

# Link Between Mixing Requirements and Dough Strength

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## ABSTRACT

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Industrial bakeries in Australia and New Zealand using the mechanical dough development (MDD) process have experienced undesirable increases in dough mixing requirements. This problem is an unwanted outcome of breeding programs that have endeavored to increase dough strength as a desirable characteristic. Research was undertaken to determine the nature of the link between dough strength and mixing requirements and its relevance to the MDD process across a wide range of wheat lines. Data from three similar trials of 20 wheat lines confirmed the existence of an apparently tight, positive correlation between mixing requirements and dough strength. Although a wide range in genotypes and environments was used, no significant outliers were found, despite the belief that the link between these quality attributes was breakable or at least flexible. This creates a dilemma, as it would be desirable to reduce work input (WI) for economic reasons but not at the expense of loaf quality, which could have a deleterious effect on product marketability. The resultant nexus between

mixing requirements, as measured by MDD WI, and dough strength measured by extensigraph resistance to extension (R<sub>max</sub>), appeared to be influenced by the application of nitrogen fertilizer during cultivation, while the nexus between mixing requirements as measured by mixograph development time (MDT) and R<sub>max</sub>, was influenced by other factors associated with crop location. The nexus between farinograph development time (FDT) and R<sub>max</sub> was affected by both location and nitrogen fertilizer application. The rates of increase in WI, MDT, and FDT against R<sub>max</sub> appeared to differ between high molecular weight glutenin subunits (HMW-GS) 5+10 or 2+12 (the *Glu-D1* alleles). The *Glu-A1* and *Glu-A3* loci also played a significant role, indicating that by manipulating them it may be possible to shift or manipulate the nexus between mixing requirements and dough strength. Alleles at these loci appeared to be additive in effect on WI, MDT, and FDT, as well as R<sub>max</sub>.

Flour breadmaking quality is often considered in terms of mixing performance and physical dough characteristics (MacRitchie 1992). In particular, mixing requirements and dough strength are recognized as key determinants in the baking process. Depending on the baking process, mixing requirements are defined as the time taken or the energy required to mix a dough to the optimum consistency to produce bread of the appropriate quality. MacRitchie et al (1990) defined dough strength as a term usually applied to describe the balance between viscous and elastic properties of a dough. It is generally recognized that this property provides the dough with sufficient resilience to withstand the mechanical action of industrial bread production. In many breeding programs, high dough strength (usually measured by extensigraph resistance to extension, extensibility, and extensigraph area under the curve taken into consideration) has been targeted as a desirable quality attribute of bread wheats.

However, the increase in dough strength of current cultivars has been accompanied by corresponding increases in dough mixing requirements. The increased mixing requirements have created difficulties for Australian and New Zealand bakeries using mechanical dough development (MDD) processes by increasing the time and energy required to prepare doughs, as well as raising the dough temperature. Consequently, bread throughput is slower, the quality is not always desirable (e.g., side-wall collapse), and production costs increase.

MacRitchie (1992) noted that baking quality was largely determined by wheat protein composition, which in turn was primarily under genetic control, and to a lesser degree affected by environmental and agronomic conditions. Furthermore, he suggested that

although mixing requirements and dough strength appeared to have similar dependencies on protein composition, the apparent nexus was not necessarily unbreakable. MacRitchie's work was based on laboratory assessment of mixing requirements, as measured by mixograph development time (MDT), and dough strength as measured by extensigraph resistance to extension (R<sub>max</sub>). Both of these tests are used by breeders in New Zealand and Australia when selecting wheat lines. However, as the mixograph has not been extensively marketed outside the United States for bakery quality assurance, the New Zealand and Australian industrial bakeries have adopted a more widely marketed farinograph to test commercial wheat lines and flour samples for mixing requirements (measured by farinograph development time [FDT]) and other physical dough properties. While there has been an absence of extensive research to establish a correlation between the mixograph or farinograph and the MDD industrial process, laboratory MDD mixers operating in New Zealand that have been designed specifically to test flours to be used in this process (Mitchell 1984) have achieved significant correlation (Wilson et al 1997). It is, therefore, important to determine the relationship between R<sub>max</sub> and the three instruments measuring mixing requirements.

Given the predominance of the MDD industrial process in the New Zealand baking industry (Mitchell 1989), 125-g MDD mixers were introduced for test baking in 1974 by the former Wheat Research Institute (since restructured to form part of Crop & Food Research, Christchurch, New Zealand) (Mitchell 1982). These continue to be used in New Zealand because they provide a direct correlation with industrial mixers for breeders' advanced wheat lines, wheat baking research, prepurchase testing of harvested wheat, and for industrially milled wheat lines or grists destined for the MDD process (Wilson et al 1997).

The principal objective of this study was to test the existence of an apparent link or nexus between MDT and R<sub>max</sub>, as described by MacRitchie (1992), and its relevance to the MDD process across a wide range of wheat lines. Additional objectives were to assess the relationship of this apparent link or nexus with any similar nexuses of other measures of mixing requirements such as MDD work input (WI), FDT, and R<sub>max</sub>, and if possible, to identify wheat cultivars and breeding lines that do not exhibit the nexus. Lines not exhibiting the nexus and with low mixing requirements and high R<sub>max</sub> values could be directly exploited in developing further bread wheat lines.

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## MATERIALS AND METHODS

### Materials

Twenty wheat cultivars and breeding lines (Table I), chosen for a wide range of physical dough properties (e.g., WI = 6–27 Wh/kg; Rmax = 103–448 BU), differences in genotype, and protein composition, were grown in the 1992 season in three trials (at two locations) with three replicates in each trial in a randomized block design. The first two trials were located at Tamworth, NSW, Australia, where trial I had no nitrogen fertilizer applied, but trial II had 60 kg/ha of nitrogen applied (Hi-Fertiliser containing 32N-20P-0K). Trial III was at Breeza, NSW, Australia, and had no nitrogen applied. As four of the 180 samples were lost during milling, the analyses are based on 176 samples.

