	-0.02	0.03	**0.50**	**0.61**															
GT28Sv	**0.35**	0.15	0.08	**0.34**	**0.44**	**-0.48**	**0.20**	**-0.37**	-0.01	0.10	-0.03														
GT25Sv	**0.24**	0.15	0.04	**0.22**	**0.37**	**-0.40**	0.16	**-0.30**	0.04	0.13	-0.02	**0.89**													
GT22Sv	0.17	0.12	0.02	0.14	**0.28**	**-0.28**	0.10	**-0.21**	0.06	0.10	-0.03	**0.71**	**0.92**												
TGW	**0.28**	0.09	-0.09	**0.28**	**0.37**	**-0.38**	0.13	**-0.33**	0.11	**0.31**	**0.20**	**0.65**	**0.58**	**0.45**											
TKW	**0.26**	0.12	-0.08	**0.25**	**0.34**	**-0.37**	0.12	**-0.37**	0.11	**0.28**	0.15	**0.61**	**0.55**	**0.43**	**0.88**										
GLength	-0.13	-0.09	**-0.24**	-0.11	0.05	-0.09	0.10	-0.06	**0.31**	**0.44**	**0.35**	**0.21**	**0.20**	0.14	**0.66**	**0.67**									
GWidth	**0.23**	0.17	-0.06	**0.21**	0.10	-0.13	0.11	-0.17	-0.15	0.08	0.01	**0.52**	**0.41**	**0.32**	**0.70**	**0.76**	**0.41**								
Wid/Len	**0.30**	**0.22**	**0.20**	**0.27**	0.02	-0.01	-0.02	-0.06	**-0.44**	**-0.41**	**-0.36**	0.17	0.09	0.09	**-0.18**	-0.15	**-0.75**	**0.30**							
Head	**-0.22**	-0.01	-0.02	**-0.23**	**-0.34**	**0.30**	0.07	**0.22**	-0.15	**-0.20**	-0.16	**-0.45**	**-0.38**	**-0.31**	**-0.42**	**-0.46**	**-0.29**	**-0.30**	0.08						
Height	0.08	0.11	0.04	0.06	**0.22**	**-0.23**	0.05	**-0.21**	-0.03	0.07	-0.02	**0.50**	**0.46**	**0.36**	**0.45**	**0.46**	**0.26**	**0.31**	-0.04	**-0.50**					
Yield	**-0.22**	-0.08	-0.10	**-0.21**	-0.14	0.09	0.15	0.04	0.08	0.03	0.02	-0.02	0.01	0.00	-0.01	0.00	0.15	-0.03	-0.18	**-0.22**	0.13				
MillEn	**-0.26**	-0.13	**-0.20**	**-0.25**	**-0.28**	**0.30**	-0.08	**0.19**	0.01	0.06	0.12	**-0.29**	**-0.25**	**-0.21**	0.02	0.02	**0.31**	-0.08	**-0.38**	0.12	0.04	**0.22**			
Moist	-0.09	-0.05	-0.05	-0.09	-0.04	-0.01	0.17	0.01	-0.12	-0.09	-0.06	-0.09	-0.12	-0.12	0.01	-0.01	0.00	0.02	0.01	**0.45**	-0.08	**-0.24**	0.09		
AnGE4	0.12	0.02	-0.05	0.13	0.06	-0.03	-0.14	-0.04	0.02	-0.05	0.02	0.06	0.10	0.10	0.14	0.14	0.06	0.06	-0.03	**-0.26**	0.10	0.16	-0.02	**-0.28**	
AnGE8	0.01	-0.04	-0.09	0.02	0.11	-0.10	-0.06	-0.03	**0.41**	**0.48**	**0.40**	**0.19**	**0.21**	**0.19**	**0.29**	**0.32**	**0.35**	0.09	**-0.30**	**-0.44**	**0.23**	0.11	0.09	**-0.31**	**0.34**

Table 4. Correlations between the traits measured on the 2000 SCRI field trial. Significant correlations given in bold.

	AnSplit	*AnGape1*	*AnGapeOv*	*AnGapeAb*	*AnLT5Sk*	*AnLT25Sk*	*AnLT50Sk*	*AnLT100Sk*	*GT28Sv*	*GT25Sv*	*GT22Sv*	*TGW*	*TKW*	*GLength*	*GWidth*	*Wid/Len*	*Head*	*Height*	*Yield*	*MillEn*	*Moist*
AnSplit																					
AnGape1	**0.64**																				
AnGapeOv	**-0.56**	**-0.94**																			
AnGapeAb	**0.29**	**0.61**	**-0.83**																		
AnLT5Sk	**-0.49**	**-0.80**	**0.86**	**-0.72**																	
AnLT25Sk	-0.15	**-0.20**	**0.22**	**-0.19**	**0.39**																
AnLT50Sk	-0.03	0.01	-0.04	0.08	0.06	**0.42**															
AnLT100Sk	0.03	-0.09	0.09	-0.08	0.07	**0.27**	**0.22**														
GT28Sv	**0.25**	**0.51**	**-0.58**	**0.53**	**-0.53**	**-0.34**	0.01	-0.17													
GT25Sv	**0.25**	**0.50**	**-0.57**	**0.54**	**-0.57**	**-0.29**	0.01	-0.12	**0.91**												
GT22Sv	**0.21**	**0.45**	**-0.52**	**0.50**	**-0.55**	**-0.25**	-0.01	-0.07	**0.75**	**0.91**											
TGW	**0.24**	**0.46**	**-0.54**	**0.52**	**-0.54**	**-0.21**	0.11	-0.08	**0.66**	**0.60**	**0.54**										
TKW	**0.27**	**0.52**	**-0.60**	**0.56**	**-0.64**	**-0.29**	0.03	-0.17	**0.73**	**0.73**	**0.68**	**0.85**									
GLength	-0.06	0.09	-0.14	**0.19**	-0.18	0.01	0.10	0.00	**0.19**	0.15	0.14	**0.56**	**0.58**								
GWidth	**0.26**	**0.39**	**-0.45**	**0.42**	**-0.49**	**-0.34**	-0.04	-0.13	**0.72**	**0.69**	**0.65**	**0.72**	**0.85**	**0.39**							
Wid/Len	**0.27**	**0.23**	**-0.23**	0.16	**-0.24**	**-0.30**	-0.13	-0.10	**0.41**	**0.43**	**0.41**	0.05	0.14	**-0.64**	**0.45**						
Head	-0.05	**-0.48**	**0.55**	**-0.51**	**0.53**	**0.25**	-0.05	0.16	**-0.63**	**-0.52**	**-0.46**	**-0.55**	**-0.57**	**-0.34**	**-0.45**	-0.05					
Height	-0.01	**0.31**	**-0.42**	**0.46**	**-0.40**	**-0.23**	0.03	-0.06	**0.51**	**0.45**	**0.44**	**0.45**	**0.51**	**0.22**	**0.48**	0.18	**-0.53**				
Yield	-0.14	-0.02	-0.02	0.08	0.00	0.04	0.00	0.07	0.09	-0.01	-0.04	**0.19**	0.09	**0.33**	0.09	**-0.26**	-0.17	0.07			
MillEn	**-0.32**	**-0.55**	**0.56**	**-0.40**	**0.51**	**0.23**	-0.06	0.05	**-0.56**	**-0.54**	**-0.51**	**-0.43**	**-0.50**	0.00	**-0.50**	**-0.41**	**0.47**	**-0.43**	0.11		
Moist	0.14	0.06	-0.05	0.06	-0.06	-0.06	-0.03	0.08	0.04	0.07	0.05	0.03	-0.01	0.03	0.00	-0.04	0.17	-0.06	0.11	0.11	
AnGE4	0.06	0.16	**-0.18**	0.13	**-0.22**	-0.07	0.13	0.01	**0.29**	**0.29**	**0.30**	**0.46**	**0.39**	**0.22**	**0.34**	0.07	**-0.31**	**0.38**	-0.05	**-0.37**	-0.12

Table 5. Correlations between the overall genotype means of the traits measured on the 1999 and 2000 SCRI field trials. Significant correlations given in bold.

	AnSplit	*AnGape1*	*AnLT25Sk*	*GT28Sv*	*GT25Sv*	*GT22Sv*	*TGW*	*TKW*	*GLength*	*GWidth*	*Wid/Len*	*Head*	*Height*	*Yield*	*MillEn*	*Moist*
AnGape1	**0.73**															
AnLT25Sk	-0.12	0.00														
GT28Sv	**0.38**	**0.51**	**-0.20**													
GT25Sv	**0.37**	**0.49**	-0.17	**0.93**												
GT22Sv	**0.33**	**0.42**	-0.14	**0.79**	**0.93**											
TGW	**0.37**	**0.47**	-0.04	**0.65**	**0.62**	**0.56**										
TKW	**0.38**	**0.49**	-0.08	**0.68**	**0.65**	**0.59**	**0.93**									
GLength	-0.07	0.08	**0.21**	0.15	0.12	0.08	**0.64**	**0.62**								
GWidth	**0.30**	**0.26**	**-0.27**	**0.68**	**0.64**	**0.58**	**0.78**	**0.84**	**0.39**							
Wid/Len	**0.30**	0.12	**-0.41**	**0.36**	**0.36**	**0.36**	-0.07	0.00	**-0.73**	**0.34**						
Head	**-0.19**	**-0.48**	0.01	**-0.54**	**-0.49**	**-0.42**	**-0.53**	**-0.56**	**-0.29**	**-0.44**	-0.03					
Height	0.14	**0.30**	-0.13	**0.52**	**0.48**	**0.44**	**0.43**	**0.46**	0.15	**0.40**	0.15	**-0.50**				
Yield	**-0.21**	-0.06	0.14	0.02	0.02	-0.01	0.10	0.08	**0.25**	0.01	**-0.25**	**-0.21**	**0.19**			
MillEn	**-0.41**	**-0.50**	0.14	**-0.53**	**-0.51**	**-0.47**	**-0.31**	**-0.35**	0.16	**-0.41**	**-0.47**	**0.37**	**-0.32**	**0.18**		
Moist	0.02	-0.06	-0.06	-0.01	-0.06	-0.09	0.01	-0.01	0.05	-0.02	-0.08	**0.33**	-0.12	-0.18	0.13	
AnGE4	0.17	0.15	0.04	**0.23**	**0.24**	**0.26**	**0.39**	**0.36**	0.17	**0.31**	0.05	**-0.32**	**0.29**	0.02	**-0.27**	**-0.27**

Components of variation

ANOVA was used to model the proportions of the phenotypic variation attributed to genetic, environmental, GxE and Error effects. The trait with the lowest proportion of genetic variation was Moist (Figure 4) and that with the highest was Glength. In the 1999 plots grain moisture varied between 16% to 44% of grain dry weight but in 2000 the range was much less (14% to 21%) reflecting a rapid drying period just before harvest. In contrast to GLength, the genetic component of Yield was less than 5% of the total, while the estimate for Glength was 65%. Past success in yield improvement indicates that the genetic potential exist for the improvement of all traits including AnLT25Sk. Apart from Moist, the largest environmental effects were seen in AnGT28Sv, AnLT25Sv and TGW with low GxE in AnLT25Sk and TGW and low error in AnGT28Sv and TGW. The large proportion of variability attributed to environmental effects for these traits reflects a marked change in grain size between 1999 and 2000 (overall mean TGW 49.8 g and 43.2 g respectively) and a relatively high correlation between TGW and GT28Sv (r = 0.65).

The ranking of the proportions of variation for sieve fractions is interesting because while the proportion attributable to GxE is not greatly different the genetic component and error increases with fraction size. The environmental effect, in contrast to the other components, is greatest for the largest fraction (AnGT28Sv). This data can be modelled by postulating that AnGT25Sv represents grain from the mid-portion of the main-stem ear. In favourable situations super-optimal grain filling at the positions of the largest grains on the main-stem ear will increase the proportion of grain in AnGT28Sv. This class of grain may be most prone to splitting if grain filling stresses the structure of the grain. Grain in the AnGT22Sv fraction is likely to be derived from the distal positions on the main-stem ear and from any position on tiller ears. Grain in these positions will be shorter as well as thinner due to competition from larger grain for carbohydrates. This class of grain is less likely to split than the average grain size. The achievement of variety improvement depends on successful selection that is best attained in traits with a high proportion of genetic variation and low environment, G x E and error components. In respect to Split there would appear to be sufficient genetic variability to permit progress, given an efficient selection process.

Multivariate analysis

Multivariate analysis (PCO) allows the relationship of many traits to be explored at the same time. While this overcomes a major limitation of uni-variate methods such as ANOVA and correlation the outcome has to be treated with care as robust methods for assessing the significance of differences are not available. From Figure 5. it can be seen that the traits fall into two main groups based on differences in the first two principle components (accounting for 39% and 17% of the total variation respectively). The third component, accounts

for 9% of the total variation, and differentiates traits within these groups e.g. height from traits related to thousand corn weight and in turn AnSplit and AnGape.

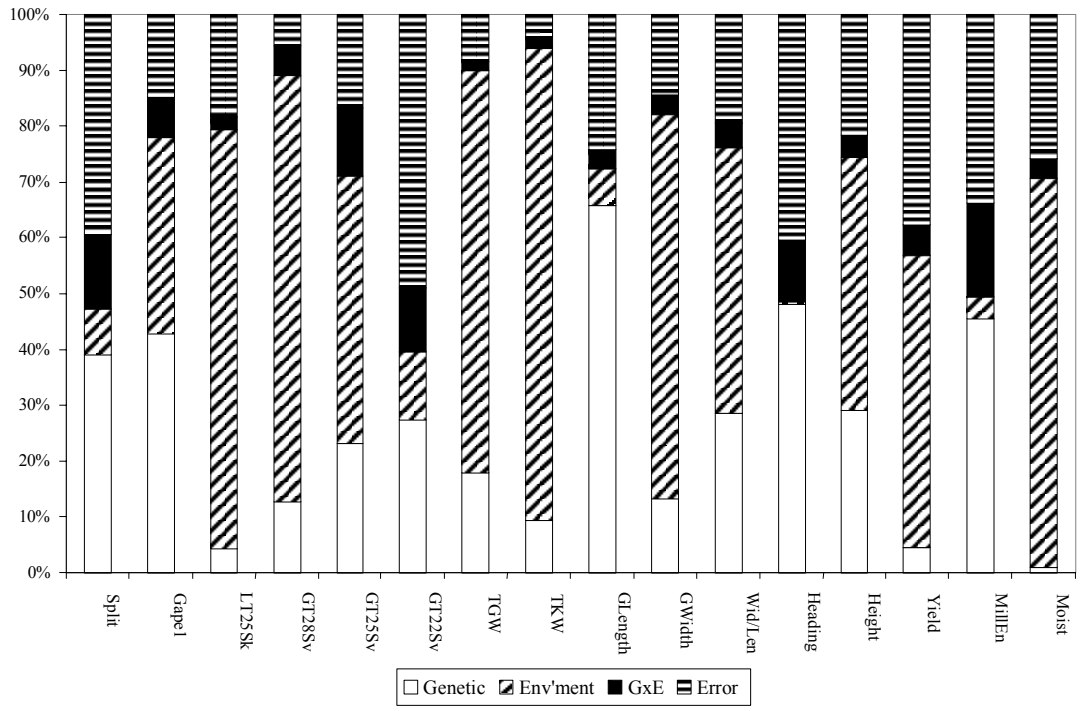


Figure 4. Proportions of phenotypic variation partitioned by ANOVA into genetic, environmental, genotype with environment interaction and error components.

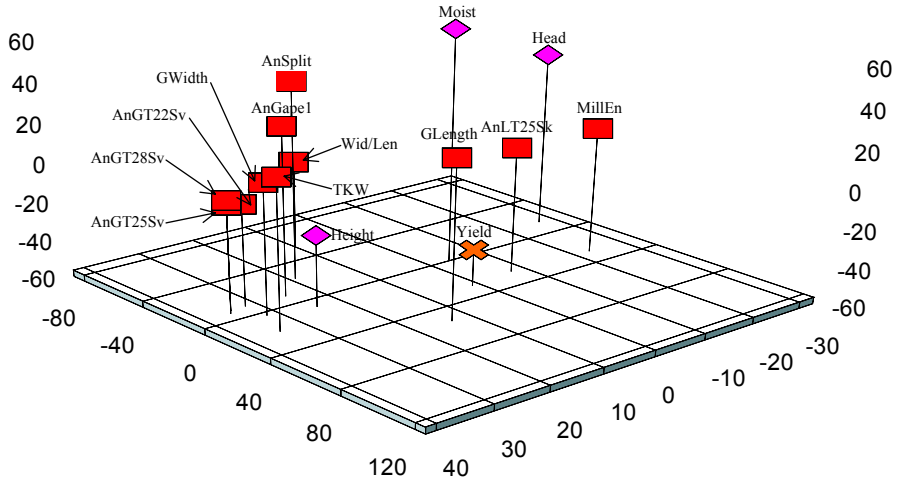


Figure 5. PCO plot for grain traits, height, yield, heading date, milling energy and grain moisture.

Comparison of Scottish Agricultural College and SCRI scores

It was necessary to compare the results of the techniques used in the two components of the programme. This depended on finding genotypes with a high and low level of splitting from the 1999 SCRI trial and the grain scores were used to identify extreme phenotypes. The chosen lines, two with low splitting and two with high splitting were grown in SAC controlled environment and glasshouse facilities. The material was grown in the glasshouse and at the estimated time of anthesis transferred to controlled environments with reduced light.

The scores of gape, skinning and splitting (Figure 6) were subject to ANOVA so that the effects of date of transfer, genotype and growing temperature could be examined (Figure 7). The three characters all showed significant effects for early compared with late transfer from glasshouse to controlled environments. In contrast, the effect of genotype, while highly significant for splitting and gape, was only just significant for skinning. The significance of the effects for growing temperature were the reverse of genotype with skinning showing a more significant effect than either splitting or gape. The highest level of splitting occurred in the Tankard x Livet line B96-76/96 and the lowest in Landlord (LSD = 2.58).

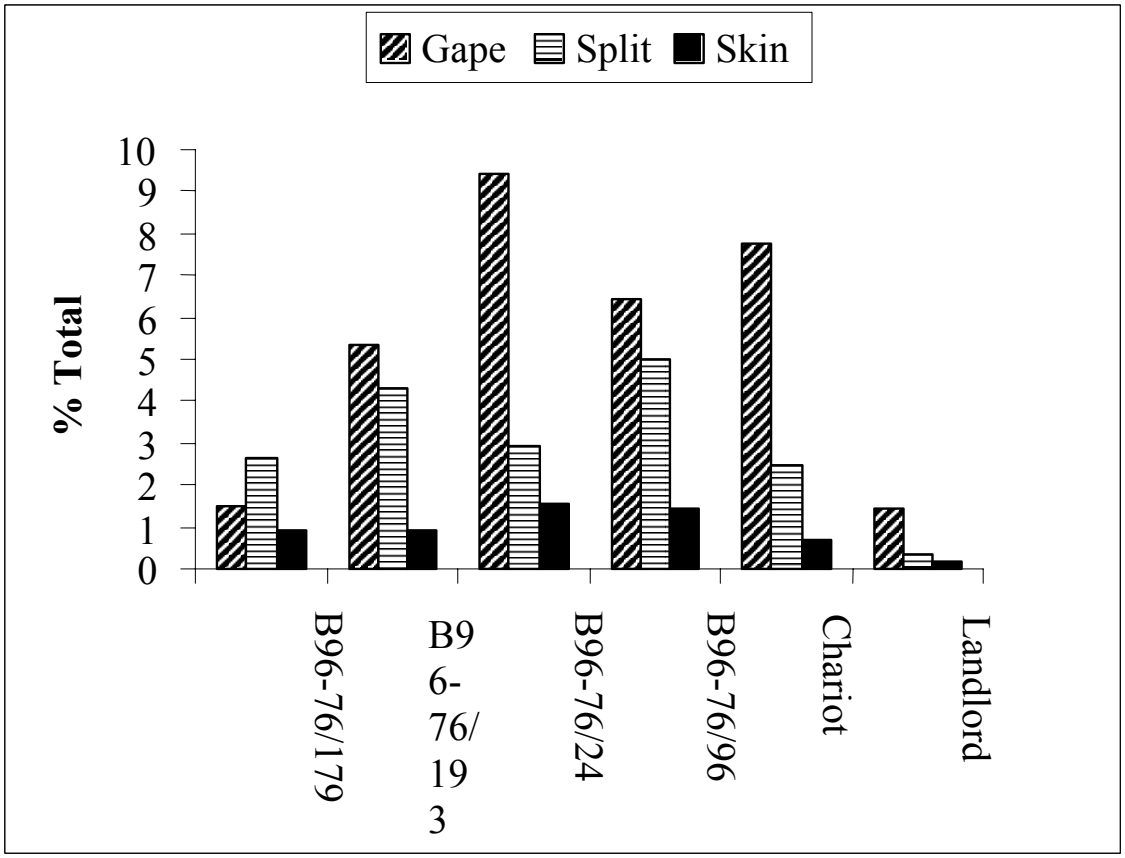


Figure 6. Mean values for traits measured on SCRI genotypes tested in SAC controlled environment and glasshouse conditions compared to selected controls.

