HGCA

PROJECT REPORT No. 218

METHODS FOR PREDICTING THE MALTING QUALITY OF BARLEY BASED ON ASSESSMENT OF CELL WALL DIGESTIBILITY

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METHODS FOR PREDICTING THE MALTING QUALITY OF BARLEY BASED ON ASSESSMENT OF CELL WALL DIGESTIBILITY

by

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ABSTRACT

Aims

The general aim of this project was to develop procedures for predicting the digestibility of the barley based on the assessment of soluble and insoluble beta-glucan, and the levels of the enzymes involved in beta-glucan solubilisation. In particular we were searching for small-scale analyses that could be used at a very early stage of breeding to enable selection of varieties most suitable for malting and thereby speed up the selection process.

The aim of the first year was to assess the levels of soluble and insoluble beta-glucan in endosperm cell walls in a wide range of barley varieties varying in malting grade and provenance.

In the second year the microstructure of the cell wall in terms of the degree of cross-linking between the cell wall components was investigated.

The final year concentrated on the relationship between the structure of the endosperm cell wall material, the development of hydrolytic enzymes and the rapidity of beta-glucan removal.

Conclusions and Implications

There are three ways in which properties of the cell wall structure may be used as an indication of malting quality:

1. Variation in quality of beta-glucan (good malting barleys are less variable).

2. Cross-linking of the cell wall structure (good malting barleys generally have few cross-linking agents in the endosperm although some non-malting varieties may also be low).

3. Digestion of the cell walls and beta glucan at G2.

Of these the beta-glucan content at day 2 of germination is likely to be the most useful indicator of malting quality.

The test is based on a commercially available test kit but the quantities of material required have been minimised.

Thus the test is ideal for:

Barley breeders

Barley quality assessors

Maltsters

SUMMARY

Key results and conclusions

The process of breeding new barleys for malting is exceedingly time consuming requiring many years of development between the initial cross and final approval as a new recommended variety. In certain cases the process is so long that some new varieties never make full approval before they are superseded.

At least part of the difficulty with this process is that the quantity of material available may be very small. At the initial stages of selection only one plant is available and much of the grain produced is needed for growing-on. For reason early analysis of the new variety does not include malting analysis. It is only when the plant has been through several growing cycles that the grain is assessed for suitability for malting.

Hence the basis of the project was as follows: The cell walls of barley endosperm cause a wide variety of problems during the brewing process and must be effectively removed during malting. Indeed the modification of these cell walls and the subsequent digestion of beta-glucan are probably the rate limiting stages of the malting process. The target for a new analysis was the cell wall material of the endosperm.

In year 1 a range of barley varieties, differing in malting grade and grown at regions in the UK, were analysed for type and quantity of beta-glucan. Those of malting quality (grade 9) had similar levels of total beta-glucan and insoluble beta-glucan compared to the non-malting (below grade 8) varieties. Total beta-glucan levels were found to be slightly higher and more varied in non-malting barley varieties than in barleys of malting quality. Insoluble beta-glucan levels followed a similar trend. However, soluble beta-glucan showed little variation between malting and non-malting barleys. Non-malting barley varieties tended to show a wider variation in levels of these beta-glucans. This suggested that the digestibility of the barley endosperm is inherently dependent upon the relative levels of soluble and insoluble beta-glucan.

Thus in year 2 the levels of cross-linking agents were examined in the cell wall of beta-glucans.

While it is possible to select barley varieties for malting on the basis of low levels of beta-glucan there is no clear relationship between beta-glucan content and malt quality; barleys with relatively high levels of beta-glucan may modify poorly, conversely, those with low

levels of beta-glucan may also modify poorly. This may be due, in part, to differences in accessibility and the level of production of the required enzymes, but it may also reflect inherent differences between varieties in terms of the structural features of the endosperm cell walls.

Cell walls contain a range of alkali-labile phenolic moieties, with ferulic acid (FA) being the most abundant. Ferulate esters undergo dimerization to provide cross-linking of cell wall polymers. The starchy endosperm of malting quality barleys had slightly lower amounts of phenolic acids compared to non-malting barleys. These differences are relatively small and thus places greater importance to the rapid synthesis of enzymes involved in the break-down of these cross-links, and on the accessibility of these enzymes within the endosperm.

Overall, of the barleys analysed, the malting varieties had slightly lower levels of FA in the endosperm compared to the non-malting barleys although these values were not significantly different. Although the degree of cross-linking within the endosperm is important, the fact that there appears to be little difference, quantitatively, between malting and non-malting barleys does indeed place greater importance on the rapid synthesis of enzymes which break-down the cross-links, and the accessibility of these enzymes within the endosperm. These results currently indicate the importance a relationship between the enzymes involved in endosperm digestibility, insoluble beta-glucan, hot water extract (HWE) and malting grade. The effect of malting on the release of soluble beta-glucan will be investigated.

The speed and effectiveness with which barleys degrade the cell walls of the endosperm correlates well with subsequent grade of malting quality. Thus the level of beta-glucan present at the second day of germination can be taken as an indicator of the likely success of this barley as a malting variety. The test for this has been developed and miniaturised using a readily available test kit.

We have shown that G2 is a critical point during malting and that the levels of beta-glucan at this stage are a reasonable indicator of the eventual malting performance of a barley variety. This method for predicating malting grade has the advantage that beta -glucan analyses are simple to perform and do not require specialist equipment or expensive materials. Also, by adapting this method so that it can be used on a small scale, breeders can assess varieties for beta-glucan without sacrificing large amounts of their seed material.

The next step in developing this test will be to assess its robustness under process trail conditions. The results presented here suggest that it will be worthwhile to proceed with this method development and BRI will incorporate this test into its malting trials.

MATERIALS AND METHODS

MATERIALS

Malting barley samples of recognised IOB varieties, together with samples of feed grade barleys, were supplied by a commercial malting company in the UK.
Each of the ten varieties were grown at five different sites in the UK (Table 1).

These barleys were sampled throughout the duration of the project and additional barley samples were provided by the National Institute of Agricultural Botany in year three.

Table 1. shows the barley varieties used for the duration of the project.

METHODS

BARLEY INTAKE

Moisture contents of the barley samples were determined according to IOB Recommended Methods. Barley samples were dried to about 12% prior to further analyses and storage.

STANDARD BARLEY ANALYSES

Total nitrogen (TN) of whole grains was analysed by the Dumas method (Leco Instruments Ltd., UK.) according IOB Recommended method.

Germinative capacity, energy and water sensitivity were assessed according to IOB Recommended Method of Analysis.

DETERMINATION OF BETA-GLUCAN CONTENT

Total beta-glucan of barley samples was measured according to the method of McCleary and Glennie-Holmes (1985) using a commercial kit supplied by Megazyme Ireland.

Hydrated barley flour in a buffer solution is treated with lichenase and filtered. An aliquot of this filtrate is then reacted to completion with beta-glucosidase: the resulting glucose level is assayed using a glucose oxidase/peroxidase reagent. A control value (not enzyme treated) is subtracted from the final result.

Water-soluble beta-glucan was analysed using an adapted method of Martin and Bamforth (1980). Milled barley flour was mixed with water and heated to 65°C, after centrifugation the supernatant is used in the McCleary total beta-glucan assay as described above.

<u>De-husking barley.</u>

Barleys were de-husked by immersing them into 50% sulphuric acid for 40 min. They were vigorously washed in running cold water until all the husk material was removed. They were then air dried prior to assay.

EXTRACTION OF INSOLUBLE BOUND PHENOLIC ACIDS

Wall bound phenolic acids were released by alkaline hydrolysis of barley cell wall material according to a modified method of Nordkvist *et al* (1984). Milled barley flour was extracted twice with 80% ethanol (100 ml/g) and twice with hexane (100 ml/g) in an ultrasonic bath. The mixture was centrifuged after each extraction. The residue was dried under nitrogen and re-extracted twice with 1 M NaOH and sonication. The alkaline extracts were pooled

following a further two washes with 1M NaOH (2 x 25 ml). The combined supernatants were acidified with concentrated HCl to pH below 2.0 and extracted three times with ethyl acetate. The combined ethyl acetate extracts were dried with anhydrous sodium sulphate, filtered and evaporated to dryness under nitrogen and re-dissolved in 50% (v/v) aqueous methanol prior to filtration and analysis by HPLC.

Analysis of esterified phenolic acids by HPLC.

Phenolics were quantified by HPLC using an Inertsil ODS-2 5U reverse phase column (25 cm x 4.6 cm Supelco Inc., USA). Elution was performed using a gradient system with increased relative amounts of methanol and acetonitrile present in aqueous 1 mM trifluoroacetic acid (TFA). All solutions were HPLC grade. The optimised gradient profile for the separation of wall bound phenolic acids was performed using solvent A (10% (v/v) aqueous acetonitrile plus 1 mM TFA), solvent B (80% (v/v) aqueous methanol plus 1 mM TFA), and solvent C (80% (v/v) aqueous acetonitrile plus 1 mM TFA) in the following programme:
initially A 90%, B 5%, C 5%; linear gradient over 25 min to A 25%, B 37%, C 37%; exponential gradient over 5 min to A 0%, B 50%, C 50%; exponential gradient over 15 min to A 90%, B 5%, C 5%; held at A 90%, B 5%, C 5% for a further 10 min. The flow rate was maintained at 1 ml/min. The phenolics were detected using a Merck LaChrom L-7450 diode array detector. Quantitation was by integration of peak areas at 280 nm with reference to calibrations made using known amounts of pure phenolic acids which included vanillic, caffeic, ferulic, *p*-coumaric cinnamic and sinapinic (Sigma, USA).

Measurement of ferulic acid by absorbance.

The method of Zupfer et al (1998) was used. Barleys were acid de-husked and milled. Barley flour (1 g) was added to 15 ml 0.2 N sulphuric acid and heated to 100°C for 1 h. The hydrolysis was terminated by cooling the mixture in an ice bath until its temperature dropped to 30°C. Sodium acetate (2.14 ml of 2.5 M concentration) containing 2% (w/v) alpha-amylase (Sigma-Aldrich, Poole, UK) was added and the mixture incubated for 1 h at 30°C. Following centrifugation the supernatant was filtered (0.45 μm) and diluted (1 in 10) prior to measuring absorbance at 340 nm in a double beam spectrophotometer. A ferulic acid standard at a range of different concentrations was treated as above and the values used for calibration.

RESULTS – YEAR 1 (1996/1997)

Variation in beta-glucan in varieties between sites.

A range of barley samples, varying in malting grade and grown in different parts of the UK were analysed for their beta-glucan content. Total, soluble and insoluble beta-glucan content were determined for each variety. Data from barleys grown at Wooton have been omitted from these graphs. Barleys grown at Wooton had unusually low levels of insoluble beta-glucan compared to those from the other sites investigated here; insoluble beta-glucan levels ranged from 0.24 to 0.81%, but those from other sites overall ranged from 0.54 to 2.39%.

Insoluble beta-glucan levels were slightly higher and more varied in the non-malting grades than in the malting barley varieties; 0.54 to 2.39% and 0.85 to 1.74% respectively. (Figure 1a).

However, soluble beta-glucan content between varieties and across all sites (except for Wooton) did not vary significantly between the non-malting and malting varieties; 0.84 to 1.71% and 0.73 to 1.34% respectively. (Figures 1b).

Total beta-glucan levels followed a similar trend to the insoluble material, being slightly higher and more varied in the non-malting grades than in the malting barley varieties; 1.61 to 4.1% and 1.74 to 2.78% respectively. (Figure 1c).

The relationship between total beta-glucan and total nitrogen.

The nitrogenous components of barley can vary with variety, growing season, soil composition and the amount of fertilizer applied by the farmer (Briggs 1978). The protein content of barley plays an important role in endosperm modification through its influence on the rate of water uptake and enzyme distribution.

Barley can be classified as either mealy or steel; these terms describe the degree of packing of proteins, cell wall polymers and starch in the endosperm. Mealy areas of the endosperm are loosely packed with open spaces between starch granules, whereas steely areas are densely packed containing a high level of cell wall polymers, proteins and small starch granules. The degree of steeliness can affect cell wall degradation. No correlation was found between inter-varietal total nitrogen content and the level of total beta-glucan. (Figure 2a). This is in agreement with findings by Alexander & Fish (1984).

The relationship between insoluble beta-glucan and total nitrogen.

Total beta-glucan values give no indication of the relative ratios of soluble to insoluble glucans. Indeed glucan-based problems in the brewery are due to the un-degraded insoluble fraction of beta-glucan. When the insoluble beta-glucan levels were plotted against total nitrogen a different picture emerged; varieties with higher insoluble beta-glucan had lower nitrogen contents. However, in those varieties with higher nitrogen levels, the insoluble beta-glucan levels became more widespread suggesting environmental factors may influence the glucan ratios. (Figure 2b).

The variation in insoluble beta-glucan between varieties across all sites.

For a given variety the percentage of insoluble ß-glucan varied depending upon growing region. (Figure 2c). This variation appeared to be more widespread in the lower malting grade varieties. As mentioned previously, varieties grown at Wooton in North East Lincolnshire had low levels of insoluble beta-glucan as a percentage of the total.

The variation in total nitrogen between varieties across all sites.

Varieties grown at the Wooton site had higher levels of total nitrogen compared to the other sites tested; one explanation could be the timing of the application of fertiliser by the farmer, although this has not been confirmed. (Figure 2d).

RESULTS - YEAR 2 (1997/1998)

Although it is claimed that barleys with lower levels of beta-glucan are more digestible, relatively high levels of the principal cell wall polymer need not be a problem provided that the barley has the capacity to develop increased amounts of enzymes involved in cell wall degradation which come into effect very early on in the germination process. While it is possible to select barleys for malting on the basis of low levels of beta-glucan (Aastrup *et al* 1985), there is no clear relationship between beta-glucan content and malt quality; barleys with relatively high levels of beta-glucan may modify poorly, conversely, those with low levels of beta-glucan may also modify poorly (Aastrup & Erdal, 1980). This may be due, in part, to differences in accessibility and the level of production of the required enzymes, but it may also reflect inherent differences between varieties in terms of the structural features of the endosperm cell walls.

It has been suggested that hemicellulosic beta-glucan are bound into the cell wall through a covalent association, probably via protein-polysaccharide linkages (Forrest & Wainwright, 1977; Martin & Bamforth, 1983), or through phenol-ester linkages between protein, ferulic acid and polysaccharide (Selvendran,1983). This may account for the insolubility of the hemicellulosic fraction of beta-glucan. Ferulic acid has been shown to be cross-linked to pentosans and may be responsible for cross-linking the polysaccharides to proteins (Ahluwalia & Fry, 1986). Esterase activity in the 'solubilase' system may be involved in the breaking of ester linkages in barley cell walls (Moore *et al* 1996). Esterases, able to release hydroxycinnamic acids (ferulic acid, *p*-coumaric, sinapic, cinnamic and caffeic), from cell walls often work in synergy with carbohydrate-degrading enzymes.
Barley endosperm cross-linkages and enzymes involved in breaking these linkages were investigated in Year 2.

Phenolic acids present in different barley grain fractions.

Phenolic compounds were analysed from a range of barley varieties of varying malting grade. Cell wall bound phenolic acids were released by sequential alkaline hydrolysis of extracted barley grain cell wall material; the esterified phenolic monomers and dimers were analysed by HPLC. Phenolic compounds were analysed from a range of barley varieties of varying malting grade. In whole grain samples the main monomeric phenolic acid released was ferulic acid (*cis* and *trans*) followed by *para*-coumaric acid; traces of cinnamic acid and ferulic acid dehydrodimers 8,5' and 8,8'were also detected. In de-husked barley samples ferulic acid with

trace amounts of *p*-coumaric acid were detected. Only *trans*-ferulic acid was found to be present in the endosperm of the barleys analysed (Table 2).

Although phenolic acids were concentrated in the cell walls of the outer layers of the endosperm (i.e. husk and pericarp) low concentrations were found in the endosperm where ferulic acid is the predominant phenolic (Figure 3a).

Only *trans*-ferulic acid was found to be present in the endosperm of the barleys analysed. (Tables 3).

Although phenolic acids were concentrated in the cell walls of the outer layers of the endosperm, that is, mainly the husk and pericarp, low concentrations were found in the endosperm where ferulic acid is the predominant phenolic. Figure 3b shows levels present in three barleys grown at the Rothwell site.

Measurement of total ferulic acid between sites.

Using the absorbance method of Zupfer et al (1998) levels of ferulic acid in a range of barley varieties between two sites was determined. Overall, levels were higher in the feed grade barley (var. Pastoral) than in barleys of malting quality. However, the variety Intro of malting grade 5 had ferulic acid levels similar to the malting quality barleys. (Figure 3c). On a relative basis this method for measuring total levels of ferulic acid is reasonably quick to perform. However, the presence of other compounds in the test solution absorbing at 340 nm must be taken into account.

Results Year 3 1998/1999

Variation in levels of total and soluble beta-glucan during malting

We have suggested that the breakdown of the cell wall during malting may proceed in the following way:

Insoluble beta -glucan ----→ soluble beta-glucan -------→ glucose

The conversion of insoluble to soluble beta-glucan is possibly catalysed by a solubilase activity. We suggested that the levels of solubilase activity may vary between malting grade (high solubilase activity) and feed grade (low solubilase activity) barley. In year 3, we decided to test this idea by extending the work in year 1, to look at the relative levels of soluble and insoluble beta-glucan during the malting process.

Initially, the barley varieties Chariot (malting grade 9) and Epic (malting grade 2) were selected to compare the changes in total and soluble beta-glucan during malting. The barleys were malted on the 300g scale with a steeping 8W/16A/24W regime selected to maximise the differences between malting and feed grade barley. Samples were taken during the air rest, at cast and on each subsequent day of germination up to day 5. The samples were freeze-dried, then analysed for total and soluble beta-glucan as described in the methods.

As expected, the levels of total beta -glucan started to decrease during malting after cast (Figure 4a), and it was clear that beta-glucan breakdown was faster and carried out to a greater extent in Chariot compared to Epic. The soluble beta-glucan in Epic remained constant throughout malting whereas it increased in Chariot at around germination day 1, then decreased in parallel with the loss of total beta-glucan (Figure 4b). This pattern might be expected if the malting grades are able to solublise beta-glucan more rapidly than the feed grades i.e. this would give rise to a peak in the levels of soluble beta-glucan in the malting grades during early germination, before beta -glucanase has been synthesised. Given that there was a noticeable difference in the appearance of soluble beta -glucan between these varieties, it seemed reasonable to establish whether this pattern was also observed in other varieties of barley. The set of samples collected from Navenby, Rothwell, Woolpit and Haughley examined in year 1 of the project were therefore used for subsequent work.

To establish if there was a relationship between the levels of soluble beta -glucan and malting grade, one set of samples from Rothwell was malted and analysed for beta-glucan content at G1, G2 and G3. In order to avoid freeze drying, which is time consuming, samples were oven dried at low temperature (40 °C) for 24 hours. In these samples, there was a good correlation between malting grade and total beta-glucan on all three days sampled. However, the best correlation was seen at G2 (R^2=0.72; fig 5a). A hypothesis that the gradient of the line was zero (ie that there was no relationship between malting grade and G2 beta-glucan) indicated a probability of 0.002. This low probability suggests that the alternative hypothesis (that there is

a relationship) is much more likely. The 95% confidence interval on the correlation was between -0.963 and -0.463 , that is not including zero, while a rho test (hypothesis: no correlation) had a probability of 0.002 also. An equally good correlation between malting grade and insoluble beta-glucan was seen at G2 (R^2=0.71;Fig 5b). In this sample set, soluble beta-glucan comprised between approximately 20-35% of the total beta-glucan, but in this case there was no clear relationship between soluble beta -glucan at G2 and malting grade (Fig 5c). The data therefore indicated that the best predictor of malting grade was to measure either insoluble or total beta-glucan at G2. In fact, levels of insoluble and total beta -glucan were closely related in these samples (R^2= 0.92; Fig 5d).

Total beta-glucan measurements are simpler to perform than insoluble beta-glucan ones (which require measurement of both total and soluble beta-glucan). This is a consideration in designing a robust predictive test for malting grade and where speed and simplicity will be a considerable advantage. Therefore, only total beta-glucan at G2 was measured in samples from the remaining 3 sites to establish if the relationship between beta-glucan and malting grade held in all cases. Figures 6a, 6b and 6c show the results from this analysis for Haughley, Navenby and Woolpit respectively. In all cases there was a good correlation between malting grade and total beta -glucan at G2 (R^2=0.81, 0.82 and 0.86 respectively) suggesting that this relationship between malting grade and total beta -glucan held for all these varieties regardless of the site at which they were grown.

However, there was some variation in total beta-glucan levels between sites for each variety. For example, in varieties such as Regina (Grade 9) total beta -glucan levels varied from 1.5 – 2.27 % dry weight (Figure 7a); whereas in other varieties such as Spice (Grade 9) the variation in beta-glucan levels was less, ranging between 1.98-2.27 % dry weight (Figure 7b). Unlike the insoluble beta-glucan levels in barley, there was no evidence that there was less site-to-site variation with malting grades compared to feed grades (Figure 7c).

The variation between sites was not unexpected and was most likely due to a combination of environmental factors, a small (approximately 10%) variation associated with the small scale malting technique (Figure 7d), and also an error of about 5-10% on the total beta-glucan measurements.

Table 5. shows a summary of the total beta-glucan levels at G2 for the ten varieties studied. Given that there was some variation between sites, maltings and total beta -glucan measurements, we derived a value for 'total beta-glucan at G2' for each variety by averaging the data from all sites (Table 5.). When these values were plotted against malting grade, the correlation between them improved over the correlation seen for these varieties within a single site (R^2=0.92,figure 8a). A hypothesis that the gradient of the line was zero (i.e. that there was no relationship between malting grade and G2 beta-glucan) indicated a probability of 10^{-5}. This very low probability suggests that the alternative hypothesis (that there is a

relationship) is much more likely. The 95% confidence interval on the correlation was between -0.9909 and -0.8373 , that is not including zero, while a rho test (hypothesis: no correlation) had a probability of 10^{-5} also.

This correlation was approximately the same when total beta-glucan at G2 values for the Chariot and Epic samples were included (R^2=0.91 Figure 8b). In corporation of these data in to the set resulted in a slight decrease in the confidence interval and a change in probability of Ho: no relationship between the variables from 10^{-5} to 10^{-6}. A significant advantage of this method is the small size of sample required for analysis. This makes the method potentially useful to breeders if it can be used with small quantities of grain. Figure 8c shows the beta-glucan at G2 verses malting grade for malts prepared on 5g scale. Such a small sample would be expected to show very large variation nevertheless the correlation obtained was –0.66 with a probability of no relationship between the beta-glucan at G2 and malting grade of p=0.039.

This would suggest that the test has potential even for very small quantities of grain.

Although site-to-site variation was identified as a significant problem for total beta-glucan analysis under our experimental conditions, that does not mean that it will be a problem for the barley breeder. In the early stages of developing new barley varieties, the barley breeder will have access to sites that are either climate controlled and also to outside plots which have been well characterised in terms of their environmental conditions. Under these circumstances, problems with site-to-site variation will become less significant.

In summary, the correlation between total beta-glucan at G2 and malting grade was high enough to indicate that this measurement alone would be a good predictor of malting grade.

VISCOSITY

Beta-glucan is a major contributor to viscosity and is well known for causing problems in the brewhouse with poor lautering rates and filterability. However there are other compounds present such as arabinoxylans that will also contribute to viscosity. In addition, the size distribution of beta-glucans which will vary between varieties, will effect their contribution to viscosity. Neither of these factors are taken into account when performing a total beta -glucan analysis by the McCleary method and it is possible that viscosity measurements, which will take all factors into account, would be a more thorough measurement of the breakdown of the complex sugars during malting. Therefore, the relationship between the viscosity at G2 with malting grade could potentially be better than the relationship between malting grade and beta-glucan content at G2.

Viscosity at G2 was determined for the samples grown at Navenby. Figure 9a shows that as expected, beta -glucan and viscosity at G2 were closely related in these samples (R^2= 0.82)

since beta -glucan is the major contributor to viscosity. There was also some correlation between malting grade and viscosity at G2, but the relationship was not as strong as seen for total beta-glucan at G2 and malting grade (Figure 9b: R^2= 0.55 vs 0.82 respectively). It is interesting that the relationship between viscosity and malting grade became weaker in the malt (Figure 9c), although in general good malting grades had lower viscosity.
These data indicate that the viscosity at G2 is not as good a predictor of malting grade as total beta -glucan measurements. It is also worth noting that for barley breeders, viscosity would not be so quick and easy to determine as total beta-glucan measurements and so for the purpose of a simple test for malting grade, total beta-glucan would also be a better suited test. This result perhaps also emphasises the importance of G2 as a key stage in germination to predict malting performance.

Hot Water Extract (HWE)

The malting grade system is used to assess brewhouse performance, and high values for HWE are essential for good malts and is in large part on how malting grades are determined. Since HWE is a continuous variable, it was possible that the correlation between HWE and total beta -glucan at G2 would be better than that between malting grade and total beta-glucan at G2. Therefore, HWE for malts from 3 sites were measured. The HWE extract values showed some site-to-site variation (Figure 10a), with some varieties such as Fighter showing more variation than others (Puffin). Using an average value of HWE for each variety, there was a weak correlation between HWE and beta-glucan at G2 (R^2=0.63). Again this correlation was not as good as that between malting grade and total beta -glucan at G2 and so no improvement in the predictive test could be made.