### Methods

Grain samples (4.5 kg) from each field plot were conditioned to 15.5% moisture for 16 hr before milling on an experimental mill. All flour samples were analyzed for flour nitrogen content (FN) at 14% mb by the Kjeldahl method (ISO 1975). High and low molecular weight glutenin subunits (HMW-GS and LMW-GS) were determined by SDS-PAGE (Gupta and MacRitchie 1991).

Physical dough properties were measured by extensigraph (Brabender OHG, Duisburg, Germany) using Approved Method 54-10 (AACC 1995). Rmax, extensibility (Ext), and extensigraph area under the curve (EAREA) measurements were made after a rest time of 45 min.

Mixing requirements measured by mixograph (National Mfg. Co., Lincoln, NE) included mixograph development time (MDT), water absorption (MWA), and mixograph peak area (MPA) using Approved Method 54-40A (AACC 1995). Mixograph results were recorded using a 5-kΩ linear taper potentiometer attached to the rotating bearing shaft of the mixer arm (Wooding and Walker 1992).

Farinograph tests (Brabender OHG, Duisburg, Germany) included farinograph development time (FDT), water absorption (FWA), stability (FST), and breakdown (FBK) using Approved Method 54-21 (AACC 1995). FBK was measured as the difference in Brabender units (BU) from the 500 BU line to the center of the curve 10 min after the start of mixing.

The MDD laboratory mixers and baking system were designed and built by Crop & Food Research, Christchurch, New Zealand. WI and water absorption (WA) were calculated using 125-g MDD mixers (Mk.II). The optimum WI is determined electronically from the torque on the paddles during mixing. The optimum WA is determined in a manner similar to farinograph water absorption, that is, the standard peak dough consistency given when the peak on a farinograph is centered on the 500 BU line (Mitchell 1989).

The MDD test-baking procedure using two lean formulations produced two doughs of each formulation: bromated (Swallow and Baruch 1986) and nonbromated from each field plot. [Author's note: For the nonbromated formulation, bromate was replaced by 0.3% Natura All Dohs and 5% Supersoy, and the amount of ascorbic acid was doubled to 200 ppm. Natura All Dohs and Supersoy (produced by Gulf-Pacific Industries Ltd., Albany, Auckland, New Zealand) contained soybean and fungal enzymes with protease, α-amylase, and cellulase activity, and contained no mineral constituents.] The first of these doughs was overmixed to calculate the WI and WA and then discarded. The remaining dough was prepared using the optimum WI and WA figures obtained from the first dough. Preliminary results had shown that the WI and WA figures calculated were very strongly correlated with values given by the traditional 125-g MDD mixers (Mk.I) with which the New Zealand industry is most familiar.

For comparative purposes, our experimental values were transformed to the traditional values by multiplying the WA values by 0.62 (standard error [SE] = 0.037) and adding 21.46 (SE = 2.300), and multiplying the WI values by 1.39 (SE = 0.033). Once mixed, the second dough was given an intermediate proof of 10 min at 32°C, a final proof of 45 min at 40°C and 80–90% rh, and was baked for 25 min at 220°C (Swallow and Baruch 1986). Bread quality was evaluated in terms of loaf volume (LV), crumb grain (CG), and bake score (BS). All MDD measurements were made on bromated and nonbromated doughs. Bromated doughs were used in the laboratory because no standard enzyme preparation has been adopted by all industrial bakeries to replace bromate. Furthermore, the response to enzyme preparations varies between bakeries, so it was necessary to retain bromate as a reference.

TABLE I  
High Molecular Weight Glutenin Subunits (HMW-GS) and Low Molecular Weight Glutenin Subunits (LMW-GS)  
of Genotypes for Wheat Cultivars and Breeding Lines Used to Determine the Extent of the Nexus  
Between Mixing Requirements and Dough Strength

