

**THE IMPACT OF SULPHUR ON THE BREADMAKING QUALITY  
OF CANADIAN WESTERN RED SPRING WHEAT IN WESTERN CANADA**

BY

CHRISTOPHER J. H. UNGER

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Submitted to the Faculty of Graduate Studies  
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## ABSTRACT

**Unger, C.J.H. M.Sc., The University of Manitoba, October, 2002. The Impact of Sulphur on the Breadmaking Quality of Canadian Western Red Spring Wheat in Western Canada. Major Professor, Dr. Don Flaten.**

Canada Western Red Spring (CWRS) wheat (*Triticum aestivum* L. cv. AC Barrie) was grown at twelve locations, over two growing seasons across western Canada, to study the impact of S fertilization on grain yield and quality of wheat. Treatments consisted of two rates of fertilizer S (0 and 20 kg ha<sup>-1</sup>) as ammonium sulphate and two rates of fertilizer N (26 and 100 kg ha<sup>-1</sup>) as urea in a factorial design. Soil and plant tissue tests were also evaluated for their ability to predict grain S concentration, grain N:S ratio, total S accumulation in the plant, and grain quality responses to S fertilization.

Analysis of grain for total S, N, and N:S ratio accurately predicted the concentration of S, N, and N:S ratio in flour. Grain S concentration and N:S ratio were weakly correlated with both absolute and relative grain yield. Grain S concentration was strongly and positively correlated with loaf height, loaf volume, and oven spring; grain N:S ratio was negatively, but more weakly, correlated with these baking parameters. The improvements in baking quality were accompanied by an increase in dough extensibility and reduction in dough strength. Grain S concentration was positively correlated with dough extensibility and negatively correlated with maximum dough resistance, mixograph peak time, and work input to peak. Grain N:S ratio was negatively correlated with dough extensibility and positively correlated with maximum dough resistance and work input to peak. The improvements in baking and dough quality were associated with

changes in the protein composition of flour. Grain S concentration was positively correlated with the proportion of soluble glutenin and negatively correlated with the ratio of insoluble to soluble glutenin in flour. Grain N:S ratio was negatively correlated with the proportion of soluble glutenin and positively correlated with the ratio of insoluble to soluble glutenin in flour.

Sulphur fertilization increased grain yield at two of seven sites used for breadmaking quality evaluation. Application of S fertilizer also frequently improved the breadmaking quality, dough quality, and flour protein composition of wheat at four of these seven sites. All four sites where grain quality improvements were observed contained  $< 40 \text{ kg SO}_4\text{-S ha}^{-1}$  prior to fertilization, a concentration of soil S regarded as marginally sufficient for grain yield. Also, at these four marginal S sites, the S concentration and N:S ratio of plant tissue samples collected at 50 % heading was  $< 0.15 \text{ \% S}$  and  $> 17:1$ , respectively. Sulphur fertilization increased the concentration of S in grain and reduced the N:S ratio in grain at all marginal S sites. The improvements in grain S nutrition were accompanied by significant improvements in loaf volume at two of the four marginal S sites when S fertilizer was applied in combination with 26 or  $100 \text{ kg N ha}^{-1}$ , and at one more site where  $100 \text{ kg N ha}^{-1}$  was applied. Sulphur fertilization increased loaf height and oven spring at three of the four sites. Application of S fertilizer also significantly increased dough extensibility at all four marginal S sites and reduced maximum dough resistance and mixograph peak time at three of four sites. Mixograph peak time was significantly reduced at the other marginal S site only in the presence of  $100 \text{ kg N ha}^{-1}$ . Furthermore, S fertilization reduced the viscoelastic ratio and mixograph work input to peak at all four marginal S sites. Sulphur fertilization increased the proportion of soluble glutenin in flour and reduced the ratio of insoluble to soluble glutenin in the flour at three of four marginal S sites. Sulphur fertilization in the presence of  $100 \text{ kg N ha}^{-1}$  only, increased the proportion of



soluble glutenin in flour and reduced the ratio of insoluble to soluble glutenin in the flour at the other marginal S site. At the three sites where soil  $\text{SO}_4\text{-S}$  concentrations were  $> 40 \text{ kg ha}^{-1}$ , no yield and few breadmaking quality improvements were observed in response to S fertilization. For these high S sites, S fertilization did not increase the S concentration in grain at any site and reduced the N:S ratio in grain at only one site.

At all four sites where grain contained  $\leq 0.17 \%$  S and an N:S ratio  $> 17:1$ , quality improvements due to S fertilization were consistently observed. At all three sites where grain contained S concentrations  $\geq 0.17 \%$  S and N:S ratios  $< 17:1$ , breadmaking quality responses to S fertilization were infrequent.

The S concentration of whole plant samples collected at the 50 % heading stage and the 4 – 6 leaf stage was poorly correlated to the S concentration in grain. However, grain N:S ratio was correlated well with the ratio of N to S in the plant tissue samples collected at the 50 % heading stage. In the absence of S fertilization, soil  $\text{SO}_4\text{-S}$  concentration to 60 cm was moderately correlated with the S concentration in grain and with total S accumulation in the plant. When fertilizer S and estimated mineralizable soil organic S were considered, in addition to soil  $\text{SO}_4\text{-S}$ , in multiple regression analysis, the predictability of grain S concentration and total S accumulation in the plant using these soil measurements was weak. Finally, when the soil N:S ratio, calculated with the soil  $\text{NO}_3\text{-N}$  and  $\text{SO}_4\text{-S}$  values, was plotted against grain N:S ratio, for the low N, zero S treatment, there was a modest correlation. When the fertilizer treatments were added to the soil  $\text{NO}_3\text{-N}$  and  $\text{SO}_4\text{-S}$  concentrations in the calculation of the soil N:S ratio, the correlation improved but was not strong.

In summary, this study demonstrated that S fertilization increased grain S concentration, reduced grain N:S ratio, and improved the breadmaking quality CWRs wheat grown in western

Canada, especially in the presence of high N fertility and marginal S fertility. These breadmaking quality improvements were probably due to decreased dough strength and increased dough extensibility creating a balance between the two, resulting from the increased synthesis of soluble glutenin and the reduction in the ratio of insoluble to soluble glutenin in the flour. Breadmaking quality responses to S fertilization were more frequent than grain yield responses to S fertilization. For processing CWRS wheat, grain with  $\leq 0.17$  % S and an N:S ratio  $> 17:1$  does not contain sufficient S for maximum grain quality and will frequently respond to S fertilization. For production of high quality CWRS wheat, S fertilizer should be applied when the soil contains  $< 40$  kg  $\text{SO}_4\text{-S ha}^{-1}$  or when plant tissue samples collected at 50 % heading contain S concentrations and N:S ratios  $< 0.15$  % S and  $> 17:1$ , respectively.

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## **FOREWARD**

This thesis has been prepared in the manuscript format in adherence with the guidelines established by the Department of Soil Science. The referencing style employed throughout this document is that of the Canadian Journal of Soil Science. Chapter 3 and Chapter 4 will be submitted to the Canadian Journal of Plant Science for publishing. Chapter 5 will be submitted as a short communication to the Canadian Journal of Plant Science. For all papers, I will be the head author and co-authorship will be designated accordingly.

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## 1. INTRODUCTION

Early work conducted at the University of Alberta demonstrated the positive impact of sulphur (S) on the quality of wheat and the bread produced from this wheat (Newton et al. 1959). In baking tests, the largest loaves of the best quality bread were obtained when wheat had been grown after legumes on plots which had received S fertilizer. The poorest bread was obtained from wheat grown on fallowed land or on plots receiving ineffective S fertilizer treatments. More recently, studies in Europe, Australia, and New Zealand confirmed that S fertilization improved the breadmaking quality of wheat (Byers et al. 1987, Fullington et al. 1987, Haneklaus et al. 1992, MacRitchie and Gupta 1993, Moss et al. 1981, 1983, Schnug et al. 1993, Wooding et al. 2000, Wrigley et al. 1984, Zhao et al. 1999a, 1999b).

Information regarding the impact of S fertilization on grain yield and quality of Canada Western Red Spring (CWRS) wheat grown in western Canadian growing conditions is limited. In addition, until relatively recently, S deficiencies were believed to be limited to the northern fringe areas of the Prairies. However, up to 30 % of cultivated soils in the Prairie Provinces are estimated to be deficient in S for both canola and legume production (Bettany et al. 1982) and marginally sufficient for cereal production. This area includes a large portion of the Gray Luvisolic soils and extends into the Dark Gray and Black Chernozemic soil zones where the soils are low in organic matter, coarse-textured, well-drained, and intensively cropped (Bailey 1987). Nyborg (1968) suggested that many of these soils known to be deficient in S for alfalfa may also provide insufficient S for optimum cereal growth.

Our studies were conducted to obtain more information about the impact of S fertilization on the yield and quality of CWRS wheat (*Triticum aestivum* L.) grown under western Canadian growing conditions. In 1999 and 2000, we conducted twelve field studies to:

1. investigate the relationship between grain S concentration, grain N concentration, and grain N:S ratio and grain yield and breadmaking quality of CWRS wheat.
2. investigate the impact of S fertilization on the grain yield and breadmaking quality characteristics of CWRS wheat. Within this objective, we wanted to examine whether the breadmaking quality of CWRS wheat was improved by S fertilization in the absence of a yield response.
3. evaluate agronomic tools (e.g. soil tests and plant tissue tests) which would aid western Canadian producers to predict the S concentration and N:S ratio in grain as well as quality responses to S fertilization.

## 2. REVIEW OF LITERATURE

### 2.1. Introduction

Sulphur is an essential nutrient for all living organisms. The needs for S by higher plants have been recognized for over two centuries (Duke and Reisenauer 1986). For wheat, S is important for maintaining maximum yields (Beaton and Soper 1986, Rasmussen and Kresge 1986, Tisdale et al. 1986) and quality (Randall and Wrigley 1986). However, western Canadian interest and research in S nutrition of wheat has lagged behind other nutrients because S deficiencies have not been as extensive as deficiencies of nitrogen (N), phosphorus (P), or potassium (K).

Deficiencies of S for yield were first identified in Canada in 1927 on Gray-wooded, Luvisolic soils in the province of Alberta (Doyle and Cowell 1993). Currently, up to 30 % of cultivated soils in the Prairie Provinces are estimated to be deficient in S for both canola and legume production (Bettany et al. 1982) and marginally sufficient for cereal production. This area includes a large portion of the Gray Luvisolic soils and extends into the Dark Gray and Black Chernozemic soil zones where the soils contain low concentrations of organic matter, are coarse-textured, well-drained, and intensively cropped (Bailey 1987). Nyborg (1968) also suggests that many soils known to be deficient in S for alfalfa may also provide insufficient S for optimum cereal growth.

Sulphur is also important for grain quality. For example, S is important for the formation of protein in wheat grain because it is an essential component of amino acids such as cysteine

and methionine (Shewry 1995, Shewry and Mifflin 1985). Disulphide bonds (S-S) that form between sulphhydryl (-SH) groups of cysteine residues play a key role in determining the structure and properties of wheat proteins (Shewry and Tatham 1997, Wall 1971). The changes in the protein composition of wheat grain as a result of S deficiency may impact the rheological properties of wheat dough, affecting the quality of bread products.

## **2.2. Impact of Sulphur on Wheat Yield**

Where S concentrations in soil are low, S fertilization generally improves the yields of crops regarded as high S-requiring, such as canola or rapeseed (Nyborg et al. 1974). Most producers recognize that these crops require the annual application of S fertilizer to maintain high yield and quality. However, the yield of small grain crops such as wheat, that have a low metabolic demand for S (Bettany et al. 1982), also responds positively to S fertilization.

Some of the earliest Canadian research on S nutrition of wheat was conducted in Alberta. At Breton, AB., out of four wheat trials established on land that had never received S fertilizer, three trials produced significant positive yield responses to S fertilization ranging from 88 to 345 %. Furthermore, out of four wheat trials established on land that had received S fertilizer on an annual basis for 20 years, three trials also demonstrated significant yield improvements due to application of additional S fertilizer, ranging from 44 to 62 % (Bentley et al. 1955). In another experiment conducted on a S-deficient, Gray Luvisolic soil near Chedderville, AB., wheat yields were improved by 10 % due to the application of 22 kg S ha<sup>-1</sup> when no other nutrients were applied. When N and P were also applied, the yield response to S fertilization increased to 22 % (Agriculture Canada 1958). More recent studies in Alberta report wheat yield improvements of 21 % (Dick 1974) and 28 % (Hennig 1986) due to S fertilization.

Very few studies in Saskatchewan and Manitoba have reported strong responses to S fertilization for wheat. However, in Loon Lake, SK., Agriculture Canada (1958) reported a 20 % yield improvement for wheat due to the application of 22 kg elemental S ha<sup>-1</sup>. In Manitoba wheat yield improvements of up to 30 % due to S fertilization have also been observed (Bradley 1986).

The nutrient status of the wheat grain may be useful in diagnosing the S status for wheat in relation to yield. In a field experiment using Australian wheat varieties, Randall et al. (1981) found a strong relationship between grain yield and grain S concentration. In addition, data for wheat receiving high rates of N fertilizer indicated a critical value (corresponding to 90 % maximum yield) of approximately 0.12 % S in the grain. For the high N treatments, this value separated plants which responded to fertilizer S from those that did not respond. However, when N was inadequate for growth and the grain S concentration was less than this critical level, the plants failed to respond in grain yield to S additions. The authors concluded that low concentrations of S in grain were associated with inadequate supplies of S or N and cannot, by themselves, be used as a diagnostic index of S responsiveness. However, further discrimination between grain from S-responsive and from non-responsive plants was examined on the basis of the total N:total S ratio in the grain. In the study, the 90 % relative yield point coincided with an N:S ratio of approximately 17:1. The overall conclusion of the study was that grain from S-responsive plants can be distinguished from unresponsive plants because the former had less than 0.12 % S and an N:S ratio wider than 17:1.



### **2.3. Amino Acid Composition of Wheat Grain**

The building blocks of wheat grain proteins are amino acids. Sulphur containing amino acids, including cysteine and methionine, are important in determining many quality related characteristics of wheat flour and dough. The disulphide bonds (S-S) that form between sulphhydryl (-SH) groups of cysteine residues play a key role in determining the structure and properties of wheat proteins (Shewry and Tatham 1997, Wall 1971).

#### **2.3.1. Impact of Sulphur on the Amino Acid Content of Wheat Grain**

The availability of soil S directly affects the amino acid content of grain. In initial research conducted in western Canada, the proportion of nine essential amino acids in wheat grain was improved by the application of S fertilizer on plots where legumes had been grown in the previous season (Newton et al. 1959). However, although the authors realized that S availability could affect the nutritional value of wheat by its effects on the amino acid content of grain, they did not establish the link between amino acid composition and breadmaking quality.

Approximately seven years later, Yoshino and McCalla (1966), at the University of Alberta, also observed that S availability could significantly affect the amino acid composition of grain. These researchers compared two samples of grain, one which had received 63 kg S ha<sup>-1</sup> and the other which received no S. The cysteine and methionine concentrations in crude gluten were much greater in the grain which had received the S fertilizer treatment. The researchers concluded that the number of potential disulphide links in the grain is affected by the availability of S and could affect the physical properties of wheat gluten.

More recent studies support these earlier conclusions. In Australian research, Wrigley et al. (1980) noted that methionine and cysteine were reduced to below half their normal

concentrations when S was deficient. These researchers also observed that the drop in cysteine and methionine concentrations was associated with a rise in grain N:S ratio due to deficiencies of S in relation to N. In the U.K., Byers and Bolton (1979) found that S deficiency significantly decreased the concentration of cysteine and methionine (expressed as a percentage of total recovered amino acids) in grain and flour. Furthermore, these researchers also observed that, in the absence of applied S, as the amount of N fertilizer increased, the amounts of cysteine and methionine (as a percent of total recovered amino acids) in the grain decreased.

#### **2.4. Protein Composition of Wheat Grain**

Proteins in wheat grain serve a storage function and are the nutrient source for the developing embryo upon germination. When wheat flour is mixed with water, these proteins combine to form gluten, which is a cohesive, extensible, rubbery network that contributes to the functional properties of wheat dough (Zhao et al. 1999c). Therefore, proteins are recognized as very important components influencing the breadmaking quality of wheat (Kaufman et al. 1986).

Traditionally, wheat proteins are classified into four groups based on their solubility (Kasarda et al. 1976). First, are the albumins, which are soluble in water. Second, are the globulins, which are soluble in salt solutions (10 % NaCl) but insoluble in water. Third, are the gliadins, which are soluble in 70 – 90 % alcohol solutions and are present as monomeric proteins, which either lack disulphide bonds completely ( $\omega$ -gliadins) or have intra-chain disulphide bonds only ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins) (Shewry et al. 1997). Finally, are the glutenins, which are insoluble in neutral aqueous solutions, saline solutions, or alcohol and consist of protein subunits present in polymers stabilized by inter-chain disulphide bonds (Shewry et al. 1997). The gliadins and glutenins are often grouped together and called prolamins (Zhao et al.

1999c) and are a major component of storage proteins in wheat grain, accounting for approximately 50 % of the total N in grain (Shewry 1995).

Prolamins (gliadins and/or glutenins) contain different concentrations of cysteine residues and are classified as S-poor, S-rich, and high molecular weight (HMW) glutenin subunits (Shewry et al. 1997). The S-poor prolamins consist mostly of  $\omega$ -gliadins, which contain low or zero amounts of cysteine and methionine. The S-rich prolamins consist of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins, and low molecular weight (LMW) subunits of glutenin. These fractions contain 2 – 3 mol % cysteine. The HMW glutenin group has an intermediate cysteine concentration of 0.5 – 1.5 mol %. In addition to the prolamins, the albumins and globulins may also be rich in S-containing amino acids such as cysteine and contribute to the functionality of wheat proteins (Zhao et al. 1999c).

#### **2.4.1. Impact of Sulphur on the Protein Composition of Wheat Grain**

Variability in the availability of S affects the protein composition of grain. Low S fertility results in the formation of S-poor polypeptides at the expense of more S-rich polypeptides. Researchers have shown that S deficiency in wheat causes increases in the relative proportions of HMW glutenin subunits and S-poor  $\omega$ -gliadins. At the same time, there is a decrease in the relative proportions of LMW glutenin subunits,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins, albumins, and globulins which are rich in S (Castle and Randall 1987, Fullington et al. 1987, MacRitchie and Gupta 1993, Moss et al. 1981, Wrigley et al. 1980, 1984).

## **2.5. Rheological Properties of Wheat Dough**

Dough is an intermediate stage in the production of bread from grain. When wheat flour is mixed with water (and other constituents including yeast, salt, etc.), the prolamins form a continuous network called gluten (Zhao et al. 1999c). Sulphydryl (-SH) groups and disulphide (S-S) bonds play a key role in determining the physical properties of gluten, therefore, on the quality of dough (Shewry and Tatham 1997, Wall 1971).

Dough that exhibits high strength (elasticity) and reasonable extensibility (viscosity) is good for making bread (Zhao et al. 1999c). The monomeric gliadins that form intra-chain disulphide bonds contribute to dough extensibility (Shewry and Tatham 1997). The polymeric glutenins, which have both intra- and inter-chain disulphide bonds, contribute mainly to dough strength (Shewry and Tatham 1997). There is a positive correlation between the content of insoluble glutenin polymers and dough strength (Gupta et al. 1993, MacRitchie 1987).

The dough properties of strength and extensibility have traditionally been evaluated with the farinograph, mixograph, and extensigraph. The farinograph and mixograph are physical dough testing tools that measure the physical properties of dough during the mixing process (Kunerth and D'apponia 1985). The extensigraph is a load-extension instrument that measures the strength and extensibility of dough during the stretching process (Shuey 1975). For a more in-depth review of these instruments, please refer to the appendix.

### **2.5.1. Impact of Nitrogen on the Rheological Properties of Wheat Dough**

High grain and flour N concentrations (high grain and flour protein concentrations) have long been associated with high breadmaking quality potential in wheat. High quality bread (e.g.

high loaf volume) is positively and directly correlated with grain and flour protein concentration (Bushuk et al. 1969, Finney and Barmore 1948).

Protein concentration also affects the performance of flour and dough in quality evaluation at functional levels. Nitrogen fertilization and subsequent improvements in grain protein concentration tends to improve dough strength. For example, the farinograph mixing parameters including farinograph stability, farinograph dough development time, and farinograph water absorption all tend to increase with rising grain/flour protein concentrations, indicating improvements in dough strength (Ayoub et al. 1994, Dexter et al. 1994, Pechanek et al. 1997). Further evidence of the relationship between grain N and dough strength is provided by Ayoub et al. (1994) who found that farinograph mixing tolerance index declined as grain protein increased, indicating that dough strength and stability improve with rising grain N concentrations.

Uthayakumanin et al. (1999) observed improvements in dough quality with increasing protein content using the mixograph and extensigraph. Both mixograph mixing time and peak resistance (mixograph peak height) increased as protein content increased (while the glutenin to gliadin ratio remained constant). An increase in mixograph peak resistance is indicative of improvements in baking quality, most namely loaf volume (Lukow 1991). These researchers also observed that as grain protein increased the extensigraph measurements of maximum resistance to extension (measuring dough strength) and extensibility increased.

### **2.5.2. Impact of Sulphur on the Rheological Properties of Wheat Dough**

Some of the first research in western Canada provided evidence that S is an important contributor to dough quality. Yoshino and McCalla (1966) found that the potential disulphide

linkages in grain protein declined when the concentration of S in grain declined, leading to a reduction in the intrinsic viscosity of gluten dispersions. These researchers concluded that protein content (N concentration x 5.7) alone is an inadequate measure of wheat quality.

Deficiency in S-rich proteins reduces the capacity for forming intermolecular disulphide bonds and might directly weaken bonds of other types. This results in a reduction of dough extensibility and an increase in dough resistance to extension (strength), ultimately leading to tough dough that is not suitable for breadmaking purposes (Moss et al. 1981). In Australia, rising concentrations of S in grain/flour were associated with increased dough extensibility and decreased maximum dough resistance to stretching (dough strength) using the extensigraph (Moss et al. 1981, 1983, Wrigley et al. 1984). Furthermore, Moss et al. (1981) also noted a negative correlation between grain S concentration and mixograph development time indicating a reduction in dough strength with rising grain S concentrations. Sulphur fertilization also significantly reduced mixograph development time, while mixograph peak height was not influenced. In New Zealand, S fertilization and subsequent improvements in grain/flour S concentrations lead to a reduction in extensigraph dough resistance to extension and an increase in dough extensibility (Wooding et al. 2000). Finally, in the U.K., Zhao et al. (1999b) observed a strong positive correlation between grain S concentration and dough extensibility and a strong negative correlation between grain S concentration and dough resistance. Sulphur fertilization treatments also significantly reduced dough resistance to extension and increased dough extensibility (Zhao et al. 1999a, 1999b).

The detrimental changes in dough extensibility and resistance, when the S supply is inadequate, are attributable to the preferential synthesis of proteins that are low in cysteine and methionine (i.e.  $\omega$ -gliadins and HMW glutenin subunits). Deficiency in S-rich proteins (i.e.  $\alpha$ -

$\beta$ -, and  $\gamma$ -gliadins and albumins) reduces the capacity for forming intermolecular disulphide bonds and might indirectly weaken bonds of other types. This results in decreased dough extensibility and increased dough resistance to extension, leading to deterioration in baking quality (Fullington et al. 1987, MacRitchie and Gupta 1993, Moss et al. 1981).