ENZYME LEVELS

Our data indicated that G2 was a key stage for predicting malting grade. Therefore we measured levels of alpha-amylase and beta-glucanase at G2 to see if there was any correlation between activity and malting grade. The Navenby and Rothwell sites were selected for these analyses.
Comparing the Navenby and Rothwell sites, there were large differences in some varieties between the sites. For example, for Puffin, Intro, Fanfare, Rifle and Halcyon, samples grown at Rothwell had much higher alpha-amylase activity than those grown at Navenby (Figure 11a). This amount of site-to-site variation suggested that it was unlikely that there would be a good correlation between alpha-amylase activity and malting grade and in fact, no correlation was seen (Figure 11b). A similar situation was seen for beta-glucanase activity. The site-to-site variation in beta-glucanase activity for varieties was significant (Figure 12a) and there was no correlation between beta-glucanase activity and malting grade (Figure 12b).

The site-to-site variation in activity of these two enzymes suggests that there is a strong environmental influence on how much and how rapidly beta-glucanase and alpha-amylase enzymes are synthesised during malting. Comparing Figures 11a and 11b, it is clear that there are similar patterns: for example in Pastoral, both enzymes were at a low levels in the Navenby samples but were about 3 fold higher in the Rothwell samples. In fact, alpha - amylase and beta-glucanase correlated with each other at Rothwell (R^2=0.83) and at Navenby (R^2= 0.52;figures 13a and 13b respectively). This result might be expected when considering the mechanism of induction of these enzymes. Although beta-glucanase is synthesised earlier during malting than alpha-amylase, both enzymes are induced by gibberellic acid and respond to the same signalling pathway. Therefore it is possible that the same environmental influence during seed formation also effects embryo and aleurone response during malting and hence the induction of both enzyme activities.

BETA-GLUCAN ANALYSIS ON SMALL SAMPLE SIZE

Of the many parameters measured during malting, it was clear that our data suggest that measuring total beta-glucan at G2 will give an assessment of malting grade. This will only be a practical technique for barley breeders if it is possible to measure beta-glucan on a small scale. Currently the McCleary assay method requires 0.5g of sample. Therefore this method was miniaturised to accommodate a smaller sample size, and is outlined in figure 14. The new method requires only 0.06g of sample for analysis and would be suitable as an analysis following micromalting. Table 6 shows that an error of about 5% is typical for this analysis which is comparable to the full scale McCleary assay.

ADDITIONAL NOTE: APPENDICES

A large number of analyses were carried out during the course of this work. Some of these were not used in the final report because they did not contribute to final out-come. Many of these have been included in Appendix 1.

Similarly some of the beta-glucan data did not yield good correlations with malting quality. A correlation matrix for these data is provided as Appendix 2.

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8b: Variation of the average total beta-glucan at G2 with malting grade for barleys grown at all four sites, and for Chariot and Epic.

8c: Beta-glucan at G2 verses malting grade for malts prepared on 5g scale

9a: Relationship between viscosity and beta-glucan at G2 for samples grown at Navenby.

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9c: Variation in malt viscosity with malting grade for samples grown at Navenby.

10a: Site-to-site variation in malt for Haughley, Navenby and Rothwell.

10b: Variation in beta -glucan content with malt HWE, averaged over Haughley, Navenby and Rothwell sites.

10c: Effect of malting regime on HWE.

11a: Site-to-site variation in alpha-amylase activity at G2 for samples grown at Navenby and Rothwell.

11b: Variation in alpha-amylase activity with malting grade for samples grown at Navenby and Rothwell.

12a: Site-to-site variation in beta -glucanase activity at G2 for samples grown at Rothwell and Navenby.

12b: Variation in beta-glucanase activity at G2 with malting grade for samples grown at Navenby and Rothwell.

13a: Correlation between beta-glucanase activity and alpha-amylase activity for samples grown at Rothwell.

13b: Correlation between beta -glucanase activity and alpha-amylase activity for samples grown at Navenby.

14: Miniaturised method for the measurement of total beta –glucan.

Table 1. Barley varieties analysed.

Barley variety	Malting grade	Each barley variety grown at five different sites :
Pastoral	2	
Intro	5	Haughley, Suffolk
Fighter	6	Navenby, S Lincoln
Sunrise	7	Rothwell, Lincoln
Halcyon	8	Wooton, NE Lincoln
Fanfare	9	Woolpit, Suffolk
Puffin	9	
Regina	9	
Rifle	9	
Spice	9	

Table 2. Alkali-extractable insoluble bound phenolic acids and beta-glucan content of three barley varieties of differing malting grade grown at Rothwell in Lincolnshire.

Phenolic acid expressed as mg per g barley flour.

	Pastoral (97/35)	**Intro** (97/34)	**Regina** (97/37)
Malting Grade	2	5	9
trans-*p*-Coumaric acid	0.1	0	0.21
cis-*p*-Coumaric acid	0	0.06	0
trans-Ferulic acid	0.63	0.59	0.58
cis-Ferulic acid	0.83	0.58	0.99
Total phenolic acid	1.56	1.23	178
% Total ß-glucan	2.54	2.69	2.33
% Insoluble ß-glucan	1.29	1.59	1.26
HWE (0.7mm)	297	300	308

Table 3. Alkali-extractable insoluble bound phenolic acids, beta-glucan and HWEvalues of dehusked barley varieties of differing malting grade grown at Rothwell in Lincolnshire.
Phenolic acid expressed as mg per g barley flour.

	Pastoral (97/35)	**Intro** (97/34)	**Regina** (97/37)	**Fanfare** (97/31)	**Rifle** (97/38)	**Spice** (97/39)
Malting Grade	2	5	9	9	9	9
trans-Ferulic acid	0.39	0.42	0.44	0.44	0.48	0.53
cis-Ferulic acid	0.2	0.33	0	0.93	0.44	0.58
Total Ferulic acid	0.59	0.75	0.44	1.37	0.92	1.11
% Total ß-glucan	2.54	2.69	2.33	2.16	2.21	2.01
% Insoluble ß-glucan	1.29	1.59	1.26	1.41	1.24	1.12
HWE (0.7mm)	297	300	308	307	311	308

Table 4. Alkali-extractable insoluble bound phenolic acids, beta-glucan and HWE values of endosperm from barley varieties of differing malting grade grown at Rothwell in Lincolnshire.
Phenolic acid expressed as mg per g barley flour.

	Pastoral (97/15)	**Pastoral** (97/25)	**Pastoral** (97/35)	**Intro** (97/34)	**Spice** (97/19)	**Fanfare** (97/21)	**Puffin** (97/26)	**Puffin** (97/36)	**Regina** (97/37)
Malting Grade	2	2	2	5	9	9	9	9	9
trans-Ferulic acid	0.06	0.048	0.06	0.1	0.07	0.1	0.03	0.049	0.03
% Total ß-glucan	2.89	2.25	2.54	2.69	2.46	1.74	2.03	2.58	2.33
% Insoluble ß-glucan	1.29	1.24	1.29	1.59	1.35	0.85	0.94	1.66	1.26
HWE (0.7mm)	293	295	297	300	303	303	298	300	308

Table 5. Summary of total beta-glucan levels (% dry weight) at G2 for all ten barley varieties at each site.

Variety	Malting Grade	Haughley (first malting)	Navenby	Rothwell	Woolpit	Haughley (second malting)	average
Fanfare	9	2	2.26	1.89	2.06	2.2	2.08
Fighter	6	2.57	2.93	2.47	2.75	2.93	2.73
Halcyon	9	2.04	2.17	2.44	1.98	2.23	2.17
Intro	5	2.6	2.95	2.64	3.02	2.99	2.84
Pastoral	3	2.81	3.21	2.85	3.24	3.07	3.04
Puffin	9	1.74	2.6	2.03	2.33	1.9	2.12
Regina	9	1.5	2.27	1.77	2	1.9	1.89
Rifle	9	1.95	2.47	2.15	2.41	2.27	2.25
Spice	9	2.05	2.23	1.98	2.16	2.27	2.14
Sunrise	8	2	2.74	1.92	2.63	2.37	2.33

Table 6. Typical results for the miniaturised McCleary assay on a sample of milled barley.

Trial no.	Weight (mg)	Dry weight (mg)	Total beta-glucan (% dry weight)
1	45	40	3.4
2	60	53	3.3
3	59	52	3.4
4	46	40	3.6

REFERENCES

Ahluwalia, B. & Fry, S.C. 1986. J. Cereal Sci., **4**, 287-295.

Aastrup, S. & Erdal, K. 1980 Carlsberg Res. Comm. 45.

Briggs, D.E. 1978. *Barley* . Chapman & Hall, London.

Forrest, I.S. & Wainwright, T. 1977. Inst. Brew., **83**, 279-286.

Martin, H.L., & Bamforth, C.W., 1980, Journal of the Institute of Brewing, **86**, 216-221.

McCleary, B.V. & Glennie-Holmes, M., 1985, Journal of the Institute of Brewing, **91**, 285-295.

Moore, J., Bamforth, C.W., Kroon, P.A., Bartolome, & Williamson, G. 1996. Biotech. Letts. **18**, 1423-1426.

Nordkvist, E., Salomonsson, A-C., & Aman, P., 1984, Journal of Science, Food and Agriculture, **35**, 657-661.

Selvendran, R.R. 1983. In: Dietary Fibre. eds: G.G Birch & K.J.Parker.

Zupfer, J.M., Churchill, K.E., Rasmusson, D.C. & Fulcher, R.G., 1998 Journal of Agriculture, Food and Science, **46**, 1350-1354.

Figure 1a. Variation in insoluble beta-glucan in varieties across sites

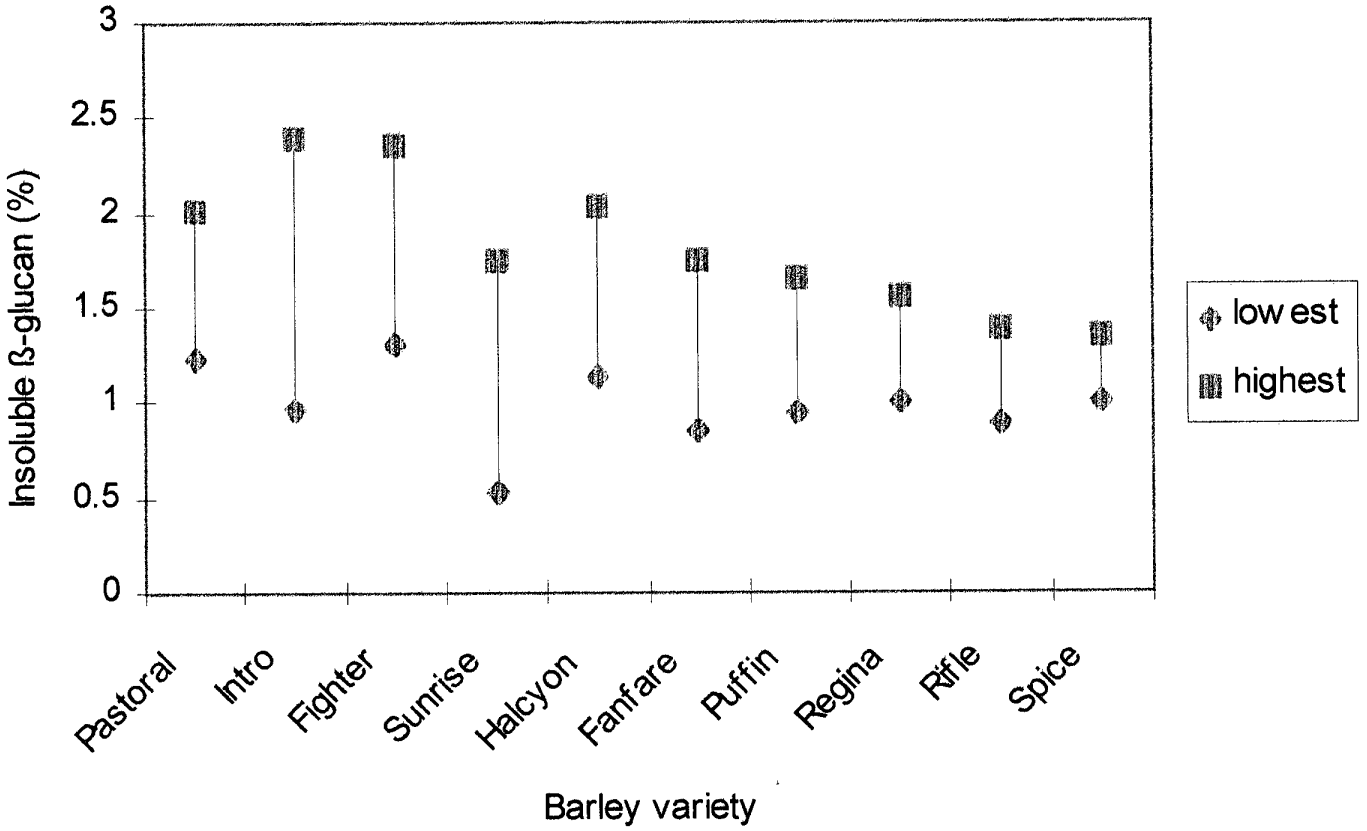


Figure 1b. Variation in soluble beta-glucan in varieties across sites

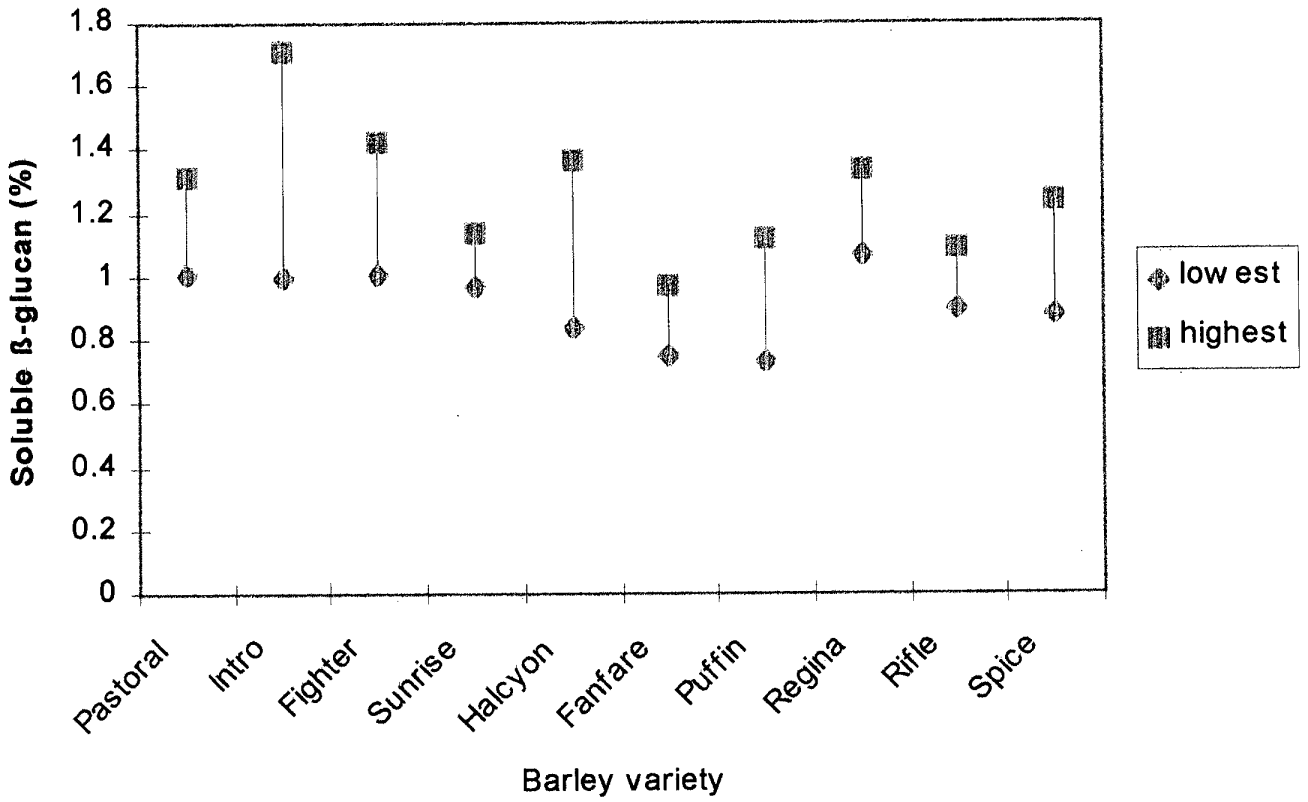


Figure 1c. Variation in total beta-glucan in varieties across sites

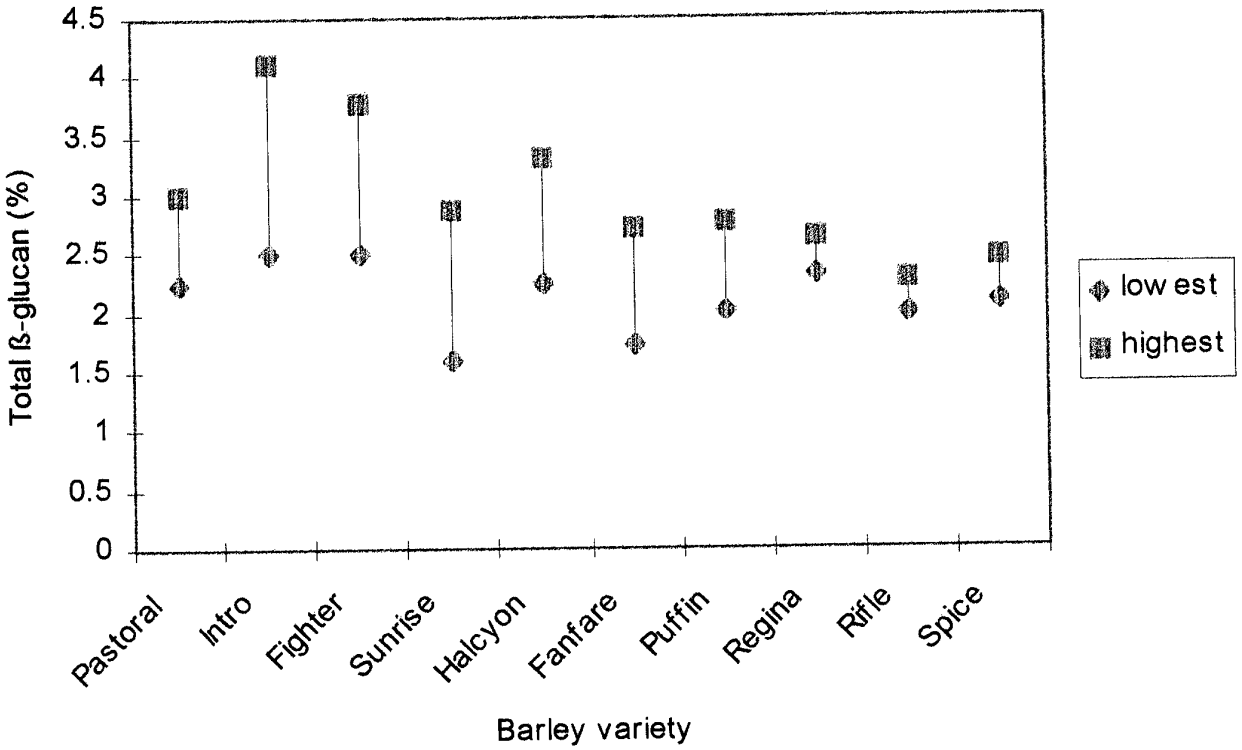


Figure 2a. Total beta-glucan verses total nitrogen for all varieties from all sites.

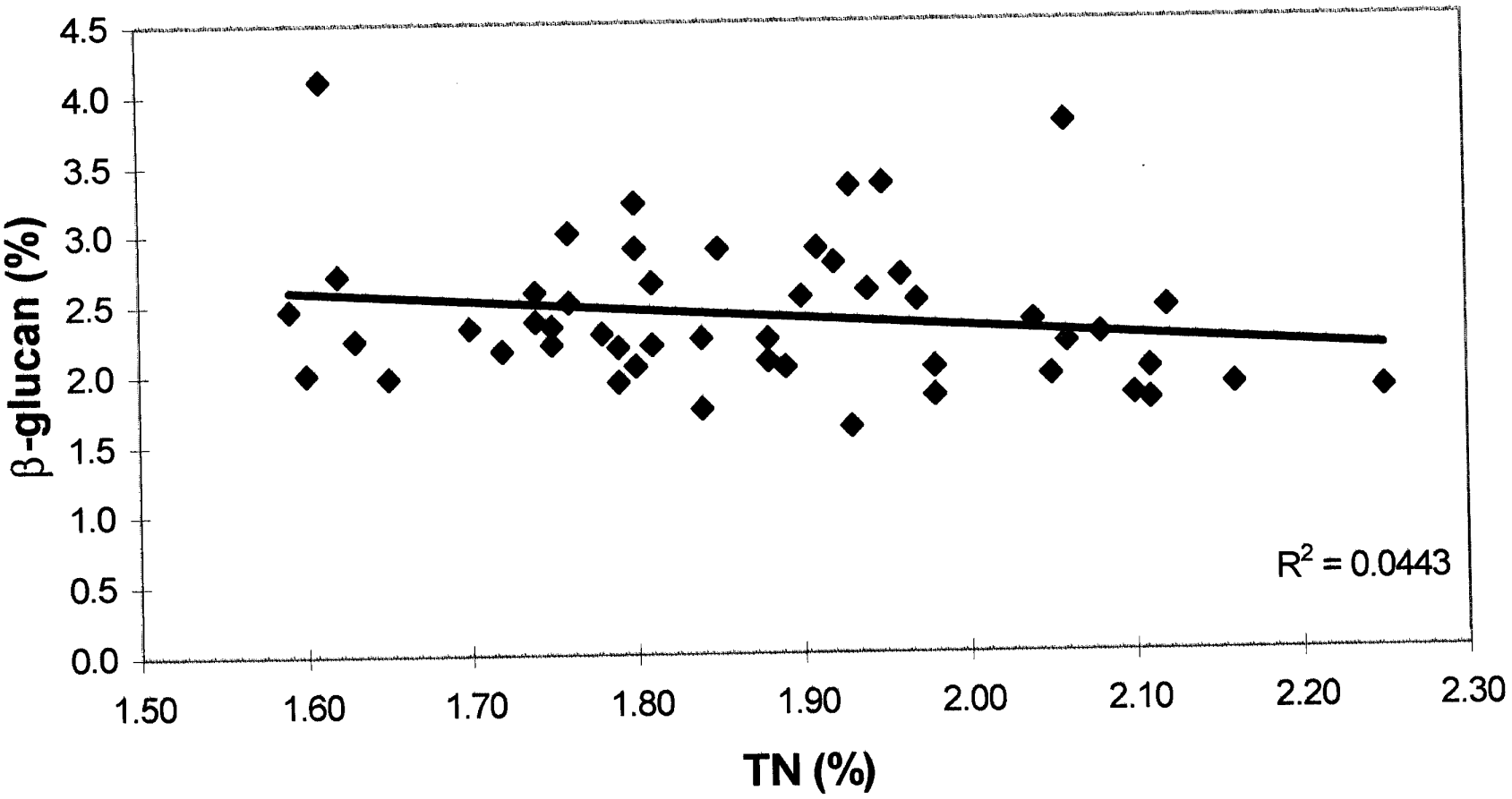


Figure 2b. Insoluble beta-glucan (as a percentage of total) and total nitrogen for all varieties from all sites.