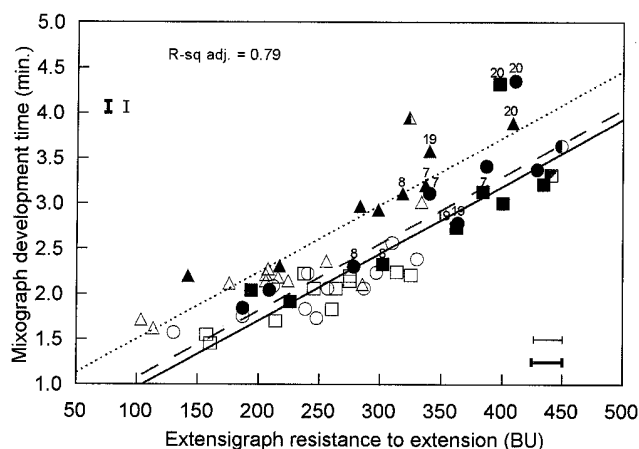
| Wheat Line | Name              | HMW-GS Alleles <sup>a</sup> |                                  |                 | LMW-GS Alleles      |                     |               |
|------------|-------------------|-----------------------------|----------------------------------|-----------------|---------------------|---------------------|---------------|
|            |                   | <i>Glu-A1</i>               | <i>Glu-B1</i>                    | <i>Glu-D1</i>   | <i>Glu-A3</i>       | <i>Glu-B3</i>       | <i>Glu-D3</i> |
| 1          | Cook              | <i>a</i> (1)                | <i>b</i> (7+8)                   | <i>a</i> (2+12) | <i>b</i>            | <i>b</i>            | <i>b</i>      |
| 2          | Sun89D            | <i>a</i>                    | <i>b</i>                         | <i>a</i>        | <i>b</i>            | sec-1 <sup>b</sup>  | <i>b</i>      |
| 3          | Sunbird 5 (mixed) | <i>a</i> (1)/ <i>b</i> (2*) | <i>b</i> (7+8)/ <i>i</i> (17+18) | <i>d</i> (5+10) | <i>b</i> / <i>c</i> | <i>b</i> / <i>c</i> | <i>b</i>      |
| 4          | Condor D4         | <i>b</i> (2*)/ <i>c</i> (-) | <i>b</i> (7+8)/ <i>c</i> (7+9)   | <i>a</i>        | <i>c</i>            | <i>b</i>            | <i>b</i>      |
| 5          | Condor D6         | <i>b</i> / <i>c</i>         | <i>b</i>                         | <i>a</i>        | <i>b</i> / <i>c</i> | <i>b</i>            | <i>b</i>      |
| 6          | Oxley             | <i>b</i>                    | <i>b</i>                         | <i>a</i>        | <i>b</i>            | <i>b</i>            | <i>b</i>      |
| 7          | Hartog            | <i>a</i>                    | <i>i</i>                         | <i>d</i>        | <i>b</i>            | <i>h</i>            | <i>e</i>      |
| 8          | Hartog 1A/1R      | <i>a</i>                    | <i>i</i>                         | <i>d</i>        | <i>e</i>            | <i>h</i>            | <i>e</i>      |
| 9          | Egret             | <i>c</i> (-)                | <i>b</i>                         | <i>d</i>        | <i>e</i>            | <i>b</i>            | <i>b</i>      |
| 10         | Halberd           | <i>a</i>                    | <i>c</i> / <i>e</i> (7+9/20)     | <i>d</i>        | <i>e</i>            | <i>c</i>            | <i>c</i>      |
| 11         | ++ MK             | <i>b</i>                    | <i>b</i> / <i>i</i> (7+8/17+18)  | <i>a</i>        | <i>c</i>            | <i>b</i>            | <i>b</i>      |
| 12         | -- MK             | <i>b</i> / <i>c</i>         | <i>b</i> / <i>i</i>              | <i>a</i>        | <i>e</i>            | <i>b</i>            | <i>b</i>      |
| 13         | Kite              | <i>b</i>                    | <i>i</i>                         | <i>a</i>        | <i>e</i>            | <i>b</i>            | <i>b</i>      |
| 14         | HT/SD 506/4       | <i>b</i> / <i>c</i>         | <i>b</i>                         | <i>a</i>        | <i>b</i>            | <i>b</i>            | <i>b</i>      |
| 15         | HT/SD 506/5       | <i>b</i> / <i>c</i>         | <i>b</i>                         | <i>a</i>        | <i>b</i>            | <i>b</i>            | <i>b</i>      |
| 16         | HT/SD 494/4       | <i>b</i> / <i>c</i>         | <i>b</i>                         | <i>d</i>        | <i>b</i> / <i>c</i> | <i>b</i>            | <i>b</i>      |
| 17         | HT/SD 494/17      | <i>b</i>                    | <i>b</i>                         | <i>d</i>        | <i>b</i> / <i>c</i> | <i>b</i>            | <i>b</i>      |
| 18         | Sundor            | <i>c</i>                    | <i>b</i>                         | <i>a</i>        | <i>b</i>            | <i>b</i>            | <i>b</i>      |
| 19         | Suneca            | <i>a</i>                    | <i>i</i>                         | <i>d</i>        | <i>d</i>            | <i>h</i>            | <i>e</i>      |
| 20         | 890719            | <i>b</i> *                  | <i>i</i>                         | <i>d</i>        | <i>b</i>            | <i>h</i>            | <i>e</i>      |

<sup>a</sup> According to Payne and Lawrence (1983).

<sup>b</sup> Lines with rye translocation (1BL, 1RS), hence with rye secalin instead of LMW-GS from the *Glu-B3* and gliadins from the *Gli-B1* locus.

## Analyses

Statistical analyses performed throughout this research used standard techniques described in Sokal and Rohlf (1981) and Ott (1988). All data were analyzed using either SAS or Genstat statistical software. All quality tests were performed on flour samples using the same factorial design and randomization with respect to the field trial, with standard flour samples included as controls. Pearson correlation coefficient matrices were used to investigate the relationships between quality attributes for each of the two locations. Regression analysis was performed on the mean data. For data from all three trials, Lund's test (Lund 1975) for outliers was



**Fig. 1.** Relationship between mixograph development time and extensigraph resistance to extension for 20 wheat lines cultivated in three trials. Wheat lines homozygous for *Glu-D1a* (2+12) (white symbols), *Glu-D1d* (5+10) (black symbols), or mixed for both alleles (bicolor symbols) are shown along with lines of best fit for trial I (continuous line and squares), II (dashed line and circles), and III (dotted line and triangles). Wheat lines 7, 8, 19, and 20 for each of the three trials are indicated by the wheat line number and symbol. Bars show 1 standard error of the mean within trials (thin) and between trials (thick).

applied to standardized residuals to determine any values that had significantly lower MDT and an Rmax value of 250–350 BU (approximate requirements of the baking industry).

## RESULTS

### Environmental and Agronomic Factors

Analyses of variance of all quality attributes showed significant main effects of trial and wheat line, along with an interaction between trial and line on mixing requirements, as measured by MDT and WI, as well as on dough strength as measured by Rmax (Table II). FN, Ext, EAREA, and all farinograph variables also varied significantly with trial and wheat line, and an interaction existed between them. Bread quality variables LV, CG, and BS showed significant interactions between trial and wheat line.

Nitrogen fertilizer application and crop location both influenced the link or nexus between mixing requirements and dough strength. The nexus between MDT and Rmax was strong (Fig. 1, Table III) but appeared to be influenced by crop location (i.e., climatic or edaphic factors) while the nexus between WI and Rmax (Fig. 2), although equally strong, was significantly influenced by the application of nitrogen during cultivation. Analysis of the relationship between MDT and Rmax showed that parallel lines could be fitted for the three trials accounting for 79% of the variation between attributes. For a given Rmax, MDT in trials I and II did not differ significantly (in spite of the nitrogen fertilizer added in trial II), but MDT in trial III was  $\approx 0.5$  min greater.