### **2.5.3. Impact of N:S Ratio on the Rheological Properties of Wheat Dough**

The physical properties of dough may deteriorate with rising grain protein concentrations possibly due to the deterioration in protein quality with higher protein concentrations (Kosmolak and Crowle 1980). Wooding et al. (2000) found that the industrial and laboratory work requirements for dough development significantly increased when N fertilizer was applied without S fertilizer. However, with combined N and S fertilization, the work requirement remained close to levels for grain grown without fertilizer, indicating that a balance between N and S is very important. Wrigley et al. (1984) demonstrated that the ratio of N to S (N:S ratio or S:N ratio) in grain or flour is an important factor contributing to the rheological performance of dough. Flour that had a high S:N ratio of 0.08 (low N:S ratio) exhibited extensibility of 270 mm and resistance of 175 BU. Flour that had a low S:N ratio of 0.052 (high N:S ratio) exhibited extensibility of 156 mm and resistance of 365 BU.

### **2.6. Breadmaking Quality of Wheat**

Bread is one of the final products of wheat production. Consumers demand bread that is of high quality and attractive appearance. Bakers have some control over the properties of dough and can modify it in such a way as to achieve optimum results in the finished loaf of bread.

However, bakers cannot compensate for the quality of flour if it is limited by low levels of S or N arising during the primary phase of grain development in the field.

### **2.6.1. Impact of Nitrogen on the Breadmaking Quality of Wheat**

One important property that has been used to determine the suitability of a wheat variety for breadmaking is the relationship of loaf volume to grain protein content (grain N concentration). Early work by Finney and Barmore (1948) demonstrated that the major factor for variation in loaf volume within a variety was protein content. For a specific wheat variety, the relationship between protein content and loaf volume was positive and linear between the limits of protein encountered (8 - 18 %); however, different varieties had distinctly different regression lines. A number of more recent studies support these earlier findings that loaf volume improves as grain protein concentration increases (Ayoub et al. 1994, Dexter et al. 1994, Kosmolak and Crowle 1980, Paredes-Lopez et al. 1985, Pechanek et al. 1997, Tipples and Kilborn 1974).

### **2.6.2. Impact of Sulphur on the Breadmaking Quality of Wheat**

Early work conducted at the University of Alberta showed the positive impact of S on the quality of wheat and the bread produced from this wheat (Newton et al. 1959). In baking tests, the largest loaves of the best quality bread were obtained when wheat had been grown after legumes on plots which had received S fertilizer. The poorest bread was obtained from wheat grown on fallowed land or on plots receiving ineffective S fertilizer treatments. In this study, the S fertilization caused changes in the quality of protein in addition to changes in the quantity of protein.



Australian work conducted by Moss et al. (1981) also provided evidence that S contributes to the breadmaking quality of wheat. Grain S concentration was strongly and positively correlated with pup-loaf volume. In addition, loaf volume was significantly improved by the application of S fertilizer.

Field trials conducted in the U.K. prior to 1990 did not produce any clear response of breadmaking quality to S fertilizers (Salmon et al. 1990). However, there were subtle indications that S may have had beneficial effects on breadmaking quality when high levels of urea were applied to wheat. These researchers suggested that the form of S fertilizer used (Thiovit) and its lack of uptake by the plant, in combination with relatively high ambient S levels, reduced the breadmaking response to S fertilization. In work by Griffiths et al. (1990), late season applications of elemental S or ammonium sulphate caused only small changes in grain S concentrations of winter wheat and did not affect bread loaf volume.

The only pre-1990 study in the U.K. that demonstrated the impact of S on the breadmaking quality of wheat was a greenhouse study conducted by Byers et al. (1987). In this study, the application of S fertilizer to wheat grown in a sand culture increased the grain S concentration from 0.9 mg g<sup>-1</sup> to 1.9 mg g<sup>-1</sup>. This improvement in grain S concentration led to an increase in loaf volume from 475 ml to 1055 ml.

After 1990, field experiments in the U.K. began to demonstrate breadmaking improvements due to S fertilization because of the increased incidence of S deficiencies mainly as a consequence of massive reductions of atmospheric S inputs (Zhao et al. 1999c). For example, Zhao et al. (1999a, 1999b) found that breadmaking quality responses to S fertilization were more common than grain yield responses. In addition, correlation and regression analysis showed that loaf volume was more closely associated with grain S concentration than grain N

concentration. The beneficial effects of S on breadmaking quality were attributed to decreased dough strength (elasticity) and increased dough extensibility.

Other post-1990 European field studies also showed the strong relationship between S and breadmaking quality of wheat. Working with German wheat varieties, Haneklaus et al. (1992) observed a strong, positive correlation between grain S concentration and loaf volume. Schnug et al. (1993) found that 46 kg S fertilizer ha<sup>-1</sup> increased the grain S concentration by 0.23 mg g<sup>-1</sup>, resulting in a loaf volume improvement of 37 ml for a 100 g loaf. These researchers also noted a very strong and positive correlation between grain S concentration and loaf volume.

### **2.6.3. Impact of N:S Ratio on the Breadmaking Quality of Wheat**

Quality losses due to severe S deficiency are probably less frequent than quality losses due to excessive N fertilization, especially where soil S supplies are marginal (Randall and Wrigley 1986). Early work in Manitoba, demonstrated that the physical dough characteristics and baking quality of wheat deteriorated when the protein content of grain was extremely high (Bushuk et al. 1978, Tipples et al. 1977). In these early experiments, the reasons for these apparent contradictory observations between protein concentration and baking quality were not described.

More recently, baking tests by Timms et al. (1981) demonstrated that the breadmaking quality of wheat increased as flour protein content increased from the lowest to intermediate levels, but the flours of intermediate and high protein contents were equivalent in breadmaking quality. In reconstituted-dough baking tests (to compare gluten baking quality independently of protein quantity, loaves were baked from 'flours' reconstituted to equivalent protein levels using isolated gluten), grain from the intensively N fertilized plots (late season application) performed

poorer than grain from the less intensively fertilized plots. These results suggested that for wheat grown where high levels of N fertilizer are applied in the absence of S fertilizer, there may be a change in the balance between available N and S, such that the available S levels become insufficient for normal grain development. As a result, the protein quantity may not be affected, but protein quality may be adversely affected due to imbalances in the N to S ratio in grain.

Much work conducted in the Europe demonstrated that grain N:S ratio impacts the breadmaking quality of wheat. In the U.K., Byers et al. (1987) observed that loaf volume decreased from 1055 to 475 ml when the N:S ratio in grain increased from 16.6 to 29.1. Zhao et al. (1999a) demonstrated similar results when stepwise regression showed that grain N:S ratio was an important parameter affecting loaf volume, with higher N:S ratios indicating a shortage of S, leading to lower loaf volumes. In Germany, Schnug et al. (1993) found that 46 kg S fertilizer ha<sup>-1</sup> reduced the grain N:S ratio from 18.3 to 15.4 resulting in an improvement in loaf volume of approximately 40 ml for a 100 g loaf.

## **2.7. Grain Composition and S and N Fertilization**

Randall et al. (1981) concluded that grain from S-responsive plants could be distinguished from grain from unresponsive plants because the former had less than 0.12 % S and the N:S ratios were wider than 17:1. These thresholds have been generally accepted as critical values for predicting S deficiency for yield of Australian wheat varieties.

It is well established that the N concentration of grain responds to N fertilization (Ayoub et al. 1994, Byers and Bolton 1979, Kosmolak and Crowle 1980, Moss et al. 1981, 1983, Pechanek et al 1997, Randall et al. 1990). For this reason, western Canadian producers apply

substantial rates of N fertilizer to their wheat to increase the grain N content in order to receive price premiums for high quality wheat.

When the soil S supply is sufficient, application of N fertilizer may also increase the S concentration in grain (Byers and Bolton 1979, Moss et al. 1981, Randall et al. 1990). Furthermore, the S content of grain also responds to S fertilization. Byers and Bolton (1979) observed that S fertilization increased the grain S concentration from 0.09 to 0.21 %. Schnug et al. (1993) found that 46 kg S ha<sup>-1</sup> increased grain S concentration by 0.23 mg g<sup>-1</sup>. Zhao et al. (1999a) observed that applications of 20 and 100 kg S ha<sup>-1</sup> increased grain S concentration by 5 to 10 % and 18 to 19 %, respectively.

Nitrogen and S fertilization also affects the ratio of N to S in grain. As would be expected, when S is deficient, N fertilization tends to increase grain N:S ratios and, in some cases, cause an imbalance between N and S in the grain. Contrarily, application of S fertilizer generally causes the ratio of N to S in the grain to decline and become more balanced. For example, in a pot experiment, Byers and Bolton (1979) found that N fertilization increased the N:S ratio of grain from 14.6 to 38.1; however, when S fertilizer was applied in addition to the N, this ratio dropped back to 14.6.

## **2.8. Occurrence of Sulphur Deficiency**

Sulphur deficiencies are becoming widespread throughout North America and the rest of the world. Approximately 30 % (11.7 million ha) of the 36 million ha of cultivated land in the Prairie Provinces of Canada is either deficient in S or potentially deficient in S (Bettany et al. 1982). In western Canada, these S deficiencies tend to be the most frequent in the Gray Luvisolic and Eutric Brunisolic soil areas of the northern agricultural fringe areas (Beaton and

Soper 1986). Within the Gray Luvisolic soil zone, Bettany et al. (1982) estimate that approximately 70 % of soils are potentially S deficient. However, S deficiencies also extend into the Dark Gray and Black Chernozemic soil zones where the soils are low in organic matter, coarse-textured, well-drained, and intensively cropped (Bailey 1987). Bettany et al. (1982) estimate that 30 % of these soils are potentially deficient in S. In Manitoba, during the period between 1979 and 1991, an average of 14 % of fields sampled and analyzed received a recommendation for S fertilizer for the production of high S-requiring crops (McGill 1991).

The overall S fertility of the agricultural land of the Prairie Provinces is declining for a number of reasons. First, due to the widespread use of high analysis, S-free, N and P fertilizers, the incidental application of S has drastically declined (Tisdale et al. 1986, Zhao et al. 1999c). This is illustrated from 1965 to 1980, in which world N and P fertilizer use increased by 3.5 times and 2.5 times, respectively; however, during the same period, S application increased only marginally (Tisdale and Bixby 1982). A more recent study by Ceccotti and Messick (1994) further demonstrates that the worldwide consumption of N doubled between 1974 and 1990, whereas the total S consumption remained the same at about 10 million tonnes per year over the same period of time.

Second, due to many national and international regulations on atmospheric pollution, S inputs from the atmosphere in precipitation, as dry deposition, or by direct absorption at the soil's surface has declined (Zhao et al. 1999c). For example, between 1970 and 1993, the United States reduced their sulphur dioxide emissions by an estimated 30 % and emissions were expected to decline by 50 % of the 1980 level by the year 2000 (Ceccotti 1996). As a result, the Sulphur Institute in Washington estimates that the annual worldwide S fertilizer deficit will increase from the current 7.5 million tonnes to over 11 million tonnes by 2010 (Ceccotti, 1996).

Third, accelerated rates of crop uptake of S from the soil, coupled with little replacement of S in the form of fertilizer, is leading to the overall net loss of S from the soil (Bettany et al. 1982, Doyle and Cowell 1993). This is especially true for the more humid Black and Gray soil zones of the Prairies where crop yields are generally higher than for the drier Brown and Dark Brown soil zones. This, in addition to the increasing acreage of high S-using crops such as canola and legumes, has accelerated the removal of S from the soil.

Fourth, leaching of soil sulphate reserves from the rooting zone has been substantial. Due to annual cropping practices, leaching of soil gypsum ( $\text{CaSO}_4 \cdot \text{H}_2\text{O}$ ) and other forms of free sulphate from the rooting zone has occurred (Doyle and Cowell 1993). As early as 1928, Wyatt and Doughty (1928) observed that sulphate salts such as gypsum occurred frequently within the root zones of crops in the Brown and Dark Brown soil zones of Alberta; however, much less frequently in the Black and Gray soil zones. This pattern is in part due to the precipitation patterns experienced in the different soil zones with the Brown and Dark Brown soil zones being much drier and less conducive for leaching than the Black and Gray soil zones.

Finally, low rates of S replenishment from humus, coupled with S losses, have contributed to soil S deficits. This is especially true in the Gray Luvisolic soils of western Canada due to the wide C:S ratios (Bettany et al. 1973) and low concentrations of ester bonded sulphate (Bettany et al. 1973, Lowe 1965) in soil organic matter. Wide C:S ratios and low concentrations of ester bonded sulphate in soil organic matter reduce the potential for soils to mineralize soil organic S (Bettany et al. 1982, Doyle and Cowell 1993, Kowalenko and Lowe 1975a).

## **2.9. The Soil Sulphur Cycle and Soil Sulphur Pools**

Sulphur occurs in the soil in many different forms, both organic and inorganic. Due to the requirements of microorganisms, plants, and animals, S is continuously being cycled between organic and inorganic forms. From the standpoint of plant nutrition, inorganic sulphate ( $\text{SO}_4\text{-S}$ ) is the most important since it is the major form that is assimilated by plant roots.

### **2.9.1. Soil Inorganic Sulphur**

Sulphur can have an oxidation number from  $-2$  (sulphide) to  $+6$  (sulphate) (Eriksen et al. 1998). In most imperfectly to well drained, non-calcareous, agricultural soils, the dominant form of inorganic S is  $\text{SO}_4\text{-S}$  because sulphide and other reduced S forms are readily oxidized under aerobic conditions (Bohn et al. 1986). This  $\text{SO}_4\text{-S}$  may be dissolved in the soil solution as free  $\text{SO}_4^{2-}$  ions, adsorbed by soil colloids, occur as relatively insoluble salts such as gypsum or  $\text{MgSO}_4$  (Freney et al. 1962), or as a co-crystallized impurity in calcium carbonate (Williams et al. 1960, Williams and Steinbergs 1962). In most agricultural soils, often less than 10 % of the total soil S pool is in the soluble or adsorbed forms (Evans 1975).

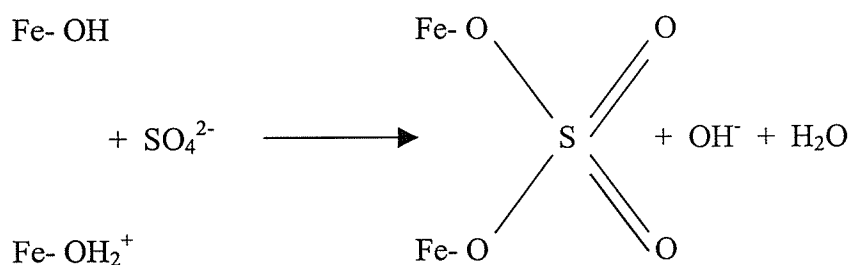
#### **2.9.1.1. Water-Soluble Sulphate**

The water-soluble  $\text{SO}_4\text{-S}$  fraction is available to plants (Bettany and Stewart 1982, Havlin et al. 1999) and is taken up in the soil solution via mass flow or diffusion. In humid areas, where soils are well drained, leaching removes  $\text{SO}_4\text{-S}$  from the surface horizons leaving small concentrations of water-soluble  $\text{SO}_4\text{-S}$  in the rooting zone. Under arid or poor drainage conditions, where leaching is not substantial, appreciable quantities of  $\text{SO}_4\text{-S}$  may be present in the surface horizons (Williams 1975).

Due to the high solubility of free  $\text{SO}_4^{2-}$  ions, large concentrations can move downwards with water and accumulate in the soil subsurface horizons (Curtin and Syers 1987). These subsoil  $\text{SO}_4\text{-S}$  reserves may provide crops with adequate supplies of S for maximum growth and production. Bole and Pittman (1984) observed that under field and controlled conditions, rapeseed and barley were both adequately supplied by subsoil reserves of  $\text{SO}_4\text{-S}$  from as deep as 54 to 72 cm in the soil profile. For this reason, soil-sampling depths of at least 60 cm should be used to make fertilizer recommendations.

### 2.9.1.2. Adsorbed Sulphate

The  $\text{SO}_4^{2-}$  adsorption capacity of soils can vary widely, depending on the inherent chemical nature of the soil. Sulphate ions are adsorbed by hydrous oxides of iron (Fe) and aluminum (Al) and by edges of clay particles (Parfitt 1978). Two mechanisms of adsorption have been proposed. First, hydrated  $\text{SO}_4^{2-}$  ions are adsorbed by purely electrostatic mechanisms, with sorption occurring in a plane distinct from the surface, but closer than the plane of sorption of nonspecifically sorbed ions such as chloride (Marsh et al. 1987). Second,  $\text{SO}_4^{2-}$  ions are chemisorbed by the formation of a binuclear bridge surface complex in which ligand displacement of  $-\text{OH}$  and  $-\text{OH}_2^+$  has taken place, as is depicted in the following scheme:



(Parfitt and Smart 1978)



There is no consensus as to which theory is more accurate. Therefore, a general definition of the mechanism of  $\text{SO}_4^{2-}$  adsorption, provided by Mott (1988), is as follows: “ $\text{SO}_4^{2-}$  ions may form ligand bonds, but may also form outer sphere complexes separated from the mineral surface by a water molecule.”

A number of soil factors influence the adsorption capacity of a specific soil. First, adsorption of  $\text{SO}_4^{2-}$  ions is affected by the nature of minerals in the soil. Soils containing large quantities of Al or Fe oxides tend to adsorb appreciable concentrations of  $\text{SO}_4\text{-S}$  (Chao et al. 1964).

Another important factor is soil pH, which affects the adsorption capacity of a soil by its effect on the net charge of Fe and Al oxides and clay edges (Eriksen et al. 1998). Bohn et al. (1986) reviewed the impact of pH on the surface charge of Fe and Al oxides and clay particles, such as kaolinite, and how this affects the adsorption of  $\text{SO}_4^{2-}$  ions. Under acidic conditions, the surface of these oxides and edges of these clays tend to gain protons resulting in the formation of a net positive charge. This net positive charge attracts the negatively charged  $\text{SO}_4^{2-}$  ions and results in the adsorption of these anions. The opposite is true under alkaline conditions where the oxides and clay tend to lose protons and become negatively charged. Under this situation, little or no  $\text{SO}_4^{2-}$  is adsorbed.

Other ions in the soil solution environment also affect the amount of  $\text{SO}_4^{2-}$  adsorbed due to competition for adsorption sites and differences in adsorptive strength between ions (Eriksen et al. 1998). The order of adsorption strength of anions in soil is: hydroxyl > phosphate > sulphate > nitrate = chloride (Reisenauer et al. 1973). For this reason, adsorbed  $\text{SO}_4^{2-}$  ions are readily displaced by phosphate and hydroxyl ions.

Adsorbed  $\text{SO}_4\text{-S}$  is plant available and, where large quantities of adsorbed  $\text{SO}_4\text{-S}$  are present, will contribute to plant nutrition (Williams and Steinbergs 1964). However, in Manitoba, adsorbed  $\text{SO}_4\text{-S}$  is not an important S fraction due to the neutral nature of the soils in this region (Anderson 1966). Furthermore, significant concentrations of adsorbed  $\text{SO}_4\text{-S}$  are only expected in soils rich in clay and Fe and Al oxides where soil pHs fall below 6 (Curtin and Syers 1990, Williams and Steinbergs 1962). Therefore, for most agricultural soils in western Canada, this fraction is probably very small and not an important source of S for plant growth.

### **2.9.1.3. Insoluble Sulphate**

Sulphate can be co-precipitated or co-crystallized as an impurity in calcium carbonates and is the most common form of “insoluble”  $\text{SO}_4\text{-S}$  (Williams and Steinbergs 1962). In calcareous soils of Australia, Williams and Steinbergs (1962) found that this fraction of inorganic S comprised a large part of the total S in soil, quite often accounting for 40 to 50 % of the total S in the subsoil horizons. Other forms of insoluble  $\text{SO}_4\text{-S}$  include barium and strontium sulphates as well as basic iron and aluminum sulphates (Williams 1975). Due to the insolubility of this S fraction, it is considered to be relatively unavailable to plants (Williams and Steinbergs 1964).

### **2.9.2. Soil Organic Sulphur**

The organic S pool, estimated as the difference between total S and inorganic  $\text{SO}_4\text{-S}$ , accounts for nearly all the S present in the surface horizons of most agricultural soils that are not gypsiferous (Freney et al 1962, Tabatabai 1982). For some Alberta soils, Lowe (1965) estimated that the organic S pool made up between 85 and 92 % of the total S pool in the soil. In some

non-gypsiferous Saskatchewan soils, Roberts and Bettany (1985) found that organic S accounted for an average of 93 % of total S in the Ap horizon, 92 % in the B horizon, and 62 % in the C horizon. Although this S pool is not readily plant-available, large fractions of this organic S may potentially be an important source for plant nutrition throughout the growing season through mineralization processes (Williams 1975).

Sulphur is closely associated with carbon (C) and nitrogen (N) in soil organic matter. Agricultural soils around the world have a mean C:N:S ratio of approximately 130:10:1.3 (Freney 1986); however, difference in C:N:S ratios exist between soils. For example, Bettany et al. (1973) noted that the C:S ratio in soil organic matter ranged from 58:1 in the Chernozemic Brown soils to 129:1 in the leached, Gray-wooded soils of the Prairies. These authors attributed the differences in C:S ratios to differences in temperature and moisture between the two soil zones. The wide C:S ratios of soil organic matter in the Gray-wooded soil zones are indicative of the low mineralization potential of those soils (Kowalenko and Lowe 1975a). Bailey (1985) also noted that the average N:S ratio of soil organic matter in Prairie Canadian soils to be 8.3:1. Differences in C:N:S ratios also arise due to differences in parent material (Williams et al. 1960), soil weathering (Walker and Adams 1959), and pasture improvement (Walker et al. 1959).

The organic S fraction is partitioned into two broad categories based on the chemical nature of each group: (i) the hydriodic acid reducible fraction (HI-S) and (ii) S which is directly bonded to C and is not reduced by hydriodic acid (Syers et al. 1987).

The HI-S fraction of organic S is composed mainly of ester sulphates (containing C-O-S linkages) and some ester sulphamates (containing C-N-S linkages) (Freney 1986). This fraction of organic S is the most labile and biologically active of the different organic S fractions (Biederbeck 1978, Lowe 1965). Hydriodic acid reduces this organic S to H<sub>2</sub>S, but will not

reduce any S that is directly bonded to C (Johnson and Nishita 1952). HI-S accounts for 30 to 70 % of organic S in soils (Tabatabai and Bremner 1972); however, the proportion of HI-S in soil organic matter tends to decline from the Chernozemic soils to the Gray-wooded soils of the Prairies. For example, Bettany et al. (1973) noted that the percentage of total S as HI-S was approximately 50 % in the Chernozemic soils in contrast to 36 % in the Gray-wooded soils. Lowe (1965) also found that the HI-S fraction was substantially higher in the Chernozemic soils (63 %) than the Gray-wooded soils (33 %). Since the HI-S fraction is considered to be the most labile organic S fraction (Biederbeck 1978, Lowe 1965), the mineralization potential of organic S declines from the Chernozemic soil zone to the Gray-wooded soil zone (Bettany et al. 1982).