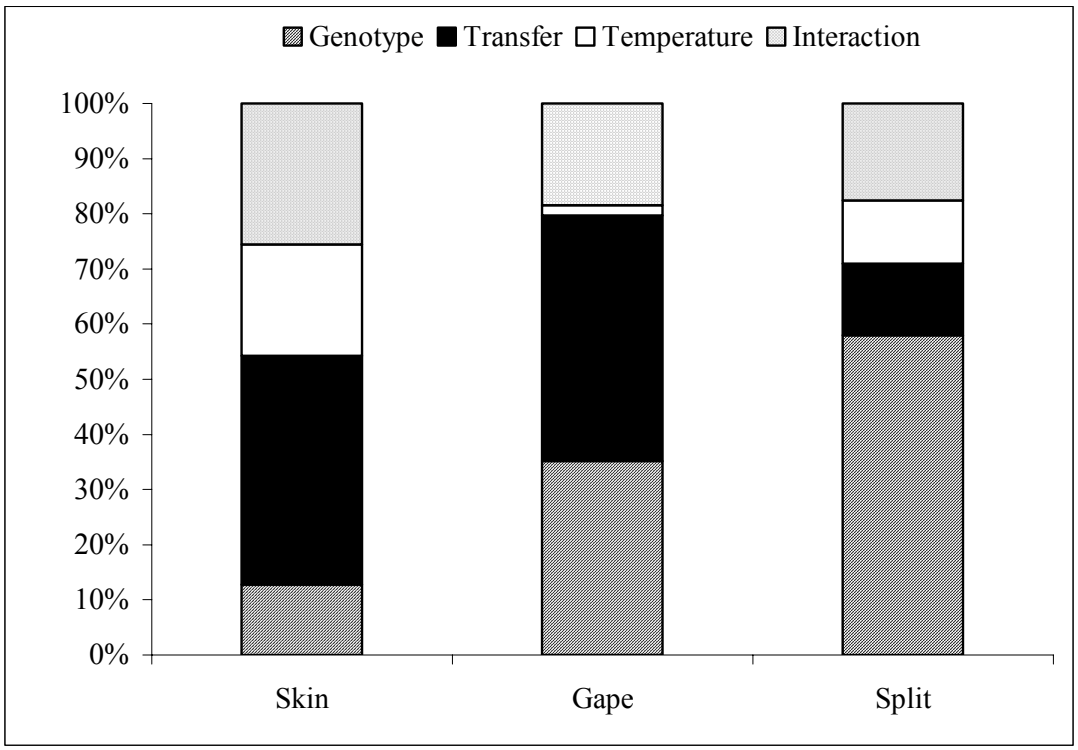
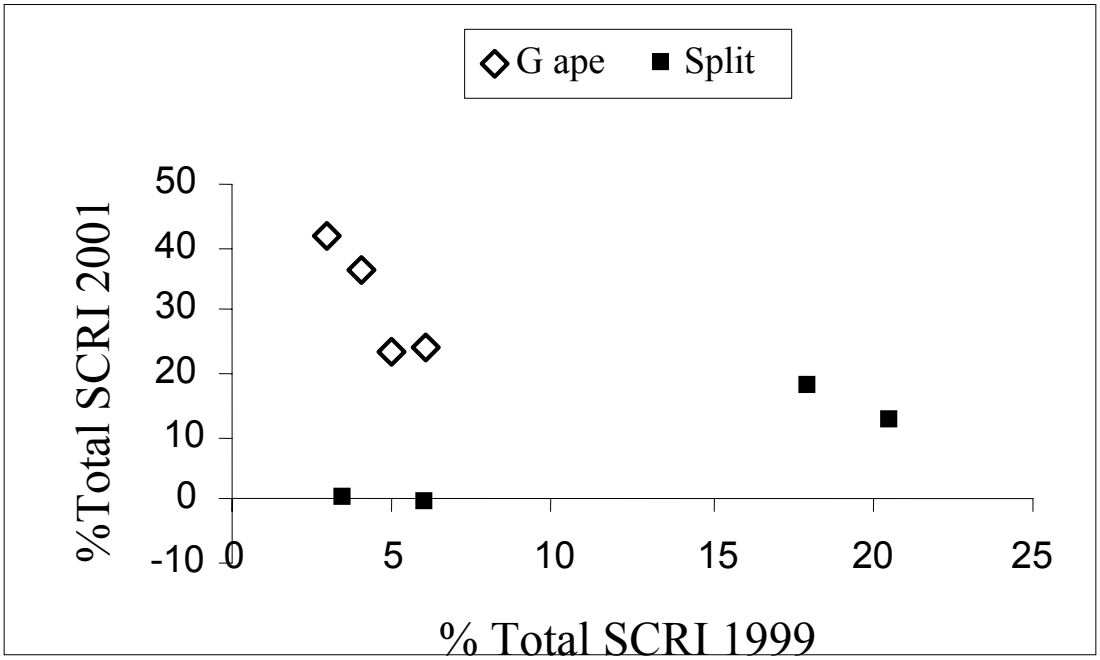


Figure 7. Proportions of phenotypic variation partitioned by ANOVA into components due to differences in time of transfer between glasshouse and controlled environments, genotype, temperature of growth and their interaction. The genotypes are those illustrated in Figure 6.

Plots grown in 2001

A comparison was made between the SCRI 1999 and 2001 scores (Figure 8) on the chosen lines and in turn, between the SCRI 2000 scores and the scores from controlled environment. Ranks between the SCRI 1999 and 2001 scores were the same as were three of the four SAC scores.

It was not possible to make a full comparison of the 2001 data with the results from the 1999 and 2000 seasons (Figure 9.) but it was obvious the overall rankings of low and high splitting genotypes was consistent to a degree that would allow successful selection. The rank correlations (r_s) between the 1999 and 2000 scores with those from the 2001 plots were both significant although the correlation was higher for 1999 (r_s = 0.70) than for 2000 (r_s = 0.48).



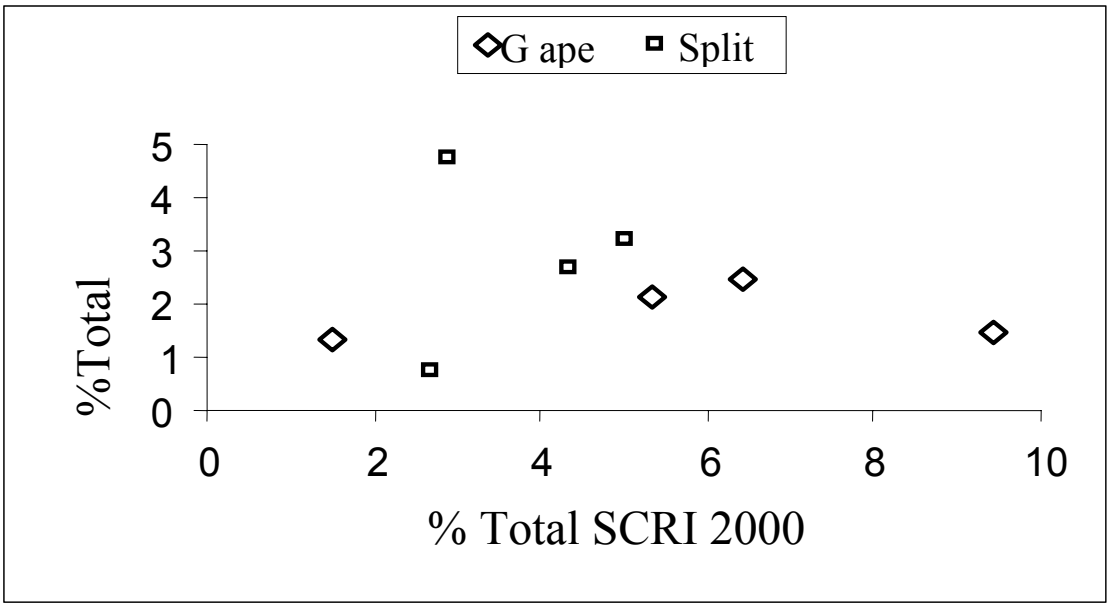


Figure 8. Comparison of mean scores for splitting and skinning in SCRI field plots in 1999 and 2000 and SAC controlled environment studies for four genotypes chosen for detailed comparison.

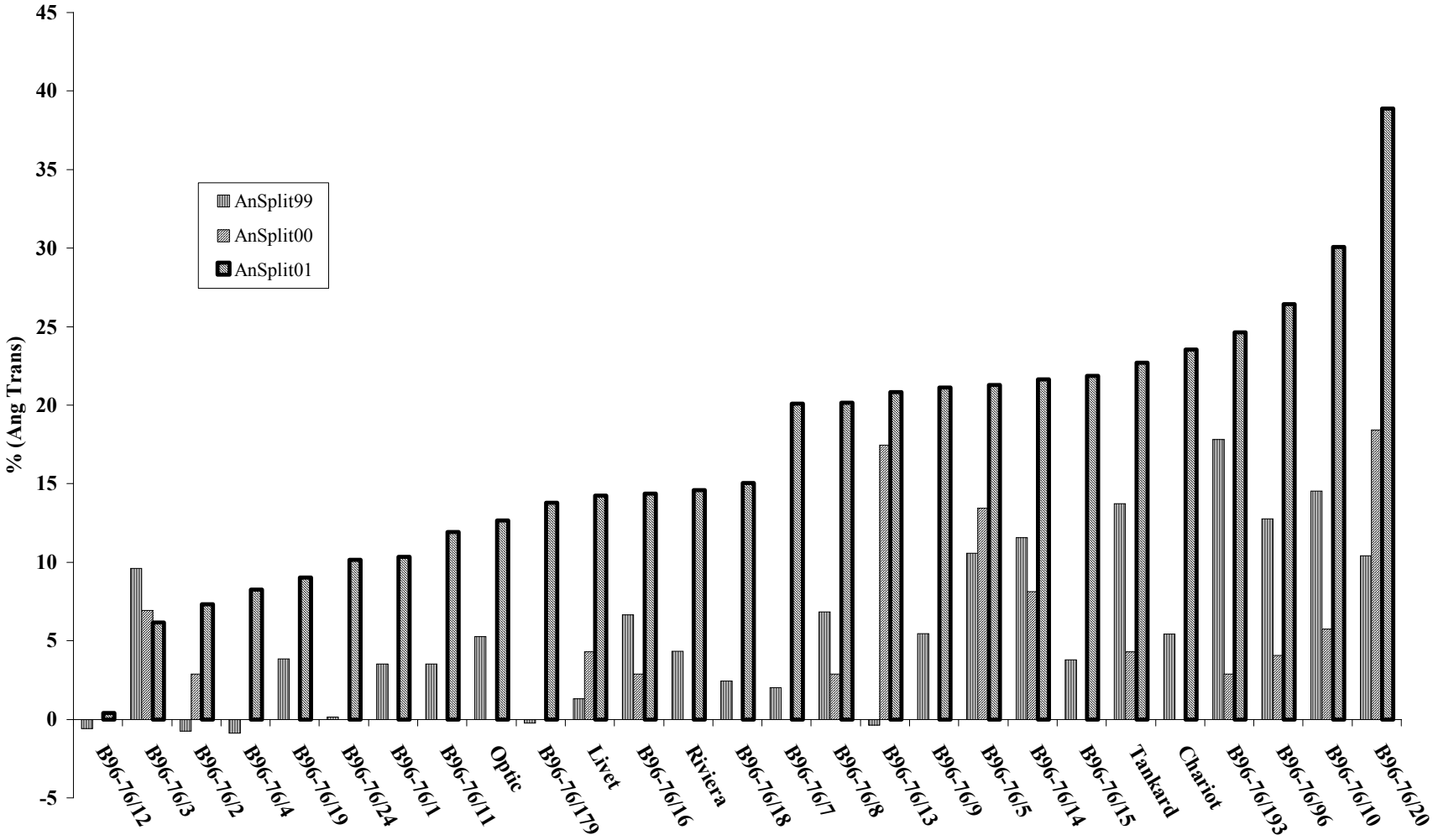


Figure 9. Results from splitting scores on selected controls and RILs from the cross Tankard x Livet from SCRI trials grown in 1999-2001. The genotypes are sorted in order of the 2001 season scores.

Exploration of Marker/Trait Associations

As the data was collected from the 1999 SCRI field trial ANOVA indicated the presence of genetic variability in traits and correlation with PCO was used to explore relationships between the variables. At this point there were insufficient polymorphic markers (Table 6.) to allow the construction of a genome map but it was necessary to explore marker trait/associations to facilitate decision taking, for example to identify a small number of lines to be assessed by the SAC controlled environment and glasshouse methods. Association between markers and traits was explored through the GENSTAT procedure REML. The traits Yield, Split, Gape1, LT25%Sk, Moist, Height, GE4, GE8 and Head were analysed to detect associations with 21 selected SSRs (Figure 10 a-c). In all, for nine traits, 52 significant associations with the markers were detected. The fewest were for Yield (2) and the most were with Split (11). This does not imply that the genetic control of yield is more or less complex than that for Split, simply that in the Tankard x Livet population of RILs that more associations were detected by this technique for splitting.

The REML method gives a rapid estimate of marker/trait association without the need for the lengthy process of forming a reliable genetic map but suffers from the disadvantage that apparently significant associations could be artefacts. For example, adjacent markers may show the same strength of association with a trait in a simple test but this does not preclude the possibility of a QTL located anywhere in the interval between the markers. The strength of Composite Interval Mapping (Jansen, 1993) is that all the information present in the map order of the markers is used in the statistically most satisfactory manner. Some of the markers used in the REML processing will not be included in the genome map produced by the use of JOINMAP (Stam and van Ooijen, 1995) because they do not satisfy the parameters of the mapping process. Despite these disadvantages the preliminary association of traits and markers was vital to decisions about which markers to use to supplement the SSRs.

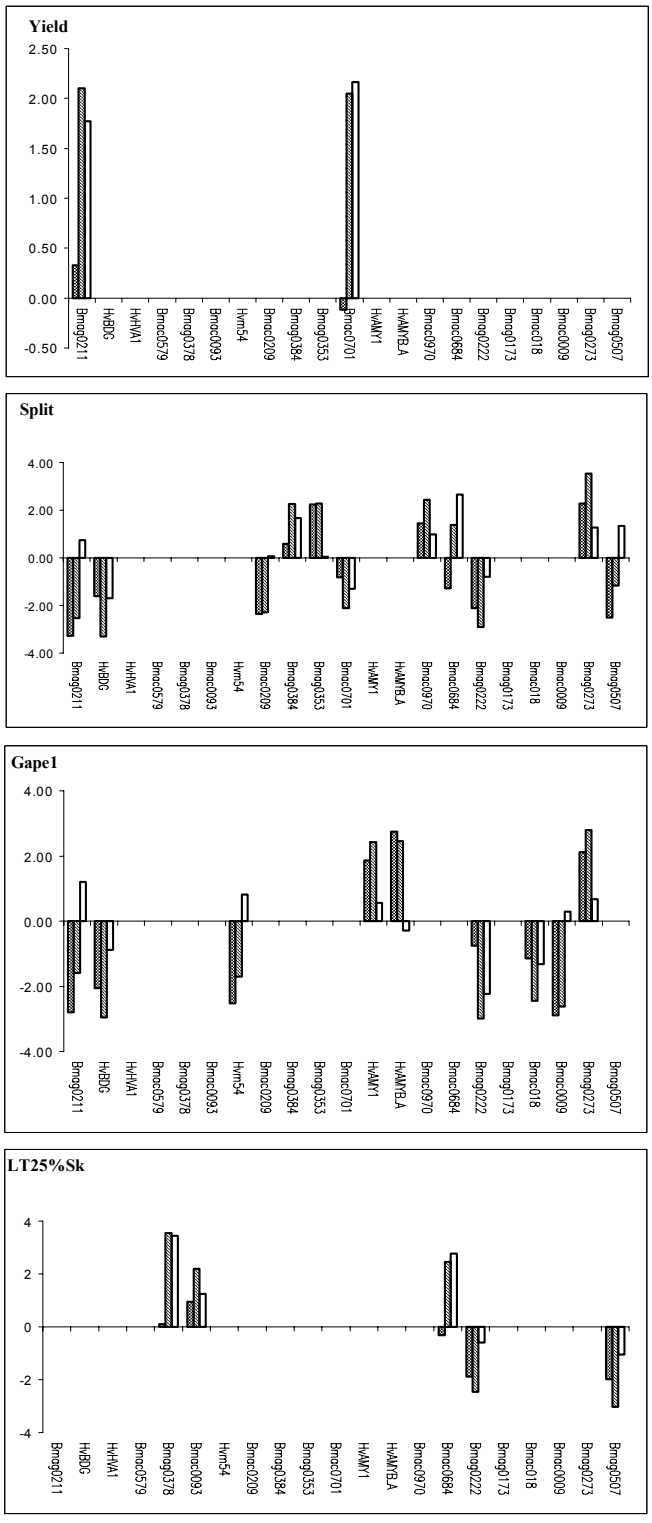


Figure 10 a. Associations detected between SSRs and grain traits by REML analysis. The histograms illustrate (l-r) the effect of the Tankard allele, the Livet allele and the effect of the heterozygote for each locus where significant associations were detected.

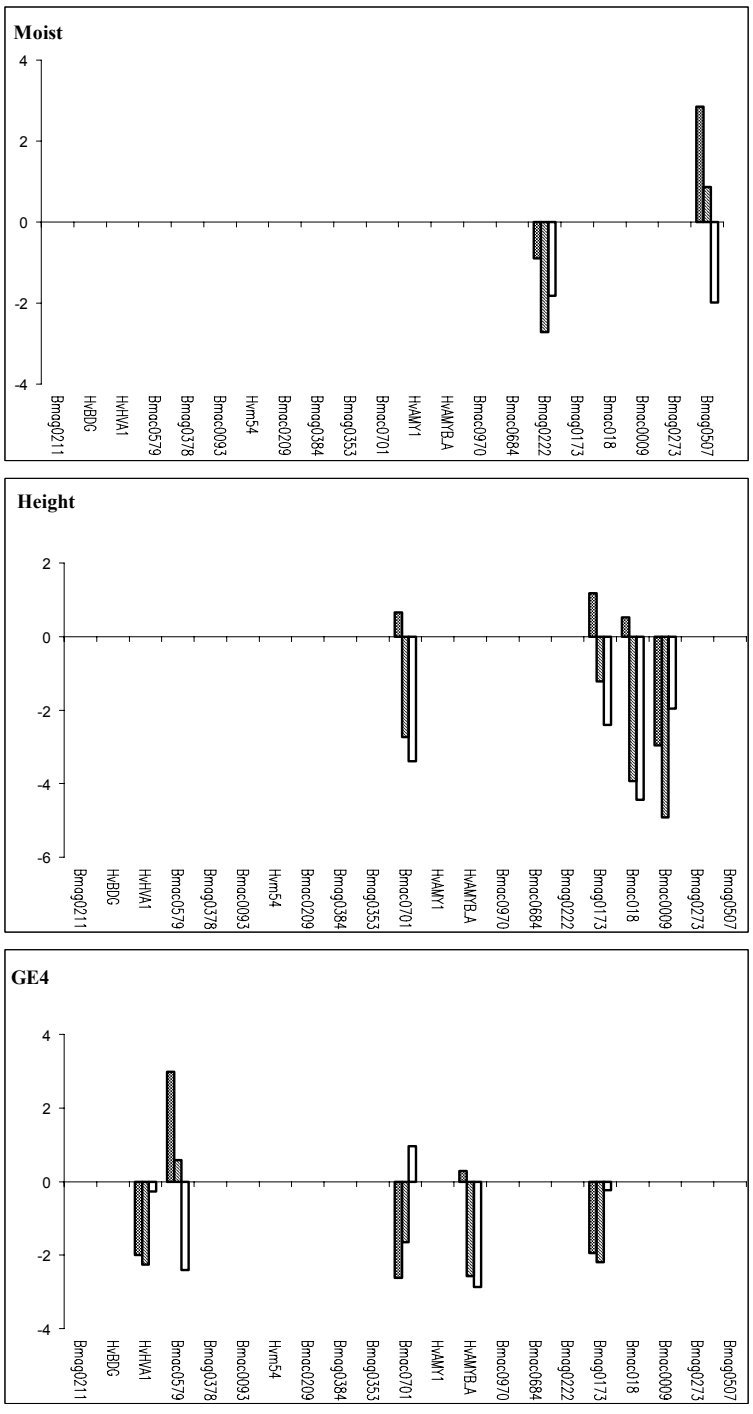


Figure 10 b. Associations detected between SSRs and traits by REML analysis.

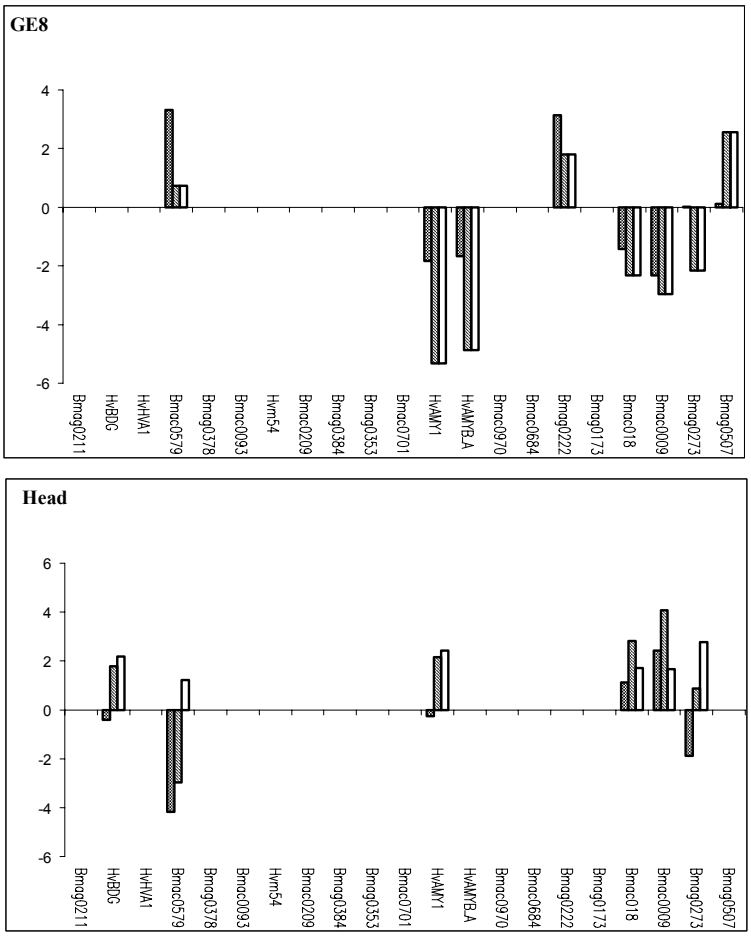


Figure 10 c. Associations detected between SSRs and traits by REML analysis

Table 6. Results from an initial screening of mapped SSRs (alignment based on the genome map of Ramsay et al., 2000) on RILs from the Tankard x Livet mapping population. For each marker italicisation indicates a lack of polymorphism while those in bold were polymorphic.