Over 80% of the variation between WI and Rmax could be accounted for by fitting parallel lines for each trial. In this case, these variables measured in trials I and III were not significantly different. But in trial II, values of WI were  $\approx 2$  Wh/kg higher at any given Rmax. For a given increase in Rmax, the proportional increase in WI was slightly greater than that for MDT. No significant outliers were found with the desirable combination of low WI and an Rmax value of 250–350 BU. Fitting individual lines with different slopes for each trial accounted for 82% of the variation between FDT and Rmax (Fig. 3).

**TABLE II**  
Summary Analysis of Variance Showing Mean Square Values for Effects of Trial and Wheat Lines on Quality Attributes

| Dependent Variable                      | Source of Variation        |           |   |                           |           |
|---|----------------------------|-----------|---|---------------------------|-----------|
|   | Trial $\times$ Rep Stratum |           | Trial $\times$ Rep $\times$ Wheat Stratum |                           |           |
|   | Trial                      | Residuals | Wheat Line                                | Trial $\times$ Wheat Line | Residuals |
| <b>Mixograph</b>                        |                            |           |   |                           |           |
| Development time (min)                  | 0.700***                   | 0.033     | 4.252***                                  | 0.152***                  | 0.048     |
| Water absorption (%)                    | 58.559*                    | 6.718     | 22.846***                                 | 3.831 ns                  | 2.547     |
| Peak area <sup>b</sup>                  | 458.29 ns                  | 172.00    | 3,577.15***                               | 116.40***                 | 38.36     |
| <b>Extensigraph</b>                     |                            |           |   |                           |           |
| Rmax <sup>c</sup> (BU)                  | 34,604*                    | 6,826     | 59,310***                                 | 3,400**                   | 1,748     |
| Extensibility (cm)                      | 24.273*                    | 3.287     | 26.051***                                 | 2.851***                  | 1.301     |
| Area under the curve (cm <sup>2</sup> ) | 4,050.6**                  | 354.8     | 5,131.4***                                | 276.2**                   | 142.4     |
| <b>Farinograph</b>                      |                            |           |   |                           |           |
| Development time (min)                  | 53.105***                  | 0.494     | 11.971***                                 | 1.615***                  | 0.559     |
| Water absorption                        | 48.438***                  | 1.261     | 35.300***                                 | 2.264***                  | 0.939     |
| Stability (10 min)                      | 384.07***                  | 6.88      | 399.31***                                 | 38.95***                  | 17.37     |
| Breakdown (BU)                          | 12,736.82***               | 109.69    | 2,753.26***                               | 331.14***                 | 65.85     |
| <b>Mechanical Dough Development</b>     |                            |           |   |                           |           |
| Work input (Wh/kg)                      | 260.968***                 | 3.355     | 160.423***                                | 5.297***                  | 2.402     |
| Water absorption (%)                    | 12.234*                    | 2.212     | 14.956***                                 | 1.171**                   | 0.600     |
| Loaf volume (cm <sup>3</sup> )          | 6,202.4 ns                 | 4,236.2   | 17,197.0***                               | 1,714.1**                 | 848.2     |
| Crumb grain <sup>d</sup>                | 4.067 ns                   | 2.492     | 2.588***                                  | 0.879**                   | 0.468     |
| Bake score <sup>e</sup>                 | 40.527 ns                  | 10.018    | 71.509***                                 | 8.415***                  | 2.912     |
| Flour nitrogen (%)                      | 0.977***                   | 0.019     | 0.126***                                  | 0.014**                   | 0.007     |
| Degrees of freedom <sup>f</sup>         | 2                          | 6         | 19  | 38                        | 110 (4)   |

a \*, \*\*, \*\*\* =  $P < 0.05, 0.01, \text{ and } 0.001$ , respectively; ns = not significant.

b % torque  $\times$  min

c Resistance to extension

d Subjective scale 0–14.

e Subjective scale 0–35.

f Total = 175 (4).

Trial III displayed the least increase in FDT over the range of Rmax values. The slope of the relationship between FDT and Rmax in trials I and II was not significantly different. Fitting parallel lines for each trial accounted for only 49.0% of the variation between MDT and FDT (Table III). No significant variation between trials was observed for the relationship between WI and FDT, with a single line accounting for 59% of the variation between these two attributes (Table III).

Parallel lines fitted to each trial for the relationship between WI and MDT accounted for 82% of the variation in the data (Fig. 4). On average, for a given value of MDT, WI was significantly lower in trial III by 3.8 and 4.9 Wh/kg, relative to trials I and II, respectively. WI for a given value of MDT was not significantly different between trials I and II.

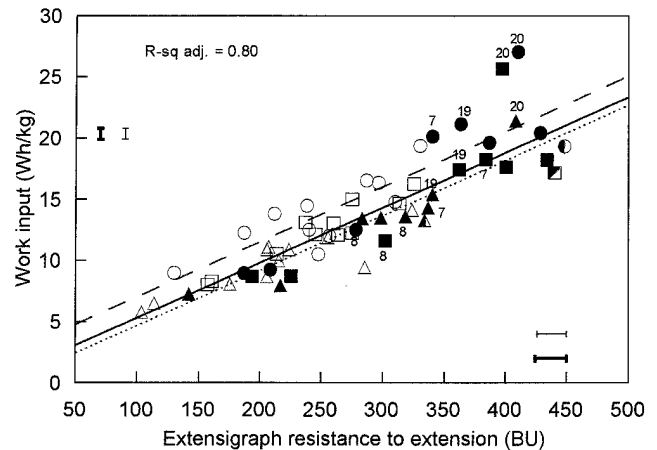
Even with differences in mixing actions, MDT and FDT, as well as WI, all exhibited a high degree of correlation (Table IV). Pearson correlation coefficients for other quality attributes, which are sometimes used to measure physical dough rheological properties, were often high, >0.9 for Rmax to EAREA and Rmax to MPA; 0.8 for WI to EAREA, for WI to FST, and for WI to MPA, as well as 0.8 for WA of bromated doughs to FWA and for WA to MPA.