Organic S which is not reduced to H<sub>2</sub>S by hydriodic acid is believed to be present in organic compounds where S is directly bonded to C. This fraction represents the difference between total organic S and that reduced to H<sub>2</sub>S by hydriodic acid and includes the S containing amino acids (e.g. cysteine and methionine), mercaptans, disulphides, sulphones, and sulphonic acids (Freney 1986).

This C-bonded organic S group can be further divided into Raney-Ni reducible S and non-reducible S (Freney 1986). Initially, it was proposed that Raney Ni could be used to measure C-bonded S (Lowe and DeLong 1963). However, the amount of S reduced by Raney Ni does not account for the difference between total organic S and HI reducible S (Freney 1986). In some soils, however, there is a good relationship between Raney Ni reducible S and the amino acid content of the soil and it may be possible to use this as an estimate of the concentration of amino acid S in the soil (Scott et al. 1981). There is also evidence that some of the C-bonded S may be inaccessible to S-reducing agents and may be in the form of aliphatic sulphonic acids or sulphones (Freney 1986).

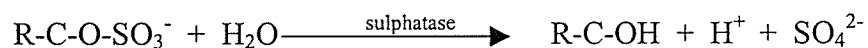
## 2.10. Mineralization and Immobilization of Soil Sulphur

Mineralization and immobilization are important for plant nutrition under agricultural conditions because  $\text{SO}_4\text{-S}$  is the only form of soil S that can be assimilated by plant roots (Bettany and Stewart 1982). However, the majority of S in the surface horizons of soils is often in the organic form and needs to be transformed to  $\text{SO}_4\text{-S}$  to become available (Freney et al. 1962, Lowe 1965, Roberts and Bettany 1985, Tabatabai 1982). Under natural conditions, mineralization and immobilization occur concurrently and it is the net change which ultimately determines how much S becomes available for plant uptake.

### 2.10.1. Mineralization

The processes and mechanisms of S mineralization are still poorly understood (Bettany and Stewart 1982, Syers et al. 1987). Bettany and Stewart (1982) proposed that the process of mineralization involves two mechanisms: (i) biochemical mineralization and (ii) biological mineralization.

The biochemical mineralization process probably involves the HI-S fraction of organic S because this fraction is the most labile and biologically active (Biederbeck 1978). This mineralization process relies upon the activity of the sulphatase (sulphohydrolase) enzyme causing the hydrolysis of HI-S to  $\text{SO}_4\text{-S}$  as follows:



(Freney 1986)

The sulphatase activity may be end-product limited, meaning that the amount of S biochemically mineralized may depend on the  $\text{SO}_4\text{-S}$  levels of the soil (Freney 1986). For

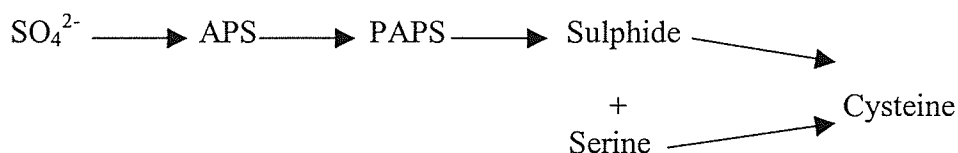
example, when the  $\text{SO}_4\text{-S}$  concentration in the soil is high, the rate of biochemical S mineralization may slow or stop.

The biological process of mineralization probably occurs as a result of the microbiological population utilizing carbonaceous residues as sources of energy, releasing inorganic  $\text{SO}_4\text{-S}$  as a byproduct. This release of  $\text{SO}_4\text{-S}$  is not specifically in response to a need for N or S by the microorganisms (Bettany and Stewart 1982).

Due to the microbiological nature of S mineralization, the rate of mineralization is influenced by the same factors that generally affect the activity and growth of microorganisms in the soil (Syers et al. 1987). These include temperature (Tabatabai and Al-Khafijii 1980), soil pH (Williams 1967), soil moisture content (Williams 1967), the presence of plant roots (Freney and Spencer 1960), and drying and heating processes (Kowalenko and Lowe 1975b).

### **2.10.2. Immobilization**

Immobilization of  $\text{SO}_4\text{-S}$  is a biological process that is performed by microorganisms and plants (Bettany and Stewart 1982). It is a two-step process that utilizes the  $\text{SO}_4^{2-}$  ion, leading to the production of the energy-rich sulphate nucleotides: APS (adenosine 5'-sulphato-phosphate) and PAPS (adenosine 3'-phosphate, 5'sulphato-phosphate) (Bettany and Stewart 1982, Syers et al. 1987). Sulphur containing amino acids (cysteine and methionine) are then formed through the synthesis of these nucleotides (Bettany and Stewart 1982). The theoretical process by which this happens probably begins with the reduction of PAPS to sulphide, which then combines with the amino acid, serine, to form cysteine (Syers et al. 1987). The entire process is summarized as follows:



(Syers et al. 1987)

The immobilization of  $\text{SO}_4\text{-S}$  to organic S can be quite rapid. Laboratory studies with Australian soils showed that 50 % of added labeled  $\text{SO}_4\text{-S}$  was incorporated into organic forms within 168 days (Wainwright et al. 1986). Similarly, research in New Zealand demonstrated that 40 % of applied gypsum was converted to organic S in only six weeks (Walker and Gregg, 1975).

### 2.11. Prediction of S Deficiency using Soil Tests

Soil testing is an approach widely used for the identification of S deficient soils. A number of soil testing methods have been proposed or used to predict S deficiencies. Many of these soil test methods for S have correlated well with plant S uptake and plant yield in greenhouse and pot studies (Anderson 1966, Hue et al. 1984, Scott 1981, Zhao and McGrath 1994). However, the ability of soil tests to predict S responses in the field, under natural conditions, has not been as successful (Jones 1986).

Measuring the water-soluble  $\text{SO}_4\text{-S}$  fraction is a widely used method of evaluating the S status of soils (Tabatabai 1982). This water-soluble  $\text{SO}_4\text{-S}$  is extractable with water, alone; however, water deflocculates the soil, making the extracts difficult to filter and leaving the extracts too turbid to analyze (Tabatabai 1982). Dilute salt solutions containing calcium chloride (e.g. 0.15 %  $\text{CaCl}_2$ ) (Williams and Steinbergs 1959) are sufficient to keep the soil particles flocculated. In addition, the  $\text{Cl}^-$  ions do not displace adsorbed  $\text{SO}_4^{2-}$  ions on the exchange (Arkley 1961).

There is conflicting evidence of the value of measuring the concentration of water-soluble  $\text{SO}_4\text{-S}$  in the soil as an indicator of the S status of soils. Anderson (1966), in greenhouse studies, demonstrated that the  $\text{SO}_4\text{-S}$  released by the mineralization of organic S was an important contributor to the nutrition of a crop. His studies also demonstrated that adsorbed  $\text{SO}_4\text{-S}$  was not important in Prairie soils and that soils with low concentrations of water-soluble  $\text{SO}_4\text{-S}$  were unlikely to mineralize appreciable quantities of S. Therefore, he observed a strong correlation ( $r = 0.75$ ) between plant uptake of S and water soluble  $\text{SO}_4\text{-S}$  in soil and concluded that the measurement of water soluble  $\text{SO}_4\text{-S}$  was a satisfactory measure of the S supply of the soil. Furthermore, Anderson (1966) also concluded that, due to the relatively modest requirements of cereals for S, approximately  $11 \text{ kg S ha}^{-1}$  in the top two feet of soil would be adequate for cereal crops. In a field experiment in Saskatchewan, Hamm (1969) also concluded that a critical level of approximately  $11 \text{ kg S ha}^{-1}$  to the two-foot soil depth at seeding was sufficient for cereals. Furthermore, this researcher also suggested that the critical maximum ratio of N to S in soil was 16:1 for barley and 12:1 for rape. Hamm et al. (1973) recommended that the total amount of  $\text{SO}_4\text{-S}$  extractable from the 0 to 60 cm soil layer by 0.01 M  $\text{CaCl}_2$  be used for diagnosis of S deficiency. In Alberta, Walker and Doornenbal (1972) found that S deficiencies for legumes were predicted with 86 % accuracy by measuring the water-extractable  $\text{SO}_4\text{-S}$  in the 0 to 30 cm soil layer. However, in Australia, Williams and Steinbergs (1959), using a pot experiment, found that the amount of free, water-soluble  $\text{SO}_4\text{-S}$  was an unsatisfactory index for predicting the yield of oats.

It is difficult to satisfactorily predict crop yield and plant uptake with water-soluble  $\text{SO}_4\text{-S}$  in the field due to the different rates of net mineralization of S during the growing season, the heterogeneous spatial distribution of soil  $\text{SO}_4\text{-S}$ , and unequal plant root distribution (Bailey



1987). Concentrations of soil  $\text{SO}_4\text{-S}$  also fluctuate temporally due to changes in the balance between inputs of S from the atmosphere and fertilizer additions and losses due to leaching, plant uptake,  $\text{SO}_4\text{-S}$  adsorption, and microbial immobilization (Eriksen et al. 1998, Tabatabai 1982). Therefore, the amount of water-soluble  $\text{SO}_4\text{-S}$  measured at a single point in time may not provide a realistic indication of the S made available to a crop throughout the growing season (Curtin and Syers 1990).

Due to the difficulties and limitations of water-soluble  $\text{SO}_4\text{-S}$  as an index of S availability, Bailey (1985, 1987) suggested that the ratio of total N to total S in soil may serve as a useful indicator of expected responses to S fertilizer since it may be a more stable and predictable value than soil  $\text{SO}_4\text{-S}$  alone. Janzen and Bettany (1984), working in a growth chamber with rape and soils from Saskatchewan, reported that the ratio of available N to available S in soil may be a good predictor of crop response to S fertilization. In this study, maximum assimilation of both N and S into the canola seed occurred when the ratio of (soil  $\text{NO}_3\text{-N}$  + fertilizer N):(soil  $\text{SO}_4\text{-S}$  + fertilizer S) was approximately 7. However, caution must be used when using N:S ratio as a predictive measure because the same N:S ratio can be obtained at totally different concentration levels of each nutrient in the soil; therefore, surplus of one nutrient may falsely indicate a deficiency of the other nutrient. Furthermore, as Bailey (1987) pointed out, the use of the ratio to estimate the optimum S fertilizer rate depends on accurate knowledge of the availability of soil N and S and on the quantity of fertilizer N and S that is available to the crop.

For soils containing large concentrations of adsorbed  $\text{SO}_4\text{-S}$ , dilute salt solutions containing  $\text{CaCl}_2$  would not be appropriate (Curtin and Syers 1987). Therefore, extractants used to determine the concentration of water-soluble  $\text{SO}_4\text{-S}$  plus adsorbed  $\text{SO}_4\text{-S}$  usually contain

phosphate or hydroxyl ions (Syers et al. 1987). Fox et al. (1964) concluded that a 0.01 M  $\text{Ca}(\text{H}_2\text{PO}_4)$  extract contains sufficient phosphate to displace most adsorbed  $\text{SO}_4\text{-S}$  from soils and provides a good means of estimating the adsorbed  $\text{SO}_4\text{-S}$  concentration of a soil. Hue et al. (1984), in a greenhouse experiment, found that both wheat yield and S uptake were highly correlated with  $\text{Ca}(\text{H}_2\text{PO}_4)$ -extractable  $\text{SO}_4\text{-S}$  containing both adsorbed and water-soluble  $\text{SO}_4\text{-S}$ . In a pot experiment, Scott (1981) found that  $\text{KH}_2\text{PO}_4$ -extractable  $\text{SO}_4\text{-S}$  correlated strongly with oat yield and S uptake. However, as previously mentioned, the adsorbed  $\text{SO}_4\text{-S}$  fraction is insignificant in Manitoba (Anderson 1966) and in most of western Canada, where soil pHs are relatively high.

Sulphate that is co-precipitated with calcium carbonates can be extracted with hydrochloric acid (Williams and Steinbergs 1962); however, the presence of barium in the calcium carbonate can lead to errors resulting in an underestimate of this fraction of S (Williams et al. 1960). The grinding process during soil preparation for analysis can also expose this fraction to chemical extraction (Havlin et al. 1999). As a result, more S may be classified as being available by a  $\text{SO}_4\text{-S}$  soil test analysis than what may actually be available under field conditions. Due to the relative inaccessibility of this S fraction for plant uptake (Williams and Steinbergs 1964), it is not usually quantified using a soil test.

Even though a number of tests are available for estimating the S status of a soil, most provide variable results and are not very reliable under field conditions. Therefore, Zhao et al. (1999c) appropriately concluded that:

Soil testing is only reliable in predicting non-responsive soils where they have high amounts of available S. For the majority of arable soils, which contain low to medium amounts of available S, soil test results are not reliable for the prediction of S responsive sites.

## 2.12. Prediction of S Deficiency using Plant Analysis

As mentioned previously, soil testing for S status has not been very successful due to the spatial and temporal variability of  $\text{SO}_4\text{-S}$  in the soil (Freney et al. 1982). Therefore, visual deficiency symptoms and plant tissue analysis have been used to diagnose the S status of plants.

Visual deficiency symptoms can be valuable in diagnosing S deficiency because in early growth stages of cereals, S-deficient plants usually remain smaller and show a lighter colour than those without symptoms. In addition, light green stripes quite often form along the margins of veins in the leaves of cereals (Voss 1993). However, a major obstacle of using visual symptoms to diagnose S deficiency is that S deficiency symptoms are often confused with deficiencies of other nutrients, most commonly, nitrogen deficiency symptoms (Schnug and Haneklaus 1998). In addition, the visual symptoms of S-deficient cereal species are much less specific than those in S-deficient dicotyledonous plants, such as canola. Two further problems of using visual diagnosis are first, that only severe deficiencies of S usually cause visual symptoms in cereals and second, that quite often the yield potential of a crop has probably already deteriorated by the time the S deficiency is corrected (Schnug and Haneklaus 1998).

Plant tissue analysis via a chemical test is another proposed means of diagnosing S deficiency in plants and is often more reliable than visual diagnosis (Zhao et al. 1999c). Several diagnostic indices have been proposed. One promising method is the determination of the proportion of total S as sulphate-S in the plant as proposed by Smith and Dolby (1977). A number of researchers have found that this index is very strongly correlated to grain yield for wheat in the field (Spencer and Freney 1980) and total plant yield in the greenhouse (Freney et al. 1978). In these experiments, this index provided a useful means of predicting S deficiency and the need for S fertilization to ensure that the maximum yield was achieved. Spencer and

Freney (1980) also noted that this index was the least affected by the age of plant or the stage of growth of the plant. However, in later work, Scaife and Burns (1986) concluded that the sulphate / total S index has two fundamental disadvantages as compared to using sulphate-S or total S alone as indices. First, the numerator (sulphate-S) is the major variable in the denominator, so the ratio is likely to be less sensitive than either of the measurements alone. For example, since both sulphate-S and total S increase with increasing S supply, dividing one by the other would likely produce an index which is less sensitive than either alone. The second disadvantage of this ratio as a means of predicting the S status of a plant is that it requires twice as much analytical work as either measurement alone. As a result, these researchers concluded that this ratio is not reliable and that tissue sulphate-S alone is the most satisfactory index.

Total S is another proposed means of diagnosing S deficiency in plant tissue. In a pot experiment, Zhao et al. (1996) found that the total S concentration of the uppermost leaf at stem elongation was a good indicator of S deficiency in wheat because it was closely related to relative dry matter yield at the stem elongation (Zadoks GS 37). Spencer and Freney (1980), in a field study, found total S concentration of whole plants to be correlated with final grain yield for wheat. However, Spencer and Freney (1980) also noted that the critical total S value for wheat yield was strongly influenced by growth stage and varied considerably with time of sampling. Despite this shortcoming, if a midseason growth stage and plant part could be used to accurately predict the S status of wheat at maturity, such a test would be a valuable tool for predicting S deficiency and the need for correction.

The use of N:S ratio in plant tissue has also been examined for its value in predicting the S status of plants. The idea of using N:S ratio is based on the fact that plants require S, like N, for the production of amino acids in the grain (Schnug and Haneklaus 1998). Spencer and

Freney (1980) found that the ratio of total N to total S in total above ground plant tissue provided a good indication of the S status in relation to grain yield for wheat. However, the major drawback of using the N:S ratio to measure the S status of a plant is that the same N:S ratio can be obtained at totally different overall concentrations of both nutrients in the tissue (Schnug and Haneklaus 1998). Therefore, a deficiency in both nutrients may falsely indicate apparent sufficiency for S, or a surplus of one nutrient may falsely indicate a deficiency of the other (Finck 1970). In addition, Spencer and Freney (1980) noted that the N:S ratio of above ground plant matter was affected by the age of plant at sampling.

A number of critical thresholds have been proposed for wheat tissue and predicting S deficiencies for grain yield. For field grown wheat, Spencer and Freney (1980) obtained critical values in whole plant shoots (at stem elongation) of  $1.5 \text{ mg g}^{-1}$  for total S, 11 % for percent of total S as sulphate-S, and 19:1 for N:S ratio. Westfall et al. (1990) found critical values of total S in whole plant of wheat to be  $2.2 \text{ mg g}^{-1}$  at plant tillering,  $1.9 \text{ mg g}^{-1}$  at stem elongation, and  $1.5 \text{ mg g}^{-1}$  at booting. These researchers also noted that a higher critical value of  $1.9 \text{ mg g}^{-1}$  value needs to be used if only the flag leaf is analyzed at booting.

In general, there is no consensus as to which method is the best to evaluate the S status of wheat and which threshold best indicates S deficiency. However, reliable diagnosis of S deficiency tends to be most accurate towards the end or at the end of vegetative growth (Zhao et al 1999c). Sampling earlier usually gives less accurate predictive value. This is problematic because S deficiencies need to be corrected prior to the end of the vegetative state to ensure that yield loss does not occur. For example, Zhao et al. (1999c) note the work of Haneklaus et al. (1995), who showed that wheat required correction of S deficiency prior to or at the second node

stage to fully recover and produce maximum yields. More work is required to develop a practical and quick tool that will accurately diagnose S deficiency in wheat and other crops.

### **2.13. Occurrence of Nitrogen Deficiency**

Nitrogen is frequently one of the most limiting nutrients in crop production. During the early part of the 20<sup>th</sup> century, agricultural soils across the Prairie Provinces (excluding the Gray-wooded soil zone) contained abundant quantities of plant-available N, supplied by mineralization of the large concentrations of soil organic matter (Cowell and Doyle 1993). Therefore, very few N deficiencies were observed and N fertilizer was seldom required for crop production.

During the latter half of the 20<sup>th</sup> century, economic responses to N fertilizer became common. This was due to the depletion of soil organic N reserves coupled with very few additions of N in organic and inorganic forms (Cowell and Doyle 1993). Over the past five decades, N has become deficient in many western Canadian soils and fertilizer N has become an integral tool for crop production. This is especially true for the production of CWRS wheat, which is renowned for its high quality.

Results from a number of research trials conducted in Saskatchewan prior to the 1970s demonstrated that the application of N fertilizer improved wheat yields by between 8 and 76 %, with an average improvement of 24 % (Warder and Ferguson 1954-1964, University of Saskatchewan 1965-1969). These yield responses were observed on both stubble and fallow land, indicating that the N release during summer fallow had diminished. Further experimentation in Saskatchewan after 1970 showed yield responses to N fertilization of between 21 and 53 %, with an average improvement of 35 % (Ukrainetz 1991, Innovative Acres 1981-1988, University of Saskatchewan 1974-1978).

## **2.14. The Nitrogen Cycle and Soil Nitrogen Pools**

Nitrogen is found in numerous forms in agricultural soils. There are three important soil N pools that contribute to plant nutrition. The largest pool that accounts for more than 90 % of the total N in surface soils is the organic N pool (Havlin et al. 1999, Scherer 1993). However, this organic fraction is relatively inaccessible to plants and is made available only when converted to inorganic N via mineralization processes. From the viewpoint of plant nutrition, the most important pools of N are nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) (Young and Aldag 1982). The latter fraction is found in the exchangeable and non-exchangeable (fixed) forms.

### **2.14.1. Water-Soluble Nitrate**

Nitrate is an inorganic form of N that is an important contributor to plant nutrition. The concentration of water-soluble  $\text{NO}_3\text{-N}$  available to plants depends on the amount applied in inorganic fertilizers or organic amendments (i.e. manure), the amount mineralized from soil organic matter and the amount lost through denitrification, leaching, and immobilization.

### **2.14.2. Exchangeable Ammonium**

Due to its cationic nature,  $\text{NH}_4^+$  is often adsorbed and retained on the surface of negatively charged soil colloidal material (Nommik and Vahtras 1982) through electrostatic attraction between the cation and negatively charged soil particles. This form of adsorption effectively protects  $\text{NH}_4^+$  from losses due to leaching but maintains its availability to plants and the microbial population (Nommik and Vahtras 1982).

### 2.14.3. Non-exchangeable Ammonium

Non-exchangeable or fixed  $\text{NH}_4^+$  is defined as adsorbed  $\text{NH}_4^+$  that is relatively unexchangeable by the usual methods of cation exchange (Osborne 1976). Ammonium ions are fixed when they penetrate and saturate the interlayers of 2:1 minerals in such a manner that they cause the crystal lattice structure to collapse. When this collapse takes place, the  $\text{NH}_4^+$  ions become trapped between the silicate sheets and are excluded from exchange reactions. More in-depth reviews of  $\text{NH}_4^+$  fixation are provided by Nommik and Vahtras (1982) and Scherer (1993).

Substantial concentrations of fixed native  $\text{NH}_4^+$  are found in many Canadian soils (Havlin et al. 1999). For five Saskatchewan soils studied by Hinman (1964), the total amount of fixed  $\text{NH}_4^+$  in 4-foot profiles ranged from 2600 to 4600 lbs acre<sup>-1</sup> and ranged from 7 % of the total N in surface soil to as much as 58 % in soils at the 4-foot depth.

Recently fixed  $\text{NH}_4^+$  plays an important role in plant nutrition. Kowalenko and Cameron (1978) found that 71 to 96 % of recently fixed fertilizer  $\text{NH}_4^+$  was made available to barley plants. Black and Waring (1972), in a pot experiment, reported that approximately 50 % of the  $\text{NH}_4^+$  fixed after fertilization was mobilized from the first crop, while successive cropping removed almost all of the recently fixed  $\text{NH}_4^+$ .

### 2.14.4. Organic Nitrogen

Over 90 % of N in the surface layer of most agricultural soils is usually in the organic form (Havlin et al 1999, Scherer 1993). From a soil fertility standpoint, this large pool is very important. Even though this pool is not directly accessible to plants over the short term, the process of mineralization transforms this pool into a source of nutrients for a growing crop over the long term.



Nitrogen mineralization is a two-step process (Havlin et al. 1999). The first step, called aminization, is the breakdown of proteins into amino acids, amines, and urea ( $\text{NH}_2$ ) by heterotrophic bacteria and fungi. The second step, called ammonification, is the production of  $\text{NH}_3$  and  $\text{NH}_4^+$  from amines and amino acids by other heterotrophic microorganisms.

### **2.15. Prediction of N Deficiency using Soil Tests**

Water alone, will extract  $\text{NO}_3\text{-N}$  quantitatively from most soils. The disadvantage of water as an extractant is that it deflocculates that soil, making the extracts difficult to filter and leaving them too turbid to analyze (Tabatabai 1982). To overcome this, most extraction methods utilize a salt solution containing  $\text{CaSO}_4$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{KCl}$ , or  $\text{CaCl}_2$  (Maynard and Kalra 1993), with  $\text{CaCl}_2$  being the most popular in western Canada.

