VALUE ADDED WHEAT CRC

PROJECT REPORT

Temperature variation during grain growth as a source of quality inconsistency for the Australian wheat industry

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Date: January 2003

VAWCRC Report No: 19
Copy No: 25

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SUMMARY

This report describes the results of several years of research on the effects on wheat quality of temperature fluctuations during grain filling. The stimulus for this research was a request from the Australian wheat-processing industry for better consistency and continuity of supply. Heat stress was considered to be a major environmental source of variation in grain quality.

The initial section describes experiments to define the problem of heat stress, drawing upon the literature from Australian and overseas researchers, and describing recent field and growth-chamber experiments, including a comparison of drought with heat stress. The dough-weakening effect of heat was found to vary between genotypes, with a minority of varieties being able to tolerate the effects of high growth temperatures with respect to dough properties.

The task of defining the problem also included elucidating the molecular basis of heat stress; several causes were identified, the major one being the decreased synthesis of very large glutenin polymers. This factor matched the tolerance or susceptibility of various genotypes to heat stress. Varieties also differed in their response to the effects of high growth temperatures on starch properties, many genotypes showing susceptibility in terms of a reduction in the proportion of large (A-type) starch granules, compared to small granules.

Research on approaches to rectifying the problem has focused on the selection of heat-tolerant genotypes. Specific marker proteins for this purpose were identified by protcome analysis. In addition, the 5+10 subunits of glutenin are often associated with heat tolerance. This information can be used in conventional breeding methods to produce more tolerant wheats. The research at the molecular level has also indicated promising directions for genetic manipulation to be applied to the production of improved tolerance. Other approaches include the prediction of regions where heat stress will present quality problems, and identifying grain that has been exposed to heat stress. NIR analysis appears promising as a means of identifying heat-stressed grain.

INTRODUCTION

A major concern of the Australian wheat industry has been the variation in grain quality due to growth conditions. This concern was expressed by marketing authorities, and by milling and baking companies at all stages of preparing the application for the Quality Wheat Cooperative Research Centre (QW CRC), during 1994. The exact wording stated a desire for "**Consistency and continuity of supply: Novel ways of reducing the impact of environmental factors (such as drought, weather damage, early maturation, heat shock) on quality determinants.**"

Accordingly, this concern became the focus of one of the research projects in the Quality Wheat CRC. The accent of this work was on the effects of temperature variation during grain filling, especially heat shock, which for the reasons explained below is seen as being a major cause for inconsistency in grain quality. This Wheat CRC project on temperature variation has been based on the previous research of the CSIRO Grain Quality Research Laboratory (GQRL). Some longer-term aspects of this research continued into the Value-Added Wheat CRC. This report summarises the findings of these studies, listing publications arising from the work, as well as providing reports of some aspects not otherwise published.

The interaction of genotype with environment (G X E) has long been recognised as being the basis determining all aspects of grain quality. For this reason, the Australian wheat grading system has been based on the specification of variety (genotype). However, it has proved much more difficult to account for the effects of growth conditions on grain quality, both for the practical issue of quality specification after harvest, and for the conduct of research to elucidate how grain quality is affected by specific aspects of environmental fluctuations.

Part 1. DEFINING THE PROBLEM

Findings resulting from this research and from other reports are summarised in Table 1 for the range of environmental factors acknowledged to affect grain quality. Of these, heat stress has been chosen to be the most significant for research attention, and it has received the main attention in the research described in this report.

Table 1. Environmental factors known to affect grain quality and dough strength

Environmental factor	Effects on quality	Possibilities for manipulation
Plant nutrition – N	Variations in protein content	Tissue testing and N-fertiliser use
Plant nutrition – S	High N:S ratio produces poor extensibility, lower loaf vol.	Test N and S in grain, use of S-containing fertiliser
Plant nutrition - Cu, micronutrients	Poorer dough and baking quality for Cu deficiency. Possibly also for other micronutrients	Fertiliser use, after soil and grain testing
Modest temperature variation (15 – 35°C)	Increase in dough strength with temperature rise in this range	Choice of growing region, based on expected growth temperatures
Heat stress (a few days of maxima >35°C)	Higher grain-protein content, significant dough weakening	Select for genotypic tolerance, predict dough-weakening based on climate details and genotype
Drought (several days of severe water stress)	Higher grain-protein content, little change in protein quality	Irrigation, select for genotypic tolerance

DEFINING THE PROBLEM #1
MOSS-RANDALL-WRIGLEY RESEARCH ON GROWTH CONDITIONS

Sulfur deficiency

Various factors can produce environmental modification of grain quality, including temperature and moisture profiles (particularly extremes of these), the duration of grain filling, and soil type and fertiliser levels (particularly nitrogen and sulfur, which affect protein content and quality). Sulfur deficiency and temperature fluctuations were identified as two major factors that could alter grain quality as a result of research conducted during the 1980s in collaborations between the North Ryde laboratories (CSIRO GQRL and BRI) and CSIRO Plant Industry (Canberra). Sulfur deficiency was shown to reduce dough extensibility, but it was considered, at the time, to be an infrequent cause of quality loss in practice, due to the sulfur adequacy of Australian soils with respect to nitrogen availability (Moss *et al.*, 1983; Randall and Wrigley, 1986; MacRitchie and Gupta, 1993).

Temperature effects

The complementary GQRL-BRI-PI research on temperature fluctuations during grain filling showed this to be the most important aspect in practice, especially for the Australian wheat industry. Randall and Moss (1990) reported that increases up to 30°C in daily mean temperature during grain filling generally increased dough strength, and that temperatures above 30°C produced weaker doughs. Their research centred on glass-house grown plants of the varieties Olympic, Hartog and Skua, and it also included averaged results for field-grown grain (four varieties) during four seasons at three sites from Victoria to northern NSW.

These findings were the stimulus for ongoing research in CSIRO GQRL on heat stress and grain quality, starting with the PhD studies of Caron Blumenthal in the early 1990s, and continuing on into the Wheat CRC research. The publications resulting from these studies are listed at the end of this report in the Section 'Publications arising from research on heat stress and grain quality, based at the CSIRO Grain Quality Research Laboratory, North Ryde' (Blumenthal *et al.*, 1991a, and many subsequent references).

DEFINING THE PROBLEM #2
RESEARCH OVERSEAS ON TEMPERATURE EFFECTS

Subsequently, the deleterious effects of high temperatures during grain filling on grain quality have been reported by many authors for wheats (both hexaploid and durum) grown in different parts of the world (Borghi *et al.*, 1995; Ciaffi *et al.*, 1996; Corbellini *et al.*, 1998; Graybosch *et* al., 1995; Gibson *et al.*, 1998; Stone and Nicholas, 1994, 1996).

For example, Lafiandra *et al.* (1999) reported that "rheological properties evaluated using the Chopin Alveograph on durum and bread-wheat cultivars, subjected to different temperatures during grain filling and grown in four different areas of wheat cultivation in Italy, were affected by temperature fluctuations during kernel development (Borghi *et al.*, 1995). The most consistent temperature effect detected at different locations was related to the modification of the Alveograph parameter P/L. Compared with the control plants which never experienced temperatures above 30°C, those subjected to temperatures up to 35°C revealed higher P/L ratio (a strengthening effect). However, plants subjected to temperatures in the range 35-40°C, even for short periods, revealed a lower P/L ratio (a weakening of dough properties). These effects were observed in both bread and durum wheats (Ciaffi *et al.*, 1996; Corbellini *et al.*, 1998; Stone and Nicholas, 1994, 1996). Most of the studies have examined the effect of extreme temperatures on dough technological properties, but a progressive increase in dough properties associated with progressively rising temperatures in the range 15-35°C has been observed (Schipper *et al.*, 1986). These findings have recently been supported by Uhlen and co-workers (Uhlen *et al.*, 1998), who have been able to demonstrate the positive effect of increasing temperature in the range 9-21°C, during kernel development."

These reports confirm the much earlier report of Finney and Fryer (1958) for growth conditions in the American mid-west: "Loaf volume and mixing time decreased with accumulated degrees Fahrenheit above 90°F [> 32 °C] during the last 15 days of the fruiting period." ... Their comment that this association was "51 to 84%, depending on variety" alludes to the subsequent finding that there is naturally occurring tolerance to the effect of heat stress on dough strength.

International interest in the general topic of heat stress and cereal grains stimulated the organisation of an international workshop on the topic "Heat Tolerance in Temperate Cereals" (Hawaii, 1994). The workshop was organised under the US/Australia Bilateral Science and Technology Collaboration Program, with considerable input from Australian scientists. Presentations from this meeting were published in a special issue of the *Australian Journal of Plant Physiology*, including several papers from researchers involved in the QW CRC research, namely, Blumenthal *et al.* (1994b), Correll *et al.* (1994), Wardlaw and Wrigley (1994) and Wrigley *et al.* (1994a).

DEFINING THE PROBLEM #3
SIGNIFICANCE FOR THE AUSTRALIAN WHEAT INDUSTRY

In parallel with the production of relevant research findings on the effects of temperature on wheat quality, there have been many 'anecdotal accounts' that temperature variations have been the basis of quality problems. Some of these have been described in the introductions to conference papers, e.g., by Blumenthal *et al.* (1990c, 1990f). The need for a solution of this type of problem is epitomised by statements by millers and bakers, such as "We can cope with many quality problems, but we do not like 'surprises' – unexpected fluctuations in quality, for example when the combination of variety and protein content gives unexpected dough properties."

Commercial evidence for the respective effects on dough quality of modest *versus* high temperatures is provided by the following examples from the statistical analysis of test results from many seasons.

- **Temperature increases in the modest range (15-30°C) cause increases in dough strength.**
    - Experimental data to this effect from the literature (described above) are seen in crop data averaged over eleven seasons for the dough properties of grain from export terminals in eastern Australia (Table 2). Similar trends were seen in the results for development time and stability in Farinograph analyses (Wrigley *et al.*, 1994).
    - Grain of the variety Condor, grown in NSW, was observed to give consistently greater dough strength, compared to samples grown in Victoria. This has been attributed to the effect of the warmer growth conditions in NSW (Archer and O'Brien, 1987).

- **Temperature shocks in the high temperature range (>30°C) cause decreases in dough strength.**
  - The dough strength (as Rmax) of Prime Hard wheat decreases with greater heat stress, based on analyses of crop reports for the 29 years from 1960/1 to 1988/9 (Figure 1) (Blumenthal *et al.*, 1991a). In this case, heat stress was determined as the cumulative hours over 35°C during the grain filling period at three sites (Moree, Myall Vale and Narrabri).
  - Heat shock caused dough weakening for crops of four varieties (Hartog, Sunelg, Sunstar and Suneca), compared to grain of the same varieties, sown earlier at the same site (Blumenthal *et al.*, 1991a). The latter grain, early sown and early harvested, escaped the heat stress of 28 October to 1 November, 1988, with daily maxima of 36°, 39°, 39°, 37° and 37°C.
  - Heat-shock episodes in late November, at four sites near Narrabri, were observed to cause considerable losses in dough strength for crops of Songlen, Cook and Kite. The loss was greatest for the latest-sown trials, which were still immature at the time of the heat episode (Table 3).
  - Dough weakening due to heat shock was again demonstrated during the 2000/1 harvest for four Prime Hard varieties in the field trials of the Plant Breeding Institute of the University of Sydney, Narrabri (Tables 4 and 5). Three-Kg samples, provided by Dr F.Ellison, were milled and dough tested at Goodman Fielders, Toowoomba. Varieties differed with respect to the extent of quality loss (Table 4). Results averaged across the four varieties showed that the heat stress had greatly reduced dough properties by several other measures of dough quality (Table 5). These losses of dough strength were matched by the biochemical result of lower % 'unextractable' polymeric protein, indicating less of large polymeric glutenin.

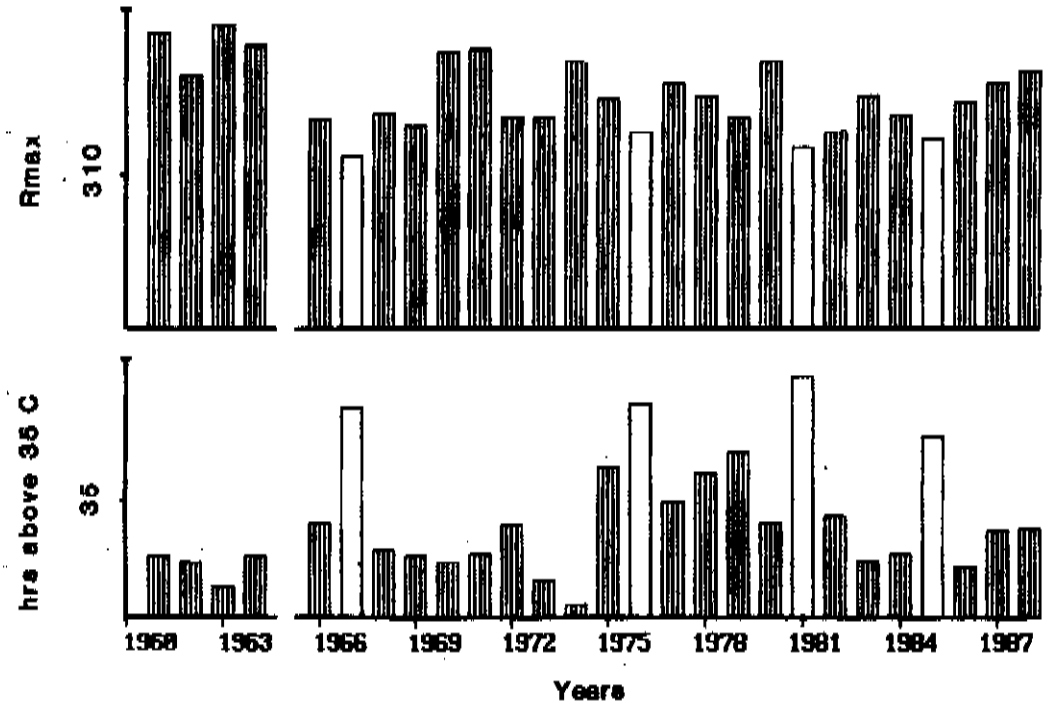
- **Temperature increases in general cause modest changes in starch properties.**
  - These appear to be mainly confined to the ration of large (A-type) to small (B) granules, increasing growth temperature reducing the proportion of B-granules (Blumenthal *et al.*, 1994b; Panozzo and Eagles, 1998).

Table 2. Wheat quality variation with latitude (and thus growth temperature). Crop data are summarised for eleven years (1982/3 to 1992/3). Entries with different letters (a, b,...) are statistically different at the 5% probability level. Adapted from Wrigley *et al.* (1994).

Export terminal (latitude range of growth region)	Protein content (%)	Extensograph Rmax
Australian Hard No 1		
Brisbane (27-29 °S)	12.4a	464a
Newcastle (30-33 °S)	12.4a	404a
Sydney/Port Kembla (34-36 °S)	12.5a	371a
Geelong/SA (37-38 °S)	12.4a	314c
Australian Standard White		
Brisbane (27-29 °S)	10.8a	465a
Newcastle (30-33 °S)	10.2ab	399ab
Sydney/Port Kembla (34-36 °S)	10.1b	354b
Geelong/SA (37-38 °S)	10.3ab	292c

Table 3. Loss of dough strength (as Rmax) with stage of grain filling due to heat stress episodes starting 28 November, 1981, near Narrabri, NSW (adapted from Blumenthal *et* al., 1991b).

Variety (*HMW alleles*)	Rmax for grain harvested before 28/11(Mean of 4 sites)	Rmax for grain near harvest ripeness at 28/11 (Mean of 2 sites)	Rmax for grain at 15-35% moisture at 28/11(Mean of 4 sites)
Stage of heat stress:-	None	At late maturity	At mid grain filling
Songlen (*a b,f a*)	391	300	227
Cook (*a b a*)	438	350	211
Kite (*b i a*)	419	343	216



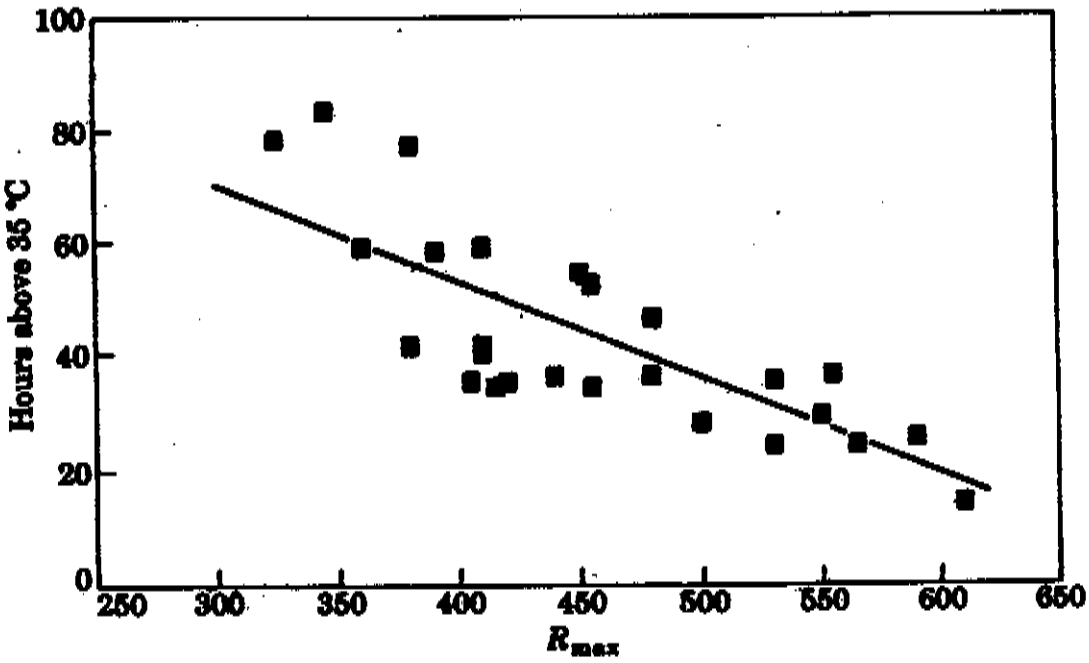


Figure 1. Variations in dough strength (Rmax) with heat stress for Prime Hard wheat for the 29 years from 1960/1 to 1988/9. The upper part of the figure is reproduced from Blumenthal *et al.* (1990a), and the lower graph is reproduced from Wrigley *et al.* (1994a). The correlation coefficient in the lower graph is –0.79 ($P<0.001$).

Table 4. Loss of dough strength (as Rmax) for grain harvested with and without three days' heat stress, at two sites in northern NSW (Spring Ridge and North Star, respectively) in the 2000/1 harvest. Grain was provided by Dr F Ellison, Narrabri. Dough tests were performed by Goodman Fielder, Toowoomba.

Variety (*HMW alleles*)	North Star **No heat stress** Rmax	Spring Ridge **Heat stressed** Rmax	**Change** in dough strength due to heat Rmax
Janz (*a b a*)	600	225	62% loss
Banks (*b b a*)	590	190	68% loss
Sunco (*a b a*)	550	205	63% loss
Sunstate (*aid*)	660	380	42% loss

Table 5. Results of grain analysis, averaged for the four varieties (listed in Table 2), grown at North Star (control) and Spring Ridge (heat stress), in field trials conducted by Narrabri/University of Sydney.

	North Star - 4 varieties	**Spring Ridge - 4 varieties**
Environment	Modest temp (control)	Heat stressed
Sowing date	Early	Later
Harvest date	6-8 Nov., 2000	4-6 Dec., 2000
Days >32^{0}C during grain filling	None	27, 28, 29, 30 Nov
Average yield, t/ha	3.10	3.81
Hectolitre weight, kg/hL	80.5	73.6
Screenings, %	3.6	14.0
Average TKW, g.	29.7	22.8
Average protein content, %	13.3	13.8
Average Rmax (45 min)	428 at Toowoomba	224
Average Rmax (90 min)	600 at Toowoomba	250
Mix time (2g Mixograph)	235 sec	186 sec
Resistance Breakdown (ditto)	4.9	11.3
Micro Extension tester, Rmax	0.38 N	0.17 N
Z-arm mixer, mix time	538 sec	128 sec
% UPP	54.3	41.5

DEFINING THE PROBLEM #4

CUMULATIVE HEAT LOAD AS A UNIFYING CONCEPT

The results described above make an important distinction between the effects of modest-to-hot growth temperatures (say, day temperatures up to about 30°C), and episodes of very high temperatures (say, a daily maximum well over 32°C). The concept of 'cumulative thermal load' was explored in an attempt to understand the relationships between the heat-shock episodes and on-going heat (Blumenthal *et al.*, 2000; Wardlaw *et al.*, 2002).

Wheat responds best to much lower temperatures (15-20°C daily maxima) than are generally experienced in most parts of the Australian wheat belt during grain filling (October to December). Added to this ongoing heat is the likelihood in the field of further complications due to short periods of heat shock (e.g. >32°C). To simulate combinations of these more moderate and extreme conditions, wheat was grown in controlled-environment facilities at each of several set temperatures throughout grain filling, namely, at daily maxima of 18°, 21°, 24°, 27° and 30°C. In addition, plants otherwise maintained at 21° were subjected to one of ten heat-shock treatments, each being approximately equivalent in terms of heat load, ranging from longer periods at only moderately high temperatures (e.g. 7 days at 27°) to shorter periods at considerably higher temperatures (e.g. 3 days at 39°).

These treatments are compared in Figure 2, the treatments being shown in the top row of histograms, which are compared on the equivalent basis of 'heat load' with degree days as the units. The histograms at top left illustrate the increasing 'heat stress' treatments, for comparison with the 'heat shock' treatments at top left. In this latter case, each based on earlier growth at 21°, the period of heat shock X shock temperature is adjusted to provide equivalent heat loads.

The second row of Figure 2 shows the effects of these heat treatments on kernel weights at maturity (an indicator of grain yield). These were progressively lower with increasing temperatures as heat loads. However, the higher heat-shock temperatures caused much greater losses, despite their equivalence as measured by heat load. Protein content was not greatly affected by the range of heat treatments, making the comparison of dough properties simpler since protein content could otherwise affect the interpretation of dough properties. Although dough strength (as time to peak mixing resistance) decreased progressively with heat generally, the greatest loss was caused by the highest heat shock treatment (four days at 39°C). Biochemically, these changes in dough quality were reflected in similar changes in %UPP, a measure of the proportion of very large polymers of glutenin. Effects were greater for Lyallpur (dark columns), considered to be more heat susceptible than the variety Trigol.

The results could further be summarised as follows:-

- Both chronic heat stress and heat shock reduced kernel dry weight at maturity.
- Heat shock over 35°C caused the greatest reductions in dough strength and in large-glutenin content.
- Chronic heat stress increased protein concentration, while there was little change due to heat shock.
- Differences in the response of grain weight to temperature between the tolerant and susceptible cultivars were most evident in the lower temperature range.
- Differences between the varieties in their response to chronic heat stress and heat shock suggest that genetic variation in grain quality may need to be assessed independently for each type of high-temperature stress.

DEFINING THE PROBLEM #5
PROSPECTS FOR FUTURE CLIMATES

The results for cumulative heat load demonstrated that shock treatments above about 36°C have the most disastrous effects on grain yield and on dough strength, even when compared with longer periods of heat of similar overall heat load. As a result, most research efforts have concentrated on this type of heat stress in this project. The need for this research has been accentuated by forecasts that the frequency of such heat-shock episodes is likely to increase with the progressive onset of global warming. For example, Figure 3 shows the extent of this increase that is expected for the year 2030.

These increases in temperature are linked to the increasing levels of atmospheric carbon dioxide (Dix and Hunt, 1995). Considerable increases in grain yield (6 - 35%) have been obtained for wheat grown in an atmosphere enriched with CO_2 to double the present level. The increased yields were due to increases in grain number, rather than grain size. Of most concern was the reduction in grain-protein content, ranging down to levels (below 8%) at which normal processing would be difficult (Rawson, 1995; Wrigley and Blumenthal, 1995; Blumenthal *et al.*, 1996a). Dough testing of the grain showed that dough properties were reduced (especially extensibility) but interpretation was difficult due to the low protein content. A dramatic change in grain composition was the considerable increase in the proportion of large (A-type) starch granules.

DEFINING THE PROBLEM #6
HEAT *versus* DROUGHT EFFECTS

Another part of the consideration of climate factors has been the extent to which moisture level is interactive with heat stress. It has been suggested that much of the effect of heat shock is due to the consequent lack of water. It was thus relevant to study the separate and interactive aspects of these two factors, with respect to both grain yield and quality. Water stress is well known as a cause of loss of grain yield. Drought has also been reported to increase protein content, but its effects on 'protein quality' have not been reported. Nor is it known if sources of genotypic tolerance are available.

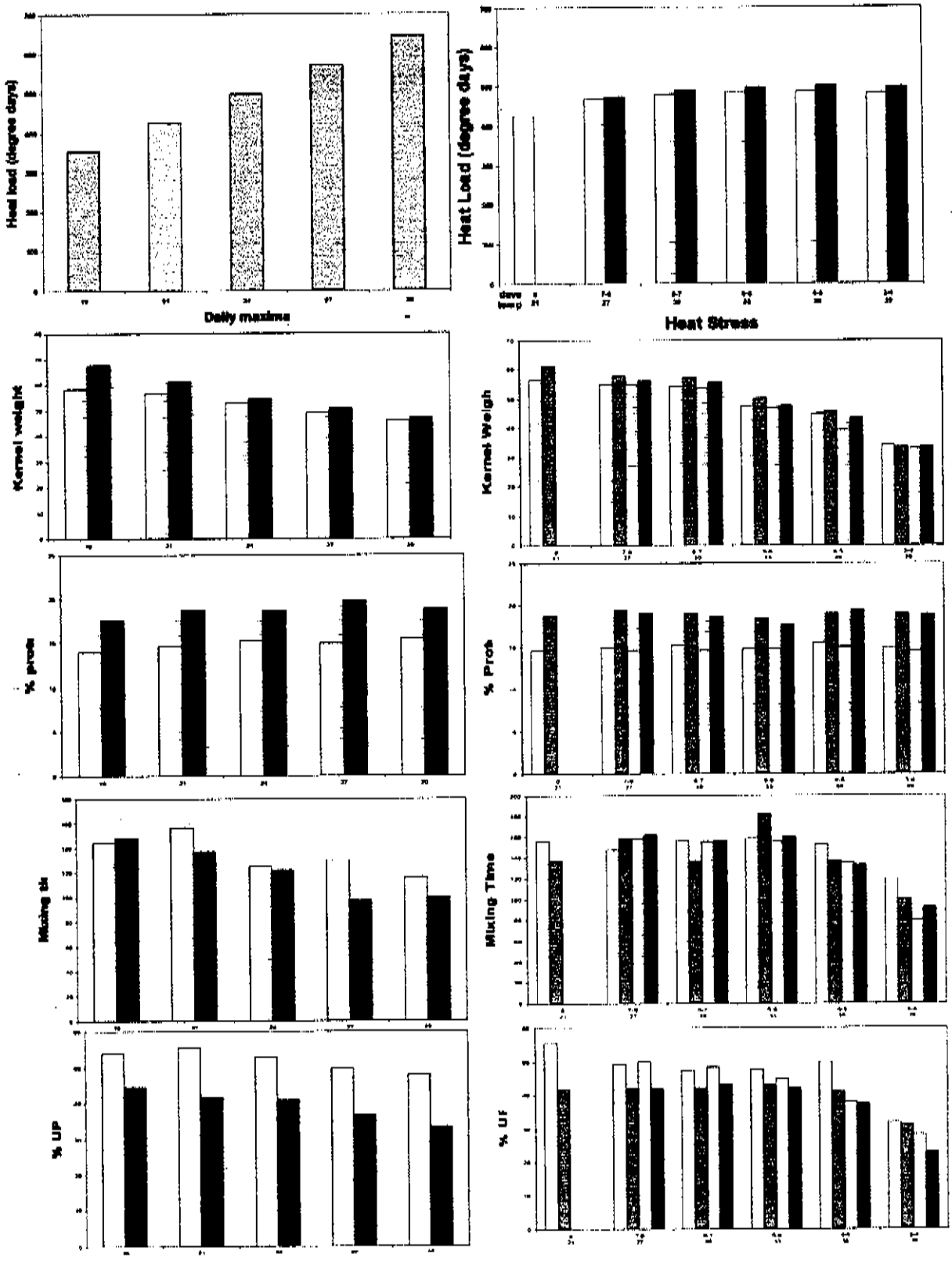


Figure 2. (Top line) Heat loads (as degree-days) for the series of heat-stress (left) and heat-shock treatments to plants of wheat varieties Trigo 1 and Lyallpur (light and dark, respectively, in pairs of columns below the first row of nomograms). Remaining nomograms (down the page) represent results for grain from the treatments at top for kernel weight, protein content, mix time and %UPP (proportion of very large glutenin). Adapted from Blumenthal *et al.* (2000).

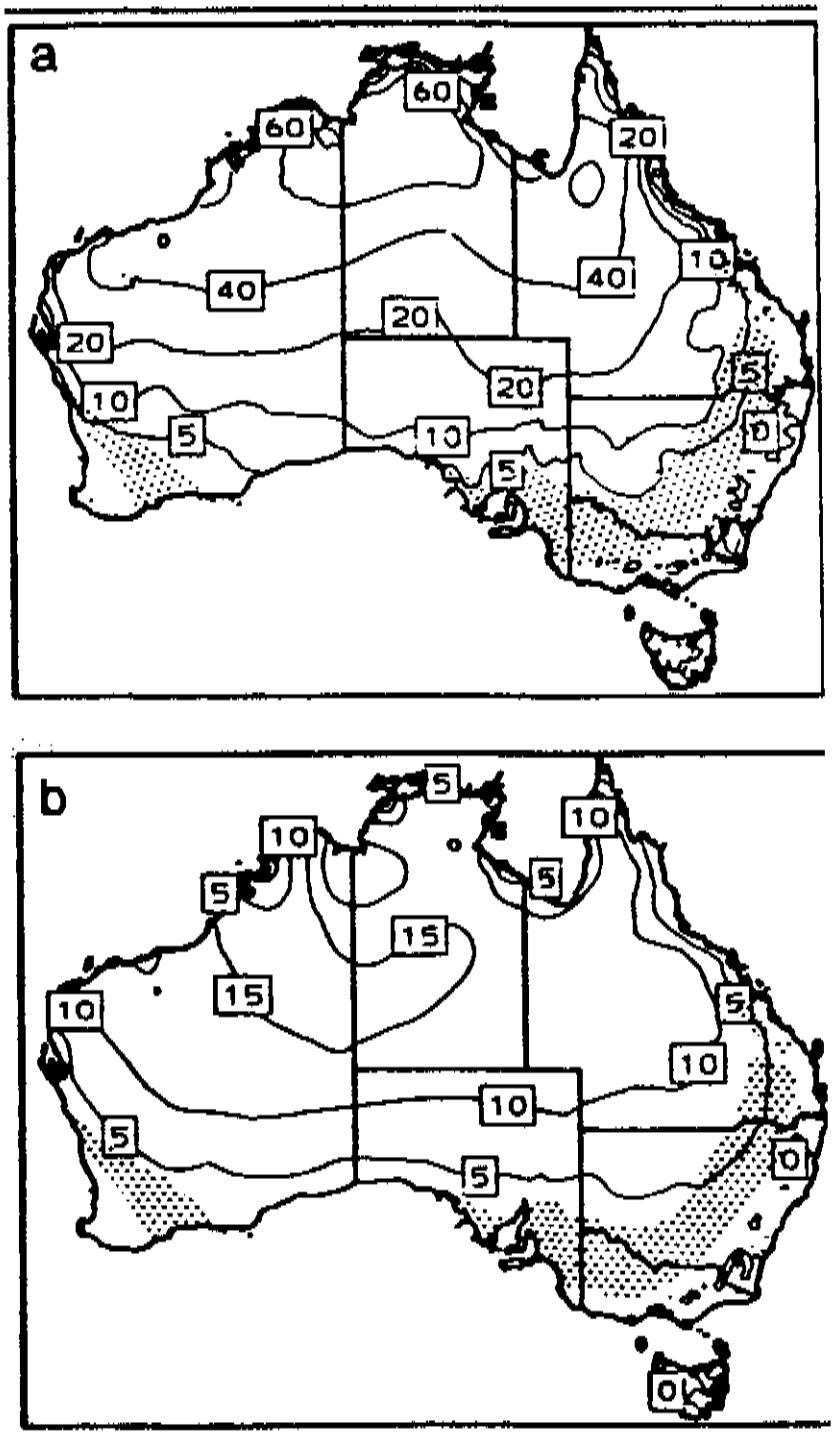


Figure 3. (a) The present average number of spring days when temperature exceeds 35°C. (b) The increase in the number of these days for a warming of about 2.5C° by the year 2030. The wheat belt is the shaded area. From Hennessy (1994).

Water stress in the field

Field experiments in water stress were conducted at three sites in Israel, in collaboration with Prof. Zvi Plaut of the Volcani Center, Israel (a Sabbatical Visitor to the Wheat CRC). The sites provided (1) no water stress, (2) moderate water stress and (3) severe water stress. Table 6 shows the yield and protein content results averaged for the 13 Israeli varieties sown at each site. These involved the following named varieties and advanced unnamed lines - Ariel, Beit Hashita, Atir, Yaniv, Nirit, Gedera, 85, Bar-Nir, 383, T-95C, T-95D, T-95K, Inbar. The results indicate that severe water stress (Site No 3) causes great yield loss (63-77%, compared to the control site, No 1).

For moderate water stress (Site No 2, versus Site No 1), yield losses were more moderate (12-42%). Increasing water stress caused progressive increases in grain protein content (Table 6) (Plaut *et al.*, 1999).

Table 6. Ranges of yield, grain size and protein content for 13 genotypes grown at three field sites in Israel differing in water stress. From Plaut *et al.* (1999).

Attribute	Control Site 1	Moderate water stress Site 2	Severe water stress Site 3
Grain yield, g/m^2	480-680	370-430	150-230
Grain size, mg	47-55	35-43	20-27
Protein content, %	10.0-12.0	11.3-13.0	14.5-16.8

Four varieties, selected from each of the field trial sites, were milled in Israel and dough tested in Australia. The results showed that water stress increased Rmax (the height of the small-scale extension-test curve) and increased Mixograph peak height, but these changes were no more than could be explained by the higher protein contents of the samples. It was thus concluded that the main effects of water stress on grain quality were primarily on protein content rather than on protein quality, the increases in protein content being primarily due to decreased starch synthesis. Apparently, water stress (*per se*) does not consistently cause dough weakening. These trends were evident in all the genotypes tested.

Water stress in the glasshouse

In a further collaboration with Prof. Plaut, grain samples were obtained from a series of glasshouse experiments in which water was restricted from plants after flowering, thus providing water stress throughout grain filling, at an early stage, and at a late stage of grain filling, each being comparable to control plants which received adequate water. Wheats sown included three Australian varieties (Batavia, Suneca, Wyuna) a CIMMYT variety (Fang), and two Israeli wheats (Nirit, Yaniv). The results in Table 7 (differences between the control and the means for all six genotypes) confirm the field trial conclusions that water stress reduces grain yield, while increasing grain protein content (Plaut *et al.*, 1999). The increases in dough strength seen for the continuously water-stressed samples are seen as reflecting the higher protein content, contrasting greatly with the effects of heat stress which can cause increased protein content together with dough weakening.

Table 7. Changes (as % of control) in average grain attributes from water-stress treatments for six genotypes grown in the glasshouse. Results quoted in bold type are statistically different from the control at the 5% level, based on standard deviations (SD). From Plaut *et al.* (1999).

Attribute	Early water stress		Late water stress		Continuous water stress	
	Difference	*SD*	Difference	*SD*	Difference	*SD*
Grain weight	- 8	*11*	-10	*17*	- 9	*22*
Protein content	+ 1	*7*	+ 2	*7*	**+23**	*8*
Extension height	- 10	*31*	-2	*17*	**+32**	*12*
Peak mix resist'ce	+ 4	*8*	0	*5*	**+15**	*9*
Mix time	0	*9*	+6	*14*	+3	*13*
Breakdown	**+12**	*3*	+13	*15*	+25	*22*

Consideration of the results for the varieties individually indicated genotypic differences in reactions to the effects of water deficit. The more drought-tolerant varieties for yield were Inbar, T-95K, Batavia and Fang. For quality changes, the more drought-tolerant varieties were Batavia, Fang and Nirit (Plaut *et al.*, 1999).

Water stress combined with heat stress

The separate and combined effects of heat and water stress were studied in glasshouse experiments, conducted at the University of Western Sydney, involving two Australian varieties, namely, Suneca (with a reputation for heat tolerance with respect to grain quality) and Batavia (heat susceptible). These experiments involved Prof. Plaut, during his sabbatical visit to Sydney, and Dr Butow, as an employee at the University of Western Sydney. The

results are provided in the paper of Butow *et* al. (2000) and in the attached unpublished Report (#1) by Butow *et al.*

Three days' heat stress (39°C for 3 days at 12 days post-anthesis) caused increases of 6% and 8% in grain-protein contents for Suneca and Batavia, respectively. The simultaneous application of both heat and water stress further increased the protein content of these cultivars (by 16% and 18 %, respectively). The changes in dough-strength characteristics varied considerably between the two cultivars; heat and water stress caused an increase in mixing time in Suneca (seen as a strengthening of dough quality), whereas heat stress alone caused dough weakening for Batavia. Water stress acted to ameliorate the negative effects of heat stress (as shorter mixing time) in Batavia and, unlike Suneca, water stress significantly increased peak resistance (PR) regardless of heat stress for Batavia. It appeared that heat and water stress acted independently, with heat stress causing loss of protein quality in the susceptible genotype, whereas water stress increased protein content with out altering protein quality.

Heat stress (alone) of Suneca caused the appearance of two proteins of molecular weights 30,000 and 40,000 in the one-dimensional SDS-gel electrophoresis patterns. These were considered to be potentially valuable as markers to identify genotypes having the heat-tolerance characteristics of Suneca. Accordingly, publication of the full report of this research (Report #1 by Butow *et al.*) was delayed and a provisional patent application was prepared (Anon, 2000a). This patent application has since been allowed to lapse, partly due to difficulties in obtaining amino-acid sequence data for these polypeptides.

Modelling the grain-filling process in heat and water stress

A mechanistic model was developed in order to analyse the daily rates of transport from vegetative organs to kernels, and its contribution to kernel weight, thereby to compare the effects of heat and water stress. This research is described in the attached Unpublished Report #2 by Plaut *et al.*

While neither water stress nor high temperature had a marked effect on the rate of kernel formation, the rate of dry matter production by kernels was significantly decreased by water stress in both cultivars. The effect of high temperature on dry-matter production was more moderate. Dry weight of vegetative organs (stems and leaves) decreased during grain filling, due to export of stored carbohydrates to the developing kernels. In decapitated plants, in contrast to intact plants, the rate of dry-weight production by vegetative organs was increased during the same period. The rate of dry-matter production by water- and temperature-stressed plants was lowered. The rates of transport from vegetative organs to kernels were much higher in Suneca than in Batavia. Water and temperature stresses reduced these rates in both cultivars, but the decrease due to water stress was much stronger in Batavia. The contribution of dry matter transported by vegetative organs was 0.40 of the total kernel weight in unstressed Suneca plants at the initiation of the treatments. It increased gradually up to 1.00 at termination of the experiment. The contribution of transported dry matter in Batavia was less, and it increased only from 0.30 to 0.60. Water stress and high temperature increased the contribution of transported dry matter to kernel growth.

In a second experiment, the final thousand-kernel weight and final kernel number per plant were determined. While kernel number was hardly affected by both stresses, thousand-kernel weight was reduced by water stress more severely than by temperature stress, more significantly in Suneca than in Batavia.

DEFINING THE PROBLEM #7

A MOLECULAR BASIS FOR CHANGES IN PROTEIN FUNCTION DUE TO HEAT STRESS

In addition to the concern of industry organisations about environmental variations in grain quality, there was the concern for better knowledge about the basis of grain quality. The exact wording, used in the lead-up to forming the Quality Wheat CRC, stated a desire for "**Knowledge of fundamentals: Better understanding of the functionality of key components of wheat for end-use, e.g. baking, noodles.**" The heat stress research has contributed to this need for basic knowledge through the aspects relating to elucidating the underlying molecular basis of the changes in dough strength due to fluctuations in growth conditions.

Glutenin composition and structure as a major determinant of dough strength

Suitable dough properties, necessary for most uses of wheat flour, are largely determined by the structure and composition of the glutenin fraction of gluten, balanced by the composition and relative amount of the gliadin fraction Gianibelli *et al.* (2001). Relevant aspects of glutenin composition are listed in Table 8. This aspect of the

growth-environment research has shown that heat-stress affects all five aspects of glutenin composition listed in Table 8. The results summarised in this section show that there are likely to be several factors responsible for the dough-weakening effects of heat shock, namely, the importance of variety (especially glutenin alleles), the actual amounts of individual and combined glutenin subunits, the size distribution of the glutenin polymers, and finally the total amounts of glutenin and gliadin, which are largely determined by grain-protein content.

Table 8. Aspects of glutenin composition and structure relevant to dough quality.

Aspect of composition	**Method of analysis**	**Significance**
HMW and LMW alleles, *Glu-1* and *Glu-3*	SDS-PAGE, RP-HPLC	Genetic potential for effective glutenin formation, partly predicted by *Glu-1* score
% of individual glutenin subunits	SDS-PAGE + scan, or RP-HPLC, after breaking SS bonds	Actual amounts of subunits for the genotype X environment combination
% of glutenin *vs* gliadin (and other proteins)	SE-HPLC, or extract ± sonication, without SS rupture	Balance of glutenin elasticity *vs* gliadin plasticising effects
Size distribution of glutenin polymers	Multi-stacking SDS-PAGE, %UPP, Field flow fractionation	Very large glutenin polymers entangle to contribute more to dough strength
Grain-protein content	NIR, Dumas analysis	Amount of gluten protein is a major factor determining suitability for processing

Abbreviations:
HMW and LMW alleles = alleles for the high- and low-molecular weight subunits of glutenin
Glu-1 and *Glu-3* = gene loci for the high- and low-molecular weight subunits of glutenin
RP-HPLC = reversed phase high-performance liquid chromatography
SDS-PAGE = sodium dodecyl-sulfate polyacrylamide gel electrophoresis
SE-HPLC = size-exclusion high-performance liquid chromatography
NIR = near infra-red spectroscopy

Increased protein content as a result of heat shock

A unique aspect of grain from plants that have been heat shocked is that grain protein content is generally increased, but that dough strength is weaker, despite the general expectation that a higher protein content would increase dough strength, as it does for example in the case of water stress. The higher protein content of grain from heat shock is evident in the Mixograms in Figure 4.

In this set of examples, both Ella and Batavia showed signs of susceptibility of to the effects of heat shock on dough quality. For the variety Ella, the Mixogram for the heat-shocked sample showed several signs of dough-weakening, namely, a lower peak resistance at a shorter time, plus a more rapid breakdown after the peak, despite the higher protein content of the heat-shocked material. The higher protein content of the heat-shocked Batavia sample is shown as a higher peak resistamce (as would normally be expected), but the weakening effect of the heat shock is shown bu the shorter time to the peak and by the more rapid breakdown after the peak.

On the other hand, the varieties Halberd and Grebe showed their tolerance to the effects of heat shock on dough strength. There was little difference between the profiles for the control and stressed Halberd samples. For Grebe, the heat-shocked sample had a higher peak (consumate with the higher protein content) but more importantly a longer development time, indicative of greater strength despite the heat shock. These four Mixograms exemplify the wider range of genotypic reactions to the effects of heat shock on grain quality shown for 45 varieties in Figure 5.

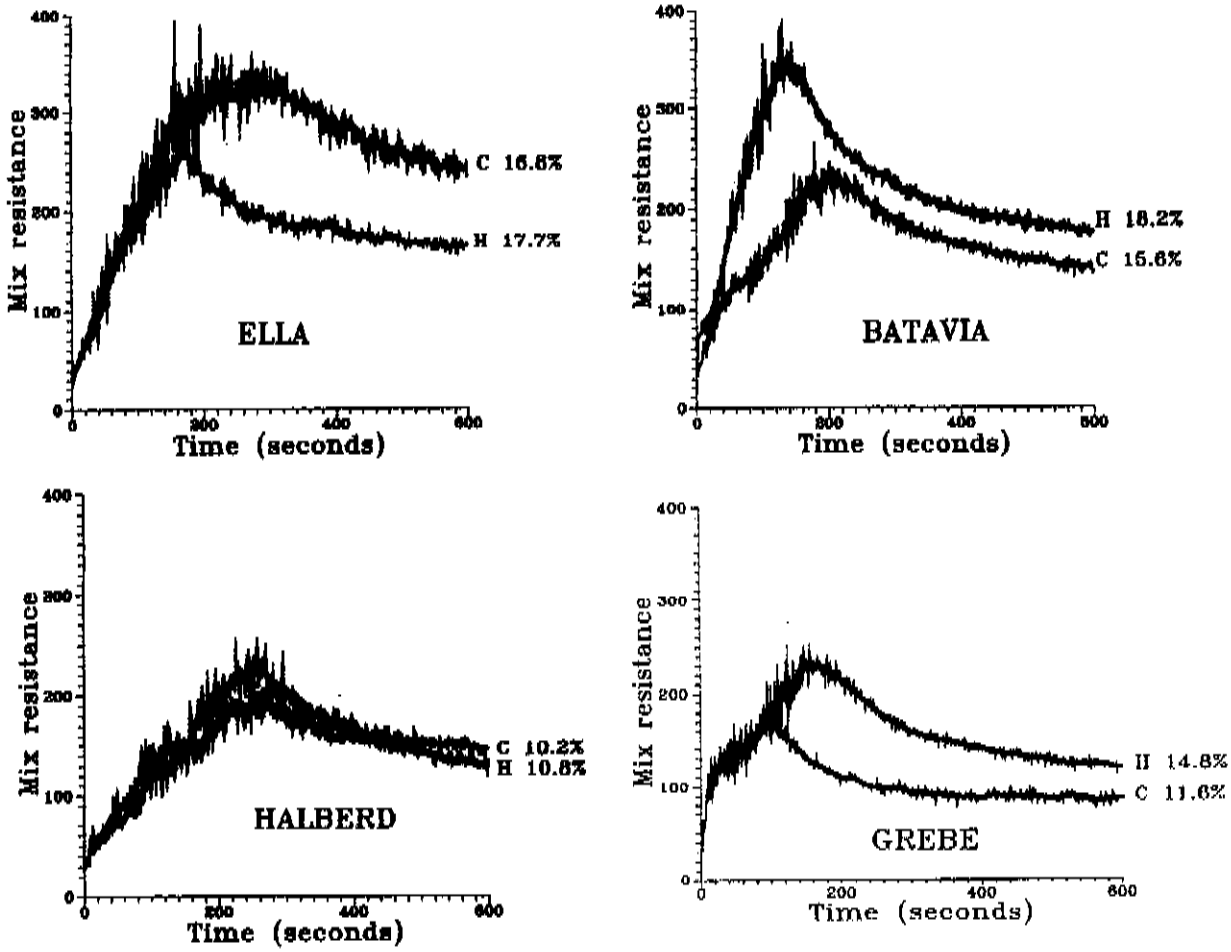


Figure 4. Mixograms for flour milled from grain of plants that had experienced heat shock (three days at about 40 °C during grain filling), designated 'H', compared to the non-shocked control (C) material. Varieties represented are Ella, Batavia, Halberd and Grebe.

Reduced synthesis of glutenin polypeptides during heat shock

An early aspect of grain composition found to be affected by heat shock related to gliadin:glutenin ratio (third aspect of glutenin composition in Table 8). This conclusion was based on the observation that gliadin sysnthesis continued at a greater rate during the heat shock than did the synthesis of glutenin (Blumenthal *et al.*, 1990d,e; 1991; 1993a). This was presumed to be due to the presence of heat-shock elements in the gene sequences of some gliadin genes, up-stream of the coding sequences. Thus, some of the gliadin proteins can be seen to act as heat-shock proteins. The consequent higher gliadin:glutenin ratio provides at least one explanation for the dough-weakening effect of heat shock.