Significant correlations were found between FN and both WI and FDT, but not MDT. WA values calculated using a MDD mixer, mixograph, or farinograph were also all significantly correlated with FN. EAREA and Rmax were highly correlated, whereas a significant but weak correlation was found between Rmax and Ext. FST and MPA were positively correlated and FBK was negatively correlated with WI, as well as MDT and FDT.

### Genotypic Factors

Despite the wide range of genotypes used, no significant outliers were found, and the nexus between mixing requirements and dough strength was not broken. For the relationship between MDT and Rmax, wheat line 2 (Sun89D) (Table I) in trial III had an Rmax in the desirable range of 250–350 BU and a high negative standardized residual (for MDT), but Lund's test showed this to be nonsignificant. The rates of increase in WI, as well as MDT and FDT with Rmax, although not significant at the 5% level,

appeared to differ in relation to whether HMW-GS 5+10 or 2+12 was present. Generally, the HMW-GS *Glu-D1d* (5+10) genotypes had long MDT and high Rmax, and the *Glu-D1a* (2+12) genotypes had short MDT and low Rmax (Fig. 1). The exceptions were wheat lines 9 and 10 (cultivars Egret and Halberd), which gave lower than expected values for MDT and Rmax for the 5+10 genotype. The lower values for Egret were probably due to the *Glu-A1* and *Glu-A3* loci being null forms and other factors associated with this cultivar being a soft wheat phenotype. Halberd also does not produce a detectable LMW-GS at the *Glu-A3* locus, and was mixed at the *Glu-B1* locus for 7+9 and 20 HMW-GS.



**Fig. 2.** Relationship between mechanical dough development work input (WI) and extensigraph resistance to extension for 20 wheat lines cultivated in three trials. Wheat lines homozygous for *Glu-D1a* (2+12) (white symbols), *Glu-D1d* (5+10) (black symbols), or mixed for both alleles (bicolor symbols) are shown along with lines of best fit for trial I (continuous line and squares), II (dashed line and circles), and III (dotted line and triangles). Wheat lines 7, 8, 19, and 20 for each of the three trials are indicated by the wheat line number and symbol. Bars show 1 standard error of the mean within trials (thin) and between trials (thick).

**TABLE III**  
Regression Coefficients for Relationships Between Quality Attributes<sup>a</sup>

| Dependent Variable | Independent Variable | Trial         | Intercept (SE) | t (df = 54–58) | t Probability | Regression Coefficient (Slope) | t (df = 54–58) | t Probability | Variation (%) <sup>b</sup> |
|--------------------|----------------------|---------------|----------------|----------------|---------------|--------------------------------|----------------|---------------|----------------------------|
| MDT                | Rmax                 | I             | 0.227 (0.164)  | 1.39           | 0.171         | 0.0074 (0.0005) <sup>c</sup>   | 14.79          | <0.001        | 79                         |
|                    |                      | II            | 0.337 (0.162)  | 2.08           | 0.042         |                                |                |               |                            |
|                    |                      | III           | 0.763 (0.144)  | 5.28           | <0.001        |                                |                |               |                            |
| WI                 | Rmax                 | I             | 0.82 (1.03)    | 0.79           | 0.432         | 0.0450 (0.0032) <sup>c</sup>   | 14.26          | <0.001        | 80                         |
|                    |                      | II            | 2.54 (1.02)    | 2.49           | 0.016         |                                |                |               |                            |
|                    |                      | III           | 0.19 (0.91)    | 0.21           | 0.838         |                                |                |               |                            |
| WI                 | MDT                  | I             | 0.899 (0.988)  | 0.91           | 0.367         | 5.471 (0.369) <sup>c</sup>     | 14.84          | <0.001        | 82                         |
|                    |                      | II            | 2.00 (1.01)    | 1.97           | 0.053         |                                |                |               |                            |
|                    |                      | III           | -2.86 (1.06)   | -2.70          | 0.009         |                                |                |               |                            |
| FDT                | Rmax                 | I             | 0.561(0.509)   | 1.10           | 0.275         | 0.01368 (0.00167) <sup>d</sup> | 8.20           | <0.001        | 82                         |
|                    |                      | II            | 0.152(0.496)   | 0.31           | 0.761         |                                |                |               |                            |
|                    |                      | III           | 1.959 (0.461)  | 4.25           | <0.001        |                                |                |               |                            |
| MDT                | FDT                  | I             | 0.613 (0.260)  | 2.36           | 0.022         | 0.3902 (0.0513) <sup>c</sup>   | 7.61           | <0.001        | 49                         |
|                    |                      | II            | 0.462 (0.288)  | 1.61           | 0.114         |                                |                |               |                            |
|                    |                      | III           | 1.315 (0.204)  | 6.45           | <0.001        |                                |                |               |                            |
| WI                 | FDT                  | I, II and III | 3.30 (1.19)    | 2.78           | 0.007         | 2.383 (0.258) <sup>e</sup>     | 9.22           | <0.001        | 59                         |

<sup>a</sup> MDT = mixograph development time; WI = work input; FDT = farinograph development time; Rmax = resistance to extension.

<sup>b</sup> Variation accounted for (adjusted  $r^2 \times 100$ ) (%).

<sup>c</sup> No significant interaction between slopes of each line.

<sup>d</sup> Lines fitted having a significantly different slope for each trial.