1H(5)		2H(2)		3H(3)		4H(4)		5H(7)		6H(6)		7H(1)	
Marker	cM	Marker	cM	Marker	cM	Marker	cM	Marker	cM	Marker	cM	Marker	cM
Bmac0032	55	*HVM36*	26	*HvLTPPB*	24	**Bmag0384**	43	*Bmag0323*	36	*Bmac0316*	6	*Bmag0021*	12
Bmag0211	62	**Bmag0378**	69	*Bmac067*	53	**Bmag0353**	43	*Bmag0337*	45	**Bmag0500**	38	*Bmag0007*	25
Bmag0382	108	**Bmac0093**	75	**Bmac0209**	54	**EBmac0701**	85	**EBmac0970**	54	**Bmag0173**	80	**HVM4**	26
HvHVA1	113	*Bmag0125*	89	*Bmag0225*	72	**HvAMYB**	177	**EBmac0684**	56	**Bmac0018**	102	*HVCMA*	79
HvBDG	117	**Hvm54**	103	**EBmac0708**	154	**HvAMY1**	180	*Bmag0223*	70	**Bmag0009**	102	**Bmac0579**	93
WMC1E8	160			*HVM62*	159			**Bmag0222**	87	*Bmac0040*	139	**Bmac0273**	93
Bmac0579	175			*Bmac0029*	179			*HvLOX*	125			**Bmag0507**	111
												Bmag0120	109
												Bmag0135	166

Quantitative trait mapping

QTLs were detected for every grain trait in the Tankard x Livet population, ranging from 5 for GT25Sv to 7 for GT22Sv and Wid/Len, with an average of over 5 (Table 7 a, b, c). After cross-validation, the detected QTLs accounted for an average of over 30% of the phenotypic variation with the highest and lowest values being for GLength and AnLT25Sk respectively. With the exception of GT25Sv, these QTLs detected over 50% of the estimated genetic variation for each of the grain traits with the highest value being found for Wid/Len. Of particular note is the fact that we detected QTLs that accounted for over 60% of the genetic variation in AnSplit and nearly 60% in AnLT25Sk (Table 7 a), which offers the prospect of developing molecular markers of real value in marker-assisted selection. For the other traits measured on the Tankard x Livet population, the numbers of QTLs detected ranged from 0 for Moist to 8 for MillEn (Table 8). Generally, the amount of phenotypic and genetic variation accounted for was much less than for the grain traits. For example, three QTLs were detected for Yield but together only accounted for 4.5% of the phenotypic and 30.8% of the genetic variation (Table 8).

Table 7. Quantitative trait loci detected in the Tankard x Livet RIL population by CIM analysis with PLABQTL (Utz and Melchinger, 1996). Interactions between QTLs are indicated by *, dominance effects by dom and the most significant QTLs for each trait in bold.

a) The grain traits splitting (Split), gape (Gape1) and skinning (LT25SK).

Character	QTL	Chromosome	Position	Left Marker	LOD	Partial R^2%	Effect of Tankard allele	%Variation (Phenotypic /Genetic)
Split	**1**	**1H**	**116**	**Bmag504**	**9.9**	**17.2**	**1.630**	
Split	2	4H	68	Bmag384	4.6	12.4	-1.596	28.9
Split	3	4H	123	EBmac701	5.3	14.1	1.477	62.7
Split	4	5H	30	EBmac970	5.9	7.9	-1.242	
Split	**5**	**5H**	**90**	**Bmag222**	**4.1**	**19.8**	**2.302**	
Split	6	5H	174	PstM36j	3.6	5.4	-0.975	
Split	2*5					3.1	0.963	
Split	3*5					6.1	-1.144	
Split	4*5					7.4	1.477	
Gape1	1	1H	112	Bmag504	5.1	11.9	3.603	
Gape1	**2**	3H	111	**Bmag10a**	**8.5**	**19.4**	**5.379**	35.0
Gape1	3	4H	123	EBmac701	5.1	13.4	3.567	51.9
Gape1	4	4H	179	HvAMYB	6.5	14.3	-4.377	
Gape1	5	5H	90	Bmag222	6.4	15.6	4.371	
Gape1	6	6H	106	Bmac18	7.3	16.0	3.893	
Gape1	7dom					8.9	27.593	
LT25Sk	1	2H	24	Ebmac684	3.5	8.8	1.181	
LT25Sk	2	3H	241	p19m36c	32	5.2	0.895	13.2
LT25Sk	**3**	5H	90	**Bmag222**	**6.9**	**15.2**	**-1.583**	57.1
LT25Sk	4	7H	64	HVM4	3.4	8.6	-0.986	
LT25Sk	5	7H	142	Bmag516	4.6	11.4	-1.325	

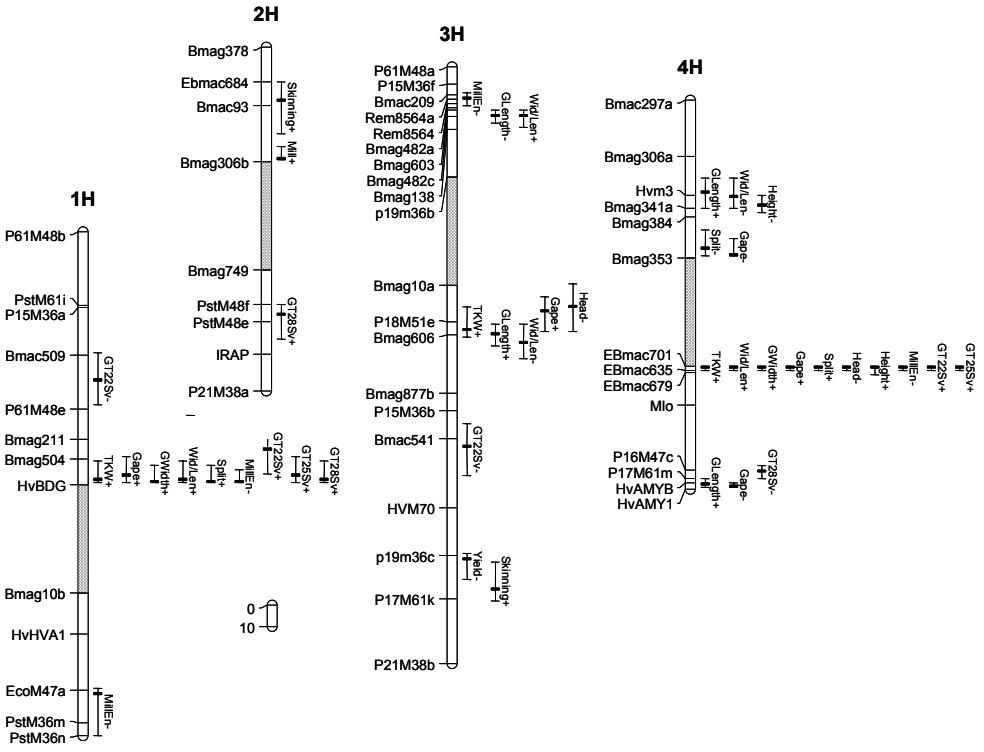
b) Grain sieving traits.

Character	QTL	Chromosome	Position	Left Marker	LOD	Partial R^2%	Effect of Tankard allele	%Variation (Phenotypic /Genetic)
GT28Sv	1	1H	114	Bmag504	4.6	12.0	1.707	
GT28Sv	2	2H	231	PstM48e	4.3	10.0	1.515	
GT28Sv	3	4H	171	P16m47c	2.9	4.1	-0.95	37.2
GT28Sv	4	5H	98	EcoM47g	4.8	12.4	1.635	67.1
GT28Sv	**5**	**6H**	**106**	**Bmac18**	**16.8**	**34.3**	**3.124**	
GT28Sv	6	7H	58	HVM4	4.2	11.3	1.866	
GT28Sv	7	7H	132	Bmag507	7.9	17.7	2.269	
GT25Sv	1	1H	112	Bmag504	3.32	5.6	0.861	
GT25Sv	2	4H	123	Ebmac701	3.21	4.5	0.666	11.0
GT25Sv	**3**	**6H**	**106**	**Bmac18**	**7.15**	**14.7**	**1.266**	26.9
GT25Sv	4	7H	58	HVM4	3.03	9.2	1.192	
GT25Sv	5	7H	134	Bmag507	3.87	9.9	1.122	
GT22Sv	1	1H	68	Bmac509	3.0	8.3	-0.689	
GT22Sv	2	1H	100	Bmag211	5.9	14.7	0.742	24.3
GT22Sv	3	3H	175	Bmac541	4.2	8.2	-0.460	63.4
GT22Sv	4	4H	123	Ebmac701	5.0	13.5	0.572	
GT22Sv	**5**	**6H**	**106**	**Bmac18**	**9.3**	**23.3**	**0.770**	
GT22Sv	6	7H	58	HVM4	3.3	8.9	0.528	
GT22Sv	7	7H	142	Bmag516	3.4	10.2	0.538	

c). Grain traits by image analysis.

Character	QTL	Chromosome	Position	Left marker	LOD	Partial R^2%	Effect of Tankard allele	%Variation (Phenotypic /Genetic)
TKW	1	1H	114	Bmag504	6.4	15.8	0.923	
TKW	2	3H	121	P18M51e	4.7	9.0	0.673	45.2
TKW	3	4H	123	Ebmac701	6.1	12.3	0.750	64.0
TKW	**4**	**6H**	**100**	**Bmac9**	**9.8**	**29.8**	**1.544**	
TKW	5	6H	136	PstM36b	3.0	8.3	0.690	
Glength	1	3H	22	Bmag603	9.5	18.5	-0.082	
Glength	2	3H	123	P18M51e	10.5	18.8	0.083	50.6
Glength	3	4H	42	Bmag306a	7.1	13.8	0.072	62.4
Glength	4	4H	177	HvAMYB	3.1	3.2	0.034	
Glength	5	5H	88	Bmag222	5.8	11.9	-0.062	
Glength	**6**	**6H**	**104**	**PstM36L**	**14.3**	**31.9**	**0.109**	
Gwidth	1	1H	116	Bmag504	7.4	18.8	0.030	
Gwidth	2	4H	123	Ebmac701	4.9	9.9	0.020	36.5
Gwidth	**3**	**6H**	**100**	**Bmac9**	**12.7**	**28.6**	**0.044**	66.3
Gwidth	4	6H	162	P15M36d	3.0	7.2	0.018	
Gwidth	5	7H	126	P34M34a	5.6	14.0	0.028	
Wid/Len	1	1H	114	Bmag504	6.2	15.1	0.004	
Wid/Len	2	3H	22	Bmag603	6.7	14.6	0.004	39.0
Wid/Len	3	3H	127	Bmag606	7.0	15.3	-0.005	67.5
Wid/Len	4	4H	44	Bmag306a	6.1	14.5	-0.004	
Wid/Len	**5**	**4H**	**123**	**Ebmac701**	**8.5**	**18.3**	**0.005**	
Wid/Len	6	5H	104	EcoM47g	4.5	11.4	0.004	
Wid/Len	7	7H	134	Bmag507	5.2	13.0	0.004	

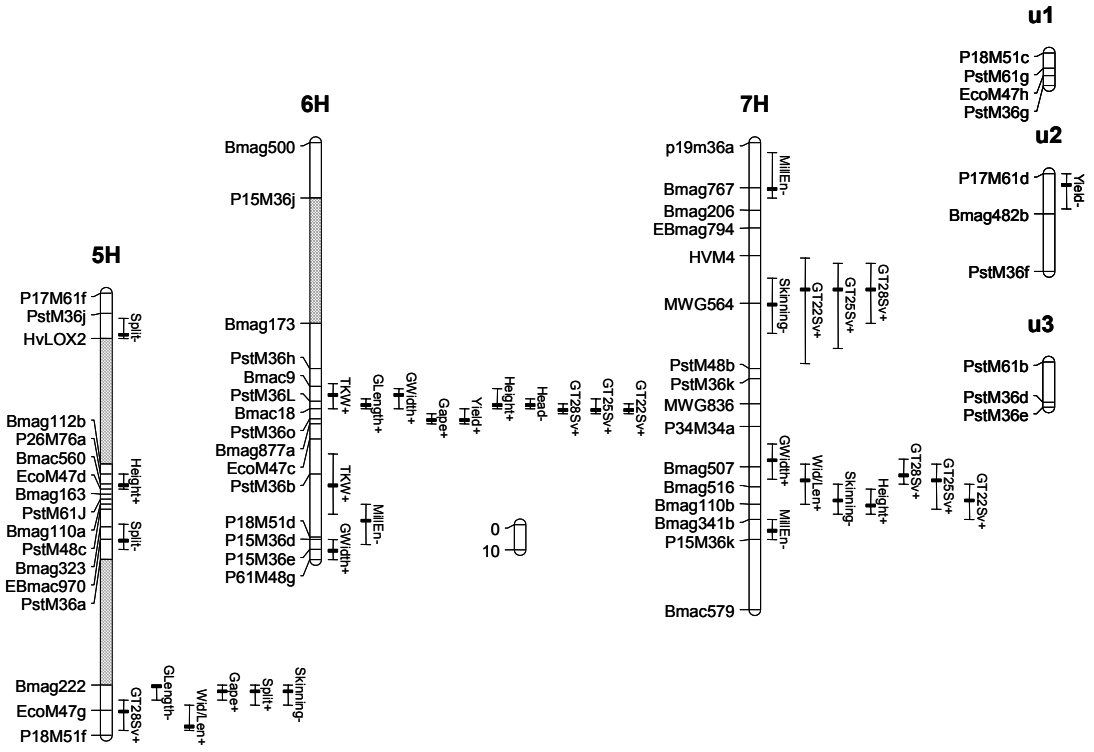
Figure 11 a. QTL Maps of chromosomes 1H – 4H for the Tankard x Livet RIL population. Thick Lines indicate QTL peaks and whiskers indicate 1 LOD confidence intervals. Hatched portions of chromosomes indicate no linkage between adjacent groups.



Considerable clustering of the QTLs can be seen in both populations (Figures 11-12) reflecting the close associations that we found through correlation and principal components analyses. Gape1, TKW and AnSplit are all highly associated. The Tankard alleles for all five QTL that we detected for TKW all increased the character and four of them were co-located with QTLs where the Tankard allele also increased Gape1. The exception was the second TKW QTL on chromosome 6H, which had few associations with other characters.

Three of the four QTL associations between TKW and Gape1 appeared to be due to an increased Wid/Len ratio, either by increases in grain width (1H and 4H) or a decrease in grain length (5H), i.e. compensatory growth mechanisms appear to be operating. Tankard QTL alleles increasing AnSplit were also found in these three QTL clusters. The QTL cluster on 6H in contrast appears to be the result of a general growth factor as Tankard alleles increase both GLength and GWidth, resulting in an association of Gape1 and TKW without an increase in Wid/Len. Interestingly, other QTLs

Figure 11 b QTL Maps of chromosomes 5H-7H for the Tankard x Livet RIL population. Thick Lines indicate QTL peaks and whiskers indicate 1 LOD confidence intervals. Hatched portions of chromosomes indicate no linkage between adjacent groups.



located in this cluster reflect this phenomenon as Tankard QTL alleles also result in increases in height and yield. There was, however, no evidence of a QTL for AnSplit in this region. The above results therefore suggest that alterations in GLength or GWidth that affect Wid/Len reflect a disruption of the appropriate grain dimensions to retain the integrity of the pericarp and/or testa and these can lead to grain splitting.

There are, however, three other QTL for AnSplit that are not co-located with any of the characters measured in the current study. One was located at the end of 4H and two were located on separate segments of 5H and, in two of these cases, the Tankard allele was responsible for a reduction in the level of splitting. Whilst this suggests that there is a tendency towards some genetic control of splitting over and above the factors controlling grain size characteristics, these QTLs were slightly less important relative to the other three.

Table 8. QTLs detected for heading date, height, yield and milling energy in the Tankard x Livet RIL population. The most significant QTLs are highlighted in bold text.

Character	QTL	Chromosome	Position	Left Marker	LOD	Partial R^2%	Effect	%Variation (Phenotypic/ Genotypic
Head	1	3H	111	Bmag10a	3.5	8.2	-0.602	21.8
Head	2	4H	123	EBmac701	4.4	10.1	-0.560	40.8
Head	**3**	**6H**	**104**	**PstM36L**	**9.5**	**20.9**	**-0.890**	
Height	1	4H	125	EBmac635	8.4	14.7	1.530	
Height	2	5H	8	Bmac560	4.8	6.1	1.126	25.4
Height	3	5H	100	EcoM47g	3.2	5.9	0.939	51.3
Height	**4**	**6H**	**102**	**Bmac9**	**9.4**	**21.8**	**1.973**	
Height	5	7H	144	Bmag516	4.6	9.1	1.268	
Height	1dom					7.4	-2.424	
Height	2dom					4.1	-1.62	
Height	4dom					2.4	-1.594	
Yield	1	3H	227	p19m36c	3.1	3.8	-0.085	4.5
Yield	2	6H	110	PstM36o	3.2	8.1	0.125	30.8
Yield	**3**	**uH**	**4**	**P17M61d**	**3.9**	**9.1**	**-0.172**	
MillEn	**1**	**1H**	**116**	**Bmag504**	**11.5**	**27.8**	**-16.534**	
MillEn	2	1H	221	EcoM47a	5.0	11.8	-10.940	36.5
MillEn	3	2H	52	Bmac306b	3.1	6.6	7.613	71.2
MillEn	4	3H	14	Bmac209	4.4	8.2	-8.227	
MillEn	5	4H	123	EBmac701	6.9	14.4	-10.443	
MillEn	6	6H	150	PstM36b	6.0	14.8	-12.251	
MillEn	7	7H	18	Bmag767	4.2	10.0	-9.302	
MillEn	8	7H	154	Bmag341b	4.6	11.5	-11.287	
MillEn	2dom					5.8	130.5	
MillEn	3dom					3.5	9.9	
MillEn	4dom					2.8	9.3	
MillEn	6dom					4.2	116.9	

Table 9. QTLs detected for grain characters measured upon the Derkado x B83-12/21/5 population from trials grown in 1995 and 1997.