Degree of glutenin polymerisation

An important aspect of the molecular basis of dough strength is known to be the size distribution of the glutenin polymers (Table 8), whose sizes can range up into the tens of millions of Daltons (Southan and MacRitchie, 1999). This aspect of glutenin function in shown in Figure 5 to relate closely to the effects of heat shock on dough strength. The correlation of dough-strength change to the heat-related change in the % of large glutenin was very highly significant (*** significance). In this case, SE-HPLC was used to separate the large glutenin from other flour proteins (Blumenthal *et al.*, 1995a).

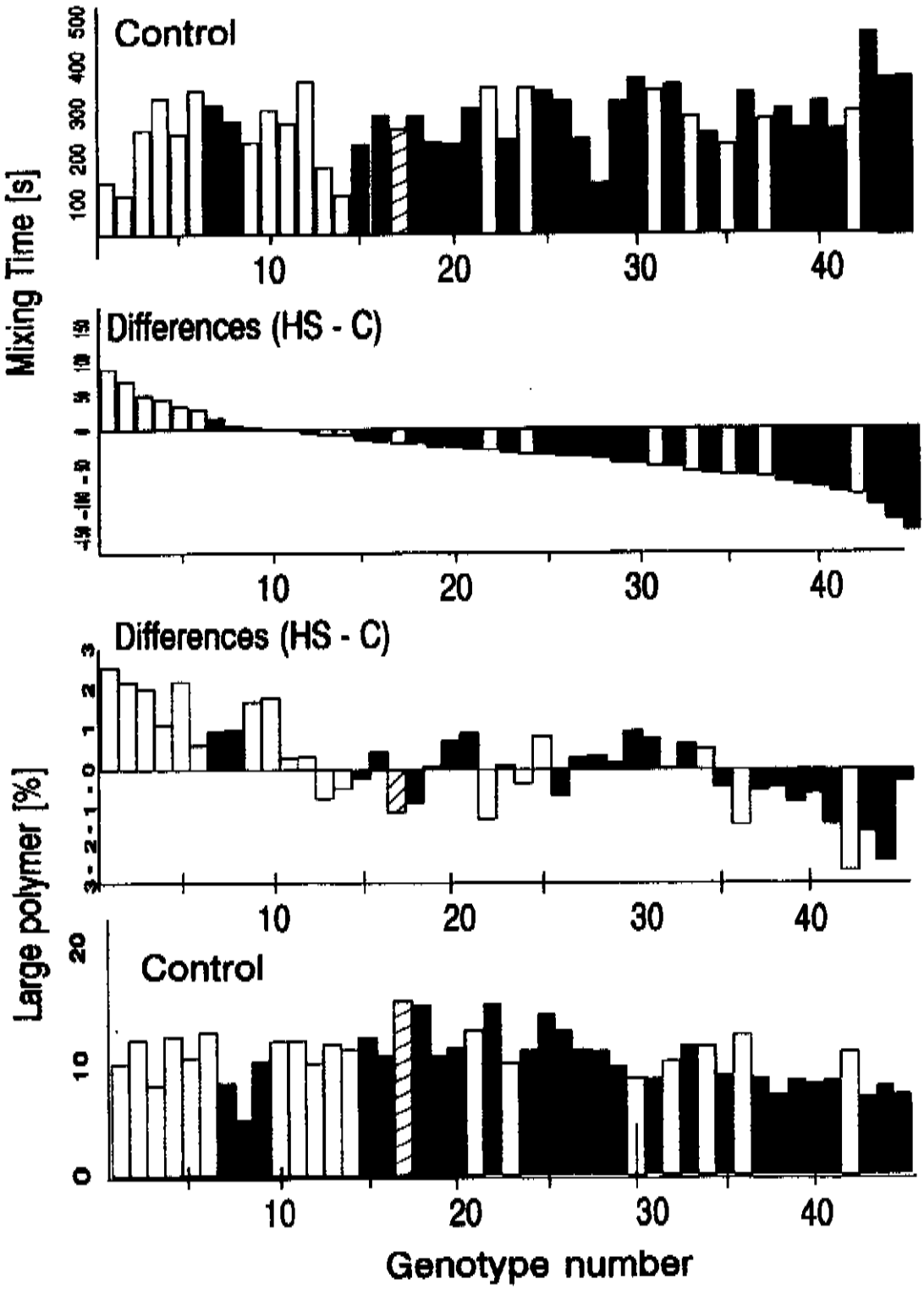
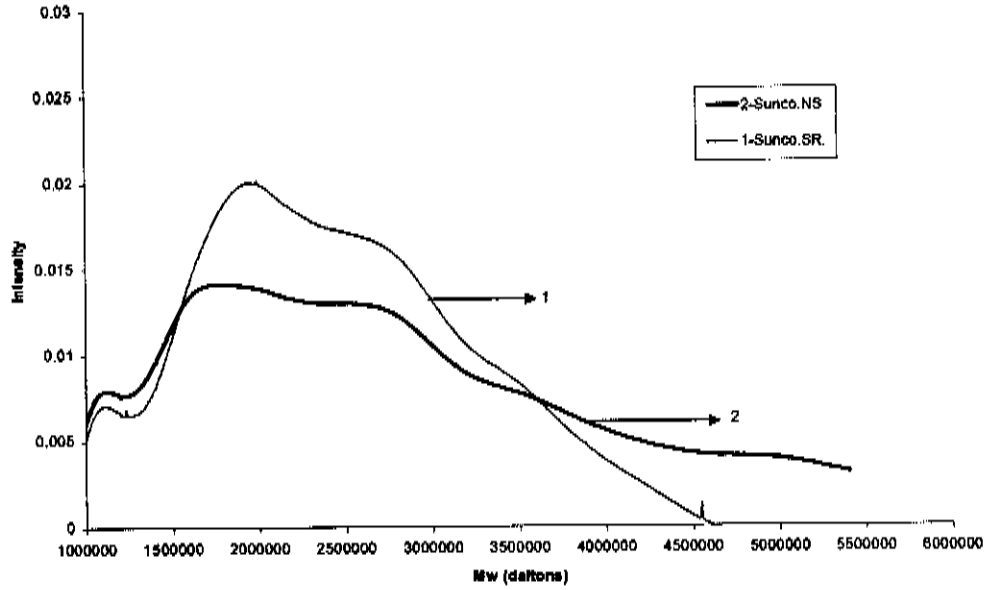


Figure 5. Changes in dough strength (as mixing time) and in the proportion of very large glutenin polymer for 45 wheat genotypes arranged from left to right in order of increasing susceptibility to heat shock. White columns indicate genotypes having HMW subunits 5+10 (the *d* allele of the *Glu-D1* locus); black columns have HMW subunits 2+12 (*a* allele). The striped column is for the durum variety Kalimaroi. From Blumenthal *et al.* (1995a).



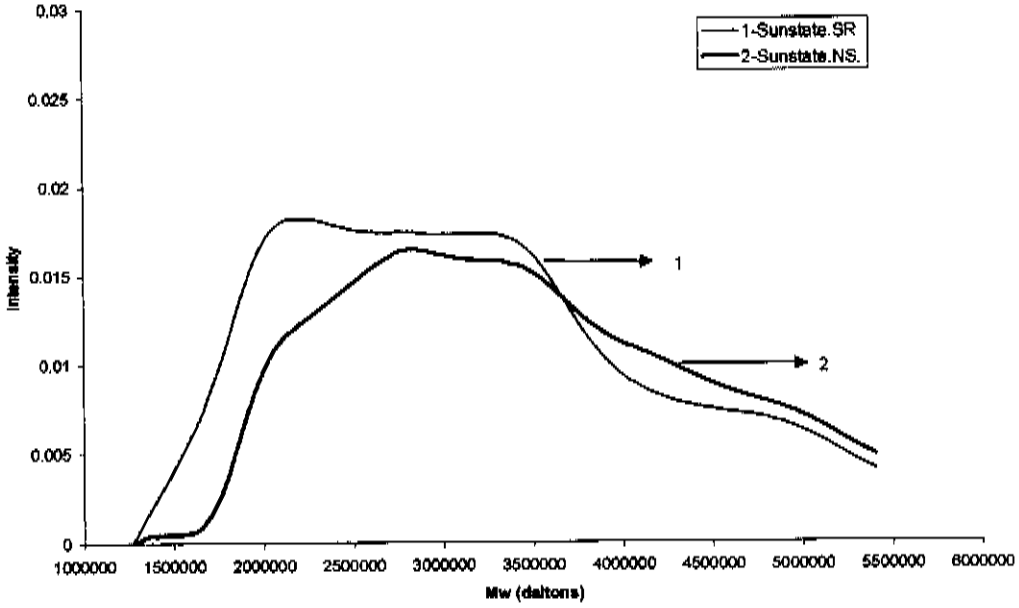


Figure 6. Field-flow fractionation of glutenin protein from cv. Sunco and the more tolerant cv. Sunstate, showing loss of the larger polymers, especially by the more susceptible variety, as a result of heat shock. Line 1 is the heat-shocked sample in each case (grown at Spring Ridge); Line 2 is for the non-stressed samples (grown at North Star). Quality details for these samples are provided in Tables 4 and 5. The figures were provided by Laila Daqiq. From Annual Report of the Quality Wheat CRC, 2000/01, Figure 2.1.

Direct evidence was obtained for the differences between glutenin from heat-shocked and control grain (Hartog variety) by preparation of the respective glutenin fractions and incorporating them into a reconstituted dough, using novel methodology (Beasley *et al.*, 2000). In the two-gram Mixograph, the dough reconstituted with heat-shock glutenin took 69 seconds to mix to peak resistance, much less than for the control (127 seconds). The

weakness of the heat-shock glutenin was also seen in its lower peak resistance (268 units *versus* 300 for control) and greater resistance breakdown (46% *versus* 26% for control).

The best procedure at present for analysing the very large glutenin polymers is field-flow fractionation (FFF), used by Laila Daqiq to characterise a range of glutenin polymer samples (Daqiq, 2002). This methodology was used to characterise the glutenin from control and heat-shocked material of the varieties Sunco and Sunstate (Figure 6), whose dough qualities are listed in Table 4. For both varieties (especially for Sunco), the FFF profiles indicated the presence of much more very large glutenin polymer (in the region of >380,000 Daltons), and less in the early parts of the profile.

Glutenin isolated from heat-shocked and from control grain was analysed by FFF for differences in average molecular size. The glutenin from heat-shocked glutenin had an average molecular size of 3.95, significantly lower than that for the control glutenin (average molecular size of 4.11) (Beasley *et al.*, 2000). These, and the results described earlier, indicate the importance of the presence of the largest glutenin polymers to maximise dough-strength potential.

Several other researchers have also reported that the effects of heat shock on dough strength are largely due to loss of the larger glutenin polymers, e.g., Ciaffi *et al.* (1996), Stone and Nicholas (1996) and Corbellini *et al.* (1998). This conclusion was reinforced by the experiments of Perrotta *et al.* (1998), who reported that the rate of production of storage-protein mRNAs was independent of temperature, but that the temperature effects on dough properties were due to 'modification of the assembly of glutenin molecules' in polymeric form. Protein disulfide isomerase (PDI) has been implicated as a critical enzyme involved in the formation of disulfide bonds to create the glutenin polymers (Shewry *et al.*, 1999). Genes for PDI have been localised on several of the wheat chromosomes (Ciaffi *et al.*, 1999). Although several isoforms of PDI were identified in our proteome analyses (Skylas *et al.*, 2000a), none of them changed in intensity as a result of heat stressing of tolerant or susceptible varieties (Skylas *et al.*, 2002a).

The loss of the largest glutenin as a result of heat shock has been attributed to the action (or lack of action) of chaperones, including heat-shock proteins (presumed to be involved in the determination of protein conformation), and of protein disulfide isomerase (presumed to be involved in disulfide-bond formation) (Shewry, 1999; Hurkman *et al.*, 1998). A major function of molecular chaperones relates to the correct folding of the polypeptide chain after its synthesis (Kolb *et al.*, 1995). This function may be disrupted by periods of heat stress (Schojjl *et al.*, 1998). Attached Review #7, by Caron Blumenthal, provides a background review of the range of heat-shock proteins and chaperones and their likely action in determining protein conformation and functional properties following the formation of primary structure.

Heat-shock proteins and protein conformation

The presence of heat-shock proteins (HSPs) as a result of heat shock has been identified in many of the heat-stress studies, namely, Blumenthal *et al.* (1990a,d; 1998), Skylas *et al.* (2002a) and Butow *et al.* (Report #1). Most prominent of the heat-shock proteins in SDS-PAGE analyses has been HSP 70, with a molecular weight of about 70,000 Daltons. The amounts of HSP 70 in the control and heat-stressed samples were determined for the set of 45 varieties (Figure 5). The amount of HSP 70 increased as a result of the heat-shock treatment for all varieties, but these increases were not clearly related to the changes in dough strength.

This result does not support the proposal of Bernardin *et al.* (1994) that the presence of HSP 70, as a result of heat shock, is a likely cause of the heat-related loss of dough strength. Further exploration of this proposal was pursued by purifying sufficient of the HSP 70 polypeptide to permit its addition to dough, to see if HSP 70 has the direct effect itself of causing dough weakening. As Figure 7 shows, there was no significant change in the Mixogram profile as a result of adding HSP 70 at a level considerably above what would be expected for the accumulation of HSP 70 in mature grain that had been heat-shocked daily during grain filling (Blumenthal *et al.*, 1998). Nevertheless, HSPs and chaperones are presumably important in determining protein structure and the polymerisation of glutenin subunits in the large disulfide-linked polymers that are shown above to be critical to dough strength (Shewry, 1999).

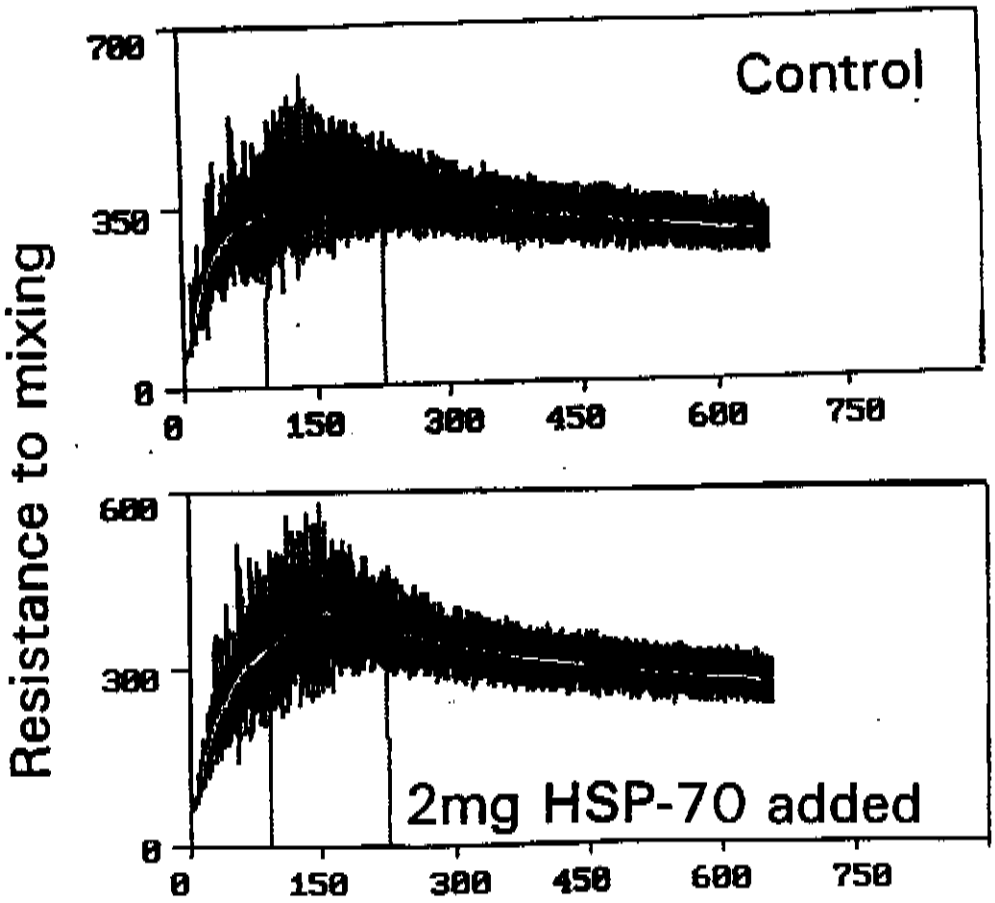


Figure 7. Mixograms for a base flour (upper) and for this same sample to which has been added 2 mg purified HSP 70. From Blumenthal *et al.* (1998).

Proteome analysis of heat-shocked endosperm

The new approach of proteome analysis offers good prospects for studying the range of polypeptides that may be involved in determining the changes in the structure of the gluten proteins as a result of heat stress (Skylas *et al.*, 2002a). In addition, this approach has the attraction of providing marker proteins that might indicate tolerance to the dough-weakening effects of heat shock, thereby avoiding the long process of growing wheat genotypes under controlled conditions and testing for dough quality.

The proteome concept involves the analysis and characterisation of all the polypeptides (after rupture of SS bonds) in a specific tissue, for a given genotype as a result of specified growth conditions. Initial experiments involved proteome analysis of immature endosperm, as well as mature grain, for grain grown under normal conditions, without any stress (Skylas *et al.*, 2000a). Two-dimensional (2-D) gel electrophoresis separated over 1,300 protein spots for endosperm 17 days after flowering. Over 300 of these were excised and submitted to N-terminal amino-acid analysis. Many of these could be identified as being similar or identical to known proteins in the data-bases searched. Particularly relevant to the formation of the very large glutenin polymers were several spots identified as isoforms of protein disulfide isomerase.

The proteome approach was then extended to compare the effects of heat shock on two wheat varieties differing in heat-tolerance, in terms of processing quality (Table 9). These two wheat varieties (heat-tolerant Fang and heat-susceptible Wyuna) were heat stressed as plants at 40^{0}C for three days during grain filling. They were subsequently analysed for dough quality characteristics and protein composition. Dough quality testing of flour confirmed the heat-susceptibility of Wyuna, whilst the heat-tolerant Fang increased slightly in dough strength.

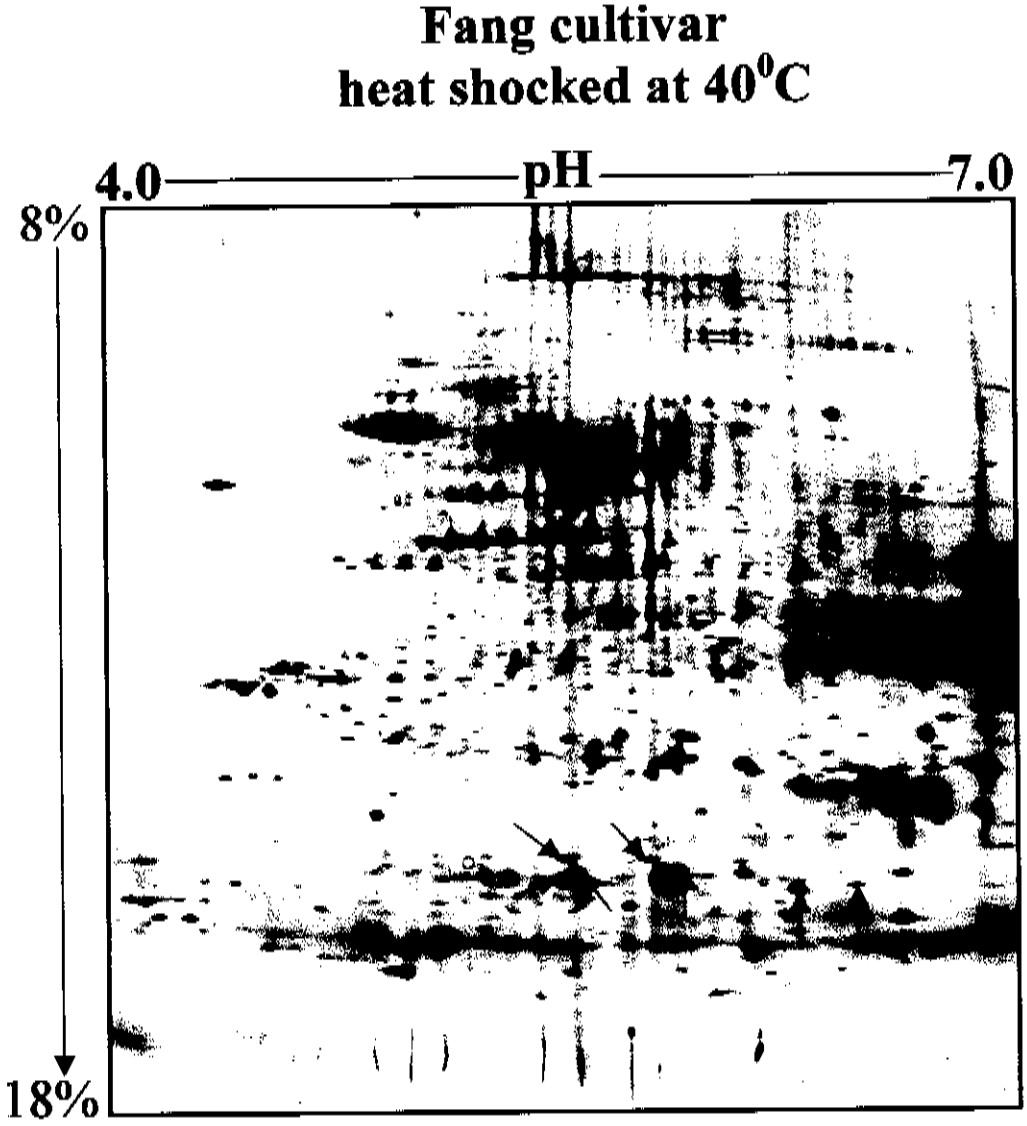


Figure 8. Proteome analysis of endosperm polypeptides from immature grain (17 days after flowering) of cultivar Fang, after heat stress of the plants, showing only the acidic range of the proteome map. Arrowed spots do not appear in the protcome map for the control sample (unstressed). From Wrigley *et al.* (2002).

These proteome studies, conducted on immature endosperm (17 days post-anthesis), showed that the heat-tolerant Fang cultivar exhibited a stronger and more diverse heat shock response than Wyuna. In total, 48 protein spots exhibiting differential expression between control and heat shock treatments, were excised from gels and analysed by mass spectrometry. The resultant tryptic-peptide mass fingerprint data was submitted to SWISS-PROT and TrEMBL databases for protein identification. The majority of heat-shock associated proteins had low molecular mass and showed database similarity to previously characterised small heat-shock proteins. Several discrete isoforms of the low molecular weight heat-shock proteins were observed as differentially expressed between the two cultivars. Seven protein spots, expressed in heat-shocked Fang but not in heat-shocked Wyuna, were further characterised utilising tandem mass spectrometry. The majority of these proteins had low molecular weights in the 16-17 kDa range, matching previously characterised small heat-shock proteins from both wheat and *Arabidopsis thaliana*. The wheat-grain endosperm proteome for the heat-tolerant Fang cultivar is shown in Figure 8. The seven heat-responsive proteins unique to the Fang cultivar are labelled with arrows. These possible marker proteins for heat-tolerance offer the prospect of assisting breeders in the selection of heat-tolerant cultivars that would not be expected to lose dough strength in such an environment. Patent applications (Anon, 2000a, 2001) were lodged to

cover these results, but they were allowed to lapse due to concern that commercial exploitation would be difficult. None of the spots identified as iso-forms of protein disulfide isomerase were among the polypeptides that altered as a result of the heat stress, so there was no evidence of this mechanism as a means of altering the molecular-weight profiles of the glutenin polymers observed to be critical to dough-quality changes.

Table 9. Effects of heat shock on the dough properties and average molecular size (Av. Mol. Size, in arbitrary units) of glutenin polymers at maturity for two wheats of contrasting heat susceptibility. From Skylas *et al.* (2001).

	Wyuna (heat susceptible)				Fang (heat tolerant)	
	Control	Heat-shocked			Control	Heat-shocked
		Early	Mid	Late		Mid
Mix time (sec.)	121	113	97*	109*	135	154*
Breakdown (%)	29	34*	33*	31	15	16
Av. molecular size	2.55	-	2.49*	-	2.52	2.50

* Significantly different (at 0.05 level) from corresponding control.

Heat stress and starch properties

Heat shock causes limited changes in the starch properties of the endosperm. The most evident changes are in the ratio of large (A-type) starch granules to the small B-type granules. Analysis of this A:B ratio for the 45 genotypes that were tested for tolerance/susceptibility to heat shock (Figure 5) showed that there were considerable genotypic differences in susceptibility for changes in this aspect of starch quality.

This variability in susceptibility is shown in Figure 9. Almost all of the 45 genotypes were susceptible to the effects of heat shock. The most tolerant genotypes (from the extreme left in Figure 9) were 6384, Ulla, 6386 and Trigo 1. The most susceptible genotypes (from the extreme right of Figure 9) were Wyuna, Machete, Grebe and Oxley. Susceptibility/tolerance to the effects of heat shock on starch-granule size did not relate consistently to susceptibility/tolerance to the effects of heat shock on dough properties. Figure 10 shows the full spread of starch-granule size distribution for Hartog grain from heat-shocked and control plants. The heat-shocked starch had 27% B-type granules, compared to 18% B-type granules for the control sample. Increased proportions of A-types starch granules following heat shock has since been reported by Panozzo and Eagles (1998), who also found modest increases in the proportion of amylose to be associated with growth temperatures of over 30°C in the first 14 days after flowering.

There were also differences in the pasting properties of starch for grain from heat-shocked plants, compared to control plants (Table 10) (Beasley *et al.*, 2000), with a general loss of pasting properties as a result of heat shock. These differences were observed for the variety Hartog only, not for a wider range of varieties, so there are no data yet on tolerance/susceptibility with respect to this aspect of starch properties. No differences were revealed by HPLC with respect to amylose:amylopectin ratios due to heat shock.

Table 10. Differences in RVA parameters of heat-shocked and control starch and flour. Results are means of duplicates; coefficients of variation were less than 4%. From Beasley *et al.* (2000).

	Flour samples				Starch samples			
	Peak	Trough	Final	Setback	Peak	Trough	Final	Setback
Heat-shocked	183	103	221	117	293	228	428	200
Control	276	121	253	133	320	206	401	195

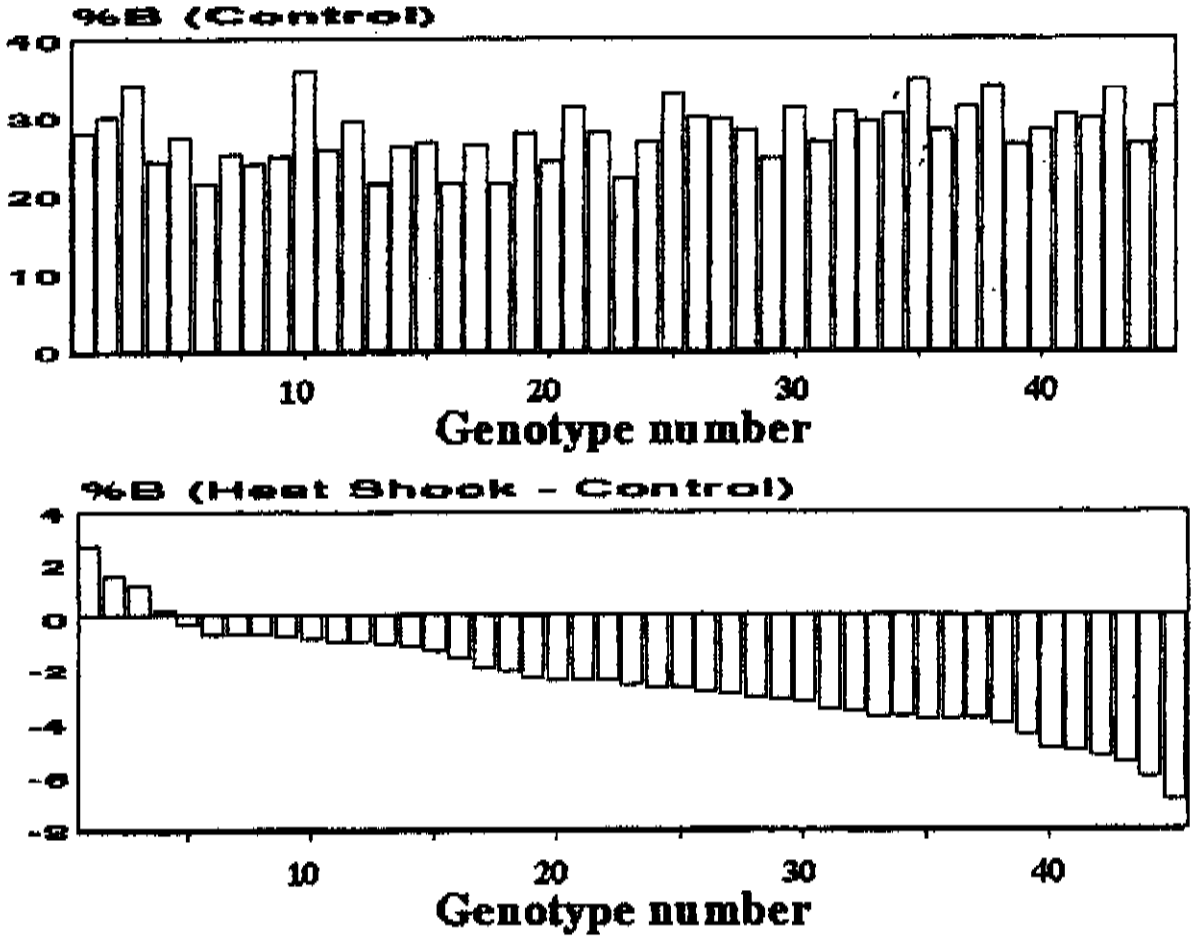


Figure 9. Proportions of small (B-type) starch granules in control grain samples (upper row) and differences due to heat shock (heat-stressed minus control) in lower row for the 45 genotypes referred to in Figure 5. From Blumenthal *et al.* (1995a).

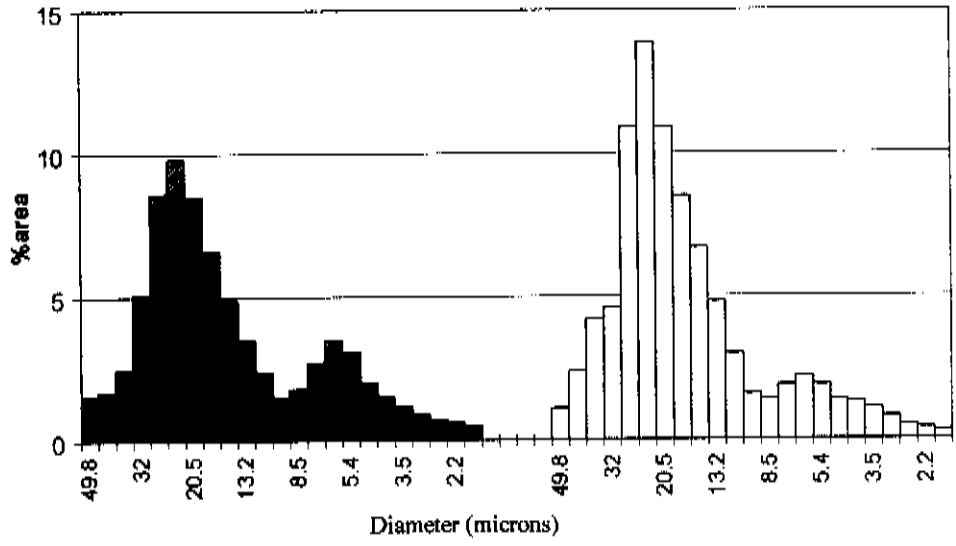


Figure 10. Differences in the ratio of A-type (>10μm) to B-type(<10μm) granules in the starch of Hartog grain, grown under control (hatched) and heat-shocked conditions. From Beasley *et al.* (2000).

Part 2. RECTIFYING THE PROBLEM

Considerable information on how to set about 'rectifying the problem' of dough-weakening due to heat stress was gathered in the research on 'defining the problem'. The various approaches are described in Part 2, namely, selecting for naturally occurring tolerance to the effects of heat shock on grain quality, using conventional or novel breeding approaches to confer tolerance, and using predictive measures to anticipate difficulties arising from the effects of heat stress so that avoidance measures can be taken.

RECTIFYING THE PROBLEM Approach #1
SELECTION FOR NATURALLY OCCURRING TOLERANCE TO HEAT STRESS

Surveying wheat genotypes for tolerance and possible marker proteins

The results obtained for many wheat genotypes during the past decade of these studies have provided evidence that there is considerable naturally occurring genotypic variability with respect to the effects of heat shock during grain filling on dough properties. The results in Figure 9 also indicate that there is naturally occurring tolerance to the effects of heat shock on starch properties. These facts have probably been a difficulty in the interpretation of results by other authors, depending on which specific genotypes have been used in experiments. Importantly, these facts offer the promise that appropriate selection of tolerant genotypes can help to solve the practical problems of heat shock with respect to grain quality. The identification of suitable markers of tolerance, such as come from the proteome studies, offer the practical advantage of permitting routine selection for tolerance, although the feasibility of doing so is yet to be proven.

The many results of the past decade of heat-shock research have already demonstrated a simple connection between a specific locus and susceptibility/tolerance, namely, the *Glu-D1* locus. In many cases, tolerance has been associated with the presence of HMW-glutenin subunits 5+10 (the *d* allele at the *Glu-D1* locus) and susceptibility has been associated with the 2+12 subunits (the *a* allele at the *Glu-D1* locus) (Blumenthal *et al.*, 1995b).

If this association proves to be general, it is likely to indicate that the effects of heat shock on Australia's premium strong wheats has been greater than in some other countries, because the HMW-glutenin subunits 2+12 (the *a* allele at the *Glu-D1* locus) have been common in the Australian Prime Hard wheats until the recent decade.

These observations for Prime Hard wheats and for the 5+10 *versus* 2+12 tolerance/susceptibility association are demonstrated in the field and glasshouse results described earlier in this report:

- Table 4, for the 2000/01 harvest, lists less dough weakening for Sunstate (*d* allele for *Glu-D1*) than for Janz, Batavia or Sunco (all having the *a* allele for *Glu-D1*).
- Songlen, Cook and Kite in Table 3 have the *a* allele for *Glu-D1*.
- Halberd and Grebe, shown to be tolerant in Figure 4 have the *d* allele for *Glu-D1*, but the two examples of susceptible genotypes in Figure 4, Batavia and Ella, have the *a* and *d* alleles for *Glu-D1*, respectively.
- The combination of Wyuna and Fang have been used as examples, respectively, of susceptible and tolerant varieties in much of the recent research. They have the *a* and *d* alleles, respectively, supporting the hypothesis that tolerance is associated with the *Glu-D1d* allele.
- Most importantly, the results for the survey of 45 wheats in Figure 5 show that most of the tolerant wheats have the 5+10 subunits (the white columns) whereas the susceptible wheats are mainly 2+12 types. Table 11 lists the actual genotypes in Figure 5, together with additional data about grain hardness, pedigree groupings, and rankings with respect to a wider range of quality results. Apart from the obvious association of tolerance to *Glu-D1* allele, no significant relationship was identified with respect to the other criteria, such as pedigree background, national origin or grain hardness (Blumenthal *et al.*, 1995a).
- Table 12 provides a statistical comparison of the results for the 2+12 lines *versus* the 5+10 lines in the set of 45 genotypes, listed in Table 11. Differences were highly significant between these two groups of genotypes with respect to the effects of heat treatment (heat-shocked minus control) on dough quality (time to peak development and rate of dough breakdown).
- Later results of other authors have indicated that there are differences in the specific susceptibilities of various genotypes. For example, Stone and Nicholas (1994, 1996) demonstrated that wheat varieties vary widely in responses to the effects of heat shock on quality. For further demonstration of these genotypic differences, they concentrated on two varieties, namely, Oxley and Egret. Egret (with HMW subunits 5+10) proved to be tolerant to heat stress, whereas Oxley (with subunits 2+12) was susceptible.

Table 11. Genotypes studied for tolerance to heat stress, as shown graphically in Figure 5, indicating their grain hardness, similarities in pedigree, allelic constitutions (*Glu-1*) for HMW glutenin subunits, and rankings (from 1 = most tolerant) for differences in attributes due to heat, together with indications (A,B,C) of significance of differences at P<0.05. Pedigree relationships are indicated according to the groupings of Wrigley *et al.* (1982).

Cultivars	Hard-Soft	Pedigree group	*Glu-1* alleles	Rankings Tolerant (1) → Susceptible					
				Mix time	Break-down	Peak height	Glu/Gli ratio	%B granules	% large polymers
6372	H	XIII	cid	3B	3B	36B	6A	7B	4A
6384	H	XIII	aid	10B	9B	22B	14A	1A	20B
6385	H	XIII	abd	13B	25B	45C	19A	5B	35B
6386	H	XIII	acd	1A	5B	23B	4A	3B	1A
Aroona	H	VIII	aca	38C	33B	25B	28B	12B	29B
Banks	H	VII	bba	16B	24B	33B	9A	26C	18B
Batavia	H	VII	aba	41C	44A	18A	18A	15B	42C
Condor	H	VII	cba	21B	35A	18A	27B	35C	11B
Croesus	S	XIII	cca	28B	23B	43B	35B	18B	21B
Cunningham	H	VII	aba	26B	15B	16A	11A	32C	22B
Dagger	H	VIII	acd	22B	13B	40B	12A	10B	40C
Dollarbird	H	XIV	aid	33C	27B	21B	7A	31C	15B
Ella	S	XIII	abd	42C	20B	44B	10A	8B	45C
Fang	H	XIII	cid	5B	6B	35B	13A	34C	2A
Grebe	S	I +sec1	ccd	2A	11B	1A	36B	42C	3A
Halberd	H	I	acd	11B	4B	38B	21B	13B	5B
Hartog	H	XI	aid	31B	8B	34B	38B	11B	14B
Janz	H	VII	aba	29B	41A	15A	31B	37C	23A
Kamilaroi	H	XII	ce-	17B	10B	43B	26B	27C	38C
Kite	H	V	bia	27B	30B	6A	30B	33C	34B
Kogat	S	XIII	cid	14B	17B	32B	5A	22B	36B
Kulin	S	XI	bia	40C	37A	14A	22B	39B	39B
Lark	H	VII	bia	39C	45A	13A	40B	45C	33B
Lyallpur	H	XIII	aid	12B	40A	26B	3A	17C	19B
Machete	H	XI	bia	32B	32B	9A	43B	23C	28B
Matong	S	III	aed	37C	42A	20B	45B	24B	31B
ME71	H	XIII	ccd	35C	7B	41B	25B	29C	32B
Meering	H	VII	bba	18B	39A	11A	41B	14C	37B
Millewa	H	XIV	cia	15B	28B	28B	2A	19C	27B
Miskle	H	VII	bia	30B	22B	5A	29B	36B	10B
Molineux	H	VIII	abd	7B	14B	31B	16A	43B	9B
Oligoculm	H	XIII	cba	23B	21B	17A	37B	36C	24B
Oxley	H	VII	bba	20B	34B	2A	33B	43C	13A
Scandia	H	XIII	bba	8B	18B	39B	34B	40C	8B
Schomburgk	H	VIII	aba	36C	16B	4A	24B	30C	41C
Sunco	H	VII	aba	25B	29B	12A	17A	41C	12B
Suneca	H	XI	aid	4B	2B	37B	15A	16B	7B
Tatiara	S	VIII	aca	9B	19B	27B	39B	28C	6B
Tincurrin	S	IV	bba	34C	38A	19B	20A	21C	17B
Trigo 1	H	XIII	cba	44C	36A	24B	8A	4B	44C
Ulla	H	XIII	abd	6B	1C	30B	1A	2B	16B
Veery	H	XIII	aid	24B	12B	29B	23B	20C	26B
Vulcan	H	VII	aia	19B	31B	3A	32B	38C	25A
WW80	H	VII	cia	43C	26B	8A	44B	25C	43C
Wyuna	S	III	bia	45C	43A	10A	42B	44C	30B

Table 12. Comparison of 2+12 and 5+10 lines in the set of 45 wheats listed in Table 11, showing means values for control samples and for heat-shocked minus control values. The significance of differences between the two groups of genotypes are shown as P values in the last column.

Attribute	*Glu-D1a* Subunits 2+12	*Glu-D1d* Subunits 5+10	P
Grain protein content (%)			
Control samples	12.1	12.8	0.18
Heat shocked – Control	2.4	1.7	0.05
Mix time (sec.)			
Control samples	263	235	0.22
Heat shocked – Control	-44	-4	0.001
Breakdown			
Control samples	14.4	20.0	<0.001
Heat shocked – Control	4.5	-0.2	<0.001
Glutenin:gliadin ratio			
Control samples	0.76	0.71	0.16
Heat shocked – Control	-0.05	-0.03	0.04

Biotypes of Kewell, Avocet, Warigal and Lance

A series of near isogenic lines, differing at the *Glu-D1* locus provided a further opportunity to evaluate the observation that tolerance/susceptibility to heat shock is associated with the HMW subunits 5+10/2+12, respectively. The four Australian wheats Kewell, Avocet, Warigal and Lance occur naturally with biotypes differing at the *Glu-D1* locus. These biotypes have been isolated, so that lines of each are available having either HMW subunits 2+12 (*Glu-D1a*) or subunits 5+10 (*Glu-D1d*). Plants of these genotypes were grown in the glasshouse, some left as controls and some heat stressed under the conditions applied to the set of 45 genotypes (Blumenthal *et al.*, 1995a).

Flour was milled from the mature grain in the Quadrumat Junior mill, and dough strength was assessed in the Mixograph as the time to reach peak resistance to mixing (Table 13). The greater strength of the 5+10 biotypes is evident for all four varieties. For all four of the 2+12 biotypes, heat shock produced a considerable loss of dough strength. For the 5+10 biotypes of two of the varieties (Kewell and Avocet), dough strength increased slightly. For the 5+10 biotype of Warigal, dough strength did not change significantly. For the 5+10 biotype of Lance, there was a significant loss of dough strength. Nevertheless, the hypothesis that the *Glu-D1d* is associated with heat tolerance was upheld for three of the four pairs of biotypes, with significance at the level of $P<0.05$ for the comparison of control minus heat-shock results.

Table 13. Dough strength (as time in seconds to peak resistance) for *Glu-D1* biotypes of four varieties, comparing the effects of heat shock during grain filling. Statistical significance figures compare the heat-shock reactions of the *Glu-D1a* biotypes *versus* that of the *Glu-D1d* biotypes, with respect to control minus heat shock values.

Variety	*Glu-D1a* Subunits 2+12		*Glu-D1d* Subunits 5+10		Significance of
	Control	Heat shocked	Control	Heat shocked	*a versus d*
Kewell	151	125	228	235	P<0.05
Avocet	191	142	242	261	P<0.05
Warigal	215	155	262	253	P<0.05
Lance	267	202	388	301	Not significant

Heat tolerance of lines lacking HMW subunits of glutenin

Given the apparent importance of the HWM subunits of glutenin to the effects of heat shock on dough quality, a set of experiments was undertaken to examine the effects of heat stress on a set of lines that are deficient for these subunits. These lines are based on a Gabo-Olympic cross, involving null alleles for the *Glu-1* loci. There are considerable losses of dough strength as a consequence of the progressive absence of the HMW subunits

(Lawrence *et al.*, 1988). Three of these lines were chosen for the heat-shock experiment, namely, the line with the full set of HMW subunits, one lacking the *Glu-D1* allele (the single-null line), and one lacking all three of the *Glu-1* alleles (the triple-null line with no HMW subunits).

These genotypes were grown together with Fang and Wyuna, as tolerant and susceptible varieties for comparison. Plants of all genotypes were heat stressed at 39°C at 18, 19 and 20 days post anthesis. Grain samples were taken at various stages of immaturity as well as at maturity. The effects of heat stress were assessed by biochemical methods, because there was insufficient mature grain to permit milling and dough testing. The results are provided in the attached Report #3 by Butow and Bariana, entitled "Heat shock of wheat genotypes lacking alleles for the HMW subunits of glutenin". Estimates of the proportion of very large glutenin polymers reflected the extreme weakness of the triple-null line. This status was further reduced by heat stress for the triple null line, but the proportion of large glutenin in the single-null line was not significantly affected by heat shock.

Heat tolerance of current Australian wheats

Sixteen of the most important Australian varieties were grown in the glasshouse and under field conditions to evaluate their tolerance to fluctuations in growth temperatures (both in the modest range and heat shock). The results are provided in two of the attached Unpublished Reports (#4 and #5). The varieties selected for evaluation were Amery, Cadoux, Carnivale, Frame, Hartog, Janz, Kallanie, Krichauff, Silverstar, Sunvale, Tailor, Wallaroi, and the unnamed advanced lines 1493, 1413, 2109, and 2024. The results were sought to permit the development of predictive measures for assessing crop quality and to plan breeding for heat tolerance, in which these wheats (or their near relatives) might be used as parents.

The controlled temperature conditions of the Canberra Phytotron were used regulate growth conditions for the results described in Report #4 by Barbara Butow, entitled "The effects of growth-temperature variations on 16 Australian wheats, assessed in the Phytotron." In addition to the imposition of heat stress (a few days at 40°C), additional experiments were conducted at modest temperatures throughout grain filling, namely, at 23, 26, 29 and 32°C. These increases in the moderate range had beneficial effects on the yield and quality of many of the varieties. The heat shock treatment permitted the ranking of the varieties from susceptible (e.g. Frame, 2109, Tailor, Janz) to tolerant (e.g. Kallanie, Krichauff, 1493, Sunvale) to the effects of shock on dough strength (as mix time in the Mixograph). The report provides comments on the individual performance of each variety.

In an attempt to obtain practical results from field trials, grain of the 16 varieties was sown early and late at sites in the regions of Narrabri, Newdegate and Wongan Hills, as described in Report #5 by Barbara Butow, entitled "Field trials to determine the effects of growth-temperature variations on 16 Australian wheats." The aim of the early and late sowings was to obtain contrasting growth temperatures, particularly the opportunity to observe the effects of heat shock for the late-sown crops. However, temperature conditions were not hot enough to provide heat-shock conditions at Narrabri and Newdegate leading to the harvest of 1998.

At Wongan Hills, there were a few days of warm weather (30 to 36°C) for the late-sown crop, permitting limited assessment of the effects of heat shock. However, it appeared that the effects of this modest heat stress was complicated by differences between the results for duplicated plots for the same sowing dates. Overall, it did not prove possible to obtain conclusive results from the field trials with respect to the relative heat-shock tolerances of the 16 varieties. This unsatisfactory conclusion highlights the problems of attempting to study the effects on yield and quality of growth conditions generally.

RECTIFYING THE PROBLEM Approach #2
CONVENTIONAL BREEDING GENOTYPIC TOLERANCE TO HEAT SHOCK

Given the evidence that genotypes differ in their tolerance to the effects of heat shock on grain quality, it is relevant to determine the extent to which this trait is heritable, to determine how to select efficiently for tolerance and to identify useful molecular markers of tolerance. Progeny were available from several crosses (some being sets of doubled-haploid lines), so the parents of these crosses were tested by heat stress to determine wheter these would provide contrasts in heat tolerance. The parents tested were from the crosses 'Mexico' X 'Israel', Chinese Spring X CD-1D Cheyenne and Halberd X Cranbrook. However, none of these combinations provided useful contrasts in heat tolerance.

Accordingly, crosses were made between varieties that were established as tolerant (Fang and Grebe) and susceptible (Wyuna and Batavia), after the re-testing of these genotypes to establish their tolerance/susceptibility (Table 14). The reassessment established their tolerance status, namely, mean heat shock minus control mix times of +14 and + 35 seconds for Fang and Grebe, in contrast to time differences of −105 and −103 seconds for Wyuna and Batavia, respectively.

Table 14. Repeat evaluation of the tolerance/susceptibility to heat shock of four varieties for use as parents in cross-breeding experiments. Dough strength is shown as time (seconds) to peak resistance in the two-gram Mixograph.