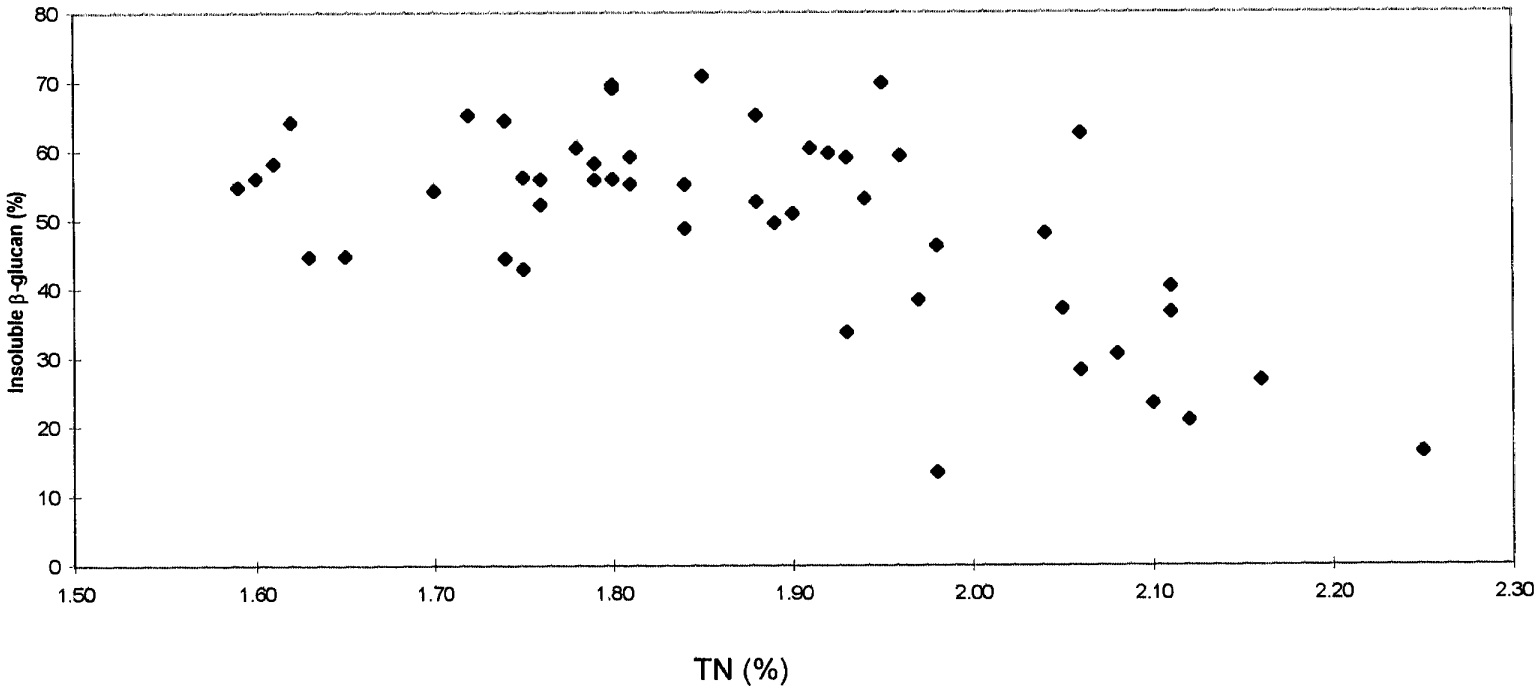


Figure 2c. Insoluble beta-glucan (as a % of total) for all varieties from all sites.

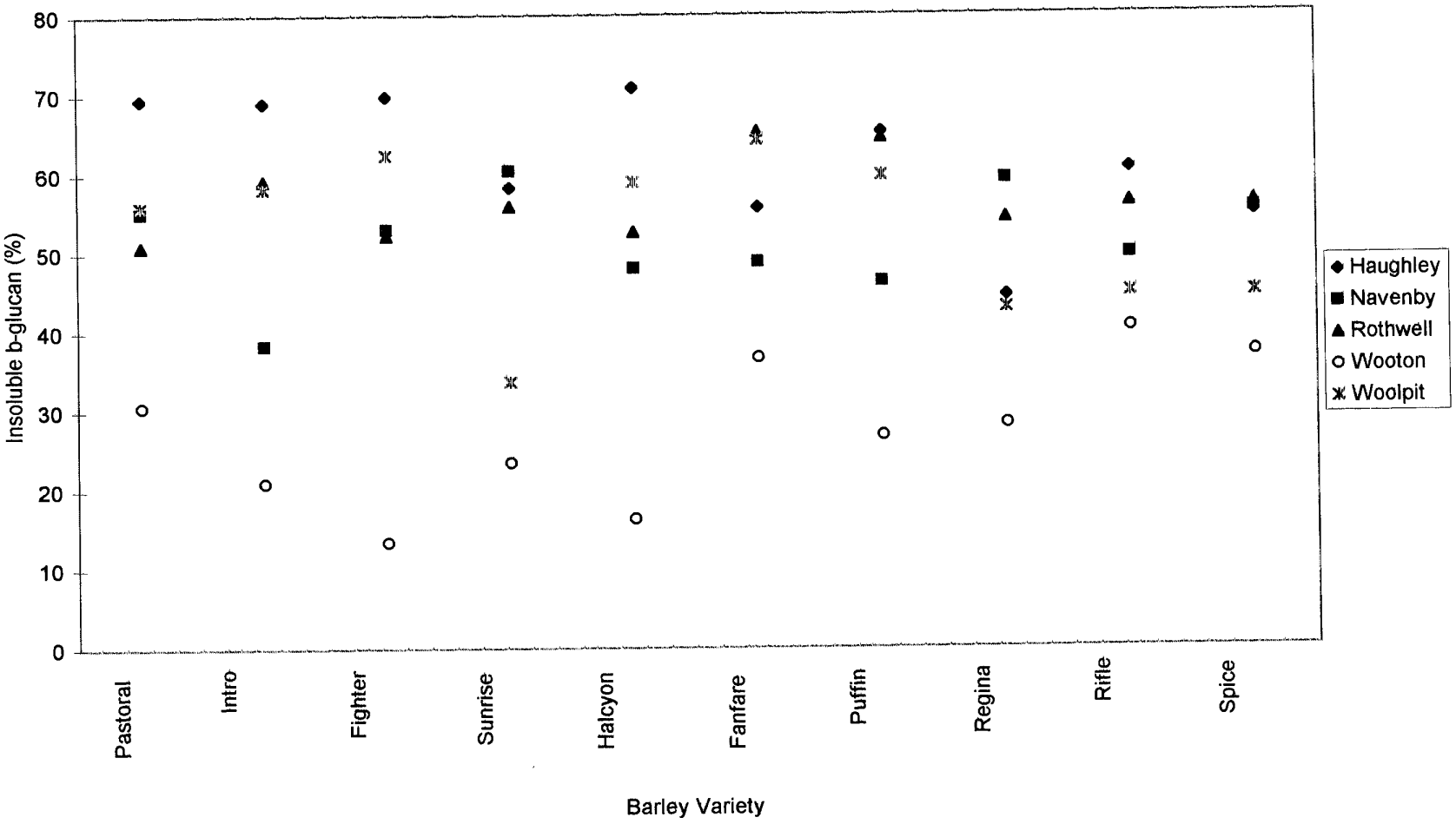
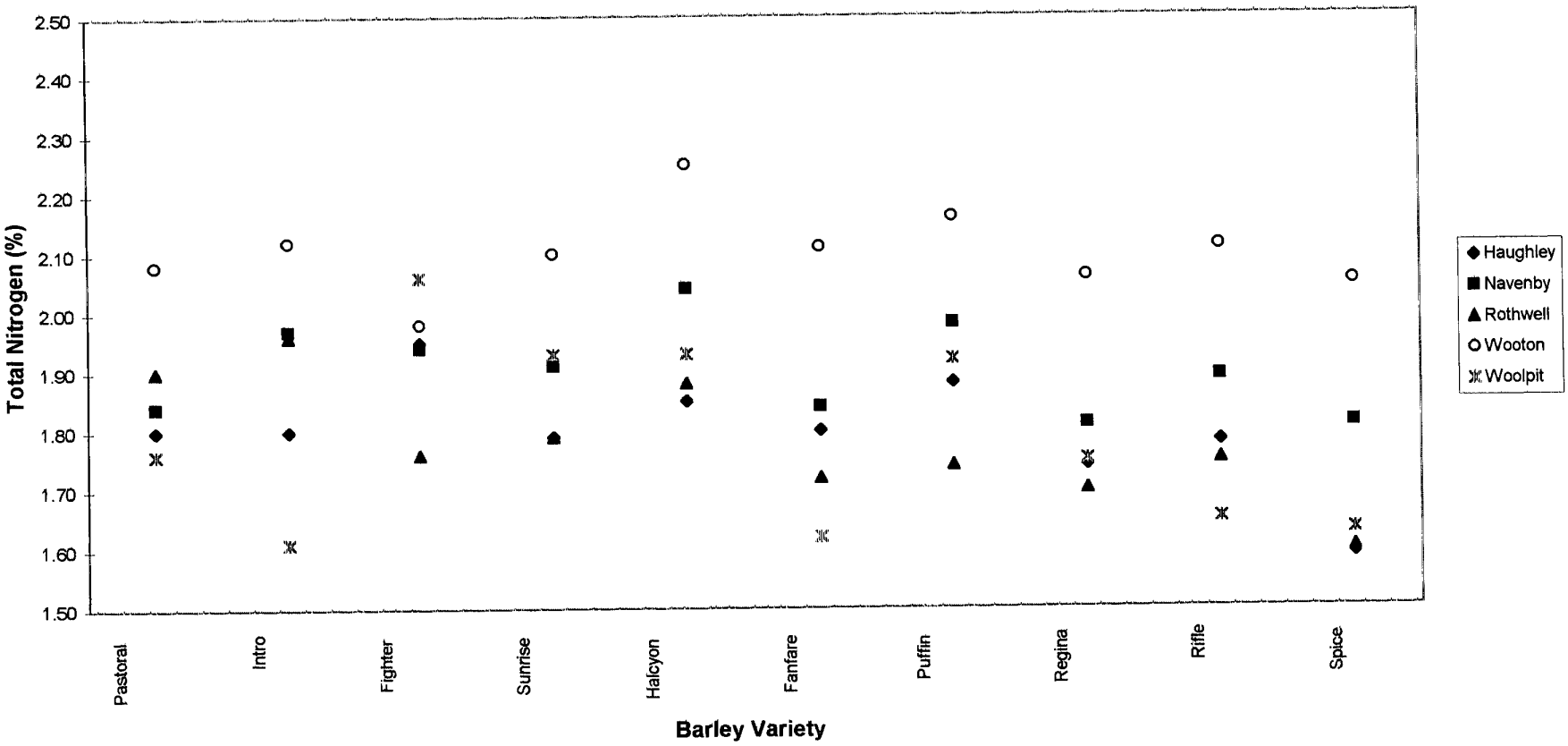
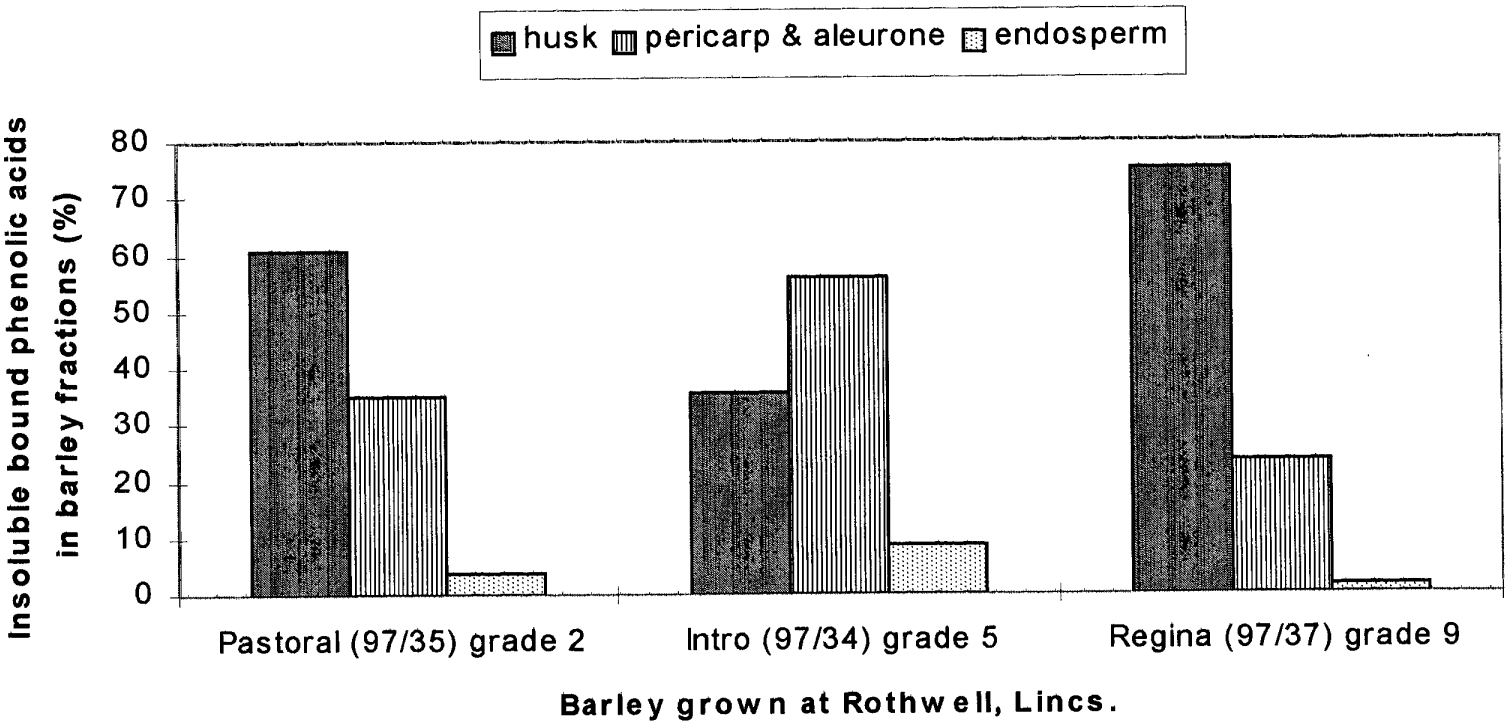


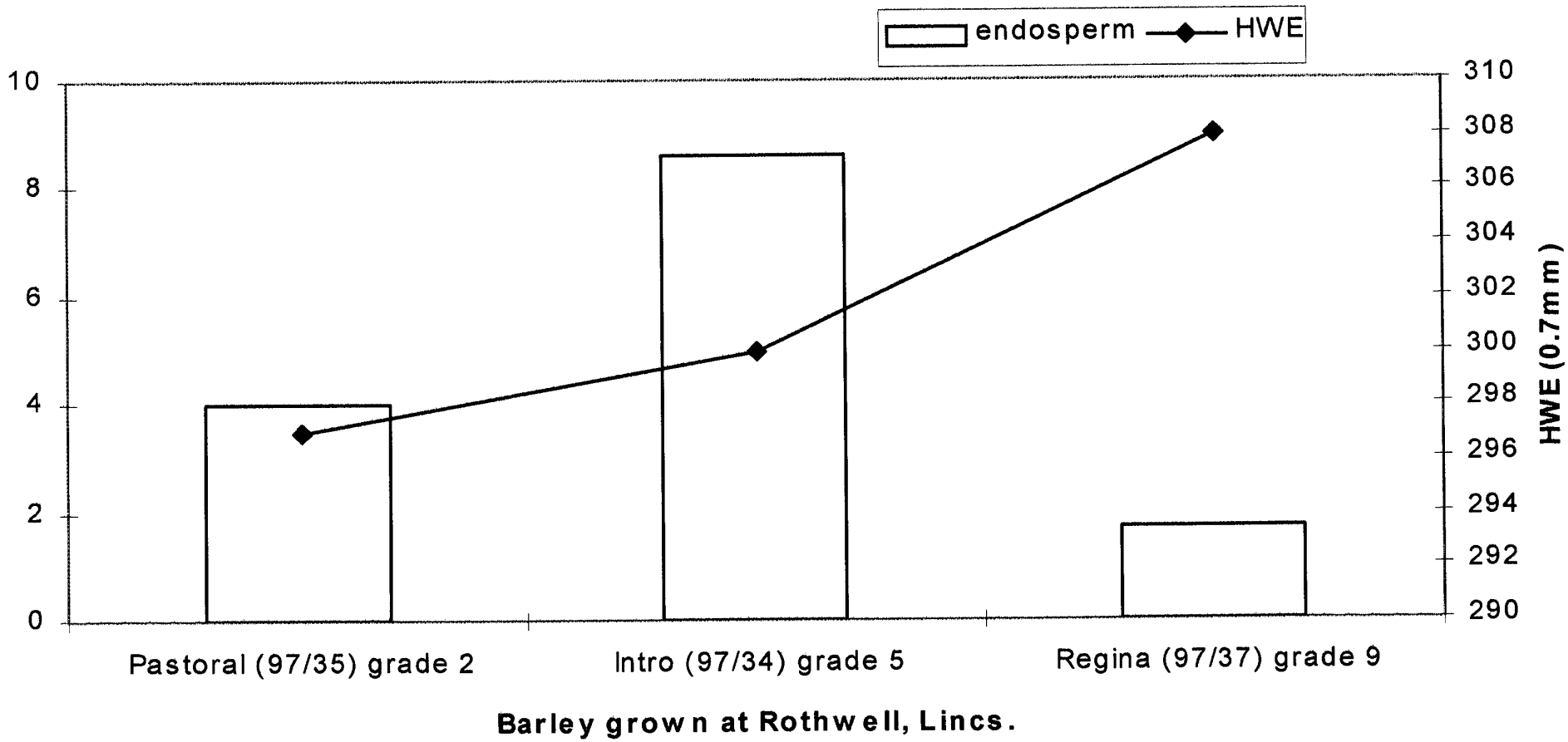
Figure 2d. Total nitrogen for all varieties from all sites.



3a. Alkali-extractable insoluble bound phenolic acids in cell walls of different fractions of the barley grain.



3b. Alkali-extractable insoluble bound ferulic acid in endosperm cell walls of barleys with different malting grade and the corresponding hot water extract (HWE) values.



Barley grown at Rothwell, Lincs.

3c. Alkali-extractable insoluble bound ferulic acid in endosperm cell walls of barleys with different malting grade and the corresponding whole grain insoluble ß-glucan content.

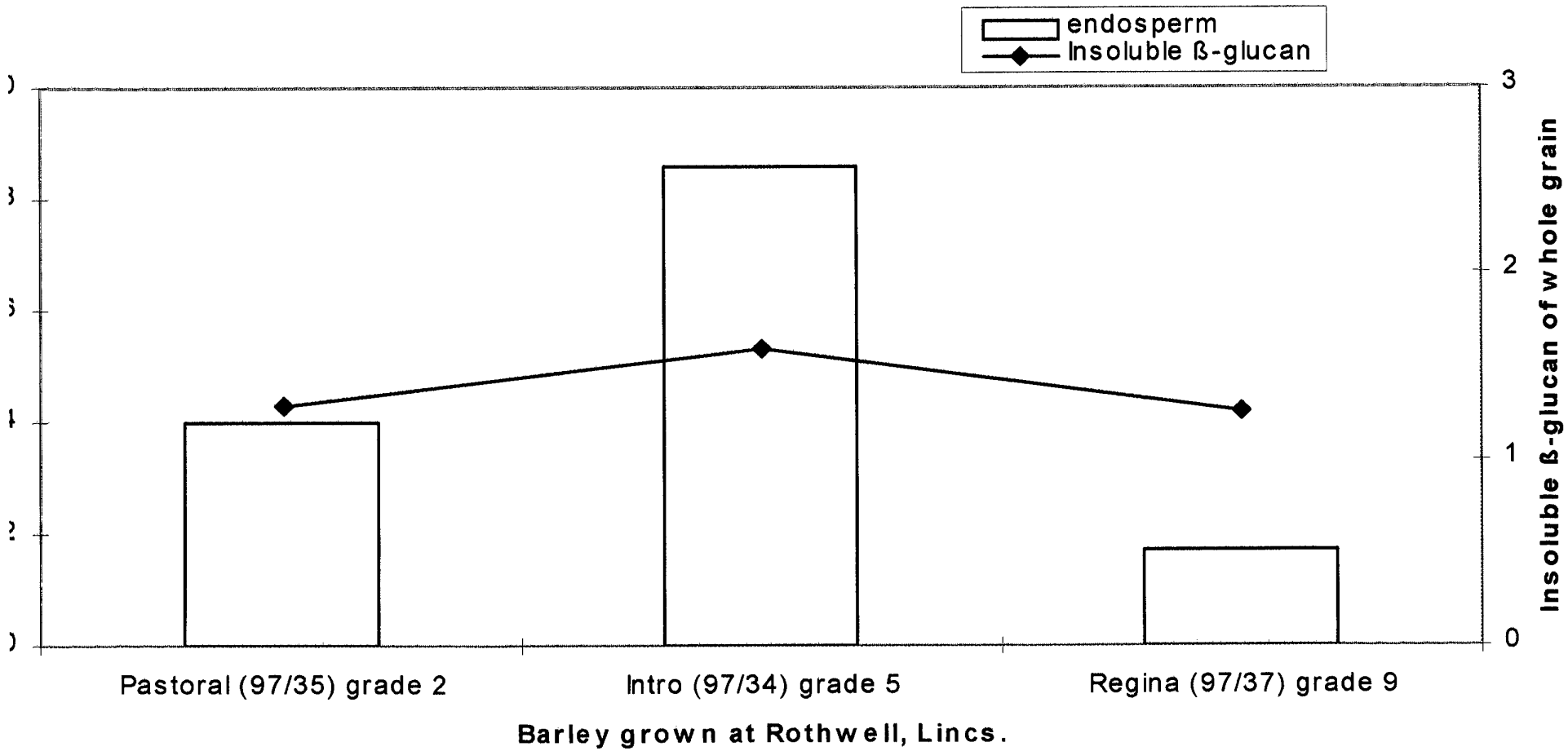


Figure 4a: Variation in total beta-glucan content with malting stage in Chariot and Epic

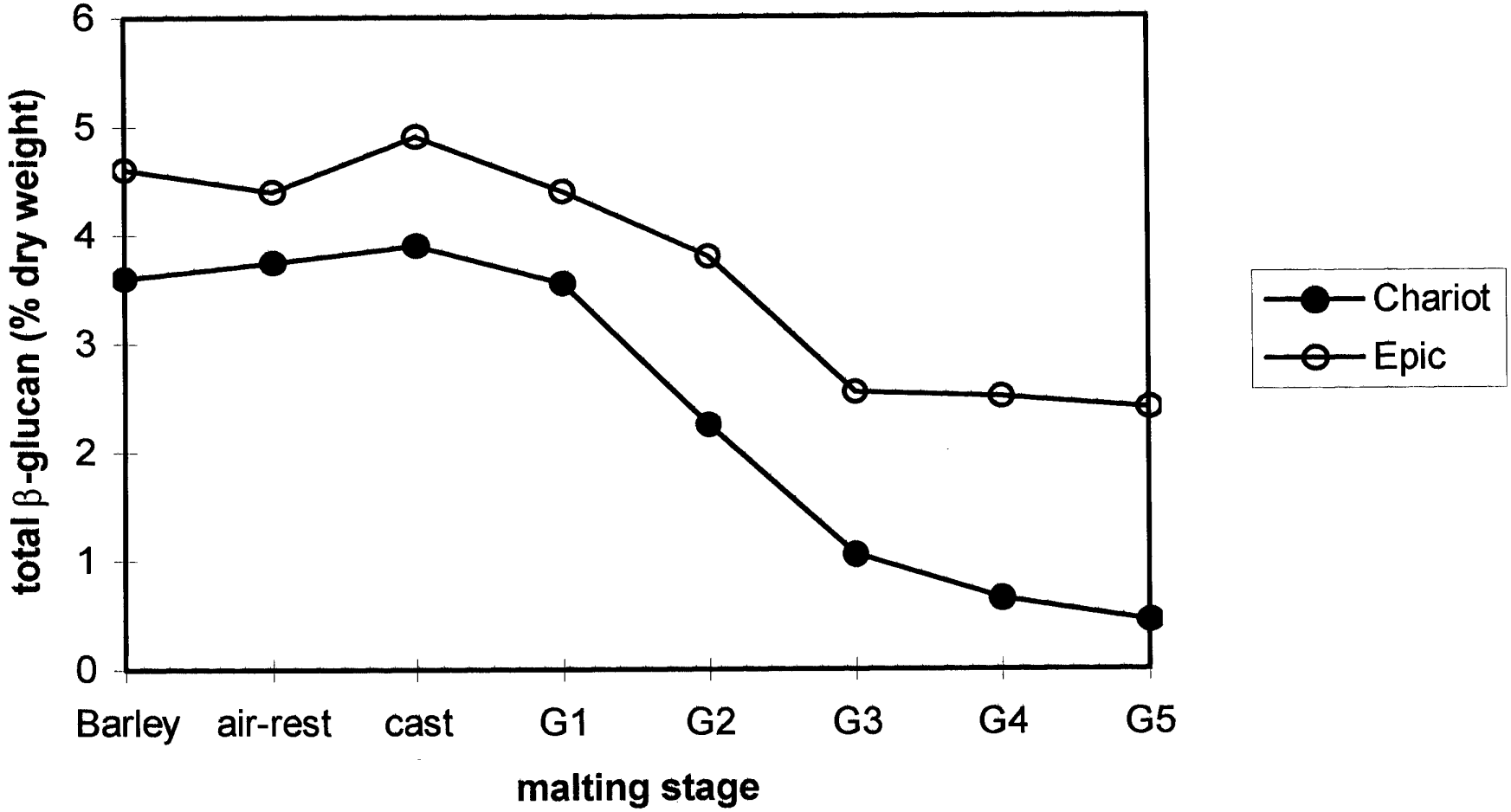


Figure 4b: Variation in soluble beta-glucan content with malting stage in Chariot and Epic