The determination of the  $\text{NO}_3\text{-N}$  concentration of the soil profile has become the standard procedure for estimating the amount of plant available soil N in western Canada and the spring wheat producing areas of the U.S.A. However, initial investigations in western Canada demonstrated that the accuracy of this method to predict crop responses to N fertilizer was poor (Cook et al. 1957, Synghal et al. 1958). This lack of success was probably due to the measurement of  $\text{NO}_3\text{-N}$  on surface samples to a depth of 15 cm only, not accounting for available  $\text{NO}_3\text{-N}$  deeper in the soil profile. In later publications,  $\text{NO}_3\text{-N}$  levels measured to a soil depth of 60 cm correlated closely with N uptake by barley (Soper et al. 1971) and % yield of rapeseed (Soper 1971). Furthermore, Dahnke and Vasey (1973) concluded that predicting N fertilizer requirements of a crop using soil  $\text{NO}_3\text{-N}$  levels was suitable for soils where percolation of water below the typical rooting depth is minimal. The relatively low levels of precipitation in the Prairies, coupled with frozen soil conditions in the winter, make the measurement of water-

soluble  $\text{NO}_3\text{-N}$  a valuable tool for measuring the N status of the soil. However, in wet years where losses of N (due to leaching, denitrification, etc.) may be substantial, or in soils that have a high capacity to mineralize organic N, the measurement of water-soluble  $\text{NO}_3\text{-N}$  may not provide a good index of available N or N that will be made available throughout the growing season.

Numerous methods have been suggested for the extraction of exchangeable  $\text{NH}_4^+$  (Maynard and Kalra 1993). The most common of these methods is an extraction by shaking a soil sample with 2.0 M KCl solution (Maynard and Kalra 1993). The  $\text{K}^+$  ion in this extraction solution displaces the  $\text{NH}_4^+$  ion on the exchange, bringing the  $\text{NH}_4^+$  ion into solution, making it extractable upon filtration.

A soil test that will accurately predict soil N mineralized during the growing season is urgently needed (Campbell et al. 1993). The water-soluble  $\text{NO}_3\text{-N}$  test that is generally used only indicates available N at the time of soil sampling. This test does not provide a reliable index of the ability of the soil to mineralize N (Campbell 1978). Two promising methods of extraction to measure potentially mineralizable N have been developed. The first method is the determination of  $\text{NH}_4^+$  produced when the soil is digested with 2.0 M KCl at  $100^\circ\text{C}$  for four hours (Gianello and Bremner 1986a). The second measures  $\text{NH}_4^+$  produced by steam distillation with pH 11.2 phosphate-borate buffer solution for eight minutes (Gianello and Bremner 1986b). In the work of Gianello and Bremner (1986b), 12 chemical extraction methods were studied for their ability to estimate available N in soils. The hot KCl and phosphate-borate methods yielded the highest correlation coefficients with the biological indices (N mineralized during an incubation period) used. Jalil et al. (1996) also found strong correlation coefficients between these two chemical extractants and N mineralized in a 24-week incubation period. These

researchers concluded that both chemical extractants may provide a quick, easy test for assessing the N-supplying capacity of the soil.

## **2.16. Research Needs**

At present, information regarding the impact of S on CWRS wheat grown under western Canadian conditions is limited. Information in the literature is based on research conducted many years ago in western Canada and more recent studies in Australia, New Zealand, the U.K., and Germany. Due to differences in wheat varieties, soils, and regional climatic and growing season differences, research conducted in Europe, New Zealand, and Australia may not be applicable to the western Canadian wheat varieties grown under Prairie growing conditions. Very little is known about the impact of S fertilization on wheat quality where soil test S concentrations are marginally sufficient for grain yield. Thus, field research in western Canada is required to determine if there is a wheat quality response to S fertilization under conditions where yield responses to S fertilizer are not observed or expected. Furthermore, additional research is required to evaluate and develop practical soil and plant tissue testing tools that would help to predict yield and quality responses to S fertilizer.

### 3. IMPACT OF GRAIN SULPHUR NUTRITION ON YIELD AND BREADMAKING QUALITY OF CANADA WESTERN RED SPRING WHEAT IN WESTERN CANADA

#### 3.1. Abstract

Canada Western Red Spring (CWRS) wheat (*Triticum aestivum* L. cv. AC Barrie) was grown at twelve different locations over two seasons in western Canada. Treatments consisted of two rates of fertilizer S (0 and 20 kg ha<sup>-1</sup>) as ammonium sulphate and two rates of fertilizer N (26 and 100 kg ha<sup>-1</sup>) as urea in a factorial design. Analysis of grain samples for total S, N, and N:S ratio accurately predicted the concentration of S, N, and N:S ratio in flour. Grain S concentration and N:S ratio were weakly correlated with both absolute and relative grain yield. Of the three measurements of N and S content, grain S concentration was most strongly and positively correlated with loaf height, loaf volume, and oven spring; grain N:S ratio was negatively, but more weakly, correlated with these baking parameters.

The improvements in baking quality with rising grain S concentrations and declining grain N:S ratios were accompanied by an increase in dough extensibility and a reduction in dough strength. Grain S concentration was positively correlated with dough extensibility and negatively correlated with  $R_{\max}$ , mixograph peak time, and work input to peak. Grain N:S ratio was negatively correlated with dough extensibility and positively correlated with  $R_{\max}$  and work input to peak.

The improvements in dough quality with rising grain S concentrations and declining grain N:S ratios were associated with an increase in the proportion of soluble glutenin in flour and a reduction in the ratio of insoluble to soluble glutenin in flour. Grain S concentration was positively correlated with the proportion of soluble glutenin and negatively correlated with the ratio of insoluble to soluble glutenin in flour. Grain N:S ratio was negatively correlated with the proportion of soluble glutenin and positively correlated with the ratio of insoluble to soluble glutenin in flour. These correlations indicated that the improvements in dough quality due to improved S nutrition were probably due to the enhanced concentration of gluten proteins rich in cysteine, improving the dough's capacity to form intermolecular disulphide and other types of bonds.

Our study, therefore, has confirmed that the S nutrition of grain, as measured by total grain S concentration and N:S ratio, is an important factor for determining the quality of CWRS wheat in western Canada.

### **3.2. Introduction**

Sulphur (S) is an essential nutrient for all living organisms. The needs of higher plants for S have been recognized for over two centuries (Duke and Reisenauer 1986) and S plays an important role in wheat yield (Beaton and Soper 1986, Rasmussen and Kresge 1986, Tisdale et al. 1986) and quality (Randall and Wrigley 1986). However, western Canadian interest and research in S nutrition of wheat has lagged behind other nutrients because S deficiencies are not as extensive as deficiencies of nitrogen, phosphorus, or potassium.

For wheat, deficiency of S can result in reduced yields. Yield responses to S fertilization have been observed in western Canada since the mid point of the 20<sup>th</sup> century. Doyle and Cowell (1993) provide an excellent review of western Canadian wheat yield responses to S fertilization. In Australia, Randall et al. (1981) found a strong correlation between grain yield and grain S concentration. In a field experiment, these researchers also found that yield data for the high rates of N fertilization indicated critical thresholds of approximately 0.12 % S and an N:S ratio of 17:1 in the grain. Grain with S concentrations below 0.12 % and N:S ratios greater than 17:1 was regarded to be deficient in S for yield.

Sulphur is important for the formation of protein in wheat grain because it is an essential component of amino acids such as cysteine and methionine (Shewry 1995, Shewry and Mifflin 1985). Disulphide bonds (S-S) that form between sulphhydryl (-SH) groups of cysteine residues play a key role in determining the structure and properties of wheat proteins (Shewry and Tatham 1997). Sulphur deficiency in wheat grain results in increased synthesis of S-poor proteins ( $\omega$ -gliadins and high molecular weight (HMW) subunits of glutenin) at the expense of S-rich proteins ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins and low molecular weight (LMW) subunits of glutenin) (Castle and Randall 1987, Fullington et al. 1987, MacRitchie and Gupta 1993, Moss et al. 1981, Wrigley et al. 1980, 1984). Deficiency in S-rich proteins reduces the capacity for forming intermolecular disulphide bonds and might directly weaken bonds of other types (Moss et al. 1981). These compositional changes in protein, due to S deficiency, are associated with a decrease in dough extensibility and an increase in dough elasticity (strength) (Moss et al. 1981, 1983, Wooding et al. 2000, Wrigley et al. 1984, Zhao et al. 1999a, 1999b).

The compositional changes in wheat protein accompanied by the deterioration of dough extensibility and elasticity associated with S deficiencies causes the baking quality of wheat to

deteriorate. A number of European and Australian studies have demonstrated strong, positive correlations between grain S concentration and loaf volume (Haneklaus et al. 1992, Moss et al. 1981, Schnug et al. 1993, Zhao et al. 1999a, 1999b). In addition, in the work of Zhao et al. (1999a), loaf volume was correlated more closely with grain S concentration than with grain N concentration.

Rising concentrations of N in grain occasionally causes the deterioration of dough physical properties, possibly due to the deterioration in protein quality (Kosmolak and Crowle 1980). There is evidence that imbalances of N to S in the grain, due to severe S deficiencies or high application rates of N fertilizer, are associated with a reduction in dough extensibility and increase in dough resistance (Wrigley et al. 1984). Wooding et al. (2000) also demonstrated that laboratory and industrial optimum mechanical dough development work input increased when N fertilizer was applied to wheat without S fertilizer; however, with combined N and S fertilization, the work input remained close to levels for grain grown without fertilizer.

Breadmaking quality losses due to severe S deficiencies is probably less frequent than quality changes due to excessive N fertilization, especially where soil S supplies are marginal (Randall and Wrigley 1986). Early work in Manitoba, demonstrated that the physical dough characteristics and baking quality of wheat deteriorated when the protein content of the grain (grain N content) was extremely high (Bushuk et al. 1978, Tipples et al. 1977). In addition, in reconstituted baking tests (to compare gluten baking quality independently of protein quantity), wheat produced on plots receiving high rates of N fertilizer produced smaller loaves than grain produced on less intensively fertilized plots (Timms et al. 1981). These results suggest that for wheat grown where high levels of N fertilizer are applied in the absence of sufficient S, there may be a change in the balance between available N and S, such that the available S levels

become insufficient for optimum grain development. As a result, grain protein quantity (N content) may not be affected, but protein quality may be adversely affected due to imbalances in the ratio of N to S in the grain, resulting in the deterioration in baking quality, most namely loaf volume (Byers et al. 1987, Schnug et al. 1993, Zhao et al. 1999a).

Due to the lack of information regarding the impact of S nutrition on the breadmaking quality of CWRS wheat, the purpose of this paper is to examine the relationship between grain S concentration, N concentration, and N:S ratio and a number of different grain yield and quality measurements. We will determine if CWRS wheat grown in western Canada demonstrates relationships with grain S concentration and N:S ratio similar to those observed in the past with European, New Zealand, and Australian wheat varieties.

### **3.3. Methods and Materials**

#### **3.3.1. Field Experiments**

Canada Western Red Spring wheat (*Triticum aestivum* L. cv. AC Barrie, a popular variety with high breadmaking quality) was grown at twelve locations across western Canada in 1999 and 2000. In 1999, field sites were located near Erickson, MB; Brandon, MB (Brandon South); Melfort, SK; Kelvington, SK; and Athabasca, AB. In 2000, field sites were located near Erickson, MB; Glenboro, MB; two sites near Brandon, MB (Brandon North and Brandon South); Rosebank, MB; Archerwill, SK; and Athabasca, AB.

The experimental design was the same for all sites in each season. Treatments consisted of factorial combinations of two fertilizer N rates (26 and 100 kg N ha<sup>-1</sup>) and two fertilizer S rates (0 and 20 kg S ha<sup>-1</sup>) and were replicated four times at each site in a randomized complete



block design. Wheat was sown between early and late May and harvested between late August and mid-October (Tables 3.3 and 3.4). Nitrogen was applied as urea, ammonium sulphate, and monoammonium phosphate. Sulphur was applied as ammonium sulphate. Phosphate was applied at a rate of 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as monoammonium phosphate. Fertilizer treatments were banded or broadcast and incorporated prior to seeding, or side-banded during the seeding operation (Tables 3.3 and 3.4). Alleys were either seeded to a cereal species or were tilled to control weeds. Wheat was seeded in border areas to reduce any border edge effects on the outside plots. During the growing season, registered herbicides were applied at recommended rates to control weed populations. In 1999, Round Up (Glyphosate) was also used to desiccate the wheat in Melfort. In 2000, Reglone (Diquat) was used to desiccate the wheat in Erickson, Rosebank, and Glenboro. In 2000, Folicur (Tebuconazole) was applied at recommended rates at Erickson, Rosebank, and Glenboro to reduce fusarium infestations (Tables 3.3 and 3.4). Previous cropping at each site is also shown in Tables 3.3 and 3.4.

### **3.3.2. Soil Sampling and Analysis**

Soil sampling consisted of three soil cores taken from each plot in the spring, just prior to fertilization and seeding. Sampling depths at most sites were 0 - 15, 15 - 30, 30 - 60, and 60 - 90 cm. For each soil depth, the three soil cores were mixed into a composite sample and immediately air-dried and ground by a high-speed mill to pass through a 2 mm screen. At Archerwill in 2000, only one composite soil sample was collected from the 0 - 15, 15 - 30, and 30 - 60 cm depths for the entire plot area prior to fertilization and seeding. Water-soluble SO<sub>4</sub>-S and NO<sub>3</sub>-N were extracted using a 0.001 M calcium chloride solution. The SO<sub>4</sub>-S concentration was determined using the automated methylthymol blue method and NO<sub>3</sub>-N was determined

Table 3.1. Physical and chemical characteristics of soils used in 1999 field studies

Characteristic	Depth (cm)	Site				
		Erickson	Brandon South	Athabasca	Kelvington	Melfort
Soil Association		Newdale	Souris	Nicot	Yorkton	Waitville
Soil Texture		clay loam	fine sandy loam	loamy sand	loam	clay loam
pH	0 to 15	6.4	7.2	6.1	7.8	7.0
Organic Matter (%)	0 to 15	2.4	2.8	1.4	7.0	2.6
NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> )	0 to 15	3.5	2.8	7.4	21.3	22.1
	15 to 30	1.9	1.5	7.5	11.9	19.7
	30 to 60	1.6	2.3	5.8	1.8*	10.9
	60 to 90	-	5.2	4.8	-	-
(Estimated kg ha <sup>-1</sup> )***	0 to 60	19 (VL)**	20 (VL)	60 (M)	91 (VH)	144 (VH+)
SO <sub>4</sub> <sup>-</sup> -S (mg kg <sup>-1</sup> )	0 to 15	4.3	4.5	4.9	7.8	5.6
	15 to 30	2.3	2.8	3.0	7.1	4.3
	30 to 60	2.4	2.1	1.8	8.8*	2.3
	60 to 90	-	2.3	1.8	-	-
(Estimated kg ha <sup>-1</sup> )***	0 to 60	26 (M)**	26 (M)	26 (M)	61 (VH+)	32 (M)
P (mg kg <sup>-1</sup> )	0 to 15	22	12	60	16	45
K (mg kg <sup>-1</sup> )	0 to 15	189	203	168	220	207
Fe (mg kg <sup>-1</sup> )	0 to 15	142	25	84	40	174
Cu (mg kg <sup>-1</sup> )	0 to 15	0.76	0.52	0.43	0.81	0.54
Zn (mg kg <sup>-1</sup> )	0 to 15	2.98	0.84	1.69	1.19	2.33
Mn (mg kg <sup>-1</sup> )	0 to 15	57	26	19	16	81

\*only measured on 30 to 45 cm depth

\*\*ratings for wheat production according to the Manitoba Soil Fertility Guide (VL = very low; L = low; M = medium; H = high; VH = very high)

\*\*\* estimated assuming a soil bulk density of 1.33 g cm<sup>-3</sup>

Table 3.2. Physical and chemical characteristics of soils used in 2000 field studies

Characteristic	Depth (cm)	Site						
		Erickson	Brandon South	Athabasca	Archerwill	Brandon North	Glenboro	Rosebank
Soil Association		Newdale	Souris	Nicot	Meota	Newdale	Stockton	Altona
Soil Texture		clay loam	fine sandy loam	loamy sand	loamy sand	clay loam	sandy loam	fine loam
pH	0 to 15	6.1	6.9	5.8	7.0	7.3	5.5	7.2
Organic Matter (%)	0 to 15	2.4	5.5	2.4	4.9	4.9	3.0	4.1
NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> )	0 to 15	6.1	13.5	6.8	7.4	6.5	5.6	9.2
	15 to 30	1.8	7.1	9.9	4.4	2.3	4.9	13
	30 to 60	1.3	3.4	13.6	3.0	2.5	4.9	5.0
	60 to 90	-	5.4	9.7	-	5.4	-	2.7
(Estimated kg ha <sup>-1</sup> )**	0 to 60	24 (VL)*	61 (M)	99 (VH)	40 (L)	30 (VL)	46 (M)	72 (H)
SO <sub>4</sub> <sup>-</sup> -S (mg kg <sup>-1</sup> )	0 to 15	4.5	10.0	5.9	4.8	7.0	12.3	18.8
	15 to 30	2.5	8.0	4.0	4.4	4.7	6.0	18.5
	30 to 60	2.1	57	2.8	3.4	4.9	3.4	14.6
	60 to 90	-	97.6	2.4	-	6.6	-	22.7
(Estimated kg ha <sup>-1</sup> )**	0 to 60	25 (M)*	296 (VH+)	35 (H)	36 (H)	48 (VH)	56 (VH)	149 (VH+)
P (mg kg <sup>-1</sup> )	0 to 15	25	15	60	16	19	42	21
K (mg kg <sup>-1</sup> )	0 to 15	170	457	130	61	263	180	240
Fe (mg kg <sup>-1</sup> )	0 to 15	186	42	72	79	47	78	28
Cu (mg kg <sup>-1</sup> )	0 to 15	0.98	1.00	0.28	0.68	1.36	0.58	0.98
Zn (mg kg <sup>-1</sup> )	0 to 15	5.70	2.46	1.40	3.12	1.20	1.86	1.24
Mn (mg kg <sup>-1</sup> )	0 to 15	48	39	17	37	26	38	22

\*ratings for wheat production according to the Manitoba Soil Fertility Guide (VL = very low; L = low; M = medium; H = high; VH = very high)

\*\* estimated assuming a soil bulk density of 1.33 g cm<sup>-3</sup>

Table 3.3. Agronomic information for the 1999 field studies

Characteristic	Site				
	Erickson	Brandon South	Athabasca	Kelvington	Melfort
Previous Crop	Canola	Barley	Fallow	Fallow	Fallow
Seeding Date	27-May	18-May	25-May	27-May	8-May
Harvest Date	27-Sep	9-Sep	20-Sep	14-Oct	4-Sep
Fertilization	pre-seed band	broadcast & incorp.	side band @ seeding	pre-seed band	broadcast & incorp.
Pre-harvest Dessicant	none	none	none	none	Round Up
Fungicide	none	none	none	none	none

Table 3.4. Agronomic information for the 2000 field studies

Characteristic	Site						
	Erickson	Brandon South	Athabasca	Archerwill	Brandon North	Glenboro	Rosebank
Previous Crop	Canola	Flax	Barley	Canola	Oats	Peas	Canola
Seeding Date	15-May	3-May	17-May	17-May	3-May	2-May	18-May
Harvest Date	12-Sep	21-Aug	25-Sep	31-Aug	21-Aug	23-Aug	23-Aug
Fertilization	pre-seed band	broadcast & incorp.	side band @ seeding	broadcast & incorp.	broadcast & incorp.	pre-seed band	pre-seed band
Pre-harvest Dessicant	Reglone	none	none	none	none	Reglone	Reglone
Fungicide	none	none	none	none	none	Folicur	Folicur

Table 3.5. Grades of grain produced in 1999 and 2000 field studies

Location	Treatments	Grade	Downgrading Factors
1999			
Athabasca	all	#1 CWRS	none
Erickson	all	#2 CWRS	fusarium head blight (0.5 - 1% damaged)
Brandon South	all	#3 CWRS	fusarium head blight (>1% damaged)
Kelvington	all	feed - #3 CWRS	frost
Melfort	all	#1 CWRS	none
2000			
Rosebank	all	#2 CWRS	fusarium head blight (0.5 - 1% damaged), ergot, midge damage
Glenboro	all	feed - #3 CWRS	fusarium head blight (>1% damaged), ergot, starch, mildew
Erickson	all	#3 CWRS	mildew, green
Brandon North	Low N treatments	#3 CWRS	ergot, fusarium head blight (>1% damaged)
	High N treatments	#2 CWRS	fusarium head blight (0.5 - 1% damaged), green, midge damage
Archerwill	all	#1 CWRS	none
Athabasca	all	feed - #3 CWRS	green, frost, smudge, sprout
Brandon South	all	#2 CWRS	fusarium head blight (0.5 - 1% damaged), green, ergot

using the automated cadmium reduction method of Greenberg et al. (1992). Physical and chemical soil properties at each experimental site are shown in Tables 3.1 and 3.2. For a more in-depth description of the methods of soil analysis, please refer to Appendix A.

### **3.3.3. Grain Yield, Nutrition, and Quality Analyses**

At maturity, absolute grain yields were determined using plot combines. Grain samples were collected for the determination of moisture content and grain yields were adjusted to dry matter basis. Similar to the calculation of Randall et al. (1981), grain yields were also calculated relative to the highest-yielding treatment at each N level at each site (relative grain yield).

Sub-samples of grain from each plot were ground with a Wiley Mill to pass a 2 mm sieve and analyzed for total N and S by combustion using a Leco CNS Analyzer (Leco Corporation 1996). Grain protein was calculated from the N concentration by multiplying by a factor of 5.7 for human food protein. The moisture content of the ground grain was determined and concentrations of N and S are expressed on a dry matter basis. Grain N:S ratio was calculated from the N and S concentrations. For a more in-depth description of the methods of tissue analysis, please refer to Appendix C.

All grain samples were graded according to the grading standards set by the Canadian Grain Commission (Table 3.5). Milling and breadmaking tests were carried out only on samples meeting #1 and #2 CWRS wheat grading standards. As a result, grain samples from Brandon South and Kelvington in 1999 were rejected due to fusarium head blight damage and frost damage, respectively. In 2000, all grain samples from Glenboro, Erickson, and Athabasca were rejected; at Brandon North, all low N treatment samples were also rejected. One replicate from Rosebank in 2000 was also rejected.

Grain samples were milled to flour using a Buhler laboratory mill after tempering to 16.5 % moisture content. During the milling process, flour yield was determined with the following calculation:

$$\text{Flour Yield} = (\text{flour out of mill} / \text{total recovered product out of mill}) \times 100 \%$$

The flour was then analyzed for total S and N by combustion using a Leco Analyzer. The moisture content of the flour was determined and concentrations of flour N and S are expressed on a dry matter basis. Flour protein was calculated from the N concentration by multiplying by a factor of 5.7 for human food protein. Flour N:S ratio was calculated from the N and S concentrations.

Sodium dodecyl sulfate (SDS) sedimentation tests were conducted, in duplicate, on 2.5 g samples of flour according to the method of Kovacs (1985).