Variety	Trial #1		Trial #2	
	Control	Heat-shocked	Control	Heat-shocked
Fang	221	256	189	182
Grebe	84	155	149	148
Wyuna	345	196	224	164
Batavia	226	133	262	149

F1 crosses between tolerant and susceptible lines were used to produce doubled haploid lines, as the most efficient means of providing a series of homozygous families for evaluation, but the resulting set of genotypes did not prove suitable for the production of doubled haploid lines. As an alternative, the crosses were selfed for several generations to produce near homozygous genotypes. The progeny of the Batavia X Fang population was selected for heat shock testing.

Grain of about 50 families of advanced lines was grown under normal conditions until mid-grain filling, when half of the plants of each line was subjected to heat-shock conditions. Unfortunately, however, the yield of grain was low for many of the lines, so that milling and dough-testing was not possible for most of them.

RECTIFYING THE PROBLEM Approach #3
GENETIC MANIPULATION TO PRODUCE GENOTYPIC TOLERANCE TO HEAT STRESS

Knowledge about the causes of heat-related loss of dough strength at the molecular level should lead to the development of strategies for rectifying the problem by appropriate genetic manipulation. The sections on "Defining the problem" indicated several likely causes for the dough-quality loss, including reduced synthesis of glutenin as a result of heat shock and reduced size of glutenin polymers. The former of these two causes is presumed to involve the lack of heat-shock elements (HSEs) upstream of the coding regions of the glutenin genes, whilst HSEs appear to be present for some of the gliadin genes. Tolerance to heat shock may thus involve the action of HSEs associated with glutenin genes. If this were found to be so, one approach to 'rectifying the problem' would be to use a transformation approach to incorporate glutenin genes having the appropriate heat-shock promoters.

Search for HSEs in glutenin gene sequences

Accordingly, the PCR approach was used in an attempt to find HSEs in the glutenin genes of heat-tolerant genotypes. Genomic DNA was extracted from leaf tissue of four genotypes, namely, Banks, ME71, Wyuna and 6386, representing a range of two tolerant and two susceptible lines, respectively (Blumenthal *et al.*, 1998). Three primers were used to isolate sequences appropriate to the up-stream regions of the glutenin genes. Sequence analysis of the large and small PCR products revealed conservation between the sequences in these four genotypes. It was thus not possible to identify differences, such as HSEs, that could be construed to explain the tolerances or susceptibilities of these genotypes. It was thus concluded that tolerance may more likely be due to the action of heat-inducible proteins, such as members of the heat-shock protein family, acting as chaperones. Evidence for this conclusion comes from earlier observations of HSPs after heat shock, and the proteome analyses comparing the polypeptides of tolerant and susceptible genotypes after heat shock (Skylas *et al.*, 2002a).

Sequencing of heat-shock promoters

An alternative approach to the genetic manipulation of heat tolerance is to obtain sequence information about heat-shock promoters that could be used in transformation experiments to provide enhanced synthesis of glutenin during heat episodes. To this end, full nucleotide sequences were obtained for the promoter region and the part coding for two genes of the heat-shock 70 family in *Triticum tauschii*, the progenitor of the D-genome of hexaploid wheat. This research is described in attached Report #6, by Caron Blumenthal, entitled "Nucleotide sequences of the promoter regions of two HSP70 genes from *Triticum tauschii*". One of these is probably heat inducible, based on its classical pattern of heat-shock elements. The gene sequence has indications of HSEs, an N-terminal intron and other important regions, such as a GC-rich cluster adjacent to the TATA box and an AT-rich region thought to be needed for transcriptional activation. It is highly homologous to both the maize and rice HSP 70 inducible genes (Figure 11). The second HSP 70 sequence is more likely to be constitutive, based on its sequence homology to prokaryotic heat-shock genes. It has a less well structured heat-shock promoter region.

N-TERMINAL SEQUENCES FOR HSP70 PROTEINS

HSP70 SOURCE																					
WHEAT			**A**	**K**	**G**	**E**	**G**	**P**	**A**	**I**	**G**	**I**	**D**	**L**	**G**	**T**	**T**	**Y**	**S**	**X**	**V**
ARABIDOPSIS	**M**	**S**	**G**	**K**	**G**	**E**	**G**	**P**	**A**	**I**	**G**	**I**	**D**	**L**	**G**	**T**	**T**	**Y**	**S**	**C**	**V**
MAIZE	**M**		**A**	**K**	**S**	**E**	**G**	**P**	**A**	**I**	**G**	**I**	**D**	**L**	**G**	**T**	**T**	**Y**	**S**	**C**	**C** **V**
CHLAMYDOMONAS	**M**		**G**	**K**		**E**	**A**	**P**	**A**	**I**	**G**	**I**	**D**	**L**	**G**	**T**	**T**	**Y**	**S**		
SOYBEAN	**M**	**A**	**I**	**K**		**E**	**G**	**K**	**A**	**I**	**G**	**I**	**D**	**L**	**G**	**T**	**T**	**Y**	**S**	**C**	**V**
CARROT	**M**	**A**	**S**	**K**	**K**	**G**	**G**	**K**	**A**	**I**	**G**	**I**	**D**	**L**	**G**	**T**	**T**	**Y**	**S**	**C**	**V**

Figure 11. The N-terminal amino-acid sequence of HSP 70 determined for wheat (*T. tauschii* in Report #6), aligned with the published sequences for *Arabidopsis*, maize, *Clamydomonas*, soybean and carrot, to show the high degree of conservation of sequence.

This pair of promoters offers access to two types of temperature-responsive regulatory elements for potential use in transformation. At the time of their sequencing in the late 1990s, heat-shock promoters of these types had not previously been available in wheat (according to data-base listings and patent search). *In situ* hybridisation was sought through collaboration with a Japanese laboratory, to determine the chromosomal locations of the genes, but results were not forth-coming.

These approaches to the production of heat tolerance have not been pursued further, largely because of the progressive resistance to the cultivation of genetically modified crops. Whilst the demonstration (or otherwise) of the success of this approach to the generation of heat tolerance would be valuable with respect to increasing our understanding of such molecular mechanisms, its practical value would be minimal given the likelihood that it could not be implemented in practice.

RECTIFYING THE PROBLEM Approach #4
PREDICTION OF QUALITY CHANGE DUE TO TEMPERATURE VARIATION

Knowledge about the causes of heat-related loss of dough strength should also lead to the development of strategies for anticipating some of the problems by predicting the extent of change in grain quality due to temperature fluctuations. Doing so requires knowledge of local climate conditions during grain filling for the crop and knowledge about the susceptibility of the varieties involved. Historical data for specific regions are available to indicate the risk of temperature fluctuations, thus to assist in forward buying from specific growers.

After harvest, the local climate details are known, so buyers are aware of the incidence of heat shock, for example. This knowledge must be coupled with information about the sowing date and maturity of the varieties involved to determine the grain-filling period, and information must be available about the sensitivity to temperature fluctuations of the varieties involved. Mechanisms are potentially available for providing all this information, but there is the need to have it incorporated into software to deliver the information in useable form.

In parallel with these predictive strategies, diagnostic methods would be valuable to identify grain samples that had been subjected to extremes of temperature, thus alerting buyers to the possibility that the grain quality might be other than what would be expected for the given combination of variety and protein content.

Mapping regions of heat stress

Climate data are available for all parts of the wheat belt, going back at least 30 years. The statistics provide a good basis for predicting future climate conditions for specific dates. However, it is obviously impossible to use these risk data to anticipate the specific dates when heat-shock conditions will occur.

For example, the Bureau of Meteorology issues a three-month seasonal climate outlook summary, to indicate for example the probability of maximum temperatures being above normal. Relevant web sites are

- http://www.bom.gov.au/climate/ahead/temps_ahead.shtml
- http://www.bom.gov.au/silo
- http://www.bom.gov.au/climate/c20thc

CSIRO Plant Industry, Canberra, has used historic temperature data to develop easy-to-use software packages (*SliDevel* and *ShowDevel*) which can predict the times of anthesis (flowering) and maturity for wheat crops, given the date of sowing, the site (as Post Code) and the variety (taking into account its maturity). The programs were originally developed to help growers in selecting the most suitable date of sowing to avoid the risks of frost at anthesis and of heat stress during grain filling. In addition, the programs can be sued to indicate the temperatures likely to be encountered during grain filling, given the sowing date, locality and variety.

The further important contribution needed for this program to be used effectively is an indication of the susceptibility of the variety to the effects of heat shock, if the temperature fluctuations indicate this risk. Obviously, this contribution is also needed in considering the likely loss of quality when, after grain filling, it is evident that the crop has been subjected to heat shock. The tolerance/susceptibility has been characterised for many wheats in the course of the past decade's research, as described in several sections above, but this information is not available for many of the wheats currently in production. The provision of this information is an on-going task.

Modelling climate-quality relationships

Initial success at modelling the effects of temperature on grain quality was demonstrated by Randall and Moss (1990), who analysed historic data for three sites (Narrabri, Wagga Wagga and Dooen) to predict grain protein content (Table 15). Their model covered actual variations in the data quite successfully, with 73 % to 82% of the variation being predicted. The most significant factor in the model (indicated as *** in Table 15) was the constant associated with maximum temperatures, indicating again the importance of heat-stress conditions to grain quality.

Table 15. Prediction of grain protein content, based on maximum (Tmax) and minimum temperatures (Tmin) at three growth sites, using the respective constants (a, b, c) in the equation %protein = a + b(Tmax) + c(Tmin). From Randall and Moss (1990).

Growth site	Constant a	Constant b	Constant c	Variation covered
Narrabri	-45	2.3***	-0.6	82%
Wagga Wagga	-19	1.1***	0	73%
Dooen	+3	0.4***	-0.2	75%

More recently, the protein content of silo receivals of wheat and of barley have been modelled for South Australia by Correll *et al.* (1994). The most important factors that determined protein content were winter rainfall)May to September) and spring heat (as days >30°C in October and November). The model narrowed the prediction for the protein content of grain being received at a specific silo from ±2%, based on the site mean, to ±0.8%, based on the climate conditions of a specific season. Thus, as a season progressed, the prediction narrowed for a specific silo as climate data were added right up to the time of harvest. This information is of great importance for the forward marketing of grain, and also for the grain-handling corporation, as it indicates the volume of grain in specific quality grades, thereby facilitating the allocation of storage as well as helping the logistics of transport and sea-board loading.

RECTIFYING THE PROBLEM Approach #5
IDENTIFICATION OF HEAT-STRESSED GRAIN BY SIMPLE METHODS
There is a need for diagnostic tests to identify grain samples that have been subjected to extremes of temperature, thus alerting buyers to the possibility that grain quality is affected. Such tests must be simple and cost-effective, preferably involving portable equipment and taking minimal time.

NIR
Near infrared (NIR) spectroscopy offers most of these advantages. This methodology has revolutionised the classification of grain according to quality. NIR analysis was applied to over 200 grain and flour samples from the set of 45 genotypes that had been subjected to heat shock in the glasshouse, together with control samples (no heat shock). They were scanned with an NIRSystems 6500 spectrometer and spectra were analysed using ISI software. NIR discriminate analysis was capable of grouping these samples on the basis of their different growth conditions (Figure 12). This approach is thus a promising possibility for the identification of grain that has a history of heat stress. Further details of these preliminary experiments are provided in the attached Report #8, by Blumenthal and Wrigley, entitled "Prediction of dough quality variations due to environmental factors; identification of environmental history by NIR", which is an application for funding to permit the involvement of the Grain Industries Centre for NIR to pursue the promising initial results to a provide a robust protocol for routine analysis. However, it did not prove possible to pursue the proposal at that time.

However, some of the low-molecular-weight heat-shock proteins, such as HSP 18, also persist in the mature grain after heat shock (Skylas *et al.*, 2002a). The use of HSP 18 as the antigen has the potential advantage over HSP 70 that it is produced only after stress conditions, so that there is no background level for control samples. There is thus the possibility of developing an immuno-assay based on other HSPs such as these, thereby avoiding complications from the Bernardin patent, which is specifically based on the presence of HSP 70. If such a kit were made available, it would allow buyers to test grain as part of the grain-assessment process, using a test-card version of the assay. Secondly, an ELISA version of the assay would serve the purpose of screening grain samples at a regional or central laboratory. In this way, processors or exporters would be alerted to the likelihood that dough properties could be less than would be expected by the combination of variety and protein content.

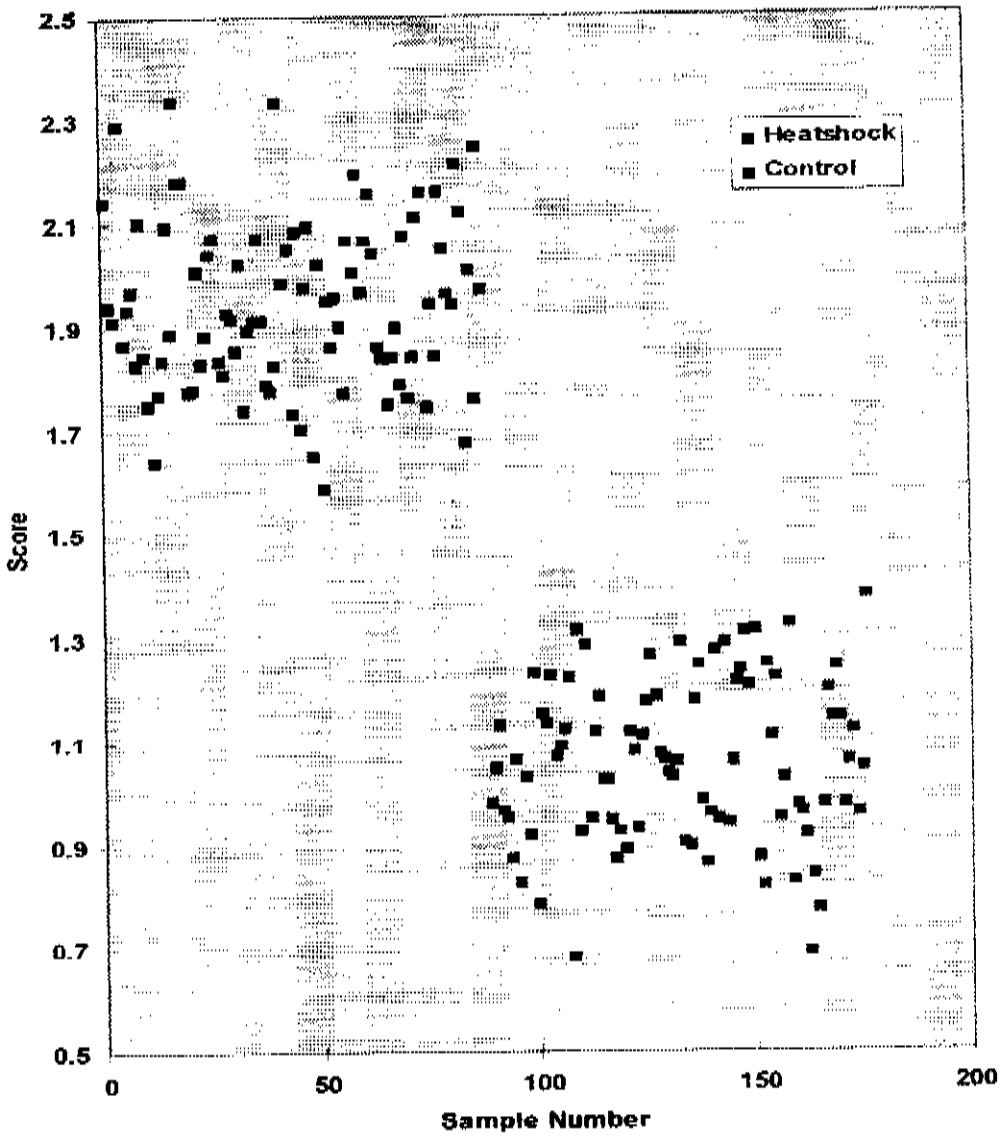


Figure 12. Distinction between grain from heat-shocked plants and control samples by NIR. (Collaboration with Dr Ian Wesley.)

OPPORTUNITIES ARISING FROM THESE STUDIES

The research described in this report goes a long way towards elucidating major sources of inconsistency in grain quality, thereby contributing towards the industry request for "Novel ways of reducing the impact of environmental factors on quality determinants." The second part of the report, "Rectifying the problem", offers several "novel ways" of reducing the impact on grain quality of temperature fluctuations in temperature during grain filling. Some of these approaches can be implemented by industry without the need for any further commercial development. Other approaches require further development effort, such as the production of user-friendly software, the characterisation of heat-tolerance of current varieties, and the production/evaluation of simple diagnostic systems. The best long-term approach is the production of heat-tolerant wheat varieties, but this requires the verification of potential markers and their use in screening progeny during breeding.

Some of the findings need to be pursued further. For example, results on the effects of temperature fluctuations on starch quality have not been extended as far as the research on dough properties. However, many of the grain and flour samples from the growth experiments are available for extending research on other aspects of grain quality. Furthermore, some of the research findings are likely to be applicable to other cereals, especially barley, for which high temperatures are known to affect malting quality.

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Wheat-grain quality under post-anthesis water and heat stress.

Report #2.
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Transport of stored carbohydrates into developing wheat kernels and its contribution to grain yield under post-anthesis water stress and elevated temperature.

Report #3.
Butow, B.J., and Bariana, H.
Heat shock of wheat genotypes lacking alleles for the HMW subunits of glutenin.

Report #4.
Butow, B.J.
Evaluation of the tolerance to heat stress during growth of 16 wheat varieties currently grown in Australia: Phytotron experiment.

Report #5.
Butow, B.J.
Field trials to determine the effects of growth-temperature variations on 16 Australian wheats.

Report #6.
Blumenthal, C.
Nucleotide sequences of the promoter regions of two HSP70 genes from *Triticum tauschii.*

Report #7.
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A review of heat-shock proteins and chaperones involved in the determination of protein conformation.

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Attached Report #1.
Wheat-Grain Quality Under Post-Anthesis Water and Heat Stress

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ABSTRACT
Two wheat cultivars, differing in heat susceptibility, were subjected to post-anthesis water and heat stress. Three days' heat stress (39°C for 3 days at 12 DPA) caused an increase of 6 % grain protein in the heat-tolerant cv. (*Triticum aestivum* L. 'Suneca') and of 8.4% protein in the heat-susceptible cv. (*Triticum aestivum* L. 'Batavia'). The simultaneous application of both heat and water stress further increased the protein content of these cultivars by 15.6% and 17.8 % respectively. The dough strength characteristics varied considerably for each cultivar, whereby heat and water stress caused an increase in mixing time (MT) in Suneca, and heat stress alone caused a weakening of dough properties for Batavia. Water stress did ameliorate the negative effects of heat stress on MT in Batavia though and, unlike Suneca, significantly increased peak resistance (PR) regardless of heat stress. When Suneca was subjected to heat stress alone, two proteins of molecular weight 30,000 and 40,000 were identified.

INTRODUCTION
The hot, dry climate inherent to the Australian wheat belt is cause for concern for both the farmer and cereal chemist alike. An understanding of the effects of temperature and water stress on protein composition and dough quality is required in order to produce reliable crop yields with flour quality that can be confidently used by industry. In the field, drought and high temperature generally occur simultaneously. It is thus difficult to distinguish the actual cause for the decrease in yield and in the various dough quality parameters. These two environmental stresses may also differ in the mode of action and mechanism of their effect on wheat. High temperature probably has a direct effect on the secondary structure of proteins (Wolkers *et al.*, 1998), protein synthesis and in the induction of heat shock proteins. Other than evaporative cooling, which is dependant upon ambient humidity and air movement, plants have no other physiological device to protect themselves from adversely high temperatures. In contrast, stomatal conductance, leaf inclination and osmotic adjustment (via accumulation of organic and inorganic solutes) play an important role in limiting dehydration and severe damage by drought.

A well-documented response of plants to high temperature stress is the rapid and transient synthesis of new proteins (Vierling, 1991; Nguyen *et al.*, 1993). Many of these are known as heat shock proteins (HSP), and they have been found in many crop plants including wheat (Weng *et al.*, 1991; Kurek *et al.*, 1999). Although their mode of action is not well known, it is claimed that they perform a chaperone-like protective function under high temperature stress (Gething and Sambrook, 1992; Hartl and Martin, 1992). They may also play an important role in the recovery of plants after a period of subjection to high temperature. Much less is known concerning the synthesis and function of special proteins induced by water stress. Dehydrins, for example, are a family of proteins induced by environmental stresses associated with dehydration, but occur mainly during seed maturation (Kermode, 1997; Colmenero-Flores *et al.*, 1999). However, no definitive role has been found for these, nor for other late embryogenesis abundant (LEA) proteins, in increasing water stress tolerance during earlier stages of development. cDNAs encoding differnet families of proteins have been reported (Nguyen and Joshi, 1993), complicating any interpretation of the molecular basis for water stress tolerance. It is even possible that plants make use of a tolerance to heat stress in order to

withstand other environmental stresses. It is known that plants exposed earlier to mild heat stress have a higher ability to survive heat shock, as compared to those not pre-adapted (Blumenthal *et al.*, 1994) . Moreover, pre-exposure to high temperature endowed protection against chilling injury in tomato plants (Sabehat *et al.*, 1998) and mung beans (Collins *et al.*, 1995).

It is well established that high temperatures (>35°C) during grain filling, have detrimental effects on yield and dough quality in certain genotypes (Wardlaw and Wrigley, 1994). Likewise, water stress is a common cause of yield loss (Jamal *et al.*, 1996) and causes considerable damage to all plant functions. For example, drought-induced damage to photosynthetic apparatus is responsible for reduced assimilate availability for grain filling (Morgan, 1984). Little research has been carried out solely on the effects of drought on dough quality, although it has recently been shown that flour protein content increased and grain yield decreased with water stress (Plaut *et al.*, 1999). The functional properties of the resulting dough were not significantly altered however, apart from the consequences of increased protein content.

In this paper we investigate the separate and combined effects of heat and drought stress on protein quality and dough properties of two Australian wheats: Suneca, which exhibits dough properties resistant to heat stress and Batavia, a heat-susceptible variety (Lawrence, 1986; Blumenthal *et al.*, 1995).

MATERIALS AND METHODS

Growth conditions

Heat-tolerant (Suneca) and heat-susceptible (Batavia) cultivars of wheat were grown under optimal conditions (irrigated up to three times per week) in a temperature-controlled glasshouse at the University of Western Sydney, Hawkesbury during a 25/18°C day/night cycle over the summer period. In contrast to field conditions, the glasshouse or growth chamber environment had no breezes to provide evaporative cooling enabling the whole plant to heat up when required. Forty 3.5L cylindrical plastic pots, each housing ten plants, were set up for each cultivar. After the plants were established, the lateral tillers were excised leaving the main tiller. Plants were grown in potting mixture with Osmocote Plus and additional $(NH_4)_2SO_4$ and were fertilised tri-weekly during growth under full drainage. Fig. 1 describes the water stress and high temperature regime deployed in this experiment. At eight days post-anthesis (DPA) a restricted water regime was applied to half of the plants; most of the available water was consumed within 3 days and the plants were re-watered only on the fourth day, shortly before reaching the permanent wilting point (PWP). Following one round of water stress, a number of water-stressed and non-stressed plants were transferred to a controlled growth chamber and subjected to heat shock conditions of 39/25 °C (14h day/10h night) for 3 consecutive days. The treated plants were returned to the glasshouse (at 25/18°C) and cycles of water stress were reapplied until harvest.

Analysis of grain composition and quality

Ears of wheat were harvested at maturity for each treatment and left to air-dry before threshing. To obtain sufficient grain for the full range of tests it was necessary to bulk grain before milling to provide one grain sample for each set of growth conditions. Grain moisture and protein content were measured using an Near Infra-Red (NIR) Grainspec (Foss UK Ltd., York.) After overnight conditioning, the grain was milled to flour in a Brabender Quadramat Junior mill. The moisture and protein contents of the flours were determined by a Foss systems 6500 NIR spectrometer with sample transport (Silver Spring, MD, USA). The nitrogen content of the flour was determined by the Dumas total combustion method using an elemental analyser (CHN-1000, Leco Inc., St. Joseph, MI, USA). Protein (%) was estimated as N x 5.7.

The size distribution of starch granules, shown as the proportion of small (B-type) granules (%B), were determined by the method of Blumenthal *et al.* (1994).

Functional dough properties of the flours were evaluated using a two-gram Mixograph (Rath *et al.*, 1990); the water absorption was estimated by Approved Methods (AACC 1995) using the calculated protein and moisture contents of the flour. Mixing was performed in duplicate and the mean time to peak dough development, known as the mixing time (MT[sec]) was calculated together with the height at peak resistance (PR [arbitrary units]), bandwidth at peak resistance (BWPR [arbitrary units]) and percentage decrease in dough resistance 3 min after the peak (RBD [%]).

Changes in grain composition during development were assessed by sampling ears immediately before heat stress (7 days PAA) and for subsequent weeks until maturity.

The proportions of gliadin and glutenin, together with the percentage of "unextractable" polymeric protein (%UPP) in endosperm protein was determined using a modified method (Larroque *et al.*, 1997) for wheat protein analysis by size exclusion HPLC (SE-HPLC) (Batey *et al.*, 1991). %UPP was calculated as (area peak1 of the insoluble extract)/ (area peak1 of the insoluble extract + area peak 1 soluble extract) x 100. Gliadin composition was further broken down into α/β; γ and ω gliadins using reverse phase HPLC (RP-HPLC, Marchylo *et al.*, 1989). Glutenin composition was also characterised using this method and expressed as relative amounts of low or high molecular weight glutenins subunits (LMW-GS and HMW-GS respectively).

SDS-PAGE was performed as described by Blumenthal *et al.* (1990) using 12.5% gels. The soluble protein fraction was first isolated by extraction in 0.5% SDS-phosphate buffer (as used for SE-HPLC) and then lyophilised before adding 200 µL SDS-PAGE extraction buffer (Laemmlli, 1970) and 9 µL mercaptoethanol, heating at 65°C for 30 min and finally centrifuging for 10min at 14,000 rpm.

Statistical Analysis

Average values and standard error bars shown in figures represent the results of duplicate or triplicate measurements for each combination of growth conditions. Analysis of variance was performed on functional dough measurements using the MSUSTAT computer package (version 4.1) (Lund., R.E.., Montana State Universisty, Bozeman, MT).

RESULTS AND DISCUSSION

Changes in grain size and composition

Both heat and water stresses caused decreased grain size for both cultivars, but the effects of water stress alone were much greater for Batavia than for Suneca (Table 1). The proportion of small starch granules increased in response to heat stress for Suneca, but not for Batavia. Water stress did not change the proportion of %B granules for Suneca but for Batavia, it resulting in a decrease (Table 1). Genotypic variations in tolerance/susceptibility to heat stress have been reported by Blumenthal *et al.* (1994) for both these characteristics, but there is no reason to assume tolerance to one attribute (e.g., kernel size) may be related to tolerance for another (eg. % B granules).

The proportion of grain protein increased for both Suneca and Batavia with each stress, respectively (Fig. 2a,b), in agreement with other studies on the effects of drought (Khanna-Chopra *et al.*, 1994; Plaut *et al.*, 1999) and heat stress (Blumenthal *et al.*, 1994). In addition, when both stresses were applied simultaneously, there was a further increase in protein content in Suneca only. It was not possible to determine the actual yields of starch and protein (in an agronomic sense of mass per unit area), but these increases in protein content are presumably indicative to a large extent of corresponding decreases in starch yield due to the stress conditions.

Functional dough properties

There was no significant change in mixing time (MT) with heat stress or with water stress alone for Suneca (Fig. 2c). However, there was a significant increase in MT when both stresses were imposed ($p<0.05$). Possibly the initial water stress predisposed Suneca to greater tolerance to ensuing heat stress, resulting in stronger dough. Conversely, heat stress significantly decreased mixing time in

Batavia ($p<0.05$) (Fig. 2d). Water stress slightly ameliorated the dough-weakening effect of heat stress on Batavia, but still caused an overall decrease in MT compared to the control.

Peak Resistance (PR) and bandwidth at peak resistance (BWPR) are functional parameters related to protein content (Uthayakumaran *et al.*, 1999). For Suneca, the small increases in protein content with either stress produced no change in PR nor with BWPR, despite the increase in protein content when both stresses were applied (Fig. 2e and 2g). This is a further indication of dough stability for Suneca. Batavia, however, showed higher sensitivity to changes in ambient water, and the higher protein levels produced under drought conditions were also reflected by significant increases in PR and BWPR ($p<0.05$; Fig. 2f and 2h). Thus increases in PR and BWPR with water stress were shown to be cultivar-specific.

The resistance breakdown (RBD) of Suneca decreased slightly under heat stress (Fig. 2i) indicating greater dough stability. Contrary to the expected behaviour of heat susceptible wheat (Wrigley *et al.*, 1994), the RBD of Batavia also decreased significantly under heat stress (Fig. 2j); this was possibly due to the high BWPR causing premature breakdown of the dough.

Endosperm protein composition (mature grain)

In mature Suneca and Batavia grains (46 and 57 days PAA, respectively), heat stress caused no significant change in %UPP (Fig. 3a and b). Water stress alone inhibited the accumulation of %UPP in Suneca, although this was not reflected by the total glutenin content, the glutenin/gliadin or the polymeric/monomeric ratio (Table 1). The %UPP was only slightly decreased in mature Suneca grains when water and heat stress were applied together. Although %UPP was not significantly affected by environmental stress in Batavia, there was a small decrease in the glutenin/gliadin and polymeric/monomeric ratios when both stresses were applied (Table 1). Changes in the glutenin/gliadin ratio and in the polymeric/monomeric protein ratio give an indication of the effects of environmental stress on dough properties (Ciaffi *et al.*, 1994) as reflected by the poorer dough strength (decreased MT) in heat- and water-stressed Batavia. For both varieties, there were less water-soluble proteins (eg. globulins and albumins) in mature grains exposed to water or heat stress (Table 1). Water-soluble proteins do not contribute to dough strength, as do the high-molecular-weight glutenin subunits (HMW-GS) (Southan and MacRitchie, 1999).

Both cultivars produced flours in the mature grain with relatively high glutenin/gliadin ratios, an aspect of composition which has been found to produce bread of good volume. The studies of Uthayakumaran *et al.* (1999) have shown that an increase in the ratio improved flour quality, but only over a certain range (0.58-1.55). Higher ratio flours may be too strong for breadmaking. To this end, it would be undesirable for this ratio to fall significantly due to stress conditions, since the higher glutenin/gliadin ratio confers a strong, non-sticky dough. The reduction in glutenin/gliadin ratio for Batavia under both water and heat stress reinforces anecdotal evidence from some Australian millers that Batavia has provided anomalous quality in some seasons.

Previous research has shown that the *Glu-3* and *Gli-1* genes encoding LMW glutenins and ω gliadins are tightly linked (Muller *et al.*, 1998). There was some evidence to show that environmental stress was found to cause both an increase in LMW-GS and in α,β gliadins (rather than in ω–gliadins) in Suneca and Batavia (Figs 4 and 5). This was inconsistent in immature Batavia samples (Figs 6b and 7b), possibly due to a differential regulation of the linked genes due to stress. The predominant gliadins for both varieties, in mature grain, were the α– and β–gliadins (Fig. 4a,b). The gliadin composition of Suneca and Batavia was similar for all treatments except that there was an increase in α– and β– gliadins with water stress which was further enhanced by heat stress in Suneca; ω–gliadins also increased when both stresses were imposed.

Only the combined effect of water and heat stress caused a slight increase in LMW-GS for Suneca (Fig. 5a); whereas water stress, regardless of temperature, caused a significant increase in LMW-GS

content in mature Batavia grain (Fig. 5b). HMW-GS composition did not alter under stress in either cultivar (Fig. 5 a,b).

Developmental aspects of protein accumulation in the endosperm

The immature grain was sampled during endosperm development in order to ascertain how water and heat stress affected the synthesis and composition of endosperm protein (Fig. 3). The timing of the water and heat stress was such that it occurred after 7 DPA, when the initiation of disulphide-linked aggregation (polymerisation) of HMW-GS and LMW-GS occurs (Gupta *et al.*, 1995). The water-soluble protein content decreased during grain maturation, possibly due to a lower rate of synthesis (% albumins, globulins in Table 1), whilst the synthesis of polymeric proteins increased. Both water and heat stress affect the water-soluble proteins early on during grain filling in Suneca, whereas in Batavia, water-soluble proteins were only affected by heat stress in immature grains.

Differences in % UPP arose within two days of heat stress (14 DPA) for Suneca, whereas Batavia was only affected by water stress at this stage, and a doubling of %UPP was observed. Suneca grain showed earlier accumulation of %UPP (Fig. 3a); at 28 DPA, there was significantly higher %UPP samples subjected to heat stress and alone and in conjunction with water stress. The decrease in %UPP for the Suneca control at 34DPA was also reflected by a correspondingly low glutenin/gliadin ratio, polymeric/monomeric protein ratio and high %water-soluble protein (Table 1). In contrast to Suneca, Batavia showed late accumulation of %UPP, except in control plants (Fig. 3b) and at 37 DPA, %UPP was significantly reduced in heat-stressed and droughted samples. Developmental differences between cultivars, regarding % UPP accumulation, have been documented by Gupta *et al.* (1996). It was shown that wheat biotypes with HMW-GS subunits 5+10 (Glu-D1d allele), such as Suneca, accumulated larger polymers more quickly than biotypes with subunits 2+12 (Glu-D1a allele), such as Batavia. Interpretation of the results must therefore take into account genotypic variability in addition to environmental effects. As these pre-maturity dates showed the greatest variation in %UPP for both Suneca and Batavia, further detail of their protein composition was determined from the gliadin and glutenin composition (Figs 6 and 7).

In immature Suneca grain (34 DPA), water stress was responsible for the increase in γ-gliadins and LMW-GS and the combined effect of water and heat stress predominantly caused an increase in α– and β–gliadins, together with an increase in LMW-GS and HMW-GS (Figs 6 and 7). Water stress alone increased α– and β–gliadins in Batavia, with no additive effect with water and heat stress as found in Suneca. These results agree with previous findings (Blumenthal *et al.*, 1994; Ciaffi *et al.*, 1994) showing how gliadin synthesis continued in heat-stressed samples of Batavia (from 37 to 57 DPA) and Suneca (from 34 DPA to 46 DPA).

The glutenin composition of Suneca and Batavia showed the most striking difference due to environmental stress. Whereas the overall glutenin content of Suneca increased with water and heat stress (Fig. 7a), as also reflected by the increasing %UPP at 34 DPA (Fig. 3b), heat stress significantly lowered glutenin content in Batavia (Fig. 7b). When water and heat stress were applied simultaneously, no change in glutenin amount or composition was found. The predominant target for water and heat stress, in both varieties, appeared to be the LMW-GS fraction, which increased for Suneca and decreased for Batavia.

The tolerance of Suneca to environmental stress was also indicated by the relatively small changes (compared to the control) of the glutenin/gliadin and polymeric/monomeric protein ratios during grain filling as compared to the fluctuations due to both heat and water stress shown by Batavia (Table1). These results confirm that the stage of polymerisation of glutenin polypeptides is critical to the establishment of dough properties (Blumenthal *et al.*, 1995).

Developmental aspects of heat shock proteins

Current research suggests that heat-shock proteins serve a vital protective role in cells at certain stages of development (Schoffl *et al.*, 1998). To this end, it was found that in the late stages of seed maturation of Suneca (34 DPA), several soluble proteins (of molecular weight [MW]) 30,000 and 40,000) appeared only under heat stress (Fig. 8). The protein corresponding to 40,000 MW was present in all samples of mature grain for Suneca, but not for Batavia at any stage of development. The 30,000 MW protein was only found at 34 DPA in developing and not mature grain of Suneca. It is proposed that this may be a specific heat-shock-related protein with a possible protective or regulatory function, associated with the presence or folding of polymeric proteins. Future analysis of these proteins by 2-D electrophoresis (Skylas *et al.*, 2000) will be employed to elucidate N-terminal sequence data in terms of revealing heat shock sequences.

There did not appear to be any additional proteins relating only to water stress at late stages of maturity; however, water stress did appear to signal a change in the proportion of monomeric gliadins and glutenin subunits synthesised, which ultimately affected inter- and intra-molecular glutenin bonds and functional dough properties.

CONCLUSIONS

These results confirmed that Suneca is a heat-tolerant variety and revealed that if arid (i.e., hot and dry) conditions are imposed, flour derived from this grain may actually show increased dough strength. The lack of change in PR, BWPR and the decrease in RBD under environmental stress reflect the stability and strength of Suneca flour/water doughs, irrespective of stress during grain filling. Heat stress alone caused a weakening of dough properties in Batavia. Water stress, on the other hand, caused a significant increase in peak resistance probably due to increased protein content.

The increase in dough strength in Suneca with both heat and water stress correlated with significant increases in LMW glutenin and smaller increases in the ω– , α– and β–gliadins. Water stress also ameliorated the effect of heat stress in Batavia, possibly due to similar changes in these glutenin and gliadin fractions.

Protein accumulation changed during grain filling, with alterations in glutenin and gliadin composition and subsequent changes in glutenin/gliadin and polymeric/monomeric protein ratios. These results showed that the protein composition in the mature grain of both varieties did not vary significantly and did not reflect the same trends seen when immature grain was examined having been exposed to environmental stress. Soluble proteins (MW 30,000 and 40,000), which developed in late-maturing seeds in heat-tolerant varieties, may have relevance as a protective or regulatory element. As such, these proteins may be useful markers for early prediction of a change in dough quality due to heat stress.

ACKNOWLEDGEMENTS

We gratefully acknowledge use of the growth facilities at the University of Western Sydney, Hawkesbury, and assistance from Assoc. Prof. J. Conroys' group.

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FIGURE LEGENDS

Fig. 1 Scheme of timing for water and heat stress. During the water-stress cycle, the well-irrigated plants were also exposed to 25°C or 39°C. After 15 Days post anthesis (DPA), all plants were maintained at 25°C; cycles of water stress were continued in those plants already subjected to water stress.

Fig. 2 Protein content and functional properties of flour milled from Suneca and Batavia grown under control conditions (25°C, well irrigated), heat stressed and/or water stressed samples.

Fig. 3. The effect of water and/or heat stress on changes in unextractable polymeric proteins (%UPP) with grain maturation in Suneca (a) and Batavia (b). Key as in Fig. 2 .

Fig. 4 The effect of water and/or heat stress on changes in total gliadin and its component parts during late stages of grain maturation in (a) Suneca (46 DPA) and (b) Batavia (57 DPA).

Fig. 5 The effect of water and/or heat stress on changes in total glutenin and its component parts during late stages of grain maturation in (a) Suneca (46 DPA) and (b) Batavia (57 DPA).

Fig. 6 The effect of water and/or heat stress on changes in total gliadin and its component parts during grain filling in (a) Suneca (34 DPA) and (b) Batavia (37 DPA).

Fig. 7 The effect of water and/or heat stress on changes in total glutenin and its component parts during grain filling in (a) Suneca (34 DPA) and (b) Batavia (37 DPA).

Fig. 8 SDS-PAGE (12.5%) showing changes in protein patterns due to heat stress at different developmental stages: a – 14 Days Post-anthesis (DPA) Suneca 25°C (Control); b - 14 DPA Suneca 39°C; c - 14 DPA Batavia 25°C (Control); d - 14 DPA Batavia 39°C; e – 34 DPA Suneca 25°C (Control); f - 34 DPA Suneca 39°C; g – broad range MW marker (BioRad); h – 37 DPA Batavia 25°C (Control); i - 37 DPA Batavia 39°C; j - 46 DPA Suneca 25°C (Control); k – 46 DPA Suneca 39°C; l - 57 DPA Batavia 25°C (Control); m - 57 DPA Batavia 39°C. Arrows indicated the presence of additional proteins appearing due to heat stress in Suneca, at 34 DPA.

Table 1: Changes in grain size (as thousand kernel weight, TKW) and in proportion of small starch granules (%B granules) for different environmental conditions.

Cultivar	Treatment	TKW	% B granules
Suneca	25°C	40.9 ± 1.17	17.6 ± 0.65
	25°C WS	39.0 ± 0.68	17.3 ± 0.65
	39°C	35.3 ± 0.85	19.4 ± 0.65
	39°C WS	34.2 ± 0.23	19.9 ± 0.65
Batavia	25°C	45.2 ± 0.45	27.9 ± 0.65
	25°C WS	31.3 ± 0.85	23.9 ± 0.65
	39°C	42.5 ± 0.11	27.4 ± 0.65
	39°C WS	37.6 ± 1.46	25.3 ± 0.65

Key: Average values shown +/- sd

"Treatments" indicates whether plants were subjected to heat stress (39°C), water stress (WS) or both.

Table 2: Effect of heat and water stress on protein composition

Cultivar	Parameter	Treatment	14 DPA		34 DPA		46 DPA	
Suneca	glutenin/gliadin ratio	25°C	0.78	0.01	0.61	0.07	0.76	0.05
		25°C WS	0.86	0.00	0.78	0.01	0.79	0.06
		39°C	0.85	0.19	0.84	0.03	0.74	0.01
		39°C WS	0.70	0.02	0.80	0.02	0.75	0.04
	polymeric/monomeric protein ratio	25°C	0.56	0.01	0.49	0.06	0.64	0.04
		25°C WS	0.66	0.00	0.66	0.01	0.67	0.05
		39°C	0.67	0.12	0.69	0.03	0.63	0.01
		39°C WS	0.57	0.02	0.67	0.02	0.65	0.03
	% water soluble proteins	25°C	17.48	0.25	12.77	0.47	9.69	0.22
		25°C WS	13.61	0.12	9.85	0.06	9.09	0.29
		39°C	12.66	1.05	10.29	0.02	8.80	0.10
		39°C WS	12.13	0.13	9.60	0.23	8.02	0.01
Batavia			14 DPA		34 DPA		46 DPA	
	glutenin/gliadin ratio	25°C	0.63	0.02	0.78	0.06	0.85	0.01
		25°C WS	0.97	0.05	0.38	0.00	0.85	0.01
		39°C	1.11	0.01	1.11	0.01	0.84	0.02
		39°C WS	0.91	0.03	0.91	0.03	0.81	0.01
	polymeric/monomeric protein ratio	25°C	0.45	0.01	0.65	0.05	0.72	0.01
		25°C WS	0.78	0.04	0.31	0.00	0.72	0.01
		39°C	0.65	0.03	0.85	0.01	0.71	0.02
		39°C WS	0.59	0.05	0.73	0.03	0.69	0.01
	% water soluble proteins	25°C	18.10	0.28	10.23	0.40	9.10	0.17
		25°C WS	18.10	0.28	11.99	0.22	8.84	0.12
		39°C	12.37	0.70	12.30	0.08	9.06	0.09
		39°C WS	12.10	0.14	11.30	0.28	8.29	0.30

Key: Average values shown +/- sd
"Treatments" indicates whether plants were subjected to heat stress (39oC), water stress (WS) or both.