Character	QTL	Chromosome & (Position)	Position	Left Marker	**LOD**	Partial R^2%	**Effect**	%Variation
TKW	1	1H	38	E52M48a	3.2	4.0	-0.451	
TKW	2	2H	168	Bare1P16e	9.9	17.2	0.961	66.2
TKW	**3**	**3H**	**0**	***sdw1***	**32.4**	**51.7**	**-2.180**	83.6
TKW	4	4H	104	P17M62d	4.0	9.9	0.776	
TKW	5	5H	0	P34M39a	5.2	3.4	0.498	
TKW	6	5H	22	*ari-e*.GP	22.9	56.2	2.928	
TKW	7	7H	112	Bmac167	5.5	9.2	0.812	
TKW	8	7H	150	HvWaxy4a	4.5	7.6	0.774	
TKW	9	7H	212	P16M47h	3.2	6.0	0.600	
GT25Sv	1	2H	168	Bare1P16e	3.24	6.7	1.444	
GT25Sv	2	3H	68	Bmag6	3.45	11.0	-1.963	63.7
GT25Sv	**3**	**3H**	**0**	***sdw1***	**29.31**	**58.9**	**-6.297**	74.8
GT25Sv	4	4H	104	P17M62d	8.68	19.5	2.872	
GT25Sv	5	5H	22	*ari-e*.GP	16.44	40.8	4.396	
GT25Sv	3*5					10.8	-1.847	
GLength	1	2H	160	P22M62a	4.6	11.6	0.079	
GLength	2	3H	20	ABG4STS	4.7	15.2	-0.090	
GLength	3	4H	104	P17M62d	3.0	9.6	0.074	75.5
GLength	**4**	**5H**	**22**	***ari-e*.GP**	**56.0**	**80.5**	**0.422**	86.8
GLength	5	7H	112	Bmac167	9.9	27.7	0.149	
GLength	6	7H	168	P17M62a	4.6	11.0	0.095	
GWidth	1	2H	170	Bare1P16e	9.8	18.0	0.037	
GWidth	**2**	**3H**	**0**	***sdw1***	**24.8**	**48.8**	**-0.073**	46.0
GWidth	3	4H	104	P17M62d	8.6	19.1	0.040	66.4
GWidth	4	5H	22	*ari-e*.GP	5.6	6.9	0.020	
GWidth	5	7H	194	P40M38b	3.3	8.7	0.025	
Wid/Len	1	1H	112	Bmac144a	3.4	12.0	0.005	
Wid/Len	2	3H	0	*sdw1*	3.4	11.7	-0.004	71.4
Wid/Len	**3**	**5H**	**22**	***ari-e*.GP**	**51.7**	**79.5**	**-0.024**	85.8
Wid/Len	4	7H	112	Bmac167	6.1	21.7	-0.008	
Wid/Len	5	7H	160	Bmag482	3.1	6.9	-0.004	
Wid/Len	2*3					5.3	-0.003	
Gape	1	3H	94	Bmag225	3.2	9.6	-1.760	2.7
Gape	2	5H	168	GMS27	3.5	7.6	1.573	36.0
Gape	**3**	**6H**	**62**	**Bmag112c**	**4.4**	**11.4**	**2.112**	
LT25Sk	1	4H	172	*mlo*	3.4	9.1	-2.162	0.4
LT25Sk	**2**	**5H**	**174**	**Bmag222**	**3.8**	**11.0**	**-2.389**	

Figure 12a. QTL Maps of chromosomes 1-4H for Derkado x B83-12/21/5 DH population. Thick Lines indicate QTL peaks and whiskers indicate 1 LOD confidence intervals. Hatched portions of chromosomes indicate no linkage between adjacent groups

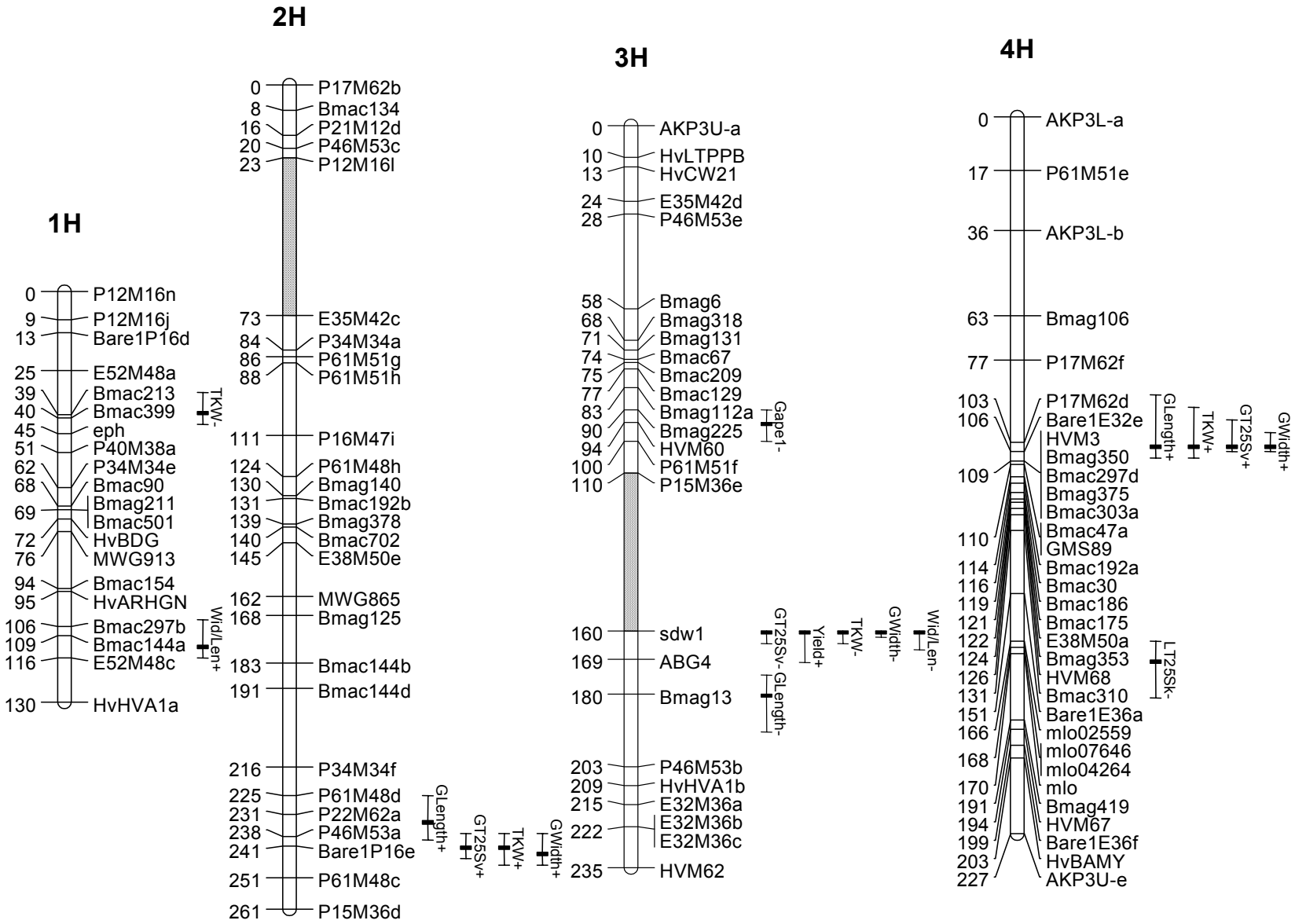
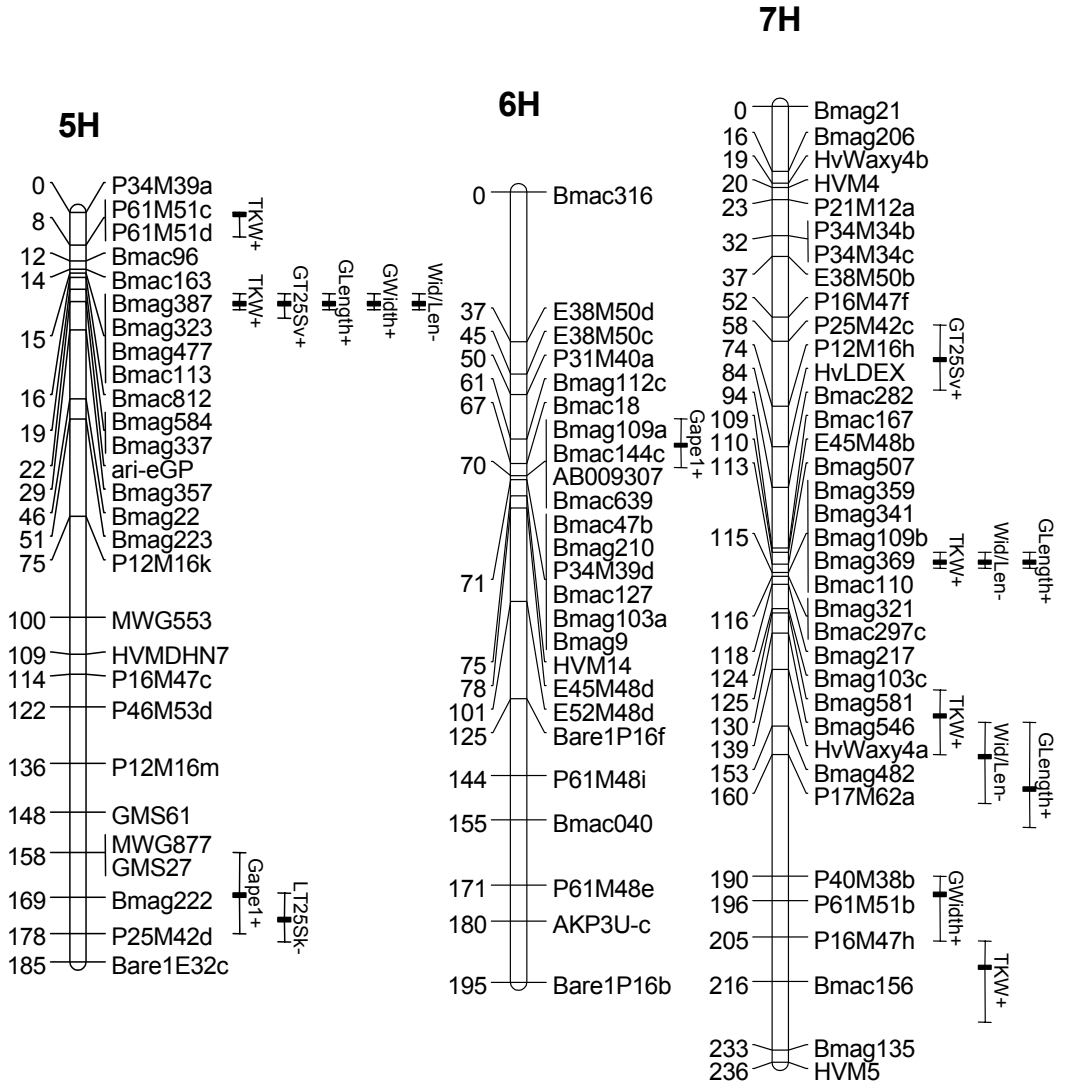


Figure 12b. QTL Maps of chromosomes 5-7H for Derkado x B83-12/21/5 DH population. Thick Lines indicate QTL peaks and whiskers indicate 1 LOD confidence intervals.



Similar numbers of QTLs were detected in the Derkado x B83-12/21/5 population for the traits pertaining to grain shape and size to those found in the Tankard x Livet population. In fact, the Derkado x B83-12/21/5 QTL accounted for a greater proportion of the phenotypic and genetic variation for each of the traits where comparable data were available (Table 9). In contrast, few QTL were detected in the Derkado x B83-12/21/5 population for the traits measuring grain damage (AnGape1, AnSplit and AnLT25Sk) and those that were significant did not account for much of the variation. In fact, no significant QTL could be detected for AnSplit, and the relatively disappointing QTL results from the Derkado x B83-12/21/5 population for the damage traits indicates that our targeting of the Tankard x Livet population was correct.

There was little evidence of epistasis in the genetic control of characters in the Tankard x Livet population or the Derkado x B83-12/21/5 population (Tables 7-9). In the former, three pairs of interacting loci were detected for splitting, which all involved the QTL with the largest effect in the region of Bmag222 on 5H. Reasons for these interactions are not clear but, whilst the detected effects are on average smaller than the additive effects, they are still substantial and were present each year. Epistasis was detected between one pair of loci for both GT25Sv and Wid/Len in the Derkado x B83-12/21/5 population. In each case, the interaction was between QTLs located in the region of the major dwarfing genes *sdw1* and *ari-e.*GP and reflects poorer performance of lines that carry both genes for GT25Sv and better performance of lines that carry the *ari.-e*.GP gene for Wid/Len.

Some comparison of QTL locations across the two populations can be made as many of the SSRs on the Derkado x B83-12/21/5 map are also represented on the Tankard x Livet map. QTLs affecting TKW and Wid/Len located in the region of Bmag606 on 3H for the latter population are in the same region as those located in the region of *sdw1* in the former. Both Tankard and Livet carry the *sdw1* allele derived from Diamant so it is surprising to detect QTLs from the RIL population in this region as previous studies have shown that recombination in the region of *sdw1* is relatively restricted. It is therefore possible that some of the effects associated with *sdw1* found in the Derkado x B83-12/21/5 population may be the result of close linkage rather than pleiotropy but further studies will be needed to establish this. A locus affecting Glength was found in both populations in the region of HVM3 on chromosome 4H. Loci affecting both Gape1 and LT25Sk were found in both populations on chromosome 5H in the region of Bmag222. The major locus for Gape1 in Derkado x B83-12/21/5 was in the same region of chromosome 6H as a locus of large effect for the same character in the Tankard x Livet population. The latter was part of a QTL cluster with effects upon TKW, Glength and Gwidth amongst others but no effects upon these characters were detected in the Derkado x B83-12/21/5 population. Finally, a QTL for Wid/Len was detected in the region of Bmag507 on chromosome 7H in both populations.

There did appear to be differences in the most important regions of the genome for the various traits measured in both populations. In Tankard x Livet, the major regions were on 1H in the region of HvBDG, 3H, in the region of Bmag606, 4H in the region of Ebmac701, 5H in the region of Bmag222, 6H in the region of Bmac9 and 7H in the region of Bmag507. Whilst fewer characters were measured, two or more QTL were detected in Derkado x B83-12/21/5 in comparable regions of 3H, 5H and 7H as noted above but there was little indication of the importance of the other regions, despite being represented on the map. This discrepancy may reflect the disrupting influence of the major dwarfing genes and a general constraint upon expression of grain size in the Derkado x B83-12/21/5 population.

Discussion

Weather

The pattern of weather at SCRI for the years 1999-2001 is summarized in Figure 13. The major effects of weather on plant development and growth in the United Kingdom are through the accumulated effects of temperature and photosynthetically active radiation (PAR) (Ellis and Kirby, 1980) and the, often more erratic, effects of rainfall. The pattern of accumulated temperature for each month between sowing and harvest was similar in each year. In contrast, the pattern of solar radiation was markedly different with low totals in May 1999 and June 1999. It is clear that the 1999 weather was markedly different from the long term average recorded (Wood, 2001) at SCRI, which shows bright sunshine hours peaking in May but air temperatures being higher in June and July. A relationship of higher solar radiation combined with lower temperatures was invoked to explain larger grain size and higher yield in Scottish plots in a study of plant development and growth (Ellis and Kirby, 1980). Grain splitting was not observed in these experiments despite a 30% increase in size of the grain from the Scottish plots between the 1976 and 1977 growing seasons. Pre-anthesis rainfall exceeded 100 mm in both years at the Scottish site.

The pattern of rainfall was more erratic with a tendency for more rain at the end of the season and a low trough in July. The long-term average for SCRI rainfall shows no particular pattern with rain occurring in every month of the year. The three seasons we consider do show a significant difference in rainfall pattern as in May 2001 there were only 12.6 mm of rain, approximately a quarter of that observed in 1999 and 2000. The rainfall for June was higher in all three years, but in 2001 the July and August rainfall totals were both higher than in 1999 and 2000. So the critical questions in this scenario are; 1) were the plots stressed by low rainfall in May 2001, 2) did the higher rainfall in July-August cause a "drought relief" effect leading to increased gape and splitting. The effect of drought, i.e. low rainfall, depends not only on the actual rainfall but also the rate of evaporation i.e. the temperature and the amount of water "stored" in the soil. Drought leads to a reduction in the size of the growing plant through a reduction in the height of the main-stem and a reduction in the number of tillers (Ellis and Russell, 1984). In turn the ability of the crop to absorb solar radiation is reduced and ultimately yield can be reduced (Russell and Ellis, 1988). In the United Kingdom the effects of drought tend to be relatively mild and short by comparison with world wide standards.

Inspection of the cultivar means for the three seasons (Figure 2) shows that the relative changes in gape and splitting were dependent on the cultivar. High levels of gape greater than 1 mm were seen in Berwick, Chariot, Cooper, Landlord, Optic and Tankard. Tankard was the only cultivar that would have failed the splitting standard in both 1999 and 2001 and showed consistently high levels of gape. However, while Chariot showed a similar level of gape to Tankard it showed high spitting only in 2001. We have to conclude

that none of the cultivars grown in 2001 were immune to the problem of splitting but lower levels were seen in Tyne and Landlord.

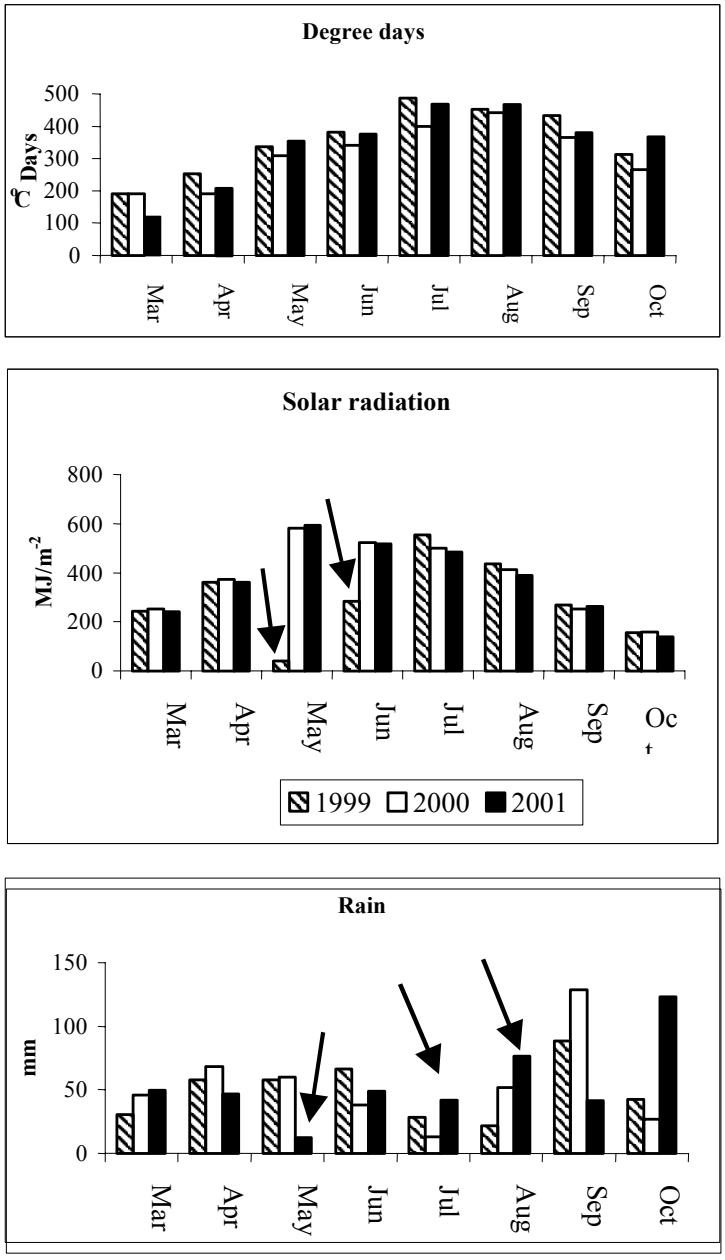


Figure 13. Meteorological data recorded at SCRI in 1999-2001. Degree days were calculated by summing daily mean temperatures for each month. Rain and solar radiation were recorded daily and then totalled for each month. Arrows indicate low solar radiation in 1999 and dry/wet rain pattern in 2001.

Genotype

Biometrical and mapping analysis of the grain spoilage characters splitting and skinning have revealed that the former is under a relatively high degree of genetic control whilst the latter is more influenced by the environment. The measures of character association, pair-wise correlations and PCO, both agree and demonstrate a high degree of inter-relationship between Split, Gape1, Wid/Len and TKW in the Tankard x Livet cross. This was reflected in the QTL mapping of the Tankard x Livet population as QTLs for Split, Gape1, Wid/Len and TKW were often co-located in the same regions of the genome. This was not apparent in the Derkado x B83-12/21/5 cross but then we did not detect any significant genetic variation for splitting. The Derkado x B83-12/21/5 cross had a much lower overall mean for TKW than the Tankard x Livet cross and, whilst this difference is confounded with environmental differences, it suggests that lines with lower TKW, particularly those with a lower Wid/Len, are less prone to grain splitting. Selection of lines with low expression of these characters is an obvious means of avoiding the problem but it would also limit expression of a yield component as well as a possible increase in screenings. Evidence for the former is not apparent from the current study as only one of the QTLs increasing Yield in the Tankard x Livet population is co-located with a QTL increasing TKW. In the Derkado x B83-12/21/5 population, the two dwarfing genes were associated with QTLs decreasing TKW but these were co-located with QTLs increasing Yield, i.e. the dwarfing genes appeared to have stimulated a yield compensation mechanism. The situation for grain sieving is much less favourable, as there is a pronounced association of TKW and GT25Sv in both crosses.

There is, however, evidence of some independent genetic control of TKW and Split in the Tankard x Livet cross, as the QTL alleles from Tankard that increase TKW, Wid/Len, Gape1 and Yield are not co-located with a QTL for Split. Conversely, three QTLs from Tankard decreasing Split that are located in the region of Bmag353 on 4H and Bmag323 and HvLOX2 on 5H are not co-located with any other QTLs. Selection of Tankard alleles at these three regions of the genome would therefore reduce overall splitting and boost TKW and Yield. This would, however, merely bring the level of splitting back to that of Tankard, which is clearly unacceptable. Further selection of other QTL alleles is therefore required to reduce the genetic potential for splitting. Selection against the Tankard allele increasing Split located in the region of Bmag222 on 5H is an attractive target as it accounts for the largest amount of variation in the multi-locus model. More importantly, whilst it is co-located with QTLs increasing Gape1 and Wid/Len, it is not associated with any effects upon TKW or Yield. It is, however, associated with a QTL decreasing LT25Sk so there would be a potential increase in skinning to be set against the reduction in splitting. Any cultivars produced by selection for decreased splitting would require careful handling in grain trading. This situation is also reflected in the Derkado x B83-12/21/5 population, where a QTL allele from Derkado decreasing LT25Sk is co-located with another Derkado QTL allele increasing Gape1 in the region of Bmag222 on 5H. This region does therefore appear to be associated with grain damage characters in European spring barley germplasm.

From the analysis of the extreme lines from the Tankard x Livet population, it can be seen that lines that are more prone to Split than Tankard can be derived. This is also reflected in the QTL analysis, as Tankard alleles in the regions of Bmag384 on 4H, and HvLOX2 and Ebmac970 on 5H decrease splitting. This is of potential concern to the malting industry as, without extensive assessment of splitting, such lines could potentially be commercialised and thus put UK malting barley supplies at risk.

Both pair wise correlation and PCO analysis do not show any close associations of LT25Sk with the other characters measured in the study of the Tankard x Livet population. This is largely reflected in the QTL mapping of the population and of the Derkado x B83-12/21/5 population where, with the exception noted above, QTLs for LT25Sk are not often co-located with other characters. As the genetic control of LT25Sk is less than that of Split and the grain shape parameters, fewer QTLs for the character were detected in the Tankard x Livet population and there were relatively few in the Derkado x B83-12/21/5 population as well.

Integration of many different genetic maps is not only technically difficult but also subject to considerable error. To circumvent these problems, (Kleinhofs et al., 1998) divided the barley genome into 10 cM segments or "bins" into which markers could be placed. This simplified the alignment of genetic maps and, with some common markers in different maps, permits the identification of homologous regions. We utilised the latest version of the "Bin" map (http://barley.genomics.wsu.edu/arnis/linkage_maps/maps-svg.html) in conjunction with maps of the Oregon-Wolfe Barley DH population (Costa et al., 2001). Lina x HS92 (Ramsay et al., 2000) and the Tadmor x ER/APM population (Teulat et al., 2001) to assign regions of interest to Bins.