Farinograph tests, using a 10 g Brabender Farinograph (Brabender Instruments Inc., South Hackensack, NJ, U.S.A.), were performed on each flour sample using the constant flour weight method (Approved Method 54-21, AACC, 2000). With this test, the water-absorbing capacity (FAB) of each flour sample was measured working to the 500 BU line. This test provides a measure of the water required to mix dough to a fixed consistency, which is used subsequently in baking tests. Other dough mixing parameters were also measured including dough development time, mixing tolerance index, dough stability, and time to dough breakdown. For a more in-depth description of the farinograph method and measured parameters, please refer to Appendix B.1.

A 2-g Micromixograph (National Mfg., TMCO, Lincoln, NE., U.S.A) was used to measure mixing characteristics of flour and dough using a modification of the method developed by Pon et al. (1989), where 2 g of flour were used instead of 10 g and using a fixed water

absorption of 65 % instead of 62 %. This test was done in duplicate. The following mixograph measurements were made during the dough mixing process: mixograph peak time, mixograph peak height, mixograph peak width, work input to peak, and total work input. For a more in-depth description of the mixograph method and measured parameters, please refer to Appendix B.2.

A 2-g Micromixograph (National Mfg., TMCO, Lincoln, NE., U.S.A) and Texture Analyzer (TA.XT2, Texture Technologies Corp., Scarsdale, NY., U.S.A. / Stable Micro Systems, Godalming, Surrey, U.K.) were used to evaluate maximum dough resistance ( $R_{max}$ ), dough extensibility, and extensigraph peak area according to the method of Suchy et al. (2000). A hook speed of 3.3 mm/second and water absorption level of FAB + 6 % were used. This test was run in duplicate. Using the  $R_{max}$  and extensibility values, the viscoelastic ratio was calculated with the following formula:

$$\text{Viscoelastic Ratio} = R_{max} / \text{Ext}$$

For a more in-depth description of the extensigraph method and measured parameters, please refer to Appendix B.3.

The optimized long-fermentation bake test (Approved Method 10-10B, AACC, 2000), at a water level of FAB – 3 %, was used to evaluate the baking potential of the flour samples. The method was based on 100-g flour samples (14 % mb). Loaf height, proof height, and oven spring were measured during the baking and preparation processes. Loaf volume determinations were made using a rapeseed displacement volumeter. Objective evaluation of the bread crumb was done by computerized image analysis system using the American Institute of Baking Crumb Scan Software, where the images of two slices of fresh cut bread were scanned and analyzed for cell size and shape and reported as crumb fineness and crumb elongation according to the



method of Wesley et al. (1999). Bread crumb firmness was determined at 25 % compression and 40 % compression according to the AACC Approved Method (Approved Method 74-19, AACC, 2000). Due to the small amount of grain available for the bake, only one replicate was carried out for each bake test for each grain sample. For a more in-depth description of the bake method and measured parameters, please refer to Appendix B.5.

The flour protein extraction protocol relied on a sequential solubility of the flour protein in various concentrations of 1-propanol (Suchy et al. 2002). Three identical samples with known flour nitrogen content and identical mass (100 mg at 14 % mb) were extracted simultaneously three times with 1.0 mL of 7.5 % 1-propanol and 0.3 M NaI at 25°C in 1.5 mL microcentrifuge tubes. The extraction was carried out in a temperature controlled-shaker with additional rapid vortexing (10 s at maximum setting using a Genie-2, Fisher Sci.) at the beginning and end of the extraction step. Tubes were centrifuged at 10,000xg (stage 1, 2 and 3). Insoluble residues were retained and actual fractions (supernatants) were used to monitor quality of extraction by SDS-PAGE. In the second stage only the residues from flour 2 and 3 were further extracted three times with the 50 % 1-propanol at 25°C. After that step, the supernatant was discarded and residue from flour 3 was extracted three times with 40 % 1-propanol and 0.2 % dithiothreitol (DTT) at 60°C. The insoluble residues from step 1, 2 and 3 were dried for 16 hours at 75°C using a solid bed heater.

Nitrogen content was determined on the insoluble residue from stage 1, 2, and 3. Nitrogen analysis was performed using combustion. The four principal flour protein solubility groups were obtained by the difference: monomeric protein (MP, flour nitrogen content minus nitrogen content of insoluble residue stage 1), soluble glutenin, (SG, nitrogen content of insoluble residue stage 1 minus nitrogen content of insoluble residue stage 2), insoluble glutenin

(IG, nitrogen content of insoluble residue stage 2 minus nitrogen content of insoluble residue stage 3), residue protein (RP, nitrogen content of insoluble residue after stage 3). The flour protein solubility fractions are expressed as a percentage of nitrogen over total flour nitrogen at 14 % mb. For a more in-depth description of the protein fractionation method, please refer to Appendix B.4.

#### **3.3.4. Data Analysis**

To examine the relationships between grain S concentration, N concentration, and N:S ratio and wheat quality and yield, linear and partial correlation coefficients were determined for the pooled data from 1999 and 2000 using the PROC CORR procedure (SAS Institute Inc. 1999).

### **3.4. Results and Discussion**

#### **3.4.1. Grain Nutrition**

Concentrations of S in the grain produced at all twelve field sites in 1999 and 2000 ranged from 0.127 % S to 0.231 % S. At the seven sites used in the quality analyses, concentrations of S in the grain ranged from 0.127 % S to 0.22 % S. According to the threshold of 0.12 % S developed by Randall et al. (1981) for Australian wheat varieties, no grain samples from our field experiments were regarded to be deficient in S for grain yield.

Concentrations of N in the grain produced at all twelve field sites in 1999 and 2000 ranged from 2.34 % N to 3.87 % N. Grain N concentrations from the seven field experiments used in the quality analyses also ranged from 2.34 % N to 3.87 % N (13.3 % to 22 % protein on

dry matter basis). According to the guidelines set by the Canadian Grain Commission, wheat containing less than 15.6 % protein (dry matter basis) is considered to be of poor quality. Therefore, in our study, very few grain samples were regarded to be of poor quality based on protein content alone.

Grain N:S ratios at all twelve field sites ranged from 13.3 to 25 and at the seven sites used in the quality analyses, from 14.4 to 25. Although the range of grain N:S ratios was quite wide over the two growing seasons, the majority of grain N:S ratios were concentrated between 14 and 17; therefore, the majority of grain samples were below the critical N:S ratio of 17 and were regarded to be sufficient in S according Randall et al. (1981) for grain yield under Australian conditions. However, N:S ratios must be interpreted cautiously. The same ratio of N to S can be obtained at totally different N and S concentration levels in grain (Schnug and Haneklaus 1998). For example, a low N:S ratio can be generated when both N and S are in surplus in the grain or when both are deficient in the grain. Furthermore, the surplus of one nutrient may falsely indicate a deficiency of the other nutrient.

#### **3.4.2. Correlation Between Grain Nutrition and Flour Nutrition**

Correlation analysis was used to determine how well the analysis of grain S concentration, N concentration, and N:S ratio predicted flour S concentration, N concentration, and N:S ratio. There was a strong, highly significant, and positive correlation between grain S concentration and flour S concentration (Table 3.6). This observation is consistent with the findings of Moss et al. (1981) who also noted a very strong correlation ( $r = 0.98$ ) between grain and flour S concentration. In our experiment, flour S concentration ( $y$ ) was related to grain S concentration ( $x$ ) by the formula:  $y = 0.9163x + 0.0055$  (Appendix Figure D.1). According to

Table 3.6. Linear and partial correlation coefficients between grain S concentration, N concentration, and N:S ratio and grain yield and quality characteristics

Variable	Simple Correlation Coefficient			Partial Correlation Coefficient	
	Grain S	Grain N	Grain N:S	Grain S at constant N	Grain N at constant S
Grain S Concentration	1.00***				
Grain N Concentration	0.50***	1.00***			
Grain N:S Ratio	-0.53***	0.46***	1.00***		
Flour S Concentration	0.95***	0.45***	-0.53***		
Flour N Concentration	0.51***	0.97***	0.42***		
Flour N:S Ratio	-0.47***	0.50***	0.97***		
Grain Yield <sup>§</sup>	0.06 <sup>ns</sup>	-0.25***	-0.28***	0.24**	-0.34***
Relative Grain Yield <sup>§</sup>	0.24***	-0.09 <sup>ns</sup>	-0.32***	0.34***	-0.27***
Flour Yield	-0.23*	-0.48***	-0.21*	0.01 <sup>ns</sup>	-0.43***
SDS Sedimentation Volume	0.25*	0.15 <sup>ns</sup>	-0.13 <sup>ns</sup>	0.21*	0.03 <sup>ns</sup>
Bake					
Loaf Height	0.82***	0.31**	-0.53***	0.81***	-0.21*
Loaf Volume	0.86***	0.49***	-0.40***	0.81***	0.13 <sup>ns</sup>
Proof Height	0.32**	0.66***	0.32**	-0.01 <sup>ns</sup>	0.61***
Oven Spring	0.43***	-0.31**	-0.74***	0.71***	-0.68***
25% Loaf Compression	-0.60***	-0.60***	-0.0002 <sup>ns</sup>	-0.43***	-0.43***
40% Loaf Compression	-0.58***	-0.64***	-0.06 <sup>ns</sup>	-0.39***	-0.50***
Crumb Fineness Score	-0.14 <sup>ns</sup>	-0.55**	-0.38***	0.18 <sup>ns</sup>	-0.55***
Crumb Elongation Score	-0.41***	-0.46***	-0.03 <sup>ns</sup>	-0.24*	-0.32**
Extensigraph					
Maximum Resistance (R <sub>max</sub> )	-0.64***	-0.07 <sup>ns</sup>	0.56***	-0.69***	0.36***
Extensibility (Ext)	0.70***	0.01 <sup>ns</sup>	-0.69***	0.80***	-0.55***
Viscoelastic Ratio (R <sub>max</sub> /Ext)	-0.02 <sup>ns</sup>	-0.10 <sup>ns</sup>	-0.08 <sup>ns</sup>	0.03 <sup>ns</sup>	-0.11 <sup>ns</sup>
Extensigraph Peak Area	-0.04 <sup>ns</sup>	-0.37***	-0.31**	0.17 <sup>ns</sup>	-0.40***
Mixograph					
Peak Time	-0.58***	-0.33***	0.28**	-0.51***	-0.06 <sup>ns</sup>
Peak Height	0.43***	0.85***	0.37***	0.02 <sup>ns</sup>	0.81***
Peak Width	0.29**	0.76***	0.41***	-0.15 <sup>ns</sup>	0.74***
Work Input to Peak	-0.27**	0.41***	0.69***	-0.61***	0.66***
Total Work Input	0.65***	0.80***	0.11 <sup>ns</sup>	0.48***	0.72***
Farinograph					
Farinograph Absorption	0.33***	0.74***	0.38***	-0.07 <sup>ns</sup>	0.71***
Dough Development Time	0.54***	0.37***	-0.20*	0.44***	0.13 <sup>ns</sup>
Mixing Tolerance Index	0.13 <sup>ns</sup>	-0.21*	-0.34***	0.27**	-0.32**
Dough Stability	-0.08 <sup>ns</sup>	0.09 <sup>ns</sup>	0.16 <sup>ns</sup>	-0.14 <sup>ns</sup>	0.14 <sup>ns</sup>
Time to Breakdown	0.32**	0.40***	0.04 <sup>ns</sup>	0.15 <sup>ns</sup>	0.29**
Protein Fractionation					
Monomeric Protein in Flour	-0.38***	0.05 <sup>ns</sup>	0.46***	-0.47***	0.30**
Soluble Glutenin in Flour (SG)	0.72***	-0.03 <sup>ns</sup>	-0.78***	0.84***	-0.63***
Insoluble Glutenin in Flour (IG)	-0.38***	-0.27*	0.11 <sup>ns</sup>	-0.29**	-0.10 <sup>ns</sup>
IG/SG ratio in Flour	-0.70***	0.03 <sup>ns</sup>	0.79***	-0.83***	0.63***
Residue Protein in Flour	-0.09 <sup>ns</sup>	0.18 <sup>ns</sup>	0.25*	-0.22*	0.26**

\*, \*\*, \*\*\* Significantly greater than 0 at P<0.05, P<0.01, and P<0.001, respectively

<sup>§</sup> Rep 1 from Erickson in 1999 excluded in correlation analysis due to deer damage

the regression formula, the concentration of S in flour tended to be lower than in the grain, probably due to the preferential loss of S through the mechanical removal of the bran and germ during the milling process. Moss et al. (1981) also found that there was the preferential distribution of S to those fractions excluded from the flour on milling, causing the concentration of S in flour to be lower than that in grain.

Flour N concentration was also very strongly, significantly, and positively correlated with grain N concentration (Table 3.6). In the correlation model, flour N concentration (y) was related to grain N concentration (x) by the formula:  $y = 0.9641x + 0.025$  (Appendix Figure D.1). The formula indicates that the concentration of N also tended to be lower in flour than in grain. Again, this is probably due to the milling process and removal of bran and germ.

The correlation between grain N:S ratio and flour N:S ratio was also very strong, highly significant, and positive (Table 3.6). As mentioned previously, the majority of grain samples in our experiment contained N:S ratios between 14 and 17. Very few grain N:S ratios extended beyond the critical 17:1 ratio as determined by Randall et al. (1981) for yield under Australian conditions.

In our study, the correlation analyses between grain and flour N concentration, S concentration, and N:S ratio provide solid evidence that grain analysis accurately predicted the N and S content of flour. The prediction is reliable even though the N and S concentrations in flour tended to be slightly less than in grain. However, the initial quality analysis of a producer's wheat is traditionally determined on grain, not on flour. Therefore, the N and S concentration in grain will be used as the basis for discussing the relationship of N, S, and N:S ratio with yield and quality parameters.

Linear correlation coefficients were used to examine the relationships between grain S concentration, N concentration, and N:S ratio and wheat yield and quality for the combined data from the two growing seasons (Table 3.6). Grain S and N concentration were positively correlated (Table 3.6). Therefore, similar to the methods of Moss et al. (1981), partial correlation coefficients were also determined for grain S concentration and grain N concentration with each dependent variable. Partial correlation coefficients make it possible to examine the relationship between grain S concentration and the measured quality variables at a constant grain N concentration and vice versa.

### **3.4.3. Correlation Between Grain Nutrition and Grain Yield**

The simple correlation coefficient between absolute grain yield and grain S concentration was not significant (Table 3.6 and Figure 3.1a). The partial correlation coefficient between the two variables was significant and positive, but very weak (Table 3.6). In an attempt to account for the variability in yield potential between field sites, grain yield was calculated as a percentage of the highest-yielding treatment at each N level at each site. However, the correlation between grain S concentration and relative grain yield was also weak, indicating that grain S concentration was a poor indicator of grain yield for our experiment (Table 3.6 and Figure 3.1b). These poor correlations were probably due to the apparent sufficiency of soil test S for yield at most of our sites (Tables 3.1 and 3.2) and is not consistent with the observations of Randall et al. (1981), who found that the S concentration in grain was positively, strongly, and significantly correlated with relative grain yield ( $r = 0.95$ ). The soils used by these researchers were probably more S deficient than the soils used in our experiment, resulting in a stronger correlation between grain S concentration and relative grain yield.

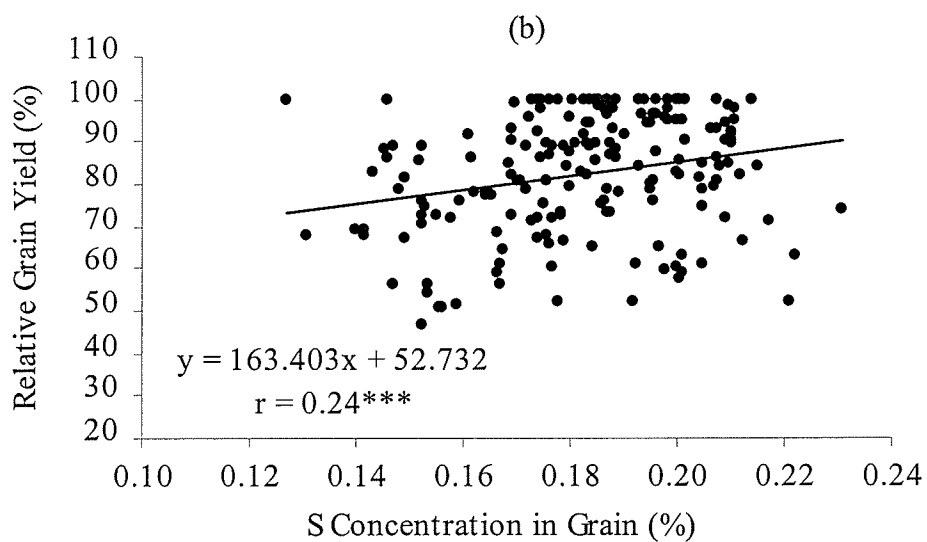
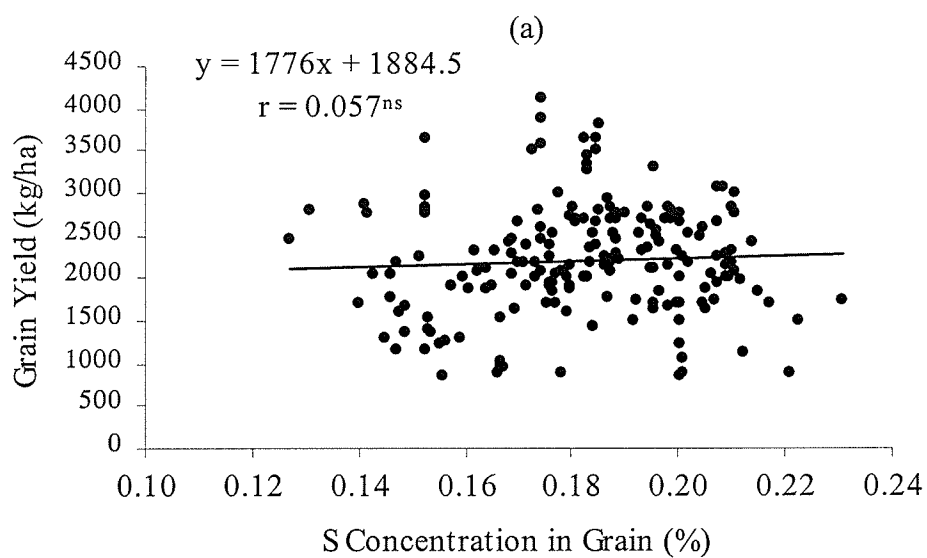


Figure 3.1. Relationship between S concentration in grain and (a) absolute grain yield and (b) relative grain yield

<sup>ns</sup> Not significantly greater than 0

<sup>\*\*\*</sup> Significantly greater than 0 at  $P < 0.001$

The simple and partial correlation coefficients for grain N concentration versus absolute grain yield and relative grain yield were also very weak (Table 3.6). However, the correlation coefficients were negative, providing a weak indication that as grain yield increased the N concentration of grain decreased, even at constant S. Grain N:S ratio was also weakly and negatively correlated with absolute grain yield and relative grain yield (Table 3.6). Randall et al. (1981) also observed an inverse relationship between grain N:S ratio and relative grain yield; however, the very weak, negative correlation coefficient between grain N:S ratio and relative grain yield in our experiment was not nearly as strong as what was observed by these researchers ( $r = -0.96$ ). Once again, the difference between our results and the results of Randall et al. (1981) may have been due to the relatively sufficient quantities of soil test S for yield at most of our sites.

#### **3.4.4. Correlation Between Grain Nutrition and Flour Yield and SDS Sedimentation Volume**

Flour yield is a measure of the milling quality of wheat. Generally, the higher the flour yield, the higher the milling quality. Sodium dodecyl sulfate sedimentation volume is a good indicator of loaf volume (Axford et al. 1979) and gluten strength (Kovacks 1985) with a high SDS sedimentation volume indicating high baking and gluten quality. Flour yield was negatively, but weakly, correlated with grain S concentration, while SDS sedimentation volume was positively, but weakly, correlated with grain S concentration (Table 3.6). However, according to the partial correlation coefficients, flour yield was not correlated to grain S concentration at constant N. The partial correlation for grain S concentration and SDS sedimentation volume remained positive, but very weak. Grain N concentration was



significantly and negatively correlated with flour yield, only; however, the simple and partial correlations were only moderate. Grain N:S ratio was not strongly correlated to flour yield or SDS sedimentation volume.

#### **3.4.5. Correlation Between Grain Nutrition and Baking Parameters**

Bread loaves of high volume and quality are desirable from a consumer viewpoint. Therefore, baking quality analyses that measure loaf quality characteristics provide an indication of the overall baking quality of CWRS wheat. In 1999 and 2000, eight baking parameters were measured on the grain/flour samples (Table 3.6). According to the simple and partial correlation coefficients, loaf volume and loaf height were most strongly and positively correlated with grain S concentration (Table 3.6 and Figure 3.2a). In previous research conducted in Australia by Moss et al. (1981), in the U.K. by Zhao et al. (1999a, 1999b), and in Germany by Haneklaus et al. (1992) and Schnug et al. (1993), loaf volume was also found to be positively correlated to grain S concentration. In our study, the simple correlation coefficients also indicate that loaf volume and loaf height were positively, but more weakly correlated with grain N concentration (Table 3.6). However, the partial correlation coefficient for grain N concentration was negative for loaf height and not significant for loaf volume (Table 3.6). Zhao et al. (1999a) also observed that loaf volume was more strongly and positively correlated with grain S concentration than with grain N concentration. However, in our study, the relatively minor effect of grain N concentration on loaf volume may have been due, in part, to the low number of grain samples containing low concentrations of N.