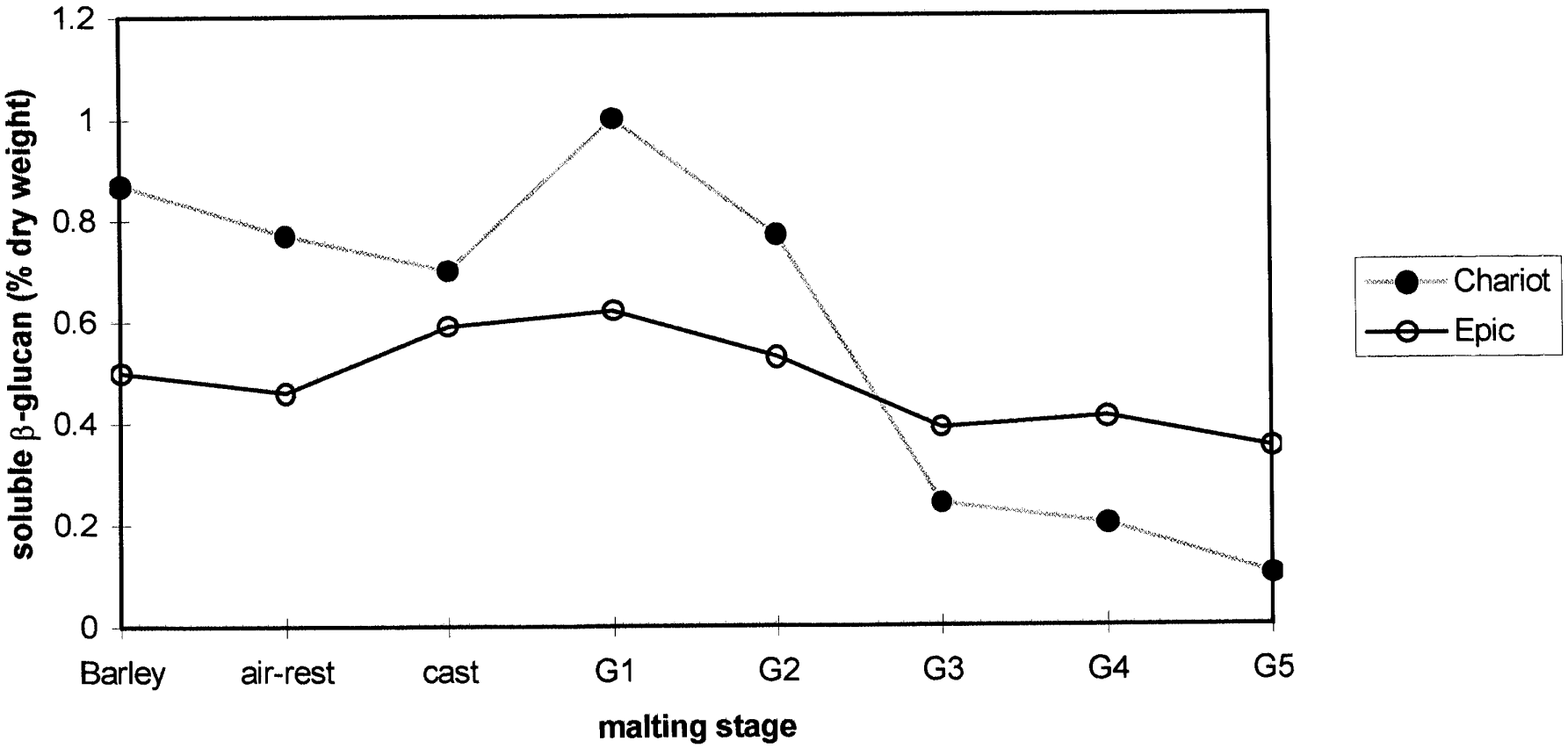


Figure 5a: Relationship between total β-glucan at G2 and malting grade in varieties grown at Rothwell

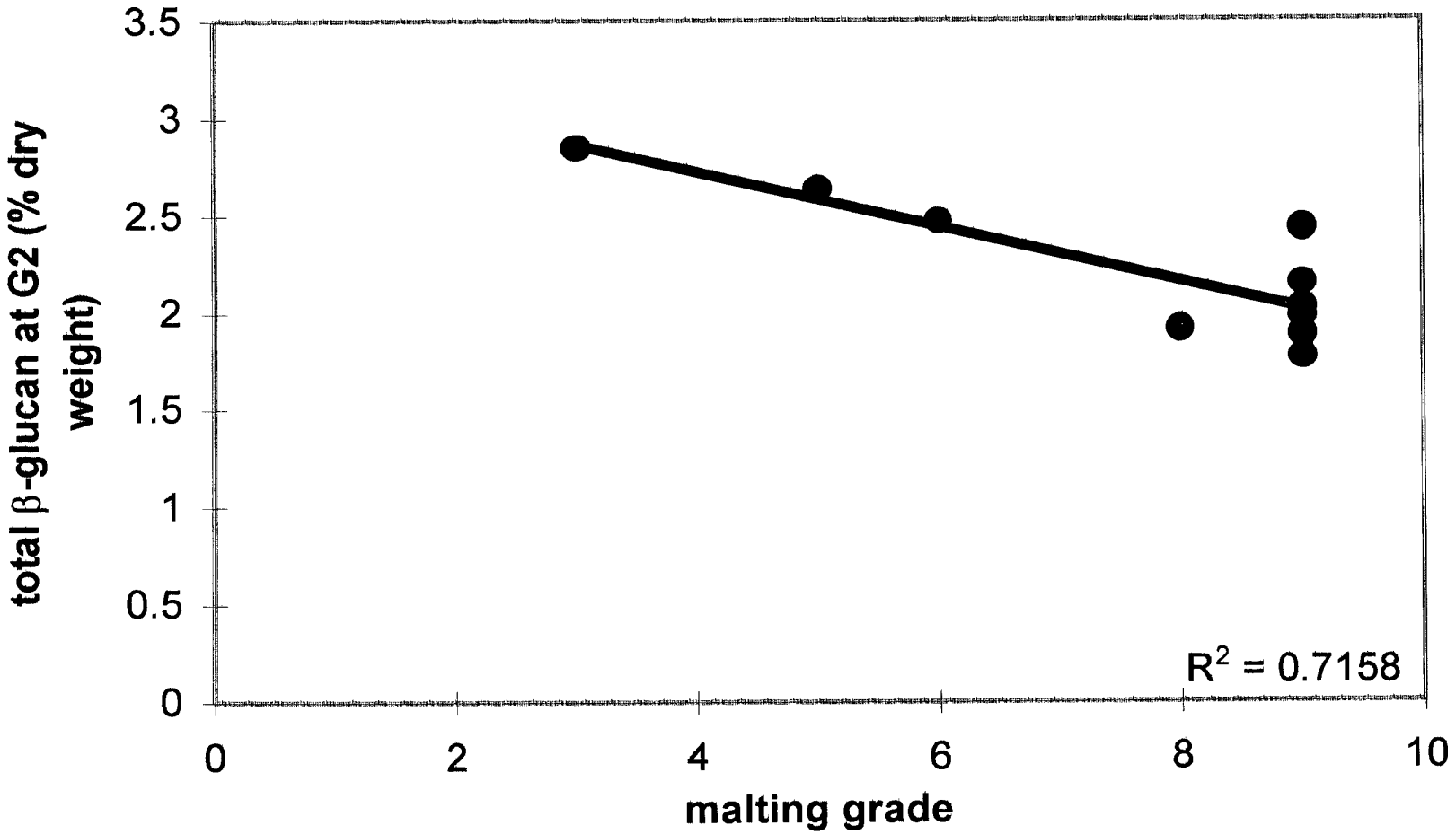


Figure 5b: Relationship between insoluble β-glucan at G2 and malting grade in varieties grown at Rothwell

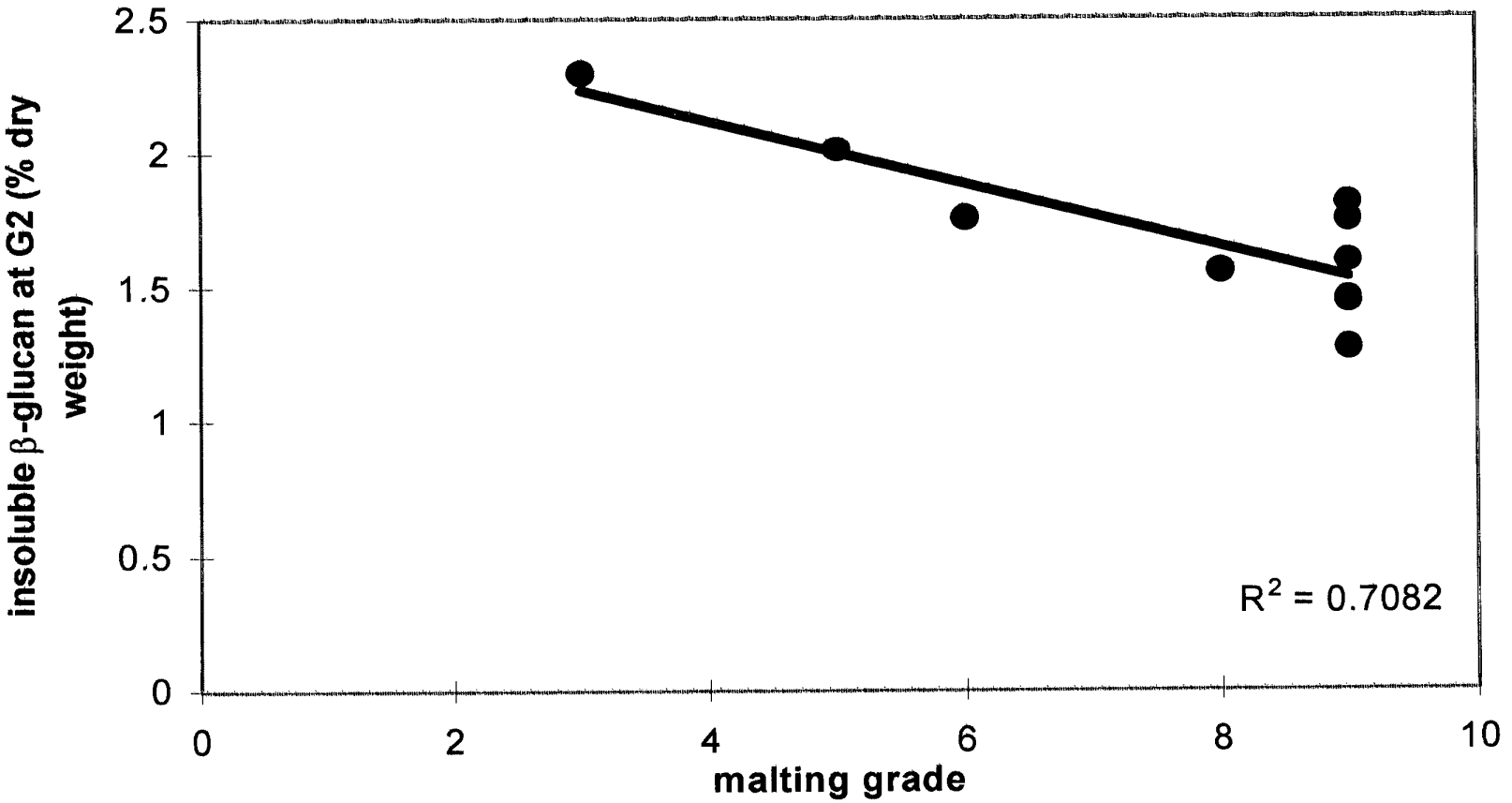


Figure 5c: Relationship between soluble β-glucan at G2 and malting grade in varities grown at Rothwell

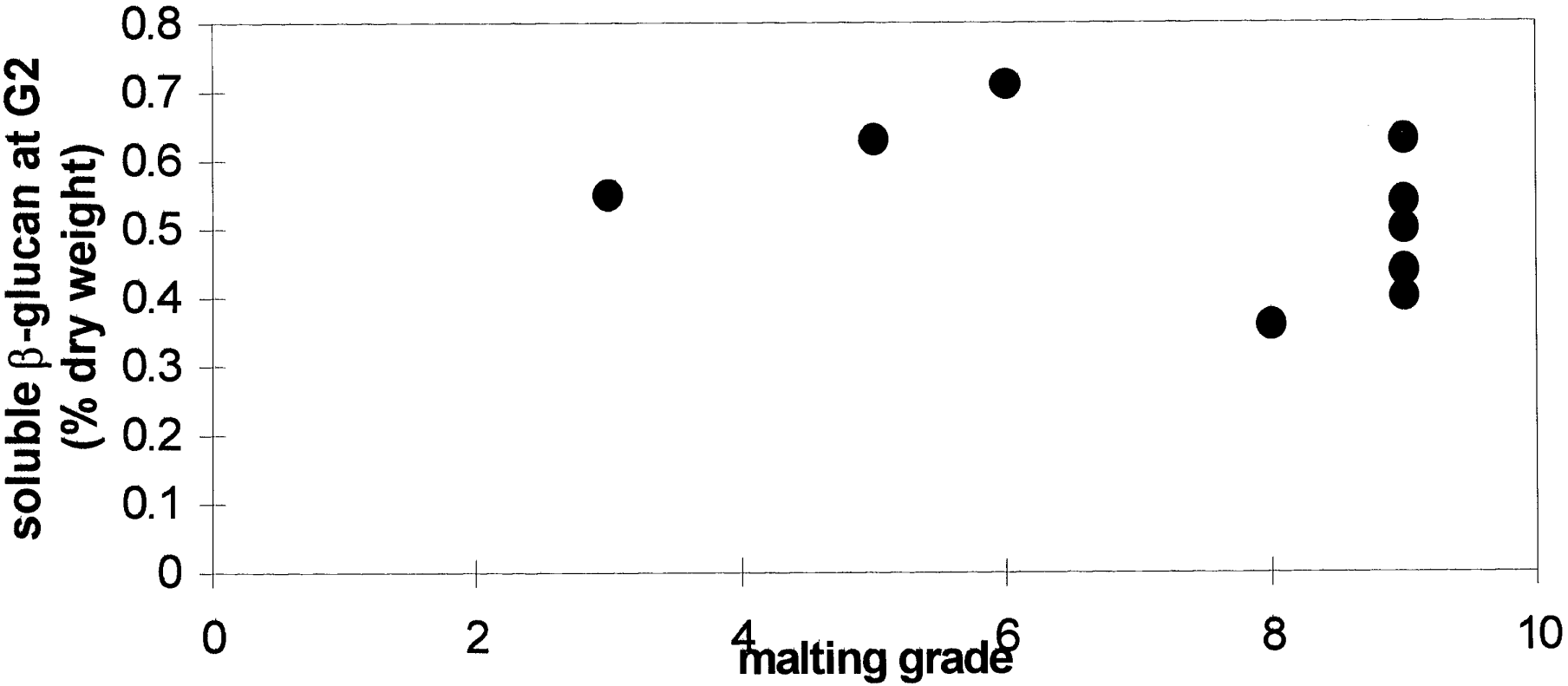


Figure 5d: Relationship between insoluble and total beta-glucan at G2 in varieties grown at Rothwell

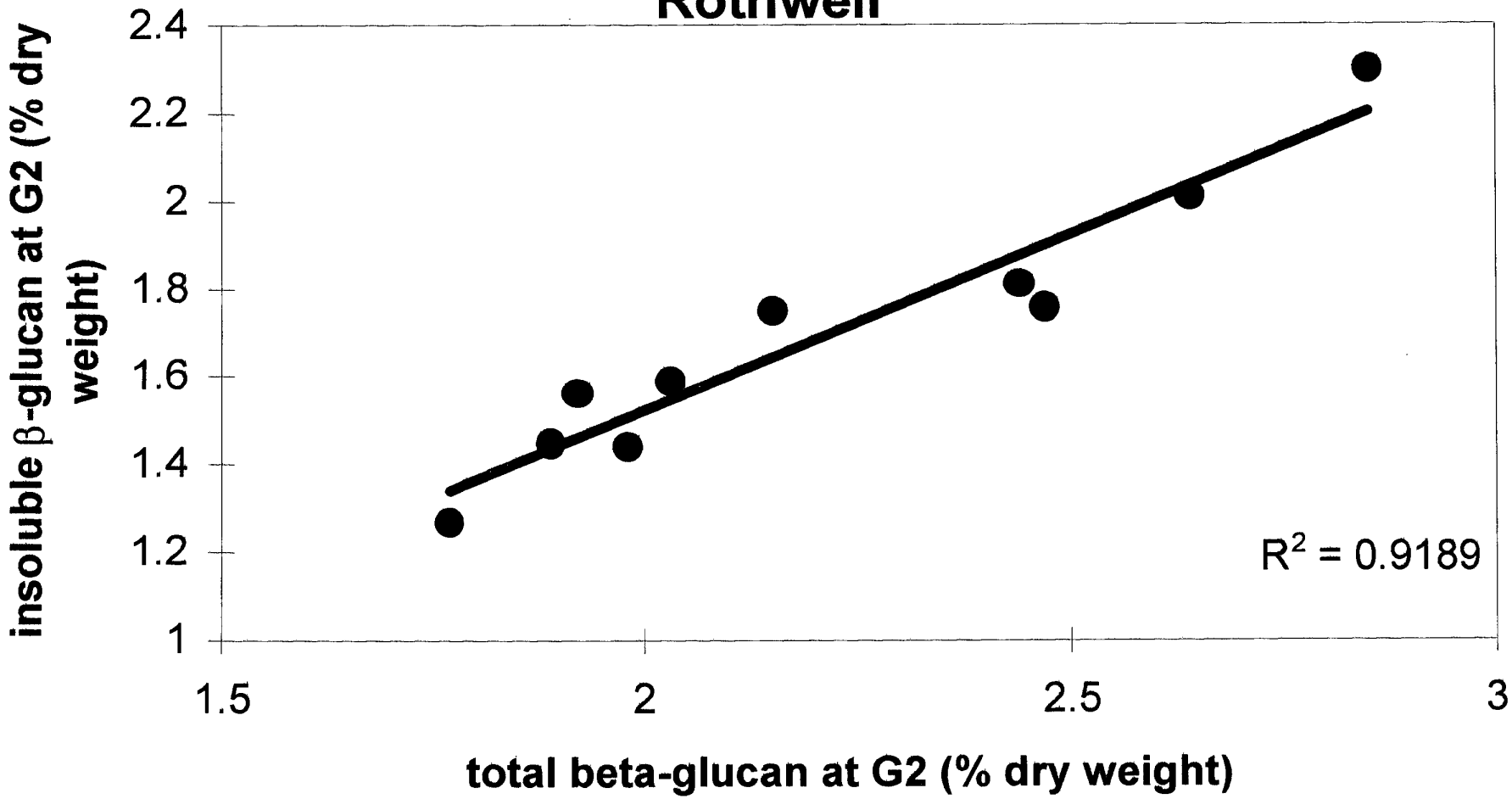


Figure 6a: Variation in total beta-glucan content at G2 with malting grade for barleys grown at Haughley

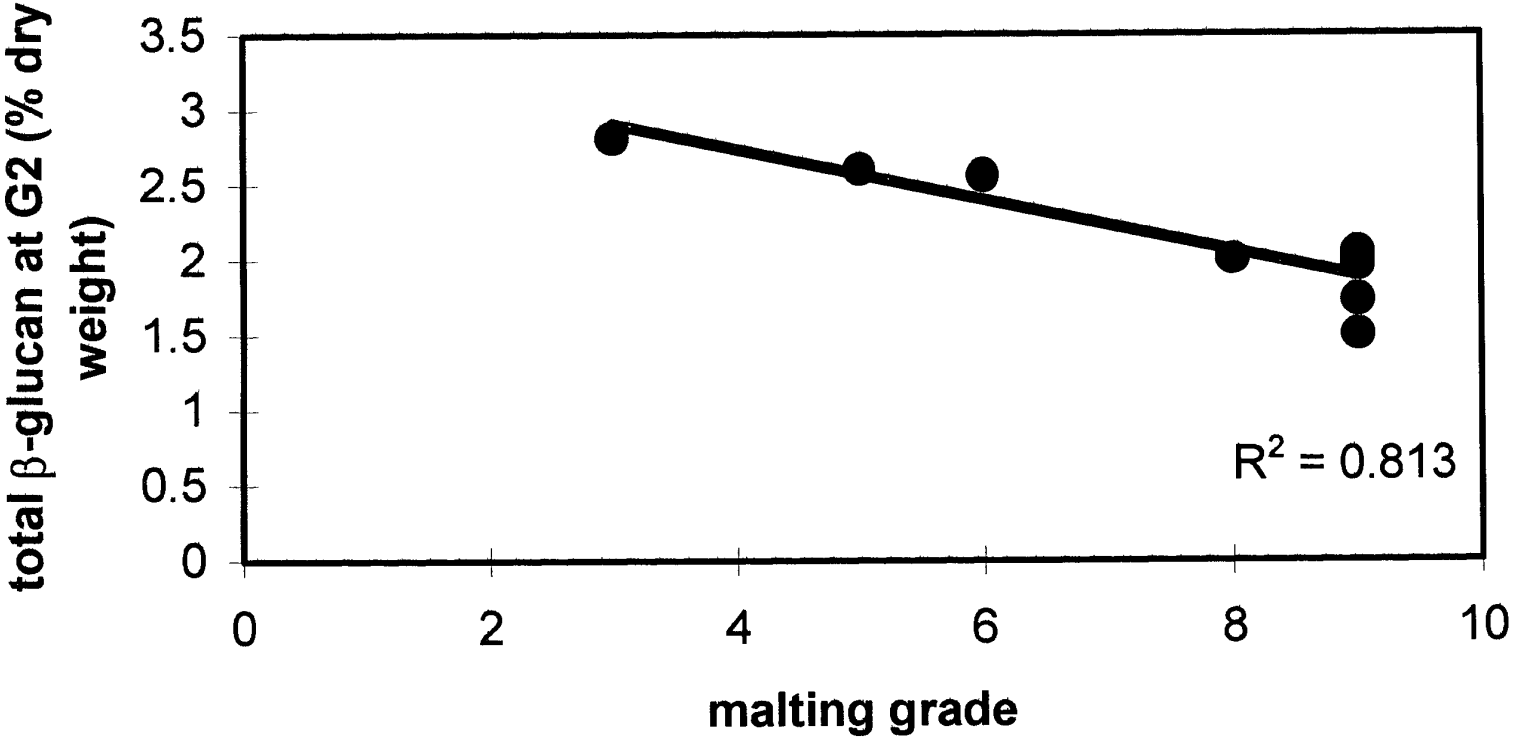


Figure 6b: Variation in total beta-glucan content at G2 with malting grade for barleys grown at Navenby

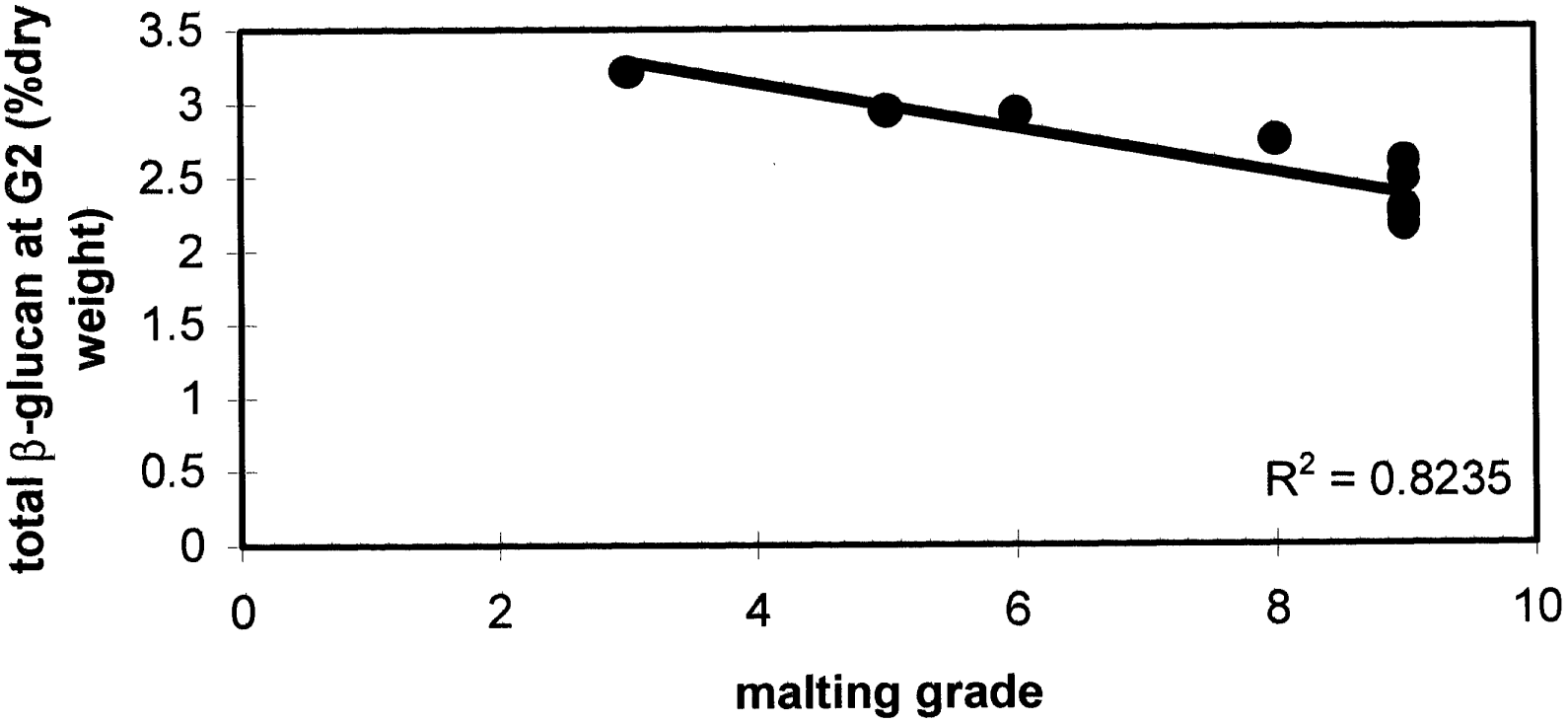


Figure 6c: Variation in total beta-glucan content at G2 with malting grade for barleys grown at Woolpit