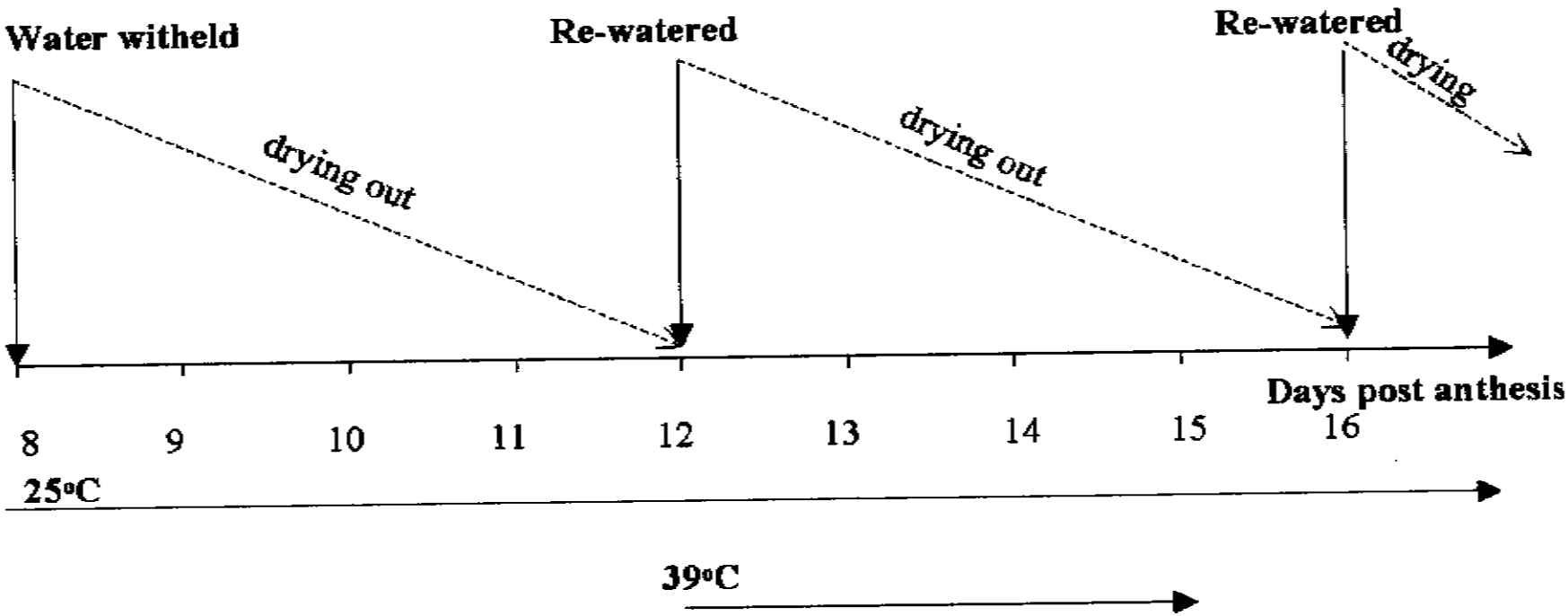

Water witheld
Re-watered
Re-watered
drying out
drying out
drying
Days post anthesis
8
9
10
11
12
13
14
15
16
25°C
39°C

Fig. 1

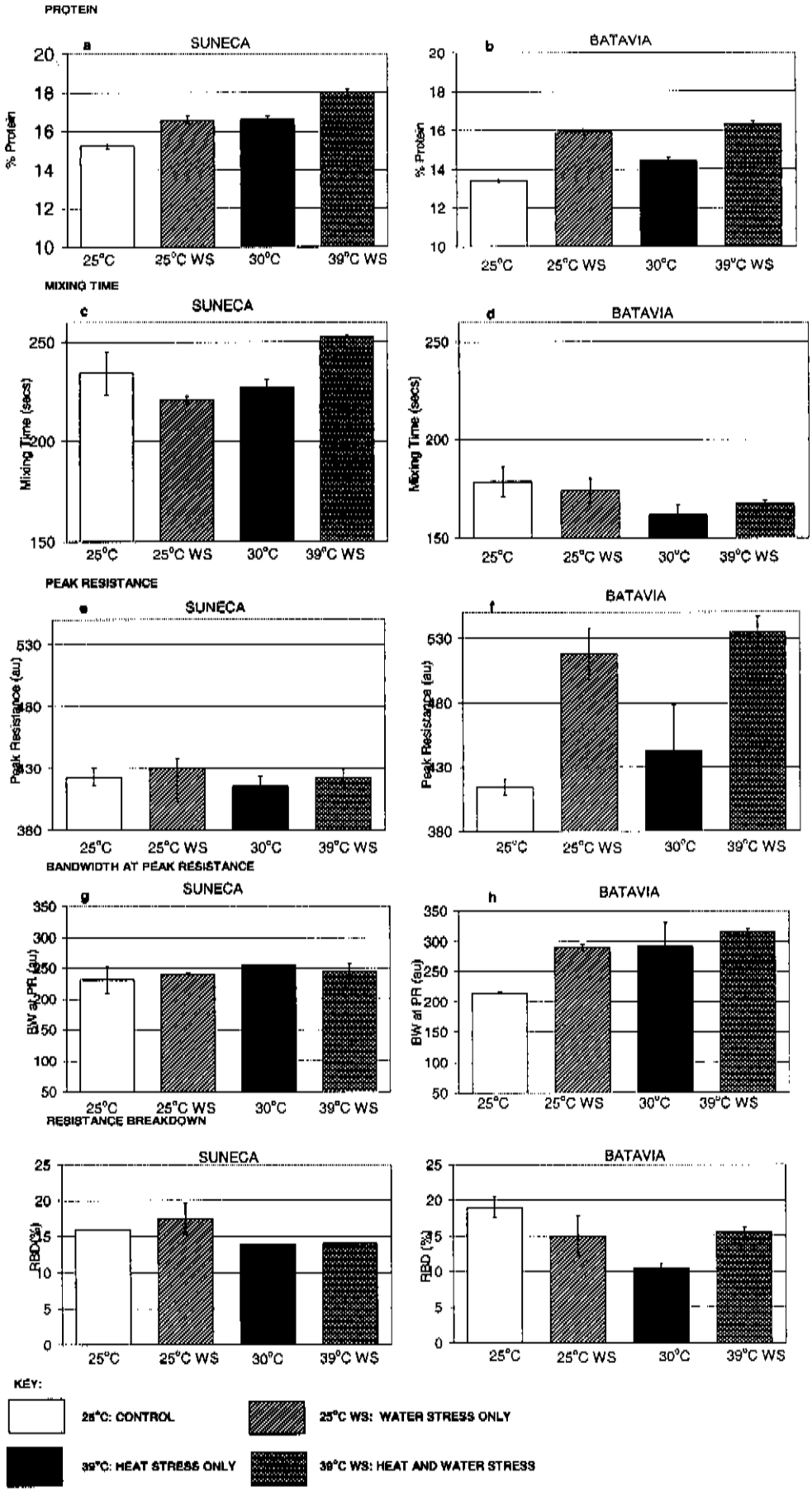
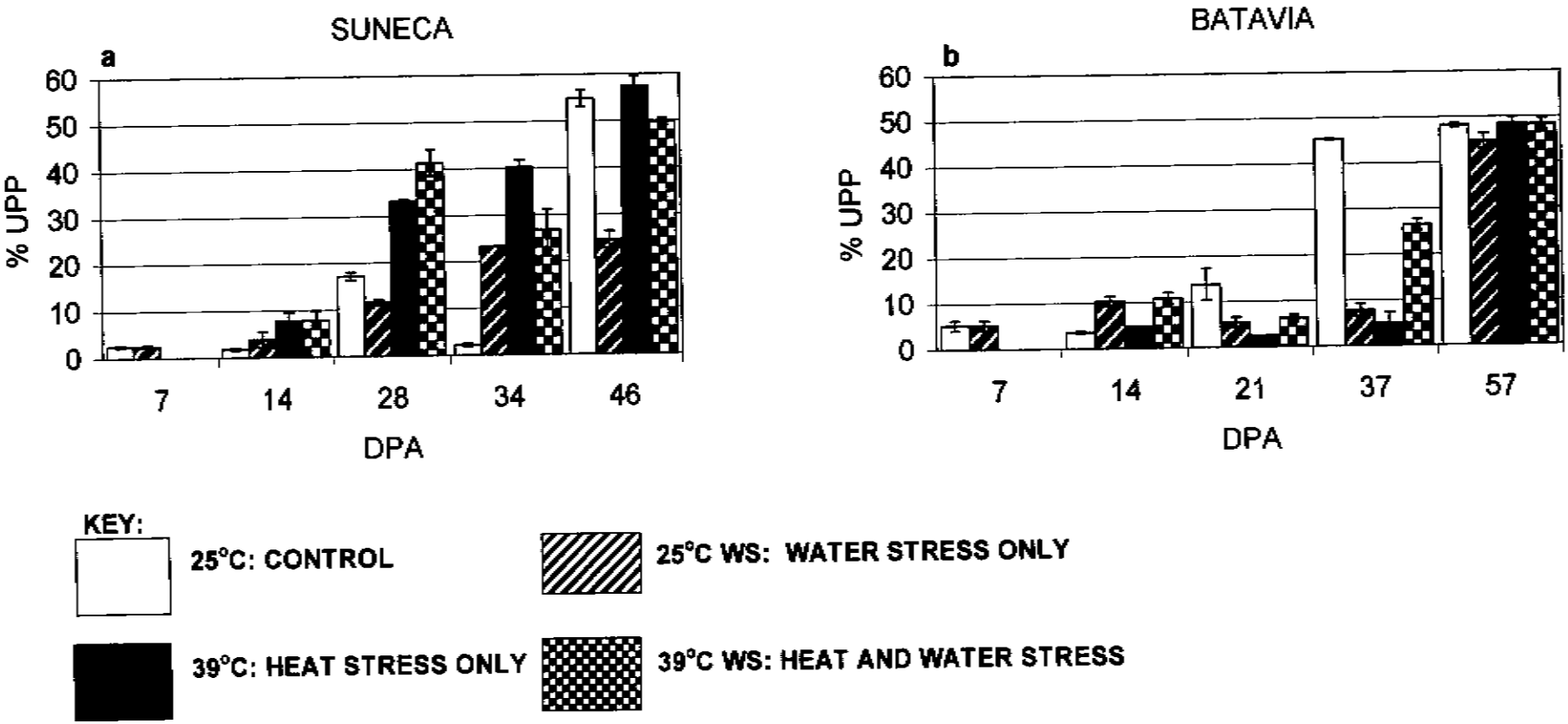

PROTEIN
a
SUNECA
b
BATAVIA
% Protein
20
18
16
14
12
10
25°C
25°C WS
30°C
39°C WS
MIXING TIME
c
SUNECA
d
BATAVIA
Mixing Time (secs)
250
200
150
PEAK RESISTANCE
e
SUNECA
f
BATAVIA
Peak Resistance (au)
530
480
430
380
BANDWIDTH AT PEAK RESISTANCE
g
SUNECA
h
BATAVIA
BW at PR (au)
350
300
250
200
150
100
50
RESISTANCE BREAKDOWN
SUNECA
BATAVIA
RBD (%)
25
20
15
10
5
0
KEY:
25°C: CONTROL
25°C WS: WATER STRESS ONLY
39°C: HEAT STRESS ONLY
39°C WS: HEAT AND WATER STRESS

Fig 2


SUNECA
a
60
50
40
30
20
10
0
% UPP
7
14
28
34
46
DPA
BATAVIA
b
60
50
40
30
20
10
0
% UPP
7
14
21
37
57
DPA
KEY:
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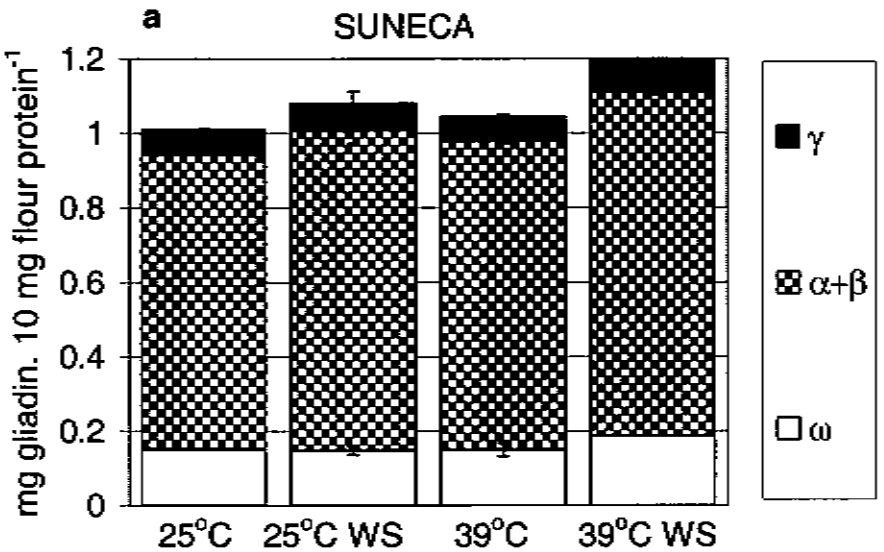
25°C WS: WATER STRESS ONLY
39°C: HEAT STRESS ONLY
39°C WS: HEAT AND WATER STRESS

Fig 3


a
SUNECA
mg gliadin. 10 mg flour protein-1
1.2
1
0.8
0.6
0.4
0.2
0
25°C
25°C WS
39°C
39°C WS
γ
α+β
ω

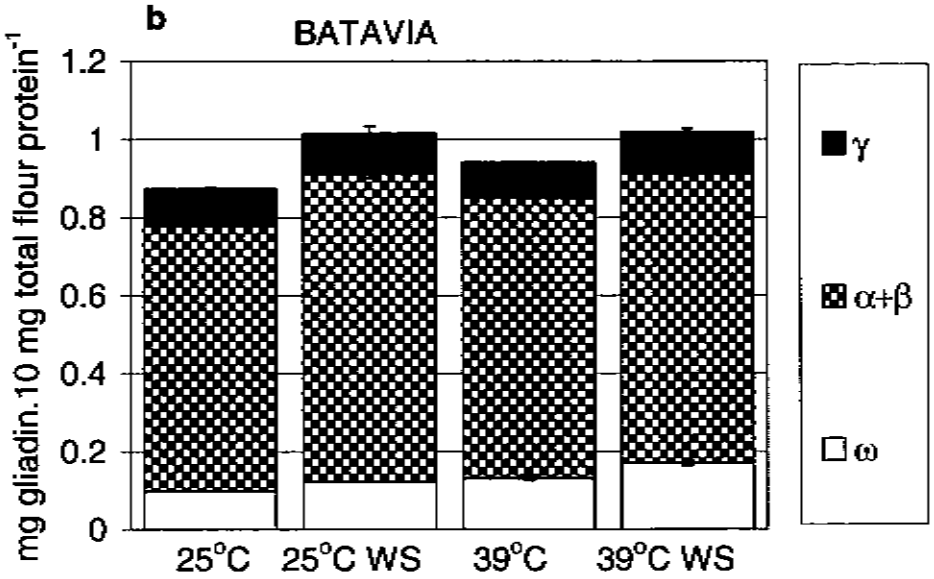
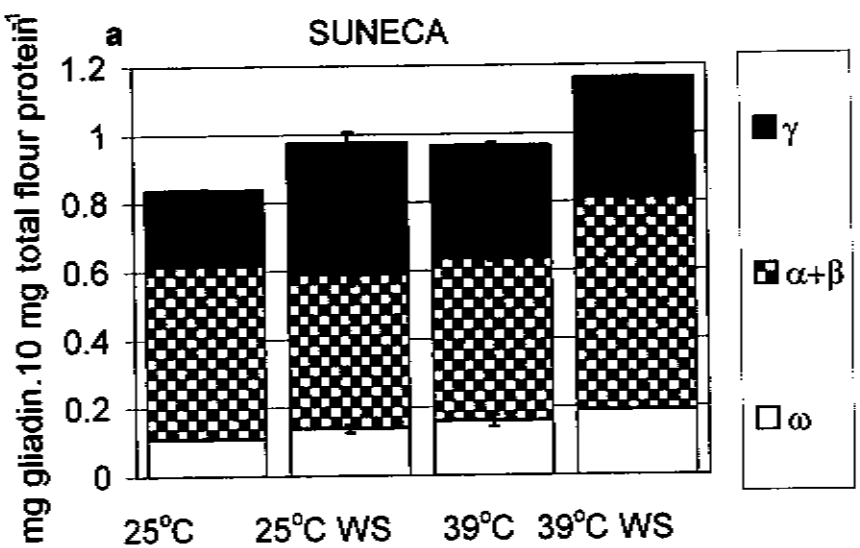
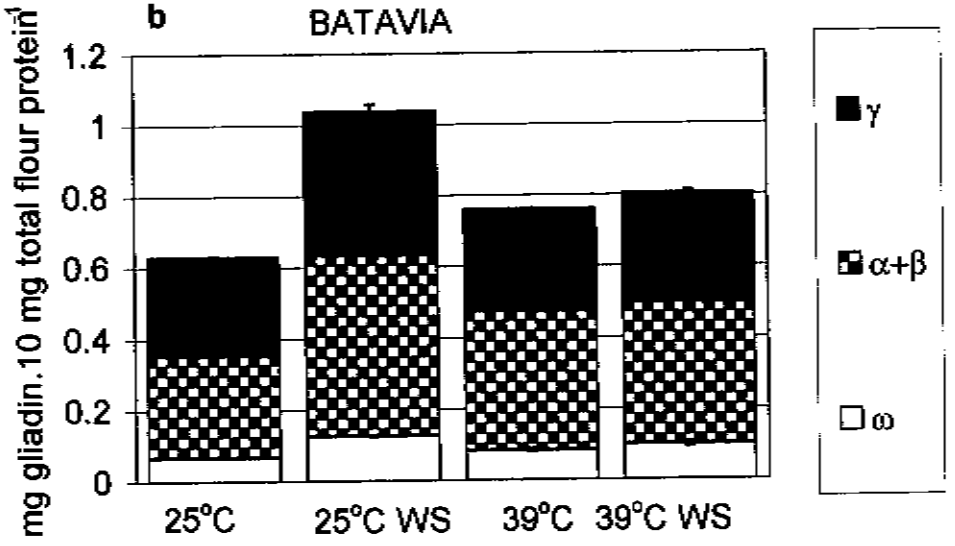

b
BATAVIA
mg gliadin.10 mg total flour protein-1
1.2
1
0.8
0.6
0.4
0.2
0
25°C
25°C WS
39°C
39°C WS
γ
α+β
ω

Fig 4

a
SUNECA
HMW-GS
LMW-GS
mg glutenin.10 mg total flour protein-1
0.8
0.6
0.4
0.2
0
25°C
25°C WS
39°C
39°C WS
b
BATAVIA
HMW-GS
LMW-GS
mg glutenin.10 mg total flour protein-1
0.8
0.6
0.4
0.2
0
25°C
25°C WS
39°C
39°C WS

Fig 5.


a
SUNECA
mg gliadin.10 mg total flour protein-1
1.2
1
0.8
0.6
0.4
0.2
0
25°C
25°C WS
39°C
39°C WS
γ
α+β
ω


b
BATAVIA
mg gliadin.10 mg total flour protein-1
1.2
1
0.8
0.6
0.4
0.2
0
25°C
25°C WS
39°C
39°C WS
γ
α+β
ω

9 of 17

a
SUNECA
HMW-GS
LMW-GS
mg glutenin.10 mg total flour protein
0.8
0.6
0.4
0.2
0
25°C
25°C WS
39°C
39°C WS
b
BATAVIA
mg glutenin.10 mg total flour protein
0.8
0.6
0.4
0.2
0
25°C
25°C WS
39°C
39°C WS

Fig 7

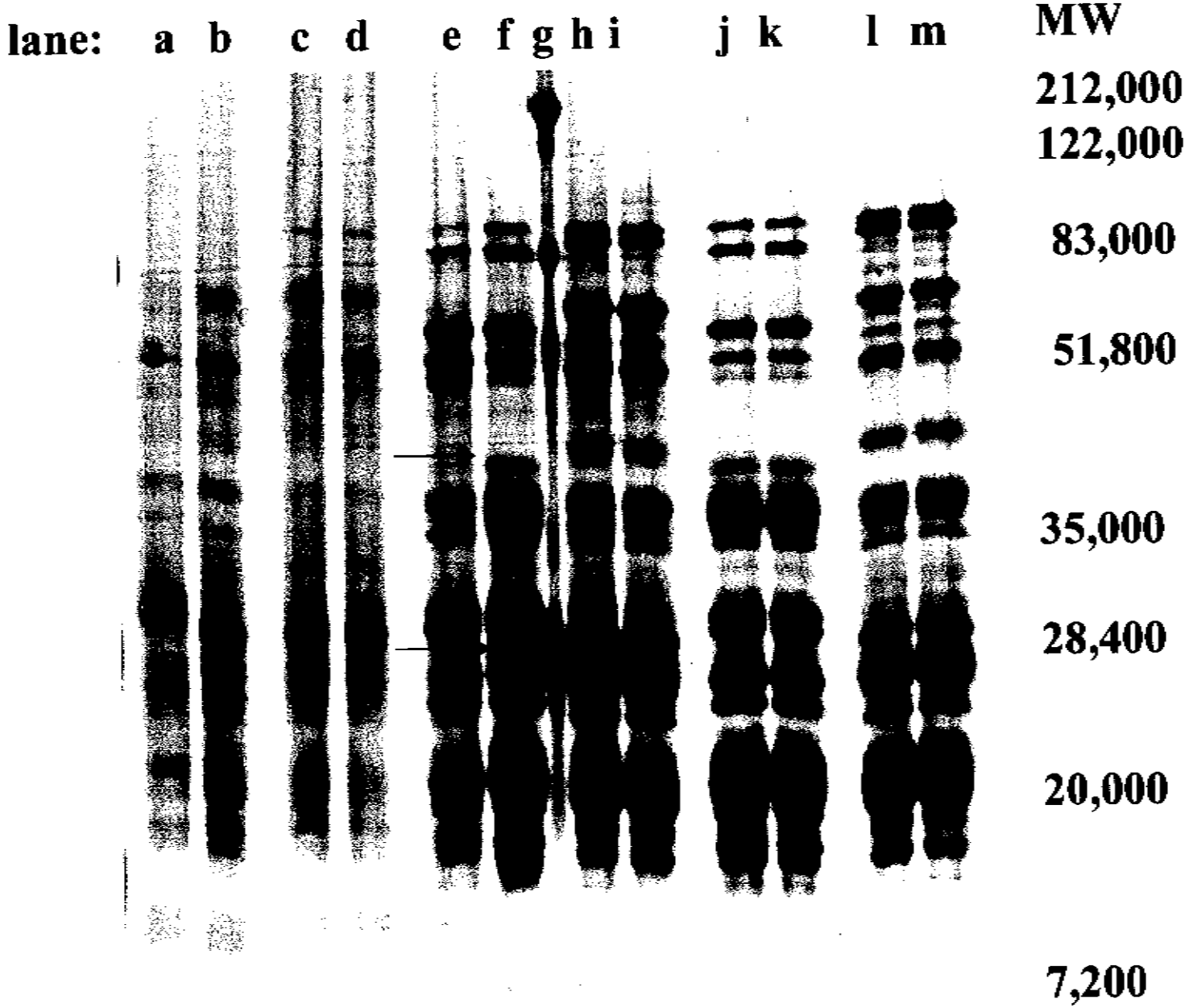

lane: a b c d e f g h i j k l m
MW
212,000
122,000
83,000
51,800
35,000
28,400
20,000
7,200

FIG. 8

Attached Report #2

Research paper intended for submission to the Journal of Crop Physiology

TRANSPORT OF STORED CARBOHYDRATES INTO DEVELOPING WHEAT KERNELS AND ITS CONTRIBUTION TO GRAIN YIELD UNDER POST-ANTHESIS WATER STRESS AND ELEVATED TEMPERATURE

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ABSTRACT

Stress-tolerant Suneca and stress-susceptible Batavia wheat cultivars were grown in pots in a temperature-controlled naturally illuminated glasshouse (March through May) under 25/18^{0}C day/night cycle. Plants were regularly irrigated and fertilized up to 8 days after anthesis (DAA), when half of the plants were water stressed. Stress was applied by withholding irrigation water for 4-6 days, until nearly all the available water was used, plants were then re-irrigated. Four such irrigation cycles were applied. Suneca plants were sub-divided into two groups, one of those was exposed to a higher temperature of 30/25^{0}C day/night cycle for 3 days toward the end of the 1st irrigation cycle, while the second and all Batavia plants were maintained at the previous temperatures. All plants were arranged in 3 groups; the first was defoliated, the second was decapitated and the third was left intact. Plants were sampled at 6-7 day intervals and dry weights of leaves, stems, ears and kernels were determined. A mechanistic model was used in-order to analyze the daily rates of transport from vegetative organs to kernels, and its contribution to kernel weight.

Neither water stress nor high temperature had a marked effect on the rate of kernel formation, as their formation was nearly terminated at the time of heat stress or the 1st application of water stress. The rate of dry matter production by kernels was significantly decreased by water stress in both cultivars. High temperature also reduced the rate of dry matter accumulation in kernels, but less than water stress. The dry weight of intact the plants' vegetative organs (stems + leaves) decreased during grain filling, probably due to export of stored non-structural carbohydrates or concurrent assimilates to the developing kernels. In decapitated plants, in contrast, dry weight of the vegetative organs increased during the same period. The rate of dry-matter loss in vegetative organs was reduced by water and temperature stresses. The rates of transport from vegetative organs to kernels were much higher in Suneca than in Batavia. Water and temperature stresses reduced these rates in both cultivars, but the decrease due to water stress was much more marked in Batavia. The contribution of dry matter transported from vegetative organs to the grains was 0.40 of the total grain weight in unstressed Suneca plants at the initiation of the treatments. It increased gradually up to 1.00 at 26-27 DAA. The contribution of dry matter transported from vegetative organs in Batavia was less and it increased only from

0.30 to 0.60. Water stress and high temperature increased the contribution of transported dry matter to kernel growth.

The final thousand-kernel weight (TKW) and final kernel number per plant were determined in a second experiment conducted simultaneously with the 1st one. While kernel number was hardly affected by both stresses, TKW was reduced by water stress more severely than by temperature stress, and more significantly in Suneca than in Batavia.

INTRODUCTION

Limited rainfall and rises in temperature occur frequently during the grain-filling stage of wheat in many wheat growing regions, thereby inducing conditions of water and heat stresses. Although water stress may promote the rate of cell division of young developing grains, it causes a marked decrease of its final mass (Evans *et al.*, 1975). Growth of the individual grains is reduced depending upon the degree of water stress and on the rate of stress development, thereby limiting final grain yield (Kobata *et al.*, 1992; Nicholas and Turner, 1992). The main effect of high temperature during grain filling was also found to be on the reduction of individual kernel mass (Wardlaw *et al.*, 1980; Parkinson, 1986: Randall and Moss, 1990 and Stone and Nicholas, 1995b). The reduction was found to be more severe when the stress occurred suddenly rather than gradually (Stone and Nicholas, 1995b), and at early stages of grain filling rather than at later stages (Stone and Nicholas, 1995a).

Grain filling of wheat depends on three main sources: current assimilates produced by photosynthesis in leaves and stems, mobilization of stored carbohydrates within these organs and subsequent transport to the ear and growing grain, and assimilates produced by the ear. The production of current photosynthesis products may become limited under conditions of water stress, since leaf stomatal conductance and net CO_2 assimilation rate decrease markedly during stress development. This is known for many species (e.g. Bradford and Hsiao, 1982) including wheat (Blum *et al.*, 1988). Other stress factors like high temperature, limited incident radiation or diseases may have similar effects. The contribution of stored carbohydrates may, thus, become the predominant source of carbohydrates (Bidinger *et al.*, 1977, Blum *et al.*, 1994). In fact, under stress conditions stored C and N contributed 64 and 81% of total grain C and N respectively (Palta *et al.*, 1994). Van Herwaarden *et al.* (1998) showed that under dry conditions the apparent contribution of stored assimilates could be 75-100% of grain yield, as compared with 37-39% under high rainfall conditions. Storage of non-structural carbohydrates may thus become an important yield-determining factor under stress conditions. In fact a high correlation was found between storage of non-structural carbohydrates of wheat stems and yield among several wheat cultivars under drought conditions (Gavuzzi *et al.*, 1997). The ability of grain filling from stored reserves was thus considered as an endogenous trait, which could serve as a tool for breeding.

The ability to support the developing grain and allow its maximal growth is thus related to the maintenance of concurrent photosynthesis, the mobilization of stored carbohydrates, and the capacity of the phloem to transport them. It will also depend on the extent of sucrose unloading, transport, metabolism and deposition of starch within the grain. As far as phloem transport is concerned, it was shown by a number of

investigators and was summarized by Evans and Wardlaw (1996) that limitations in phloem translocation was commonly not a rate-limiting step in transport of assimilates to the grain. Moreover, it was shown that in 22 wheat cultivars there was phloem spare translocation capacity to meet with maximal sink demand. Phloem unloading and post-phloem transport of sucrose into the endosperm cavity of the grain were found to continue over a wide range of sap osmolality and sucrose concentrations (Wang and Fisher, 1994). This may serve as a good indication that stress conditions which may affect sap osmolality, may only have a limited impact on phloem unloading and transfer to the endosperm cavity. Starch deposition was not a limiting factor of grain growth as the amount of sucrose located in the endosperm cavity was found to be equivalent to only 4 hrs of starch deposition (Ugalde and Jenner, 1990). Sucrose must therefore be continuously imported into the grain and its supply, rather than its metabolism seems to be a more important rate-limiting factor.

Non-structural carbohydrates are stored within the stem, leaf sheath and leaves, and fructans are probably the most abundant stored carbohydrate source for kernel filling (Kohbauch and Thorne (1989). Willenbrink *et al.* (1998) demonstrated a decrease in fructan content in the wheat peduncle during grain filling, which was more pronounced under source-limiting conditions, but was increased under sink-limiting conditions. Under conditions of water stress, stem fructans were decreased while fructose was increased, associated with a rise in fructan exohydrolase and acid invertase (Wardlaw and Willenbrink, 2000). Hydrolyzed fructans may, however, also play an important role in osmotic adjustment of the stem and leaves under conditions of water stress. Plants, which are exposed to water stress or salinity, have a tendency to perform osmotic adjustment in order to avoid dehydration and wilting (Wyn Jones and Gorham, 1983; Plaut, 1989). Both a decrease in water and an increase in solute content lead to this adjustment. While ions mostly contribute to this adjustment in the case of salinity, sugars and amino acids are significant contributing factors under water stress (Plaut, 1989; Plaut and Federman, 1991). Competition may thus exist in wheat leaves and stems between two sinks, namely the developing kernels and the leaves, which are adjusting to stress, for current photosynthates as well as for hydrolyzed reserve carbohydrates. Investigators have not always considered the need of hydrolyzed carbohydrates for adjusting to stress and have neglected such a competition. For instance, Xu and Ishii (1990) even claimed the opposite, namely, that a late drought will lower the water potential of vegetative tissue, which may then wilt, while grains will maintain a high water potential and will continue to grow.

Thus the purpose of the present study was to determine the ability to utilize stored non-structural carbohydrates for grain filling in wheat plants exposed to post-anthesis water and heat stresses.

Materials and Methods

Two experiments were conducted simultaneously on two Australian wheat varieties, Suneca and Batavia. Studies on the effects of heat stress during grain filling had shown that Suneca and Batavia are respectively tolerant and susceptible to the effects of heat shock on dough properties (Blumenthal *et al.*, 1995), but these studies did not directly examine their respective susceptibilities to the effects of heat shock on grain yield. It was expected that cultivars of higher heat tolerance would

also be better adapted to water stress, as no information on cultivars of higher water stress tolerance was available. Plants were grown in a temperature-controlled, naturally illuminated glasshouse (March through May) during a 25°/18°C day/night cycle. Ten plants were grown in 5-L cylindrical plastic pots in potting mixture with 'Osmocote Plus' and additional $(NH_4)_2SO_4$ as fertilizer. Plants were irrigated daily and fertilized twice weekly during growth. The quantity of applied water was always in excess, up to full drainage.

Eight days after anthesis (DAA) half of the plants were exposed to water stress, while the others were maintained as unstressed controls, and were irrigated as before. Water stress was applied by withholding irrigation water until about 80% (±10%) of the available water in the growing mixture was consumed, when plants were re-irrigated. The content of available water was pre-determined by pot weighing at the end of free water leaching and at the stage of PWP (permanent wilting point). Four irrigation cycles were applied during a period of 22 days in the case of Suneca (ending at 30 DAA), and 21 days in the case of Batavia (ending at 29 DAA). Such cycles had to be applied as the volume of available water in the pots was small and plants would wilt under continuous withholding of irrigation water. The duration and timing of these cycles is outlined in Table 1. During the last 3 days of the first drying cycle, a number of water stressed and non-stressed Suneca plants were transferred to a controlled growth chamber at 30°/25°C (day/night, 14h day/10h night). These pots were then returned to the original greenhouse at temperatures of 25/18°C. The higher temperature increased the rate of water loss to some extend, but the drying cycle had not to be shortened. Batavia plants were exposed to water stress only and were not transferred to different temperatures.

In the main experiment, plants of both treatments were sub-divided into three groups at 8 DAA, when the stress treatments were initiated: (1) Leaf blades were removed, leaving only the main stem and leaf sheaths (defoliation). (2) Ears were detached, leaving only vegetative organs (decapitation). (3) Intact plants, which remained untouched. These treatments were evenly spread over all the pot; mostly 3 plants from each group were located in every pot. This resulted in exposure of plants from the different groups to similar stress intensities. Tillers were removed before and after anthesis leaving only main stems. Plants of the different groups and treatments were sampled throughout the period of stress application (sampling time is outlined in Table 1). Five plants were removed from each treatment at each sampling (generally two pots per treatment), and separated between leaves, stems, kernels and residue of the ear, roots were not collected. All samples were dried at 65°C and weighed.

A mechanistic model was used in-order to analyze the collected data and calculate the potential mobilization and transport of stored compounds in the stem and leaves to the developing kernels. All the presented data for this experiment is of daily changes in dry mass, which were calculated on the basis of difference between two consecutive measurements (assuming linearity during these short periods), divided by the number of days and plotted for the median day. The data is plotted against days elapsed from the day of anthesis.

1. $M_{e\,i} = A_{e\,i} - R_{e\,i} + F_{st>e\,i}$
2. $M_{e\,d} = A_{e\,d} - R_{e\,d} + F_{st>e\,d}$

Assuming that ($A_{e\,i}$ - Re i) = ($A_{e\,d}$ - $R_{e\,d}$) (see Results and Discussion), and subtracting equation 2 from 1 gives:

3. $M_{e\,i} - M_{e\,d} = F_{st>e\,i} - F_{st>e\,d}$
4. $M_{s\,i} = A_{s\,i} - A_{s\,c} - F_{st>e\,i}$
5. $M_{s\,c} = A_{s\,c} - R_{s\,c}$

Assuming that ($A_{s\,c} - R_{s\,c}$) = ($A_{s\,i} - R_{s\,i}$) (see Results and Discussion), and subtracting equation 5 from 6, will give:

6. $M_{s\,c} - M_{s\,i} = F_{st>e\,i}$

The subtraction of equation 3 from equation 6 will now give:

7. $M_{s\,c} - M_{s\,i} - M_{e\,i} + M_{e\,d} = F_{st>e\,d}$
8. $M_{e\,i} = M_{g\,i} + M_{er\,i}$ and as well: $M_{e\,d} = M_{g\,d} + M_{er\,d}$
9. C_i is defined as $M_{g\,i} / F_{st>e\,i}$ and C_d as $M_{g\,d} / F_{st>e\,d}$

The symbols are as following:

$M_{e\,i}$, $M_{e\,d}$ = Changes in ear dry weight of intact or defoliated plants.
$M_{s\,i}$, $M_{s\,c}$ = Changes in shoot (stem + leaves) dry weight of intact or decapitated (ear removed) plants.
$M_{g\,i}$, $M_{g\,d}$ = Changes in grains dry weight of intact or defoliated plants.
$M_{er\,i}$, $M_{er\,d}$ = Changes in ear residue (excluding grains) dry weight of intact or defoliated plants.
$A_{e\,i}$, $A_{e\,d}$ = Sum of daily ear assimilation (assimilate product) in intact or defoliated plants.
$R_{e\,i}$, $R_{e\,i}$ = Sum of daily ear maintenance respiration in intact or defoliated plants.
$A_{s\,i}$, $A_{s\,c}$ = Sum of daily shoot assimilate production in intact or decapitated plants.
$R_{s\,i}$, $R_{s\,c}$ = Sum of daily shoot maintenance respiration of intact or decapitated plants.
$F_{st>e\,i}$, $F_{st>e\,d}$ = Sum of daily dry matter flux from stem to ear in intact or defoliated plants.
C_i and C_d are the relative contribution of transported substances out of total assimilates utilization in intact and in defoliated plants.

The objective of the second experiment was to study the combination of both stresses - water and heat stress and their effect on final grain weight and number. Both experiments were conducted simultaneously, but plants of both cultivars were transferred at the end of the first drying cycle to the two temperature regimes as outlined for the first experiment and then were all maintained at 25/18°C until harvest. Water stress treatments were also applied as outlined before and for the same duration, and were then suspended. Ears were sampled at maturity for each cultivar and left to air-dry. The yield was determined as kernel weight per ear, kernel number per ear and thousand-kernel weight (TKW). Grains from each treatment were assessed by near infra-red analysis for moisture content.

Results and Discussion

The effects of heat and water stresses cannot be separated under natural conditions, since the minimal rainfall and the high temperatures which usually prevail during grain filling enhance the rate of evapotranspiration, and result in extreme water deficits. It is

possible that water stress affects grain filling more or less intensively or by a different mechanism than high temperature. The separation between the two stresses may, thus, add information on specific effects.

Withholding of irrigation water started only at 8 DAA, when 65-75% of total kernels was already formed in Suneca and 62-66% in Batavia (Figure 1). It is also very likely that a major portion of the 20-27% of Suneca and the 30% of Batavia kernels were formed during the initial days of 8-18 DAA, when stress did not yet prevail (see Figure 1). The rate of kernel formation decreased very sharply with time in all treatments, and only about 5% of total kernels were formed thereafter. These were either due to delayed kernel development or kernels of overlooked tillers, which had not been removed. Grain formation was neither affected by water stress nor by exposure to high temperatures, and was also not much different in both cultivars (Figure 1). Defoliation at 8 DAA did also hardly affect kernel formation (data not presented). The total number of kernels calculated from Figure 1 for the different cultivars and treatments was very close to their final number as counted in the second experiment (Table 2). This may indicate that the sampled plants indeed represent the total population. It is, however, interesting that in Suneca the number of kernels calculated from Figure1 always exceeded their number in Table 2 by 5.0 –7.6%, while in Batavia it was lower by 7-8%. This might be due to different distribution of plants between the two experiments, but resulted in a larger number of kernels in Batavia as compared to Suneca in Table 2 but not in Figure 1.

In contrast to kernel number, the rate of kernel dry weight increase in Suneca was markedly affected by water stress in intact plants of both cultivars and at both temperatures (Figure 2). The daily increase in kernel dry weight shortly after anthesis was approximately 25 mg per plant in Batavia but 35-60 mg in Suneca. As sink size (number of kernels) and probably also sink demand increased, the rate of kernel filling increased, but in Suneca the rate became more than twice of that in Batavia at the two later samplings. The rate decreased in the water-stressed plants of both cultivars, but was still higher in Suneca. Plant exposure to the high temperature had minor effects on their response to water stress, and the high temperature as such caused only a slight decrease in the rate of kernel filling. It is suggested that an interpretation of the observed stress tolerance of Suneca may be due to the high potential of its kernel growth. Thus, although being reduced under stress conditions, grain yield may still be higher than in other cultivars. The effect of water stress on the rate of kernel filling in defoliated plants was qualitatively similar to that in the intact plants, but the maximal daily increase in dry weight in unstressed Suneca plants was only 43 mg per defoliated plant as compared to 95 mg per intact plant. Batavia plants were more severely affected by defoliation, as no increase in the rate of kernel dry weight accumulation with time could be found, evens in unstressed plants. On the contrary, the rate increased significantly with time in intact Batavia plants, although the number of kernels in intact and defoliated plants was similar (data not presented).

The removal of the ear (decapitation) had a very significant effect on dry matter content of the vegetative organs – leaves and stems. The change in dry matter content of these plants was always positive, regardless of cultivar, treatments, age or duration of the applied treatment (Figure 3). This was not the case in the intact plants, in which the ear served as an active sink not only for currently produced assimilates but for stored compounds as well. This resulted in a decrease of dry

weight of the vegetative organs, which was mainly conspicuous in the unstressed plants of both cultivars, except for the first set of measurements in Suneca. The rates became more negative with time in the unstressed plants of both cultivars, especially at the high temperature in Suneca. This indicates that with time there was an increase in sink strength of the ear. In water-stressed plants of both cultivars, there were relatively small changes of dry weight throughout the entire period (except 5 days after anthesis in Suneca). The decrease in rates of dry weight production in decapitated plants by water stress and high temperature was, probably a result of the effect of stress on growth. Suneca seemed to be a faster growing cultivar than Batavia and the steeper decrease of dry matter production rate with time is, possibly, an indication of faster senescence of Suneca. The rate of dry matter production by vegetative organs of intact unstressed plants, was presumably similar to that, but was not sufficient to furnish sink demand of the developing kernels. Stored carbohydrates were thus transported to the kernels, which resulted in negative dry matter accumulation. In stressed plants one would expect a more marked negative accumulation. In fact, the negative accumulation was less, suggesting that more stored carbohydrate were retained in leaves and stems and were not transported.

The model, which was used to calculate the transport rate of stored assimilates under the different environmental conditions, was based on two main assumptions:

1. The rates of assimilation and maintenance respiration of the ear are autonomous, and independent on the rates of assimilation and maintenance respiration in the vegetative organs. This can easily be accepted, because post-anthesis assimilation rates of the ear are low (Evans *et al.*, 1975), and the high respiration rates of the developing grains reduce the net rate of ear photosynthesis very considerably (Evans and Rawson, 1979). Therefore $(\Sigma A_{e\,i} - \Sigma R_{e\,i}) = (\Sigma A_{e\,d} - \Sigma R_{e\,d})$, and the removal of leaves is expected not to intervene.

2. $(\Sigma A_{s\,i} - \Sigma R_{s\,i}) = (\Sigma A_{s\,c} - \Sigma R_{s\,c})$, namely the rates of assimilation and maintenance respiration by shoots of intact plant and decapitated plants were not much different. This can be concluded from earlier studies on wheat (Apel *et al.*, 1973; Austin and Edrich, 1975). It is also based on earlier findings in our laboratory, which showed that photosynthetic carbon fixation rates in source wheat leaves were reduced only 12-14 days after sink removal, and was mainly due to build-up of CO_2 diffusion resistances by accumulated starch granules (Mayoral, 1982). King *et al.*, (1967) found, however, a significant decline in net photosynthesis of flag leaves shortly after removal of the ear.

Figure 4 shows that maximal daily rate of dry matter was transported from vegetative organs to kernels in unstressed intact Suneca plants grown at 25^0C. Water stress and higher temperature decreased this rate markedly. This suggests that assimilates which are located in leaf sheaths were used for osmotic adjustment of water stressed plants, rather than being transported to the grains. There was, however, no additive effect of both stresses, namely in water-stressed plants the higher temperature hardly decreased the rate any further. No SE of means are given in Table 4 as both $F_{st>e\,i}$ and $F_{st>e\,d}$ had to be calculated on the basis of averages. Maximal daily transport rate of intact unstressed Batavia wheat plants was only about 80 mg per plant as compared with 125 mg per plant in Suneca. Batavia was also more sensitive to stress and the decrease in the rate of transport was very low in water-stressed plants. It is

assimilates, as leaves had been removed, but not to lower availability of assimilates, as those were mainly located in the sheaths. It should be noted, that even if the 2nd assumption is not accepted (so that $F_{st>e\,i}$ and $F_{st>e\,d}$ cannot be calculated), the sharper decline of shoot dry weight in unstressed as compared to stressed Suneca (Figure 3) is still a good indication for limited export to grains. This is not so evident in Batavia, which is possibly less stress tolerant.

Defoliation of control unstressed plants reduced transport rates of dry matter much more in Suneca than in Batavia. This may explain the difference between the two cultivars in the effect of defoliation on grain dry weight, which was by 3-fold drop in unstressed Suneca and only by 1.8-fold drop in Batavia (Figure 2). As far as the changes with time are concerned, It seems that there was nearly a steady state in transport rate in Batavia and at 30°C in Suneca (both intact and defoliated). The high rates of transport in intact Suneca plants at 25°C are a good indication for the availability of assimilates under optimal growing conditions, which contributes to kernel filling. It may also explain the high productivity of this cultivar, which was indicated earlier.

The relative contribution of dry matter from vegetative organs (stem and leaves) to grain dry weight was nearly 0.40 in unstressed intact plants of both cultivars at early stages after anthesis (Figure 5; SE of the means could also not be presented here as in Figure 4). King *et al.* (1967) presented similar values for different wheat cultivars. At this developmental stage, the ear was still photosynthetically active and could contribute assimilates for the filling of grains. At the second sampling, the vegetative organs contributed already about 0.80 and at the third sampling (27DAA) nearly the entire dry weight accumulated in the grain. It should be noticed that roots could also serve as sinks for assimilates, altering somewhat the distribution of dry weight transported out of stems+leaves. It was shown that roots are a limited sink at the studied growth stage (King *et al.*, 1967), so that their elimination could not introduce a real error. Moreover, as tillers were constantly removed, these could also not serve as a sink. The relative contribution in water and high temperature stressed plants was much higher at the first two samplings. It seems that stress enhanced plant senescence so that photosynthetic activity of the ear was reduced considerably earlier. In defoliated Suneca plants, the contribution of the vegetative organs was low at the first sampling, as may be expected. However, since most of the transported substances are stored in the stem and sheath, their contribution became significant with time. In Batavia, the relative contribution of transported dry matter from vegetative organs in intact unstressed plants increased much less with time as compared with Suneca and even at 26 DAA, it was less than 0.60. In stressed Batavia plants it was, however, nearly 1.00 throughout. The relative contribution of stored non-structural carbohydrates and leaves in defoliated Batavia plants was lower as compared to intact plants. The supply of carbohydrates by vegetative organs became, however, very significant at later stages, even in the stressed Batavia plants.

The final kernel yield is presented as thousand-kernel weight (TKW) (Table 2), as total grain yield has limited meaning in pot-grown plants, and at different densities. The response of TKW to water stress was much stronger than the response to high temperature, and was more remarkable in the sensitive cultivar Batavia. It should be

noted that plant exposure to the different temperatures was for 3 days only, while the different irrigation regimes were applied for about 20 days. But, since water stress was evident only on the last day of every irrigation cycle, the two types of stress are of similar durations. A quantitative comparison between the two types of stress is difficult due to several reasons: 1. Their intensities are not known, and are difficult to compare. 2. They were applied differently - heat stress continuously and water stress in cycles, as a constant water stress cannot be applied continuously. 3. Heat stress was applied during11-13 DAA, while the cycles of water stress continued up to 30 DAA. The effect of the two stresses on TKW was only partly additive in both varieties. Moreover, in both cultivars water stress decreased TKW more severely in plants which were constantly at 25^{0}C (21.7% and 25.9% in Suneca and Batavia, respectively) as compared to those which were exposed to higher temperature(17.3% and 22.1%). Whether this was due to some resistance to water stress achieved by early exposure to heat stress is not known, but is worth further investigation.

Conclusions

The present study confirms earlier findings that the contribution of non-structural carbohydrates from vegetative organs is a very significant source for grain filling in unstressed plants. In water-stressed plants, however, carbohydrates stored in vegetative organs are a limited source for grain filling, as these are retained within the vegetative organs, probably to sustain osmotic adjustment under stress conditions. Both water and high-temperature stress reduced the daily rate of dry matter transport from vegetative organs to grains, but there was no additive effect of both stresses. The inhibitory effect of heat stress on the rate of kernel growth was much less than the effect of water stress. This could be due to the fact that the intensity of grain filling was still very high after being exposed to the temperature stress.

The lower sensitivity of Suneca to stress can be interpreted by its much higher rate of grain filling under unstressed conditions. In fact, the higher rate of dry matter production of vegetative organs in Suneca as compared to Batavia may explain the large resources for this rate. Thus, even if grain filling was inhibited by stress, a notable rate was still retained.

The sources of transported carbohydrates to kernels in Batavia were mostly in the stem and leaf sheaths, while in Suneca current assimilates produced by the leaves served also as an important source.

Concurrent assimilates of the ear can contribute limited amounts of assimilates for grain filling under water stress, due to the effect of stress on assimilation rates. The relative contribution of assimilates for kernel growth that was produced by the ear was higher in unstressed Batavia than in Suneca.

Acknowledgement

The authors wish to thank Prof. J. Conroy of the University of Western Sydney for providing the growth chamber and greenhouse space and for helpful suggestions and to Dr. S. Fishman for her suggestions in formulating the equations. Helpful comments on the manuscript were provided by Dr I Wardlaw. The support of Wheat CRC to the study is gratefully acknowledged.

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Legends to Figures

Figure 1: Rates of daily grain formation of Suneca and Batavia intact plants (SE of the means are presented as vertical bars). NS and S = unstressed control and water stressed respectively. 25^0C= not exposed to higher temperature, 30^0C= exposed to 30^0C.

Figure 2: Rates of daily increase in kernel dry weight per ear of Suneca and Batavia intact and defoliated plants (SE of the means are presented as vertical bars). NS, S, 25^0C and 30^0 C are as outlined in Figure 1.

Figure 3: Rates of daily changes of vegetative organs (Stem + leaves) of Suneca and Batavia intact and decapitated (ear removed) plants (SE of the means are presented as vertical bars). NS, S, 25^0C and 30^0 C are as outlined in Figure 1.

Figure 4: Rates of daily dry matter transport from vegetative organs to grains. Rates are $F_{st>e\,i}$ and $F_{st>e\,d}$ calculated from equations 6 & 7. NS, S, 25^0C and 30^0 C are as outlined in Figure 1.

Figure 5: Relative contributions of transported dry matter from vegetative organs to grain dry weight in Suneca and Batavia intact and defoliate plants (C_i and C_d calculated according to equation 9. NS, S, 25^0C and 30^0 C are as outlined in Figure 1.

Table 1: Durations of drying cycles, sampling and median days (for plotting in graphs).

All given as DAA for Suneca and Bata cultivars.

Cultivar		Pre-stress	Cycle 1	Cycle 2	Cycle 3	Cycle 4
Suneca	Drying cycle	0-8	8-13	13-18	18-24	24-30
	Sampling	8		18	24	30
	Median day	4		13	21	27
Batavia	Drying cycle	0-8	8-12	12-17	17-23	23-29
	Sampling	8		17	23	29
	Median day	4		12	20	26

Table 2: Effect of water and heat stress on final thousand-kernel weight and number of kernels per ear.