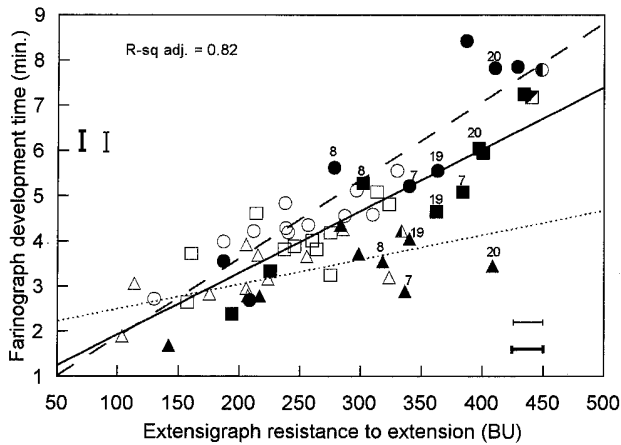
<sup>e</sup> Single line fitted as no significant differences between slopes or intercept for each trial.

## DISCUSSION

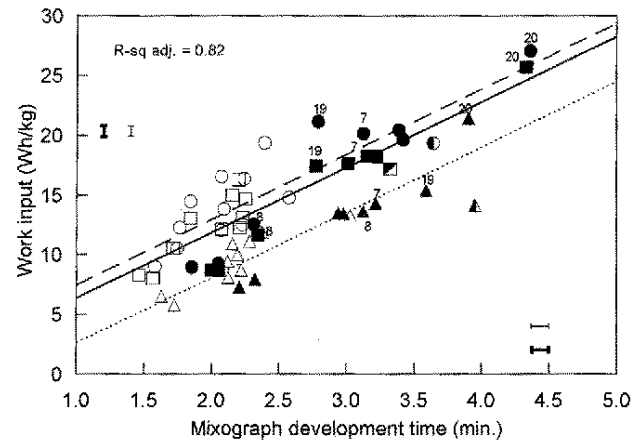
While not as influential, alleles at other loci appeared to be additive in their effect on MDT and Rmax. For example, MDT and Rmax values for wheat line 20 (890719) were significantly greater than for wheat line 8 (Hartog 1A/1R) (Fig. 1 and Table V). These wheat lines differed at the *Glu-A1* and *Glu-A3* loci: wheat line 20 had alleles *b\** and *b* and wheat line 8 had *a* and *e* (null), respectively (Table I). In contrast, no significant differences were observed between wheat lines 19, 8, and 7, differing only at the *Glu-A3* locus, nor between wheat lines 20 and 7, differing only at the *Glu-A1* locus (Fig. 1). No significant differences were also observed for WI and FDT and Rmax between wheat lines 20 and 7, where the *Glu-A1* alleles differed, and between wheat lines 19 and 7, where only the *Glu-A3* alleles differed (Figs. 2 and 3). As with MDT, wheat lines containing the HMW-GS 5+10 generally had high WI, and those with HMW-GS 2+12 had low values, with the exception of wheat lines 9 and 10. Alleles at other loci also appeared to be additive in their effect on WI.

This study of environmental and agronomic effects on dough quality attributes and flour protein composition has yielded valuable insights into protein functionality in terms of the breadmaking quality of wheat lines. The cultivation, in three different trials, of the 20 wheat lines chosen for this research achieved the desired effect of producing flours with a very wide range in mixing requirements, dough strength, and bread quality, and the WI range observed corroborated industry concerns about energy requirements. The spread between maxima and minima for quality attributes of the test materials formed a suitable base from which to make a robust assessment of the interrelationships among several measures of these important attributes.

Significant differences were observed in the three measurements of mixing requirements and dough strength among the three environments. MDT was significantly higher at a given Rmax at



**Fig. 3.** Relationship between farinograph development time and extensigraph resistance to extension for 20 wheat lines cultivated in three trials. Wheat lines homozygous for *Glu-D1a* (2+12) (white symbols), *Glu-D1d* (5+10) (black symbols), or mixed for both alleles (bicolor symbols) are shown along with lines of best fit for trial I (continuous line and squares), II (dashed line and circles), and III (dotted line and triangles). Wheat lines 7, 8, 19, and 20 for each of the three trials are indicated by the wheat line number and symbol. Bars show 1 standard error of the mean within trials (thin) and between trials (thick).



**Fig. 4.** Relationship between mechanical dough development work input (WI) and mixograph development time for 20 wheat lines cultivated in three trials. Lines homozygous for *Glu-D1a* (2+12) (white symbols), *Glu-D1d* (5+10) (black symbols), or mixed for both alleles (bicolor symbols) are shown along with lines of best fit for trial I (continuous line and squares), II (dashed line and circles), and III (dotted line and triangles). Wheat lines 7, 8, 19, and 20 for each of the three trials are indicated by the wheat line number and symbol. Bars show 1 standard error of the mean within trials (thin) and between trials (thick).

**TABLE IV**  
Correlation Coefficients Between Quality Attributes for 20 Wheat Lines Cultivated in Two Trials at Tamworth and One Trial at Breeza<sup>a</sup>