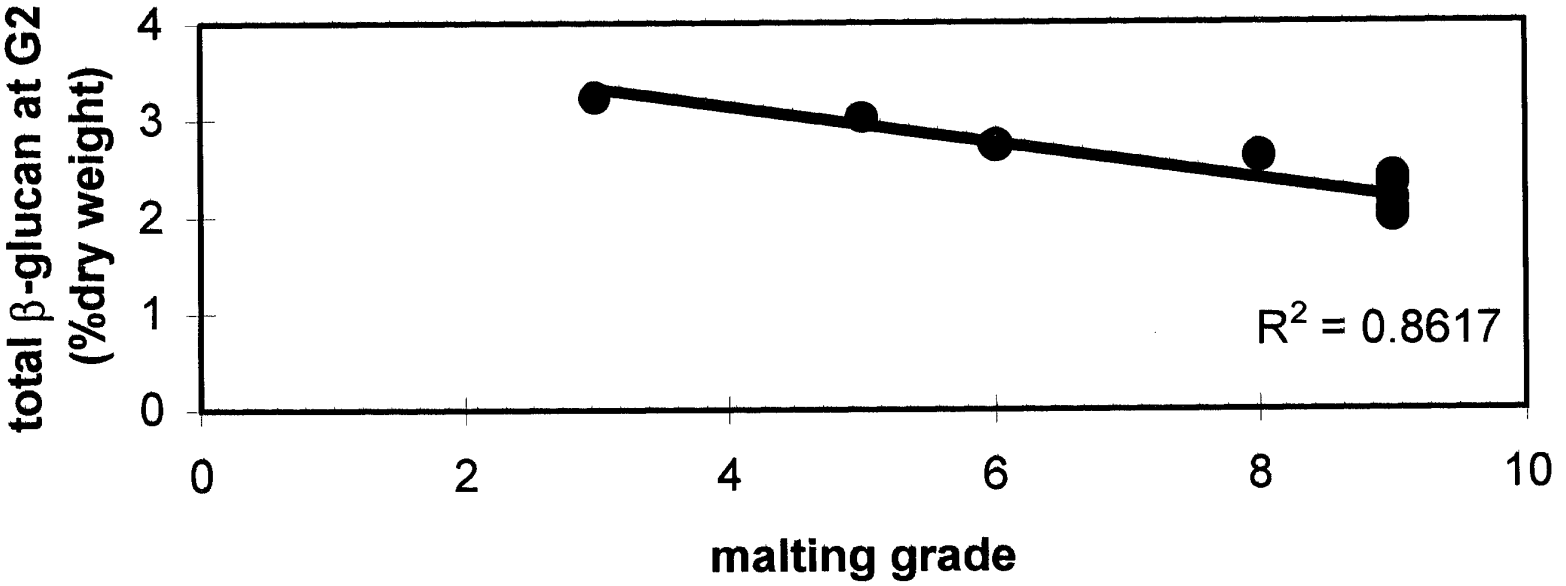


Figure 7a: Total beta-glucan at G2 for Regina at all four sites

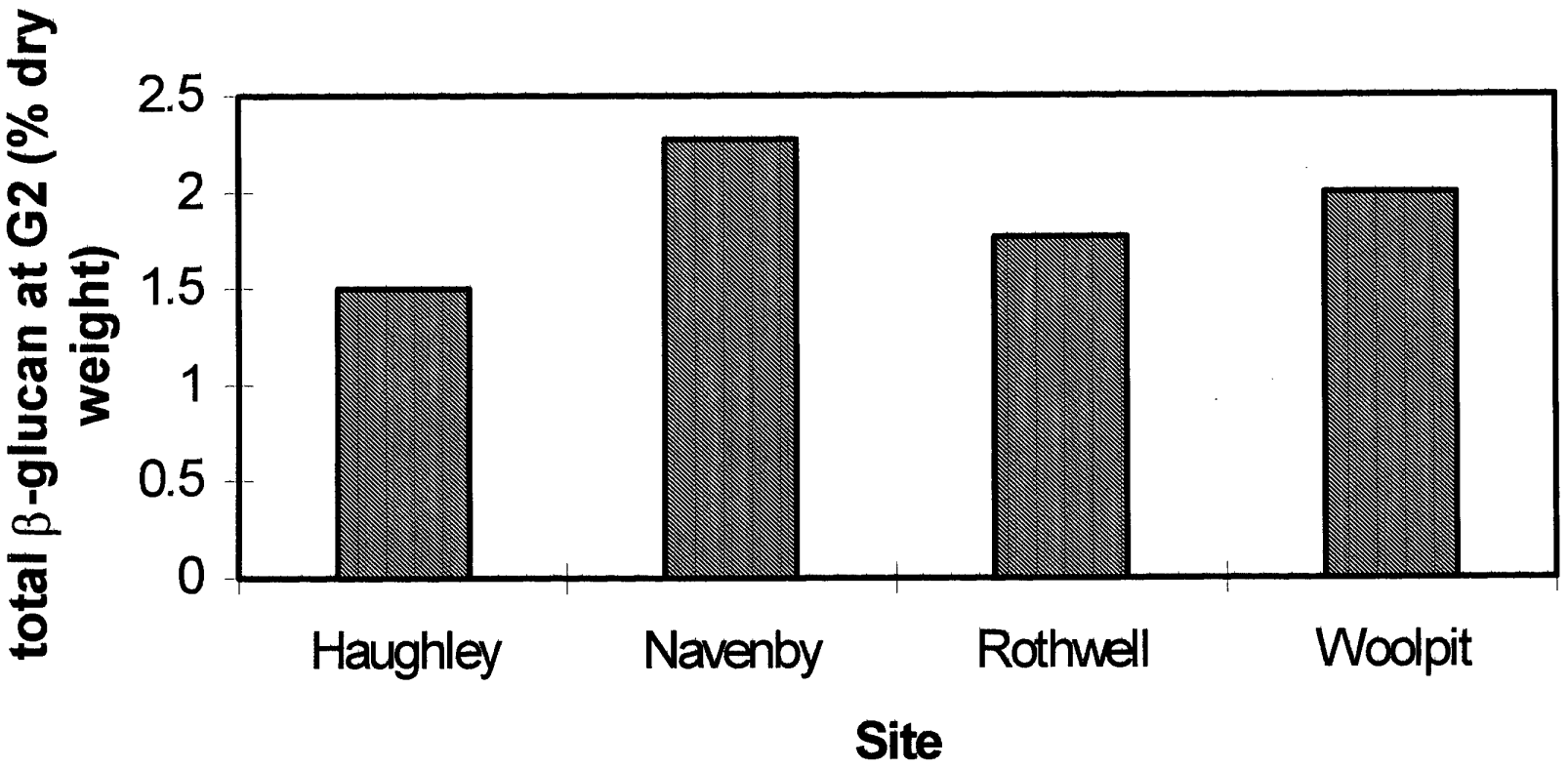


Figure 7b: Total beta-glucan at G2 for Spice at all four sites

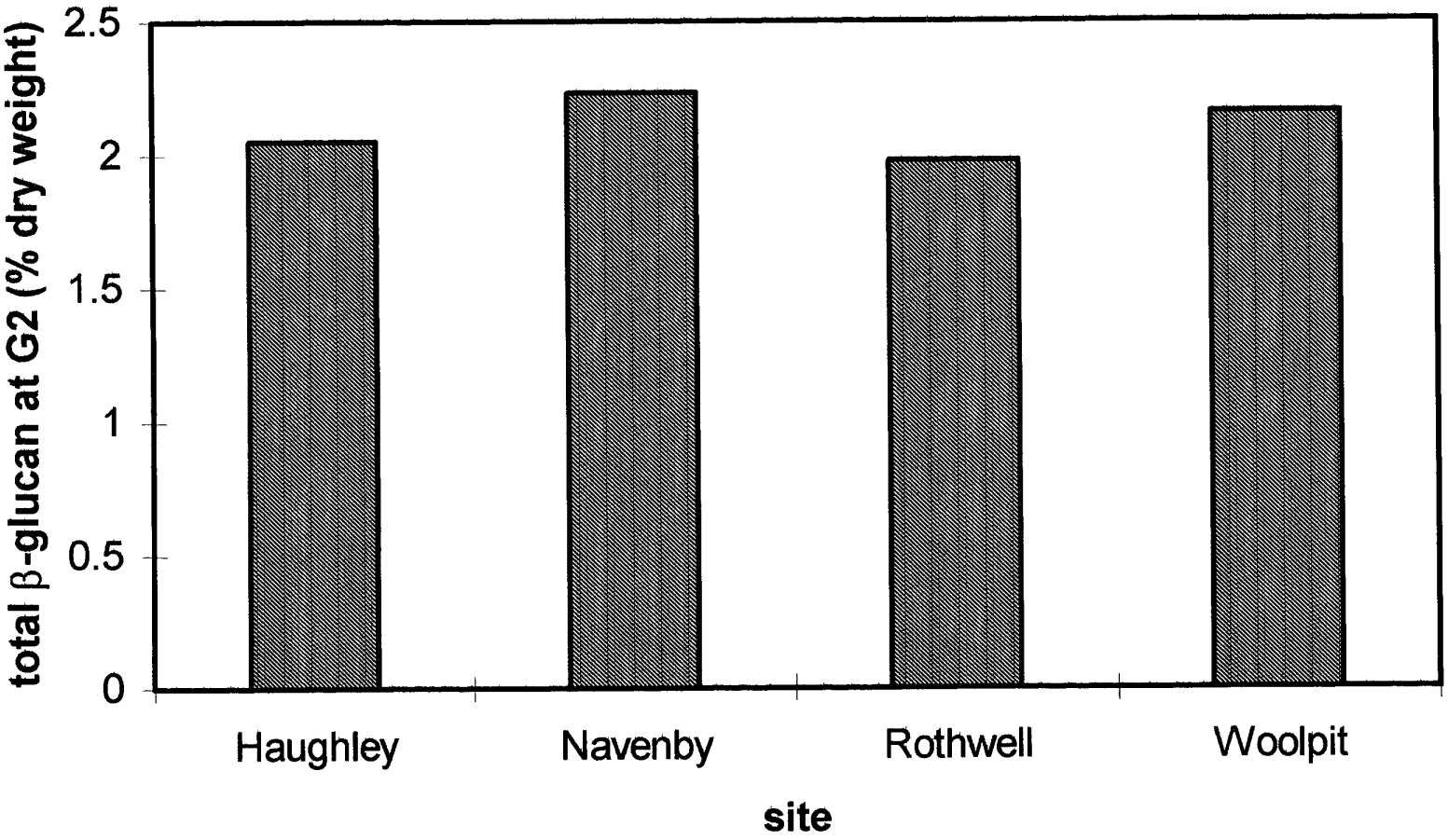


Figure 7c: variation in total beta-glucan content at G2 for barley varieties grown at four sites

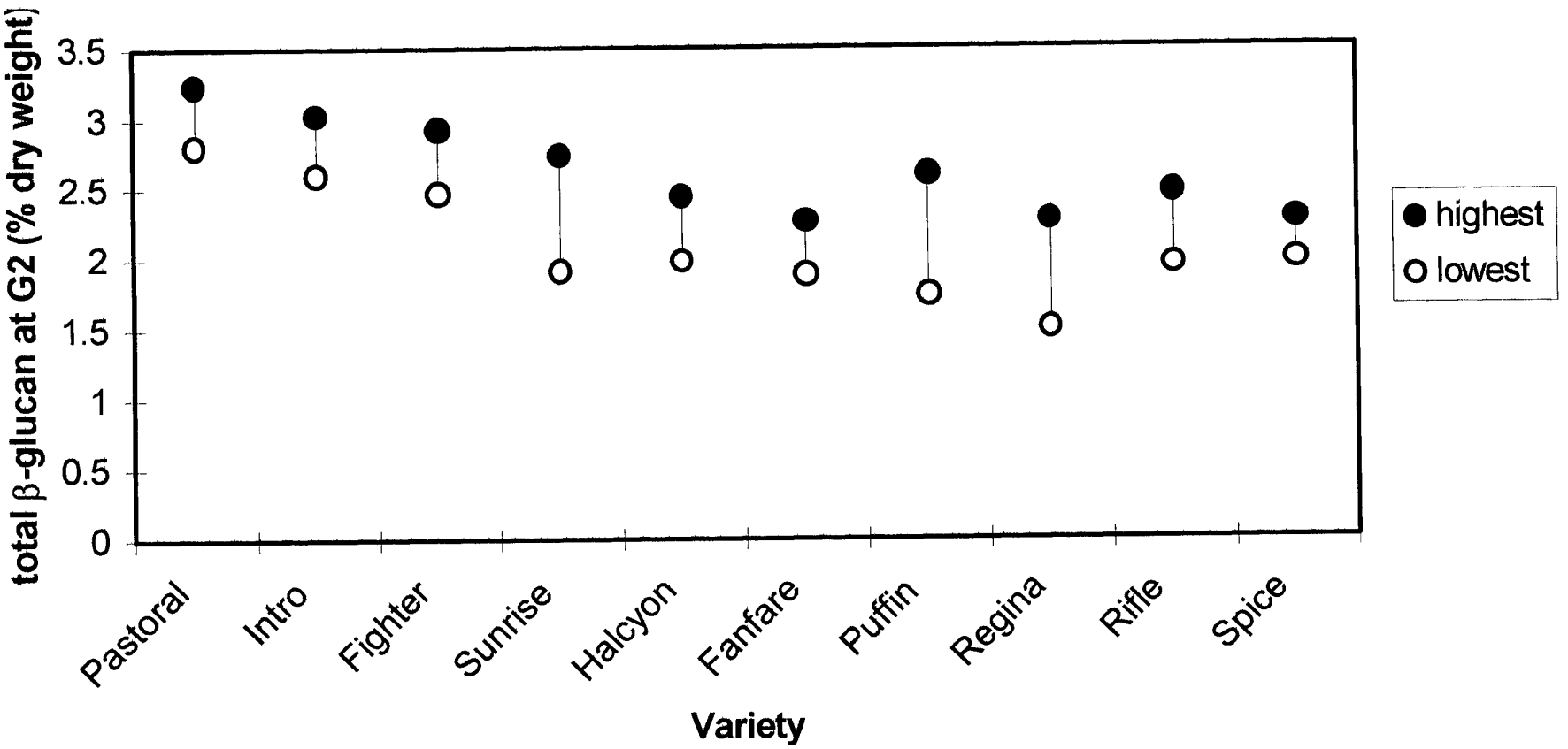


Figure 7d: Comparison of total beta-glucan levels at G2 for two separate maltings (Haugley site)

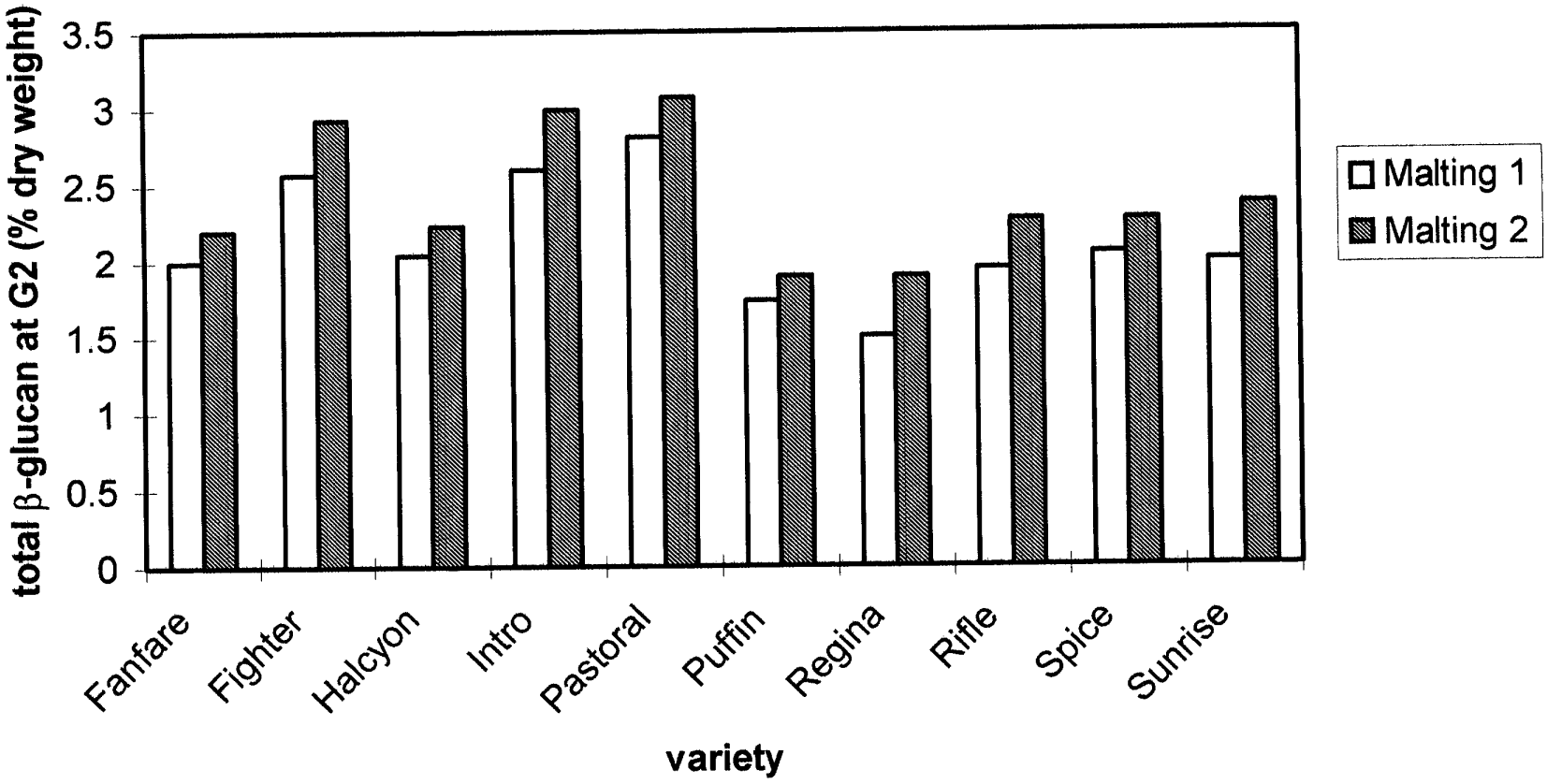


Figure 8a: Variation of the average total beta-glucan at G2 with malting grade for barleys grown at all four sites

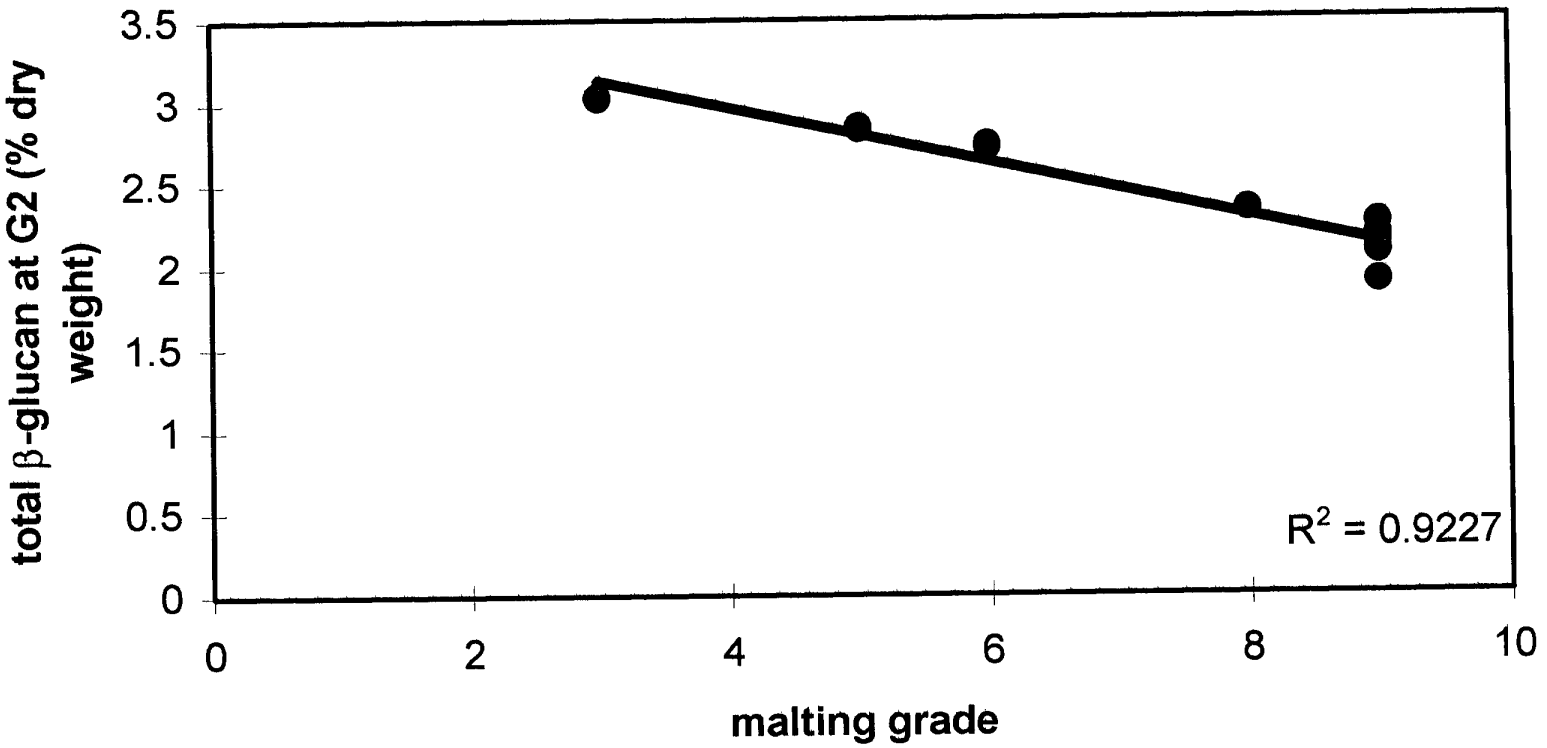


Figure 8b: Variation of the average total beta-glucan at G2 with malting grade for barleys grown at all four sites, and for Chariot and Epic