			25^0 C		30^0 C	
	Cultivar	Water stress	Cultivar	Water stress		
Thousand Kernel Weight (g)	Suneca	Unstressed	53.87	1.17	47.32	0.42
		Stressed	42.16	0.67	39.14	1.05
	Batavia	nstressed	42.20	0.45	38.38	0.65
		tressed	31.28	0.85	29.91	0.67
Grain Number (number/ ear)	Suneca	Unstressed	31.12	1.82	30.97	1.59
		Stressed	33.31	2.03	28.67	1.98
	Batavia	Unstressed	46.72	3.56	41.51	4.11
		Stressed	45.42	2.06	39.52	3.87

Figure 1

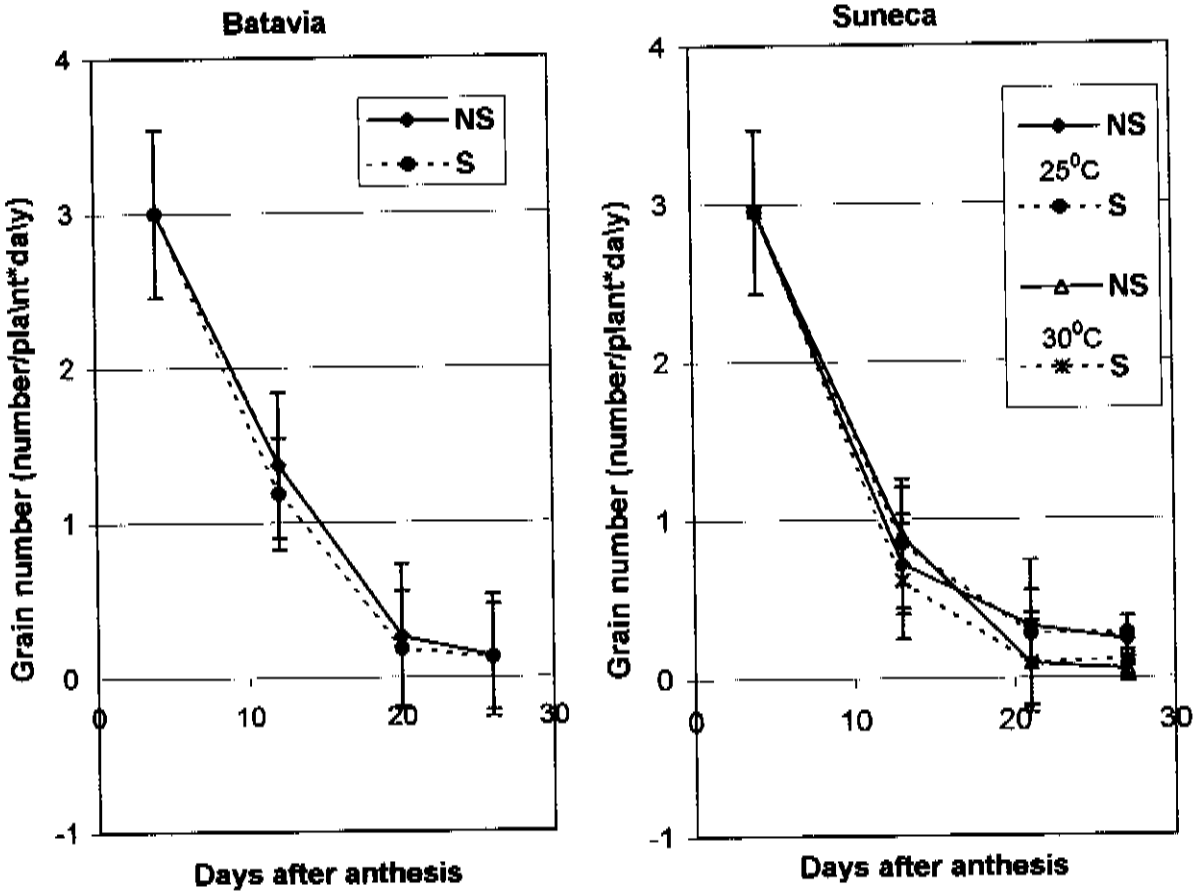

Batavia
Suneca
NS
S
25°C
30°C
Grain number (number/plant*daly)
Days after anthesis
4
3
2
1
0
-1
0
10
20
30

Figure 2

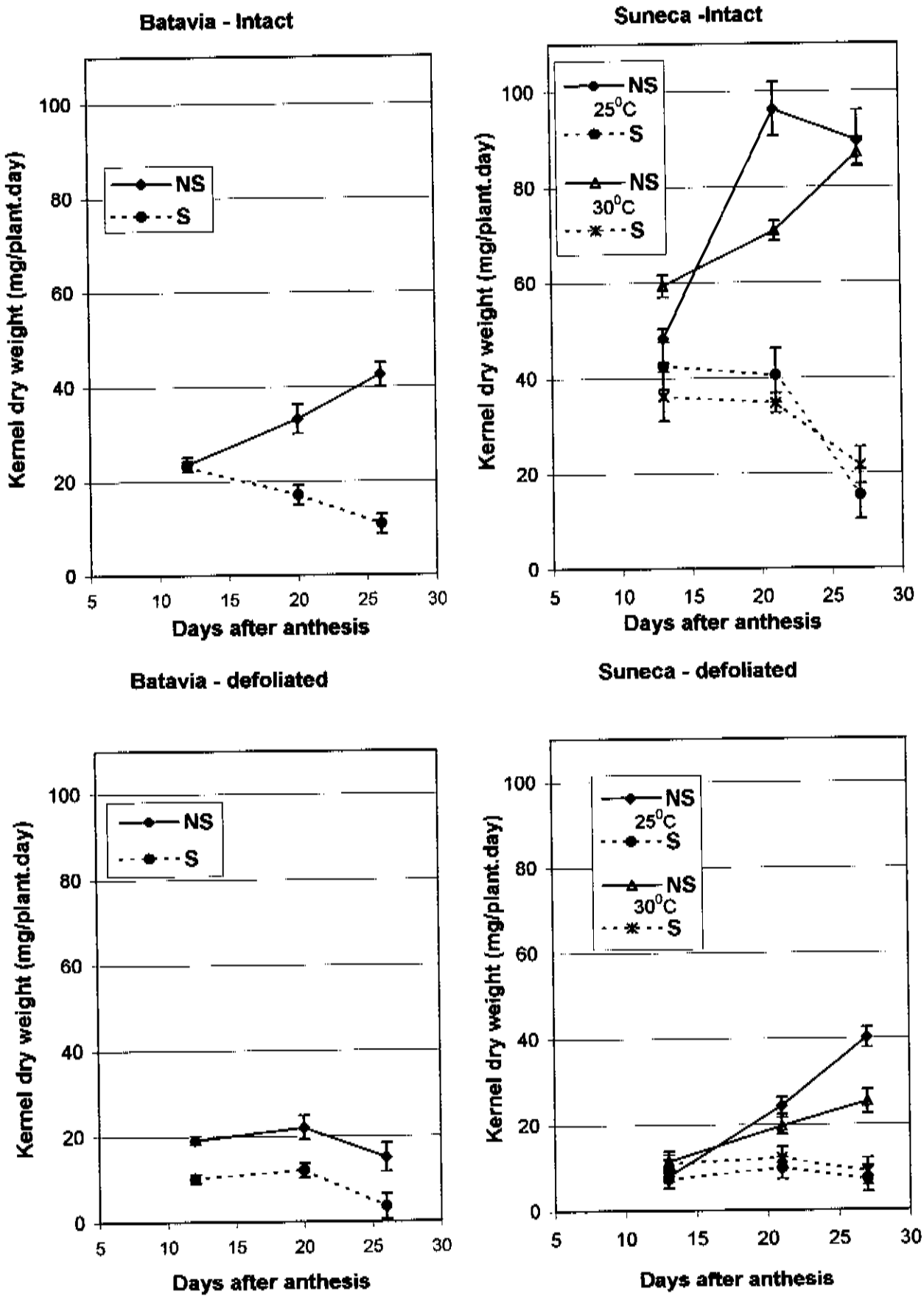

Batavia - Intact
Kernel dry weight (mg/plant.day)
NS
S
Days after anthesis
Suneca -Intact
NS
25°C
S
NS
30°C
S
Batavia - defoliated
Suneca - defoliated

Figure 3

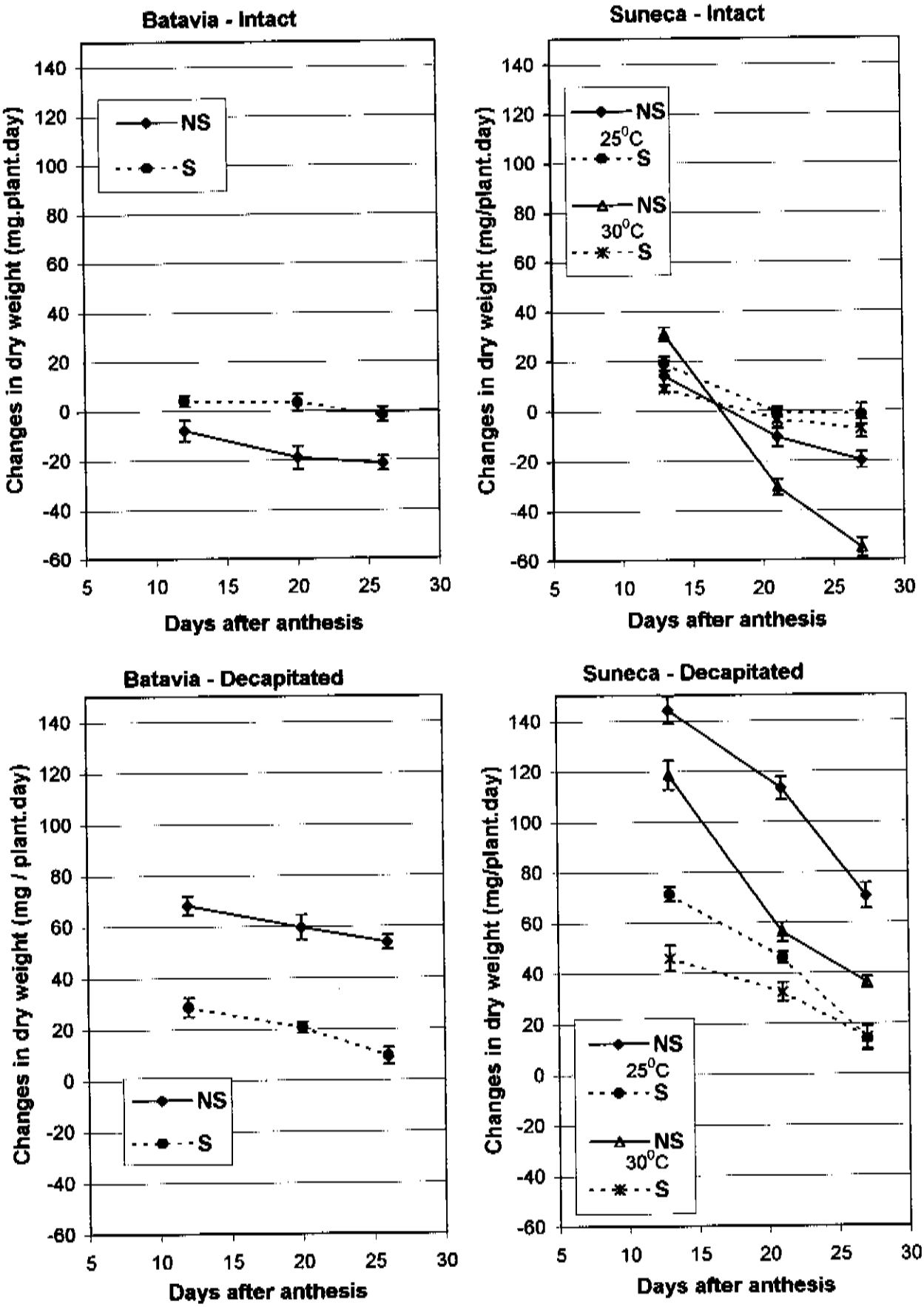

Batavia - Intact
Changes in dry weight (mg.plant.day)
Days after anthesis
NS
S
Suneca - Intact
Changes in dry weight (mg/plant.day)
Days after anthesis
NS
25°C
S
NS
30°C
S
Batavia - Decapitated
Changes in dry weight (mg / plant.day)
Days after anthesis
NS
S
Suneca - Decapitated
Changes in dry weight (mg/plant.day)
Days after anthesis
NS
25°C
S
NS
30°C
S

Figure 4

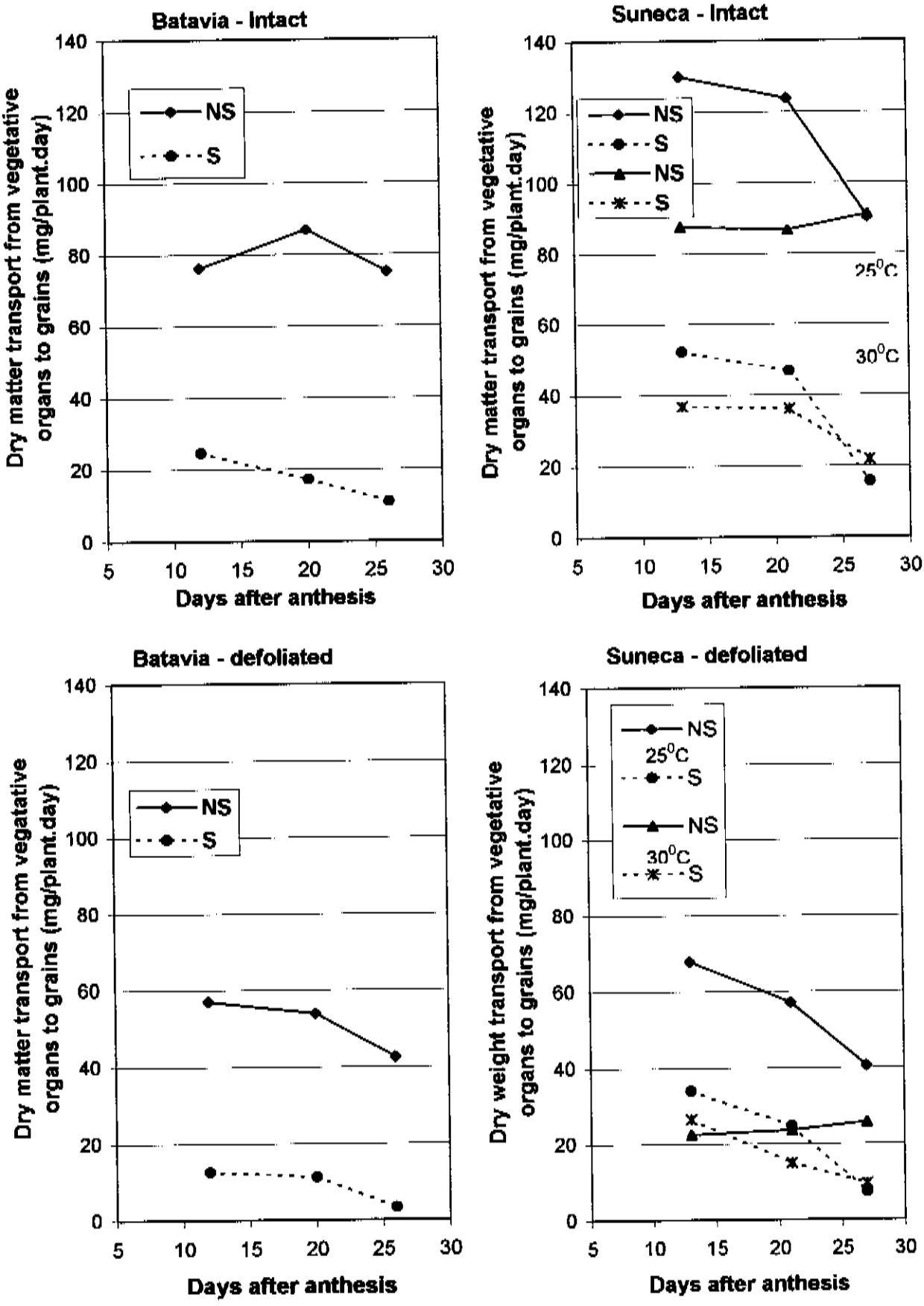
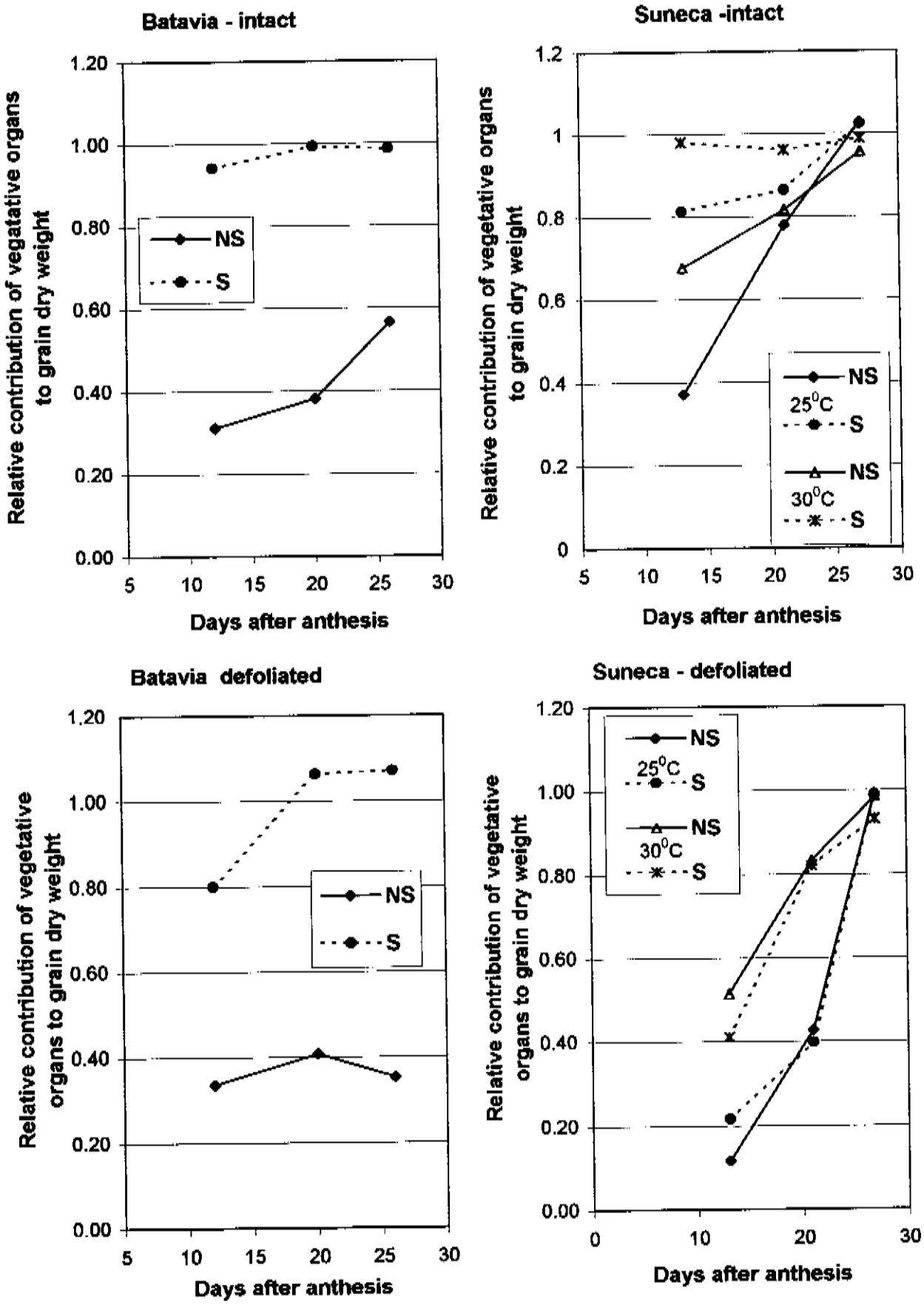

Batavia - Intact
Dry matter transport from vegetative organs to grains (mg/plant.day)
NS
S
Days after anthesis
Suneca - Intact
NS
S
NS
S
25°C
30°C
Batavia - defoliated
Suneca - defoliated
Dry weight transport from vegetative organs to grains (mg/plant.day)

Figure 5


Batavia - intact
Relative contribution of vegatative organs to grain dry weight
1.20
1.00
0.80
0.60
0.40
0.20
0.00
5
10
15
20
25
30
Days after anthesis
NS
S
Suneca -intact
Relative contribution of vegetative organs to grain dry weight
1.2
1
0.8
0.6
0.4
0.2
0
NS
25°C
S
NS
30°C
S
Days after anthesis
Batavia defoliated
Relative contribution of vegetative organs to grain dry weight
Days after anthesis
NS
S
Suneca - defoliated
Relative contribution of vegetative organs to grain dry weight
0
10
20
30
Days after anthesis
NS
25°C
S
NS
30°C
S

Attached Report #3
Heat shock of wheat genotypes lacking alleles for the HMW subunits of glutenin

B.J.Butow and H.Bariana

Introduction

There is evidence that some of the HMW subunits of glutenin (especially subunits 5+10) may contribute more than others to the tolerance of some genotypes to the effects of heat stress on dough quality. This observation has been based on the statistical observations of reactions of the set of 45 genotypes, and of biotypes that differ in their *Glu-1* alleles (see the main report). The multi-null series of genotypes of Gabo-Olympic lines offered the opportunity of further evaluating this proposition, due to their possession of specific combinations of HMW subunits (Table 1). Only three genotypes from this series was used, namely those with all the HMW subunits, with none of them, and with only the D-genome subunits missing. In addition, the varieties Fang and Wyuna were included as 'benchmarks' of heat-tolerant and susceptible genotypes.

Table 1. HMW subunits in the Gabo-Olympic lines used in these experiments, as described originally by Lawrence *et al.* (1988) J. Cereal Sci. 7, 109-112.

Code for genotype	*Glu-A1*subunit	*Glu-B1*subunits	*Glu-D1*subunits
+ + +	1	17+18	5+10
+ + -	1	17+18	Absent
- - -	Absent	Absent	Absent

Materials and Methods

The three Gabo-Olympic lines, Wyuna and Fang were planted in growth chambers to achieve a final yield of 100 ears per cultivar. Plants were grown at 21°C/16°C - 16h/8h day/night cycles. Heat stress was applied at 18, 19 and 20 days post anthesis (DPA) by changing to a temperature regime of 39°/C/25°C for 3 days. All plants were watered frequently during the heat stress period. Grain samples were taken during developmental stages for Fang, Wyuna and Gabo null lines for both controlled and heat stressed plants.

Unfortunately, samples were only provided for the following developmental stages; Gabo: 35, 41 and 45 (mature) DPA; Wyuna: 23, 26, 30, 45 DPA; Fang: 39, 46 and 50 DPA. Furthermore, there was insufficient grain for quality analyses, so protein analysis was carried out on the samples provided. SE-HPLC was used to measure effects on polymeric and monomeric proteins and as Wyuna was the only susceptible cultivar, RP-HPLC was used in addition to ascertain if there were any significant effects on the high molecular weight glutenins in particular.

Results

Detailed figures of experimental design and of results are attached.

Endosperm protein during grain filling

Endosperm maturation occurs from about 30 – 50 days post anthesis (DPA). The overall pattern of changes in endosperm-protein composition differed during this period for the three cultivars investigated. For **Fang**, the % unextractable polymeric protein (%UPP) content increased from 22% to 45% during maturation (39 – 50 DPA). However, little change was noted for polymeric/monomeric protein ratio or glutenin/gliadin ratio.

Gabo-Olympic lines, with a full complement of HMW-GS (i.e. "+++"), had %UPP values of 45% by 35 DPA, and although these values were reduced at 41 DPA, they recovered to 47% by maturation at 45 DPA. The null Gabo line lacking all HMW-GS only had 5%UPP at maturation and the "++-" null line showed an increase of 10 to 20% UPP during grain maturation. The polymeric/monomeric protein ratios and glutenin/gliadin ratios for the "+++" and "++-" Gabo-Olympic lines were similar at maturation, but were significantly reduced for the "---" null line.

Wyuna showed earlier maturation of protein development during grain filling and reached 22% UPP by 23 DPA. The %UPP continued to increase to 43% until maturity at 45 DPA. There was a steady decrease in polymeric/monomeric protein ratio and glutenin/gliadin ratio from the cell growth phase 17 – 30 DPA until maturation.

Effects of heat stress

Fang: Heat stress applied at 18 DPA caused a significant increase in %UPP, polymeric/monomeric protein ratio and glutenin/gliadin ratio in endosperm protein expressed at 39 DPA only. By full maturity, at 50 DPA, there was no discernible difference in %UPP or the other protein parameters between control and heat stressed grain.

Wyuna: Heat stress applied at 16 DPA caused significant decreases in % UPP at 23, 26, 30 and 36 DPA, however by maturity (45 DPA) there was no discernible difference in %UPP between the control and heat stressed samples. Decreases in the polymeric/monomeric protein ratios and glutenin/gliadin ratios, due to heat stress, were only found at 23 and 26 DPA. The effects of heat stress on HMW-GS composition were further investigated (by RP-HPLC) with this cultivar as it was found to be the most susceptible of the three cultivars investigated. The HMW/LMW ratio was significantly lower in heat stressed samples throughout most of the cell elongation and maturation stages of grain filling. Furthermore, a significant decrease in the proportion of *Glu-B1* 17x and 18y alleles was found in mature grain, with a corresponding increase in the proportion of *Glu-A1* 2*x.

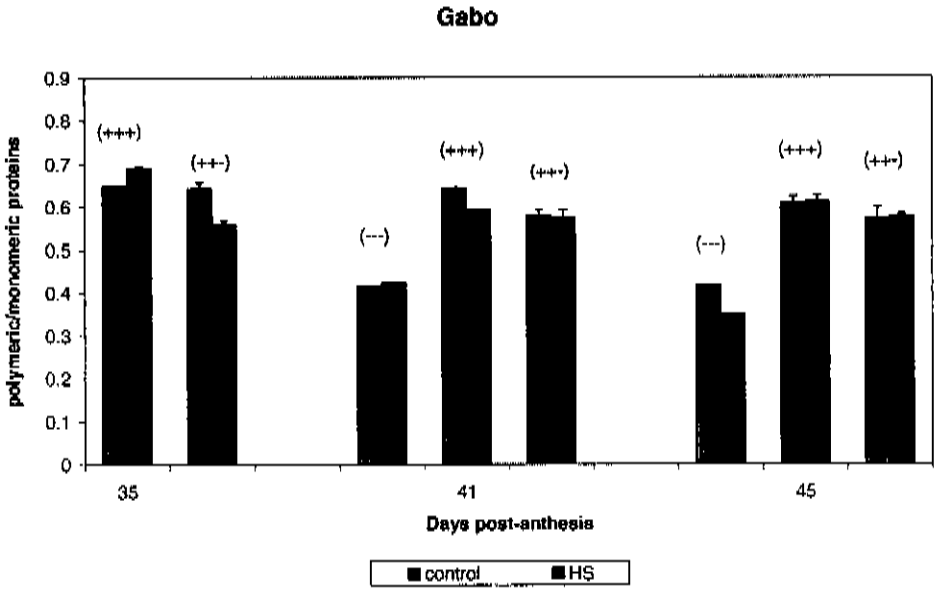
Gabo-Olympic lines: Heat stress, applied at 15 DPA, caused no significant changes in % UPP the late stages of maturation, regardless of whether the Gabo-Olympic line was null for one of the HMW glutenins. However, heat stress did decrease the polymeric/monomeric ratio and glutenin/gliadin ratio at 35 DPA for the "++-" Gabo-Olympic line and for the "+++" line at 41 DPA, but no effect was seen at full maturation (45 DPA). Heat stress appeared to have a net negative effect on the polymeric/monomeric ratio and glutenin/gliadin ratios at full maturation of the null Gabo-Olympic line for all the HMW glutenins ("---").

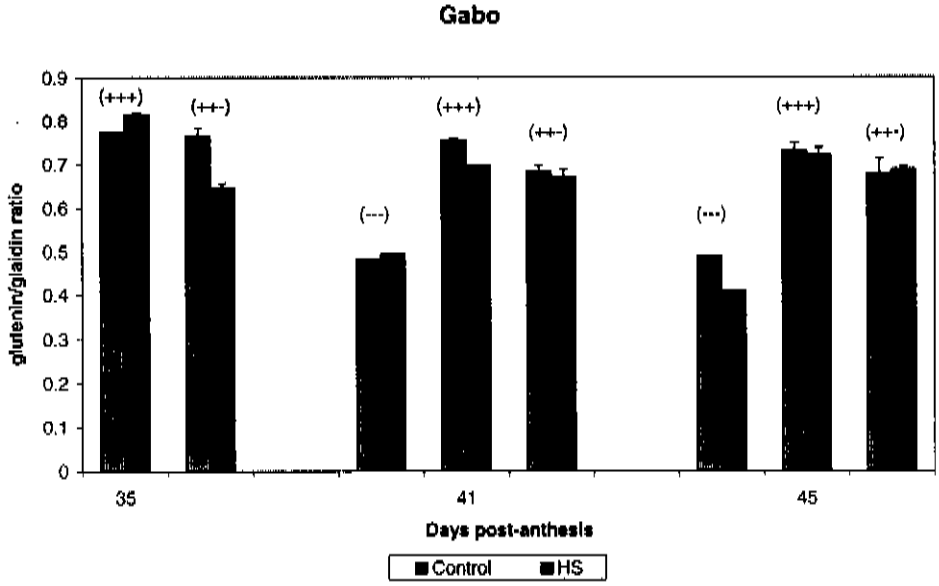
Conclusions

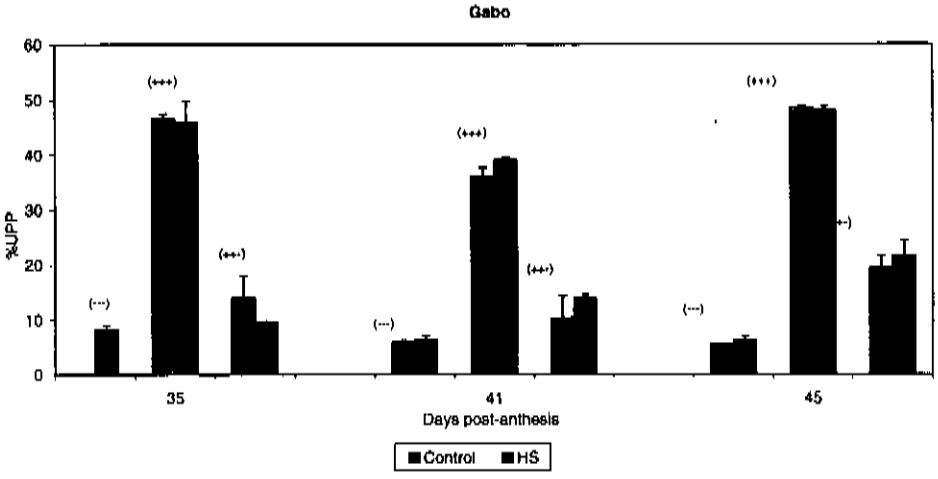
This set of experiments indicated the differential maturation of common Australian cultivars, Gabo-Olympic lines, Fang and Wyuna, and their susceptibility to heat stress.

Endosperm proteins of Fang were found to be tolerant to heat stress and were even shown to respond positively to the stress in late stages of maturation. Wyuna endosperm proteins, however, were susceptible to heat stress as shown by the decrease in %UPP during the maturation phase of endosperm development, although this was not evident at full maturation.

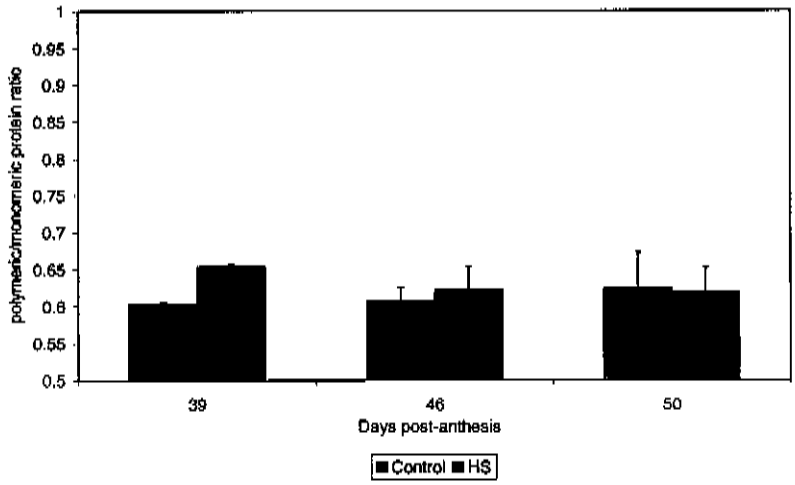
The RP- HPLC data did reveal a cause for dough weakening though, as heat stress produced a significant decrease in HMW/LMW ratio and a large decrease in *Glu-1* Bx17 and By18. These are considered to be strength-conferring alleles in this cultivar. Gabo-Olympic lines were generally heat tolerant for all the lines investigated. The results showed the greater contribution of *Glu-D1* to %UPP; this was calculated as 30% UPP from the difference in %UPP shown by "+++" and "++-" lines at maturation. However, these results also showed that gliadins and LMW glutenin subunits, in the full null Gabo-Olympic line ("---"), were also susceptible to heat stress.


Gabo
polymeric/monomeric proteins
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
(+++)
(++-)
(---)
(+++)
(++-)
(---)
(+++)
(++-)
35
41
45
Days post-anthesis
control
HS

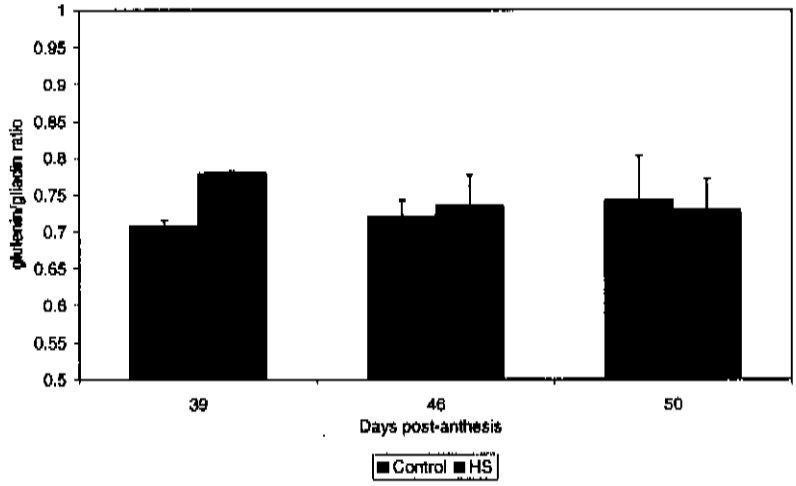

Gabo
glutenin/gliadin ratio
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
(+++)
(++-)
(---)
(+++)
(++-)
(---)
(+++)
(++-)
35
41
45
Days post-anthesis
Control
HS


Gabo
%UPP
60
50
40
30
20
10
0
(+++)
(++-)
(---)
(+++)
(++-)
(---)
(+++)
(---)
35
41
45
Days post-anthesis
Control
HS

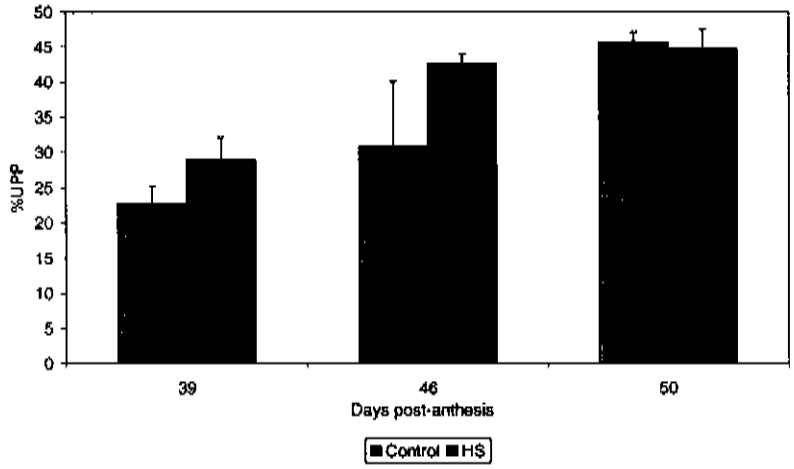
FANG

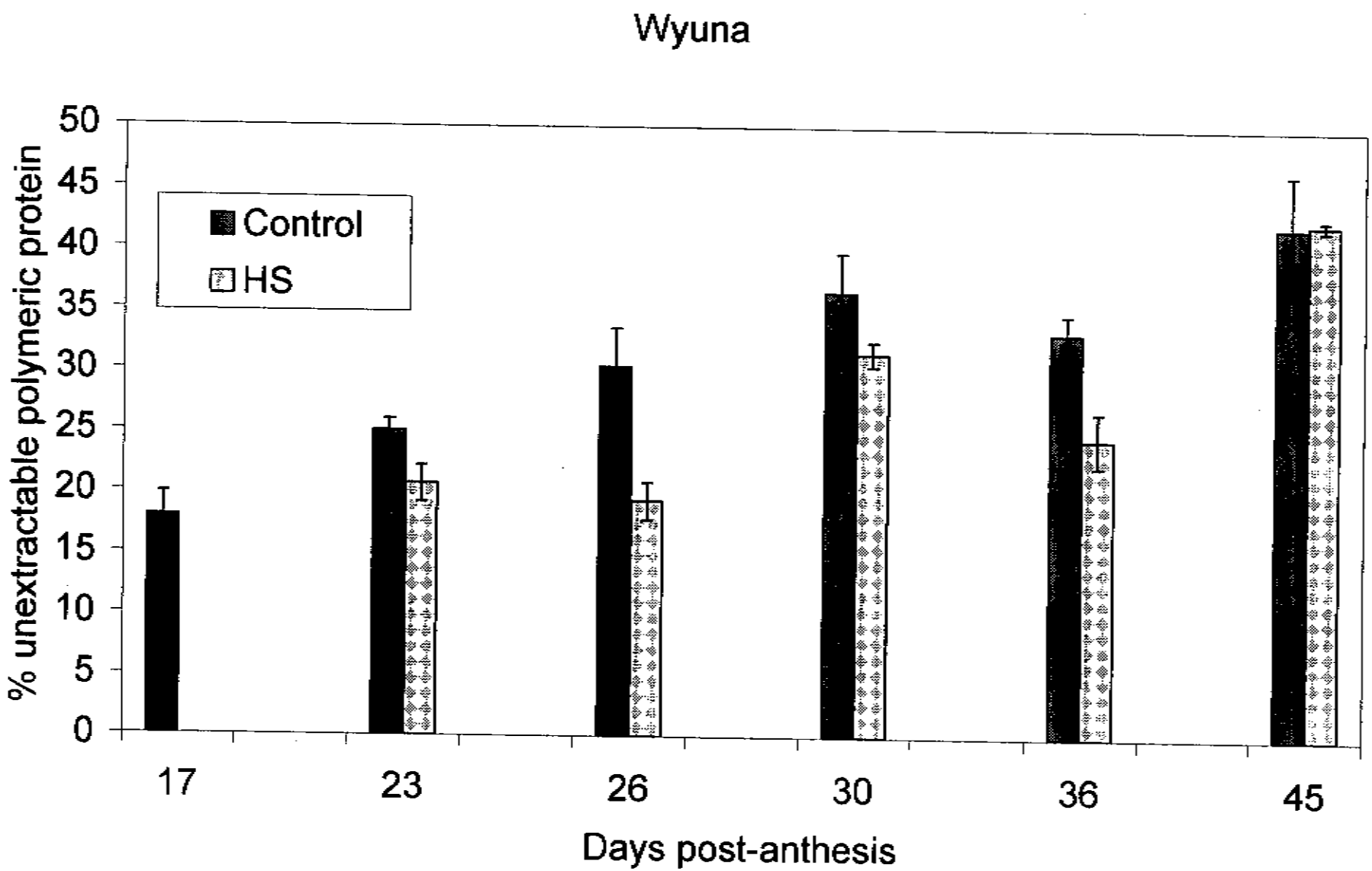


FANG

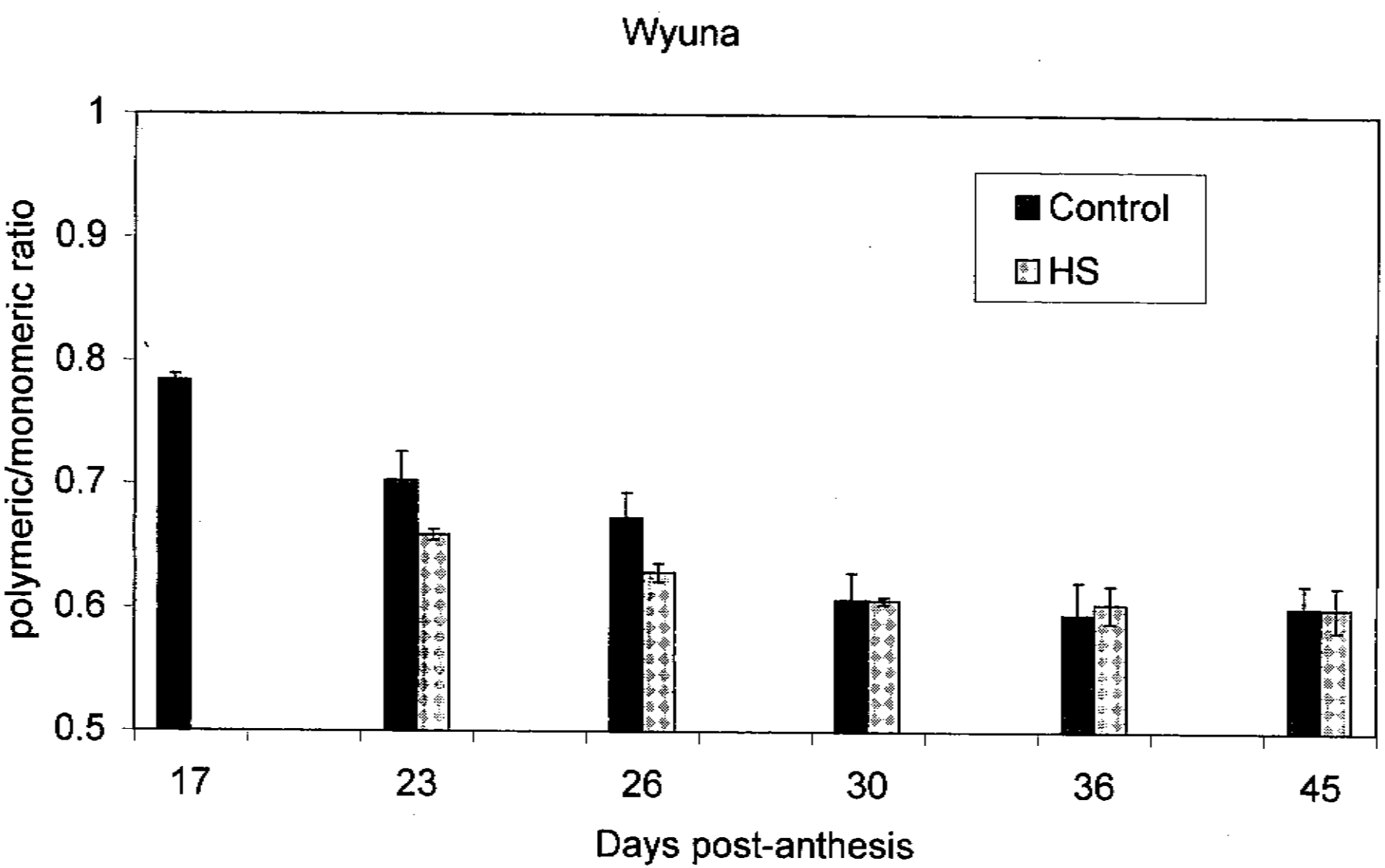


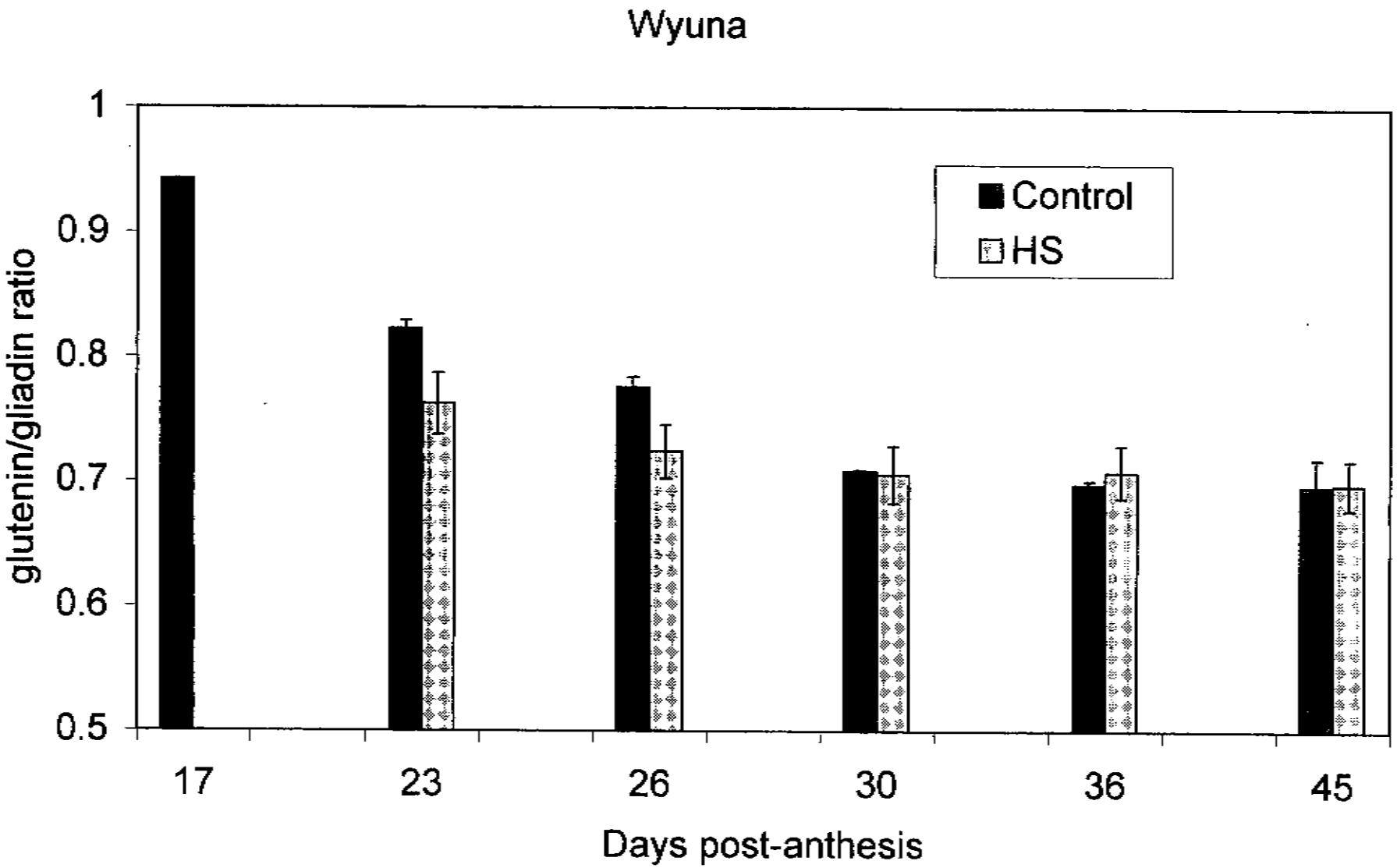
FANG





HS applied at 16


Wyuna
polymeric/monomeric ratio
1
0.9
0.8
0.7
0.6
0.5
Control
HS
17
23
26
30
36
45
Days post-anthesis


Wyuna
1
0.9
0.8
0.7
0.6
0.5
glutenin/gliadin ratio
Control
HS
17
23
26
30
36
45
Days post-anthesis

Sample DPA - t'ment	% Glutenin		% Gliadin		% alb/glob.		% UPP	
	average	sd	average	sd	average	sd	average	sd
23 control	41.268	0.806	50.173	0.552	8.559	0.254	24.963	0.995
23 HS	39.720	0.164	52.107	0.242	8.174	0.078	20.643	1.491
26 control	40.216	0.725	51.849	0.497	7.935	0.228	30.334	3.151
26 HS	38.613	0.287	53.340	0.225	8.046	0.063	19.277	1.512
30 control	37.765	0.862	53.315	0.512	8.919	0.350	36.510	3.226
30 HS	37.831	0.013	53.516	0.112	8.654	0.125	31.451	1.002
36 control	37.858	0.266	53.428	0.030	8.713	0.236	33.164	1.545
36 HS	36.890	0.470	53.165	0.559	9.945	0.089	24.475	2.231
mature contro	37.532	0.749	53.909	0.443	8.559	0.306	41.984	4.375
mature HS	37.518	0.698	53.795	0.697	8.687	0.001	42.241	0.400

Sample DPA - t'ment	HMW/LMW	average	% of total HMW Glu-A1x	Glu-B1x	Glu-B1y	Glu-D1x	Glu-D1y
23 control	0.62		16.18	30.31	26.04	15.84	11.63
	0.65	**0.64**	14.67	30.95	30.34	13.27	10.78
23 HS	0.51		16.58	34.89	25.54	13.65	9.34
	0.52	**0.52**	16.02	33.71	25.71	14.29	10.28
26 control	0.63		17.10	34.60	25.38	13.15	9.77
	0.61	**0.62**	17.33	34.18	25.20	13.65	9.64
26 HS	0.59		16.32	35.07	25.49	13.56	9.56
	0.67	**0.63**	16.65	33.78	25.45	14.03	10.09
30 control	0.66		16.81	34.50	25.22	14.16	9.32
	0.61	**0.63**	15.24	36.81	24.58	14.18	9.19
30 HS	0.58		16.62	35.91	24.07	13.87	9.53
	0.51	**0.54**	27.63	37.84	19.22	10.68	4.63
36 control	0.62		20.77	38.18	22.29	11.31	7.46
	0.53	**0.58**	18.05	38.04	23.84	12.86	7.21
36 HS	0.54		17.76	36.07	24.43	12.60	9.15
	0.53	**0.54**	17.85	37.22	24.40	11.86	8.66
mature contro	0.53		18.70	34.80	25.37	12.62	8.51
	0.55	**0.54**	34.11	24.27	21.79	11.77	8.06
mature HS	0.46		38.42	23.66	18.84	10.63	8.45
	0.46	**0.46**	40.96	22.40	19.87	9.93	6.84

Attached Report #4
Evaluation of the tolerance to heat stress during growth of 16 wheat varieties currently grown in Australia: Phytotron experiment

B.J.Butow

Aim
To determine the effect of heat stress (40°C) compared to moderate and high temperatures (23, 26, 29, 32°C) on functional dough properties, under controlled conditions.