|       | WI     | WA     | MDT    | MWA    | MPA    | Rmax   | Ext    | EAREA  | FDT    | FWA    | FST    | FBK    | LV    | CG    | BS    |
|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|-------|-------|
| WA    | 0.270  |        |        |        |        |        |        |        |        |        |        |        |       |       |       |
| MDT   | 0.776  | -0.005 |        |        |        |        |        |        |        |        |        |        |       |       |       |
| MWA   | 0.492  | 0.551  | 0.367  |        |        |        |        |        |        |        |        |        |       |       |       |
| MPA   | 0.866  | 0.861  | 0.197  | 0.330  |        |        |        |        |        |        |        |        |       |       |       |
| Rmax  | 0.830  | 0.257  | 0.796  | 0.429  | 0.935  |        |        |        |        |        |        |        |       |       |       |
| Ext   | 0.548  | 0.193  | 0.267  | 0.402  | 0.679  | 0.398  |        |        |        |        |        |        |       |       |       |
| EAREA | 0.863  | 0.275  | 0.743  | 0.485  | 0.150  | 0.953  | 0.657  |        |        |        |        |        |       |       |       |
| FDT   | 0.719  | 0.418  | 0.526  | 0.442  | 0.402  | 0.751  | 0.313  | 0.720  |        |        |        |        |       |       |       |
| FWA   | 0.229  | 0.854  | -0.059 | 0.510  | 0.817  | 0.111  | 0.044  | 0.106  | 0.373  |        |        |        |       |       |       |
| FST   | 0.804  | 0.176  | 0.782  | 0.335  | 0.849  | 0.724  | 0.243  | 0.675  | 0.733  | 0.163  |        |        |       |       |       |
| FBK   | -0.655 | -0.538 | -0.390 | -0.560 | -0.570 | -0.642 | -0.397 | -0.658 | -0.655 | -0.514 | -0.484 |        |       |       |       |
| LV    | 0.382  | 0.231  | 0.199  | 0.278  | 0.293  | 0.458  | 0.498  | 0.541  | 0.354  | 0.034  | 0.137  | -0.460 |       |       |       |
| CG    | 0.349  | 0.087  | 0.324  | 0.348  | 0.382  | 0.429  | 0.306  | 0.453  | 0.319  | 0.074  | 0.248  | -0.525 | 0.366 |       |       |
| BS    | 0.423  | 0.219  | 0.255  | 0.334  | 0.352  | 0.509  | 0.517  | 0.589  | 0.395  | 0.047  | 0.182  | -0.542 | 0.963 | 0.590 |       |
| FN    | 0.484  | 0.577  | 0.039  | 0.462  | 0.262  | 0.385  | 0.457  | 0.467  | 0.578  | 0.532  | 0.259  | -0.669 | 0.432 | 0.307 | 0.465 |

<sup>a</sup> WI = mechanical dough development (MDD) work input (Wh/kg); WA = MDD water absorption (%); MDT = mixograph development time (min); MWA = mixograph water absorption (%); MPA = mixograph peak area (% torque × min); Rmax = extensigraph resistance to extension (BU); Ext = extensigraph extensibility (cm); EAREA = extensigraph area under the curve (cm<sup>2</sup>); FDT = farinograph development time (min); FWA = farinograph water absorption (%); FST = farinograph stability (10 min); FBK = farinograph breakdown (BU); LV = MDD loaf volume (cm<sup>3</sup>); CG = MDD crumb grain (subjective scale 0–14); BS = MDD bake score (subjective scale 0–35); FN = flour nitrogen (%).

Breeza than at Tamworth, but the application of nitrogen fertilizer at Tamworth had no significant effect on this relationship. This suggests that weather conditions (such as rainfall), or soil conditions (such as ability to retain water or heat from the sun), or mineral deficiencies (such as a lack of sulfur) have a greater influence on grain quality than the application of nitrogen fertilizer where the level of soil nitrogen is already sufficient for normal plant growth and grain filling. In contrast, WI was not significantly influenced by location but was significantly increased for a given Rmax when 60 kg/ha of nitrogen fertilizer was applied, compared to no nitrogen applied at the same location. FDT was significantly affected by both location and variation in the application of nitrogen more than the other two mixing requirement indicators, WI and MDT. Consequently, its relationship with dough strength was more varied between trials. These results indicate the importance of ensuring that the laboratory testing of samples destined for a specific industrial process is attuned to factors that will affect that process.

As protein concentration and composition play dominant roles in determining mixing requirements and dough strength (Tipples et al 1982, MacRitchie 1987, Gupta et al 1992), the nexus might also be broken by using strategies to vary the ratio of LMW to HMW glutenin subunits, the ratio of glutenin to gliadin, the proportion of total glutenin to total grain protein, the ratio of polymeric to monomeric protein, or the molecular size distribution of polymeric protein. For example, it has been shown (MacRitchie 1992), using genetic variant lines (HMW null lines and rye translocation lines), that MDT varied more steeply as a function of Rmax when the amount of HMW-GS was varied than when the amount of LMW-GS was varied. Therefore, decreasing the ratio of HMW-GS to LMW-GS may reduce MDT, but as it would also tend to reduce Rmax, it may be necessary to compensate by increasing the proportion of glutenin in the total grain protein (MacRitchie 1992).

Results suggest that, although the *Glu-D1* locus was largely responsible for the variation in protein functionality and, consequently, physical dough characteristics and breadmaking quality,

the *Glu-A1* and *Glu-A3* loci also played a significant role in determining these properties. Valuable information on genetic effects on the nexus could be obtained from the evaluation of numerous progeny of a cross between parents differing in mixing requirements and dough strength. This approach differs from the traditional one of evaluating quality only in the small number of high-quality progeny from a given cross and will be covered in a subsequent report.

Nitrogen variability is generally recognized as mainly affecting flour protein content, whereas sulfur variability affects protein composition. In particular, the effect of nitrogen and sulfur on yield and protein quantity, and to a lesser extent on physical dough characteristics, has been of significant interest (Finney et al 1957; Fernandez and Laird 1959; Campbell et al 1977; Bushuk et al 1978; Spencer and Freney 1980; Timms et al 1981; Wrigley et al 1984a,b). It has been suggested that in some countries, a corresponding increase in mixing requirements and dough strength may be due in part to a reduction in the use of combination fertilizers containing sulfur and increased use of pure nitrogen fertilizers such as urea. It would, therefore, seem an appropriate strategy to manipulate the nitrogen and sulfur availability during crop cultivation to alter the protein composition in terms of one of the aforementioned protein ratios in such a way as to reduce mixing requirements while maintaining dough strength and other desirable properties. This strategy has been tested and will be covered in a subsequent report.