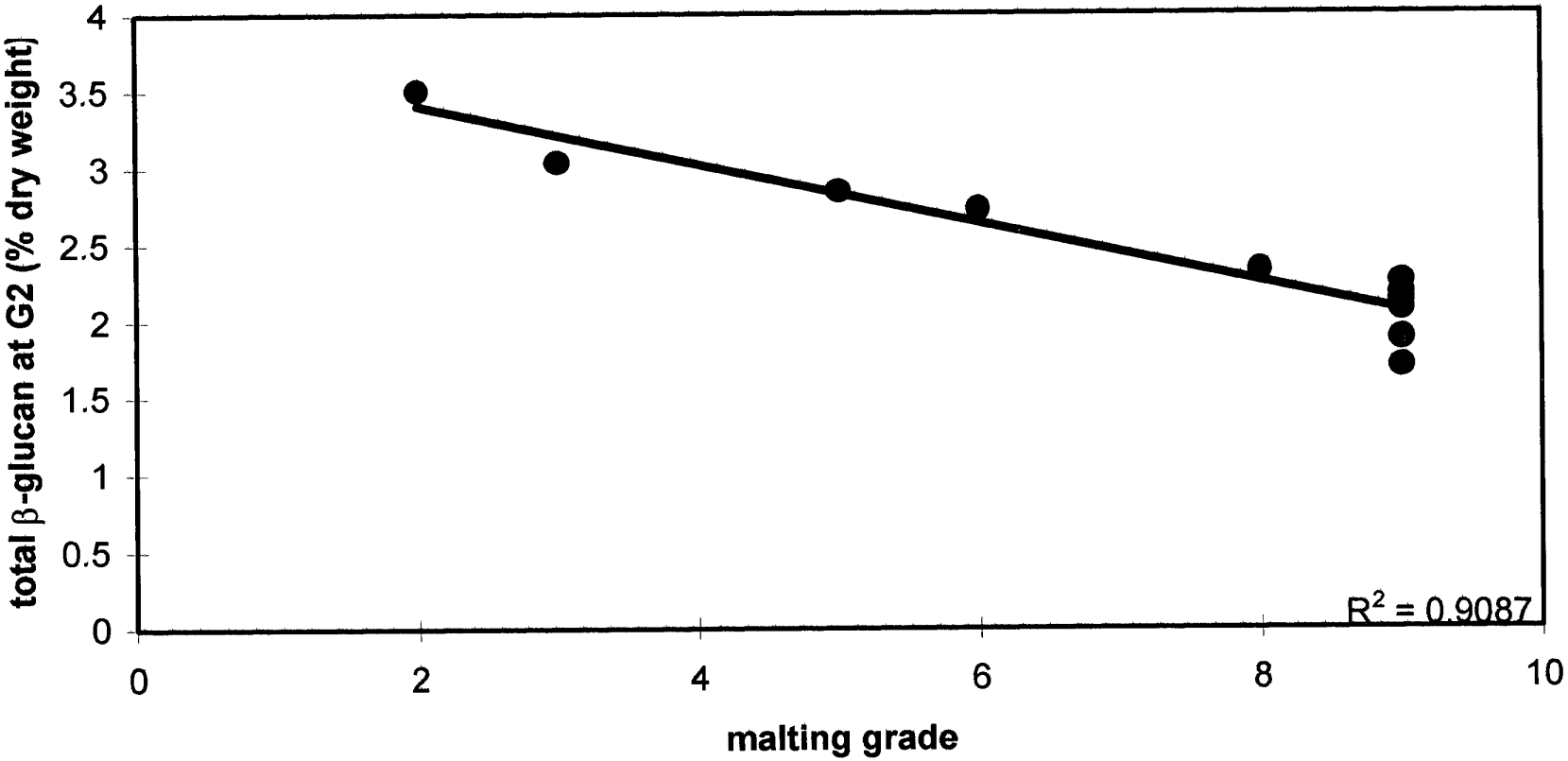


Figure 8c: Beta-glucan at G2 verses malting grade for malts prepared on 5g scale

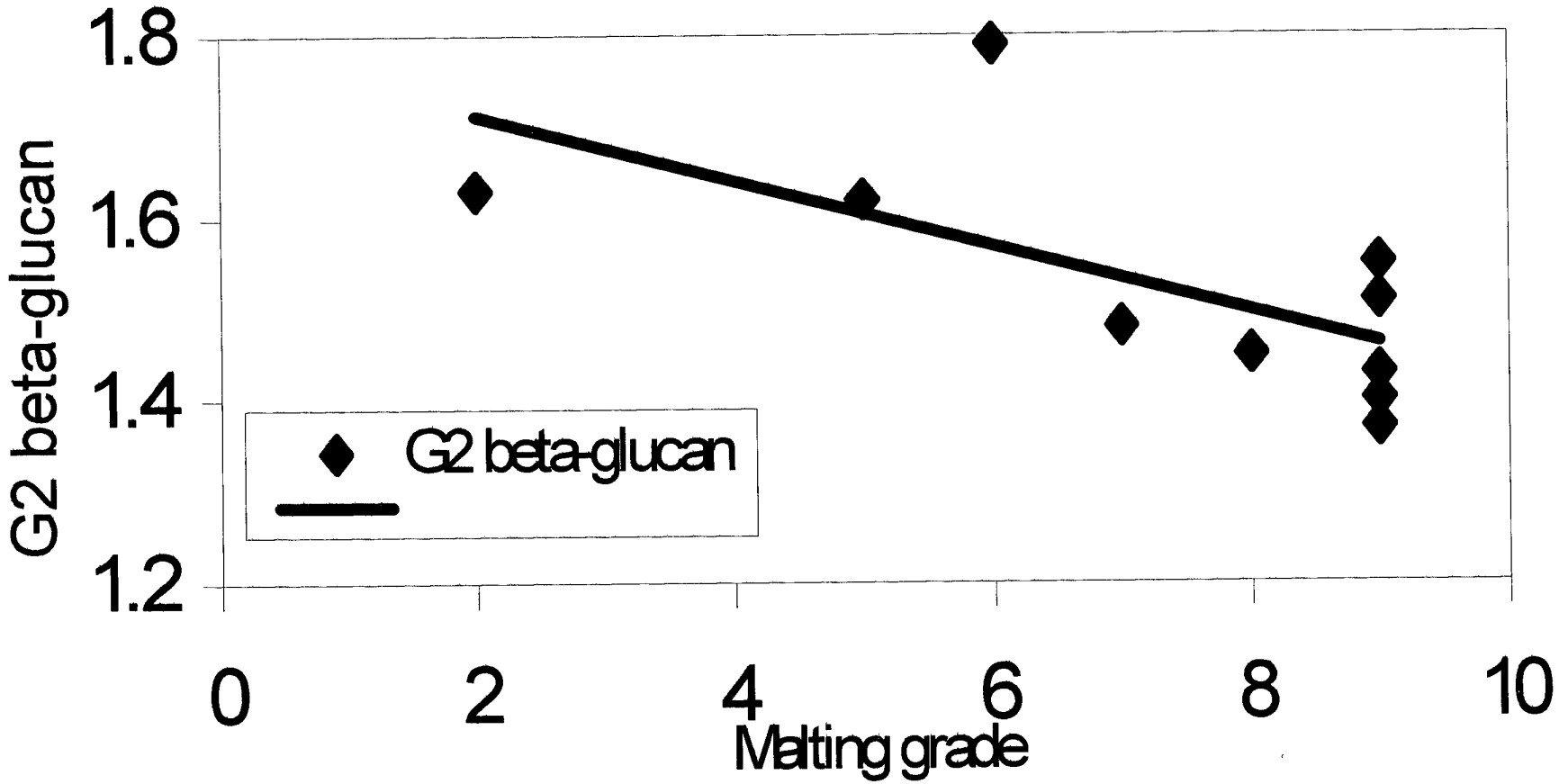


Figure 9a: Relationship between viscosity and beta-glucan at G2 for samples grown at Navenby

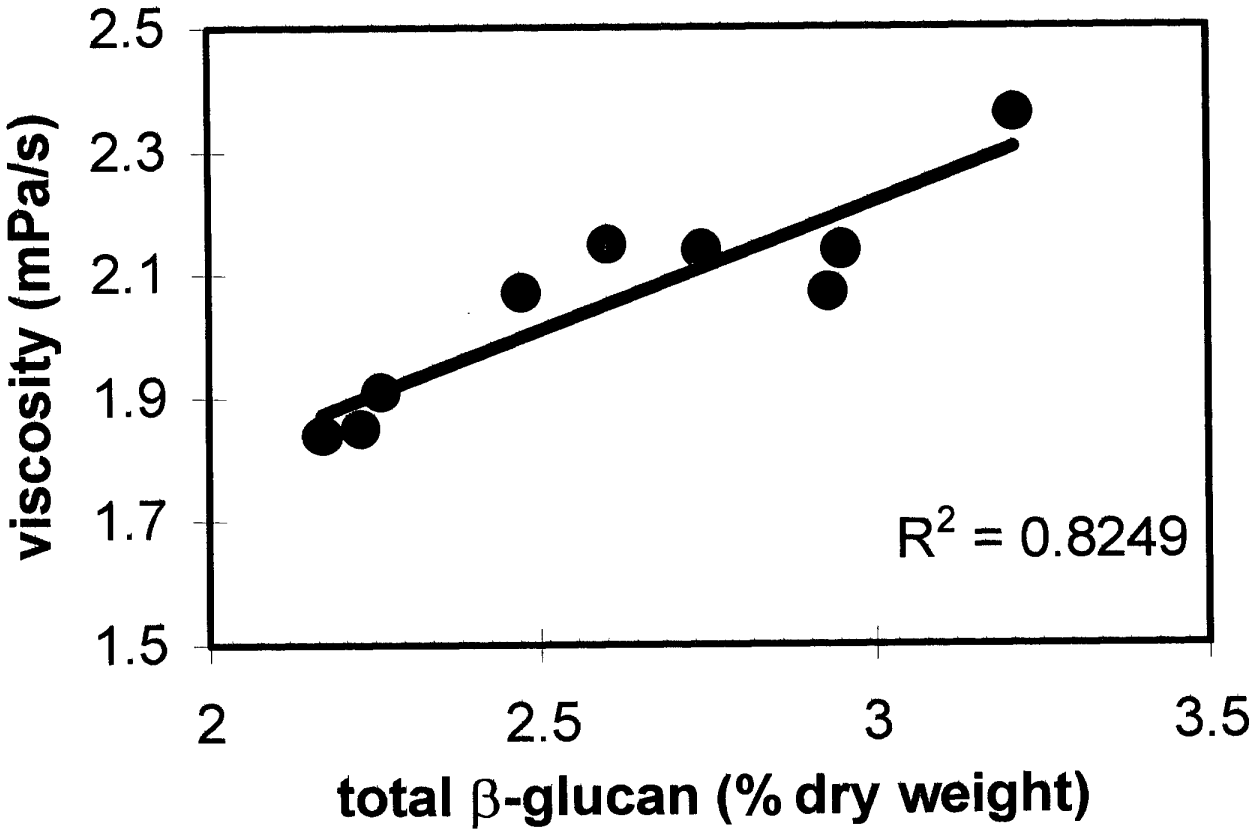


Figure 9b: Variation in viscosity at G2 with malting grade for samples grown at Navenby

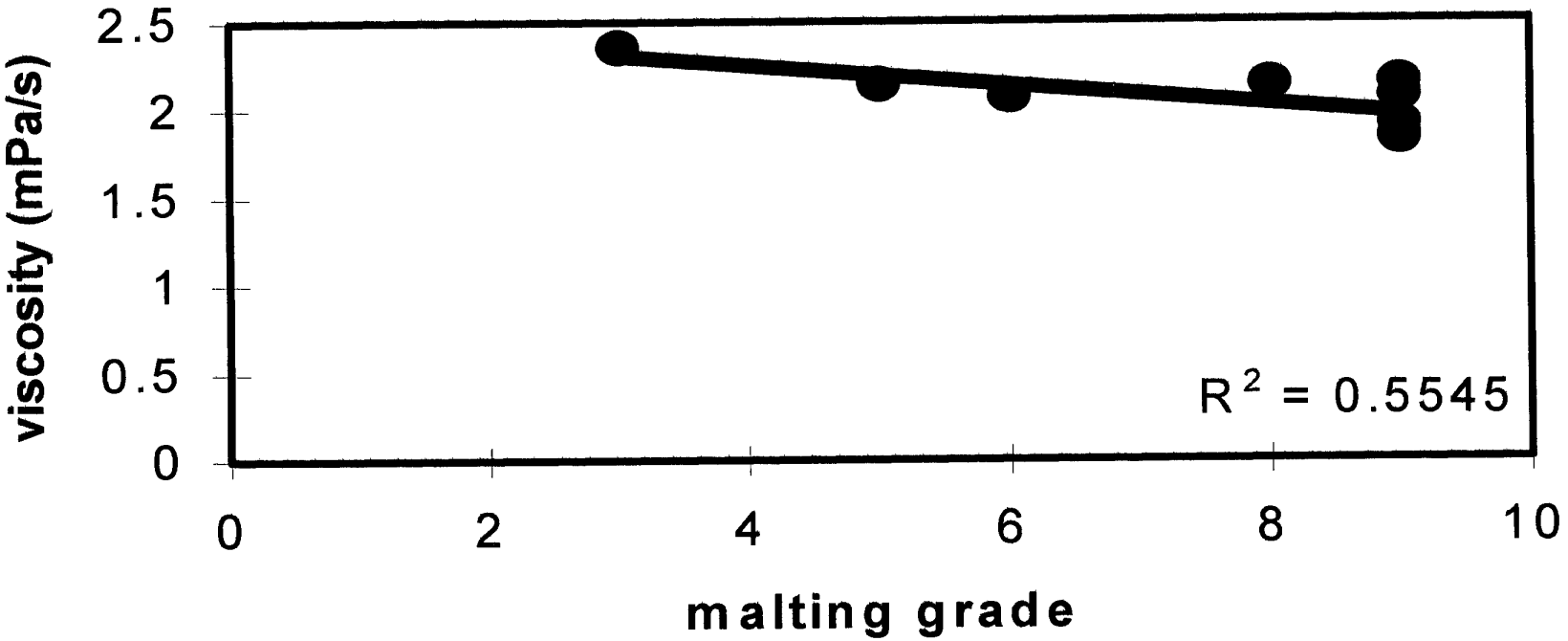


Figure 9c: Variation in malt viscosity with malting grade for samples grown at Navenby

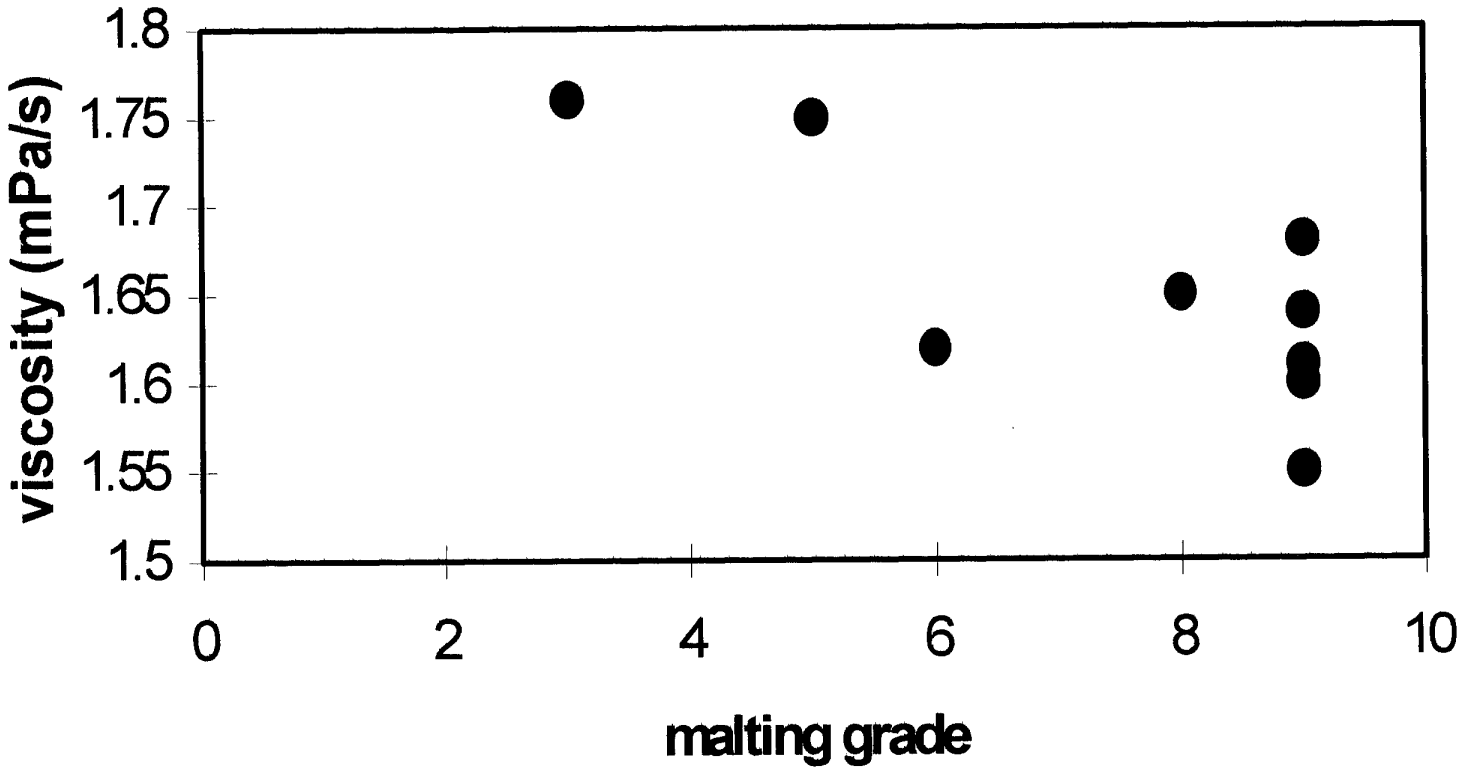


Figure 10a: Site-to-site variation in malt HWE for Haughley, Navenby and Rothwell

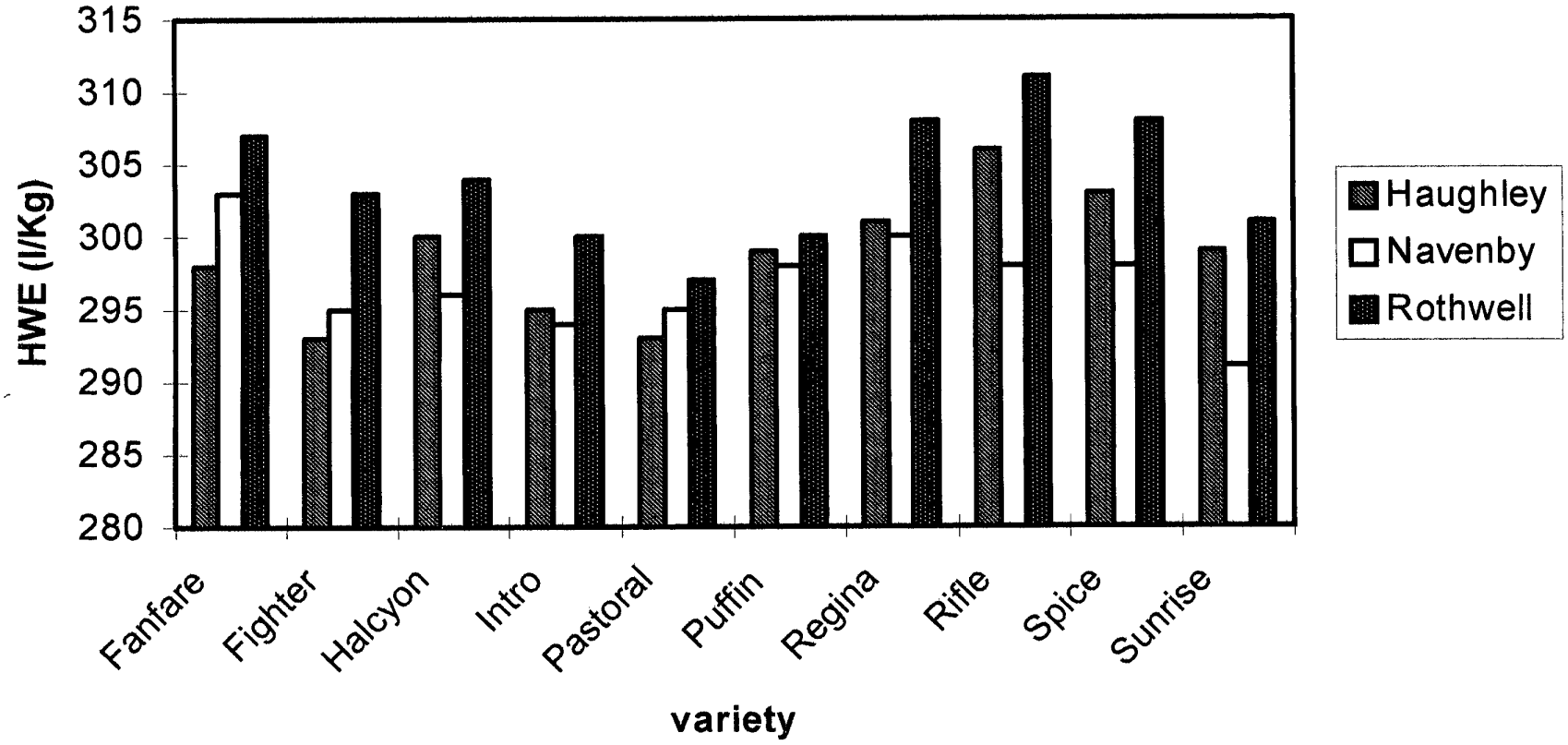


Figure 10b: Variation in beta-glucan content with malt HWE, averaged over Haughley, Navenby and Rothwell sites.

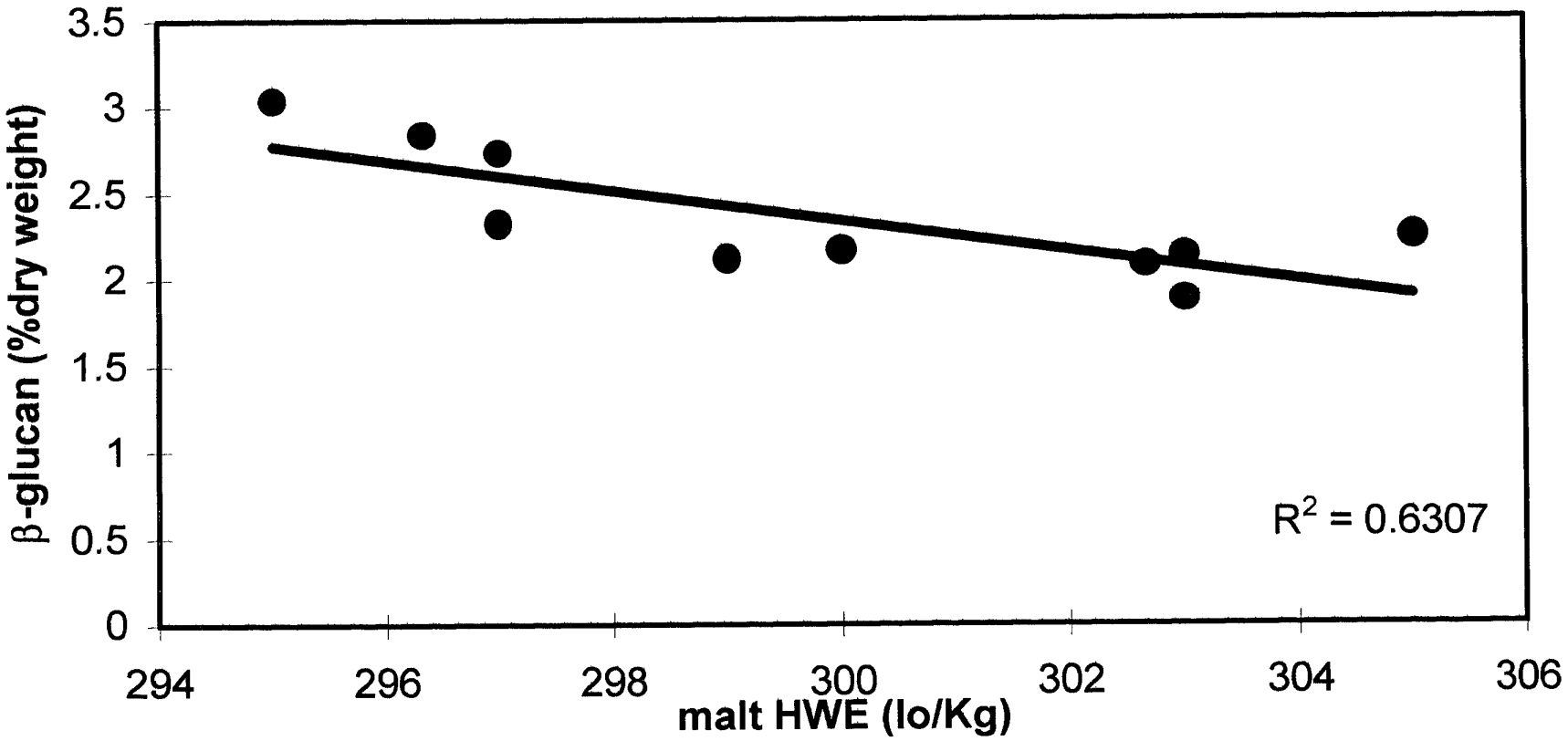


Figure 10c: Effect of malting regime on HWE

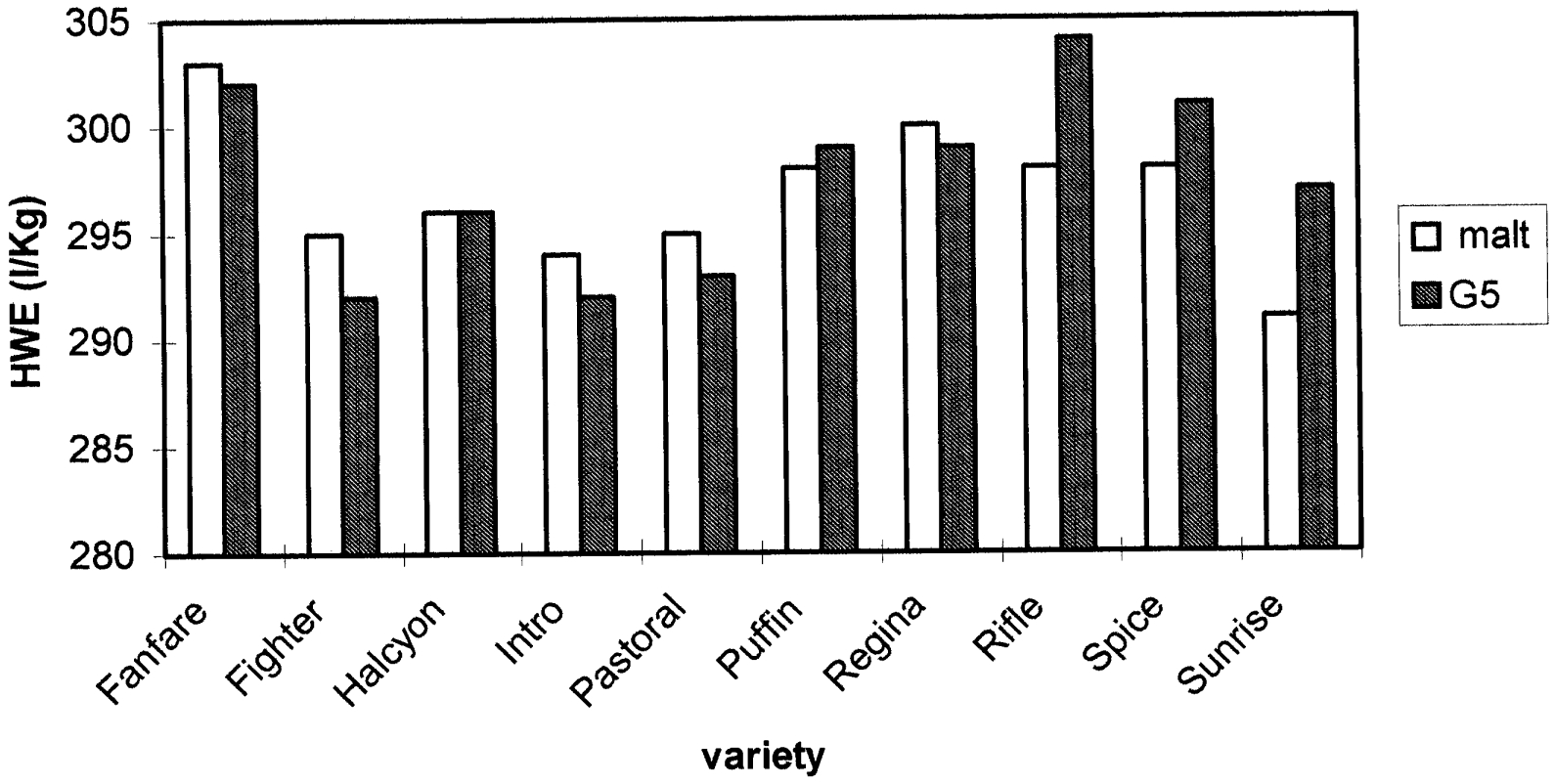


Figure 11a: Site-to-site variation in alpha-amylase activity at G2 for samples grown at Navenby and Rothwell