According to the simple correlation coefficients, grain N:S ratio was negatively correlated with loaf volume and loaf height (Table 3.6), providing evidence that as the ratio of

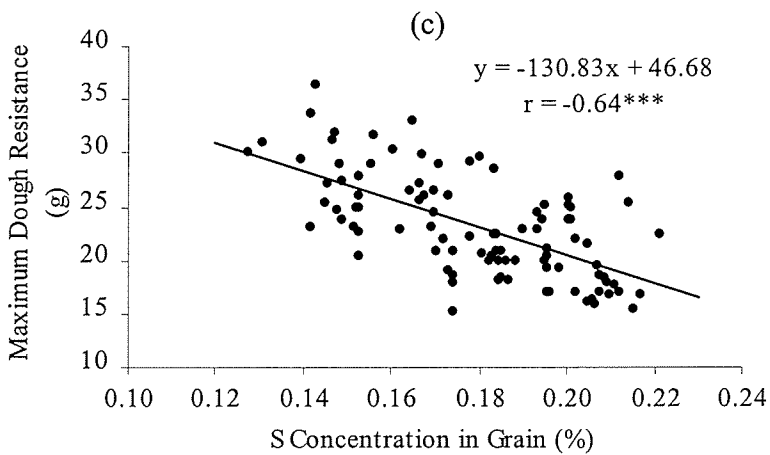
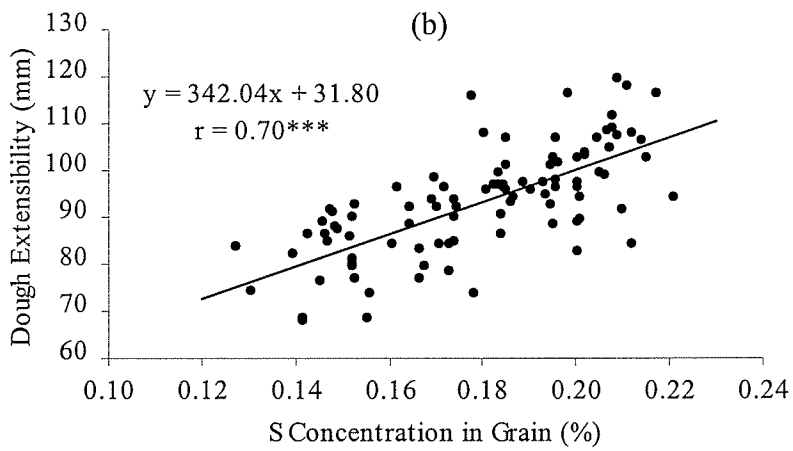
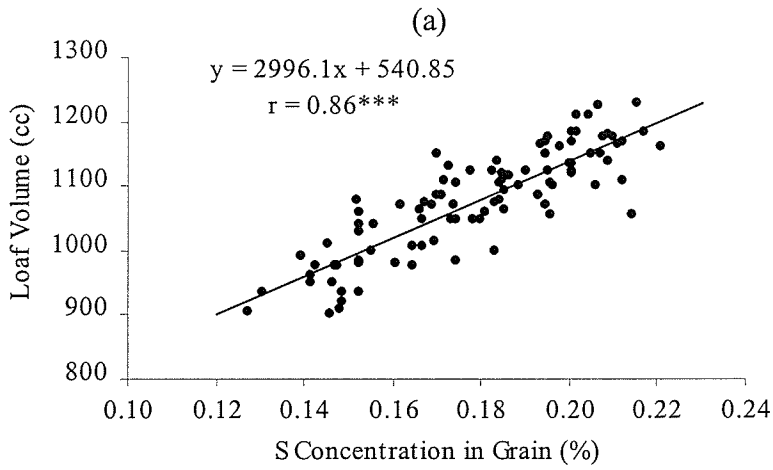


Figure 3.2. Relationship between S concentration in grain and (a) loaf volume, (b) dough extensibility, and (c) maximum dough resistance ( $R_{max}$ )  
 \*\*\* Significantly greater than 0 at  $P < 0.0001$

grain N to S widened, the baking quality of wheat deteriorated. The simple correlation coefficient between grain N:S ratio and loaf volume was only moderate, but of similar strength to the correlation coefficient ( $r = -0.45$ ) determined for the same two variables by Zhao et al. (1999a). Other European studies also provided evidence that a widening in the ratio of N to S in grain was associated with the deterioration of baking quality in wheat; however, correlation analysis on the data was not conducted in those studies (Byers et al. 1987, Schnug et al. 1993).

The correlation between grain S concentration and oven spring as well as between grain N:S ratio and oven spring reinforce the observations made for loaf volume and loaf height. As the concentration of S in the grain increased and the ratio of N to S decreased, the dough tended to rise more during the baking process, resulting in loaves of greater height and volume. This is supported by the strong, positive partial correlation coefficient determined for grain S concentration versus oven spring and the strong, negative correlation coefficient observed between grain N:S ratio and oven spring (Table 3.6).

According to the simple and partial correlation coefficients, there was a negative association between grain N concentration and oven spring (Table 3.6). The negative relationship provides further evidence to support the earlier observation of the negative relationship between grain N concentration and loaf height (Table 3.6). It is evident that as the concentration of N in the grain increased, the rise in loaf height during the baking process tended to decrease, leading to the deterioration in loaf height.

Proof height was not significantly correlated with grain S concentration according to the partial correlation coefficient for grain S concentration (Table 3.6). Grain N concentration was positively correlated to proof height according to the simple and partial correlation coefficients

for grain N concentration (Table 3.6). Proof height was also positively, but weakly correlated with grain N:S ratio.

Bread crumb firmness at 25 % and 40 % compression were negatively correlated with grain S concentration and grain N concentration according to the simple correlation coefficients (Table 3.6). These observations are supported by the partial correlation coefficients, which were also negative, providing evidence that as the concentration of N and S in grain increased, the loaves tended to become softer. Grain N:S ratio was not significantly correlated with the bread crumb firmness parameters.

The crumb fineness score, according to the simple and partial correlation coefficients, was not significantly correlated with grain S concentration (Table 3.6). The crumb elongation score was negatively correlated with grain S concentration; however, the simple and partial correlations were both weak, indicating that the quality of loaf, as measured by this parameter, was not negatively impacted by increasing concentrations of S in the grain.

Grain N concentration was negatively correlated with the crumb fineness and crumb elongation scores, according to the simple correlation coefficients (Table 3.6). The negative relationships observed between grain N concentration and these crumb scores were further demonstrated by partial correlation coefficients for each parameter, which were also negative (Table 3.6). A reduction in the crumb fineness and elongation scores with rising grain N concentrations provides evidence that as the concentration of N in grain increased, loaves tended to become denser with poorer cell formation.

Grain N:S ratio was not significantly correlated with the crumb elongation score (Table 3.6). However, grain N:S ratio was weakly and negatively correlated with crumb fineness score.

### 3.4.6. Correlation Between Grain Nutrition and Extensigraph Parameters

The extensigraph is a load-extension instrument that measures the strength and extensibility of dough during the stretching process (Shuey 1975). According to the simple and partial correlation coefficients, dough extensibility was most strongly correlated with grain S concentration (Table 3.6 and Figure 3.2b). The correlation between grain S concentration and dough extensibility was strong and positive according to the simple and partial correlation coefficients, indicating that grain containing higher concentrations of S tended to be more pliable and extensible than grain containing lower concentrations of S. These observations are consistent with the earlier findings from Australia (Moss et al. 1981, 1983, Wrigley et al. 1984) and the U.K. (Zhao et al. 1999a, 1999b). However, in the work of Moss et al. in 1981, the simple correlation coefficient for the relationship between grain S concentration and dough extensibility was significantly greater ( $r = 0.95$ ) than what was observed in our study. The soils used by Moss et al. (1981) were probably more S deficient than the soils used in our experiment, resulting in a stronger correlation between grain S concentration and dough extensibility.

The simple correlation coefficient for the relationship between grain N concentration and dough extensibility was not significant (Table 3.6). However, according to the partial correlation coefficient, there was a negative correlation between grain N concentration and dough extensibility, indicating that if the concentration of S in grain remained constant and the concentration of N increased, the dough became less extensible.

Grain S concentration demonstrated a negative simple and partial correlation with  $R_{\max}$  providing evidence that as the concentration of S in grain decreased, dough strength or toughness increased (Table 3.6 and Figure 3.2c). Moss et al. (1981) also observed a negative partial correlation of similar magnitude ( $r = -0.64$ ) for the relationship between grain S concentration

and  $R_{\max}$ . Further studies in Australia (Moss et al. 1983, Wrigley et al. 1984) and the U.K. (Zhao et al. 1999a, 1999b) also demonstrated the negative relationship between grain S concentration and  $R_{\max}$ .

Grain N concentration, according to the partial correlation coefficient, was positively, but weakly correlated with  $R_{\max}$  (Table 3.6).

Grain N:S ratio was negatively correlated with dough extensibility and positively correlated with  $R_{\max}$  according to the simple correlation coefficients (Table 3.6). These observations provide evidence that as the balance between grain N and S widened, dough quality deteriorated, and supports the observations of Wrigley et al. (1984) who found that dough became tough and inextensible with rising ratios of N to S in grain.

Extensigraph peak area was not correlated with grain S concentration and was negatively, but weakly correlated with grain N concentration and N:S ratio (Table 3.6). The viscoelastic ratio ( $R_{\max}/\text{Extensibility}$ ) was not correlated with grain S concentration, grain N concentration, or grain N:S ratio (Table 3.6).

#### **3.4.7. Correlation Between Grain Nutrition and Mixograph Parameters**

The mixograph is a dough-mixing instrument that records a number of dough mixing characteristics for the evaluation of dough strength (Kunerth and D'apponia 1985). For the simple and partial correlations, mixograph peak time, which is an estimate of dough strength (Shuey 1975), was negatively correlated with grain S concentration, whereas total work input was positively correlated with grain S concentration (Table 3.6). Furthermore, according to the partial correlation coefficients, work input to peak was also negatively correlated with grain S concentration (Table 3.6). The negative correlations observed for grain S concentration versus

mixograph peak time and work input to peak again indicate that rising concentrations of S in grain are associated with a reduction in dough elasticity and support the observations made on the extensigraph, where rising concentrations of S in grain were associated with lower  $R_{max}$  (Table 3.6). Furthermore, these observations are consistent with the observations of Moss et al. (1981) who also observed that mixograph peak time and work input both correlated negatively with grain S concentration.

Mixograph peak height and peak width were positively, but weakly correlated with grain S concentration, according to the simple correlation coefficients (Table 3.6). However, according to the partial correlation coefficients for grain S concentration, neither parameter was significantly correlated with grain S concentration at constant N.

According to the simple and partial correlations, all mixograph parameters except peak time were more strongly and positively correlated with grain N concentration than with grain S concentration (Table 3.6). The strongly positive correlation between mixograph peak height and grain N concentration was consistent with observations made by Uthayakumanin et al. (1999). Furthermore, in the work by Uthayakumanin et al. (1999), mixograph peak time was also strongly and positively correlated with grain N concentration (while the glutenin to gliadin ratio remained constant). However, in our study, the correlation between these two parameters was very weak and negative.

It is apparent from the positive partial correlations observed between grain N concentration and the mixograph parameters that dough containing high concentrations of N tended to be stronger than grain containing lower concentrations of N. Furthermore, the positive correlations observed for these mixograph parameters are consistent with the earlier observation on the extensigraph where  $R_{max}$  increased with rising concentrations of N in grain (Table 3.6).

Grain N:S ratio was strongly and positively correlated with work input to peak (Table 3.6), providing evidence that as the ratio of N to S in the grain increased, the dough tended to become tougher. Again, these observations are similar to what was observed on the extensigraph, where grain N:S ratio was positively correlated with  $R_{\max}$  (Table 3.6).

#### **3.4.8. Correlation Between Grain Nutrition and Farinograph Parameters**

The farinograph is a dough-mixing instrument that records a number of dough mixing characteristics for the evaluation of dough strength and stability (Preston and Kilborn 1990). Most farinograph measurements were poorly correlated with grain S concentration (Table 3.6). According to the simple and partial correlation coefficients, dough development time was the only parameter that was positively correlated with grain S concentration. Mixing tolerance index, dough stability, and time to breakdown, which provide an index of dough stability (Shuey 1990), were not strongly correlated with grain S concentration (Table 3.6). Therefore, the S concentration of grain did not appear to have a large impact on measures of dough stability on the farinograph.

The simple and partial correlation coefficients for grain N concentration and farinograph absorption were strong and positive. In a number of previous studies, grain N concentration was also found to be positively correlated to farinograph absorption (Ayoub et al. 1994, Dexter et al. 1994, Pechanek et al. 1997). In addition, these earlier studies also demonstrated that dough stability and dough development time increased with rising grain/flour nitrogen concentrations. However, in our study, according to the partial correlations, the relationship between grain N concentration and these latter farinograph parameters was not significant (Table 3.6).



Grain N concentration was weakly correlated with mixing tolerance index and time to breakdown (Table 3.6). The simple correlation coefficient was weakly negative and positive for mixing tolerance index and time to breakdown, respectively. The same trend for mixing tolerance index was observed by Ayoub et al. (1994). These correlations, although quite weak, provide evidence that increasing concentrations of N in grain were associated with more stable dough.

Weak correlations between grain N:S ratio and farinograph absorption, dough development time, and mixing tolerance index were also observed (Table 3.6). The correlation for farinograph absorption was positive, whereas for dough development time and mixing tolerance index, the correlations were negative. Dough stability and time to breakdown were not significantly correlated to grain N:S ratio.

#### **3.4.9. Correlation Between Grain Nutrition and Protein Fractionation**

Flour protein fractionation provides an indication of the compositional make-up of flour. Of the five measured protein fractions, the concentration of soluble glutenin in flour was most strongly and positively correlated with grain S concentration (Table 3.6). The strong relationship between these two variables was demonstrated by both the simple and partial correlation coefficients. The soluble glutenin fraction is comprised primarily of LMW glutenin subunits as well as a small amount of HMW glutenin subunits and residual gliadins (Suchy 2002). According to Zhao et al. (1999c), the LMW glutenin subunits contain 2 to 3 mol % of cysteine; therefore, this fraction is considered to be composed of S-rich soluble glutenin subunits. The positive association between grain S concentration and the concentration of soluble glutenin in flour is, therefore, consistent with a number of previous studies that have demonstrated that S

deficiency in grain causes the relative proportions of LMW glutenin subunits (as well as  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins, albumins, and globulins), which are rich in S, to decline (Castle and Randall 1987, Fullington et al. 1987, MacRitchie and Gupta 1993, Wrigley et al. 1980, 1984).

According to the simple correlation coefficient, the concentration of soluble glutenin in flour was also strongly and negatively correlated with grain N:S ratio (Table 3.6). The concentration of soluble glutenin in flour was negatively correlated with grain N concentration, according to the partial correlation coefficient (Table 3.6).

The simple and partial correlation coefficients for grain S concentration versus the concentration of insoluble glutenin were negative and weak (Table 3.6). The fraction of insoluble glutenin is comprised of both LMW and HMW glutenin subunits that are soluble only in the presence of a reducing agent (Suchy 2002). Past research has demonstrated strong evidence that low concentrations of S in grain are associated with a rise in the relative proportion of HMW glutenin subunits (Castle and Randall 1987, MacRitchie and Gupta 1993, Wrigley et al. 1980, 1984). However, according to Zhao et al. (1999c), the HMW glutenin subunits contain moderate concentrations of cysteine. Therefore, due to the presence of both S-rich, LMW and S-poor, HMW glutenin subunits, the relatively weak correlation was expected. Grain N concentration and N:S ratio had very little influence, if any, on the insoluble glutenin fraction (Table 3.6).

The soluble glutenin fraction (SG) is composed primarily of LMW glutenin subunits and the insoluble glutenin fraction (IG) is composed of both LMW and HMW glutenin subunits (Suchy 2002), so the IG/SG ratio provides an estimate of the ratio of HMW to LMW glutenin subunits. The ratio of insoluble glutenin to soluble glutenin was highly correlated to all grain nutrition variables (Table 3.6). According to the simple correlation coefficients, the IG/SG ratio

was negatively correlated with grain S concentration and positively correlated with grain N:S ratio (Table 3.6). The strongly negative association between the IG/SG ratio and grain S concentration was also observed for the partial correlation coefficient for grain S. Our results are consistent with the work of MacRitchie and Gupta (1993) who demonstrated that a decrease in grain S concentration was associated with a rise in the ratio of HMW/LMW subunits of glutenin.

According to the simple correlation coefficient, grain N concentration was not correlated to the IG/SG ratio; however, the partial correlation coefficient for grain N concentration demonstrated that the correlation between the IG/SG ratio in flour and grain N concentration at constant S was highly significant and positive (Table 3.6).

The monomeric protein fraction and grain S concentration were negatively correlated according to the simple and partial correlations (Table 3.6). The monomeric fraction is composed of gliadins ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins and  $\omega$ -gliadins), albumins, and globulins (Suchy 2002). According to Zhao et al. (1999c), the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins are considered to be S-rich and the  $\omega$ -gliadins are considered to be S-poor. For example, as the concentration of S in the grain increases, the concentration of S-rich,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins increases (Castle and Randall 1987, Fullington et al. 1987, MacRitchie and Gupta 1993, Moss et al. 1981, Wrigley et al. 1980, 1984). Furthermore, rising concentrations of S in grain cause a decrease in the concentration of S-poor,  $\omega$ -gliadins. However, because all forms of gliadins are included in the monomeric fraction measured in our study, the relationship between grain S concentration and the monomeric protein fraction is not precisely clear. Further fractionation of the protein into the different groups of gliadins would be necessary to reach firm conclusions regarding the relationship between S nutrition and the monomeric protein composition.

Grain N concentration was positively, but weakly correlated with the concentration of monomeric protein in flour; grain N:S ratio was positively correlated with this protein fraction.

The residue protein, the surplus residue that does not fit into any of the previously discussed protein fractions was poorly correlated with all of the grain nutritional parameters (Table 3.6).

In Australia, Moss et al. (1981) demonstrated that a deficiency of S in grain resulted in a deficiency in proteins that were rich in cysteine (LMW glutenin subunits, and  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins). This reduced the dough's capacity to form intermolecular disulphide bonds and might have indirectly weakened bonds of other types. These changes in protein composition associated with the deficiency of S in grain causes a reduction in dough extensibility and an increase in dough resistance (strength), leading to the deterioration in the overall baking performance of wheat grown in Australia and the U.K. (Castle and Randall 1987, Fullington et al. 1987, MacRitchie and Gupta 1993, Moss et al. 1981, Wrigley et al. 1980, 1984, and Zhao et al. 1999a, 1999b). Furthermore, an imbalance between LMW and HMW glutenin subunits, associated with low S concentrations in grain, has also been found to cause dough extensibility to decline and dough strength to increase in Australian and British studies (MacRitchie and Gupta 1993, Moss et al. 1981, Wrigley et al. 1984, Zhao et al. 1999a, 1999b). In our study, the same is true for CWRS wheat; low S containing grain was inextensible and tough, probably due decreased synthesis of LMW glutenin subunits,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins and an imbalance between LMW and HMW glutenin subunits.

### 3.5. Summary and Conclusions

Our study demonstrated that both grain S concentration and N:S ratio were poor predictors of grain yield probably because no soils were highly deficient in S. However, our study confirmed that grain S concentration and N:S ratio were important indicators of breadmaking quality of CWRS wheat grown in western Canada, similar to observations for wheat in Europe, New Zealand, and Australia. Grain containing high concentrations of S or low ratios of N to S produced dough that was less tough (strong) and more extensible and ultimately produced bread loaves of higher quality than grain containing low concentrations of S or high N:S ratios. The improved baking quality of high S containing wheat was demonstrated by the strong, positive correlations between grain S concentration and loaf volume, loaf height, and oven spring and the negative correlations between grain N:S ratio and loaf volume, loaf height, and oven spring (Table 3.6). The associated increase in dough extensibility with improved S nutrition in grain was demonstrated by the strong, positive correlation between grain S concentration and dough extensibility and the negative correlation between grain N:S ratio and dough extensibility (Table 3.6). The reduction in dough toughness (strength) with improved grain S nutrition was demonstrated by the negative correlations between grain S concentration and  $R_{\max}$ , mixograph peak time, and work input to peak and the positive correlations between grain N:S ratio and  $R_{\max}$ , and work input to peak (Table 3.6).

The improvement in dough rheological and baking properties with rising concentrations of S in the grain and declining grain N:S ratios was probably due to changes in the composition of protein in the flour. The soluble glutenin content in flour protein, comprised primarily of S-rich, LMW glutenin subunits, as well as a small amount of HMW glutenin subunits and residual

gliadins (Suchy 2002), was positively correlated with grain S concentration and negatively correlated with grain N:S ratio (Table 3.6). The associated rise in proportion of soluble glutenin with rising grain S concentrations and declining grain N:S ratios led to the negative correlation observed between grain S concentration and the ratio of insoluble glutenin, composed of both LMW and HMW glutenin subunits (Suchy 2000), to soluble glutenin in flour and the positive correlation between grain N:S ratio and the ratio of insoluble to soluble glutenin in flour (Table 3.6). These observations indicate that rising concentrations of S in grain and reductions in grain N:S ratio probably resulted in increased concentrations of S-rich, LMW glutenin subunits balancing the ratio between LMW and HMW glutenin subunits in the flour protein.

The concentration of N in grain was poorly correlated with a number of important breadmaking parameters including loaf volume, probably, in part, because most grain samples in our experiment contained sufficient N. In addition, for a number of quality parameters, including loaf height, dough extensibility, and  $R_{max}$ , rising concentrations of N in grain were associated with the deterioration of breadmaking quality.

In conclusion, our study showed that high grain S concentrations and low grain N:S ratios increased the concentration of soluble glutenin in flour and reduced the ratio of insoluble to soluble glutenin in flour. As a result, there were probably more cysteine residues available for the production of disulphide and other types of bonds, producing dough that was more extensible and pliable, ultimately producing bread loaves of better quality. According to these results, it is evident that the S nutrition of grain, measured as total S concentration and N:S ratio should be considered, in addition to the concentration of N, in the quality evaluation of CWRS wheat grain grown in western Canada. However, in the evaluation of grain for quality, N:S ratio should be used with caution because the same ratio of N to S can be obtained at totally different N and S

concentrations in grain and the surplus of one nutrient may falsely indicate a deficient of the other nutrient. Furthermore, a rapid test for the estimation of S in grain, similar to the determination of protein in grain, is required. The combustion method used in our experiment, although accurate, is not a suitable method for use at local grain elevators where the initial determination of grain quality is made.

#### 4. PREDICTION OF BREADMAKING QUALITY RESPONSES TO SULPHUR FERTILIZATION FOR CANADA WESTERN RED SPRING WHEAT IN WESTERN CANADA

##### 4.1. Abstract

Canada Western Red Spring wheat (*Triticum aestivum* L. cv. AC Barrie) was grown at twelve locations over two growing seasons across western Canada to study the impact of S fertilization on grain yield and quality of wheat. Treatments consisted of two rates of fertilizer S (0 and 20 kg ha<sup>-1</sup>) as ammonium sulphate and two rates of fertilizer N (26 and 100 kg ha<sup>-1</sup>) as urea in a factorial design. Application of 20 kg S ha<sup>-1</sup> significantly improved grain yield at only two of twelve sites. Sulphur fertilization significantly increased the concentration of S in grain at six of twelve sites and reduced the N:S ratio in grain at eight of twelve sites.

Sulphur fertilization increased grain yield at two of seven sites used for breadmaking quality evaluation. Application of S fertilizer also improved the breadmaking quality, dough rheological quality, and protein composition of wheat at four of these seven sites. All four sites where quality improvements were observed contained < 40 kg SO<sub>4</sub>-S ha<sup>-1</sup> prior to fertilization, a concentration of soil S regarded as marginally sufficient for grain yield. Also, at these four marginal S sites, the S concentration and N:S ratio of plant tissue samples collected at 50 % heading was < 0.15 % S and > 17:1, respectively. Sulphur fertilization increased the concentration of S in grain and reduced the N:S ratio in grain at these four sites, even though S fertilization improved grain yield at only two of the sites. The improvements in grain S nutrition



were accompanied by significant improvements in loaf volume at two of the four marginal S sites when S fertilizer was applied in combination with 26 or 100 kg N ha<sup>-1</sup>, and at one more site where 100 kg N ha<sup>-1</sup> was applied. Sulphur fertilization increased loaf height and oven spring at three of the four sites. Application of S fertilizer also significantly increased dough extensibility at all four marginal S sites and reduced  $R_{max}$  and mixograph peak time at three of four sites. Mixograph peak time was significantly reduced at the other site only in the presence of 100 kg N ha<sup>-1</sup>. Furthermore, S fertilization reduced the viscoelastic ratio and mixograph work input to peak at all four marginal S sites. Sulphur fertilization increased the proportion of soluble glutenin in flour and reduced the ratio of insoluble to soluble glutenin in the flour at three of four marginal S sites. Sulphur fertilization in the presence of 100 kg N ha<sup>-1</sup> only, increased the proportion of soluble glutenin in flour and reduced the ratio of insoluble to soluble glutenin in the flour at the other marginal S site. Finally, at the three sites where soil SO<sub>4</sub>-S concentrations were > 40 kg ha<sup>-1</sup>, no yield and few breadmaking quality improvements were observed in response to S fertilization. At these high S sites, S fertilization did not increase the S concentration in grain and reduced the N:S ratio in grain at one site.