Few other reports of genetic studies of grain damage parameters have been published and very little mapping work has been done. (Kanatani et al., 1998) mapped QTLs for "hull-cracked grain", which they defined as being the exposure of the caryopsis through the lemma and palea, in the North American two-row spring barley population Harrington x TR306. We interpret this trait as being equivalent to Gape1 and (Kanatani et al., 1998) detected three QTL in the regions of ABG609B, MWG502 and MWG511 on chromosomes 3H, 5H and 7H respectively, although the QTL on 5H was not significant in a multi-locus model. By comparison with the bin maps of (Kleinhofs et al., 1998), we can locate these loci in the region of Bins 15 and 16 on 3H, Bin 1 on 5H, and Bin 7 on 7H. In the current study, we did not detect any QTLs for Gape1 in these regions, although we did detect QTLs for Wid/Len, a possible determinant of Gape1, in both Derkado x B83-12/21/5 and Tankard x Livet that were located in Bin 7 of 7H. As Lox1a is located in Bin 1 of 5H, we predict that HvLOX2 is also located in Bin 1 but we do not have any other marker data to validate the prediction. It would mean, however, that the QTL that we detect for Split in the Tankard x Livet population that is associated with HvLOX2 on 5H is in the same region as the QTL for Gape1 detected by (Kanatani et al.,

1998). There is therefore some supporting evidence that QTLs in the region of Bin 1 on 5H and Bin 7 on 7H are important in the genetic control of grain damage.

Collins et al., (2000) published the results of a study of some grain and malt characters in a cross between the Australian spring barley Galleon and the Japanese spring barley Haruna Nijo. They found that QTLs for husk content and skinnings were co-located in a region corresponding to Bin 4 on 2H and that the allele for high husk content was associated with the allele for low skinnings. In the current study, we did not detect any polymorphism in this region of the genome in either the Derkado x B83-12/21/5 or the Tankard x Livet populations.

In another Australian study, seed shape parameters length, width and length:width ratio were analysed in a population from a cross between the Australian spring barley Chebec and the Canadian spring barley Harrington. A range of other characters was also analysed, including TKW (Langridge et al., 1996). The more significant effects for TKW were located in regions equivalent to Bin 3 on 1H, between Bins 5 and 8 on 2H, Bins 6 and 8 on 4H, and Bin 9 on 6H. For seed width, the more significant effects (P <0.01) were located between Bins 9 and 10 on 2H, Bin 13 on 3H, Bin 8 on 4H, and between Bins 3 and 7 on 5H. Regions affecting Length:Width ratio were co-located with the region affecting seed width on 5H and another was located in Bin 7 on 7H. No highly significant (P<0.01) regions affecting seed length were found. TKW has been studied in a number of crosses, the most relevant to the current study being Harrington x TR306 (Tinker et al., 1996), Blenheim x E224/3 (Powell et al., 1997), Blenheim x Kym (Bezant et al., 1997) and Tadmor x ER/APM (Teulat et al., 2001). Six primary QTLs for TKW in Harrington x TR306 were located in regions corresponding to Bins 4 and 10 on 4H, Bins 1 and 11 on 5H and Bins 1 and 7 on 7H, with a secondary QTL being located in Bin 3 on 6H (Tinker et al., 1996). No primary QTLs for TKW were detected in Blenheim x E224/3 but secondary QTLs were located the region of Bins 5 and 8 on 2H, 6 and 13 on 3H and Bin 11 on 5H (Powell et al., 1997). (Bezant et al., 1997) detected QTLs for TKW in Blenheim x Kym that were located in the region of:- Bins 3 and 14 on 2H, Bin 13 on 3H, Bin 11 on 4H, Bin 13 on 5H, Bin 10 on 6H, and Bin 2 and a region which is impossible to assign to a Bin on 7H. Over a range of environments, QTLs for TKW in the Tadmor x ER/APM population were detected in regions corresponding to:- between Bins 8 and 9 on 1H, Bin 8 on 2H, Bin 6 on 3H, Bin 13 on 4H, Bins 11 and 15 on 5H and Bins 6 and 14 on 6H.

Considering the grain shape parameters width, length and their ratio, there is reasonable concurrence between the results of the current study and that of (Langridge et al., 1996). The Bin 13 region of 3H and Bin 7 region of 7H was detected in Chebec x Harrington as well as Derkado x B83-12/21/5 and Tankard x Livet. In addition, the regions detected around Bin 8 on 4H and between Bins 3 and 7 on 5H in Chebec x Harrington were also detected in the Tankard x Livet and Derkado x B83-12/21/5 populations respectively.

The only other region detected in Chebec x Harrington where the probability was less than 0.01 was in the region of Bins 9 and 10 on 2H. We therefore have good evidence to substantiate the results from the current study for mapping grain shape parameters.

It is clear from the previous studies quoted above that regions of the genome affecting TKW in two-row spring germplasm are detected on all seven chromosomes. There is evidence from other crosses to substantiate most of the QTLs for TKW detected in this study (Table 10).

Table 10. Estimated Bin location of QTLs for TKW detected in selected other studies and the current study. Numbers in bold indicate Bins in common or adjacent to those detected in the current study.

Cross	1H	2H	3H	4H	5H	6H	7H
Harrington x TR306				4 + 10	1 + 11	3	1 + **7**
Blenheim x E224/3		5 + 8	6 + **13**				11
Blenheim x Kym		3 + **14**	**13**	11	13	10	2 + ??
Tadmor x ER/APM	**8/9**	8	6	13	11 + 15	**6** + 14	
Chebec x Harrington	**3**	5-8		**6**		9	
Derkado x B83-12/21/5	**4**	**14**	**13**	**6**	5/6		**7** + **8** + **12**
Tankard x Livet	**9**		**13**	8		**6** + **7?**	

The only QTLs that are unlikely to have been detected in the previously published studies are those in the region of Bin 8 on 4H in Tankard x Livet and between Bins 5 and 6 on 5H in Derkado x B83-12/21/5.

Given the problem of environmental variation for Split, the ability to use molecular markers as a means of selecting lines resistant to Split that is environmentally independent would be of great advantage to plant breeders and/or official testing authorities. We therefore need to demonstrate that the markers that we have identified as being associated with Split would provide such a method of identifying lines resistant to the character. This is best evaluated by either mapping in an unrelated population or by genotyping a range of lines of known splitting phenotype. We attempted the former means of validation through the analysis of

data from the Derkado x B83-12/21/5 population but we did not observe any significant genetical variation for Split. Whether the absence of genetic effects in the population was due to lack of suitable environments or its small-grain nature is not clear but we cannot use the population to draw any conclusions about the suitability of the markers that we had identified from the Tankard x Livet population. As an alternative, we can use the phenotypic data that we had collected on a limited number of control lines over 1999-2001. Some of these lines had been genotyped in an independent project and we can utilise the data from it to estimate the predicted level of Split for these lines. By comparing the genotypes of the lines in the regions around the QTLs for Split with those of Tankard and Livet, we can estimate the relative degree of Split in each control. We can then compare this data with that observed over the three years of trial to determine whether or not the use of the marker information would aid selection. Unfortunately, a number of the key markers identified in Tankard x Livet population were not represented in the genotypes of the controls in question and resources did not permit further testing so we cannot derive a true picture of the predicted genetic potential for Split. With this limitation and ignoring the epistatic effects, we obtained a correlation of +0.3 between predicted and observed Split. Whilst this is not as high as would be desired, it does indicate that there is potential in utilising the markers that we detected for selection. If this approach is adopted for Gape1 where the degree of genetic control is similar but the error variation much less (Figure 4) the correlation between predicted and observed gape is 0.4. Thus, even when the genetic control is relatively high, we are not getting a high correlation between predicted and observed performance probably because the available resources did not permit sufficient genotyping of the controls. Had we been able to do so, we have expected to find a closer agreement between predicted and observed performance. We can also utilise the phenotypic scores obtained from the lines grown in trial in 2001 (Figure 9) to provide some test of the validity of the predictions. By using the genotype at the marker closest to each of the 6 QTLs detected in the Tankard x Livet population in conjunction with the estimated QTL effect, we can predict the overall splitting performance of each of the 22 lines and compare it to the observed. The marker genotypes correctly identified eight of the worst 11 lines for splitting and could therefore be used in direct selection with a good level of confidence. This is not an independent test, however, and further work is required to assess the value of these markers in selection. Derivation of more closely linked, and even direct, gene markers would vastly improve the potential to use molecular markers for selection.

Milling energy, in contrast to shape parameters, depends on grain composition as well as shape and size (Camm et al. 1990). Selection for malting quality has reduced both the hardness of the endosperm and the thickness of the husk. The whole grain value for milling energy depends on the hardness of the starchy endosperm as this is the largest tissue of the grain (70% dm). This is despite the fact that on a weight for weight basis the milling energy of the husk can be three times that of the starchy endosperm. It is an

interesting implication, of the negative correlations between milling energy and gape and splitting, that milling energy reflects the resistance of the outer tissues to damage.

The pattern of relations between the QTLs suggests that alterations in GLength or GWidth that affect Wid/Len reflect a disruption of the appropriate grain dimensions to retain the integrity of the pericarp and/or testa and these can lead to grain splitting. We have identified the extent of genetic control for the trait grain splitting and have defined marker/trait associations in this particular cross. It is possible that if genes underlying the QTLs can be identified then the markers would have potential to be developed as diagnostics for use in marker assisted selection and in cultivar testing. In the broader sense, grain damage involves a complex interaction of genotypic and environmental factors that require to be carefully unpicked.

The critical factor in grain splitting and gape is likely to be the growth of the lemma and palea. The development of the lemma has been analysed with two series of mutants, *calcaroides* (*cal*) and *leafy lemma* (*lel1*) that map to loci on chromosomes 1H, 2H, 3H and 5H (Pozzi et al. 2000). More grain size may not be matched by the mechanical strength of the outer grain tissues. In addition, it is possible that global climate change, i.e. increased variability of rainfall, is occurring so fast that selection programmes are not keeping pace. Our results represent a significant step in the definition of the precise genetic control of grain traits important to maintenance of potential malting performance.

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TECHNICAL PAPER 3

CAUSES OF SKINNING IN GRAINS OF SPRING MALTING BARLEY. I. REPORT OF TRIALS IN 1999 AND II. REPORT OF TRIALS IN 2000

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I. Report of Trials 1999

Summary

A field experiment was conducted at the ADAS Bridgets Research Centre, Winchester, Hampshire in 1999. A malting quality spring barley, cv Chariot, was subjected to a range of treatments aimed at influencing skinning. Treatments included misting of plots with water using overhead sprays, creating 'sink' limited crops by removing half ears, maximising photosynthesis and 'source' by use of late strobilurin fungicides, souce limiting by crop shading and finally use of seed sealant spray. There were six treatments, including a control and three replicates of each treatment. Individual plot size was 4 m x2 m. Ears were sampled from each plot by hand and threshed using a laboratory thresher. Approximately 2 m² from each plot was harvested. The remaining plot area was harvested using a Sampo 20-25 plot combine. The concave was set to the minimum recommended for barley of 7 mm and the drum speed at maximum for the crop of 1250 rpm. These settings were chosen to maximise the opportunity for abrasion and skinning. Grain samples from both harvest methods was assessed for skinning using a standard technique. Percent skinning in the hand harvested samples was greatest for the misting treatment, reaching 20%, although overall there were no significant differences between treatments. In the ex-combine samples percent skinning was significantly greatest in the misted (12.3%) and sink limited treatments (14.8%) which were significantly greater than for all other treatments (7.8 - 9.6%).

Objective

To investigate whether field treatments can be used to induce high levels of grain skinning in malting barley, variety Chariot.

Treatments, assessments and records

There were six treatments fully randomised within each replicate block. There were three blocks and individual plots, 4 m x 2 m were marked out in a commercial crop of spring barley cv Chariot within Arizona field at ADAS Bridgets (See field plan in Appendix 2)

Except where specified below, all treatments (herbicide, nitrogen, fungicides, etc.) were applied across the experiment according to standard commercial practice for spring barley at the site (see site records in Appendix 1).

Experimental treatments

1. Control (no special treatment, single fungicide spray)
2. 'Sprinkled' with water using overhead sprays
3. 'Sink limited' by removal of half-ears (half the ear from each ear)
4. 'Clean ear' - Strobilurin/triazole fungicide applied at GS 55/59
5. 'Source limited' by shading during grain-filling
6. Desikote spray at GS 83/85

Details of experimental treatments:

2. 'Sprinkled' - plots were watered using overhead sprays following the onset of grain-filling (GS71-87). Water was taken from an adjacent mains supply. The amounts and frequency of water applied attempted to simulate the wetting and drying cycles observed in the 1997 season when skinning was a serious and significant problem.

3. 'Sink limited' - The top half of each ear was manually excised from the entire plot at GS 59-69.

4. 'Clean ear' - these plots were sprayed with a second fungicide using a mixture of strobilurin and triazole fungicides at GS 55/59 to maintain green canopy, maximising source availability and keeping ears as free as possible of fungal infections.

5. 'Source limited' - plots were shaded (target is 30% reduction using shade) using shade cloth from GS 61. Shading will permit rainfall to reach the crop.

6. Desikote. A spray treatment was used in an attempt to preserve grain quality.

Harvesting and sample collection

Barley was hand harvested (ears only) from approx. 2m² per plot. This was achieved by taking 2 x 1m² quadrats (double this for half ears treatment). Ears were weighed and placed in cloth bags. Samples were then despatched to ADAS Rosemaund for skinning assessment (see Appendix 3).

Skinning was expressed as the proportion by weight of grains which have lost their husk. The remainder of the plot was harvested using a plot combine (for yield measurement although data reliability on the remaining small area was acknowledged as low), the harvested seed was despatched to Rosemaund.

Combine settings

Plots were harvested on 29 July 1999 using a Sampo 20-25 plot combine harvester. The concave was set at 7 mm, the lowest recommended setting for barley and the drum speed increased to 1250 rpm, the maximum for barley.

Plot size was small for this harvest method, longer plots are preferred to minimise grain carryover from plot to plot.

Results and comment

Table 1. Treatment application details

Treatment	Details
1- Control	None
2- Sprinkled	Sprinkling began on 21 June 1999 - see separate details in table 2
3- Ear removal	Half ears were removed on 14 June 1999
4- Extra fungicide	Amistar at 1l/ha + Punch C at 0.625 l/ha was applied on 8 June 1999 at GS59
5- Shaded	Shading was erected on plots on 14 June.
6- Desikote	Desikote was applied on 30 June at 0.8 l/ha at GS83/85

Table 2. Sprinkling Schedule for treatment 2

Date	Sprinkling Schedule	Rainfall (mm)	Irrigation (mm)	Total precipitation
21 June	0900-1100, 1200-1400, 1500-1700	1.4	10.0	11.4
22 June	0900-1100, 1200-1400, 1500-1700	0	8.0	8.0
23 June	0900-1100, 1200-1400, 1500-1700	0	8.0	8.0
24 June		0		0
25 June		0		0
26 June		0.6		0.6
27 June		3.6		3.6
28 June	0900-1100, 1200-1400, 1500-1700	11.6	12.4	24.0
29 June	0900-1100, 1200-1400, 1500-1700	4.8	7.2	12.0
30 June	0900-1100, 1200-1400, 1500-1700	1.2	1.8	3.0
1 July		0.2		0.2
2 July		12.2		12.2
3 July		0		0
4 July		0		0
5 July		1.0		1.0
6 July		0		0
7 July		0		0
8 July		0		0
9 July		0		0
10 July		0		0
11 July		0		0
12 July	1500-1700	0		0
13 July	0900-1100, 1200-1400, 1500-1700	0	6.0	6.0
14 July	0900-1100, 1200-1400, 1500-1700	0	6.0	6.0
15 July	0900-1100, 1200-1400, 1500-1700	0	3.8	3.8
16 July	0900-1100, 1200-1400, 1500-1700	0	3.8	3.8
17 July	0900-1100, 1200-1400, 1500-1700	0	3.8	3.8
18 July	0900-1100, 1200-1400, 1500-1700	0	3.8	3.8
19 July	Sprinkler removed	4.8		4.8
20 July		0		0
21 July		0		0
22 July		0		0
23 July	Hand harvest	0		0

Results from the assessment of hand harvested and ex-combine harvested samples are shown below.

Table 3. Percent skining

Treatment	% skinning Hand harvest	Ex-combine
Untreated (control)	4.0	9.7
Sprinkling/Misting	20.7	12.3
Half ears ('sink' limited)	9.5	14.8
Extra strobulirin fungicide	9.7	8.8
Shading ('source' limited)	0.7	7.8
Grain sealant	5.6	9.3
SED (10df)	7.54	1.35
Prob	0.225	0.004
CV%	110.7	15.8

- Percent skinning in the hand harvested samples showed a wide range, 0.7 to 20.7%. The raw data was skewed and there was a suggestion of some influence to plots in the prevailing wind from misted plots.

- Overall there was no significant difference between treatments for the hand harvested samples.

- Percent skinning in the ex-combine samples ranged from 7.8 to 14.8%. Percent skinning was significantly greater in the misted and sink limited plots. The combine settings used were chosen to maximise abrasion. The TGW of grain from sink limited plots was higher than for other treatments (see Table 4).

Table 4. Thousand Grain Weight (g at 100% DM) of ex-combine and hand harvested samples.

	TGW (g)	
Treatment	Hand harvest	Ex-combine
Untreated (control)	42.0	40.3
Sprinkling/Misting	41.2	40.2
Half ears ('sink' limited)	44.9	41.9
Extra strobulirin fungicide	41.9	40.7
Shading ('source' limited)	39.0	37.4
Grain sealant	42.6	40.3
SED (10df)	0.57	0.68
Prob	<0.001	0.002
CV%	1.7	2.1

- TGWs were higher for hand samples than ex-combine
- TGW was significantly greater for the half ear treatments (sink limited). This may explain the higher % skinning figures recorded for this treatment after combining, relative to the value recorded after hand harvest.
- TGWs were significantly less for shaded grain (source limited)

Acknowledgements

The authors thanks Mr Rick Kane, ADAS Bridgets Research Centre for technical assistance and Ms Sonia Brunton, ADAS Starcross for skinning asssessments.

Appendix 1.

Details of site and crop on trial plots

SITE

Site name	:	ADAS Bridgets
Field name	:	Arizona
Soil texture	:	Silty clay loam
Drainage	:	Good
Soil analysis (199?)		
PH	:	8.2
P index	:	14 (1)
K index	:	171 (2)
Mg index	:	28 (1)
Copper	:	0.75
Previous cropping		
1998	:	Maize
1997	:	Spring Linseed
1996	:	Winter Wheat
1995	:	Winter Oilseed Rape
Previous crop residue	:	Ploughed under
Previous cultivations	:	Ploughed and drilled

CROP

Cultivar	:	Chariot
Sowing dates	:	04-02-99
Seedrate	:	160 kg/ha
Fertilizer		
(i)	:	0:20:30 at 300 kg/ha product 10-03-99
(ii)	:	Sulphan (30%N 19SO_3) at 300 kg/ha product 22-03-99
Herbicide		
(i)	:	Harmony M at 50 g/ha + Starane at 0.4 l/ha 30-04-99

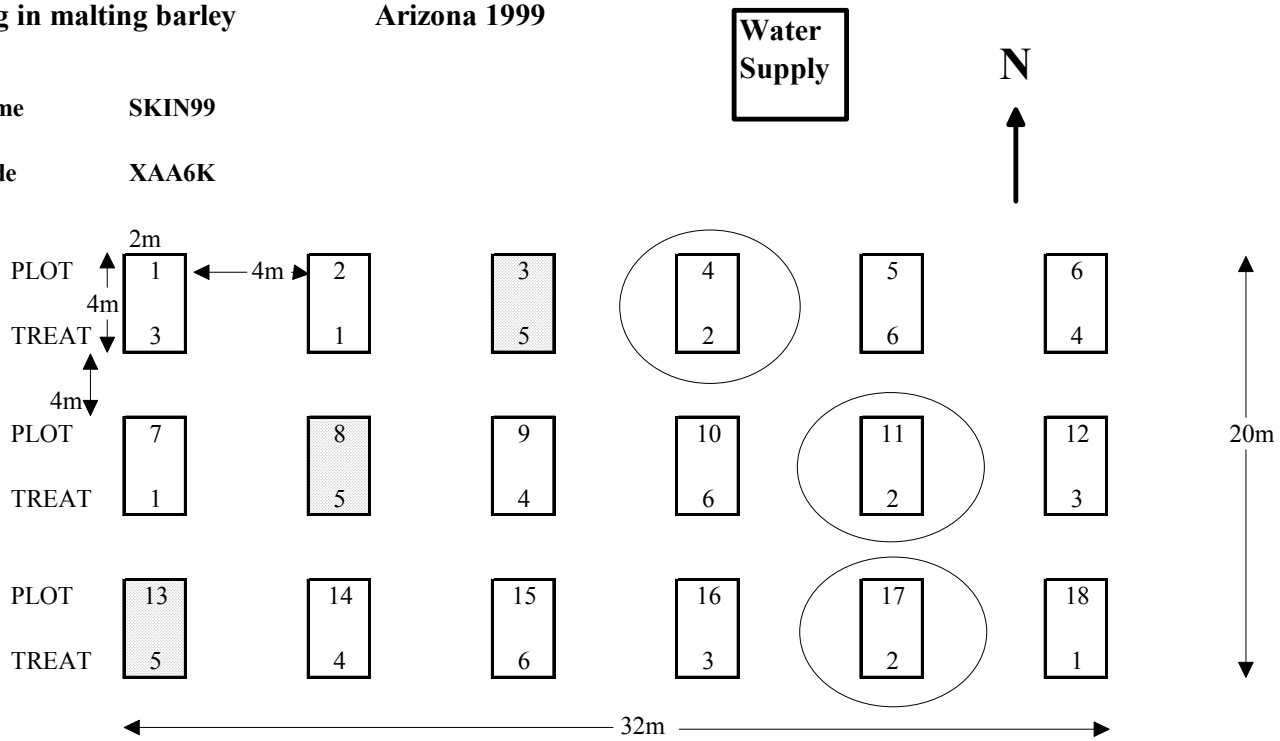
Growth regulator		
(i)	:	None
Insecticide		
(i)	:	None
Fungicide		
(i)	:	Landmark 1.0 l/ha + Mistral 0.25 l/ha 14-05-99
Harvest date	:	23 July 1999 for hand harvest
	:	29 July 1999 for combine harvest

Appendix 2. Experiment plan ADAS Bridgets 1999 season.