Cultivars
Amery, Cadoux, Carnivale, Frame, Hartog, Janz, Kallanie, Krichauff, Silverstar, Sunvale, Tailor, Wallaroi, 1493, 1413, 2109 and 2024.

Conditions
All cultivars were grown in duplicate in pots maintained at 20/12°C until anthesis. At 7 days post anthesis all pots, except those undergoing extreme heat stress, were moved to the new temperature and grown there until harvest i.e. 23/ 15°C; 26/18°C; 29/21°C and 32/24°C. At 23 days post anthesis, plants destined for extreme heat stress were moved to a 40/26°C regime for 3 days and then returned to 20/12°C until harvest.

Parameters
Thousand Kernel Weight; % Protein; Functional dough parameters.

Results
Detailed figures of results are attached.

These experiments clearly show inter-cultivar variation with respect to the effects of different temperatures on varying parameters (Figs. 1a – d and summarised in Fig. 2). In many cases, moderate increases in temperatures had beneficial effects on both dough quality and wheat yield. However, the extreme of the heat stress (HS) treatment did have significant consequences on these parameters. In a review (below) of the effect of HS compared to control temperatures, only two of the sixteen cultivars trialed could be considered as heat-stress tolerant regarding both quality and yield.

Review of Susceptible vs. Tolerant cultivars:

Susceptible

- **Frame**: Although %P was barely changed by HS, TKW dropped quite significantly and dough strength and stability were greatly reduced.
- **2024**: %P was slightly reduced under HS and although the dough strength did not change, the dough stability increased. HS did cause a significant decrease in TKW .
- **Krichauff**: Although HS gave very significant increases in dough strength and stability, the decrease in TKW was equally significant. %P dropped slightly.
- **Silverstar**: Little change in %P or dough strength was shown but there was a significant decrease in dough stability and a slight increase in TKW under HS.
- **2109**: There was a significant decrease in dough strength although dough stability (or %P) were hardly altered. HS did significantly reduce TKW.
- **Hartog**: Similar to 2109; little change in %P or dough stability and a slight decrease in dough strength. Again, TKW was significantly decreased by HS.

- **Tailor:** Dough strength was significantly decreased with HS even though stability increased. TKW was also significantly decreased.
- **Carnivale**: Similar to Tailor, except dough strength slightly increased with HS.
- **Wallaroi:** Greatly increased dough stability with HS and slight increase in %P and dough strength. However HS adversely affected TKW.
- **Janz:** Although there was a slight increase in %P and dough stability with HS, dough strength and TKW decreased.
- **1493:** Despite a significant increase in dough strength (and slight increase in %P) a weaker dough was formed due to HS and TKW was also lower.
- **Cadoux:** Although no change was found in TKW and a significant increase in %P was shown with HS, the dough was weakened slightly and was less stable.

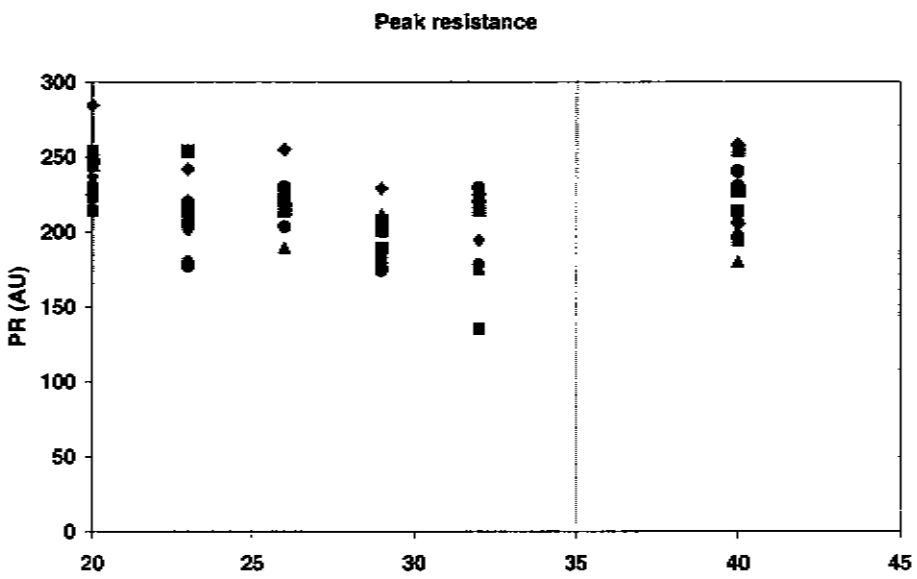
Tolerant

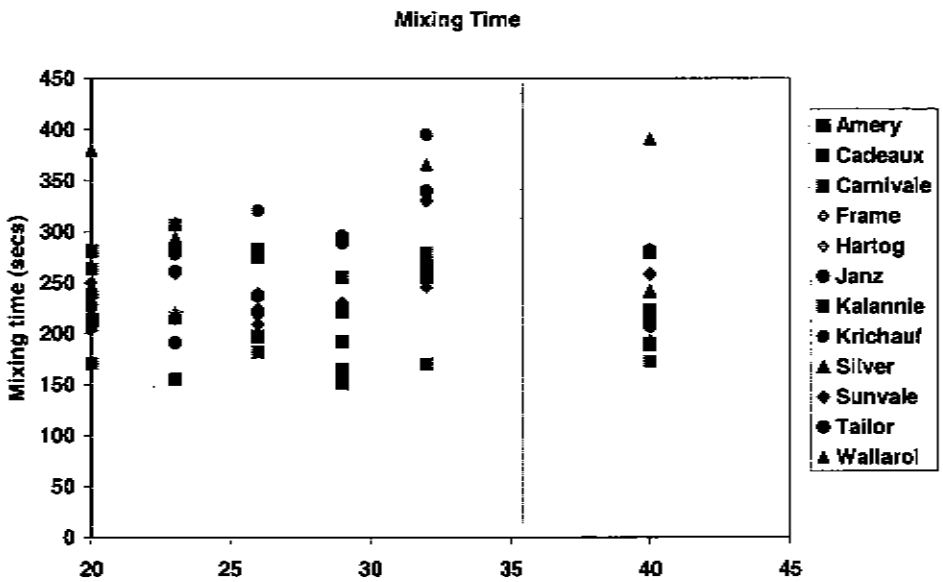
- **Amery:** despite a significant decrease in %P, dough strength and stability were maintained or even increased under HS. TKW was only slightly reduced by HS.
- **Sunvale:** similar results as Amery, however an increase in TKW was achieved under HS.
- **1413:** No significant changes were seen with %P or dough quality (a slight increase in dough stability was found), but a significant increase in TKW was shown.
- **Kalannie:** included as tolerant as only a slight decrease in TKW given with HS. Otherwise get a significant increase in %P, very large and significant increase in dough strength and moderate increase in stability.

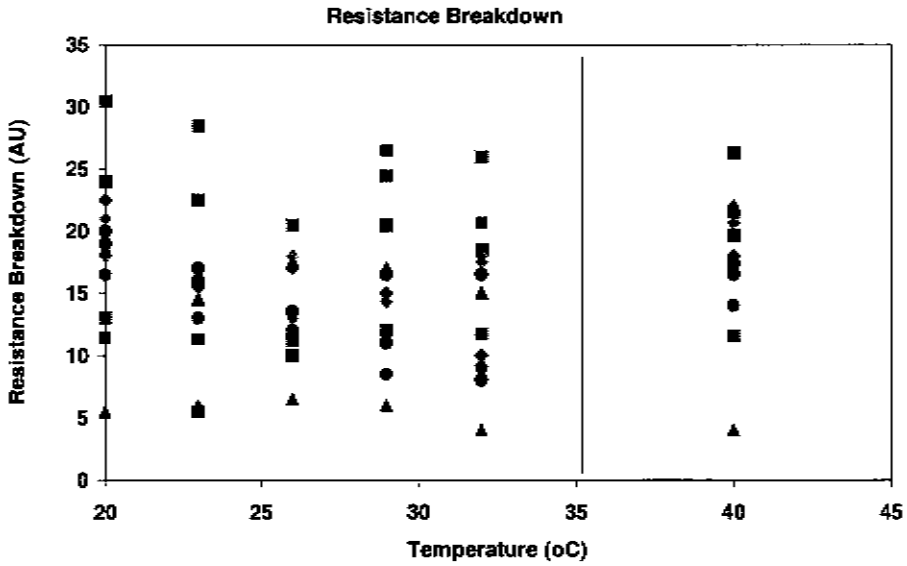
Conclusions

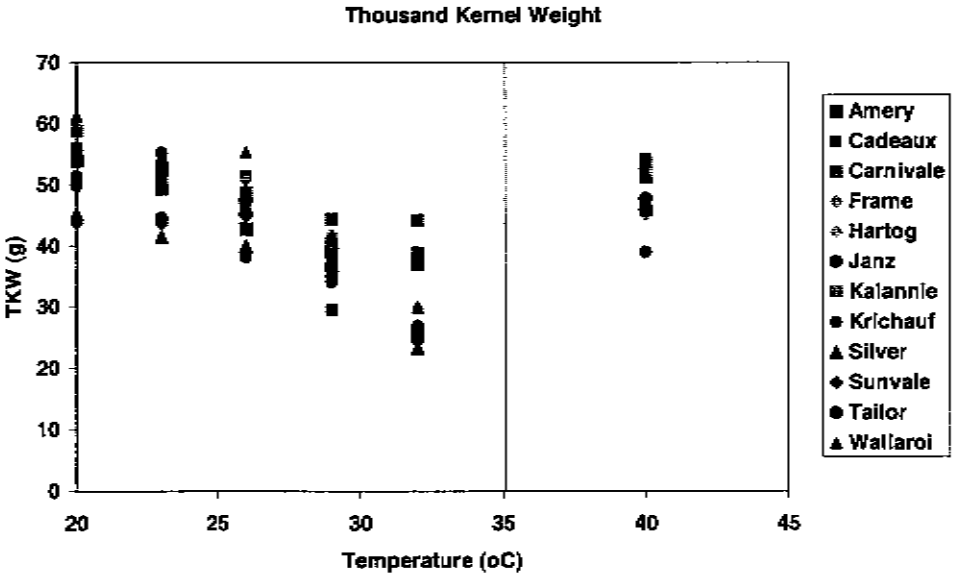
Within the confines of pot experiments, we found large cultivar-specific variations. Some cultivars favoured increases in post anthesis temperatures; for example, Janz showed significantly stronger and more stable dough even when left to mature at 32°C. Other cultivars, such as Amery, showed little change in dough quality with increasing post anthesis temperatures and some cultivars (Cadoux and 1493), showed diminished dough strength and stability at 29°C, for example. Overall, the detrimental effect of increased post anthesis temperature on grain yield was more consistent compared to effects on dough quality.

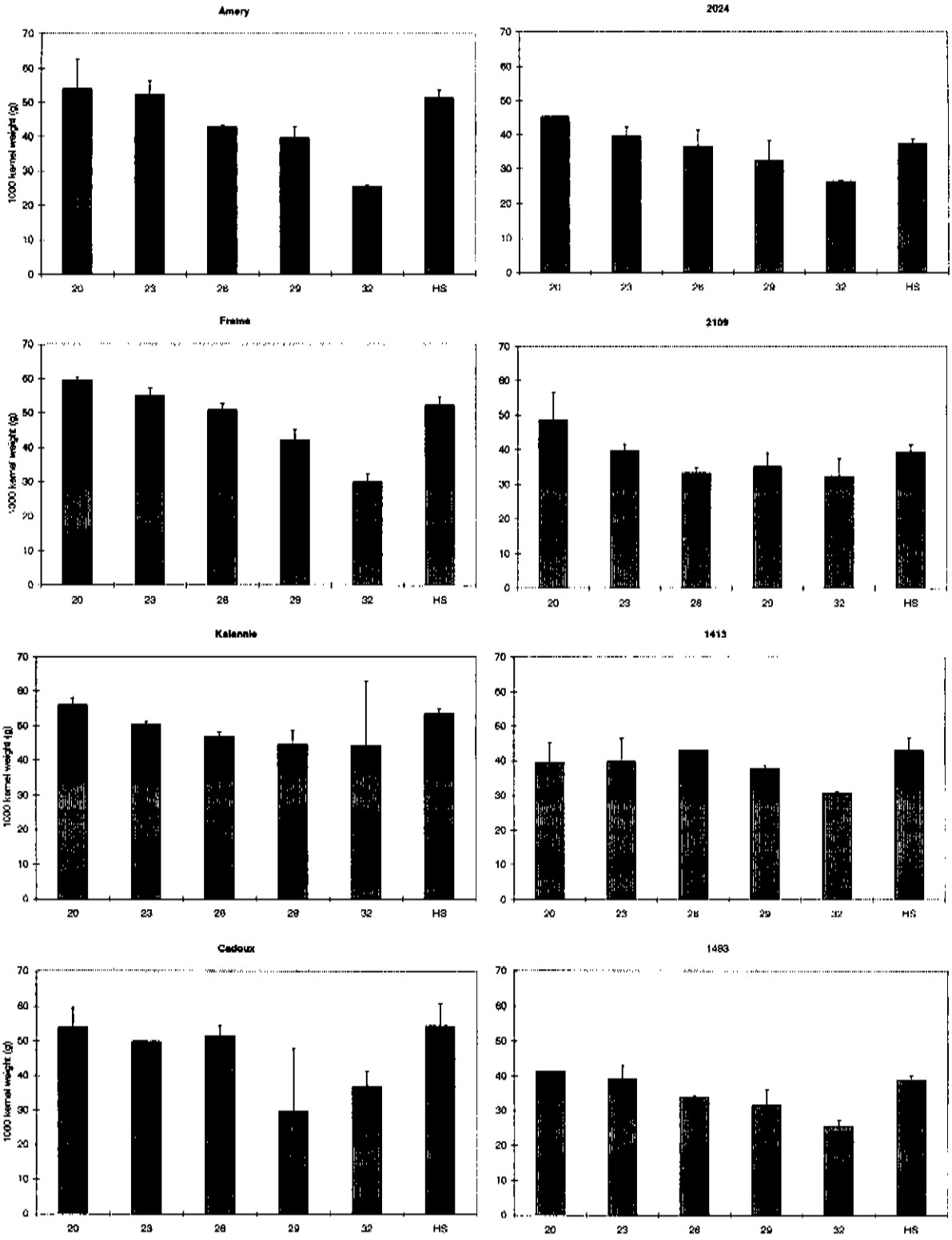
For all cultivars, the accumulated effects of post anthesis heat stress, i.e., increased temperature throughout grain maturation was significantly more detrimental to TKW than the short duration of the HS temperature (3 days at 40°C). This was shown by the incremental increases in temperature (up to 32°C) causing a corresponding decrease in TKW. Most cultivars maintained their final TKW having been subjected to temperatures up to 26°C, the final yield then generally dropped above this temperature. Conversely, a constant post anthesis temperature of 32°C resulted in an increase in %P in many cultivars; this high temperature would most likely have caused a complete metabolic reorganisation for example in the partitioning of starch and protein within the endosperm, starch biosynthetic enzymes possibly being more sensitive to temperature.

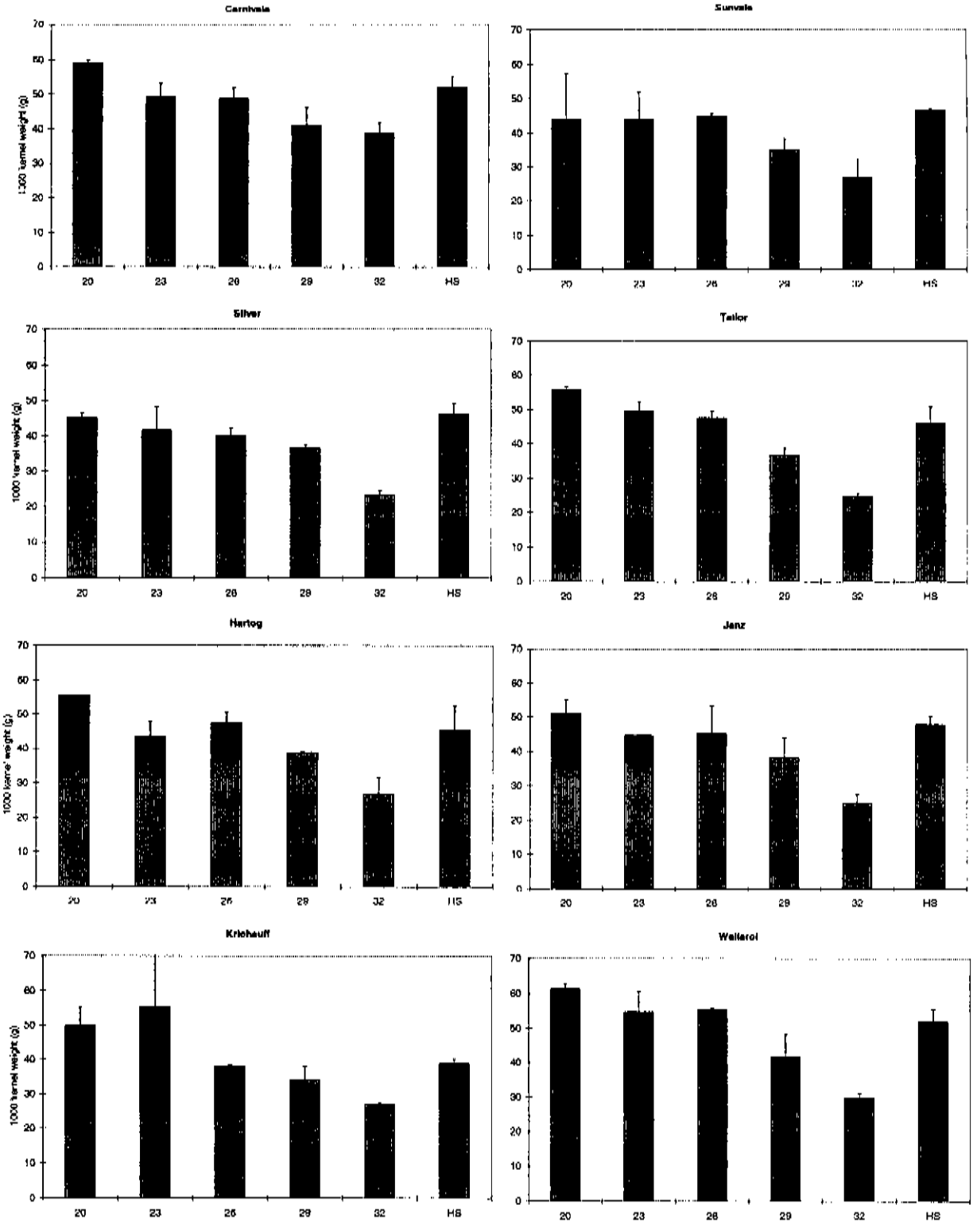

Peak resistance
PR (AU)
300
250
200
150
100
50
0
20
25
30
35
40
45


Mixing Time
Mixing time (secs)
450
400
350
300
250
200
150
100
50
0
20
25
30
35
40
45
Amery
Cadeaux
Carnivale
Frame
Hartog
Janz
Kalannie
Krichauf
Silver
Sunvale
Tailor
Wallaroi

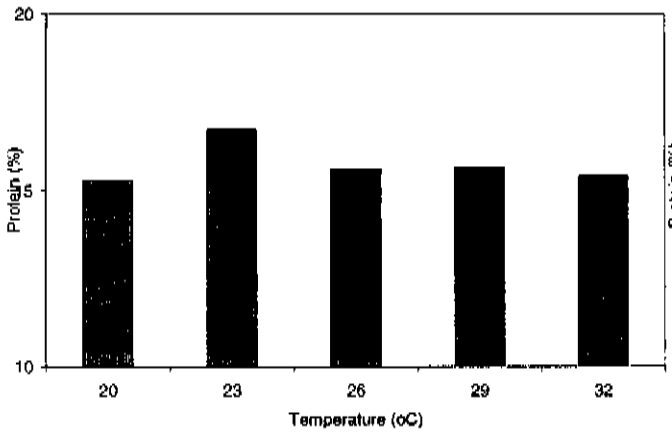

Resistance Breakdown
Resistance Breakdown (AU)
35
30
25
20
15
10
5
0
20
25
30
35
40
45
Temperature (oC)


Thousand Kernel Weight
TKW (g)
70
60
50
40
30
20
10
0
20
25
30
35
40
45
Temperature (oC)
Amery
Cadeaux
Carnivale
Frame
Hartog
Janz
Kalannie
Krichauf
Silver
Sunvale
Tailor
Wallaroi

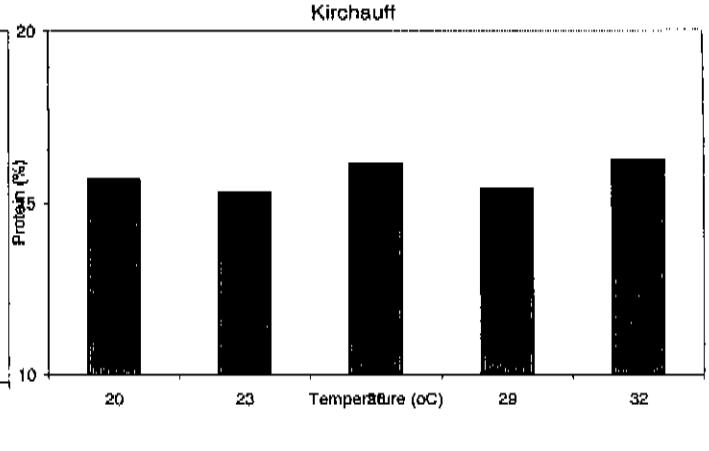

Amery
2024
Franka
2109
Kalannie
1413
Cadoux
1483
1000 kernel weight (g)
0
10
20
30
40
50
60
70
20
23
26
29
32
HS


Carnivale
Sunvale
Silver
Tailor
Hartog
Janz
Krichauff
Wallaroi
1000 kernel weight (g)
0
10
20
30
40
50
60
70
20
23
26
29
32
HS
Temperature oC)

Carnivale



Kirchauff



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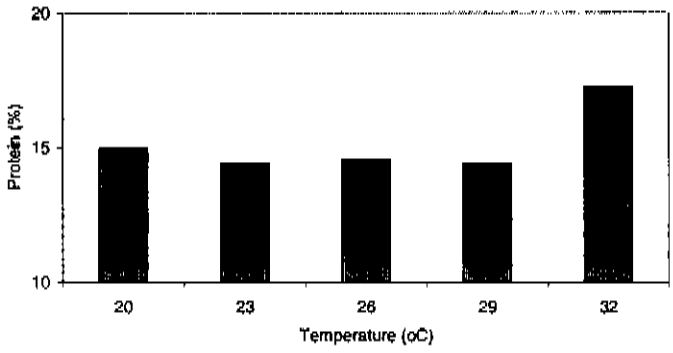

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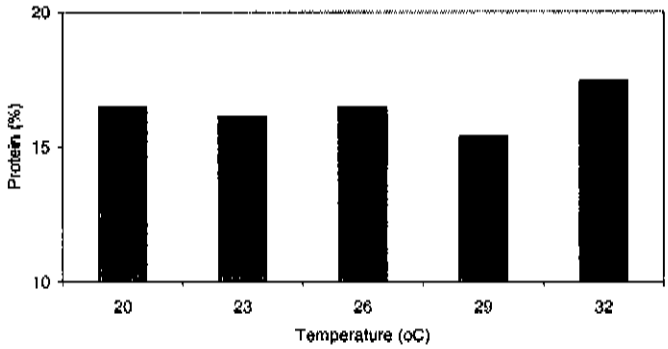
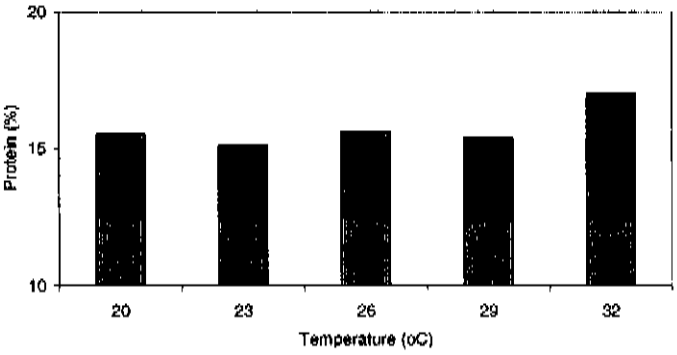


Hartog



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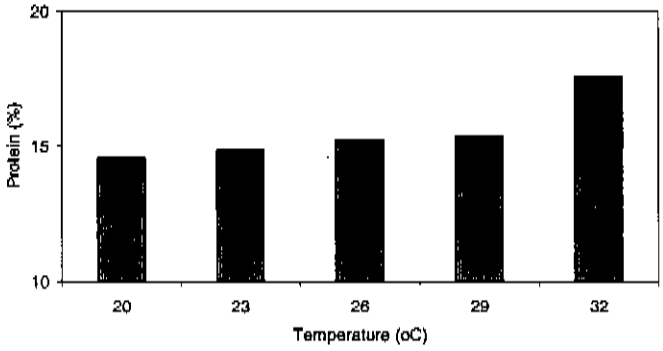
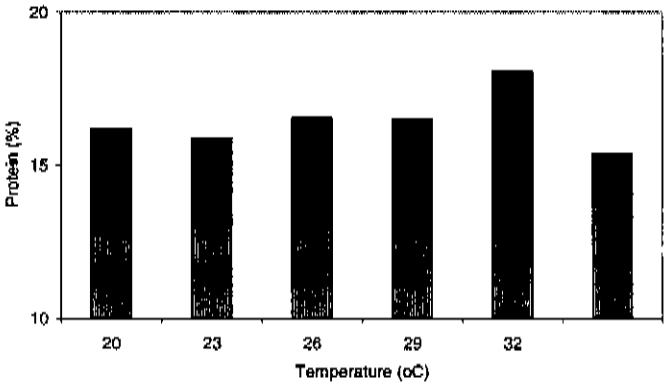


2024



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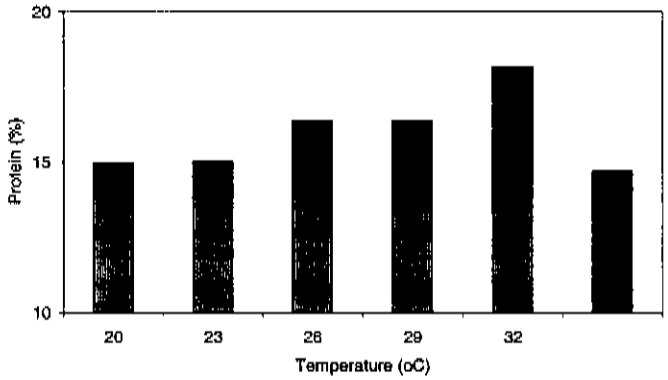

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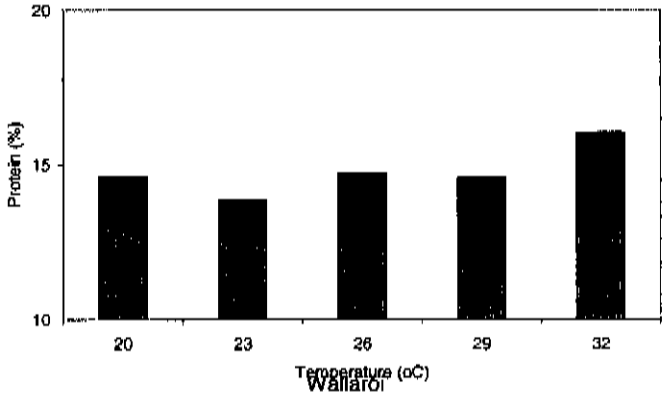

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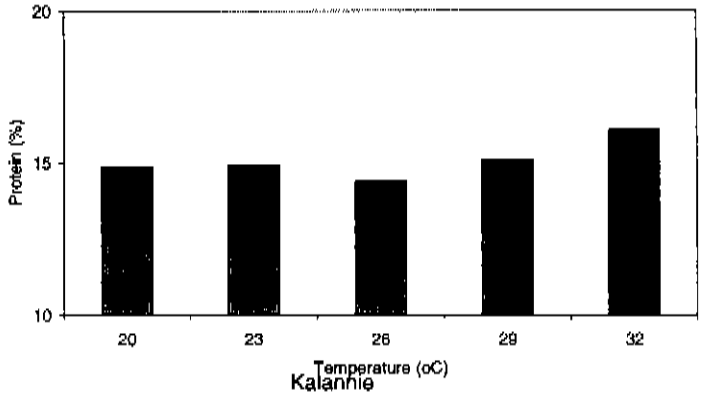

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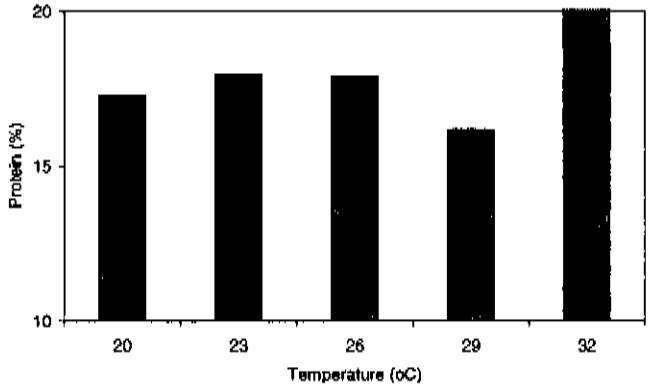
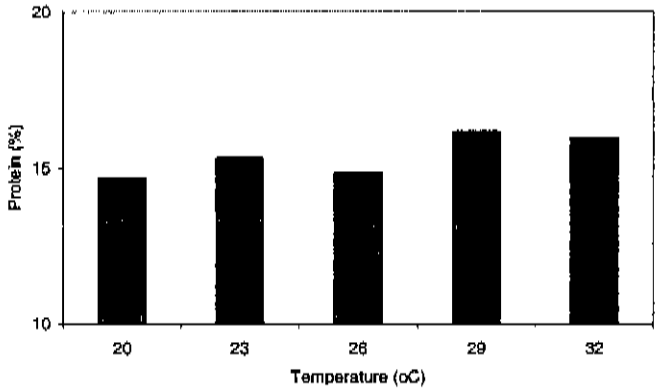


Kalannie



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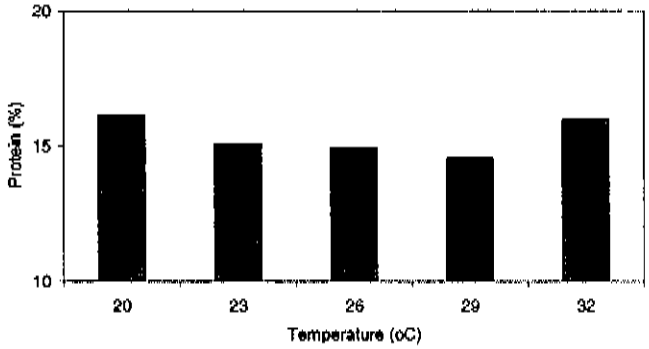
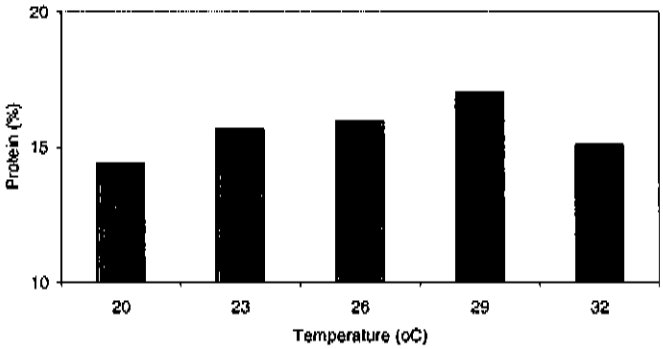
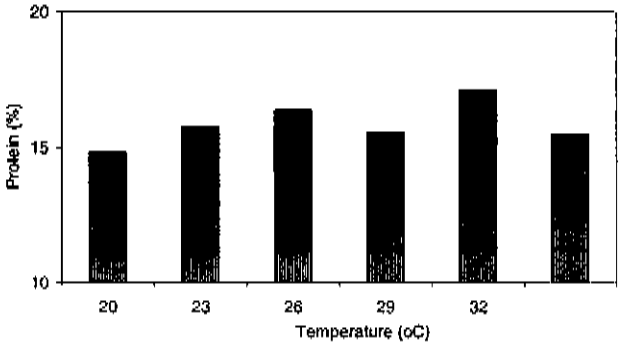


Cadoux



1493



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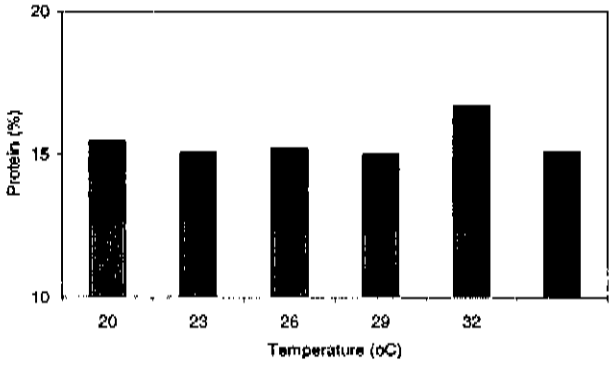

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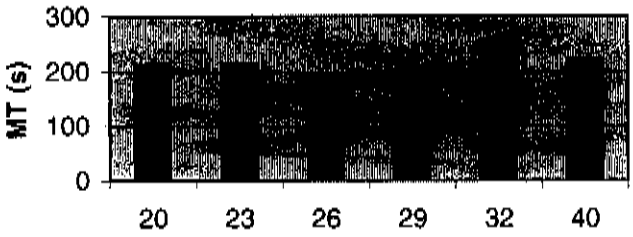

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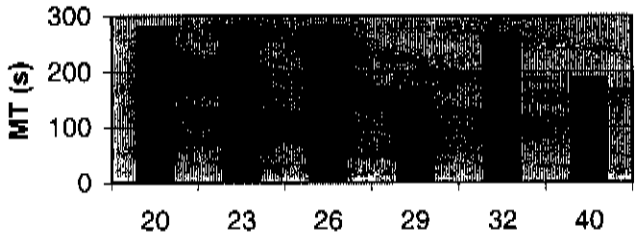
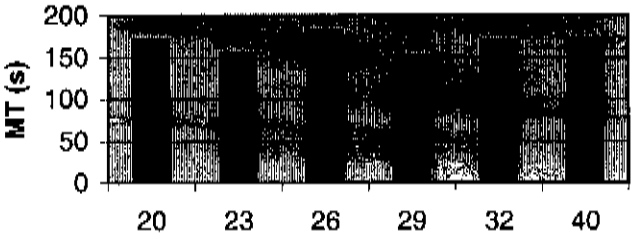


Carnivale



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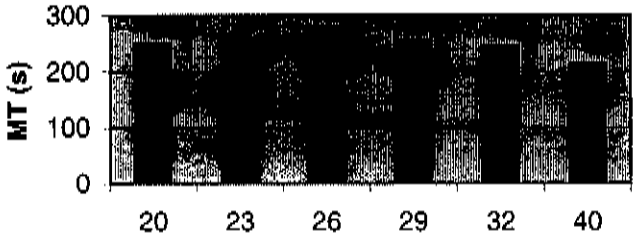

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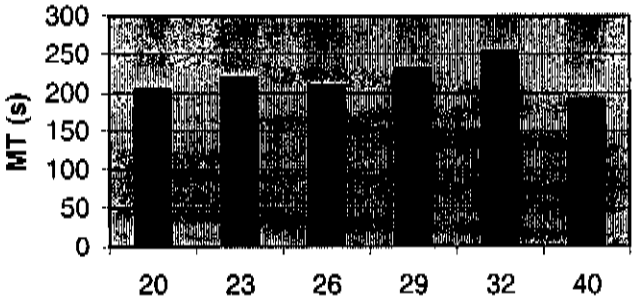

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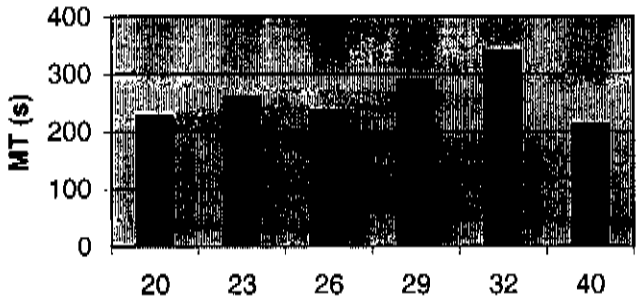
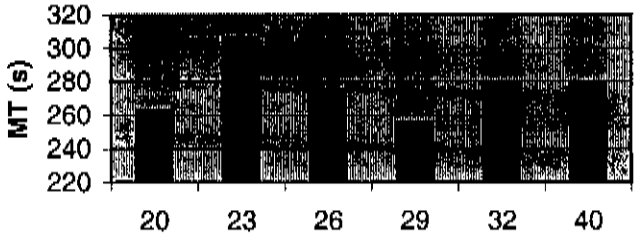
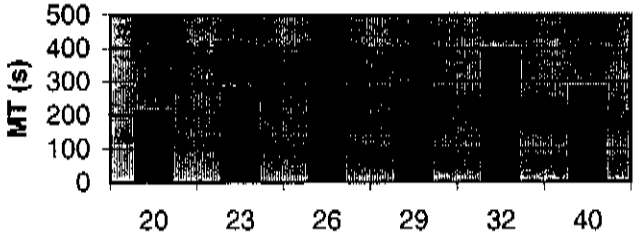
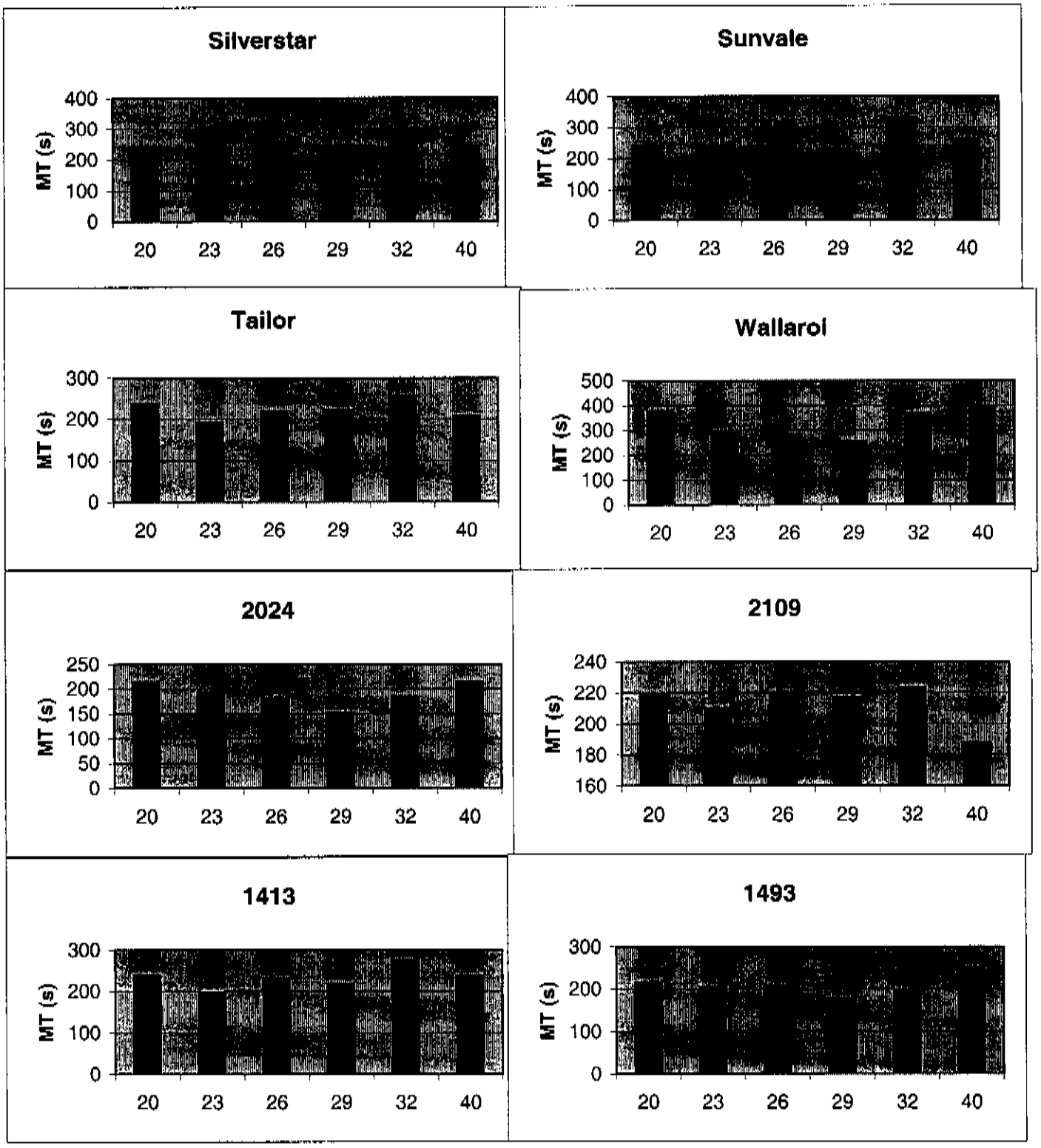


Kalannie

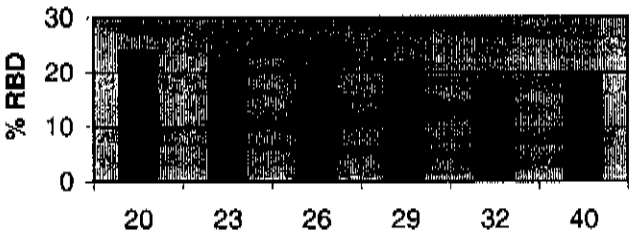


Krichauff

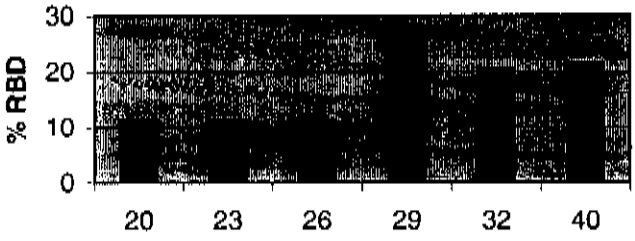



Silverstar
MT (s)
400
300
200
100
0
20 23 26 29 32 40
Sunvale
MT (s)
400
300
200
100
0
20 23 26 29 32 40
Tailor
MT (s)
300
200
100
0
20 23 26 29 32 40
Wallaroi
MT (s)
500
400
300
200
100
0
20 23 26 29 32 40
2024
MT (s)
250
200
150
100
50
0
20 23 26 29 32 40
2109
MT (s)
240
220
200
180
160
20 23 26 29 32 40
1413
MT (s)
300
200
100
0
20 23 26 29 32 40
1493
MT (s)
300
200
100
0
20 23 26 29 32 40

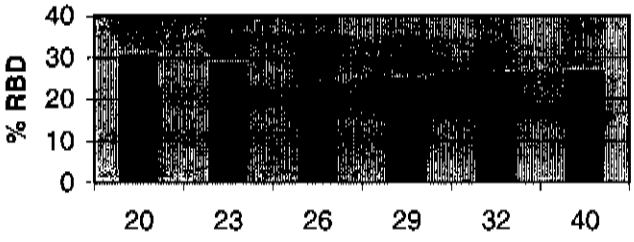
Amery



Cadoux



Carnivale



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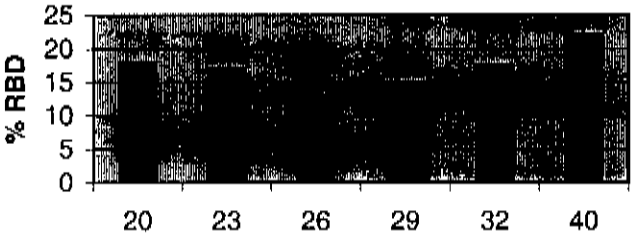
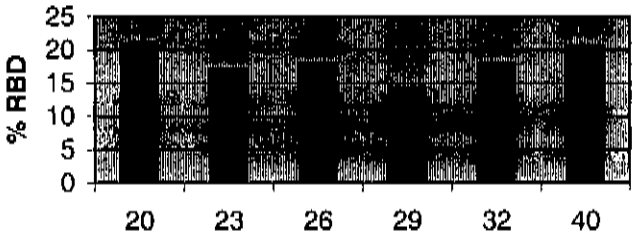
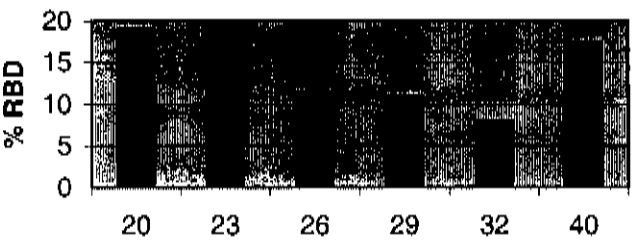


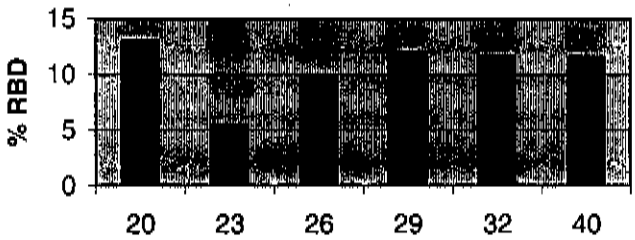
Hartog



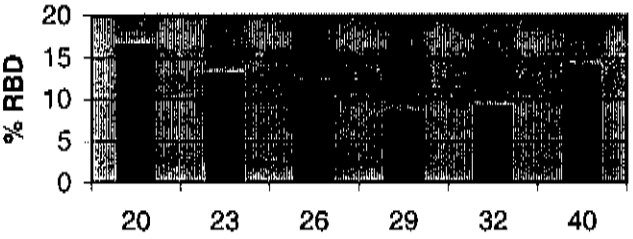
Janz



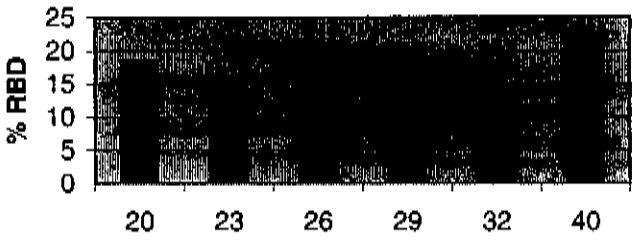
Kalannie



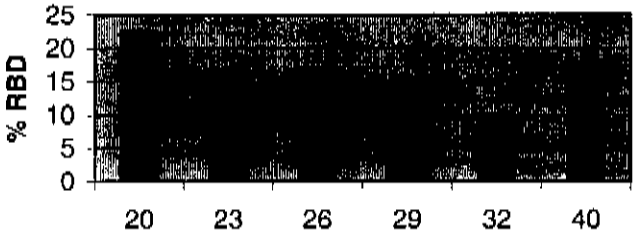
Krichauff



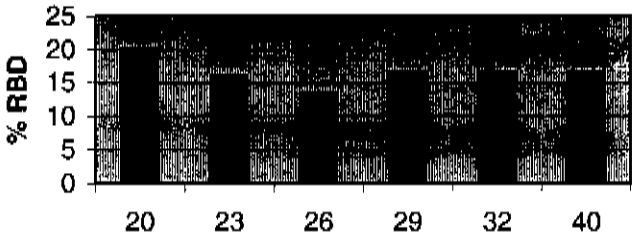
Silverstar



Sunvale



Tailor



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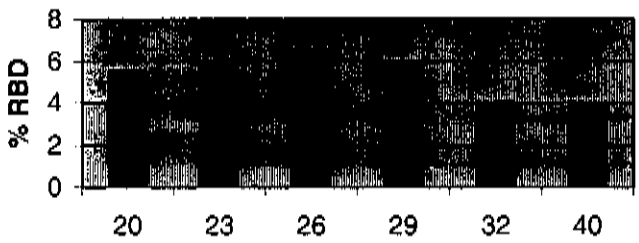
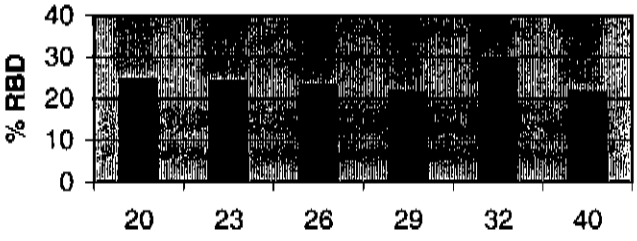
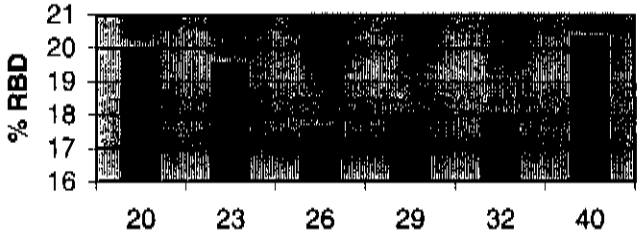


2024



2109



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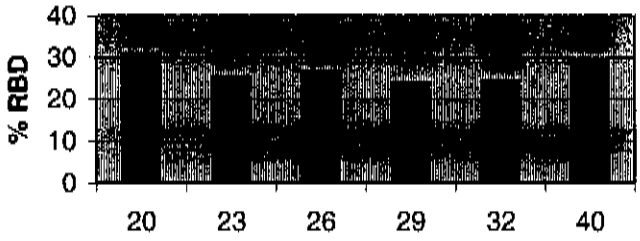

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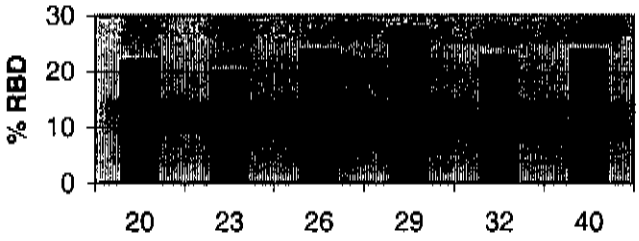


Variety	% protein content % (HS-C)/C
Amery	-11.59
Sunvale	-9.15
2024	-4.88
Silver	-3.14
Krichauff	-2.42
1413	-2.33
2109	-2.00
Frame	-0.94
Hartog	0.90
Tailor	1.44
Carnivale	2.68
Janz	2.80
Wallaroi	2.86
1493	4.39
Cadoux	7.02
Kalannie	9.68

Variety	RBD (s) % (HS-C)/C
Wallaroi	-27.27
Sunvale	-20.00
Amery	-18.05
Tailor	-17.50
Krichauff	-15.15
Carnivale	-13.66
Kalannie	-13.46
2024	-12.24
Janz	-8.79
1413	-4.84
Hartog	-1.59
2109	1.66
Cadoux	7.50
1493	9.09
Silver	18.92
Frame	22.22

Variety	Mixing time (secs.) % (HS-C)/C
Frame	-14.80
2109	-13.91
Tailor	-12.82
Janz	-6.40
Hartog	-5.49
Cadoux	-0.79
1413	-0.63
Silver	0.21
2025	0.23
Carnivale	0.78
Wallaroi	3.03
Amery	4.14
Sunvale	6.61
1493	15.97
Krichauff	33.02
Kalannie	40.59

Variety	Peak Resistance (s) % (HS-C)/C
Wallaroi	-22.91
2109	-20.92
Krichauff	-14.53
Silver	-14.49
1413	-11.95
Sunvale	-9.14
Amery	-8.70
Cadoux	-8.06
Frame	-7.24
2024	-6.00
Janz	-4.44
Hartog	-2.95
Carnivale	0.00
Tailor	2.44
Kalannie	3.35
1493	25.52

Variety	TKW % (HS-C)/C
Krichauf	-21.79
Hartog	-18.79
2109	-18.65
Tailor	-17.56
2024	-17.11
Wallaroi	-15.56
Frame	-12.22
Carnivale	-11.44
Janz	-6.90
1493	-6.14
Amery	-4.97
Kalannie	-4.23
Cadoux	0.54
Silver	1.73
Sunvale	6.40
1413	8.65

Attached Report #5
Field trials to determine the effects of growth-temperature variations on 16 Australian wheats

B.J.Butow

Introduction

Sixteen cultivars were chosen as representing current and coming varieties of interest to the Australian wheat industry. They were grown under controlled conditions at three sites (Newdegate and Wongan Hills in Western Australia, and at Narrabri, NSW), with two sowing dates designed to provide an early harvest (no heat stress) and a late harvest (after heat stress would be expected). In this way, realistic field conditions were planned for elucidating the effects of naturally occurring heat stress on TKW, %P and functional dough properties for a range of significant varieties. These field trials were designed to complement the experiment in the Canberra Phytotron described in Report #4.