Where the concentration of NO<sub>3</sub>-N in soil was low, the majority of quality improvements due to S fertilization occurred only when 100 kg N ha<sup>-1</sup> was applied. Where soil NO<sub>3</sub>-N concentrations were medium to high, quality responses to S fertilization were observed when either 26 or 100 kg N ha<sup>-1</sup> was applied, providing evidence that grain quality responses to S fertilization are enhanced at high concentrations of plant available N due to the balancing effect of S fertilization on the N:S ratio in grain.

At all four sites where grain contained ≤ 0.17 % S and an N:S ratio > 17:1, quality improvements due to S fertilization were consistently observed. At the three sites where grain

contained S concentrations  $\geq 0.17$  % S and N:S ratios  $< 17:1$ , breadmaking quality responses to S fertilization were infrequent.

In summary, S fertilization, especially in the presence of high N fertility and marginal S fertility, increased grain S concentration, reduced grain N:S ratio, and improved the breadmaking quality CWRS wheat grown in western Canada. These breadmaking quality improvements were due to decreased dough strength and increased dough extensibility resulting from the increased synthesis of soluble glutenin and the reduction in the ratio of insoluble to soluble glutenin in the flour. Breadmaking quality responses to S fertilization were more frequent than grain yield responses to S fertilization. For processing CWRS wheat, grain containing  $\leq 0.17$  % S and an N:S ratio  $> 17:1$  should be regarded as deficient in S for maximum grain quality. For production of high quality CWRS wheat, S fertilizer should be applied when the soil contains  $< 40$  kg  $\text{SO}_4\text{-S}$   $\text{ha}^{-1}$  or when plant tissue samples collected at 50 % heading contain S concentrations and N:S ratios  $< 0.15$  % S and  $> 17:1$ , respectively.

## **4.2. Introduction**

Deficiencies of S were first identified in Canada in 1927 on Gray-wooded, Luvisolic soils in the province of Alberta (Doyle and Cowell 1993). Up to 30 % of cultivated soils in the Prairie Provinces are now estimated to be deficient in S for both canola and legume production (Bettany et al. 1982) and marginally sufficient for cereal production. The majority of S deficiencies are found in the Gray Luvisolic soil zone but extend into the Dark Gray and Black Chernozemic soil zones where the soils contain low concentrations of organic matter, are coarse-textured, well-

drained, and intensively cropped (Bailey 1987). Nyborg (1968) suggested that many of the soils known to be deficient in S for alfalfa may also provide insufficient S for optimum cereal growth.

The incidence of S deficiency on the Prairies is increasing as a result of a number of factors. Reduced S inputs from the atmosphere (Zhao et al. 1999c), less indirect application of S in N and P fertilizers (Tisdale et al. 1986, Zhao et al. 1999c), coupled with accelerated rates of crop uptake by high yielding S using crops (Bettany et al. 1982, Doyle and Cowell 1993), leaching of sulphate from the rooting zone (Doyle and Cowell 1993), and less mineralization of organic S from soil organic matter (Doyle and Cowell 1993) have contributed to increased S deficiency.

For wheat, severe S deficiencies can reduce yields. However, according to Anderson (1966), due to the relatively modest requirements of cereals for S, approximately 11 kg S ha<sup>-1</sup> in the top two feet of soil would be adequate for maintaining maximum yields of cereal crops. In a field experiment, Hamm (1969) also concluded that a critical level of approximately 11 kg S ha<sup>-1</sup> to the two-foot soil depth at seeding was sufficient for cereals. A number of critical thresholds have also been proposed for wheat tissue in relation to grain yield. For grain yield of field grown wheat, Spencer and Freney (1980) obtained critical values in whole plant shoots at stem elongation of 1.5 mg g<sup>-1</sup> for total S, 11 % for percent of total S as sulphate-S, and 19:1 for N:S ratio. Westfall et al. (1990) found critical values of total S in whole plant of wheat for grain yield to be 2.2 mg g<sup>-1</sup> at plant tillering, 1.9 mg g<sup>-1</sup> at stem elongation, and 1.5 mg g<sup>-1</sup> at booting. These researchers also noted that a higher critical value of 1.9 mg g<sup>-1</sup> value needs to be used if only the flag leaf is analyzed at booting. Finally, in Australia, Randall et al. (1981) found that grain with a S concentration less than 0.12 % S and an N:S ratio greater than 17:1 was deficient in S for yield.

Sulphur is important for wheat quality. Low S fertility results in the formation of S-poor polypeptides at the expense of more S-rich polypeptides. Researchers have shown that S deficiency in wheat causes increases in the relative proportions of HMW glutenin subunits and S-poor  $\omega$ -gliadins. At the same time, there is a decrease in the relative proportions of LMW glutenin subunits,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins, albumins, and globulins which are rich in S (Castle and Randall 1987, Fullington et al. 1987, MacRitchie and Gupta 1993, Moss et al. 1981, Wrigley et al. 1980, 1984). For wheat grown in Australia and the U.K., these changes in protein composition reduced the dough's capacity to form intermolecular disulphide bonds and weakened bonds of other types causing the deterioration of dough extensibility and an increase in dough strength (Fullington et al. 1987; MacRitchie and Gupta 1993; Moss et al. 1981, 1983; Wrigley et al. 1984; Zhao et al. 1999a, 1999b). A number of studies report significant increases in dough extensibility and reductions in dough strength due to S fertilization (Moss et al. 1981, Wooding et al. 2000, Zhao et al. 1999a, 1999b).

Due to the compositional changes in wheat protein and the subsequent deterioration in dough quality as a result of S deficiencies, baking quality of wheat also declines. In tests conducted at the University of Alberta, the largest loaves of the best quality bread were obtained when wheat had been grown after legumes on plots which had received S fertilizer. The poorest bread was obtained from wheat grown on fallow land or on legume stubble where no effective S fertilizer was applied (Newton et al. 1959). More recently, greenhouse and field experiments conducted in Europe and Australia provided additional evidence that S fertilization improves loaf volume (Byers et al. 1987, Haneklaus et al. 1992, Moss et al. 1981, Schnug et al. 1993, Zhao et al. 1999a, 1999b). Zhao et al. (1999a, 1999b) also found that responses of breadmaking quality (loaf volume) to S fertilization were more common than responses in grain yield.

Breadmaking quality losses due to severe S deficiency are probably less frequent than quality changes due to excessive N fertilization, especially where soil S supplies are marginal (Randall and Wrigley 1986). There is evidence that imbalances of N to S in the grain, due to S deficiencies or high rates of N fertilization, are associated with a reduction in dough extensibility and increase in dough resistance (Wrigley et al. 1984). Wooding et al. (2000) demonstrated that laboratory and industrial optimum mechanical dough development work input increased when N fertilizer was applied to wheat without S fertilizer; however, with combined N and S fertilization, the work input remained close to levels for grain grown without fertilizer. Early work in Manitoba also demonstrated that the physical dough characteristics and baking quality of wheat deteriorated when the N content of the grain was extremely high (Bushuk et al. 1978, Tipples et al. 1977). In addition, reconstituted baking tests (to compare gluten baking quality independently of protein quantity) performed by Timms et al. (1981) demonstrated that wheat produced on plots receiving high rates of N fertilizer produced smaller loaves than grain produced on less intensively fertilized plots. These results suggest that for wheat grown where high levels of N fertilizer are applied in the absence of S fertilizer, there may be a change in the balance between available N and S, such that the available S levels become insufficient for normal grain development. As a result, grain protein quantity may not be affected, but protein quality may be adversely affected due to imbalances in the ratio of N to S in the grain. Research in Europe has also demonstrated that deterioration in loaf volume occurs as a result of rising ratios of N to S in grain (Byers et al. 1987, Schnug et al. 1993, Zhao et al. 1999a).

The general objective of this part of our study was to investigate the impact of S fertilization on the dough rheological and breadmaking properties of CWRS wheat grown under the conditions and agronomic practices generally used in western Canada. The first specific

question we attempted to answer in this particular study was: does S fertilization improve the breadmaking quality of CWRS wheat where the soil S fertility is sufficient for grain yield? The second specific question we attempted to answer is: under what conditions should western Canadian producers apply S fertilizer to improve the breadmaking performance of CWRS wheat?

### **4.3. Methods and Materials**

The methods and materials used in the field experiments and quality analyses are described in Chapter 3 (section 3.3.). In addition, in 1999 and 2000, fifteen whole plants were collected from each plot at the 50 % heading stage (Feekes 10.3 stage). At Rosebank, Glenboro, and Erickson in the 2000 growing season only, fifteen whole plants were also collected from each plot at the 4 – 6 leaf stage (Feekes stages 1.4 to 1.6). The plants were cut at the base, just above the soil surface. These plants were dried at 60°C for 12 – 24 hours. Immediately after drying, the samples were ground with a Wiley Mill to pass a 2 mm sieve and analyzed for total N and S by combustion using a Leco CNS Analyzer (Leco Corporation 1996). The moisture content of the ground samples was also determined and the N and S concentrations were converted to dry matter basis. The ratio of N to S was calculated from the N and S concentrations.

Analysis of variance (ANOVA) and calculation of least significant difference values (LSD's) were conducted using the PROC GLM procedure (SAS Institute Inc. 1999). Single degree of freedom contrasts were used to further analyze treatment effects. In the ANOVA for the treatment effects on grain yield, all yields from replicate 1 of Erickson in 1999 were

discarded due to severe deer damage. At Brandon South, the ANOVA for treatment effects on grain quality was conducted only for the S treatment in combination with the high rate of N fertilizer because the low N fertilizer treatments were downgraded to a #3 CWRS grade. Only three replicates from Rosebank in 2000 graded #1 or #2 CWRS and were used in the ANOVA for treatment effects on grain quality.

#### **4.4. Results and Discussion**

##### **4.4.1. Site Characterization and Growing Conditions**

Table 3.1 summarizes the average  $\text{SO}_4\text{-S}$  and  $\text{NO}_3\text{-N}$  concentrations in soil prior to fertilization at the field sites for 1999. None of the sites would be regarded as S-deficient based on the traditional soil  $\text{SO}_4\text{-S}$  test to a sampling depth of 60 cm. Athabasca, Erickson, Brandon South, and Melfort contained medium supplies of soil S for the production of spring wheat. Kelvington was the only site that was very high in S fertility. The N fertility of Brandon South and Erickson was very low; Athabasca contained medium concentrations of N; and Kelvington and Melfort contained high concentrations of N.

In 1999, overall growing conditions were excellent at Melfort. Brandon South, Kelvington, and Erickson had good growing conditions. However, at Kelvington, poor separation between the seed and pre-plant banded urea fertilizer, in combination with the high soil pH, resulted in seedling damage due to ammonia toxicity under the high N treatments. This toxicity delayed grain maturity by approximately two weeks and, even though the growing conditions were quite good, frost damaged the crop late in the season. Finally, growing conditions at Athabasca were poor due to lack of precipitation and drought conditions.

In 2000, no sites tested low in S fertility (Table 3.2) and Erickson was the only site that contained medium levels of S. Soil SO<sub>4</sub>-S concentrations tested high to very high at the remaining sites, although, at Athabasca and Archerwill, the concentrations of SO<sub>4</sub>-S were just above the medium S range for the production of spring wheat. The range in N fertility of the sites in 2000 was wide (Table 3.2). Erickson and Brandon North contained very low concentrations of N; Archerwill contained low levels of N; Glenboro and Brandon South contained medium levels of N; and Rosebank and Athabasca contained high concentrations of N.

Growing conditions were good at all locations during the 2000 growing season; all sites received adequate precipitation and heat. However, at Rosebank, poor separation between the seed and pre-plant banded urea fertilizer resulted in some initial effects of ammonia toxicity on the seedlings grown under the high N treatments. Later in the growing season, the wheat seemed to compensate for the damage and, at maturity, there were no visual symptoms of any ammonia toxicity effects.

#### **4.4.2. Grain Grades**

As mentioned in the Materials and Methods section of Chapter 3 (section 3.3.3), all grain samples were graded according to the grading standards set by the Canadian Grain Commission (Table 3.5). Milling and breadmaking tests were carried out only on samples meeting #1 and #2 CWRS wheat grading standards. Therefore, only grain samples from Erickson, Melfort, and Athabasca in 1999 and from Archerwill, Brandon South, Brandon North (only high N treatments), and Rosebank in 2000 (only three of four replicates) were analyzed for breadmaking quality.



#### 4.4.3. Grain Yield

Significant, positive grain yield responses to S fertilization occurred at only two of twelve sites in 1999 and 2000 (Appendix Table E.1), including two of seven sites where breadmaking quality was measured (Table 4.1). At Athabasca and Melfort in 1999, there were yield improvements of 142 kg ha<sup>-1</sup> and 515 kg ha<sup>-1</sup>, respectively. However, at both locations, the overall significant positive yield response to S fertilization was mostly due to the yield response when S fertilizer was applied with the high rate of N fertilization (Appendix Table E.1).

According to the soil tests (Table 3.1), Athabasca and Melfort in 1999 both contained medium concentrations of soil SO<sub>4</sub>-S; therefore, yield responses at these sites were not expected. For example, both sites contained significantly greater concentrations of SO<sub>4</sub>-S than 11 kg S ha<sup>-1</sup> in the top two feet of soil, which Anderson (1966) and Hamm (1969) found to be sufficient for cereal production. Grain yields at Erickson and Brandon South in 1999 were not improved by S fertilization even though the SO<sub>4</sub>-S concentrations at these sites were also in the same medium range for wheat production. The Kelvington site in 1999 contained very high soil SO<sub>4</sub>-S levels; therefore, no yield response was expected or observed at this site. The lack of positive yield responses in 2000 was probably due to the relatively high concentrations of SO<sub>4</sub>-S in the soil at all sites (Table 3.2). The only 2000 site that contained medium concentrations of soil SO<sub>4</sub>-S was Erickson; all other sites contained high to very high concentrations of SO<sub>4</sub>-S.

Over the two growing seasons, N fertilization increased grain yield at four of the seven sites where breadmaking quality was evaluated (Table 4.1). Grain yield responses to the increased rate of N fertilization were also observed at Kelvington in 1999 and Erickson in 2000 (Appendix Table E.1). The yield responses were positive at Erickson in 1999 and 2000, Brandon North in 2000, and Archerwill in 2000 because soil NO<sub>3</sub>-N concentrations were low to very low

(Tables 3.1 and 3.2). Positive grain yield responses to N fertilization were also expected at Brandon South in 2000, where concentrations of NO<sub>3</sub>-N were medium. At Brandon South and Athabasca in 1999 as well as Glenboro in 2000, where the soil N fertility was also low to medium, grain yield responses were also expected but not observed. A reduction in yield occurred at Kelvington in 1999 due to ammonia toxicity effects. Finally, as the soil test predicted, grain yield was not improved by N fertilization at Melfort in 1999 and Athabasca and Rosebank in 2000, where soil NO<sub>3</sub>-N concentrations were very high for the production of wheat.

One of the main objectives of this study was to determine if grain quality responses to S fertilization occurred when yield responses to S fertilization did not. Table 4.1 summarizes the analysis of variance for the fertilizer treatment effects for the measured yield and quality parameters for the seven sites where grain quality was measured. In an attempt to answer this question systematically, sites were grouped together in the table according to the S fertility of the site and the observed overall response of grain yield and quality to S fertilization. The “adequate S” group consisted of sites that contained > 40 kg SO<sub>4</sub>-S ha<sup>-1</sup> and included Brandon North, Rosebank, and Brandon South from 2000. Sulphur fertilization resulted in no significant yield increases and very few quality improvements at these sites. The “marginal S” group consisted of sites containing < 40 kg SO<sub>4</sub>-S ha<sup>-1</sup> and included Athabasca, Erickson, and Melfort from 1999 and Archerwill from 2000. The marginal S group was further divided into two sub-groups; one made up of Athabasca and Melfort where grain yield responses to S fertilization were observed (marginal S, yield responsive). The other sub-group was made up of Erickson and Archerwill, where no yield responses to S fertilization were observed (marginal S, yield unresponsive).

Table 4.1. Summary of the analysis of variance for the N and S fertilization effects and the N x S interactions on the different yield and quality parameters for sites where quality analyses were conducted in 1999 and 2000

Variable	Sites With Marginal Soil Test S (< 40 kg SO <sub>4</sub> -S ha <sup>-1</sup> )												Sites With Adequate Soil Test S (> 40 kg SO <sub>4</sub> -S ha <sup>-1</sup> )									
	Yield Responsive						Yield Unresponsive						Yield Unresponsive									
	Athabasca (1999)			Melfort (1999)			Erickson (1999)			Archerwill (2000)			Brandon N (2000)			Rosebank (2000)			Brandon S (2000)			
Soil SO <sub>4</sub> -S to 60 cm (kg ha <sup>-1</sup> )	26 (M) <sup>†</sup>			32 (M)			26 (M)			36 (H)			48 (VH+)			149 (VH+)			296 (VH+)			
Soil NO <sub>3</sub> -N to 60 cm (kg ha <sup>-1</sup> )	60 (M) <sup>†</sup>			144 (VH+)			24 (VL)			40 (L)			30 (VL)			72 (H)			61 (M)			
	N	S	N*S	N	S	N*S	N	S	N*S	N	S	N*S	N	S	N*S	N	S	N*S	N	S	N*S	
Grain Yield	ns	* (+)	ns	ns	* (+)	ns	* (+)	ns	ns	** (+)	ns	ns	*** (+)	ns	ns	ns	ns	ns	ns	* (+)	ns	ns
Grain S Concentration	ns	*** (+)	ns	ns	*** (+)	ns	ns	* (+)	ns	ns	*** (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Grain N Concentration	*** (+)	** (+)	ns	* (+)	ns	ns	*** (+)	ns	ns	*** (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Grain N:S Ratio	ns	*** (-)	ns	* (+)	*** (-)	ns	* (+)	** (-)	ns	*** (+)	*** (-)	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Flour S Concentration	ns	*** (+)	ns	ns	*** (+)	*	ns	* (+)	ns	* (+)	*** (+)	***	ns	** (-)	ns	ns	ns	ns	ns	ns	ns	ns
Flour N Concentration	** (+)	** (+)	ns	* (+)	ns	ns	** (+)	ns	ns	** (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	*** (+)	* (+)	ns
Flour N:S Ratio	* (+)	*** (-)	ns	* (+)	*** (-)	ns	* (+)	* (-)	ns	*** (+)	*** (-)	***	ns	ns	ns	ns	ns	ns	ns	* (+)	ns	ns
SDS Sedimentation Volume	* (-)	*** (+)	ns	ns	* (+)	ns	ns	ns	**	ns	*** (+)	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Flour Yield	ns	* (+)	ns	ns	* (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Loaf Height	ns	*** (+)	ns	ns	** (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Loaf Volume	ns	*** (+)	ns	ns	** (+)	ns	ns	ns	ns	*	ns	*	ns	ns	ns	ns	ns	ns	ns	* (-)	ns	ns
Oven Spring	* (-)	*** (+)	ns	ns	** (+)	ns	ns	ns	ns	** (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Crumb Fineness Score	* (-)	ns	ns	ns	ns	ns	ns	ns	ns	ns	*** (+)	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Crumb Elongation Score	ns	* (-)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
25% Loaf Compression	ns	** (-)	ns	ns	ns	ns	*** (-)	ns	*	** (-)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
40% Loaf Compression	ns	* (-)	ns	ns	ns	ns	*** (-)	ns	ns	** (-)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Proof Height	ns	ns	ns	ns	ns	ns	ns	ns	ns	** (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Extensibility (Ext)	ns	*** (+)	ns	ns	** (+)	ns	ns	** (+)	ns	ns	** (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Maximum Resistance (R <sub>max</sub> )	* (+)	** (-)	*	* (+)	*** (-)	ns	ns	* (-)	ns	* (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R <sub>max</sub> /Ext	* (+)	*** (-)	*	* (+)	*** (-)	*	ns	* (-)	ns	ns	* (-)	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
Extensigraph Peak Area	ns	* (+)	ns	ns	ns	ns	ns	ns	ns	** (+)	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mixograph Peak Height	*** (+)	*** (+)	***	ns	ns	ns	** (+)	ns	ns	*** (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	** (+)	ns	*
Mixograph Peak Time	ns	*** (-)	ns	ns	*** (-)	**	ns	ns	**	ns	*** (-)	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mixograph Work Input to Peak	* (+)	*** (-)	ns	* (+)	*** (-)	*	** (+)	* (-)	ns	** (+)	*** (-)	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mixograph Total Work Input	* (+)	*** (+)	**	ns	ns	ns	** (+)	ns	ns	*** (+)	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mixograph Peak Width	ns	*** (+)	ns	ns	ns	ns	* (+)	* (-)	ns	*** (+)	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Farinograph Absorption	ns	*** (+)	ns	ns	ns	*	* (+)	ns	ns	*** (+)	* (-)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dough Development Time	ns	*** (+)	ns	ns	* (+)	ns	ns	ns	ns	*** (+)	** (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mixing Tolerance Index	ns	ns	ns	ns	*** (+)	ns	ns	ns	ns	ns	* (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dough Stability	ns	ns	ns	ns	*** (-)	ns	ns	ns	ns	ns	* (+)	ns	ns	ns	ns	ns	ns	ns	ns	** (-)	ns	ns
Time to Breakdown	ns	*** (+)	ns	ns	ns	ns	ns	ns	ns	** (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	* (+)	ns	ns
Monomeric Protein Content	ns	*** (-)	ns	ns	*** (-)	**	ns	ns	ns	*** (+)	ns	ns	ns	ns	ns	ns	ns	ns	ns	* (+)	ns	ns
Soluble Glutenin Content (SG)	ns	*** (+)	ns	ns	*** (+)	ns	ns	ns	ns	* (+)	* (-)	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Insoluble Glutenin Content (IG)	** (-)	** (+)	ns	ns	ns	*	ns	ns	ns	*** (-)	*** (+)	***	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
IG/SG	ns	*** (-)	ns	ns	** (-)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Residue Protein Content	** (+)	** (-)	ns	ns	ns	ns	ns	ns	ns	*** (+)	*** (-)	**	ns	* (+)	ns	ns	ns	ns	**	ns	ns	ns

<sup>†</sup> ratings for wheat production according to the Manitoba Soil Fertility Guide (VL = very low; L = low; M = medium; H = high; VH = very high)  
 \*, \*\*, and \*\*\* Significant at the 0.05, 0.01, and 0.001 levels, respectively  
 note - symbol in parenthesis represents direction of change in the measurement as a result of N and S fertilization

Table 4.2. Summary of contrasts for the effect of sulphur fertilization at the different levels of N fertilization for selected quality parameters in 1999 and 2000