Skinning in malting barley **Arizona 1999**

Study Name **SKIN99**

Study Code **XAA6K**



Treatments

1 Control (no special treatment)
2 'Sprinkled' using overhead sprays 22-23/06/99, 26-27/06/99, 12-16/07/99 (6-10mm/day)
3 'Sink limited' by removal of half ears 14/06/99 (GS69)
4 'Clean ear' - Amistar? applied at GS 55/59 08/06/99 (GS59)
5 'Source limited' by shading during grain-filling 14/06/99 (GS69) - 19/07
6 Desikote 30/06/99 GS83

Appendix 3. Assessment of skinned corns in a barley sample.

Materials and equipment

Good light source (preferably daylight)

Balance with a resolution of 0.01 g or better

Sealable polythene bags

Sheet of A3 white paper

Forceps

Procedures

1. Screen grain using a 2.2 mm slotted sieve. Discard all material falling below this size.

2. Thoroughly mix the screened sample of grain for analysis. This should be preferably done using a sample divider but where this is not available place the sample in a bucket and mix thoroughly by hand.

3. Weigh a 50 ± 0.05 g sample and spread out on a sheet of A3 white paper.

4. Ensure a good light source is directed on the sample.

5. Select those grains which have lost >20/25% of their husk. Remove them from the sample using the forceps and collect them in a separate beaker. Include broken grains in the assessment where it is obvious that the husk has been lost. Weigh the separate samples of skinned and intact grains.

6. Calculate the % skinning as the proportion by weight of grains which have lost their husk compared to the total sample weight.

7. Present results in the following table format in Excel:

Sample id (Variety, location or treatment)	Total sample wt., (g)	Wt. intact grains, (g)	Wt. Skinned grains, (g)	% (by wt.) skinned grains
e.g. Chariot, Cockle Park				

II. Report of Trials 2000

Summary

A field experiment was conducted at the ADAS Bridgets Research Centre, Winchester Hampshire in 2000. Two malting quality spring barley varieties, Chariot and Optic, were subjected to a range of treatments aimed at influencing skinning. Treatments included misting of plots with water using overhead sprays, creating 'sink' limited crops by removing half ears, maximising photosynthesis and 'source' by use of late strobilurin fungicides and 'source' limiting by crop shading. There were five treatments, including a control and three replicates of each treatment. Individual plot size was 4 m x 3 m. Ears were sampled from each plot by hand and threshed using a laboratory thresher. Approximately 2 m² from each plots was harvested, 4m² on sink limited plots. The remaining plot area was harvested using a Sampo 20-25 plot combine. The concave was set to the 9 mm and the drum speed at maximum of 1200 rpm. These settings were chosen to maximise the opportunity for abrasion and skinning without causing excessive grain cracking. Grain samples from both harvest methods were assessed for skinning by visual scoring of a 50 g sub sample. Skinning % in the hand harvested crops was low, at 1.2 to 3.7%, but there were significant treatment differences. Percent skinning was greatest for the sprinkled and half ear treatments. Percent skinning in the ex combine grain samples were much higher, ranging from 7.3 to 14.9%. This reflected the deliberately high abrasive nature of the combine settings (mean 11.3% for the control treatment) used to harvest plots. Percent skinning was greatest for the half ear 14.9% and sprinkled (12.2%) treatments. The skinning assessments in ex combine grain samples demonstrated the effect of combine settings on grain skinning. Percent skinning was increased for the sink limited treatment (half ears) presumably as a consequence of the larger grains being exposed to greater abrasion during combining. In the ex combine samples there was marginally more skinning in Chariot than Optic, although this difference was not significant at the 5% level of probability. Compared to the 1999 season, which was predominately dry, the weather during the summer period of 2000 was milder and wetter, therefore the exposure to wet/cool, hot/dry cycles was not as pronounced. This may explain the smaller differences in % skinning between treatments recorded in 2000, although the pattern of treatment effects on skinning was the same.

Objective

To investigate whether field treatments can be used to induce high levels of grain skinning in malting barley, varieties Chariot and Optic.

Treatments, assessments and records

There were five treatments fully randomised within each replicate block. There were three blocks and individual plots, 4 m x 3 m were drilled with varieties Chariot and Optic within California field at ADAS Bridgets.

Except where specified below, all treatments (herbicide, nitrogen, fungicides, etc.) were applied across the experiment according to standard commercial practice for spring barley at the site (see site records in Appendix 1).

Experimental treatments

1. Control (no special treatment, single fungicide spray)
2. 'Sink limited' by removal of half-ears (half the ear from each ear)
3. 'Clean ear' - additional Strobilurin/triazole fungicide applied at GS 55/59
4. 'Source limited' by shading during grain-filling
5. 'Sprinkled' with water using overhead sprays

Details of experimental treatments:

2. 'Sink limited' - The top half of each ear was manually excised from the entire plot at GS 59-69.

3. 'Clean ear' - these plots were sprayed with a second fungicide using a mixture of strobilurin and triazole fungicides at GS 55/59 to maintain green canopy, maximising source availability and keeping ears as free as possible of fungal infections.

4. 'Source limited' - plots were shaded (target is 30% reduction using shade) using shade cloth from GS 61. Shading will permit rainfall to reach the crop.

5. 'Sprinkled' - plots were watered using overhead sprays following the onset of grain-filling (GS 71-87). Water was taken from an adjacent mains supply. The amounts and frequency of water applied attempted to simulate 5 day cycle of wetting and drying for a period of 20 days . Poor weather (rain) dictated a less uniform pattern of misting in 2000 compared to 1999 seasons. (see Table 2)

Harvesting and sample collection

Barley was hand harvested from approx. 2m² per plot. This was achieved by taking 2 x 1m² quadrats (double this for half ears treatment). Ears were weighed and placed in cloth bags. The ears were threshed in a stationary Wintersteiger Ear thresher as gently as possible. Samples were then despatched to ADAS Starcross for assessment of skinning. Skinning was expressed as the proportion by weight of grains which have lost their husk.

The remainder of the plot was harvested using a plot combine (for yield measurement although data reliability on the remaining small area was acknowledged as low), the harvested seed was despatched to ADAS Starcross.

Combine settings

Plots were harvested on 11 August 2000 using a Sampo 20-25 plot combine harvester. The concave was set at 9 mm (the limit of excess grain cracking) and the drum speed increased to 1200 rpm, the maximum recommended for barley.

Materials and methods

Table 1. Treatment application details

Treatment	Details
1- Control	None
3- Ear removal	Half ears were removed on 28/06/00
4- Extra fungicide	Amistar Pro at 2l/ha was applied on 20/06/00 at GS59
2- Sprinkled	Sprinkling began on 3 July - see separate details in Table 2
5- Shaded	Shading was erected on plots on 23 July.

Table 2. Sprinkling Schedule for treatment 2

Date	Hours Of Irrigation	Irrigation (mm) & Rainfall (mm)	Maximun Temp °C	Minimum Temp °C	Solar Radiation (W/m²)
03/07/00	14.92	66.096	24.97	8.59	4119
04/07/00	15.50	88.168	18.52	10.44	4978
05/07/00	0	0	22.37	12.74	1205
06/07/00	11.45	97.92	24.26	13.1	2876
07/07/00	0	0	19.33	12.84	3615
08/07/00	0	0	17.59	7.12	4340
09/07/00	0	0	19.63	12.17	2264
10/07/00	0	4.08	16.81	10.44	3592
11/07/00	0	0	19.92	8.15	2816
12/07/00	0	3.264	18.95	3.118	4633
13/07/00	0	0	23.63	12.85	2947
14/07/00	0	0	19.2	12.41	4466
15/07/00	0	0	22.98	5.286	3799
16/07/00	0	3.264	23.04	5.173	5949
17/07/00	14.92	69.36	26.82	5.845	6208
18/07/00	0	0	27.96	5.845	6212
19/07/00	0	0	29.61	7.7	6031
20/07/00	0	0	29.21	8.82	6494
21/07/00	0	0	28.72	9.27	5623
22/07/00	0	0	24.18	11.76	6284
23/07/00	0	0	22.77	12.59	6561
24/07/00	0	0	17.5	12.34	3679
25/07/00	15.5	73.44	21.05	11.74	2214
26/07/00	0	0	25.36	10.5	*
27/07/00	15.66	88.944	23.64	10.16	*
28/07/00	0	0	23.43	12.96	*
29/07/00	0	0	23.45	10.67	*
30/07/00	0	0	26.12	11.66	4620
31/07/00	15.58	83.232	25.99	12.84	5179
01/08/00	15.58	86.496	25.30	10.27	6450

* Solar Radiometer taken out of service for routine maintenance.

Results and comments

Grain Samples

Table 3a. Percent skinning in 50 g subsample of barley, hand sampled material, by weight.

	Control	Half ear	Clean ear	Shaded	Sprinkled	Mean
	(SED=0.58)					(SED=0.26)
Optic	1.2	2.9	1.9	1.5	2.9	2.1
Chariot	1.7	2.5	1.5	1.3	3.7	2.2
	(SED=0.41)					
Mean	1.5	2.7	1.7	1.4	3.3	

CV%= 33.7%

- Percent skinning was low.

- Only management treatment had a significant effect on % skinning (P<.001). Variety had no effect on percent skinning.

- Skinning was greatest for the sprinkled treatment at 3.3% and marginally less for the half ears (sink limited) treatment at 2.7%. Percent skinning for all other treatments was similar.

Table 3b. Thousand Grain weight (g) in barley, hand sampled material.

	Control	Half ear	Clean ear	Shaded	Sprinkled	Mean
	(SED = 0.68)					(SED = 0.31)
Optic	48.0	49.7	50.2	45.1	47.3	
Chariot	42.0	43.1	41.4	38.2	40.4	
	(SED = 0.48)					
Mean	45.0	46.4	45.8	41.6	43.9	

CV% = 1.9 %

- Thousand grain weight was significantly greater in Optic than Chariot ($P<0.001$) and was also affected by treatment ($P<0.001$). The interaction between variety and treatment was almost significant ($P=0.093$).
- Thousand grain weight was least for the shaded (source limited) treatments and greatest for the half-ears, (sink limited) treatments.

Table 4a. Percent skinning in 50 g subsample of ex-combine grain by weight.

	Control	Half ear	Clean ear	Shaded	Sprinkled	Mean
	(SED=2.13)					(SED=0.95)
Optic	12.1	11.4	8.4	7.9	11.1	10.1
Chariot	10.5	18.4	10.8	6.7	13.3	12.0
	(SED=1.51)					
Mean	11.3	14.9	9.6	7.3	12.2	

CV%= 23.6%

- Only management treatment had a significant effect on % skinning (P=0.001), although variety (P=0.083) and the interaction (P=0.072) were almost significant at the 5% level of probability.

- Skinning was greatest for the half ears (sink limited) treatments, which also had the largest grain size (see table 3a below). Skinning was least for the shaded treatment (smallest grain size).

- Skinning was marginally greater in Chariot than Optic, despite its smaller grain size, although this was not significant. With the exception of sink limited, the sprinkled treatment gave the greatest level of skinning overall, but differences were not significant.

Table 4b. Thousand Grain weight (g) in ex-combine grain by weight.

	Control	Half ear	Clean ear	Shaded	Sprinkled	Mean
	(SED=0.66)					(SED=0.29)
Optic	48.0	50.2	50.2	45.1	47.3	48.2
Chariot	42.0	43.1	41.4	38.2	40.4	41.0
	(SED=0.46)					
Mean	45.0	46.6	45.8	41.6	43.9	

CV%= 1.8%

- Thousand grain weight was significantly greater in Optic than Chariot (P<0.001) and was also affected by treatment (P<0.001). The interaction between variety and treatment was almost significant (P=0.096).

- Thousand grain weight was least for the shaded (source limited) treatments and greatest for the half-ears, (sink limited) treatments.

Table 5a. Combine Harvest Yield Data @ 15% Moisture

	Control	Half ear	Clean ear	Shaded	Sprinkled	Mean
	(SED=0.566)					(SED=0.253)
Optic	4.52	3.02	4.83	4.00	4.44	4.16
Chariot	5.36	3.79	4.96	4.13	4.89	4.63
	(SED=0.400)					
Mean	4.94	3.40	4.90	4.06	4.67	

CV%=15.8

- Statistical confidence in yield data was low due to the small plot size.

- There was no significant difference in the yield of the two varieties (P=0.081).

- Yield were significantly affected by crop management (P=0.005), with the lowest yields recorded for the half ear (35% yield reduction) and shaded treatments (20% yield reduction) compared to the control.

- Hand harvested yields (from quadrats) indicated an identical pattern, although yields were lower.

Acknowledgements

The author thanks Mr Stuart Faithful and Mr Rick Kane, ADAS Bridgets Research Centre for technical assistance and Ms Sonia Brunton, ADAS Starcross for skinning asssessments.

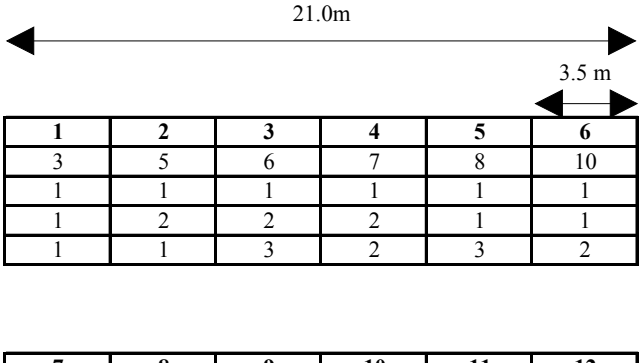
Appendix 1.

Details of site and crop on trial plots

SITE

Site name	:	ADAS Bridgets
Field name	:	California
Soil texture	:	silty clay loam
Drainage	:	Good
Previous cropping		
1999	:	Grass
1998	:	Grass
1997	:	Grass
1996	:	Grass
Previous crop residue	:	ploughed under
Previous cultivations	:	ploughed and drilled
CROP		
Cultivar	:	Chariot & Optic
Sowing date	:	10/03/00
Seedrate	:	169 kg/ha (Chariot) 200 kg/ha (Optic), all 400 seeds m²
Fertilizer		
(i)	:	Sulphan (30%N 19SO_3) at 300 kg/ha product 22/04/00
Herbicide		
(i)	:	Harmony M at 46 g/ha + Starane at 0.6 l/ha 01/05/00
Growth regulator		
(i)	:	None
Insecticide		
(i)	:	None
Fungicide		
(i)	:	Landmark 0.5 l/ha
Harvest date	:	11 August 2000 for hand harvest
	:	11 August 2000 for combine harvest

Appendix 2. Trial plan for California field site 2000



MARKED PLOT	**1**	**2**	**3**	**4**	**5**	**6**
STATISTIC PLOT	3	5	6	7	8	10
BLOCK	1	1	1	1	1	1
TREAT1	1	2	2	2	1	1
TREAT2	1	1	3	2	3	2

MARKED PLOT	**7**	**8**	**9**	**10**	**11**	**12**
STATISTIC PLOT	11	13	14	17	18	20
BLOCK	2	2	2	2	2	2
TREAT1	1	1	1	2	2	2
TREAT2	2	3	1	1	3	2

MARKED PLOT	**13**	**14**	**15**	**16**	**17**	**18**
STATISTIC PLOT	21	22	25	27	28	29
BLOCK	3	3	3	3	3	3
TREAT1	2	1	2	1	1	2
TREAT2	3	1	2	2	3	1

36m

MARKED PLOT	**19**	**20**	**21**	**22**	**23**	**24**
STATISTIC PLOT	1	9	12	19	26	30
BLOCK	1	1	2	2	3	3
TREAT1	2	1	1	2	2	1
TREAT2	4	4	4	4	4	4

7.0m (Last Block only)

MARKED PLOT	**25**	**26**	**27**	**28**	**29**	**30**
STATISTIC PLOT	2	4	15	16	23	24
BLOCK	1	1	2	2	3	3
TREAT1	1	2	2	1	2	1
TREAT2	5	5	5	5	5	5

Treatments

Treat 1

1	Optic	(400 seeds/m²)
2	Chariot	(400 seeds/m²)

Treat 2

1	Control (single Fungicide Spray)
2	Sink limited (Half the Ear From Each Plant removed)
3	Clean Ear (Strobulurin/Triazole fungicide @ GS 55/59)
4	Source Limited (Shaded)
5	Sprinkled (Overhead Spray GS 71-87)

Appendix 3. Weather data for the trial growing season.

Month	Mean Max. Temp. °C	Mean Min. Temp. °C	Mean 10 cm Soil Temp °C	Mean 50 cm Soil Temp °C	Mean Soil Surface Temp °C	Mean Solar radiation W/m²	Mean Wind Speed m/s	Total Rainfall mm
January	8.01	0.1	3.9	5.4	-1.6	883.2	2.9	18.6
February	10.0	2.0	5.4	6.2	0.7	1528.8	3.4	64.0
March	12.0	2.5	6.2	7.1	0.3	2376.6	2.7	10.4
April	12.8	3.6	7.1	7.6	2.3	3222.5	*	144.8
May	18.6	7.5	12.7	11.9	9.4	4648.7	1.4	65.6
June	21.8	9.9	13.5	12.8	11.7	4723.7	2.2	15.0
July	22.9	10.1	14.4	14.0	11.3	3834.8	1.9	43.6
August	24.4	10.8	17.3	16.7	11.4	4449.0	2.1	29.2

TECHNICAL PAPER 4

HUSK ADHERENCE IN MALTING BARLEY

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Introduction

This paper reports some of our more detailed observations into husk adherence that may help us to understand the structural causes of skinning. The work described here supports part of the wider investigation into the physiology of grain development in relation to skinning, gape and splitting in which comparisons were made between the cultivars Chariot and Landlord (i.e. Experiments I and II in Technical Paper 1, in this Report).

The barley grain at harvest is composed of a caryopsis enclosed in a husk. The caryopsis consists of the embryo and the endosperm together enclosed in the testa which is fused to the pericarp except at the ventral crease. The husk is made up of two (fertile) glumes, the palea on the ventral (adaxial) side and the lemma on the dorsal (abaxial) side. Both the lemma and the palea adhere to the surface of the pericarp except at the apical or distal end and along their somewhat hyaline edges where the lemma usually overlaps the palea. The adherence of the husk to the caryopsis is of considerable significance in both malting and brewing (Palmer, 1989). If, in a batch of barley there are grains without husks, these grains will germinate more rapidly than those with firmly adhering husks, thus giving rise to uneven malting. However, sometimes, grains without husks are likely to sustain embryo damage which prevents germination and gives rise to mould growth. In grains with a loosely adhering husk, the growth of the plumule (acrospire) tends to be more vigorous than in grains with tightly adhering husks and this leads to handling problems and to greater malting losses. In brewing, the husk plays a vital role in filtration in the mash tun. Malting barley is therefore rejected by maltsters if it contains an undue proportion of grains that have undergone skinning (known as 'peeling' in Canada) during harvesting, and have either no husk or an incomplete husk. In a discussion of the Canadian malting barley varieties of the future, Edney (1999) puts hull (husk) adherence top of his list of desirable traits. Australian barley breeders are also concerned that any barleys introduced into their breeding

programme should not produce cultivars with a level of skinning higher than that of the malting barleys well adapted to Australian conditions (Roumeliotis *et al*. 2001). Analyses in Canada of crosses involving Harrington, a cultivar prone to peeling (skinning), indicated that heritability of hull peeling was relatively low to moderate and that much of the variability observed in this trait was due to environmental factors (Aidun *et al*. 1990). In Australia, Roumeliotis *et al*. (2001) also found that environmental factors affected skinning levels but they concluded that it may be possible to breed barley varieties with low husk content, good husk adherence and high malt extract.

In the early stages of grain development i.e. up to about 18 days after anthesis in plants grown under glasshouse conditions at approximately 15ºC, the husk does not adhere to the pericarp. Then, within a day or two it becomes impossible to remove the lemma and palea from the caryopsis without tearing them or removing the epidermis of the pericarp. The nature of the material that cements the husk to the pericarp was investigated by Gaines et al. (1985). They concluded that the cementing layer was produced by the pericarp only, that it could be observed on the surface of the pericarp as early as two days after flowering, and that the husk adhered to the pericarp from 10 days after flowering onwards. Using several staining methods they demonstrated that the cementing layer did not contain detectable amounts of carbohydrate or protein. They suggested that the cementing layer may be cuticular in origin. Using Sudan IV 'staining' of hand-cut sections of fresh caryopses, Cochrane and Duffus (1979) demonstrated the presence of a lipid layer on the outside of the pericarp epidermis of immature grains. They compared the ultrastructure of this cuticular layer with that of the cuticular layers of the testa and nucellus. (Freeman and Palmer (1984) identified a cuticular layer approximately 0.3µm thick on the outside of the pericarp of mature grains and a similar layer on the outside of the husk. Palmer (1989) concluded that the cementing layer between the pericarp and the husk is cuticular in origin.