Limitations of the field trials

Wheat grown at Wongan Hills in 1998 experienced a sustained period (2 – 3 days) of high temperatures ranging from 30° to 36°C in between the first and second harvests. This potentially provided the 'desired' heat stress required for this field experiment (see attached graph of temperatures at Wongan Hills).

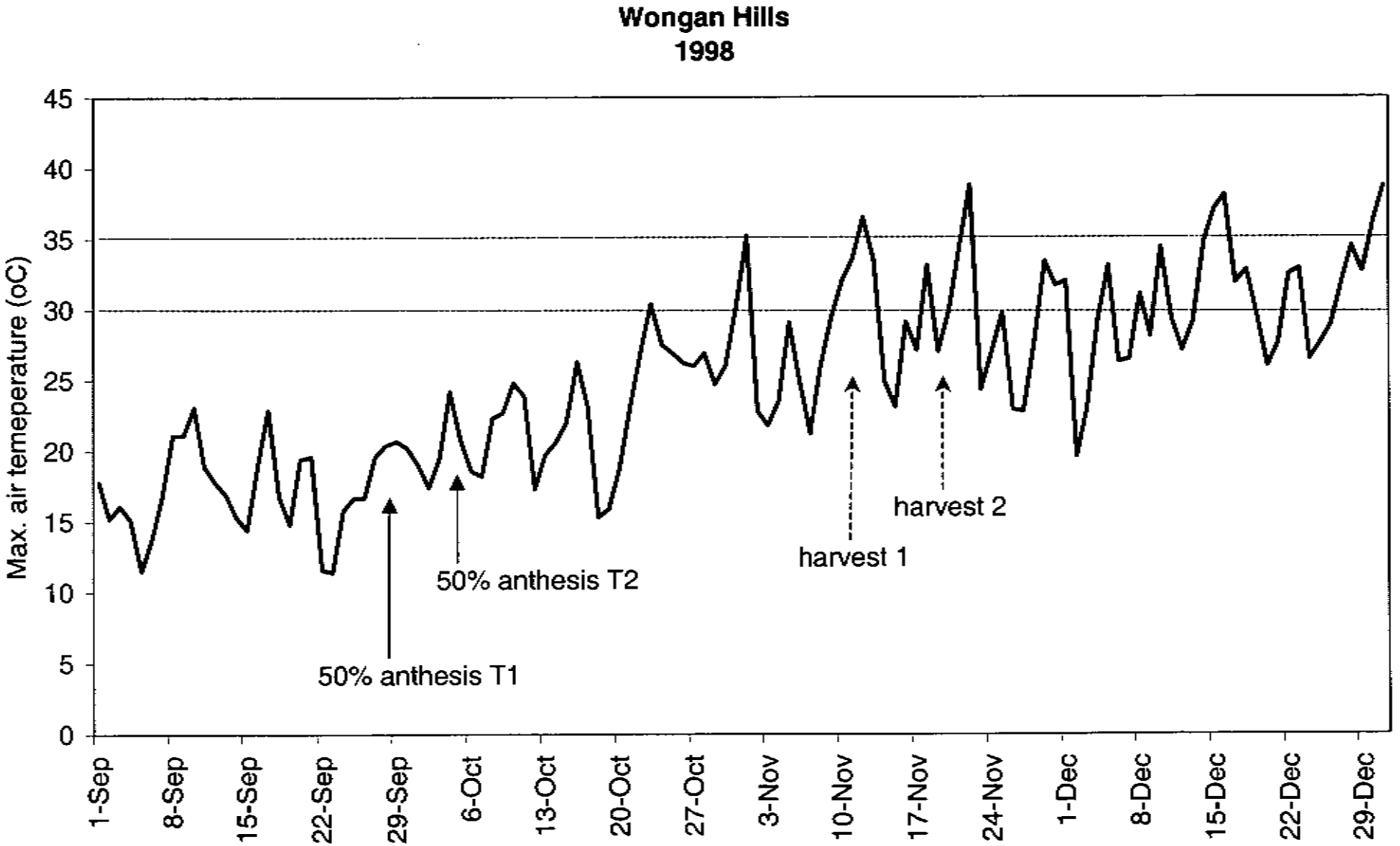
However, weather conditions during the grain-maturation period at Newdegate and Narrabri were such that the heat stress incurred by the plants was not as severe as that provided by the Phytotron experiments (Report #4), nor the conditions at Wongan Hills. Furthermore, the high temperatures (over 32°C) that were attained did not continue for three consecutive days (see attached graphs of temperature conditions). Thus the field experiments at Newdegate and Narrabri did not provide adequate conditions of heat stress to permit conclusions about heat tolerance/susceptibility to be drawn at these sites.

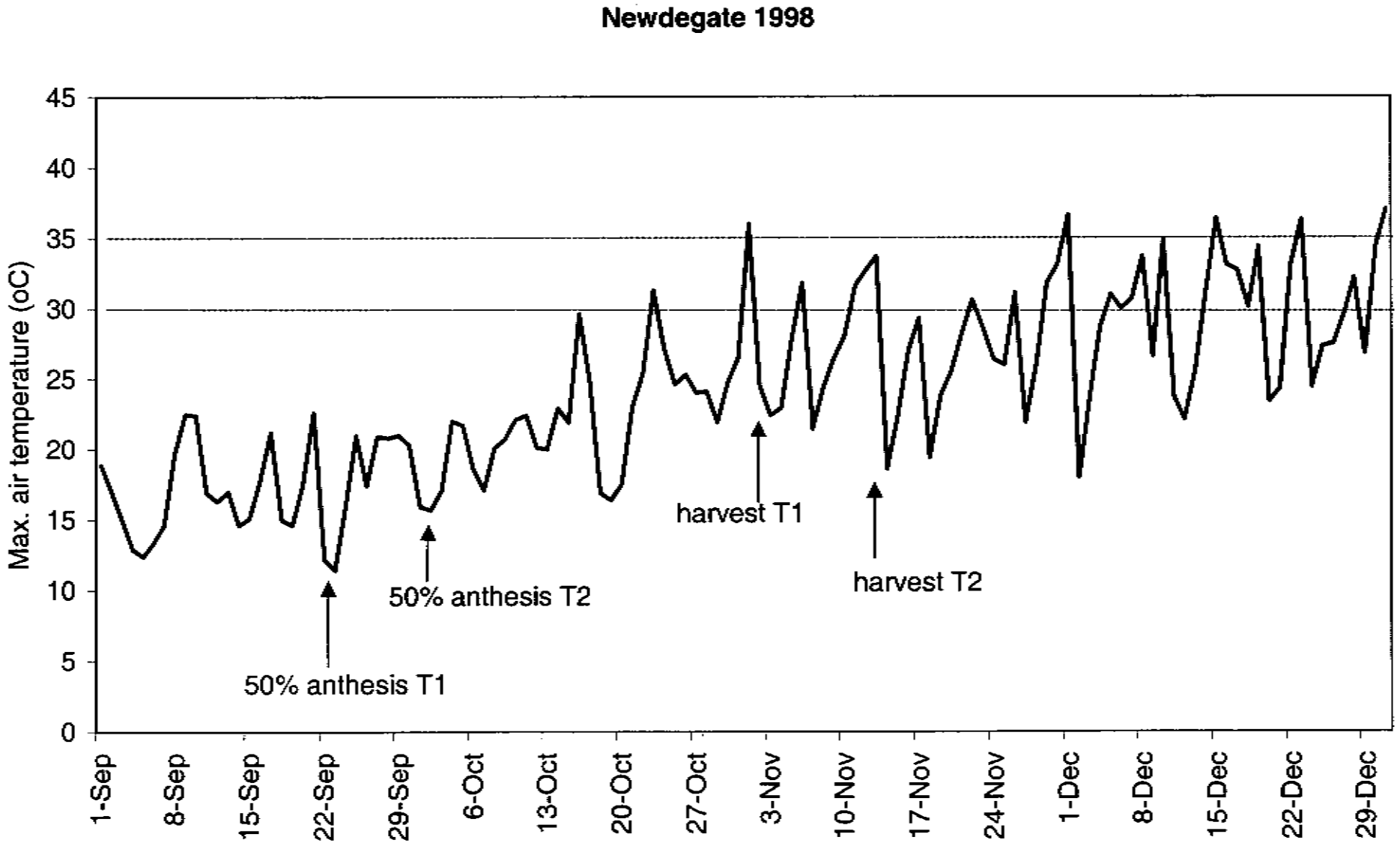
Conclusions from the Wongan Hills field trials

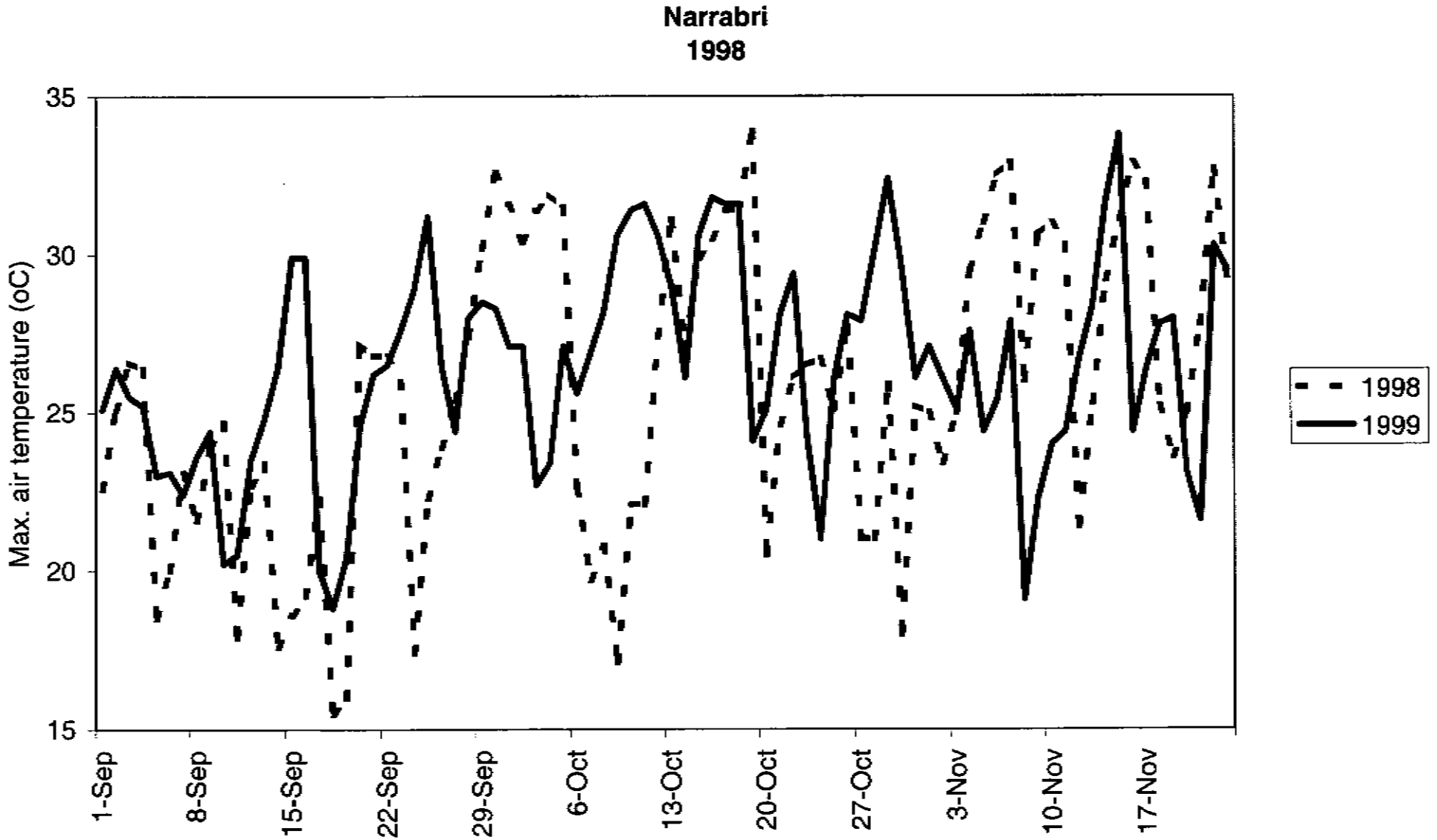
Wheat grown at Wongan Hills was sent for pilot-scale analysis (as a CRC In-Kind Contribution) to Agrifood Technology, Werribee. See detailed results attached. The same cultivar, sown at two sowing dates gave vastly different dough properties, in some cases. This indicated the important effects of this aspect of environmental conditions, but these results did not support the conclusions about individual varieties that were obtained in the Phytotron experiment (Report #4), where heat stress and control conditions were (obviously) more closely controlled.

Small-scale functional analyses also showed cultivar-specific variation, due to the two sowing dates. Evidence of the effect of sowing plot was also evident and was superimposed on these results. For example, Janz grown at Plot B showed a greater decrease in yield at the second sowing date (SD2) (inferring a decrease due to heat stress) as compared to the lesser, yet significant, decrease shown by Janz sown at Plot C. Yield appeared to be more sensitive to sowing date in the case of Janz, as no significant change in dough strength and stability (for Plot A only) with late sowing, but also a slight, yet significant, decrease in yield.

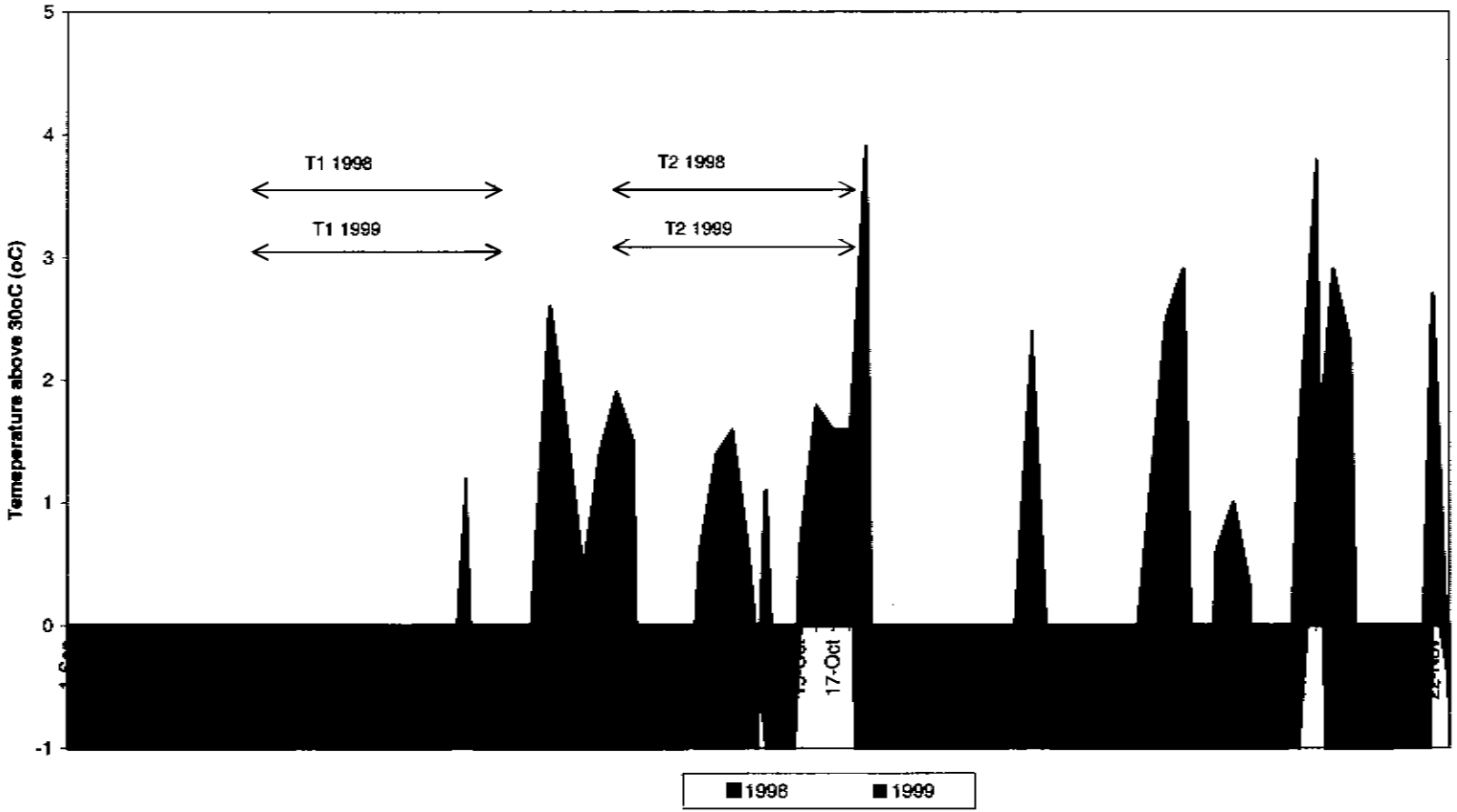
Taking into account the variations in results that we obtained for the same cultivars within field plots, it is not surprising that a comparison between field trials and the Phytotron experiments is tenuous. Hartog yield, for example, may have been highly susceptible to heat stress in the Phytotron experiment, but it was unchanged in this field experiment.


Wongan Hills
1998
Max. air temeperature (oC)
45
40
35
30
25
20
15
10
5
0
1-Sep
8-Sep
15-Sep
22-Sep
29-Sep
6-Oct
13-Oct
20-Oct
27-Oct
3-Nov
10-Nov
17-Nov
24-Nov
1-Dec
8-Dec
15-Dec
22-Dec
29-Dec
50% anthesis T1
50% anthesis T2
harvest 1
harvest 2


Newdegate 1998
Max. air temperature (oC)
45
40
35
30
25
20
15
10
5
0
1-Sep
8-Sep
15-Sep
22-Sep
29-Sep
6-Oct
13-Oct
20-Oct
27-Oct
3-Nov
10-Nov
17-Nov
24-Nov
1-Dec
8-Dec
15-Dec
22-Dec
29-Dec
50% anthesis T1
50% anthesis T2
harvest T1
harvest T2


Narrabri
1998
Max. air temperature (oC)
35
30
25
20
15
1-Sep
8-Sep
15-Sep
22-Sep
29-Sep
6-Oct
13-Oct
20-Oct
27-Oct
3-Nov
10-Nov
17-Nov
1998
1999

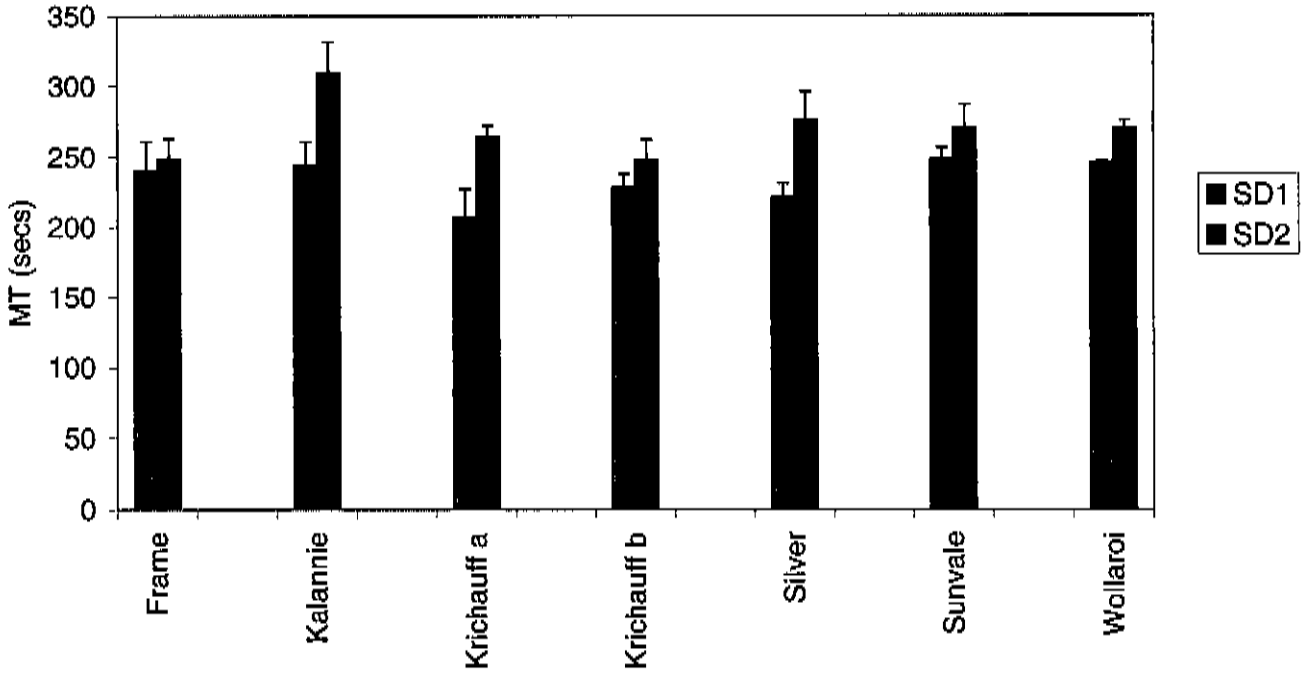
Graph showing duration and amount of heat stress (over 30oC) for field-grown wheat 1998 and 1999.



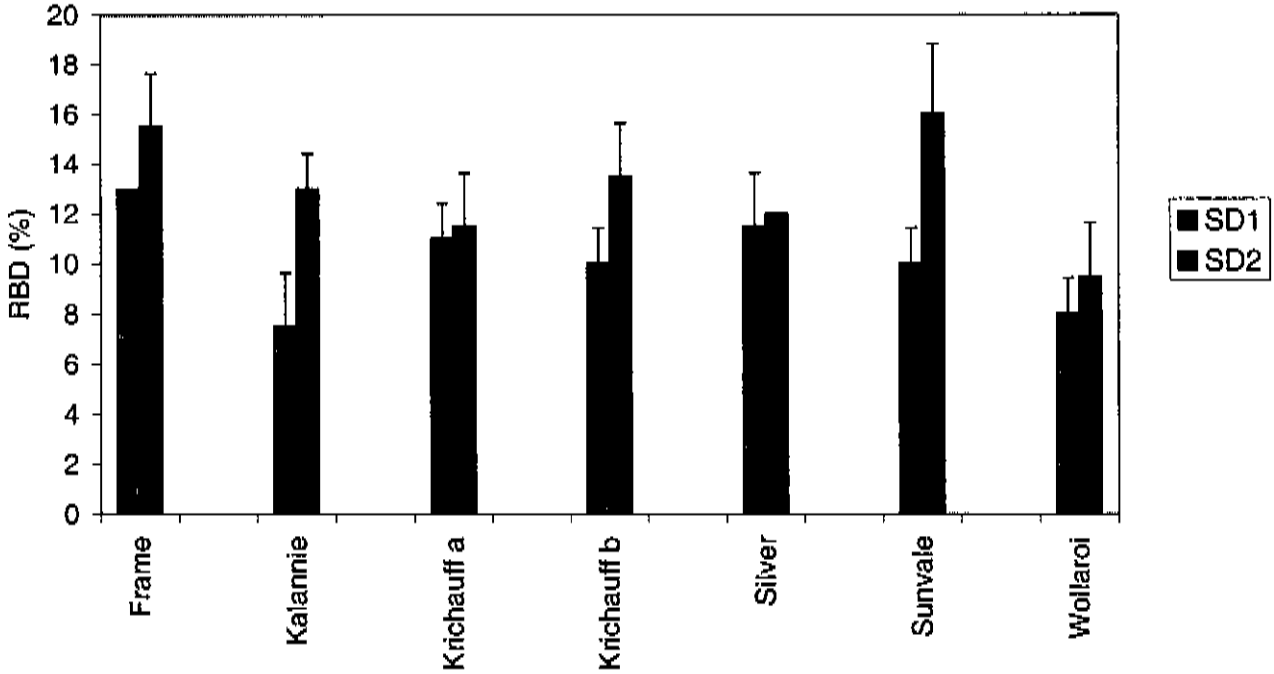
Results from CRC in-kind work by Agrifood - Wongon-Hills grown grain

	Frame		Hartog		Sunvale		Silverstar		Janz		Cadoux		Carnamah	
Farinograph	SD1	SD2	SD1	SD2	SD1	SD2	SD1	SD2	SD1	SD2	SD1	SD2	SD1	SD2
Water absorption	61.2	62	61.5	60.2	60.4	60	61.8	60	60.5	61.8	52.6	53.7	61.5	60.3
Development time (min)	8.4	18.5	3.7	19.4	12.7	8.2	5.4	7.2	6	7.3	4.2	4.7	5.5	7.2
Stability (min)	>15	>15	>15	>15	>15	>15	9.6	>15	10.3	>15	11.6	11.5	>15	>15
Breakdown (B.U.)	13	33	16	39	12	7	26	10	20	10	35	30	20	10
Extensograph (45min)														
Extensibility (cm)	20.7	16.8	17.9	21.5	24	20	18.6	20.9	17.4	23.3	19.1	22.9	18.3	20.8
Maximum height (B.U.)	462	514	540	571	607	645	535	570	535	560	495	460	535	525
Area (sq. cm)	132	121	136	168	195	174	138	162	132	179	129	146	135	147

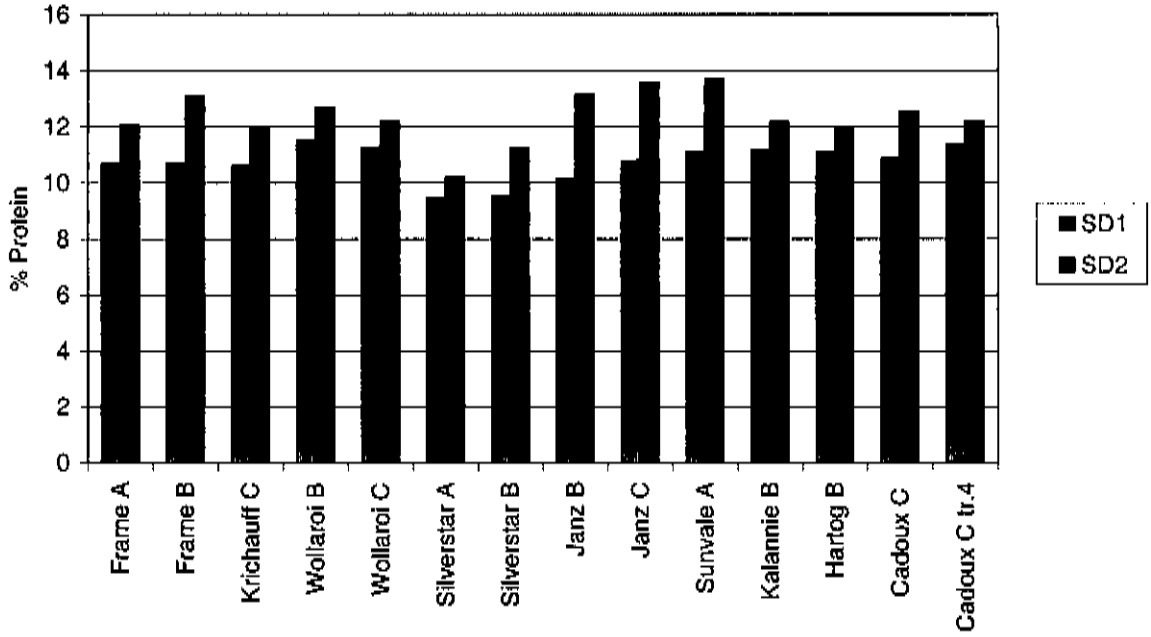
Wongan Hills
Mixing Time



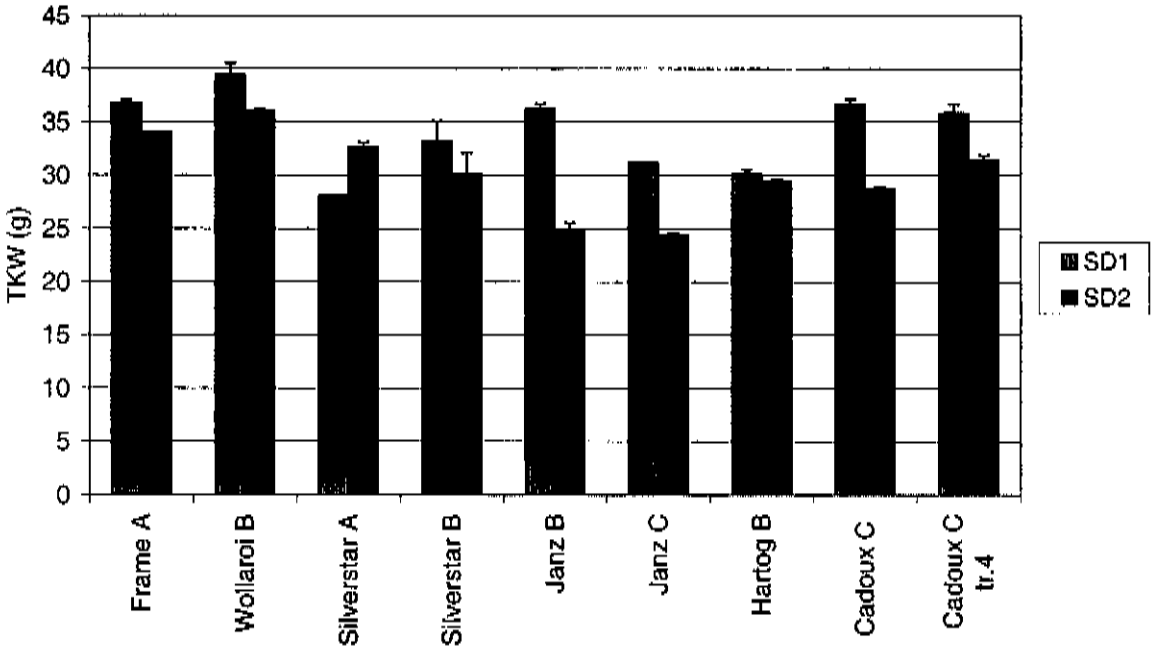
Wongan Hills
Resistance Breakdown



PROTEIN

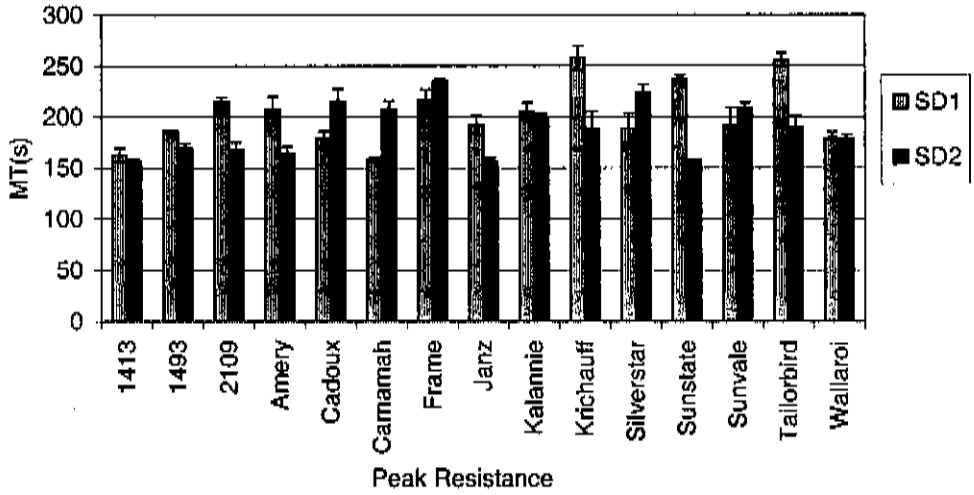


THOUSAND KERNEL WEIGHT

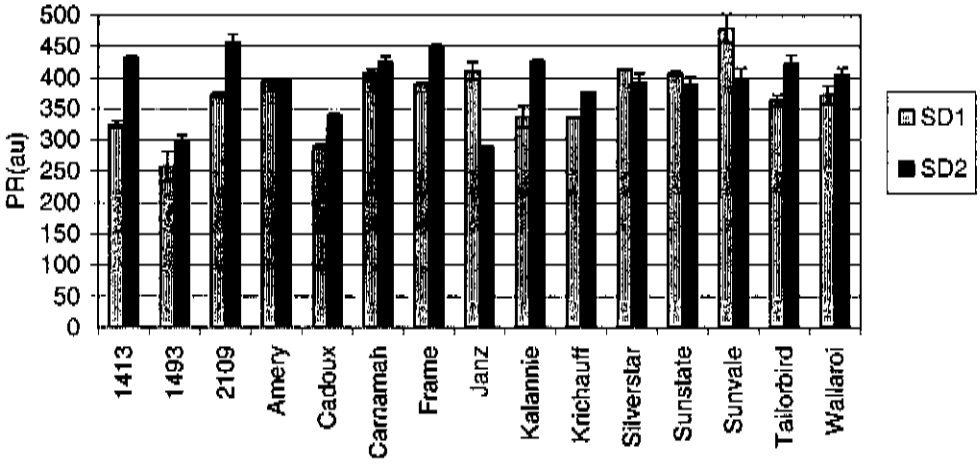


NARRABRI 1999

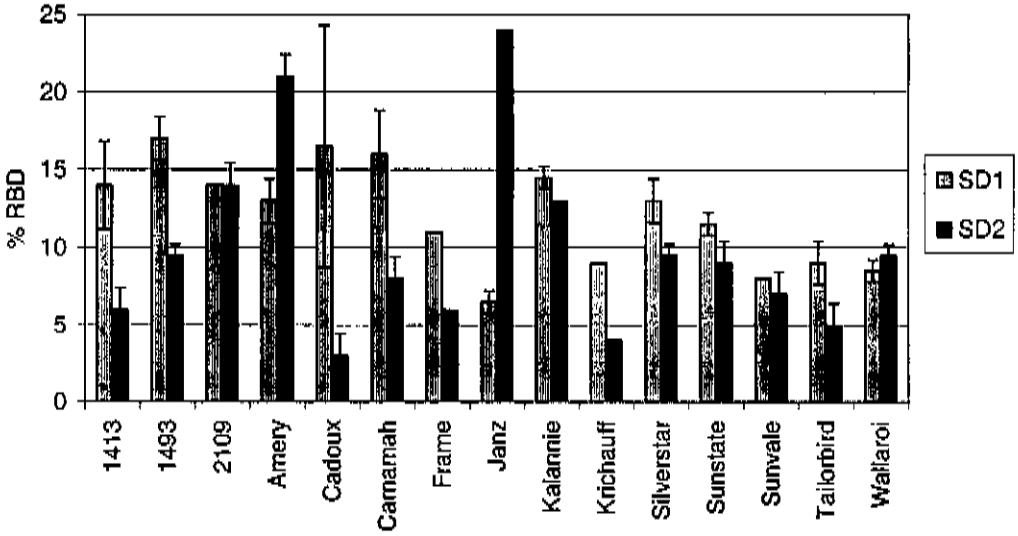
Mixing Time



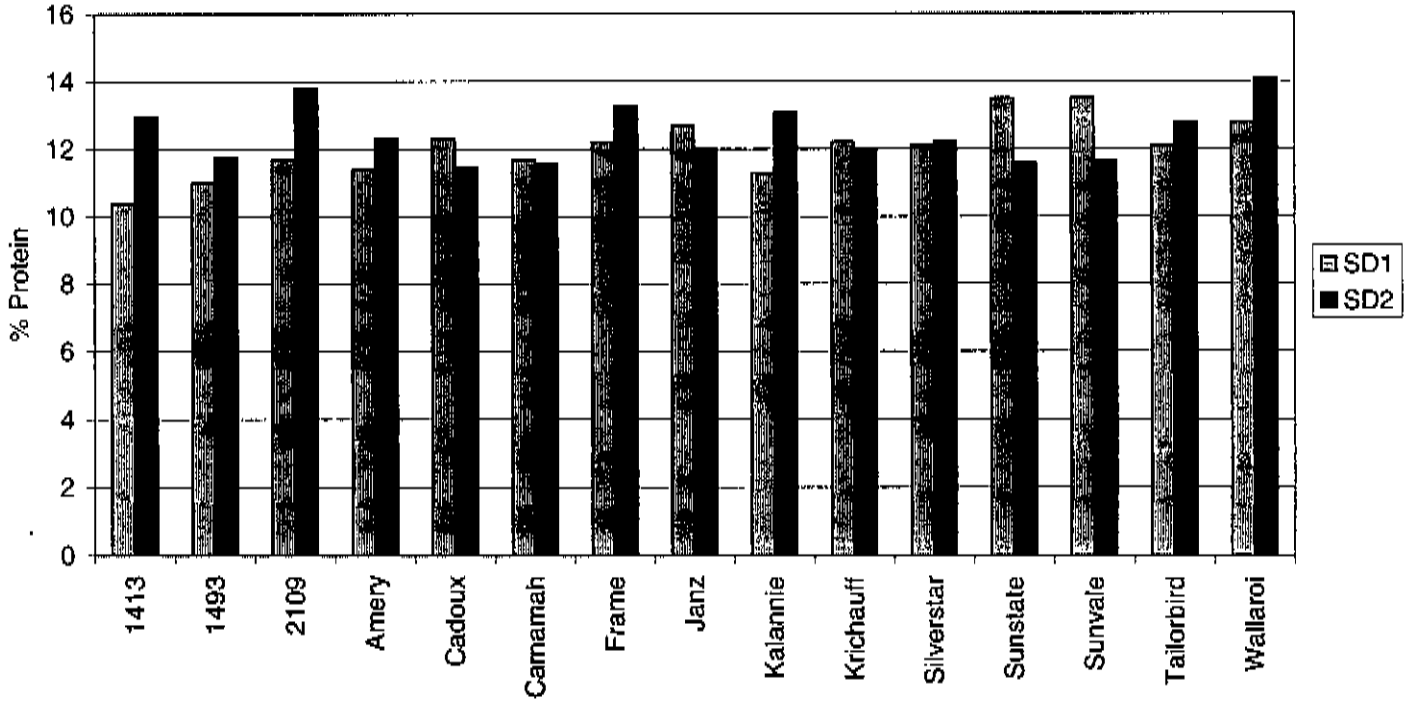
Peak Resistance



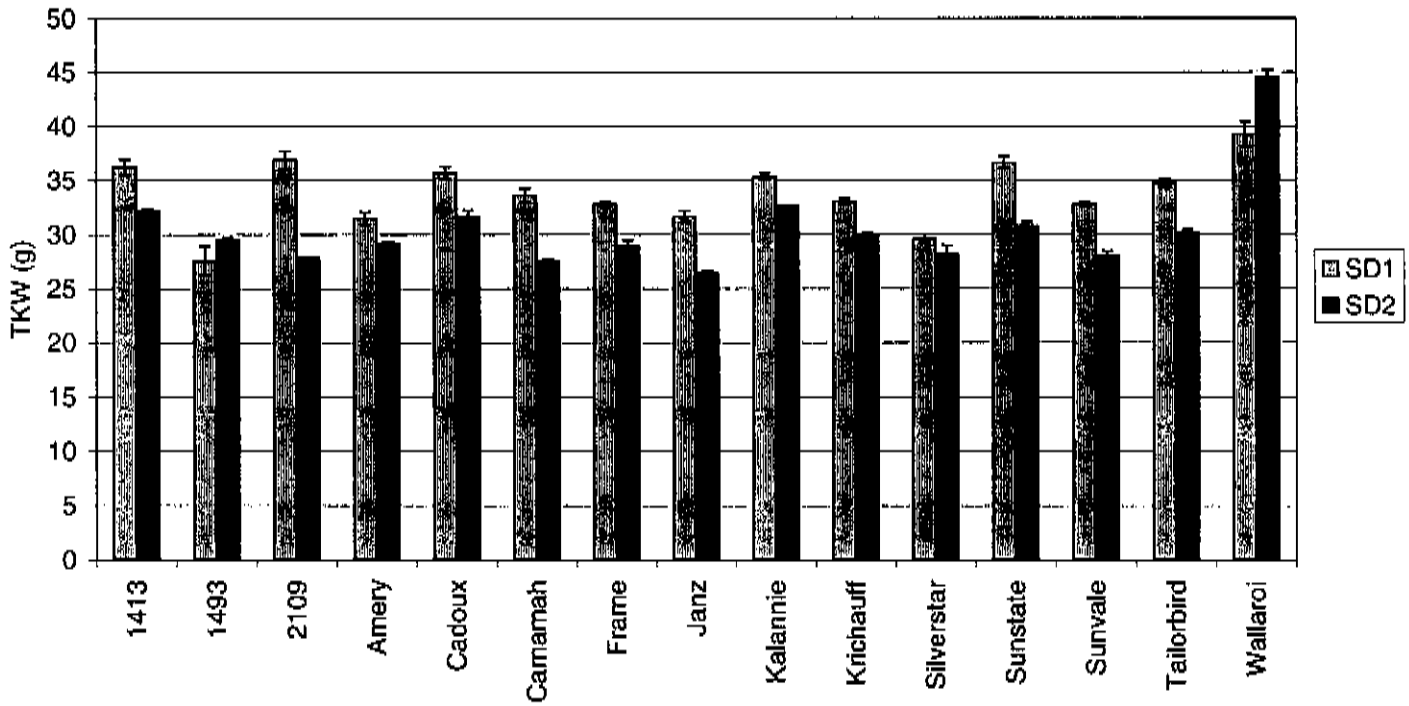
Resistance Breakdown

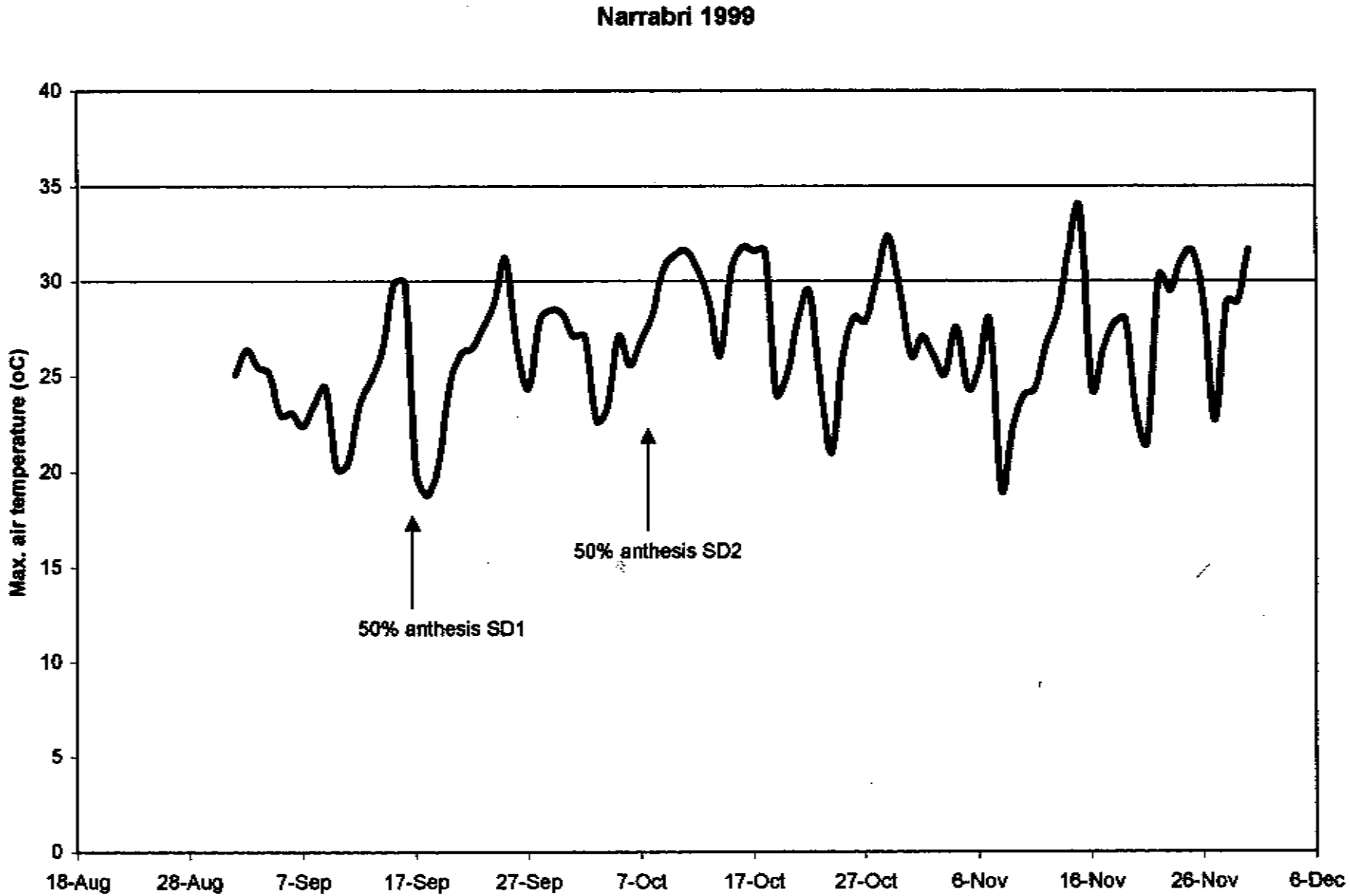


PROTEIN
1999



Thousand Kernel Weight
1999




Narrabri 1999
Max. air temperature (oC)
40
35
30
25
20
15
10
5
0
18-Aug
28-Aug
7-Sep
17-Sep
27-Sep
7-Oct
17-Oct
27-Oct
6-Nov
16-Nov
26-Nov
6-Dec
50% anthesis SD1
50% anthesis SD2

Attached Report #6.
Nucleotide sequences of the promoter regions of two HSP70 genes from *Triticum tauschii*

C.Blumenthal and C.W.Wrigley

The exposure of all organisms to higher temperatures and a range of other stresses elicits the induction if a set of heat-shock proteins (HSPs) which range in molecular weight from 10 to 110 kD (Kimpel and Key, 1985; Heikkila *et al.*, 1984; Guy *et al.*, 1985; Lin *et al.*, 1984). In higher plants, as opposed to other organisms, the synthesis of the low-molecular-weight HSPs are most prominent in response to temperature stress (Lin *et al.*, 1984). Proteins of the HSP 70 family, also strongly enhanced in response to heat stress, have been found in all species and are highly conserved in terms of amino-acid and nucleotide sequence in diverse organisms (Craig, 1985). They are coded for by a multi-gene family (Wu *et al.*, 1988). In eukaryotic cells, distinct members are found in various sub-cellular compartments including the cytoplasm, endoplasmic reticulum, mitochondria and chloroplast. They are involved in protein folding, intracellular targeting, and disaggregating proteins denatured by high temperatures (Campbell *et al.*, 1997). Experiments were set up to provide basic information on the use of heat-tolerant genotypes for use in breeding for consistent dough quality, involving study of the heritability of this trait. In parallel, we are identifying nucleotide sequences that could be used if transformation were to be the chosen route to developing heat-tolerant genotypes.