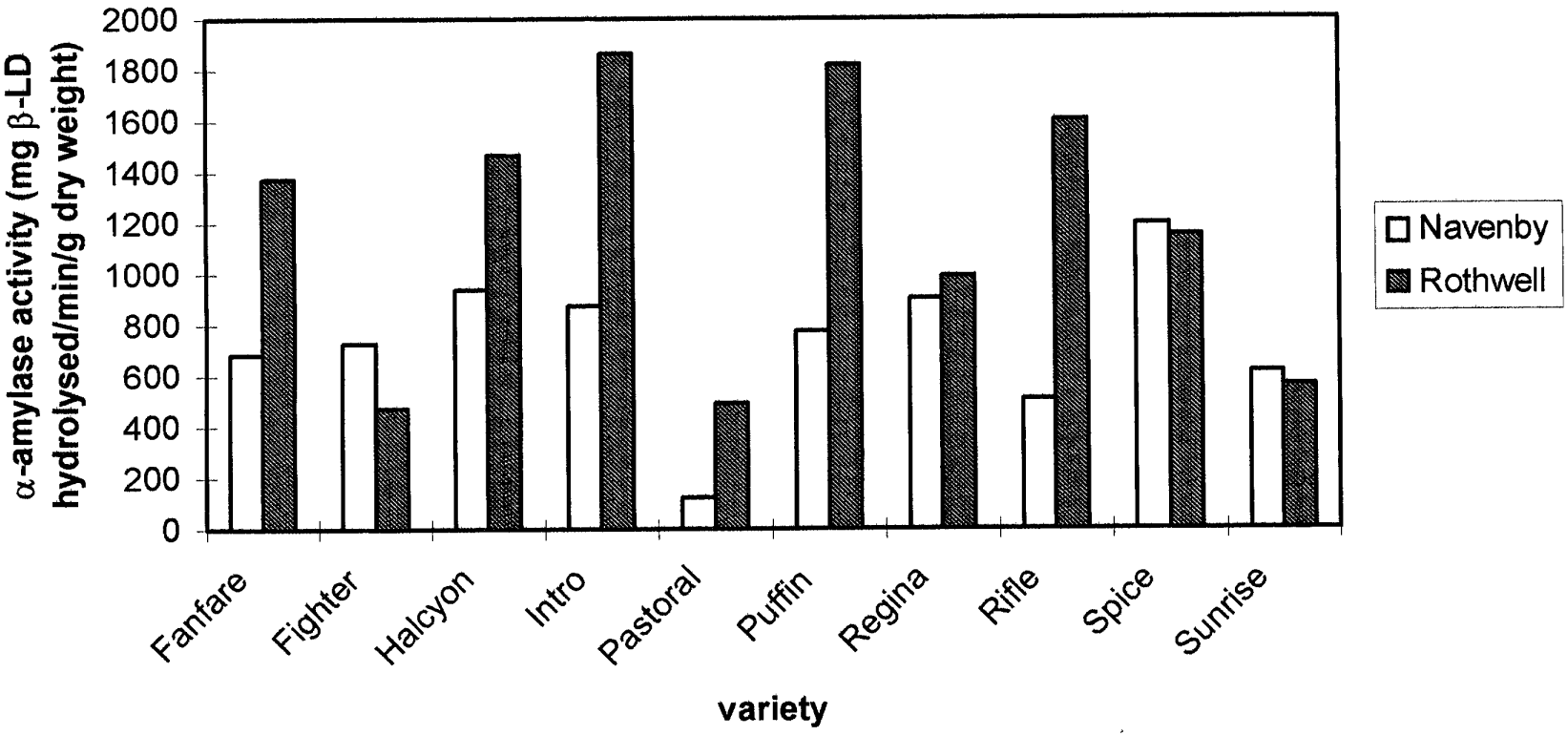


Figure 11b: Variation in alpha-amylase activity at G2 with malting grade for samples grown at Navenby and Rothwell

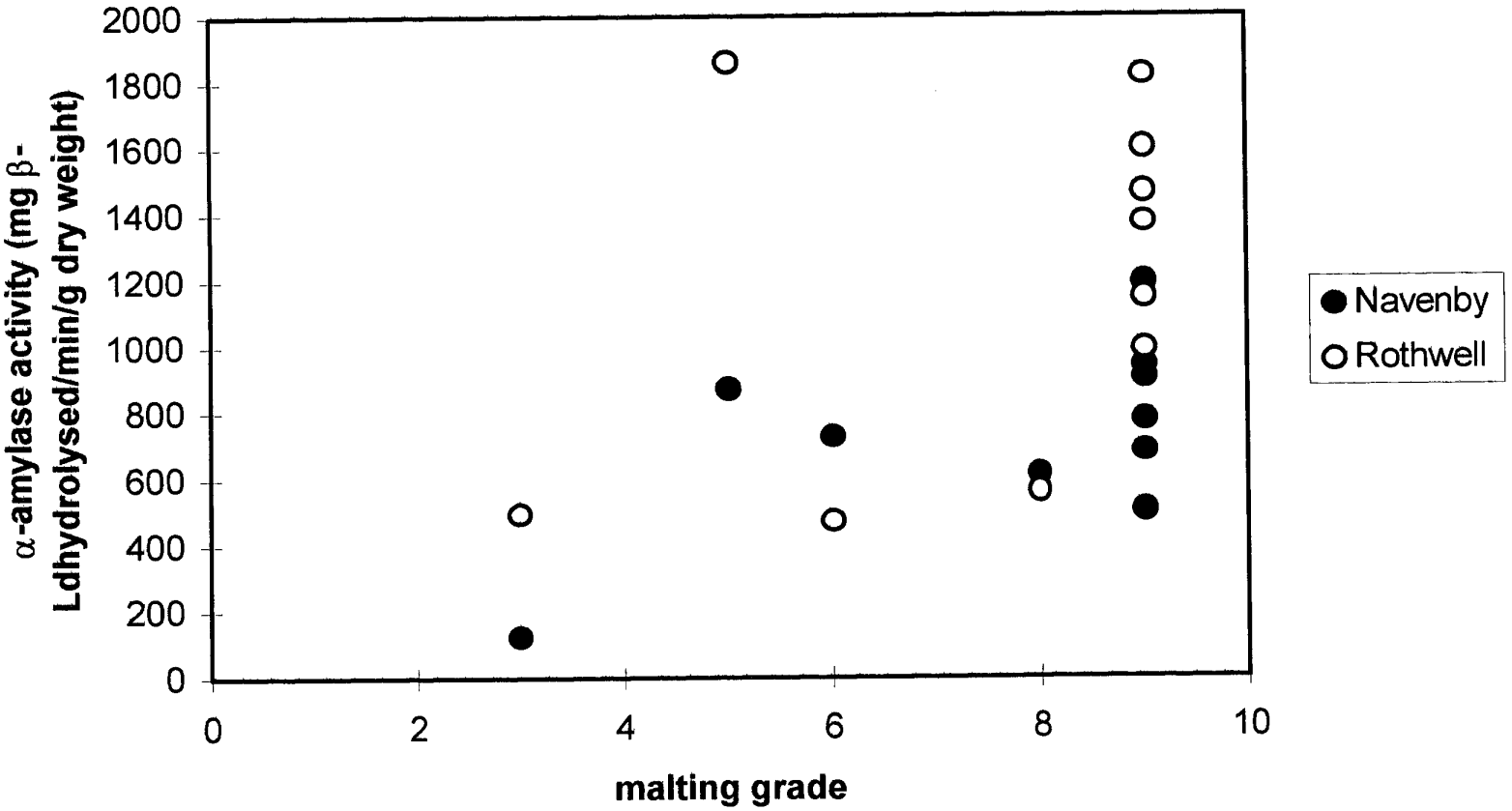


Figure 12a: Site-to-site variation in beta-glucanase activity at G2 for samples grown at Rothwell and Navenby

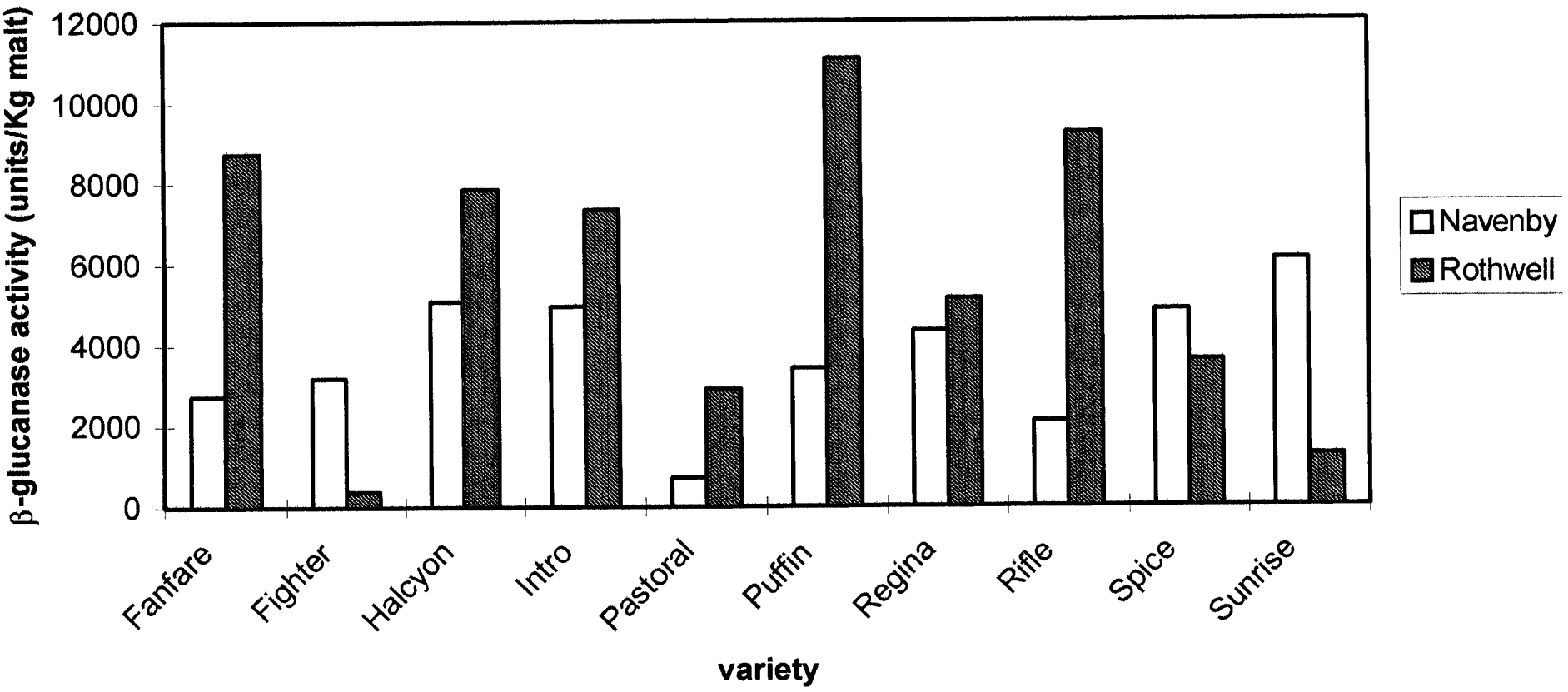


Figure 12b: Variation in beta-glucanase activity at G2 with malting grade for samples grown at Navenby and Rothwell

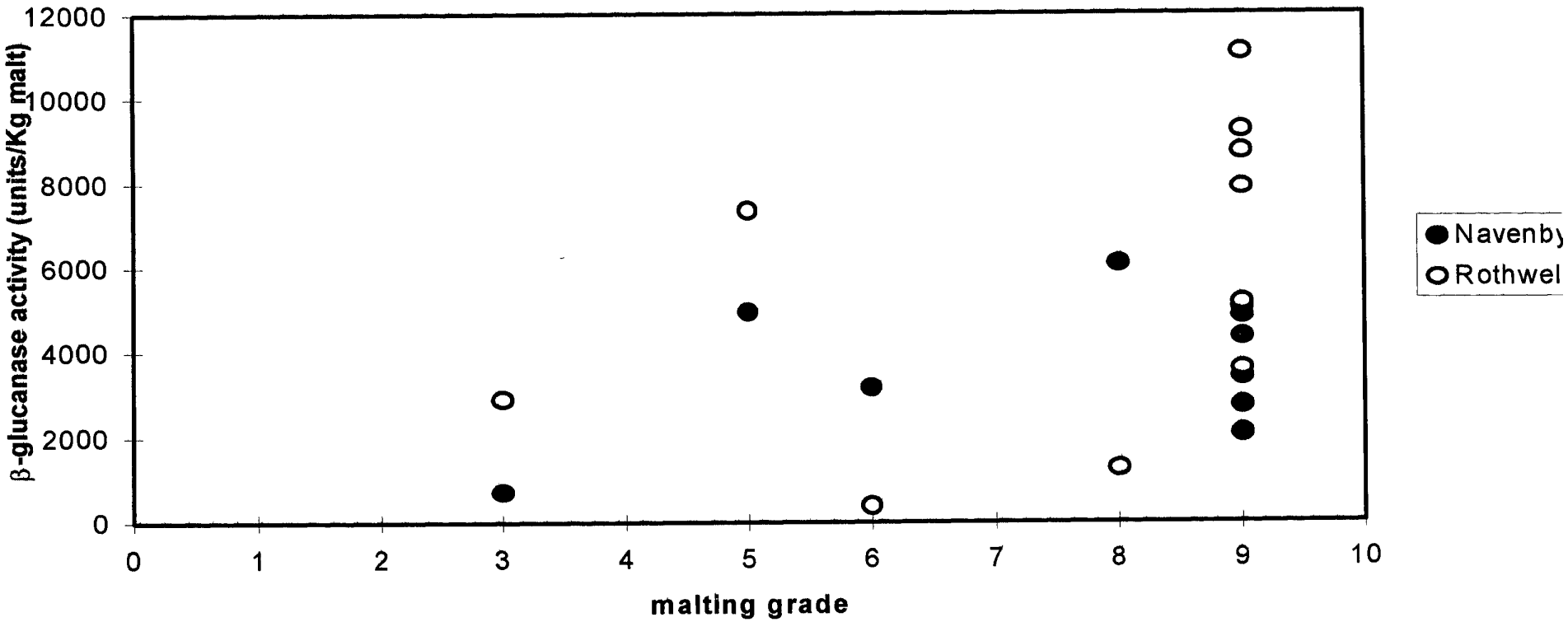


Figure 13a: Correlation between beta-glucanase activity and alpha-amlyase activity for samples grown at Rothwell

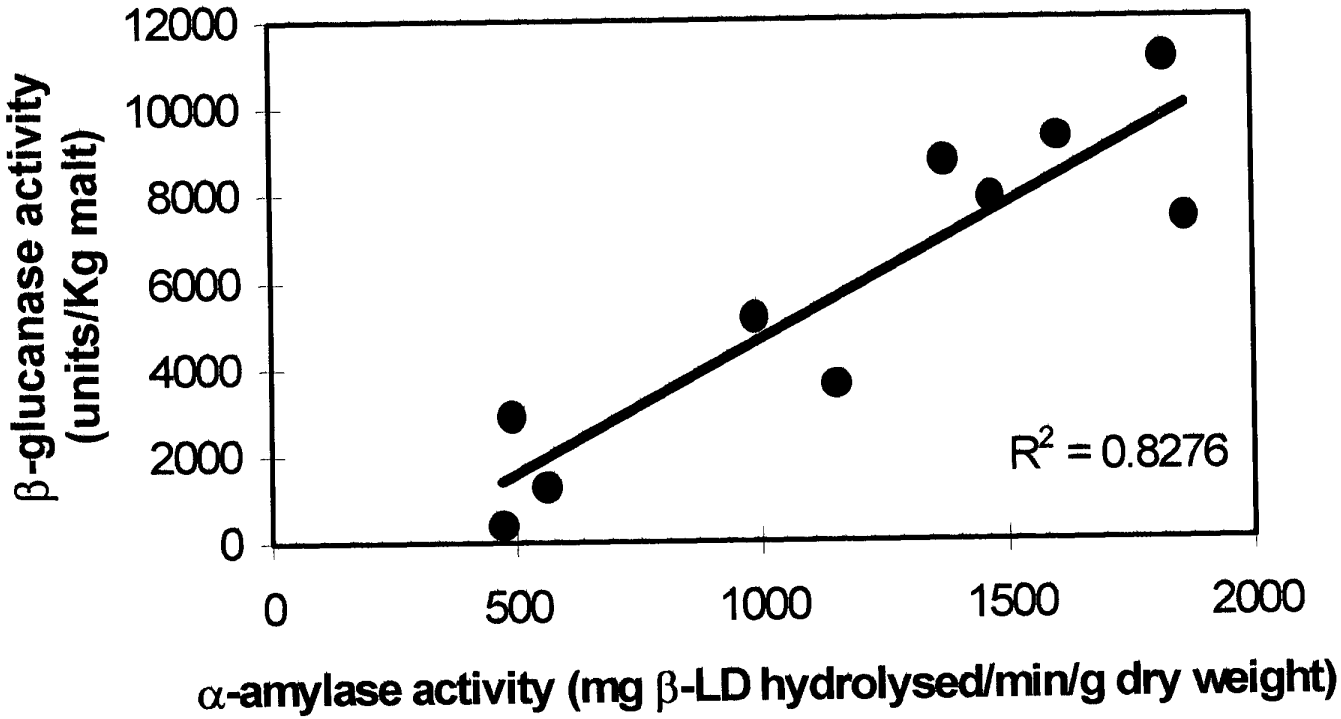


Figure 13b: Correlation between beta-glucanase activity and alpha-amylase activity for samples grown at Navenby

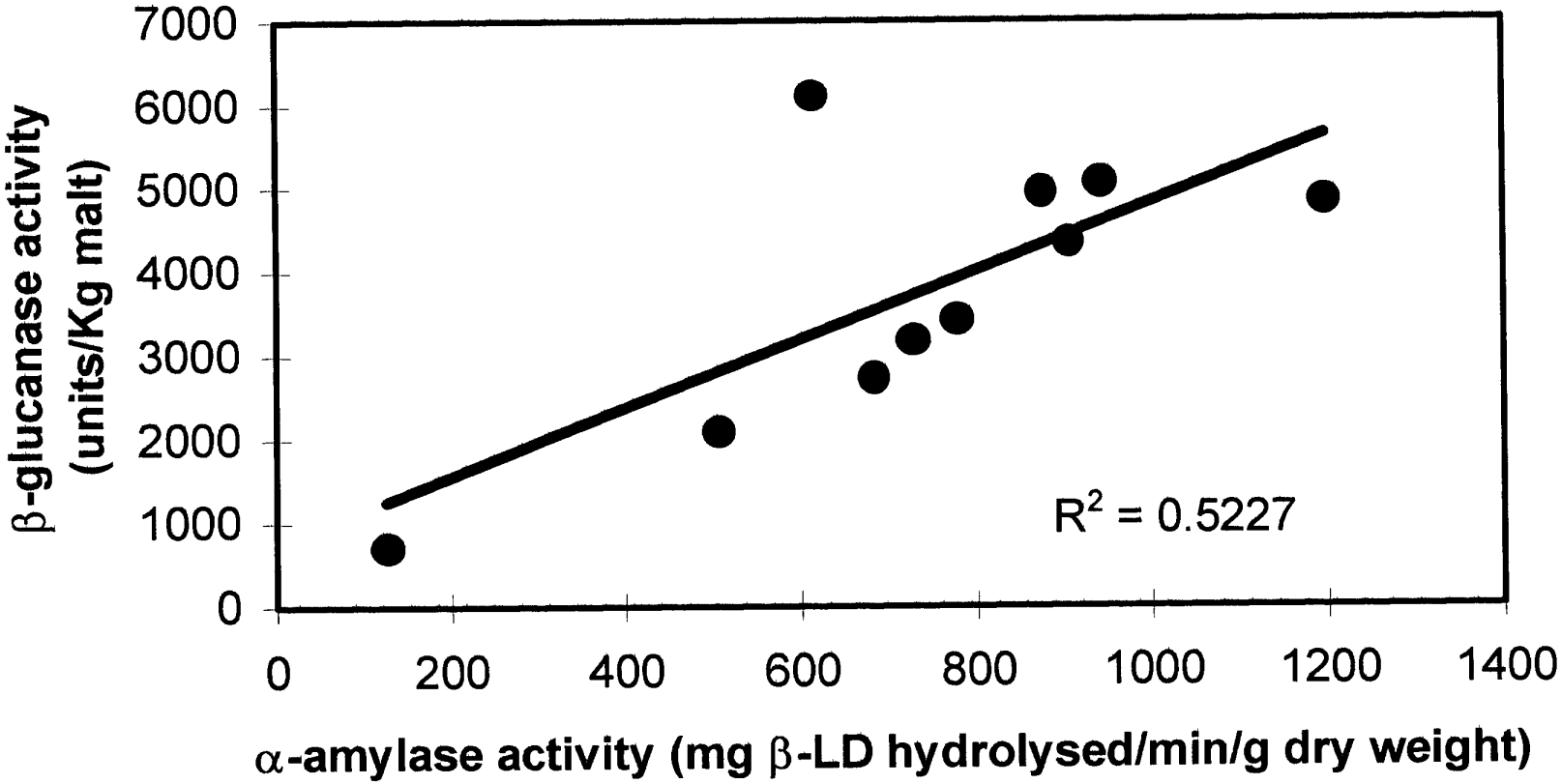


Figure 14: Miniaturised method for the measurement of total β-glucan

1. ca 0.06g milled barley (or G2) into a 2 ml microcentrifuge tube
2. Add 0.125 ml 50% Ethanol + 0.63 ml 20mM Sodium Phosphate buffer.
3. Boil for 2 min mixing every 45 s
4. Add 0.63 ml chilled water and 25 μl lichenase. Incubate 1h at 40C.
5. Spin at 13,000 x *g* for 1 min to remove solids.
6. Dilute 0.2ml supernatant with 0.34 ml 50 mM sodium acetate buffer
7. Pipette 10 μl diluted supernatant into wells of microtitre plate and add either 10 μl β-glucosidase or the same volume of acetate buffer (blank)
8. Cover microtitre plate and incubate at 40 °C for 15 min.
9. Add 200 μl Trinder glucose assay reagent (Sigma). Incubate 20 min and read absorbance at 490 nm.

All buffers and enzymes are made up according to the standard McCleary method.