The adherence of the palea to the caryopsis also occurs in *Bromus,* a genus closely related to *Hordeum*. Smith (1989) showed that it was possible to separate the palea from the caryopsis in *Bromus* spp. by incubating the grains in solutions of EDTA or of pectinase enzymes. He therefore concluded that in this species, one layer of the cementing material was composed of pectinaceous material. A rather more drastic procedure, steeping in 50% sulphuric acid, has been used to remove the husk from barley grains (Palmer, 1989). However, as this process is reported to remove most if not all of the pericarp as well as the husk, it cannot be concluded that the cementing layer in barley is soluble in strong acid.

The observations reported herein are from light microscopy, electron microscopy and enzyme treatments in grains sampled from Experiment II, in Technical Paper 1, in this Report.

Materials and Methods.

Growth of plants

Plants of barley cvs Chariot and Landlord were grown in pots in peat-based compost in a glasshouse in a series of experiments in which shading was used (see Technical Paper 1). In Experiment II of this series, plants were divided into two groups. One group was shaded from GS25-31 to completion of anthesis. The other group was shaded after anthesis until the grains were harvested. Ears were tagged at anthesis (mid- to late-July 1999).

Light microscopy using hand-cut sections

At 24, 31, 38 and 45 days after anthesis grains were sampled from the middle of ears cut from each of the two groups of plants in Experiment II. Hand-cut transverse sections from mid-grain were stained in Fluorol Yellow (Brundrett *et al*. 1991), and examined and photographed as described previously (Cochrane *et al*. 2000)

Transmission electron microscopy

Transverse slices 1 mm thick were cut from the middle of grains under a fixative containing 2.5% glutaraldehyde in 0.025M Na/Na phosphate buffer pH 7.15. After immersion in the fixative for 4h at room temperature, the tissue slices were dehydrated in an ethanol series and embedded in LR White resin. Sections of the resin-embedded material were stained using uranyl acetate and lead citrate and examined in a Philips CM120 Biotwin transmission electron microscope.

Scanning electron microscopy

Grains from ears of cvs Chariot and Landlord grown from anthesis in shaded and unshaded conditions were harvested 17 days after anthesis. They were fixed overnight at 4°C in 2.5% glutaraldehyde in 0.025M Na/Na phosphate buffer pH 7.1, dehydrated in an acetone series and either dried in air or by critical point drying, coated with gold and viewed in a Cambridge S250 scanning electron microscope.

Enzyme treatments

Intact grains and transverse slices of grains cut at mid-grain were incubated with and without gentle agitation, for periods up to two days at room temperature or at 37°C in buffered solutions containing

pectinase, and/or cellulase, and in solutions of sodium ethylene diamine tetra-acetic acid (EDTA). Control samples of grains or grain slices were incubated in the appropriate buffers. Enzymes were supplied by Sigma, UK. Pectinase (3.2.1.15) was used in citric/phosphate buffer pH4 at concentrations up to 180 units ml^{-1}. Cellulase (3.2.1.4) was used in citric/phosphate buffer pH5 at concentrations up to 25 units ml^{-1}. NaEDTA was used in borate buffer pH10 at a concentration of 0.07M.

Results

Light microscopy

In hand-cut sections of fresh grains, a narrow band of material between the pericarp epidermis and the inner epidermis of the lemma and palea fluoresced after staining in Fluorol Yellow in the same way as did the cuticular layers on either side of the testa (Figures 1 and 2). Where the lemma or palea has separated from the caryopsis during specimen preparation, a brightly fluorescent layer is visible on the outside of the pericarp but not along the inside of the palea or lemma. Cuticular material is not visible on the outer epidermis of the palea or lemma (i.e. glumes).

Transmission electron microscopy

Mature grains of barley are notoriously difficult to section for transmission electron microscopy but sections were obtained in which it was possible to see the three cuticular layers identified by light microscopy. In Figure 3, a section of a grain fixed 45 daa, a cuticular layer is sandwiched between the thin-walled cells of the of the inner epidermis of the lemma and the crushed cell walls of the pericarp. A much thicker cuticular layer lies between the pericarp and the crushed remains of the testa. Traces of a thin cuticular layer can be seen outside the aleurone, adjoining the nucellus, but there is no evidence of a cuticular layer on the outer epidermis of the lemma. At higher magnification (Figure 4), the outermost cuticular layer, i.e. that between the pericarp and the lemma, has an irregular, 'bubbly' outline on the side next the pericarp, and a smooth, slightly electron dense edge next the lemma. In a section from another grain (Figure 5), however, the edge of the cuticular layer adjoining the lemma was also irregular, though somewhat less so than the edge adjoining the pericarp. The reticulation that is a prominent feature of the cuticular layers of the testa and nucellus (Figure 6) is absent from the pericarp cuticle. The pericarp cuticular layer is approximately the same thickness as the nucellus cuticular layer and about one-third as thick as the testa cuticular layer. In Figures 4, 5

and 7 it is possible to see a double line of slightly electron dense material, running parallel to the cell walls, in the otherwise uniform cuticular material. In the separation of husk and pericarp in Figure 7, the cementing layer appears to have split between the two lines. The thickness of the cementing layer was approximately the same at 24 daa as it was at 45 daa, thus indicating that deposition of cuticular material ceased well before grain-filling was completed.

Scanning electron microscopy

The surface of the pericarp of grains fixed 17 daa was examined. Only one or two stomata were found on each grain. These were located on the ventral side just behind the hairs at the apex (Figures 8 and 9). No differences between the cultivars Chariot and Landlord were detected in number or distribution of stomata.

Enzyme treatments

None of the enzyme treatments used on mature, immature, or sliced grains separated the husk from the caryopsis. Incubation in EDTA also failed the remove the husk, but some loosening of the husk did occur when grains were incubated at 37°C for 19 hours in borate buffer, pH10. When slices of immature and mature grains were incubated in pectinase it was found that in the conditions used, the starchy endosperm of the immature grains was completely disintegrated, and that of the mature grains was partially disintegrated, but in both cases the husk remained firmly attached to the pericarp. No disintegration of endosperm tissues was observed in control slices incubated in buffer.

Discussion

Evidence from fluorescence microscopy of hand-cut sections of fresh grains and from transmission electron microscopy of sections of resin-embedded grains confirms that the cementing layer between the pericarp and the husk of barley grains is largely, if not entirely, composed of cuticular material. The cuticular membrane of the testa appears to resemble closely the generalised model of the mature cuticular membrane put forward by Jeffree (1996), except for the absence of cuticular wax on the surface and of lamellae in the cuticle proper. Jeffree interprets the 'bubbly' interface with the secondary cell wall as evidence of the deposition of globular masses of cutin in the cell wall and concludes that this process takes place after the formation of the cuticle proper. The pericarp cuticle is somewhat different from the cuticular membrane of the testa. It has a well-developed 'bubbly' boundary with the pericarp epidermal cell wall but lacks reticulation and is almost amorphous

throughout. The boundary with the inner epidermis of the glumes shows little if any evidence of the deposition of cutin globules in the cell wall. It is possible therefore, that all the cuticular material in the cementing layer originates in the pericarp epidermis as proposed by Gaines *et al.* (1985). However, if this were the case, the inner surface of the glumes would be without a cuticular layer for the first two to three weeks of grain growth and would thus be unprotected from water loss and invasion by micro-organisms. A more likely explanation is that early in their development, the cells of the inner epidermis of the glumes produce a cuticle proper but do not proceed further along the pathway of cuticular membrane formation described by Jeffree. The cementing layer would thus be formed by the fusion of the two cuticles. The presence of slightly electron dense layer inside the otherwise amorphous almost electron-lucent cementing layer would seem to provide evidence of this fusion.

The difference between the cuticular membrane of the testa and the cementing layer between the pericarp and husk in their response to ruthenium red may be related to the presence of reticulation in the former but not in the latter. It is possible that no pectin lamella forms outside the secondary cell wall of the pericarp epidermis. Alternatively, it is also possible that pectinaceous material is deposited under the cuticle proper of the pericarp epidermis very early in grain development but that as the cuticular membrane matures, the pectinaceous material is enveloped in cutin. It thus becomes unavailable either to stains such as ruthenium red or to enzymes in aqueous solutions. Cuticular membranes have been isolated from the leaves of many species using pectinase, but in some cases this can only be achieved before the leaves are fully developed (Jeffree, 1996). The separation of the husk from the caryopsis in *Bromus* using pectinase (Smith, 1989), would suggest that in this genus, a pectin lamella is present in the cementing layer.

When the husk separates from the caryopsis i.e. when skinning takes place, the cementing layer is thought to separate from the husk and remain attached to the pericarp (Gaines *et al.*, 1985). Our observations on grains fixed 24 daa suggest that the separation may occur along the electron-dense line in the amorphous layer (Figure 7). There would thus be two different interpretations of the cause of skinning. It could be due to a failure of the cuticular material to adhere to the surface of the inner epidermis of the glumes (palea or lemma), or it could be due to inadequate fusion of the cuticle proper of the pericarp epidermis with that of the inner epidermis of the glumes. Whichever is the cause, the critical processes appear to take place very early in grain development, possibly even before anthesis, and to involve the synthesis of cuticular material. The identification of a particular chemical pathway responsible for the phenomenon of skinning is an impossible task at this time. The composition of cutin differs not only from species to species but also from organ to organ in any one plant. It is also influenced by environmental factors (Kolattukudy, 1996).

The results in Technical Paper 1 indicated that the cultivars Landlord and Chariot differed in the rate of water loss from the grains during the final stages of grain maturation. Previous observations on the morphology of barley grains (Cochrane and Duffus, 1979) had shown that there are stomata in the pericarp epidermis. Our results confirm the presence of stomata. It is possible that stomata might play a part in grain dehydration. However, there was no evidence to suggest that the number and/or distribution of pericarp stomata differed between two cultivars.

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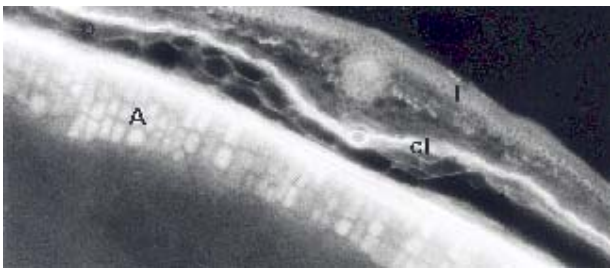

Figure 1. The dorsal side of a hand-cut transverse section from the middle of a fresh grain of barley cv. Chariot harvested 38 days after anthesis. The section was stained in Fluorol Yellow and photographed using fluorescence microscopy. cl, cementing layer; l, lemma; p, pericarp; A, aleurone; arrow, testa cuticular layer. × 130.

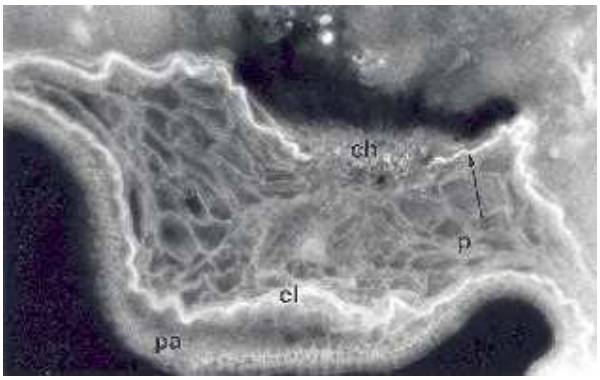

Figure 2. The ventral side of a hand-cut transverse section from the middle of a fresh grain of barley cv. Chariot harvested 45 days after anthesis. The section was stained in Fluorol Yellow and photographed using fluorescence microscopy. cl, cementing layer; pa, palea; p, pericarp; ch, chalaza; arrow, testa cuticular layer. × 130.

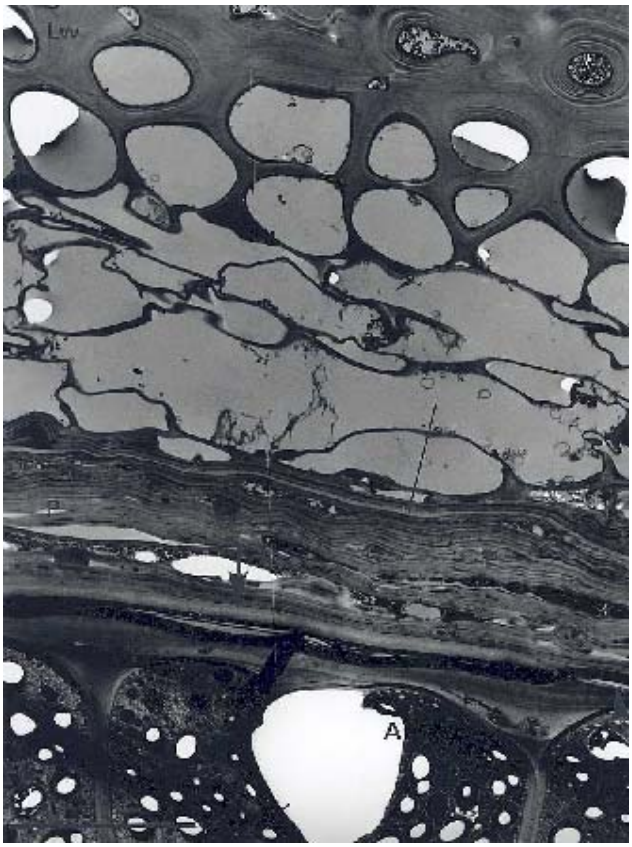

Figure 3. Transmission electron micrograph of a transverse section cut from the middle of a grain of barley, cv. Chariot, fixed 45 days after anthesis (unshaded before anthesis, shaded after anthesis). Lw, lignified walls of the cells of the outer epidermis of the lemma; P, pericarp; A, aleurone; long arrow, cementing layer; short arrow, testa cuticular membrane; arrowhead, nucellus cuticular membrane. Bar represents 10μm.

Figure 4. Transmission electron micrograph of a transverse section cut from the middle of a grain of barley, cv. Chariot, fixed 45 days after anthesis (shaded before anthesis, unshaded after anthesis). L, lemma; P, pericarp; arrow, cementing layer; b, bubbly boundary, thought to be globular deposits of cutin. Bar represents 500nm.

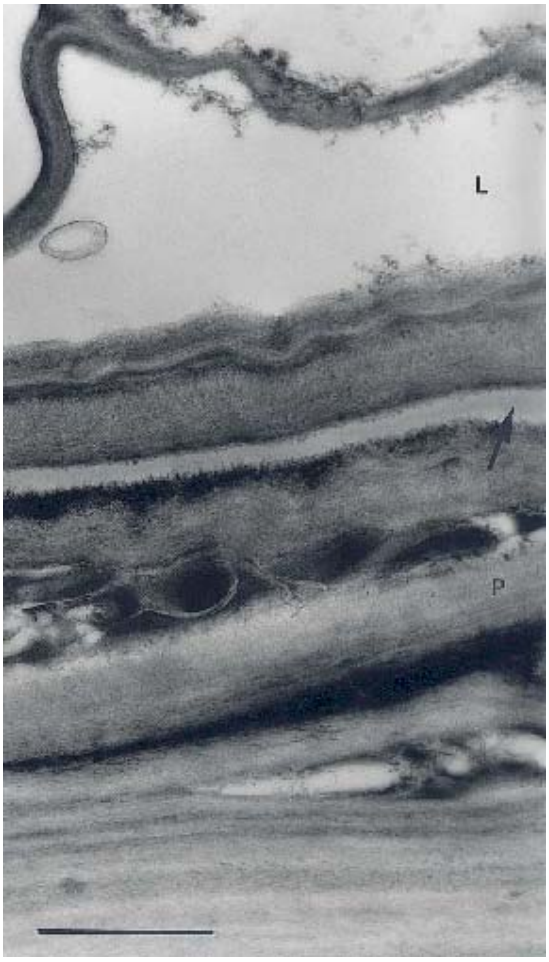

Figure 5. Transmission electron micrograph of a transverse section cut from the middle of a grain of barley, cv. Chariot, fixed 45 days after anthesis (unshaded before anthesis, shaded after anthesis). L, lemma; P, pericarp; arrow, cementing layer. Bar represents 1μm.

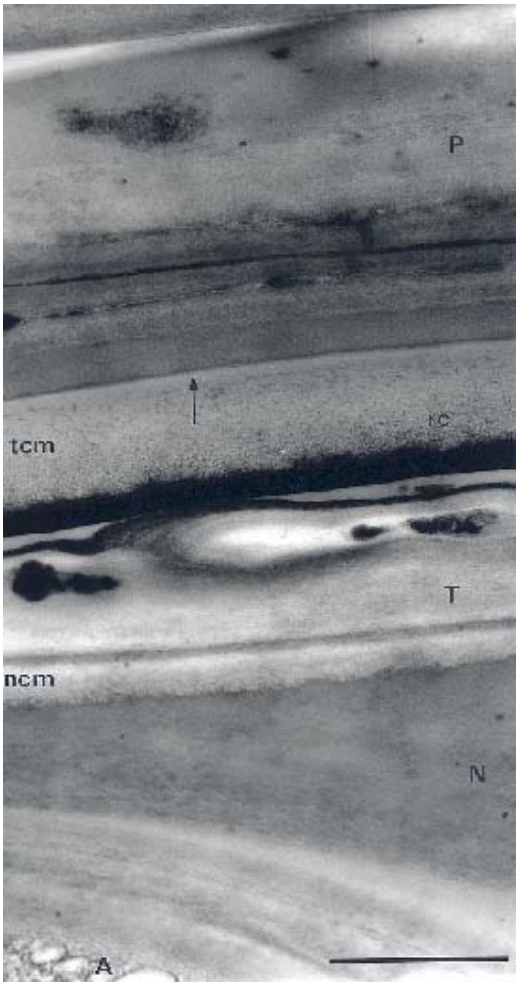

Figure 6. Transmission electron micrograph of a transverse section cut from the middle of a grain of barley, cv. Chariot, fixed 45 days after anthesis (unshaded before anthesis, shaded after anthesis). P, pericarp; T, testa; N, nucellus; A, aleurone; ncm, nucellus cuticular membrane; tcm, testa cuticular membrane; rc, reticulate component; arrow, cuticle proper. Bar represents 1μm.

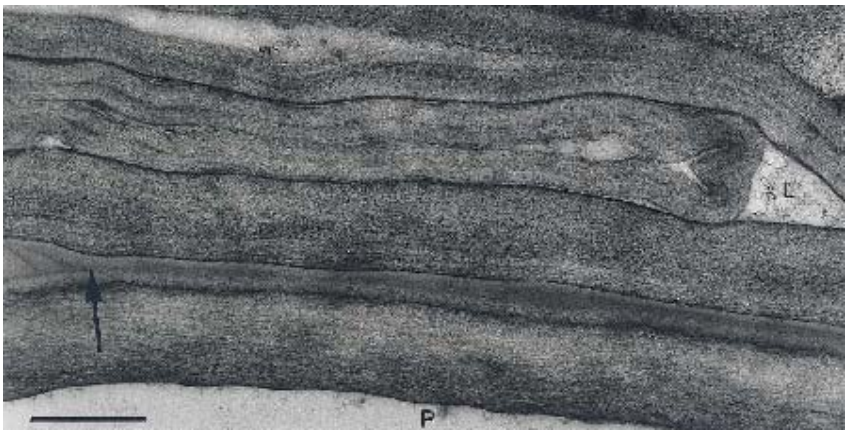

Figure 7. Transmission electron micrograph of a transverse section cut from the middle of a grain of barley, cv. Chariot, fixed 24 days after anthesis (unshaded before anthesis, shaded after anthesis). L, lemma; P, pericarp; arrow, cementing layer. Bar represents 500nm.

Figure 8. Scanning electron micrograph of the outer surface of an area of the pericarp epidermis near the apex of a caryopsis of barley, cv. Chariot, fixed 17 days after anthesis. s, stoma; arrow, apical hair. Bar represents 40μm.

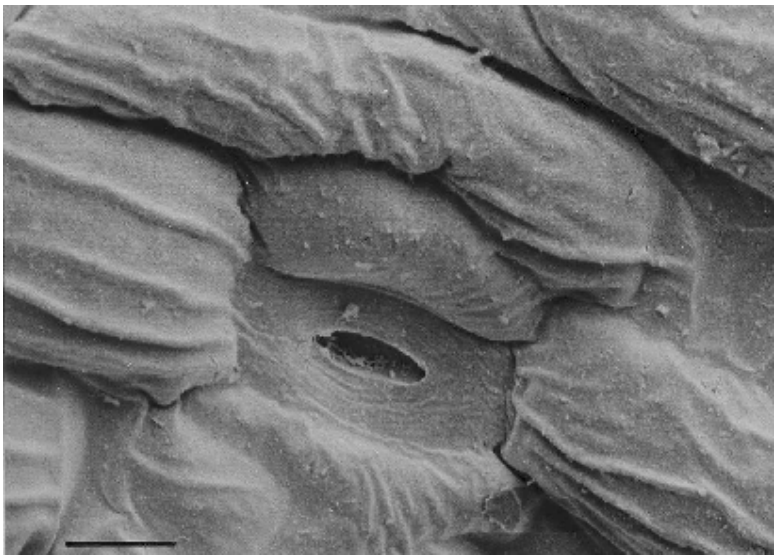

Figure 9. As Figure 8, showing detail of stoma. Bar represents 10μm.

TECHNICAL PAPER 5

DEFINITIONS AND MEASUREMENTS OF GAPE, SPLITTING AND SKINNING IN GRAINS OF MALTING BARLEY

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Introduction

The malting industry uses a number of definitions for damaged barley grains; ranging from degrees of damage to the caryopsis (also referred to as a kernel) e.g. broken or chipped, split or skinned grains and other conditions such as germinated or mouldy grains.