## CONCLUSIONS

Statistical analysis over the wide range of wheat lines has validated the assertion of MacRitchie (1992) that a nexus exists between mixing requirements (as measured by MDT) and dough strength (as measured by Rmax). This nexus was also shown for the MDD process where mixing requirements are measured by WI. However, in both cases the relationship was shifted by environmental or agronomic factors that varied between trials. The relationship between FDT and Rmax was also strong but varied more from trial to trial. Despite different mixing actions, WI and MDT and

**TABLE V**  
Differences in Mixograph Development Time (MDT), Mechanical Dough Development (MDD) Work Input (WI), and Extensigraph Resistance to Extension (Rmax) Between a Selection of Paired Wheat Lines Differing in Alleles at *Glu-A1* and *Glu-A3* Loci<sup>a</sup>

| Trial | Quality Attribute | First Wheat Line |            |               |               | Second Wheat Line |            |               |               | t (df)       |           |
|-------|-------------------|------------------|------------|---------------|---------------|-------------------|------------|---------------|---------------|--------------|-----------|
|       |                   | No.              | Mean Value | <i>Glu-A1</i> | <i>Glu-A3</i> | No.               | Mean Value | <i>Glu-A1</i> | <i>Glu-A3</i> |              |           |
| I     | Rmax (BU)         | 8                | 301.67     | <i>a</i>      | <i>e</i>      | 20                | 396.67     | <i>b*</i>     | <i>b</i>      | 3.73* (4)    |           |
| II    |                   |                  | 278.33     |               |               |                   | 410.00     |               |               | 5.97**       |           |
| III   |                   |                  | 318.33     |               |               |                   | 408.33     |               |               | 3.39*        |           |
| I     |                   | 20               | 8          | 301.67        | <i>a</i>      | <i>e</i>          | 19         | 360.00        | <i>a</i>      | <i>d</i>     | 1.94 (3)  |
| II    |                   |                  |            | 278.33        |               |                   |            | 363.33        |               |              | 2.46*     |
| III   |                   |                  |            | 318.33        |               |                   |            | 340.00        |               |              | 1.05      |
| I     |                   |                  | 396.67     | <i>b*</i>     | <i>b</i>      | 360.00            | <i>a</i>   | <i>d</i>      | 1.03 (3)      |              |           |
| II    |                   |                  | 410.00     |               |               | 363.33            |            |               | 1.02          |              |           |
| III   |                   |                  | 408.33     |               |               | 340.00            |            |               | 0.81          |              |           |
| I     | WI (Wh/kg)        | 8                | 11.70      | <i>a</i>      | <i>e</i>      | 20                | 25.73      | <i>b*</i>     | <i>b</i>      | 14.03*** (4) |           |
| II    |                   |                  | 12.57      |               |               |                   | 27.07      |               |               | 13.78***     |           |
| III   |                   |                  | 13.67      |               |               |                   | 21.50      |               |               | 9.78**       |           |
| I     |                   | 20               | 8          | 11.70         | <i>a</i>      | <i>e</i>          | 19         | 19.95         | <i>a</i>      | <i>d</i>     | 3.41* (3) |
| II    |                   |                  |            | 12.57         |               |                   |            | 21.20         |               |              | 7.54**    |
| III   |                   |                  |            | 13.67         |               |                   |            | 15.47         |               |              | 5.10**    |
| I     |                   |                  | 25.73      | <i>b*</i>     | <i>b</i>      | 19.95             | <i>a</i>   | <i>d</i>      | 2.03 (3)      |              |           |
| II    |                   |                  | 27.07      |               |               | 21.20             |            |               | 2.33          |              |           |
| III   |                   |                  | 21.50      |               |               | 15.47             |            |               | 6.17**        |              |           |
| I     | MDT (min.)        | 8                | 2.34       | <i>a</i>      | <i>e</i>      | 20                | 4.33       | <i>b*</i>     | <i>b</i>      | 8.71*** (4)  |           |
| II    |                   |                  | 2.31       |               |               |                   | 4.36       |               |               | 29.69***     |           |
| III   |                   |                  | 3.12       |               |               |                   | 3.90       |               |               | 10.48***     |           |
| I     |                   | 20               | 8          | 2.34          | <i>a</i>      | <i>e</i>          | 19         | 2.74          | <i>a</i>      | <i>d</i>     | 2.42* (3) |
| II    |                   |                  |            | 2.31          |               |                   |            | 2.79          |               |              | 5.97**    |
| III   |                   |                  |            | 3.12          |               |                   |            | 3.59          |               |              | 1.72      |
| I     |                   |                  | 4.33       | <i>b*</i>     | <i>b</i>      | 2.74              | <i>a</i>   | <i>d</i>      | 6.76** (3)    |              |           |
| II    |                   |                  | 4.36       |               |               | 2.79              |            |               | 35.39***      |              |           |
| III   |                   |                  | 3.90       |               |               | 3.59              |            |               | 1.29          |              |           |

<sup>a</sup> Significance levels: \* = 0.147, \*\* = 0.193; df = 174.

FDT all exhibited a high degree of correlation; WI and MDT had the best predictive relationship with each other.

The general view that the wheat genotype plays a significant role in determining physical dough characteristics, including mixing requirements and dough strength, was reinforced. Although a wide range of genotypes was used, no significant outliers were found and the nexus was not broken.

Despite the apparent strength of the nexus between mixing requirements and dough strength, it may be possible to shift or manipulate it genetically. Although the *Glu-D1* locus was responsible for a large part of the variation in protein functionality and, consequently, in breadmaking quality, the *Glu-A1* and *Glu-A3* loci also played a significant role and could be manipulated to try and break the nexus between mixing requirement and dough strength.

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