Appendix 1 - Barley Analysis

SITE: Haughley, Suffolk

Bri No.	Malting Grade	Barley Variety	Total β-glucan (%)	Soluble β-glucan (%)	Insoluble β-glucan (%)	Moisture (%)
97/11	9	Fanfare	1.85	0.87	0.98	13.5
97/12	6	Fighter	3.01	0.97	2.04	13.4
97/13	8	Halcyon	2.60	0.81	1.79	13.7
97/14	5	Intro	2.90	0.96	1.94	13.4
97/15	2	Pastoral	2.61	0.85	1.76	13.4
97/16	9	Puffin	1.88	0.70	1.18	13.5
97/17	9	Regina	2.14	1.27	0.87	13.1
97/18	9	Rifle	2.06	0.87	1.19	13.3
97/19	9*	Spice	2.22	1.07	1.15	13.3
97/20	7	Sunrise	1.75	0.78	0.97	13.2
		Control (4.4%)	3.60	-	-	10.4

SITE: Navenby, S. Lincoln

Bri No.	Malting Grade	Barley Variety	Total β-glucan (%)	Soluble β-glucan (%)	Insoluble β-glucan (%)	Moisture (%)
97/21	9	Fanfare	1.59	0.86	0.73	13.6
97/22	6	Fighter	2.36	1.17	1.19	13.6
97/23	8	Halcyon	2.15	1.18	0.97	13.3
97/24	5	Intro	2.29	1.49	0.80	13.4
97/25	2	Pastoral	2.05	0.97	1.08	13.4
97/26	9	Puffin	1.85	1.05	0.80	13.3
97/27	9	Regina	2.41	1.04	1.37	13.4
97/28	9	Rifle	1.86	0.99	0.87	13.5
97/29	9*	Spice	2.01	0.95	1.06	13.6
97/30	7	Sunrise	2.63	1.10	1.53	13.6
97/20		Control (1.75%, 0.78%)	1.77	0.90	0.87	13.2

SITE: Rothwell, Lincoln

Bri No.	Malting Grade	Barley Variety	Total β-glucan (%)	Soluble β-glucan (%)	Insoluble β-glucan (%)	Moisture (%)
97/31	9	Fanfare	2.60	1.05	1.55	13.4
97/32	6	Fighter	3.01	1.67	1.34	13.1
97/33	8	Halcyon	2.69	1.48	1.21	13.3
97/34	5	Intro	3.23	1.53	1.70	13.0
97/35	2	Pastoral	3.05	1.74	1.31	13.4
97/36	9	Puffin	3.10	1.28	1.82	13.3
97/37	9	Regina	2.80	1.49	1.31	13.2
97/38	9	Rifle	2.65	1.35	1.30	13.1
97/39	9*	Spice	2.41	1.23	1.18	13.4
97/40	7	Sunrise	2.63	1.35	1.28	13.4
97/20		Control (1.75%, 0.78%)	2.33	1.13	1.20	13.2

SITE: Wooton, N. E. Lincolnshire

Bri No.	Malting Grade	Barley Variety	Total β-glucan (%)	Soluble β-glucan (%)	Insoluble β-glucan (%)	Moisture (%)
97/41	9	Fanfare	2.20	0.87	1.33	13.5
97/42	6	Fighter	2.24	1.21	1.03	13.4
97/43	8	Halcyon	2.28	1.19	1.09	13.2
97/44	5	Intro	3.00	1.48	1.52	13.2
97/45	2	Pastoral	2.77	1.20	1.57	13.0
97/46	9	Puffin	2.32	1.06	1.26	13.2
97/47	9	Regina	2.70	1.21	1.49	13.0
97/48	9	Rifle	2.47	0.92	1.55	13.5
97/49	9*	Spice	2.42	0.95	1.47	13.6
97/50	7	Sunrise	2.24	1.07	1.17	13.4
97/20		Control (1.75%, 0.78%)	2.38	0.62	1.76	13.2

SITE: Woolpit, Suffolk

Bri No.	Malting Grade	Barley Variety	Total β-glucan (%)	Soluble β-glucan (%)	Insoluble β-glucan (%)	Moisture (%)
97/51	9	Fanfare	2.04	0.72	1.32	12.8
97/52	6	Fighter	2.84	1.05	1.79	12.8
97/53	8	Halcyon	2.50	1.01	1.49	13.0
97/54	5	Intro	3.09	1.27	1.82	13.1
97/55	2	Pastoral	2.26	0.98	1.28	12.8
97/56	9	Puffin	2.09	0.83	1.26	12.8
97/57	9	Regina	1.76	0.99	0.77	12.8
97/58	9	Rifle	1.49	0.81	0.68	12.9
97/59	9*	Spice	1.69	0.92	0.77	13.0
97/60	7	Sunrise	1.21	0.79	0.42	12.8
97/20		Control (1.75%, 0.78%)	1.46	0.60	0.86	13.2

SITE: Haughley, Suffolk

Bri No.	Total nitrogen (%)	Germinative Energy (%)	Germinative Capacity (%)	Water Sensitivity (%)	Sieve Analysis >2.8	2.5-2.8	2.2-2.5	<2.2
97/11	1.80	92	99	21	42.4	44.8	9.8	3.0
97/12	1.95	95	99	42	58.6	32.4	6.2	2.8
97/13	1.85	99	99	40	31.5	44.7	18.4	5.4
97/14	1.80	97	96	43	81.8	13.4	2.5	2.3
97/15	1.80	92	100	51	43.9	45.0	8.1	3.0
97/16	1.88	99	98	37	55.2	36.7	5.7	2.4
97/17	1.74	98	98	50	57.7	32.9	7.4	2.0
97/18	1.78	96	98	66	50.9	37.0	9.0	3.1
97/19	1.59	98	99	52	32.8	46.6	15.6	5.0
97/20	1.79	94	98	48	44.7	43.0	9.1	3.2
	-	-	-	-	-	-	-	-

SITE: Navenby, S. Lincoln

Bri No.	Total nitrogen (%)	Germinative Energy (%)	Germinative Capacity (%)	Water Sensitivity (%)	Sieve Analysis >2.8	2.5-2.8	2.2-2.5	<2.2
97/21	1.84	92	97	14	27.7	52.5	16.3	4.5
97/22	1.94	92	94	36	37.9	46.2	12.4	3.5
97/23	2.04	96	97	42	17.9	39.7	30.5	11.9
97/24	1.97	97	94	31	67.8	23.8	6.2	2.2
97/25	1.84	91	97	31	61.0	28.1	7.8	3.1
97/26	1.98	98	98	24	50.6	36.6	9.9	2.9
97/27	1.81	93	97	41	68.9	24.3	5.3	1.5
97/28	1.89	96	98	44	62.2	30.1	5.6	2.1
97/29	1.81	96	97	42	17.7	47.4	27.3	7.6
97/30	1.91	93	98	19	42.8	45.0	9.5	2.7
97/20	1.79							

SITE: Rothwell, Lincoln

Bri No.	Total nitrogen (%)	Germinative Energy (%)	Germinative Capacity (%)	Water Sensitivity (%)	Sieve Analysis >2.8	2.5-2.8	2.2-2.5	<2.2
97/31	1.72	98	99	31	79.4	13.8	4.2	2.6
97/32	1.76	96	98	47	85.8	10.7	2.2	1.3
97/33	1.88	97	99	37	61.3	29.2	6.3	3.2
97/34	1.96	93	98	40	90.3	6.8	1.8	1.1
97/35	1.90	91	98	50	85.3	10.6	2.9	1.2
97/36	1.74				94.1	4.0	1.1	0.8
97/37	1.70				86.1	8.6	3.2	2.1
97/38	1.75				84.5	10.9	3.3	1.3
97/39	1.60				80.7	13.3	3.5	2.5
97/40	1.79				78.1	15.1	4.8	2.0
97/20	1.79							

SITE: Wooton, N. E. Lincolnshire

Bri No.	Total nitrogen (%)	Germinative Energy (%)	Germinative Capacity (%)	Water Sensitivity (%)	Sieve Analysis >2.8	2.5-2.8	2.2-2.5	<2.2
97/41	2.11				43.2	29.7	16.8	10.3
97/42	1.98				63.4	30.7	4.3	1.6
97/43	2.25				32.8	30.1	21.7	15.4
97/44	2.12				82.1	14.1	3.0	0.8
97/45	2.08				69.4	22.0	5.3	3.3
97/46	2.16				59.2	28.1	9.5	3.2
97/47	2.06				78.3	16.4	3.8	1.5
97/48	2.11				71.3	23.0	4.1	1.6
97/49	2.05				39.2	33.4	16.7	10.7
97/50	2.10				45.6	38.7	11.9	3.8
97/20	1.79							

SITE: Woolpit, Suffolk

Bri No.	Total nitrogen (%)	Germinative Energy (%)	Germinative Capacity (%)	Water Sensitivity (%)	Sieve Analysis >2.8	2.5-2.8	2.2-2.5	<2.2
97/51	1.62				45.0	42.4	8.8	3.8
97/52	2.06				23.9	55.4	17.2	3.5
97/53	1.93				20.8	45.1	24.4	9.7
97/54	1.61				84.0	11.2	2.0	2.8
97/55	1.76				52.0	29.5	6.0	2.5
97/56	1.92				42.0	40.7	13.1	4.2
97/57	1.75				68.6	25.6	4.3	1.5
97/58	1.65				60.3	31.6	6.0	2.1
97/59	1.63				34.2	43.4	15.3	7.1
97/60	1.93				38.0	42.9	13.0	6.1
97/20	1.79							

Appendix 2 - Whole data sheet of different 14 samples

				TOTAL β-glucan(%)				SOLIBLE β-glucan(%)						germination			
			Malting	Barley		CAST	G2	Barley		CAST	G2						
our no.	bri no.	Barley variety	Grade	Kamini	Ave	our ave	our ave	Kamini	Ave	our ave	our ave	moisture G2	(%)	Cast	G1	G2	G5
1	97/17	Regina	9	2.37	3.56	3.45	1.51	1.32	0.85	0.43	0.44	45.6	1.74	60	85	97	97
2	97/27	Regina	9	2.64	3.54	3.23	2.27	1.08	0.93	0.43	0.57	46.1	1.81	34	64	76	97
3	97/31	Fanfare	9	2.16	3.47	3.11	1.89	0.75	0.64	0.48	0.44	45.0	1.72	6	72	96	99
4	97/32	Fighter	6	2.51	4.07	3.47	2.47	1.2	0.83	0.69	0.71	44.3	1.76	30	85	98	98
5	97/33	Halcyon	8	2.24	3.85	3.72	2.44	1.06	0.81	0.58	0.63	45.1	1.88	26	22	94	97
6	97/34	Intoro	5	2.69	4.08	3.93	2.64	1.1	0.87	0.74	0.63	44.9	1.96	59	92	91	99
7	97/35	Pastoral	2	2.54	3.66	3.28	2.85	1.25	0.6	0.46	0.55	44.8	1.90	13	48	99	97
8	97/36	Puffin	9	2.58	3.6	2.90	2.03	0.92	0.68	0.55	0.44	44.9	1.74	27	90	97	96
9	97/37	Regina	9	2.33	3.55	3.05	1.77	1.07	0.96	0.65	0.50	44.9	1.70	49	78	100	97
10	97/38	Rifle	9	2.21	3.53	3.44	2.15	0.97	0.65	0.49	0.40	46.4	1.75	34	77	92	97
11	97/39	Spice	9	2.01	3.23	3.14	1.98	0.88	0.44	0.45	0.54	45.7	1.60	22	87	93	96
12	97/40	Sunrise	7	2.19	3.49	3.37	1.92	0.97	0.57	0.43	0.36	44.2	1.79	16	84	94	99
13	97/47	Regina	9	2.2	3.6	3.40	1.50	1.58	0.87	0.61	0.50	45.6	2.06	61	82	98	98
14	97/57	Regina	9	2.34	3.54	3.31	2.00	1.34	0.91	0.55	0.50	45.8	1.75	48	70	82	92
15		Epic	1		4.16	3.88	3.52		0.24	0.25	0.25						
		Chariot	9		3.51	3.35	1.75		0.50	0.37	0.37						

Correlation of 10 samples

				TOTAL β-glucan(%)				SOLIBLE β-glucan(%)						germination			
			Malting	Barley		CAST	G2	Barley		CAST	G2						
our no.	bri no.		Grade	Kamini	Ave	our ave	our ave	Kamini	Ave	our ave	our ave	moisture G2	(%)	Cast	G1	G2	G5
Malting	grade		1.00	-0.60	-0.50	-0.41	-0.85	-0.77	-0.04	-0.13	-0.43	0.41	-0.72	0.01	0.21	-0.14	-0.26
Total b-glucan	barley	Kamini	-0.60	1.00	0.75	0.26	0.62	0.62	0.52	0.64	0.42	-0.40	0.65	0.46	0.14	0.19	0.12
		ave	-0.50	0.75	1.00	0.70	0.69	0.66	0.70	0.81	0.74	-0.40	0.72	0.49	-0.12	-0.12	0.37
	Cast	ave	-0.41	0.26	0.70	1.00	0.63	0.43	0.34	0.46	0.53	-0.01	0.74	0.43	-0.26	-0.64	0.46
	G2	ave	-0.85	0.62	0.69	0.63	1.00	0.76	0.15	0.31	0.66	-0.13	0.79	0.12	-0.39	-0.11	0.07
Solible b-glucan	barley	Kamini	-0.77	0.62	0.66	0.43	0.76	1.00	0.44	0.46	0.62	-0.33	0.59	0.35	-0.27	0.25	-0.03
		ave	-0.04	0.52	0.70	0.34	0.15	0.44	1.00	0.88	0.47	-0.27	0.38	0.71	-0.08	0.22	0.21
	Cast	ave	-0.13	0.64	0.81	0.46	0.31	0.46	0.88	1.00	0.68	-0.24	0.37	0.81	0.15	-0.03	0.19
	G2	ave	-0.43	0.42	0.74	0.53	0.66	0.62	0.47	0.68	1.00	-0.25	0.37	0.33	-0.24	-0.04	0.01
Total / Solible b-glucan	barley	Kamini	-0.48	0.00	0.25	0.35	0.46	0.78	0.16	0.09	0.45	-0.09	0.23	0.11	-0.44	0.15	-0.16
		ave	0.15	0.35	0.45	0.12	-0.09	0.29	0.95	0.75	0.25	-0.18	0.18	0.67	-0.07	0.35	0.11
	Cast	ave	0.11	0.56	0.50	-0.06	-0.03	0.27	0.81	0.85	0.45	-0.26	-0.01	0.67	0.29	0.35	-0.07
	G2	ave	0.23	-0.05	0.25	0.03	-0.10	0.11	0.46	0.59	0.68	-0.17	-0.30	0.33	0.03	0.15	-0.13
□¢S/T b-glucan	barley	G2-Kamini	0.57	-0.03	-0.04	-0.28	-0.47	-0.60	0.18	0.33	0.08	-0.04	-0.40	0.13	0.40	-0.02	0.06
		G2 - ave	0.09	-0.37	-0.17	-0.08	-0.01	-0.15	-0.42	-0.11	0.43	-0.01	-0.46	-0.29	0.09	-0.17	-0.22
	Cast	ave	-0.09	0.17	-0.08	-0.28	0.11	-0.11	-0.48	-0.09	0.18	-0.06	-0.31	-0.20	0.50	-0.11	-0.28
	G2	ave	0.18	-0.60	-0.17	0.10	-0.09	-0.12	-0.20	-0.08	0.43	0.04	-0.37	-0.23	-0.25	-0.15	-0.09
G2 moisture (%)			0.41	-0.40	-0.40	-0.01	-0.13	-0.33	-0.27	-0.24	-0.25	1.00	-0.29	0.12	-0.04	-0.41	-0.47
T - N (%)			-0.72	0.65	0.72	0.74	0.79	0.59	0.38	0.37	0.37	-0.29	1.00	0.26	-0.40	-0.16	0.41
germination		cast	0.01	0.46	0.49	0.43	0.12	0.35	0.71	0.81	0.33	0.12	0.26	1.00	0.31	-0.22	0.01
		G1	0.21	0.14	-0.12	-0.26	-0.39	-0.27	-0.08	0.15	-0.24	-0.04	-0.40	0.31	1.00	-0.18	0.16
		germ.speed	0.07	0.39	0.27	0.22	-0.08	0.03	0.33	0.58	0.08	0.07	0.01	0.79	0.80	-0.38	0.19

Correlation of 15 samples

				TOTAL β-glucan(%)				SOLIBLE β-glucan(%)						germination			
			Malting	Barley		CAST	G2	Barley		CAST	G2						
our no.	bri no.		Grade	Kamini	Ave	our ave	our ave	Kamini	Ave	our ave	our ave	moisture G2	(%)	Cast	G1	G2	G5
Malting	grade		1.00	-0.46	-0.68	-0.56	-0.87	-0.16	0.53	0.28	0.12	0.52	-0.41	0.23	0.21	-0.23	-0.28
Total b-glucan	barley	Kamini	-0.46	1.00	0.62	0.18	0.54	0.19	0.48	0.34	0.43	-0.18	0.32	0.24	0.05	-0.25	0.03
		ave	-0.68	0.62	1.00	0.76	0.72	0.27	-0.05	0.20	0.18	-0.44	0.52	0.26	-0.11	0.09	0.30
	Cast	ave	-0.56	0.18	0.76	1.00	0.58	0.31	-0.14	0.00	0.05	-0.04	0.57	0.37	-0.20	-0.10	0.29
	G2	ave	-0.87	0.54	0.72	0.58	1.00	-0.15	-0.51	-0.28	-0.04	-0.25	0.23	-0.36	-0.42	-0.16	0.08
Solible b-glucan	barley	Kamini	-0.16	0.19	0.27	0.31	-0.15	1.00	0.55	0.25	0.22	0.13	0.61	0.67	-0.04	0.01	-0.24
		ave	0.53	0.48	-0.05	-0.14	-0.51	0.55	1.00	0.71	0.60	0.12	0.35	0.72	-0.07	-0.28	-0.15
	Cast	ave	0.28	0.34	0.20	0.00	-0.28	0.25	0.71	1.00	0.73	-0.32	0.38	0.47	0.14	0.20	0.12
	G2	ave	0.12	0.43	0.18	0.05	-0.04	0.22	0.60	0.73	1.00	-0.21	0.27	0.11	-0.29	-0.13	0.05
Total / Solible b-glucan	barley	Kamini	0.05	-0.21	0.01	0.23	-0.38	0.92	0.36	0.12	0.04	0.21	0.49	0.59	-0.04	0.11	-0.24
		ave	0.65	0.34	-0.25	-0.29	-0.64	0.53	0.98	0.61	0.49	0.26	0.23	0.71	-0.05	-0.34	-0.26
	Cast	ave	0.44	0.28	-0.05	-0.36	-0.44	0.13	0.70	0.93	0.63	-0.34	0.12	0.34	0.24	0.29	-0.02
	G2	ave	0.66	-0.12	-0.33	-0.30	-0.74	0.54	0.75	0.64	0.65	0.09	0.16	0.59	0.13	0.11	-0.04
□¢S/T b-glucan	barley	G2-Kamini	0.13	0.19	0.05	-0.23	0.21	-0.83	-0.15	0.05	0.17	-0.21	-0.52	-0.40	0.13	-0.07	0.28
		G2 - ave	0.12	-0.46	-0.15	-0.07	-0.25	0.04	-0.20	0.14	0.31	-0.17	-0.07	-0.09	0.19	0.45	0.23
	Cast	ave	-0.47	-0.15	0.29	0.07	0.46	-0.44	-0.68	0.01	-0.10	-0.50	-0.15	-0.47	0.22	0.54	0.25
	G2	ave	0.53	-0.31	-0.40	-0.12	-0.64	0.47	0.45	0.12	0.36	0.32	0.08	0.38	-0.03	-0.08	-0.02
G2 moisture (%)			0.52	-0.18	-0.44	-0.04	-0.25	0.13	0.12	-0.32	-0.21	1.00	-0.09	0.33	-0.05	-0.51	-0.45
T - N (%)			-0.41	0.32	0.52	0.57	0.23	0.61	0.35	0.38	0.27	-0.09	1.00	0.36	-0.24	0.05	0.32
germination		cast	0.23	0.24	0.26	0.37	-0.36	0.67	0.72	0.47	0.11	0.33	0.36	1.00	0.33	-0.09	-0.17
		G1	0.21	0.05	-0.11	-0.20	-0.42	-0.04	-0.07	0.14	-0.29	-0.05	-0.24	0.33	1.00	0.11	0.14
		germ.speed	0.24	0.29	0.13	0.21	-0.35	0.41	0.49	0.35	-0.02	0.29	0.14	0.84	0.71	-0.26	-0.12

Appendix 2 - V

our no.	bri no.	Barley variety	germination speed (N/D)	G2 total / barley total	barley total - G2 total	(barley - G2)/ barley	HWE(%)	Total cast-Total G2
1	97/17	Regina	40	0.42	2.06	0.58	301	1.95
2	97/27	Regina	37	0.64	0.33	0.41	300	0.97
3	97/31	Fanfare	31	0.54	0.24	0.34	307	1.23
4	97/32	Fighter	38	0.61	0.35	0.34	303	1.00
5	97/33	Halcyon	29	0.63	0.28	0.33	304	1.28
6	97/34	Intoro	43	0.65	0.28	0.33	300	1.29
7	97/35	Pastroal	30	0.78	0.38	0.21	297	0.44
8	97/36	Puffin	36	0.56	0.32	0.33	300	0.87
9	97/37	Regina	37	0.50	0.35	0.54	308	1.28
10	97/38	Rifle	36	0.61	0.28	0.30	311	1.30
11	97/39	Spice	35	0.61	0.28	0.22	308	1.17
12	97/40	Sunrise	34	0.55	0.29	0.30	301	1.45
13	97/47	Regina	39	0.42	0.47	0.58	299	1.90
14	97/57	Regina	39	0.56	0.41	0.46		1.31
15		Epic		0.85	0.00	0.07		0.36
		Chariot		0.50	0.00	0.29		1.60

Correlation of 10 s

our no.	bri no.		germination speed (N/D)	G2 total / barley total	barley total - G2 total	(barley - G2)/ barley	HWE	Total cast-Total G2
Malting grade			0.07	-0.81	-0.55	0.42	0.76	0.61
Total b-glucan	barley	Kamini	0.39	0.36	0.51	0.10	-0.70	-0.48
		ave	0.27	0.30	0.25	0.15	-0.46	-0.10
	Cast	ave	0.22	0.40	-0.19	-0.16	-0.18	0.29
	G2	ave	-0.08	0.90	0.39	-0.46	-0.61	-0.56
Solible b-glucan	barley	Kamini	0.03	0.60	0.80	-0.01	-0.51	-0.47
		ave	0.33	-0.23	0.27	0.79	-0.03	0.18
	Cast	ave	0.58	-0.09	0.20	0.55	-0.12	0.12
	G2	ave	0.06	0.42	0.31	-0.02	-0.26	-0.24
Total / Solible b-glucan	barley	Kamini	-0.25	0.46	0.61	-0.08	-0.09	-0.19
		ave	0.27	-0.40	0.26	0.92	0.14	0.24
	Cast	ave	0.50	-0.35	0.35	0.74	-0.02	-0.02
	G2	ave	0.16	-0.28	0.09	0.44	0.26	0.15
□¢S/T b-glucan	barley	G2-Kamini	0.32	-0.59	-0.46	0.37	0.26	0.27
		G2 - ave	-0.09	0.10	-0.14	-0.42	0.12	-0.07
	Cast	ave	0.22	0.20	0.05	-0.54	-0.26	-0.43
	G2	ave	-0.28	-0.02	-0.22	-0.16	0.35	0.22
G2 moisture (%)			0.07	0.07	-0.34	-0.17	0.68	0.15
T - N (%)			0.01	0.59	0.16	-0.14	-0.66	-0.19
germination		cast	0.79	-0.14	0.13	0.53	0.08	0.31
		G1	0.80	-0.44	-0.12	0.12	0.10	0.21
		germ.speed	1.00	-0.27	-0.09	0.28	0.05	0.33

Correlation of 15 s

our no.	bri no.		germination speed (N/D)	G2 total / barley total	barley total - G2 total	(barley - G2)/ barley	HWE	Total cast-Total G2
Malting grade			0.24	-0.81	0.28	0.68	0.51	0.69
Total b-glucan	barley	Kamini	0.29	0.37	0.04	0.01	-0.61	-0.43
		ave	0.13	0.50	-0.20	-0.32	-0.35	-0.37
	Cast	ave	0.21	0.41	-0.03	-0.29	-0.17	-0.03
	G2	ave	-0.35	0.96	-0.49	-0.81	-0.23	-0.84
Solible b-glucan.	barley	Kamini	0.41	-0.29	0.40	0.60	-0.56	0.37
		ave	0.49	-0.60	0.33	0.84	-0.22	0.54
	Cast	ave	0.35	-0.39	-0.06	0.47	-0.03	0.34
	G2	ave	-0.02	-0.08	-0.01	0.23	-0.21	0.08
Total / Solible b-glucan	barley	Kamini	0.31	-0.45	0.36	0.60	-0.31	0.55
		ave	0.49	-0.67	0.37	0.89	-0.14	0.59
	Cast	ave	0.27	-0.50	-0.07	0.54	0.07	0.30
	G2	ave	0.39	-0.77	0.42	0.80	-0.06	0.70
□¢S/T b-glucan	barley	G2-Kamini	-0.16	0.23	-0.25	-0.36	0.35	-0.38
		G2 - ave	-0.08	-0.24	0.14	-0.01	0.08	0.26
	Cast	ave	-0.30	0.45	-0.55	-0.71	0.19	-0.51
	G2	ave	0.22	-0.63	0.62	0.65	-0.10	0.70
G2 moisture (%)			0.29	-0.11	0.17	0.23	0.34	0.24
T - N (%)			0.14	0.05	-0.08	0.19	-0.64	0.16
germination		cast	0.84	-0.53	0.46	0.77	-0.17	0.63
		G1	0.71	-0.45	0.16	0.19	0.06	0.30
		germ.speed	1.00	-0.47	0.34	0.55	-0.14	0.51