The conditions gape, splitting and skinning were defined in ways to complement assessment procedures that might be used within the malting industry, as well as in variety testing and research studies. The evaluation and acceptance of grain is based on many criteria and it is possible to identify varying degrees of gape, splitting and skinning. Therefore, definitions need to be appropriate across a range of end user requirements. Each character is described in terms of a standard definition and a range of categories or variations from the standard. This provides a base from which industry standards could be standardised.

Definitions

The intact barley grain

Barley grains have an adherent husk which is composed of two parts from the flower, the palea and the lemma (Fig. 1a). The palea covers the ventral side of the grain which is characterised by a central crease and the lemma covers the dorsal side of the grain (Plate 1). In most grains, the lemma overlaps the palea along the sides of the grain. Several layers of tissues (pericarp, testa and aleurone) separate the husk from the endosperm, which comprises about 80 % of the mature grain (Fig. 1b). Immediately beneath the husk lies the pericarp or ovary wall, which protects and supports the growing endosperm and embryo. The caryopsis is the term used to describe all the tissues beneath the husk, including the endosperm. As the grain matures, the palea and lemma become cemented to the pericarp by "glue" that is secreted from the pericarp. This glue is absent in the *nud* or naked mutant located on chromosome 7H and the grain then freely threshes as in wheat. From about two weeks after anthesis, the husk becomes very difficult to remove from the caryopsis.

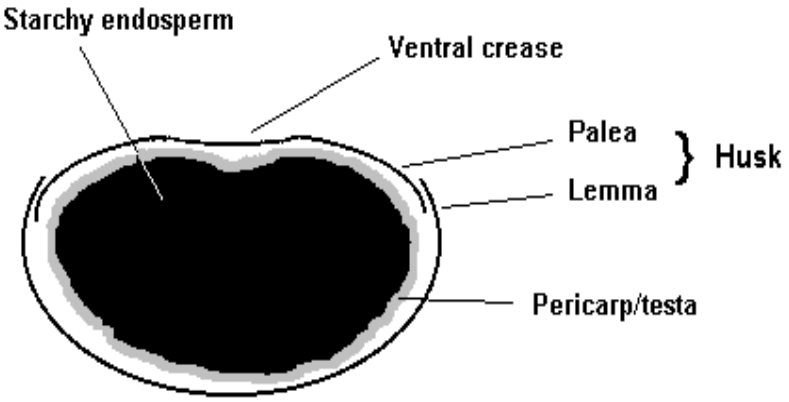
Gape

In a normal grain the lemma overlaps the palea (Fig. 1a). Gape occurs when the palea and lemma do not cover the caryopsis fully and there is a gap between them. A gap of up to 2 mm can occur on one or both sides of the grain. The presence of a gap exposes the pericarp, which may become discoloured as a result of weathering. For assessments, gape is defined as a gap of 0.5 mm or more between the palea and lemma in the middle third of the grain (Plates 2 and 3). When describing and measuring gape there are two other categories to consider:
(1) 'overlapping' is used to describe a grain in which the palea and lemma overlap along its entire length, and (2) 'abutting' occurs when the palea and lemma meet without overlapping or leaving a gap. Abutting is a very fine contact between the palea and lemma and can appear as a wavy margin either laterally across the surface of the caryopsis or when the lemma loses contact with the palea or caryopsis, and lifts upwards from the grain. Even when the palea and lemma appear to overlap, especially when viewed from the side, the palea and lemma may become separated if the lemma lifts outwards from the grain. Sometimes the irregular nature of abutting can expose small areas of the pericarp, though these areas are only visible with a binocular microscope or magnifying glass (x 4 to x 10 magnification) and are not considered as a serious problem in grain quality.

In our own validation of gape assessments scores for gape could vary widely depending on the categories used. For clarity of assessments within the industry it is recommended that a standard gap between the palea and lemma is measured (e.g. > 0.5 mm), or at least a gap that is indicated by the assessor.

Figure 1. (a) Cross section of a grain showing how the husk (palea and lemma) covers the grain and the starchy endosperm within. (b) Schematic diagram of the main grain tissues (not to scale). The husk overlays the pericarp/testa which surrounds the caryopsis (or kernel) which is comprised of an outer aleurone layer and the starchy endosperm.

(a)



(b)

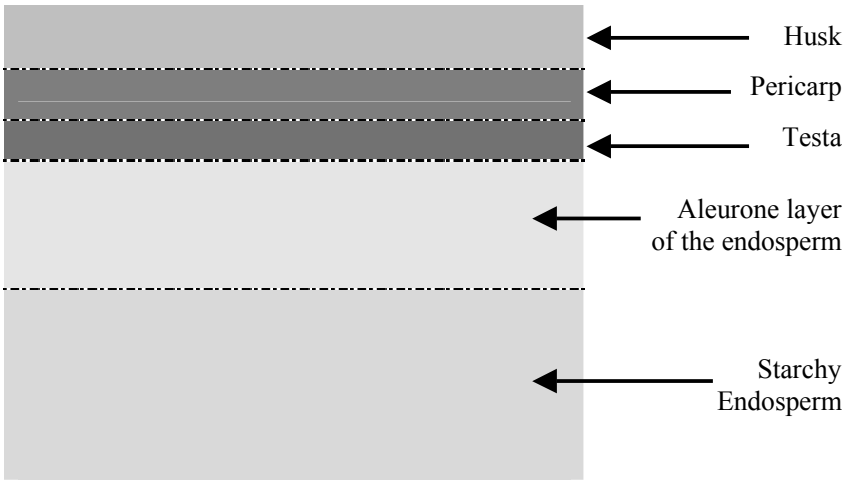


Plate 1. Ventral view of an intact barley grain.

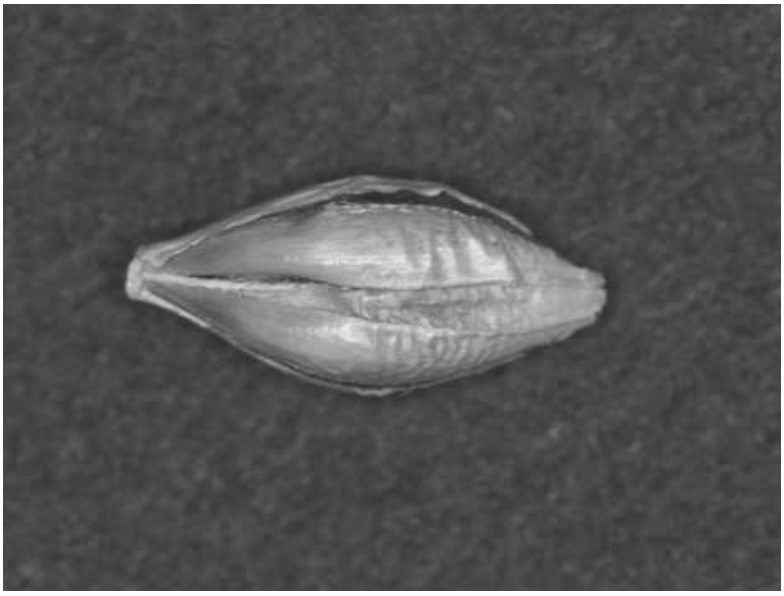


Photo 2. A grain showing gape of more than 0.5 mm.

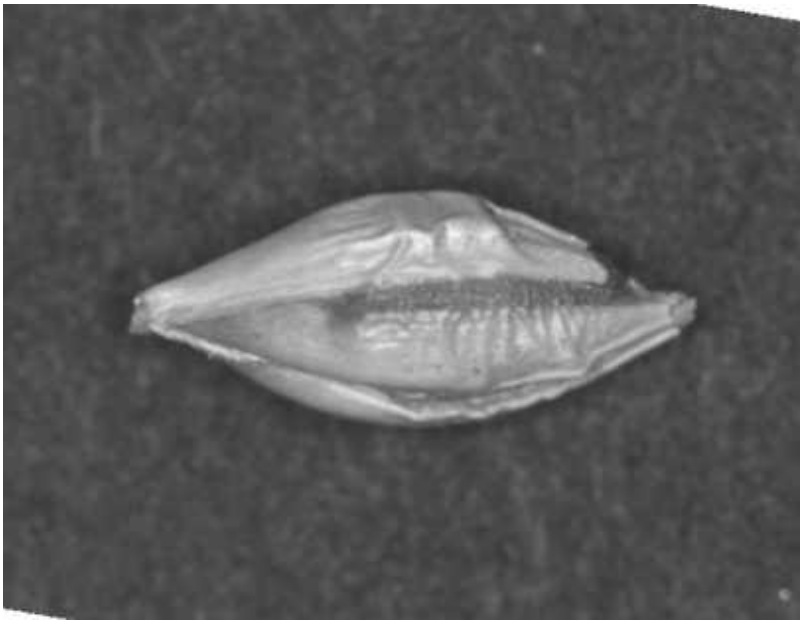


Plate 3. Gape clearly visible to the naked eye.

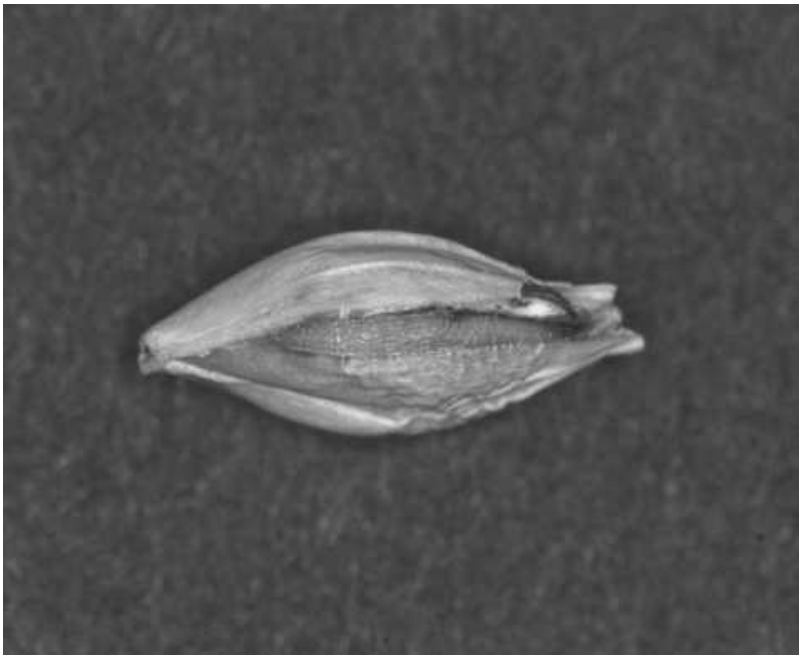


Plate 4. Wide gape revealing where the grain has also split laterally.

Splitting

Splitting refers to the process in which a crack is formed in the testa/pericarp/aleuorne layers exposing starchy endosperm. Splitting commonly can occur on the ventral (front), dorsal (back) or lateral (side) faces of the grain: each can be regarded as causing the same degree of damage. Lateral splitting is most often associated with gape which exposes a crack or opening in the pericarp/testa/aleurone which encloses the endosperm (Plates 4 and 5). In ventral (Plate 6) and dorsal splitting the husk adheres to the pericarp and lesions in both the husk and the pericarp/testa/aleurone expose the starchy endosperm. In some cases a split along the ventral crease can be wider and deeper than that along the lateral or dorsal surfaces. Splitting can also occasionally occur across the grain.

For assessment of splitting all types of cavity or exposure of the endosperm can be scored equally, though in some cases in may be appropriate to categorise the condition into lateral, ventral and dorsal.

Skinning

Skinning is a loss of the husk as a result of grip between the husk and pericarp (Plates 7 and 8). Skinning occurs during harvesting and subsequent handling and is influenced by grain moisture content and the amount of abrasion to the grain. Skinning can occur across any part of the grain and can range from the loss of a few percent of the husk to complete husk detachment. The threshold for skinning is when 25 % or more of the husk (palea and/or lemma) has failed to adhere to the caryopsis.

Skinning can be further defined as dorsal (removal of the lemma), ventral (removal of the palea) or lateral (removal of a longitudinal strip of the palea and/or lemma). A pearled grain is one in which the entire husk has been removed. Skinning can also occur at the ends of the grain, especially at the distal end when there has been damage to, or removal of, the awn resulting in a loss of husk from the end towards the mid-grain. A level of 5 percent skinning is common in barley because of this type of damage to the awn. In assessments of skinning, the threshold (e.g. 25 %) will comprise of any of the types described above.

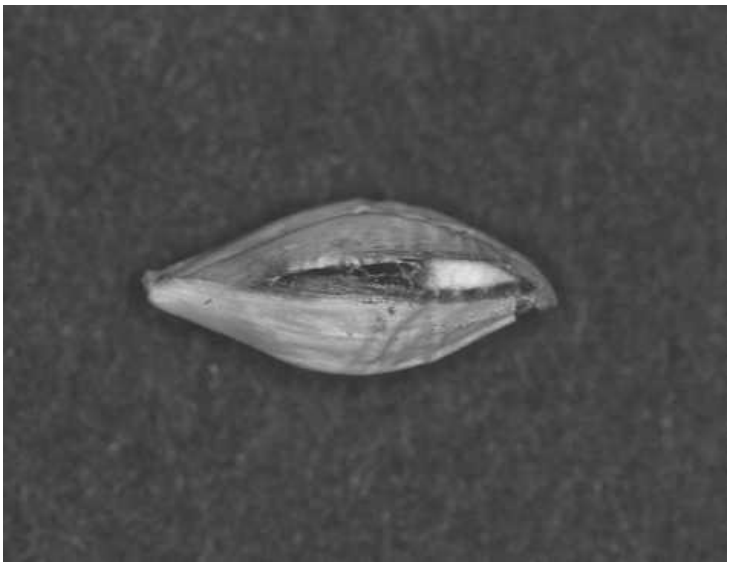


Plate 5. A deep lateral split: the black stained area is where the endosperm has been stained with a dye, the white area is a deeper cavity beneath the stained area.

Plate 6. A deep ventral split.

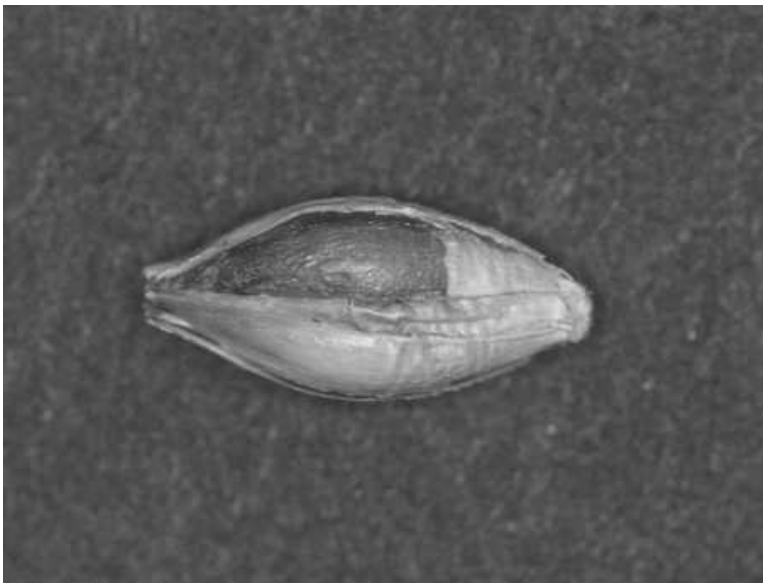


Plate 7. A partially skinned grain in which 20 % of the husk has been lost.

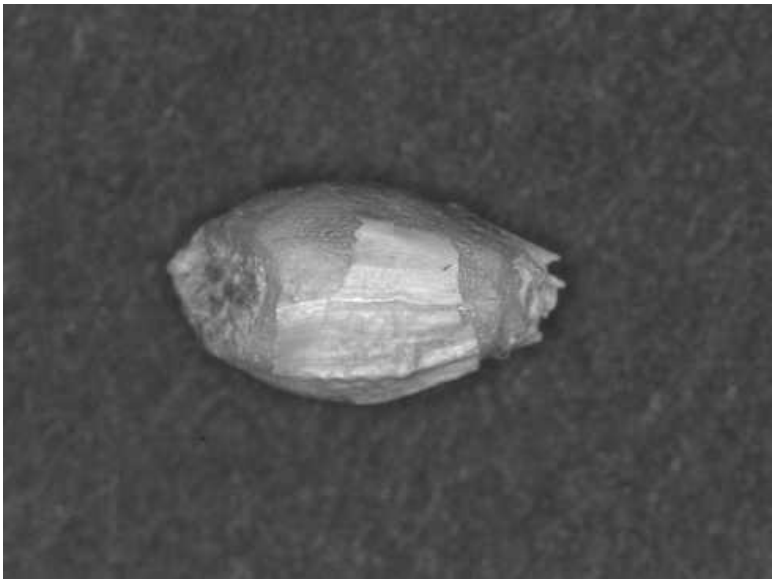


Plate 8. Dorsal view of a grain that has lost 80 % of its husk: on the ventral side the husk had been completely removed.

Other conditions

There are several other conditions that may be used to describe damage to grains e.g. chipping or breaking. However, these conditions are distinct from gape, splitting and skinning and are a consequence of mechanical rather than physiological processes.

Assessing grain for gape, splitting and skinning

Assessments of gape, splitting and skinning can be carried out on the same sample of grain. It is recommended that at least 3 replicate samples of one hundred grains are used to in each assessment. Scores are expressed as a percentage of each condition.

Materials

Good light source (preferably daylight)
Seed counter (optional)
Binocular microscope or magnifying glass (x 4 to x 10 magnification)
Small beaker (for immersing sample in stain e.g. 50 cm^3 beaker)
Small container (e.g. Petri dish or weighing boats)
Sealable polythene bags or plastic, screw-top, pots
Sheet of A3 white or light blue paper
Forceps
Iodine / potassium iodide solution (for measuring grain splitting)

Methods

Screen grain using a 2.2 mm or 2.5 mm slotted sieve. Discard all material falling below this size. Thoroughly mix the screened sample of grain for assessment. Take at least 3 replicates of 100 grains from the sample (this can be done with a seed counter).

Gape

Ensure a good light source is directed on the sample. Take each sample of grains and record the number of grains that gape with a gap of 0.5 mm or more between the palea and lemma in the middle third of the grain. Express gape as the average % of the replicate samples.

Splitting

Splitting can be assessed with or without the use of a iodine-based dye to stain the areas of exposed endosperm. Although the use of a dye is more time consuming than an assessment on unstained grains, the blue/black stain makes the identification of split grains easier.

Grains are dyed with a solution of iodine (I) in potassium iodide (KI). Two g of KI is dissolved in 100 cm^3 water and 0.2 g of I is dissolved into the KI solution. One hundred grains are placed into a 50 cm^3 beaker and immersed in approximately 20 cm^3 of KI/I solution. The dish is shaken gently to ensure that all grains are thoroughly soaked by the solution. After 10 min the solution is poured off and the grains rinsed with water and placed into a Petri dish. Each grain is examined against a white background using magnification (x 4 to x 10). Grains that have stained black or blue-black are scored as split.

Skinning

Skinning is scored as the percentage of grains with more than 25 % of the entire husk missing.

Recording and data sheets

Although various types of data sheets can be used, it is recommended that the industry is familiar with a standardised recording sheet that enables the scores for gape, splitting and skinning to be clearly recorded and presented. Two styles of recording sheet are presented below. Figure 2 was used by Rajasekaran *et al.* (Technical Paper 2, in this Report) to score different categories of gape, splitting and skinning in a single sample of 100 grains. Figure 3 is adapted from SAC recording sheets and shows how categories of gape, splitting and skinning can be recorded for a large number of samples: the level of detail added to either Figure 2 or Figure 3 will depend on the requirements or priorities of the assessor. Figure 4 is adapted from an ADAS guide and indicates diagrammatically how levels of skinning can be assessed.

Sample	Gape			Splitting		
Skinning	Overlap	Abutting	Gape 1mm	Ventral	Dorsal	Lateral
0%						
5%						
25%						
50%						
100%						

Figure 2. SCRI recording grid for gape, splitting and skinning.

	Gaping			**Split**				**Skinned**
Sample No. (100 grains)	Overlap	Abutting	Gap (>0.5 mm)	Ventral	Dorsal	Lateral	Total Split	>25%
1*	67	11	**22**	2	4	4	**10**	**5**
2								
3								
4								
5								
Etc								

Figure 3. SAC recording sheet for gape, splitting and skinning based on levels of each condition. *Sample 1 indicates that 22 % of grains had gaped (with a gap of 0.5 mm or more), 10 % had split and 5 % had skinned (at a threshold of 25 % or more of the husk lost).

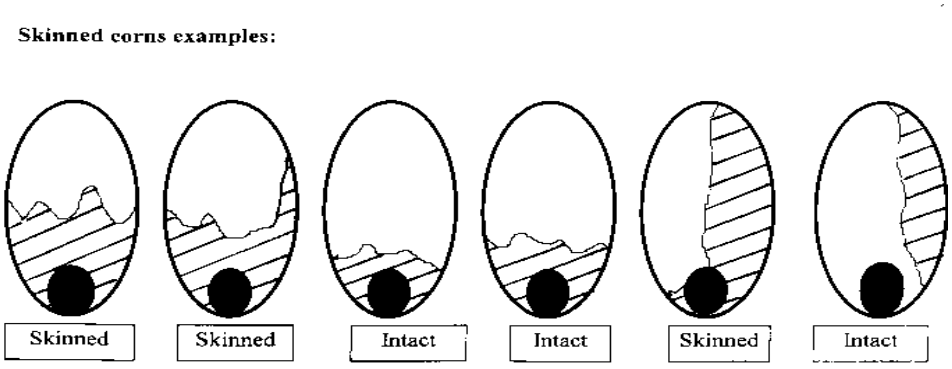


Figure 4. Examples of different levels of skinning. The threshold for a skinned grain is 25 % loss of the husk. The shaded area represents the skinned area view from one side of the grain (adapted from a figure supplied by ADAS).