There are a few short regions of the HSP 70 sequence that are almost completely conserved in all species. Using this information, Galley *et al.* (1992) described a general PCR-based approach to enable cloning genes from the HSP 70 family. Based on this method, degenerate oligonucleotide primers were designed and synthesised:

Forward Primer: 5' AAT TC CAR GCN CAN AAR GAY GCN GG 3'
Reverse Primer: 5' AAT TCC GCN CAN GCY TCR TCN GGR TT 3'

The third codon position was kept degenerate to allow for different codon usage in *T. tauschii*. N in the nucleotide sequence refers to A, C, T or G; Y refers to either T or C; R refers to either A or G.

PCR amplification was carried out in 10ul reaction volumes (Blumenthal *et al.*, 1998). Amplification of a 0.65 kb fragment was obtained, sub-cloned into a plasmid vector, and the nucleotide sequence was determined. The 0.65 kb fragment was used as a probe to screen a *T. tauschii* library. Two classes of clones hybridising to the probe were identified. These constitute members of two of the three HSP 70 gene families. They were identified and characterised, using Sac I and Eco RI respectively, for sub-cloning. Type one was designated cat2 (see attached sequence as Figure 1). It is presumably a HSC (heat-shock cognate) based on the presence of a conserved 5' intron. It is likely to be heat inducible, as most plant HSPSs are inducible regardless of the presence of introns, which render other eukaryotic heat-shock genes constitutive. It is highly homologous to the maize HSC 70 gene, which has a 30-40 fold increase in mRNA upon heat stress.

The other designated car64.con or cat1 (see attached sequence as Figure 2) is likely to be one of the organelle (mitochondrial or chloroplast) HSP 70 gene, based on the high degree of sequence homology to DnaK and other prokaryotic heat-shock genes. Full nucleotide sequences are available for the promoter region and part of the coding region for both of these genes.

Car64.con is almost certainly heat inducible, based on its classical pattern of heat-shock elements (HSEs). The position in the gene of heat-shock elements, N-terminal codon and intron are documented below. Other important regions, such as a GC-rich cluster adjacent to the TATA box as well as an AT rich region thought to be needed for transcriptional activation, are evident in the gene sequence. The translation product of 301 amino acids is attached. It is highly homologous (95%) to *Oryza sativa* HSP 70-inducible protein.

CAT1 (Car64.con):
Nucleotide position of HSE No 1 (heat shock element) 1329 TTC--GAA--GA
HSE No 2 1355 GAA--TTC--GAA

TATA signal 1383 AAATAAAA
CDS 1540 - 1752; ATGGCG.....
2519 - 3219 ATGCCA.....

Possible G-box like motif 808
Possible CAAT box 1143

The other HSP 70 gene sequenced is more likely to be constitutive, based on its sequence homology to prokaryotic heat-shock genes. It has 86% homology in terms of amino-acid sequence to the *Hordeum vulgare* region.

Nucleotide position of HSE No 1 766 GAA-GAA--GAA--CAA (some degeneracy is allowed as regards heat inducibility).

TATA signal 970 TATATATA
CDS 1101 onward ATGCCA..... No N-terminal intron is present.

Note that from amino acid No 17 – 46, there is a very highly conserved region in comparison with most HSP 70 amino-acid sequences.

This pair of promoters therefore offers access to two types of temperature-responsive regulatory elements for potential use in transformation. Heat-shock promoters of these types have not been previously available from wheat (according to database listings and patent search).

Proof of concept in terms of heat inducibility of these promoters would be the next step. It has been suggested that transformation into rice of a construct utilising a wheat storage HMW glutenin gene driven by these promoters would be the most logical option. *In situ* hybridisation has been requested for performance in Japan to determine the chromosomal locations of the genes.

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Kimpel, J. A. and Key, J.L. (1985) Trends Biochem. Sci., 10: 353-357.
Lin, C.Y., Roberts, J.K. and Key, J.L. (1984) Plant Physiol 74: 152-160.
Wu, C.H., Caspar, T., Browse, J., Lindquist, S. and Sommerville, C. (1988) Plant Physiol. 88: 731-740.

Figure 1. Sequence of the gene for a type-one heat-shock protein 70, designated cat2.

CAR 70 · CON
CAT 2

Fig. 1

DSP4.tmp

Length: 2678 February 13, 1998 16:43 Type: N Check: 9637 ..

```
   1 ATCTCCTCCT GGCTATTCTG GCCGCCCnCG TTnGTGATGG TGATCTTCTC
  51 CGACTTGCCG nTGCCCTTGT CCGCCGCCTT CACGTGCAGG ATGCCGTTCA
 101 CGTCCACCTC GAACGTCACC TCGATCTGCG GTGCGCCCCT CGGAGCTGGT
 151 GGGATTCCAG AGAGATCAAA CTTGCCGAGC AGCCTGTTGT CCTTGGTCAT
 201 GCTCCTCTCG CCCTCGTAGA CCTTTATGGA CACGGTGGTC TGCnTGTCCT
 251 GGTACGTCGT GAACACCTGC GTCTTCTTCG TCGGCACCAC CGTGTTCCTC
 301 GGGATCAGCT TCGTCATCAC ACCGCCGACC GTCTCCAGGC CTAGCGTAAG
 351 CGGCGCGACA TCGATCAGGA TCATTTTCTT GGTGTTCTCG TCGACGTGCC
 401 CGCTCACGAT GCTGGCCTGC ACGGCGGCGC CGTACGCGAC GGCCTCGTCG
 451 GGATTGACCC CCTTGTTGGG CTCCTTGCCG TTGAAGTAGT CCTTGAGGAG
 501 CTGCTGCACC TTGGGAATCC TGGTGCTGCC GCCGACnAGC ACGATCTCGT
 551 CGATGTCAGC CTTGCTGAGC CCAGCGTCCG CCATGGCCTT CTTCACCGGC
 601 ACCATCGTCT TGCGGAAAAG GTCGTTGTTG AGCTCCTCGA ATCGTGCCCG
 651 GGTGAGCGGC TCCGAGAAGT CGACGCCGTC GAAGAGCGAT TCGATCTCGA
 701 CGCGCACCTG GTGCTGGTTG CTGAGCGCAC GCTTGGCGCG CTCGCACTCC
 751 CGGCGCAGTT TCCCCAGCGC GCGCAGGTCG CCGCTGATGT CCCGGCCGTG
 801 CTTCCGCTTG ATCAGTTTCA TGAAGTAGTC CATCAAGCGC TGGTCGAAGT
 851 CCTCTCCGCC GAGGTGGGTG TCGCCGTTGG TGGCCAGGAC CTCGAACACG
 901 CCATTGTCGA TGGACAGCAC GCTGACATCA AAGGTGCCGC CGCCAAGGTC
 951 GAACACCAGG ACGTTCTTCT CGGGCCCCTT CTCGTCGATG CCGTAGGCGA
1001 TTGCCGCCGC TGTGGGCTCG TTGACGATGC GGAGGACGTT GAGCCCGGCG
1051 ATGACGCCAG CGTCCTTGGT GGCCTGGCGC TGCGCGTCGT TGAAGTAGGC
1101 CGGCACGGTG ACCACGGCGT GCGTGACCTT CTCGCCGAGG TAGGCCTCGG
```

```
1151  CGGTCTCCTT CATCTTGGCA AGCACCATGG CGCTGACCTC CTCGGGGCTG

1201  AAGACGCGCA CGTCGCCGTC CTTGACCCGC ACCTGAATGT GCGGCTTGCC

1251  CTTGCGGTCG ACGACGGAGT AGGGCAGCAG CTTGATGTCC CGCTGCACGA

1301  CGGCGTCGGC GAAGTCCCGG CCGATGAGGC GCTTGGCGTC GTAGATGGTG

1351  CGCTCCGGGT TGGCGGCCGC CTGGTTCTTG GCGGCCTCGC CGATGAGCCG

1401  CTCGCCGCCG TCAGCGAAGG TGACCCAGGA GGGGGTGATC CgGGTTGCCC

1451  TGTTCGTTGG CGATGATCTC GACGCGGCCG TTCCGGTAGA CGCCGACGCA

1501  CGAGTAGGTG GTGCCGAGGT CGATGCCGAT CACGGTGCCC GTGTCGCCGC

1551  CCGTGGGCGC AGCGGAAGCG GCTGGCATCA CGAGAAGCAA TGCCGCGACG

1601  AGCGTAGCGA ACATCCACAT CGGCGCACGA GCCATCCCGA CTGACTCGTT

1651  GTGCTTGGTT TGATTTGATT TGATTGGTCT GTTTTGCTTT GCCGTCGTTC

1701  GTATATATAG CGGCATAGCG TGCGTGCTTC ATGATGAATC GGAGGCGGTC

1751  TCCCACTCCG ATCGTCGTGG GACGTCTGGA CGCGTCCGTC CGGTGATCTC

1801  AGCCGTGCGT TTTGCTGATC TGACGGCTAA GAAGATGCCC TGACGTGTCG

1851  TATGCATGCT GACTGATCTC GGTAGGAACG TCGATGTGTT TTGTTTTCGG

1901  TTTCCCTTCG TTCATGGGCC TTTCAGGGCC CTTTGTTCTG AGTGGGCCTC

1951  ACGATTTTTT TTACGGAATA TCTAACTGAC GAACGTCAGG TATTTGATGA

2001  TCTCCAGCGA ACGCACCAAT AACCCTTGGA TCTTGCTCAT GAATCTTGAA

2051  GCAGGGAGAG GTTACACGTC AGCTACAGAA AAACGTTCGC TACATTCACA

2101  TTCCTGGCGA ACAGTTTTTT TTTACTTTCC ACAAAAATAT GGCAACCTCA

2151  TGCAGAAATG AGGGCGGGCT CACCACTTGG ATCGGAAGCT GGTTGTGGAC

2201  ATTTCAGATC AAACTTGTCA TATTTTTTCA GCTAAAATGA ATTATTTTTT

2251  CATTTGAAAT TTTCAACTGA AAAAAATCCA TAAATTCCAA AAAGATTCCA

2301  TACTTTAAAA GAACAAAAAT CCGTACAAAT CCAAAAGGTT GCCATGCTTT
```

```
2351  TcTAGAGAAC ATGTTTAAAA AAAAATTCCC ATGTGCAAAT TATATTTGTT

2401  TGTTTGCCAT GTACTCCTTC CGTAAACTAA TATAAGAGCG TTTAGATCAT

2451  TACTTTAGTT ATCTAAACAT TTTTATATTA GTTTACAGAG GGAGTACCTT

2501  TGTTTAGAGG GGAATTTTTC TAAACTTTCC ATGTGCAATT ATATTATAGG

2551  TAGCATGGCA TTTTCGTGTA GGAACCATGT CATTTTTTTA GAAACCATGG

2601  AATTCTGTTT TGAACGGTTG GTTGAAATGG GTTGTACTTT TAACAAACTG

2651  TAAATGGGCT GTAGTAAATT CCATTAGA
```

Figure 2. Sequence of the gene for an organelle heat-shock protein 70, designated cat1.

CAR64.con
CAT 1

Fig. 2

DSP4.tmp
Length: 3219 March 2, 1998 22:20 Type: N Check: 4561 ..

```
   1  CAAGCGTGGT CGCCTGTAGT GAAGAAAAGG AGGAGAGGAG GCGCGAGGGn
  51  CCnnGGATCG CCGGCGTGAG GTTCGGCAAC CGATAGGTGG CGGTTGCGAC
 101  GTGCATGGCA CgAACGCCaT GGTGGCGGCT CGGTGCTATC GGCTCGCCGT
 151  TCTTGCCGCC TCTCTTTCTC CGACCTGGCG GCAAAGCAGA CCTCGGTGCG
 201  GGCTCCCGCA GCGGCGCATC GGTGGCCGGA CAGTTAGGTC GGTGAGCCCC
 251  TTGGCGCATC TTTCATCCGT CCACTAGATC GTTGATAGCG CGCGTTGCCG
 301  CGCCAGTCCG TTTTTATGAT AAAATAGTAA TAGTTTTCAT AGATATTGTG
 351  CATAAATAAA GTGTGAAAAG ACGGGTTGAT TTTTCTGATT AAAATTTAAA
 401  TTGTACAAAA ATGTATAAAG TATTGCACTA CTTAGCCCTA GTAAGTAGGG
 451  TTTTTATAAT TTATGCCAGT AGTTATGTCA CACTACTCAG TTTTGCCATT
 501  AAAACTTACA ACTGCTAAAA AATACAATCG CTACGTTAGA TGCTTGCGCT
 551  GGAATGCCAT TAGACACCTA AAAGATGTAA TAGCATCGTT AGATGTTTGC
 601  TAAAAAATGG CATTAAACAT TGTTACTATC AGGTCAAACC CCGTTGATCA
 651  TGTTATATGA TCAAAATACC CTTTGACCCA CATGTCAGCT CTCTCTATCT
 701  CACAATGATA AGTGTGGCCC TACTTGTTAA GCTTAAGCAA GAGAAGAATT
 751  CTATAGGAAA ATAAGAACAA TGTTGGAGAT CATGTGGGAC CCACACTTTA
 801  CCGTAGTGAT ATAACGGGGG AACTGACATG TGGGTCCATG GAAATTTTGG
 851  CCATATGGCA TGGTAAACGG GATTTGAGCT AGCAGTAATG GTGTCTAGTG
 901  ACATTTTTAA GCATGCGGCT AACGGAGTGA TGACATTGTT GAGCAGTTGA
 951  AACTCCTAAT GGCAAAACTG AGTAACGGAG CACAACGAGT GGCATAAATA
1001  AAAAATGTTA AAAACACTTT CATTTATTTA TTTTTGAGGG GCAAAAAAAC
1051  GCTTTCATAT GACCGCAGAT TTCATTTTCC TATTTTCAAC AAAAATGTTT
1101  TTTTTCAGAA ACATTTTTCA ACAAAAATTT ATGACTGGTC TTCAATAATG
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1151  GGCCATATAT TTTTTTACCC AAAAGGAGAG AAAACCCAGC CCACCAAGAA

1201  AGCCCAGCAG CCGGAACATA GCCGTTCGCT GTAGAACCTA CGAGACAGGG

1251  TCAAAGGCTG GTCTCACCGG TCCGCGTCTT CCTCCTCTCC AACGCCAACC

1301  GTCAGGCATA CCGCACCACT CGAGCGGCTT CGAGAACAGA GCGCGCCCCT

1351  CCCCGAACAT TCTCGAAAAA TCCCAGCATC CCAAATAAAA CCCGCGCCCC

1401  GTGTTCGTCC CTAGGATCCA TCAGTTCCAT CGCGATTCCC AACGCGTTCT

1451  AGCAATCAAG CGATTTCCCT CAGATCTCGA GTTCTTAGCT CCGATTTCAT

1501  CTTGGAGTTC CCGTTTCCCC GTTCGAGTTT AGAGAGGAGA TGGCGAAGGG

1551  AGGAGAGGGG CCGGCGATCG GCATCGACCT GGGCACCACC TACTCGTGCG

1601  TCGGCGTGTG GCAGCACGAC CGGGTGGAGA TCATCGCGAA TGACCAGGGC

1651  AaCCGCACCA CGCCCTCCTA CGTCGGCTTC ACCGACACCG AGCGCCTCAT

1701  CGGCGACGCC GCCAAGAACC AGGTCGCCAT GAACCCCACC AACACCGTCT

1751  TCGGTACGTC CTCCTGCACC GCTCCTCTTC TACTAGCTTA GATAGATGGA

1801  TCATCTCGTG TTCGTGCTTG GCTTGCCACT GATTCTGTTT GGACCCAATT

1851  CCTCGTAGGG ACGCTAGTAT CGCAGTGCAA ATCAATTTGT TTACTGGGCT

1901  TAACAAGTAG CAGAGGAATG TAAAACCAAC TGGACATGTG TCTAATCGCT

1951  GGCATTGTCC TATTCTGCAT CGTCGTGTAT TCGACAAACT GACGGAAATA

2001  TATGCAATTA ATCTGGTCTA AGCTTAAAAT CCCCTTAGTT CCAGTAGTAT

2051  TCTGTCAAAA GCTTTGCTGA TTAAGATTTC TATGTTGAAA ACTGTTCACG

2101  GGTCAACAAT GGATAGGATG ACAACTCCTT GGGTTGGCTG TTTCTGTTGA

2151  AACAATTGTT TGGGGACAGG AGAACATCGT GGGTAAATTT CAGTTTCCTG

2201  TTTGGAACTG TAATGCTGTT GCTCTGAATT TTGTTTGTCT AAATTTGTTT

2251  GCTGGGCTTA ACGAGTACTC CTAGCAGAGA TACGAGAAAA CAATGGGTCA

2301  ACAATGGAAA GGAAGATAAC TTGTTGGGTT GGCTGTTTCT GTTGAAACTA
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2351 TTTGTTTTGG GGCAGGACAG CATTGTGGGT AAATTTCAGT TTACTGTTTG

2401 GAGCATGTCA AAACTCTGCT GTTGCTCTGC TTATTTGCTT TTTGTTCCCA

2451 GATGAGTTTG ATTAATTTGA GCGCAAATGG AACAGTTTGA CTCACAGTCT

2501 TGTTATATCA CTATTGCAGA TGCCAAGCGG CTGATCGGCA GGCGGTTCAC

2551 CGACCCGTCG GTGCAGAGCG ACATGAAGCT GTGGCCGTTC AAGGTCATCC

2601 CAGGCCCTGC TGACAAGCCG ATGATCGTCG TCCAGTACAA GGGCGAGGAG

2651 AAGCAGTTCG CCGCCGAGGA GATCTCCTCG ATGGTGCTCA TCAAGATGAA

2701 GGAGATCGGC GAGGCCTACC TCGGCACCAC CATCAAGAAC GCCGTCGTCA

2751 CGGTGCCGGC CTACTTCAAC GACTCGCAGC GGCAGGCCAC CAAGGACGCC

2801 GGGGTCATCG CCGGCCTCAA CGTGATGCGC ATCATCAACG AGCCCACTGC

2851 CGCCGCCATA GCCTACGGCC TGGACAAGAA GGCCAGCAGC TCCGGCGAGA

2901 AGAACGTGCT CATCTTCGAC CTTGGTGGCG GCACCTTCGA TGTGTCGCTG

2951 CTCACCATCG AGGAGGGCAT CTTCGAGGTG AAGGCCACCG CCGGmGACAC

3001 cmACcTCGGC GGCGAGGACT TCGACAACCG CATGGTGAAC CACTTnGTCC

3051 AGGAGTTCAA GCGCAAGCAC AAGAAGGACA TCAGCGGCAA CCCTCGCGCG

3101 CTGCGCCGGC TGCGCACGGC CTGTGAGCGC GCCAAGCGCA CCCTGTnGTC

3151 CACCGCCCAG ACCACAATCG AGATCGACTC GCTGTACGAG GGCAnCGAnT

3201 TnTACTCnAA ACATCACCA

Report #7.

A review of heat-shock proteins and chaperones involved in determining protein conformation: research opportunities

C.Blumenthal

Wheat endosperm protein comprises an intricate complex made up of two major fractions - gliadins and glutenins. Gliadins are monomeric proteins; glutenins are polymeric aggregates made up of low (LMW) and high molecular weight (HMW) polypeptides held together by disulphide bonds. This protein complex is responsible for the unique dough-mixing properties of wheat. Understanding the mechanisms of disulfide-bond formation and folding in gluten proteins will ultimately permit the manipulation of the functional properties of dough.

Protein polymerisation and folding are mediated by an array of proteins that act either as foldases or molecular chaperones. Foldases include protein disulphide isomerase (PDI), peptidyl prolyl isomerase (PPI) and heat-shock protein 90 (HSP90). These catalyse the rearrangement of disulphide bonds or the isomerisation of peptide bonds around proline residues, respectively. Levels of isomerase activity increase 3 to 4-fold between 20 and 40 days post anthesis.

Molecular chaperones, such as heat shock proteins (HSP) 16-30, 60, 70, 90 and 100, are proteins that bind to and stabilise an otherwise unstable protein conformation. They are responsible for a wide range of processes including protein folding and unfolding, oligomerisation, subcellular localisation and proteolytic removal. High temperatures during grain filling disrupt the normal process of glutenin aggregation and polymerisation. The amounts of glutenin aggregates of very high molecular weight decrease under these conditions, thereby reducing dough strength.

A variety of stresses, including heat stress, induce the action of molecular chaperones. They have been detected during heat stress in the wheat endosperm and it has been postulated that the detrimental effects that heat stress has on the gluten matrix is mediated via these foldases and chaperones.

Two cultivars of wheat have been studied in depth, Fang (tolerant to the effects of heat stress) and Wyuna (susceptible). Wheat has been grown at 21/16°C (day/night temperatures) for both cultivars, and a heat stress has been imposed on a subset midway during grain filling. Grain samples have been collected throughout grain grain filling.

Levels of expression of the chaperones and foldases need to be monitored, using antibodies, under control and stress conditions, with reference to the expression and accumulation of the various classes of endosperm storage proteins. A range of antibodies to the various chaperones and foldases are commercially available. There is a high degree of conservation in terms of the amino acid and nucleotide sequence of the various classes of HSPs, and these antibodies are likely to prove useful even though they have been raised against species other than wheat. For example, the antibody to the mammalian HSP10 has been successfully used to monitor the levels of HSP10 in mature wheat endosperm in response to heat stress during grain filling. The level of expression of the various HSPs may differ in the tolerant and susceptible cultivars, which may in fact be due to differing levels of expression of the heat shock transcription factors.

Chaperones and foldases shown to be elevated under stress conditions need to be studied in more depth to ascertain what role they play in the reduction of gluten polymer size in response to heat stress. This would involve their purification by using relevant technology such as affinity chromatography (antibody, ATP, etc.) or by bacterial expression. The gene for HSP18 in wheat has been cloned, and depending on its expression during endosperm development and heat stress, it may be a candidate for characterisation and use in bacterial expression.

Alternatively, in order to ascertain which gene/s to target, the gene expression of the heat-induced chaperone/heat-shock proteins in wheat at the mRNA level may be analysed. This involves the isolation

of cytosolic polysomes, as heat-induced mRNAs can be shown by the *in vitro* translation of the isolated polysomes.

The actions of the purified chaperones and foldases need to be assessed using *in vitro* polymerisation assays. The oxidative polymerisation behaviour of purified individual HMW glutenin subunits is studied by measuring the kinetics of polymerisation *in vitro*, using different oxidants ($KI0_3$, $KBrO_3$, $KMn0_4$, O_2 and H_2O_2) so that the resulting protein polymers can be analysed by multi-stacking SDS gel electrophoresis. Catalytic amounts of the purified chaperones and foldases could then be added to the *in vitro* system to monitor the polymerisation of individual HMW glutenin subunits.

NMR spectroscopy (possible collaboration with Wollongong University) may be used as an alternative to the *in vitro* method mentioned above. It is possible to follow the aggregation and precipitation of subunits via the formation of a high molecular weight complex using NMR spectroscopy. The presence of certain of the small heat shock proteins (smHSPs, 16-30kD) has been shown to prevent the precipitation of certain polymeric proteins. From these studies, the conformational state of proteins when they act in the presence of HSPs will be ascertained.

An understanding of these processes will allow a more precise genetic manipulation of factors affecting dough quality as a result of heat stress during grain development and will complement work aimed at rendering a HMW-glutenin gene heat-inducible.

Information emanating from this work may result in the development of an antibody assay yielding information to buyers and processors as to whether the wheat grain purchased was exposed to elevated temperatures during grain development. Information may also be made available to buyers and processors as to the origins and thus environmental growing conditions of grists of wheat. Heat stress (and possibly) water stress yield high protein, but weak dough, that is often unsuitable for its proposed end use. The ability to characterise a mixture of grain grown under a range of environmental conditions would be an advantage. The HSP 18 antibody is a likely candidate for use in an assay (in contrast to HSP70) as it is only produced under stress conditions and not constitutively, thus yielding no background level in normal grain.

Background information on important chaperones and HSPs

(1) Peptidyl-prolyl-*cis-trans*-isomerases

Peptidyl-prolyl-*cis-trans*-isomerase (PPI, E.C 5.2.1.8) is a conserved and abundant protein located in the cytosol and organelles of eukaryotic cells and in the cytoplasm and periplasm of bacteria. PPIs catalyse the *cis-trans* isomerisations of Xaa-Pro peptide bonds which are involved in the refolding reactions of many proteins. PPIs were originally described by researchers who were working with short oligopeptides from porcine kidney and other tissues. Further work showed PPI to catalyse the cis-trans isomerisations in larger proteins. In general, proteins are able to reach their final folded state *in vitro* without the assistance of PPI activity, but the actual cis-trans isomerisation step is very slow (rate-limiting) with a high activation energy because there is rotation around a partial double bond. This reaction is significantly accelerated by PPI catalysis, up to 300-fold *in vitro*, but these rates may not apply *in vivo*.

Prolyl peptide bonds

Peptide bonds are planar and can exist in the cis or trans states with respect to the two C-alpha positions. For peptide bonds that do not contain proline residues the trans state is the favoured conformation, with cis content of around 0.1% found. Peptide bonds between proline residues and the N-terminal preceding residue (Xaa-Pro) often exist in the cis or trans state in solution. Cis contents of around 10-30% are found.

PPI classification

There are two known unrelated classes of PPI. The first subclass are known as cyclophilins which are specifically inhibited by the immuno-suppressive cyclic peptide cyclosporin A. This subclass occurs in all organisms within all organelles, but mainly in the cytoplasm, mitochondria and endoplasmic reticulum. The second subclass is the FK506 binding proteins (FKBPs). These proteins are inhibited by

the immuno-suppressants FK506 and rapamycin. There is no sequence homology between these two PPI subclasses but they catalyse the same cis-trans isomerisation reaction in protein folding.

Evidence of a role for PPI in cellular protein folding

Indirect evidence supporting PPI involvement in cellular protein folding is provided in two ways:

(1) cyclophilins from different origins all have a conserved characteristic which is catalytic involvement in protein folding, and

(2) the *in vivo* maturation of two proteins (collagen and transferrin) is slightly retarded in the presence of the known PPI inhibitor cyclosporin A.

The folding reactions of small proteins are decelerated from the time range of milliseconds to the time range of seconds and minutes when incorrect prolyl isomers are present in the protein chains. Aggregation of unfolded proteins could be minimized by shortening the time of exposure of interactive surfaces in folding intermediates, i.e., by catalysing critical slow folding steps such as prolyl isomerisations. It is not known whether the main role of PPIs is in signal transduction pathways or protein folding.

(2) Protein disulfide isomerases

Protein disulfide isomerase (PDI, E.C. 5.3.4.1) is a soluble protein found in the lumen of the endoplasmic reticulum. PDI has been characterised relative to its catalytic, cellular and molecular properties. PDI has been shown to be involved in the biosynthesis of secretory proteins (disulfide bond formation). PDI was also shown to be the J3-subunit of prolyl 4- hydroxylase as well as a component of the triglyceride transfer complex. As well as this, PDI has been shown to be a glycosylation binding protein, a thyroid hormone-binding protein and an iodothyronine 5'-monodeiodinase. PDI has been identified and purified from bovine liver.

In vitro studies showed that all the information needed for denatured proteins to refold is contained in the amino-acid sequence, as denatured proteins refolded upon removal of the denaturant. These *in vitro* studies showed that the time taken to refold denatured proteins (hours) was much greater than the rates of folding *in vivo* (minutes). Although the *in vivo* role of PDI is still poorly understood, over expression of PDI in *Saccharomyces cerevisiae* can increase secreted yields of certain heterologous proteins in the 10 to 24-fold range or the two-fold range in *E. coli*. *In vitro* experiments show that foldases often can act synergistically to increase both the rate and yield of folded end product.

Enzymatic properties

PDI accelerates reactions at a molar concentration of less than 1% substrate, requiring disulfides and thiols to make or break disulfide bonds. It was shown that PDI does not catalyse the renaturation of proteins that do not contain disulfide bonds.

PDI in developing wheat endosperm

A proportion of wheat storage proteins are present in the developing seed as disulfide-linked aggregates. These aggregates are known for there bread-making qualities (dough elasticity). PDI has been detected in mature seeds of wheat and both the germ and endosperm fractions. The fact that it is located in the developing endosperm of wheat is consistent with its functional role of forming disulfide bonds in the wheat storage proteins. PDI was shown to be absent at germination but was detected in the developing seed over 10-50 days after anthesis, the period when storage proteins are synthesised and deposited into the endosperm.

Recent studies on PDI

Recently, a family of endoplasmic reticulum -specific proteins sharing active-site homology with PDI has been identified. A number of these proteins are stress inducible, specifically by agents which affect endoplasmic reticulum functioning, such as, malfolding of proteins, tunicamycin and Ca^{2}+ ionophores . Genes for PDI of rat, murine, human, yeast and *Aspergillus niger* have been cloned and expressed.

To further elucidate the structural/mechanistic basis of the isomerase and chaperone activities of PDI, it would be necessary to construct an expression/purification system for PDI. The wild-type human PDI

(rhPDI) and a mutant human PDI (mhPDI) have been expressed with an extra 10 N-terminal amino acids in *E. coli*. As a result, the mhPDI was expressed with the highest yield, purified and demonstrated no loss of any enzyme activity as compared to the rhPDI.

(3) HSP 104

Most eukaryotic cells produce proteins in the 100-110 kDa range after high temperature exposure. The HSP 104 gene is a member of the highly conserved HSP 100 gene family. HSP 104 has been reported to be a member of the highly conserved ClpA/ClpB protein family first identified in *E. coli*.

Research involving *Saccharomyces cerevisiae* demonstrated that HSP 104 is essential for tolerance to heat, ethanol as well as other stresses. Mutagenesis of two putative nucleotide-binding sites in HSP 104 indicated that both sites are essential for function in thermotolerance. HSP 104 was reported to mediate the resolubilisation ofheat inactivated luciferase. In this experiment cells were pretreated at 37°C, heat shocked at 44°C, and then allowed to recover at 25°C in the absence of new protein synthesis. Wild type cells recovered p0% of initial luciferase activity, but HSP 104 mutant cells failed to reactivate the enzyme. These results suggest that HSP 104 functions to promote the proper renaturation and reactivation of damaged proteins.

One essential process in cells that is temperature sensitive in many organisms is the splicing of intervening sequences from mRNA precursors. The role of heat-shock proteins has been investigated in reducing the toxic effects of heat on mRNA splicing. Results showed that there were no differences between the wild type and cells containing mutations in HSP 26, HSC 82 and HSP 82 in the recovery of heat disrupted mRNA splicing. However, mRNA splicing is heat disrupted to the same extent in both wild type and HSP 104 mutant cells, but heat-disrupted mRNA splicing recovers much faster in the wild type than in the HSP 104 mutant cells. These results implicate HSP 104 with a role in the repair of heat-disrupted mRNA splicing.

Rice seedlings accumulate stainable amounts of HSP 104 and HSP 90 in response to high temperature stress. Highly specific polyclonal antisera against both HSP 104 and HSP 90 have been purified and raised. HSP 104 and HSP 90 accumulated to varying degrees in rice seedlings after being subjected to NaCl, water stress, low-temperature stress and exogenous abscisic acid application. It was also shown that seedlings of *Triticum aestivum, Sorghum bicolor, Pisum sativum, Zea mays, Brassica juncea* and mycelium of *Neurospora crassa* showed accumulation of immunological homologues of both HSP 104 and HSP 90 in response to high-temperature stress.

(4) HSP 90

The proteins of the HSP 90 class are highly conserved and range in size from approximately 80 to 94 kDa. In addition to cytoplasmic forms of HSP 90, vertebrates have a homologue located in the endoplasmic reticulum also called GRP94 that is expressed in response to glucose starvation. HSP 90 is the second most commonly expressed heat shock protein in animal and microbial systems, with the most common being HSP 70. HSP 90 is an important protein as mutant yeast cells with an impaired capacity to express HSP 90 were unable to grow at higher temperatures. In mammalian cells, HSP 90 is thought to act like a molecular chaperone as it appears to maintain steroid hormone receptors in their correct conformation. As mentioned earlier in the HSP 104 section, HSP 90 accumulates in many plants as a result of various stresses, including high temperature.

(5) HSP 70

HSP 70 was one of the first eukaryotic genes cloned and has been extensively studied in many organisms. HSP 70s are highly conserved ATPases in all species. There has been much work done on this heat shock protein already; in fact the crystal structure has been determined and resembles a folding structure to two other ATP-binding proteins: globular G-actin and hexokinase. HSP 70 gene diversity is partly accounted for by the presence of distinct homologues located in the cytoplasm, the lumen of the endoplasmic reticulum and the matrix of the mitochondria. The homologues located in the endoplasmic reticulum are called binding protein, BiP, or glucose-regulated protein, GRP.

They have critical roles in protein metabolism under stress and non-stress conditions, including functions in protein folding, membrane translocation, the degradation of misfolded proteins as well as other regulatory processes. Basically, HSP 70s bind and release hydrophobic segments of an unfolded polypeptide chain in an ATP-hydrolytic reaction cycle.

(6) HSP 60

This class was the first to be called molecular chaperones. Eukaryotic HSP 60 homologues are highly conserved and are nucleus-encoded proteins found in mitochondria and chloroplasts, which remain abundant even in the absence of heat stress. HSP 60s are shown to assist in protein folding, transport and assembly of oligomeric proteins. Genes encoding chloroplast-HSP 60 have been reported from wheat.

(7) Small heat-shock proteins

Small heat-shock proteins (smHSPs) are ubiquitous in nature but are abundant and diverse in higher plants as compared to other eukaryotes. The smHSPs range in size from around 17-30 kDa. These smHSPs share a C-terminal domain of about 100 amino acids with the alpha-crystallin proteins. The smHSPs dominate the protein synthesis profile of many plants during heat stress, as well as this certain smHSPs can accumulate to over 1.0% of total leaf or root cell protein under certain heat stress conditions. Plants have at least six nuclear gene families encoding smHSPs, while non-plant eukaryotes on average have one to four single genes for smHSPs. Proteins encoded by the various smHSP gene families are targeted to different cellular organelles, which include the cytosol, chloroplasts, mitochondria and endoplasmic reticulum. The smHSP diversification is unique to plants and are the only eukaryotes in which organelle-specific smHSPs have been described. The smHSPs are also quite stable following heat stress, with approximate half-lives between 30 and 50 hours which may implicate a functional role involved in the recovery period.

(8) Ubiquitin

Ubiquitin (Ub) is a highly conserved 76-amino acid protein that exists in cells either free or covalently linked to other proteins marking them for degradation. Ub-dependant pathways play essential roles in many biological processes such as cell differentiation, the cell cycle, embyogenesis, apoptosis, signal transduction, DNA repair, transmembrane and vesicular transport, stress response and nervous system functioning. A characteristic of Ub-dependant proteolysis is that it can destroy a subunit of an oligomeric protein selectively, leaving intact the rest of the proteins subunits. Wheat roots treated at 42°C showed a 30% decrease in free ubiquitin and a corresponding increase in ubiquitin conjugated to other proteins.

Report #8.

PREDICTION OF DOUGH QUALITY VARIATIONS DUE TO ENVIRONMENTAL FACTORS; IDENTIFICATION OF ENVIRONMENTAL HISTORY BY NIR

Submission to Quality Wheat CRC for addition to 2.1.4 ..... January, 1998.

Caron Blumenthal and Colin Wrigley

LONG-TERM AIM

- To predict changes in dough quality due to environmental variations, including both growth and storage conditions.

INITIAL AIMS

- To determine whether specific aspects of environmental history are associated with consistent changes in the NIR spectra of mature wheat grain and flour, with an accent on temperature profile and drought.

- To optimise NIR methodologies for practical characterisation of wheat samples according to their environmental history.

- To determine the chemical basis for the observed distinctions in the spectra associated with differences in environmental history

- To determine how the prediction of these environmental factors can be used to benefit industry.

OUTCOME

- Provision of a protocol to determine whether grain or flour samples have been subjected to environmental stresses, and are thus likely to exhibit unexpected variations in quality.

BACKGROUND

The combination of variety and protein content has been used traditionally in Australia to specify grain quality, but on many occasions this combination has proved to be inadequate, largely due to the effects of growth and storage conditions. We are starting to understand which of these environmental fluctuations have the greatest effect on dough quality and what effects they are likely to produce. It would therefore be valuable if screening methodology were available to identify those samples that have been subjected to environmental stresses likely to cause unexpected deviations from the normal basis of quality prediction. Most obvious of these stresses are heat and drought, both of which have been shown in CRC-based research to reduce dough strength.

This proposal therefore provides a basis that enables the Quality Wheat CRC to capitalise on these research findings and thus to provide an immediate benefit for Australian industry partners.

PROMISING INITIAL RESULTS

We already have promising results, based on the NIR analysis of approximately 200 flour samples, to indicate a consistent change in NIR behaviour due to heat stress. These samples came from control and heat stressed plants of 45 varieties grown in the Canberra Phytotron and characterised for variations in dough strength due to growth conditions. These samples were scanned on a NIRSystems 6500 spectrometer and spectra were analysed using ISI software. Discriminant Analysis segregated the spectra into two groups which corresponded to the known growth conditions, thereby identifying samples having control or heat stressed histories with about 93% accuracy (see Figure 1, appended). This discrimination was not due merely to variations in protein content because the distinction could be

made for samples where protein content was uniform. The specific basis of the discrimination is not yet known.

Furthermore, analyses of 74 wheat samples from the 1995 'Prime Hard in the South' trial produced results that fell into two categories (Figure 2). This distinction was not due to either variety or protein content.

METHODOLOGY

1. NIR analysis of further samples

In addition to >200 well-characterised samples of many varieties, there are further sets of grain and flour samples with known environmental histories that have been characterised for quality attributes, plus many samples currently growing under controlled conditions and available from the current harvest. These include ...

- water- and/or heat-stressed samples generated by Prof Zvi Plaut, a scientist visiting at North Ryde for six months (funded by a GRDC Fellowship) (>100 samples),
- a range of samples from John Oliver, including grain from the 1997 harvest of the 'Prime Hard in the South' trials (about 200 samples),
- samples provided by Dr Frank Ellison (from the 1997/98 harvest) from the Moree and Narrabri regions, which were subjected to 2 to 3 days of 38-40° temperatures during grain filling (>100 samples),
- samples grown at either moderate or heat-stress temperatures generated in the phytotron in Canberra (Blumenthal and Wardlaw) (>60 samples), and
- samples stored under a range of atmospheric conditions.

2. Evaluation and optimisation of NIR identification systems

Several approaches will be explored with the aim of determining the most appropriate and practical NIR procedure for the identification of environmental history. These will include both linear calibration (discreet wavelength and full spectrum) and cluster analyses. The possibility of improving history identification by the provision of varietal identity will also be considered.

3. Molecular basis of history identification

Aspects of the chemical composition of these samples (or sub-sets) will be used to elucidate chemical reasons that may explain the ability of NIR to distinguish them according to environmental history; ie what is the chemical link between samples that have similar spectral characteristics. The relationship between environmental history and genotype will also be explored.

4. Application of optimised protocol to new samples

Later in the project further sets of samples will become available and these will be subjected to the near-optimised protocols with a view to further improvement.

5. Later approaches to expanding data collection

With the completion of the NIR Centre's networking project, it will become possible to obtain NIR spectra for thousands of wheat samples for which the environmental history can be readily obtained. This will provide an ideal opportunity to test, further develop and apply the protocol developed in the earlier stages of this project. It is therefore important that these stages are completed in time for application via the network.

RESOURCES NEEDED

... FOR METHODOLOGY ITEMS 1-3, during calendar 1998

... FOR METHODOLOGY ITEMS 3-5, during financial 98/99

	1997/8	**1998/9**
Labour - casual, for NIR analyses and data processing	6,000	10,000
Freight - transport of samples to North Ryde	900	1,200
Consumables - lab analysis of quality and composition	2,000	4,000
Travel - interaction with collaborators	1,200	1,900
	10,100	**16,100**

INDUSTRY BENEFITS

An 'instant' screening method to determine the environmental history of wheat samples and thus to identify samples likely to have unexpected quality characteristics.

A primary request of Australian industry collaborators has been for greater consistency of processing quality or at least for better predicability of quality attributes. With progress in our knowledge of the effects of environmental stresses on grain quality, there has been a request from industry for simple screening procedures to identify samples with a history that is likely to provide anomalous quality properties. This application of NIR would fulfil this requirement.

COLLABORATIONS

This project relies on the availability of many samples that have been characterised for growth and storage conditions and for quality and composition. It therefore involves close collaboration with several researchers able to provide samples, and also organisations likely to use the outcomes. Most grain samples analysed thus far have come from activities of the Quality Wheat CRC. An important aspect of the proposal is the development of collaborations with a wider range of sample providers.

Figure 1 - Discrimination of Samples According to Environment

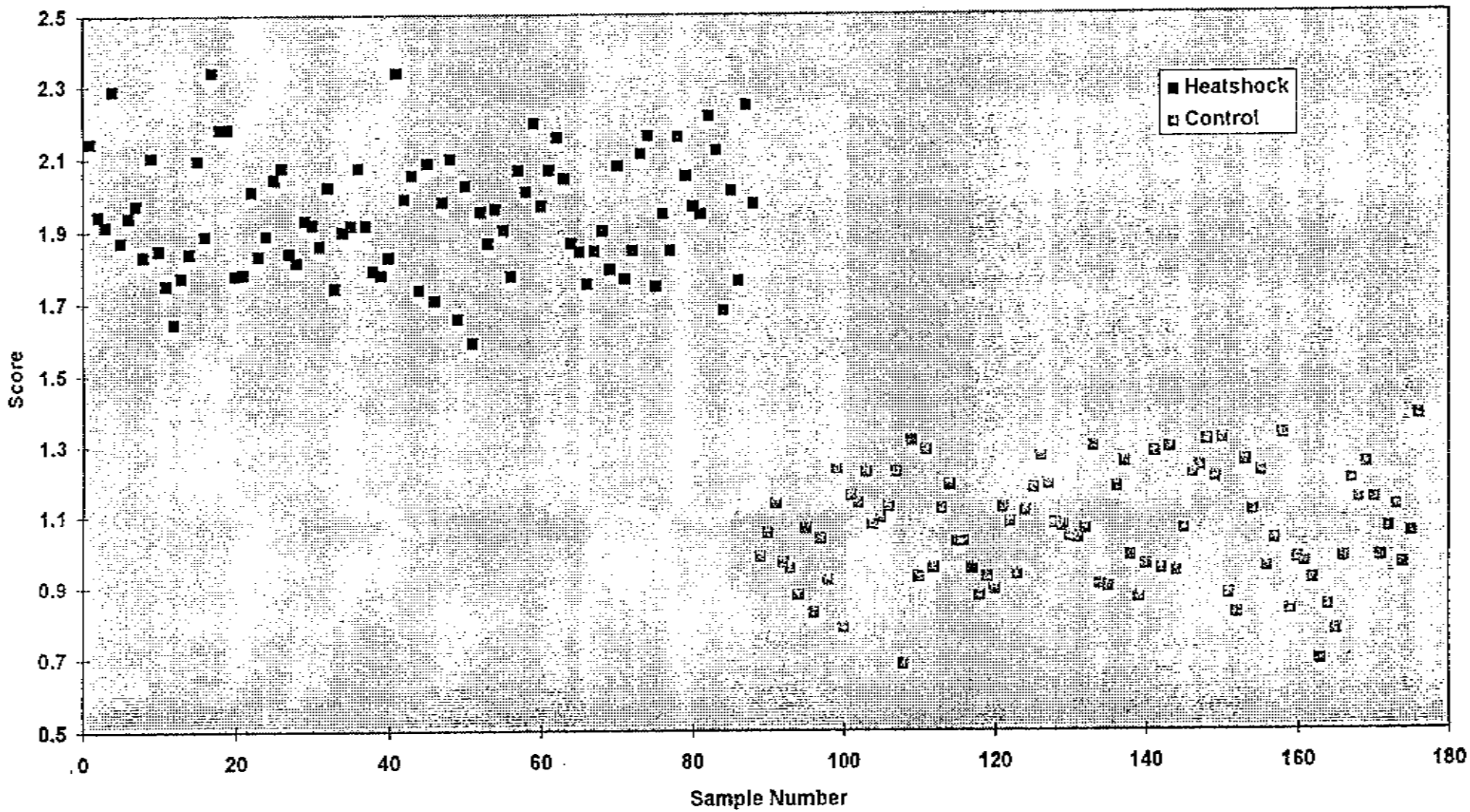

Figure 2 - Analysis of Prime Hard in the South 1995 Samples