Quality Variable	Treatments Contrasted	Marginal S, Yield Responsive Sites		Marginal S, Yield Unresponsive Sites		Adequate S, Yield Unresponsive Sites		
		Athabasca (1999)	Melfort (1999)	Erickson (1999)	Archerwill (2000)	Brandon N (2000)	Rosebank (2000)	Brandon S (2000)
Soil SO <sub>4</sub> -S to 60 cm (kg ha <sup>-1</sup> )		26 (M) <sup>†</sup>	32 (M)	26 (M)	36 (H)	48 (VH+)	149 (VH+)	296 (VH+)
Soil NO <sub>3</sub> -N to 60 cm (kg ha <sup>-1</sup> )		60 (M) <sup>†</sup>	144 (VH+)	24 (VL)	40 (L)	30 (VL)	72 (H)	61 (M)
Grain S Concentration (%) <sup>†</sup>		0.17	0.14	0.17	0.14	0.17	0.20	0.21
Grain N:S Ratio <sup>†</sup>		22.8	22.4	17.9	21.1	16.2	15.6	16.3
S Conc. of Whole Plants at 50 % Heading (%) <sup>†</sup>		0.10	0.12	0.14	0.11	0.10	0.20	0.15
N:S Ratio of Whole Plants at 50 % Heading <sup>†</sup>		22.5	18.2	17.8	20.9	12.7	13.6	11.9
Pr>F								
Grain S Concentration	0 S vs 20 S @ 26 N	0.0001**	0.012*	0.31	0.23	0.61	0.030*	0.88
	0 S vs 20 S @ 100 N	0.0001**	0.0008**	0.025*	0.0001**	0.65	0.97	0.94
Grain N Concentration	0 S vs 20 S @ 26 N	0.0053**	0.42	0.14	0.57	0.24	0.12	0.44
	0 S vs 20 S @ 100 N	0.22	0.83	0.15	0.18	0.54	0.45	0.78
Grain N:S Ratio	0 S vs 20 S @ 26 N	0.0003**	0.012*	0.17	0.094	0.031*	0.79	0.056
	0 S vs 20 S @ 100 N	0.0001**	0.001**	0.0076**	0.0001**	0.015*	0.44	0.45
Flour S Concentration	0 S vs 20 S @ 26 N	0.0001**	0.0063**	0.35	0.077	-	0.16	0.14
	0 S vs 20 S @ 100 N	0.0001**	0.0001**	0.007**	0.0001**	0.57	1.00	0.11
Flour N Concentration	0 S vs 20 S @ 26 N	0.013*	0.84	0.15	0.63	-	0.35	0.74
	0 S vs 20 S @ 100 N	0.0063**	0.53	0.56	0.72	0.61	0.33	0.68
Flour N:S Ratio	0 S vs 20 S @ 26 N	0.0001**	0.019*	0.18	0.34	-	0.52	0.39
	0 S vs 20 S @ 100 N	0.0001**	0.0006**	0.020*	0.0001**	0.055	0.034*	0.36
SDS Sedimentation Volume	0 S vs 20 S @ 26 N	0.0001**	0.34	0.07	1.00	-	0.61	0.65
	0 S vs 20 S @ 100 N	0.0001**	0.029*	0.011*	0.0001**	0.61	0.44	0.72
Loaf Height	0 S vs 20 S @ 26 N	0.0022**	0.048*	0.89	0.75	-	0.083	0.91
	0 S vs 20 S @ 100 N	0.0018**	0.0042**	0.25	0.003**	0.20	0.50	0.48
Loaf Volume	0 S vs 20 S @ 26 N	0.0003**	0.11	0.49	0.65	-	0.032*	0.77
	0 S vs 20 S @ 100 N	0.0001**	0.0037**	0.37	0.027*	0.12	0.53	0.15
Oven Spring	0 S vs 20 S @ 26 N	0.0007**	0.021*	0.80	0.04*	-	0.13	0.48
	0 S vs 20 S @ 100 N	0.0008**	0.03*	0.19	0.0001**	0.77	0.097	0.48
Extensibility	0 S vs 20 S @ 26 N	0.01**	0.043*	0.053	0.65	-	0.068	0.41
	0 S vs 20 S @ 100 N	0.0006**	0.0024**	0.023*	0.0007**	0.21	0.40	0.40
R <sub>max</sub>	0 S vs 20 S @ 26 N	0.19	0.015*	0.62	0.71	-	0.15	0.49
	0 S vs 20 S @ 100 N	0.0012**	0.0011**	0.018*	0.17	0.28	0.032*	0.074
R <sub>max</sub> /Ext	0 S vs 20 S @ 26 N	0.021*	0.0094**	0.27	0.84	-	0.90	0.33
	0 S vs 20 S @ 100 N	0.0002**	0.0002**	0.011*	0.011**	0.23	0.23	0.41
Mixograph Peak Time	0 S vs 20 S @ 26 N	0.0006**	0.0018**	0.18	0.28	-	0.87	0.48
	0 S vs 20 S @ 100 N	0.0002**	0.0001**	0.007**	0.0001**	0.29	0.30	0.62
Mixograph Work Input to Peak	0 S vs 20 S @ 26 N	0.0034**	0.015*	0.84	0.054	-	0.69	0.77
	0 S vs 20 S @ 100 N	0.01**	0.0004**	0.012*	0.0001**	0.14	0.34	0.70
Monomeric Protein in Flour	0 S vs 20 S @ 26 N	0.0001**	0.35	0.84	0.78	-	0.41	0.61
	0 S vs 20 S @ 100 N	0.0002**	0.0001**	0.02*	0.002**	0.87	0.80	0.52
Soluble Glutenin in Flour	0 S vs 20 S @ 26 N	0.0001**	0.017*	0.66	0.46	-	0.043*	0.47
	0 S vs 20 S @ 100 N	0.0001**	0.0005**	0.025*	0.0001**	0.11	0.25	0.70
IG/SG in Flour	0 S vs 20 S @ 26 N	0.0001**	0.16	0.84	0.66	-	0.0071**	0.79
	0 S vs 20 S @ 100 N	0.0001**	0.011*	0.05*	0.0001**	0.031*	0.088	0.66

<sup>†</sup> ratings for wheat production according to the Manitoba Soil Fertility Guide (VL = very low; L = low; M = medium; H = high; VH = very high)

<sup>†</sup> Means for the 100 kg N ha<sup>-1</sup> and 0 kg S ha<sup>-1</sup> treatment; S concentrations reported on dry matter basis

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

#### 4.4.4. Grain Nutrition

Over the two growing seasons grain S concentrations ranged from 0.127 % S to 0.231 % S. Therefore, no grain contained < 0.12 % S, the critical threshold that was determined by Randall et al. (1981) for the maintenance of grain yield in Australia. Sulphur fertilization significantly increased grain S concentration at six of twelve sites over the two growing seasons (Appendix Table E.2), including four of the seven sites where breadmaking quality was evaluated (Table 4.1). Sulphur fertilization increased the S concentration in grain at the marginal S, yield responsive sites of Athabasca and Melfort and at the marginal S, yield unresponsive sites of Erickson and Archerwill. At the latter two sites, where the soil N fertility was low, the increase in S concentration in grain was due primarily to S fertilization in combination with the 100 kg N ha<sup>-1</sup> treatment (Table 4.2).

Over the two growing seasons, application of 100 kg N ha<sup>-1</sup> increased the S content of grain at five of twelve sites (Appendix Table E.2), including only one site, Rosebank, where quality evaluation was conducted (Table 4.1). Nitrogen fertilization did not decrease the S concentration of grain at any site.

The ranges of grain N concentration were similar in both seasons, varying between 2.34 and 3.87 % N. The highest N concentrations were observed at Athabasca in 1999 where lack of precipitation limited grain yield, resulting in the production of extremely high grain N concentrations. The lowest N concentrations were produced in Archerwill in 2000. However, most grain samples contained sufficient N to meet the grain N standard of 2.7 % N (15.6 % protein on dry matter basis) set by the Canadian Grain Commission for quality.

The only site where S fertilization significantly increased the overall N concentration of grain was Athabasca in 1999 (Appendix Table E.3 and Table 4.1); this improvement was

significant at only the low rate of N fertilization (Table 4.2). Furthermore, at nine of twelve sites, S fertilization caused a slight, insignificant reduction in average grain N concentration. Application of 100 kg N ha<sup>-1</sup> significantly increased the concentration of N in the grain at ten of twelve sites (Appendix Table E.3), including five of the seven sites where quality was evaluated (Table 4.1).

Grain N:S ratios ranged from 13.3 to 25.0 over the two growing seasons. Grain produced at Athabasca in 1999 contained very high N:S ratios due to drought conditions and the resulting high grain N concentrations. At Melfort in 1999, high grain N:S ratios were produced from the low S treatments. At Archerwill in 2000, high grain N:S ratios were produced only from the high N, low S treatment. Most grain produced at the remaining sites in 1999 and 2000 contained N:S ratios < 17:1, the threshold developed by Randall et al. (1981) for grain yield in Australia.

Over the two growing seasons, S fertilization significantly reduced N:S ratios in grain at eight of twelve sites (Appendix Table E.4), including five of the seven sites where breadmaking quality evaluation was conducted (Table 4.1). At the marginal S, yield responsive sites of Athabasca and Melfort and at the marginal S, yield unresponsive sites of Erickson and Archerwill, S fertilization resulted in large reductions in grain N:S ratio. At Brandon South in 1999 as well as Glenboro and Brandon North in 2000, S fertilization, although significant, led to small reductions in grain N:S ratio (Appendix Table E.4). For five of the eight sites where an overall reduction in grain N:S ratio was observed, the reduction was due primarily to S fertilization on plots where the rate of N fertilization was high. These sites included Brandon South in 1999 and Glenboro and Erickson in 2000 (Appendix Table E.4) as well as Erickson in 1999 and Archerwill in 2000 (Table 4.2). Soil N concentrations at these sites ranged from very low to medium (Tables 3.1 and 3.2). Therefore, in spite of the lack of a significant interaction

between N and S fertilization in the analysis of variance at most sites, high rates of N fertilizer applied onto N deficient soil appeared to increase the impact of S fertilization on the N:S ratio in grain.

Application of 100 kg N ha<sup>-1</sup> caused grain N:S ratios to significantly increase at seven of twelve sites (Appendix Table E.4), including three of the seven sites where quality was evaluated (Table 4.1). At Kelvington in 1999, ammonia toxicity effects in the high N treatments caused an overall reduction in grain N:S ratio.

#### **4.4.5. Flour Nutrition**

Flour S concentrations varied between 0.123 % S and 0.210 % S over the two growing seasons. Sulphur fertilization significantly increased the S concentration in flour at all of the marginal S, yield responsive sites; at all of the marginal S, yield unresponsive sites; and at one adequate S site (Table 4.1). However, at the adequate S site of Brandon South, the increase in S nutrition of flour was small and not likely to have practical value (Appendix Table E.5). At the marginal S sites, the increases in flour S concentration were significantly larger. Furthermore, the overall increase in flour S concentration at Erickson and Archerwill, where the soil N fertility was low, was due mainly to the increase in flour S concentration when S fertilizer was applied at the high rate of N fertilization (Table 4.2).

Increasing the N fertilization rate from 26 to 100 kg N ha<sup>-1</sup> did not affect flour S concentration at any sites in 1999. However, in 2000, increasing the rate of N fertilization significantly increased flour S concentration at three sites (Table 4.1). Furthermore, the interaction between the N and S treatments was significant at Melfort and Archerwill. At both sites, increasing the rate of N fertilization decreased the concentration of S in flour in the absence

of applied S fertilizer, but increased the S concentration in flour in the presence of 20 kg S ha<sup>-1</sup> (Appendix Table E.5).

Flour N concentrations varied between 2.28 % N to 3.82 % N over the two growing seasons. The concentration of N in flour was significantly increased by the application of 100 kg N ha<sup>-1</sup> at all six sites where breadmaking quality and the impact of N fertilization were measured (Table 4.1). Sulphur fertilization increased flour N concentration at only Athabasca.

Flour N:S ratios ranged from 14 to 25.5 over the two growing seasons. Sulphur fertilization significantly reduced the N:S ratio in flour at the marginal S, yield responsive and marginal S, yield unresponsive sites (Table 4.1). At the adequate S sites, there is also evidence that S fertilization reduced flour N:S ratios, however, these reductions were not significant (Appendix Table E.7). In addition, at Erickson and Archerwill, where the soil N fertility was low, the overall significant effects of S fertilization on flour N:S ratio was due mainly to the reduction in flour N:S ratio at the 100 kg N ha<sup>-1</sup> rate (Table 4.2). Finally, over the two seasons, application of 100 kg N ha<sup>-1</sup> significantly increased the N:S ratio in flour at four of the six sites where breadmaking quality and N fertilization effects were measured (Table 4.1).

#### **4.4.6. Flour Yield and SDS Sedimentation Volume**

Flour yield is a measure of the milling quality of wheat. Generally, the higher the flour yield, the higher the milling quality. There was little impact of N and S fertilization on flour yield, except at Athabasca and Melfort, where S fertilization significantly increased flour yield (Table 4.1). The general lack of response of flour yield to S fertilization is consistent with the observations of Zhao et al. (1999b) who found that S fertilization significantly increased flour



yield for only one of three varieties. Increasing the rate of N fertilization had no effect on flour yield at any site in 1999 and 2000 (Table 4.1).

In a previous study, SDS sedimentation volume was found to correlate positively and strongly with loaf volume for a number of different wheat varieties (Axford et al. 1979). Furthermore, high SDS volumes are indicative of high gluten strength and quality (Kovacks 1985). The overall effect of S fertilization on SDS sedimentation volume was significant at three of seven locations over the two growing seasons (Table 4.1). At the marginal S, yield responsive sites and the marginal S, yield unresponsive site of Archerwill, S fertilization significantly increased SDS sedimentation volume. The increase in SDS sedimentation volume at Melfort and Archerwill was due mainly to S fertilization in combination with the high rate of N fertilization (Table 4.2). Furthermore, at Erickson, where the interaction between the S and N treatments was significant, S fertilization also significantly increased SDS sedimentation volume when combined with the high rate of N fertilization (Table 4.2). Therefore, the positive impact of S fertilization on SDS sedimentation volume at four of seven sites indicates potential improvements in breadmaking quality of grain.

Increasing the rate of N fertilization from 26 to 100 kg ha<sup>-1</sup> had no consistent effects on SDS sedimentation volume. At Athabasca, increasing the rate of N fertilization significantly reduced sedimentation volume (Table 4.1). At Rosebank, increasing the rate of N fertilization significantly increased sedimentation volume. At all other sites, increasing the rate of N fertilization had no significant impact on SDS sedimentation volume.

#### 4.4.7. Baking Quality

Bread loaves of high volume and quality are desirable from a consumer viewpoint. Therefore, baking quality analyses that measure loaf quality characteristics provide an indication of the overall baking quality of CWRS wheat. Loaf height ranged from 102 to 126 mm over the two growing seasons. Sulphur fertilization increased loaf height slightly at six of seven sites over the two growing seasons. However, significant increases in loaf height were observed at only the marginal S, yield responsive sites of Athabasca and Melfort and at the marginal S, yield unresponsive site of Archerwill (Table 4.1). At these three sites, S fertilization increased loaf height by 7.6 mm, 6.0 mm, and 3.2 mm, respectively. Furthermore, at Archerwill, where the soil N fertility was low, the interaction between the N and S fertilizer treatments was also significant and the overall increase in loaf height was mainly at the high rate of N fertilization (Table 4.2).

Increasing the N fertilization rate to 100 kg N ha<sup>-1</sup> had less effect on loaf height than adding 20 kg S ha<sup>-1</sup>. In addition, responses to the increased N fertilization rate were inconsistent over the two growing seasons. Of the six sites where breadmaking quality and the effect of the high rate of N fertilization were measured, one site responded with a significant reduction in loaf height and one site responded with a significant increase in loaf height (Table 4.1).

Loaf volume varied between 900 and 1230 cc over the two years. Similar to the observed responses of loaf height to S fertilization, application of S fertilizer slightly increased loaf volume at six of seven sites over the two years. The overall impact of S fertilization, however, was statistically significant at only the two marginal S, yield responsive sites (Table 4.1). At Athabasca and Melfort, the increases in loaf volume were 113 and 71.8 cc, respectively. Furthermore, at Melfort the response to S fertilization at the high N fertilization rate was responsible for most of the overall significant improvement in loaf volume (Table 4.2). At the

marginal S, yield unresponsive site of Archerwill, where the soil N fertility was low and where the overall impact of S fertilization on loaf volume was nearly significant, S fertilization increased loaf volume by 49 cc where the high rate of N fertilization was applied and was highly significant (Table 4.2). The increases in loaf volume due to S fertilization in our study are consistent with previous research from western Canada (Newton et al. 1959), Australia (Moss et al. 1981, 1983), and Europe (Byers et al. 1987, Zhao et al. 1999a, 1999b) where S fertilization also increased loaf volume.

Increasing the rate of N fertilizer from 26 to 100 kg N ha<sup>-1</sup> had less effect on loaf volume than applying 20 kg S ha<sup>-1</sup> (Table 4.1). Over the two growing seasons, the only site where loaf volume was improved by the high rate of N fertilization was Archerwill.

Proof height was not significantly impacted by S fertilization any of the sites (Table 4.1).

Oven spring is the rise in loaf height from the beginning to the end of the baking process. Some of the increases observed for loaf height and loaf volume in response to S fertilization are a result of increases in oven spring. At the same marginal S sites where loaf height and volume increased due to S fertilization, including the two yield responsive sites and the yield unresponsive site of Archerwill, oven spring was also significantly increased by S fertilization (Table 4.1). At the remaining four sites, S fertilization also increased oven spring; however, the increases were not statistically significant.

Application of 100 kg N ha<sup>-1</sup> reduced oven spring at five of six sites where breadmaking quality and the effect of the high rate of N fertilization were measured, with Athabasca demonstrating the only statistically significant reduction (Table 4.1). In addition, the interaction between the N and S fertilization treatments was highly significant at Archerwill where, in the absence of applied S fertilizer, the increased rate of N fertilization caused oven spring to decline.

However, in the presence of applied S fertilizer, the increased rate of N fertilization caused oven spring to increase at this site (Appendix Table E.12).

No other baking parameters demonstrated consistent responses to the S or N treatments. The crumb fineness score was not significantly affected by S fertilization at any site in either year (Table 4.1). The crumb elongation score was significantly reduced by S fertilization at only Athabasca (Table 4.1). From these crumb evaluation scores, it is evident that S fertilization had no consistent impact on the overall quality of the crumb, even though loaf height and volume were significantly increased at three of seven sites.

Crumb firmness at 25 % compression and 40 % compression were generally unaffected by the fertilization treatments. Sulphur fertilization significantly reduced the 25 and 40 % compression values at Athabasca, only (Table 4.1). In addition, for the 25 % compression value, the interaction between the N and S treatments was significant at Erickson, where firmness was significantly reduced by the application of S fertilizer only when the high rate of N was applied (Appendix Table E.15). At all other sites, S fertilization had no overall impact on the compression values.

#### **4.4.8. Dough Quality on the Extensigraph**

The extensigraph is a load-extension instrument that measures the strength and extensibility of dough during the stretching process (Shuey 1975). Sulphur fertilization had a stronger influence on dough extensibility over the two growing seasons than increasing the rate of N fertilization from 26 to 100 kg N ha<sup>-1</sup>. Over the two growing seasons, dough extensibility was significantly increased by S fertilization at the marginal S, yield responsive and marginal S, yield unresponsive sites (Table 4.1). Sulphur fertilization increased dough extensibility by 14.9,

10.9, 16.4, and 6.7 mm at Athabasca, Erickson, Melfort, and Archerwill, respectively. Furthermore, at Erickson and Archerwill, where the soil N fertility was low, most of the increase in dough extensibility due to S fertilization was at the high rate of N fertilization (Table 4.2). The observed increase in dough extensibility due to S fertilization is consistent with the observations made in Australia (Moss et al. 1981), New Zealand (Wooding et al. 2000), and England (Zhao et al. 1999a, 1999b) where S fertilization also increased dough extensibility.

At all sites in both seasons, increasing the N fertilization rate from 26 to 100 kg ha<sup>-1</sup> did not significantly affect dough extensibility (Table 4.1). These observations were not consistent with the observations of Zhao et al. (1999a), who noted that N fertilization significantly increased dough extensibility.

Maximum dough resistance is a measure of dough strength (Preston and Hosney 1991). Strong dough generally produces high  $R_{\max}$  values and weak dough produces low  $R_{\max}$  values. A number of previous studies conducted in Europe, Australia, and New Zealand have shown that S fertilization reduced dough resistance (Moss et al. 1981, Wooding et al. 2000, Zhao et al. 1999a, 1999b). In our study, over the two growing seasons, S fertilization reduced  $R_{\max}$  at all sites but significantly at only three of seven sites (Table 4.1). These included both marginal S, yield responsive sites and the marginal S, yield unresponsive site of Erickson. Furthermore, at Athabasca and Erickson, where the N fertility was medium and very low, respectively, S fertilization also had a much larger and more significant impact on  $R_{\max}$  when the high rate of N fertilizer was applied (Table 4.2).

The effect of N fertilization on  $R_{\max}$  was opposite to that observed for S fertilization. In 1999, increasing the rate of N fertilization from 26 to 100 kg N ha<sup>-1</sup> significantly increased  $R_{\max}$

at Athabasca and Melfort (Table 4.1). In 2000, the increased rate of N fertilization significantly increased  $R_{\max}$  at Archerwill.

The viscoelastic ratio, which is the ratio of  $R_{\max}$  to extensibility, provides a good indication of the overall strength and extensibility characteristics of dough (Preston and Hosney 1991). Dough with a high viscoelastic ratio tends to be quite strong (tough) and have poor extensibility properties. Dough with a low viscoelastic ratio tends to be relatively weak and highly extensible. Over the two growing seasons, S fertilization significantly reduced the viscoelastic ratio at all four marginal S sites (Table 4.1). At Erickson and Archerwill, where the N fertility was low, the overall reduction in viscoelastic ratio due to S fertilization occurred mainly at the high rate of N fertilization (Table 4.2).

Increasing the rate of N fertilization from 26 to 100 kg N ha<sup>-1</sup> significantly increased the viscoelastic ratio at Athabasca and Melfort (Table 4.1). The interaction between the N and S fertilization treatments at Athabasca and Melfort was also significant. In the absence of applied S fertilizer, increasing the rate of N fertilization increased the viscoelastic ratio substantially at both sites. In the presence of S fertilizer, increasing the rate of N fertilization, only slightly increased the viscoelastic ratio at both sites (Appendix Table E.20).

Sulphur fertilization had no consistent effect on extensigraph peak area (Table 4.1). At Athabasca and Brandon South, S fertilization significantly increased extensigraph peak area. At the five remaining sites, S fertilization reduced extensigraph peak area; however, the reductions were not statistically significant. Increasing the rate of N fertilization from 26 to 100 kg ha<sup>-1</sup> significantly increased extensigraph peak area at Archerwill and Rosebank. The interaction between the S and N treatments at Brandon South, Archerwill, and Rosebank was also significant, but did not follow any consistent pattern.

It is also important to note that dough produced from Erickson was weaker and more extensible than dough from the other marginal S locations (Appendix Tables E.18, E.19, and E.20). Although it is desirable to have moderately strong, extensible dough, the infection of fusarium head blight at Erickson (Table 3.5) probably weakened the dough too much, reducing the impact of S fertilization on the baking parameters. Dexter and Nowicki (in press) noted that the increase in concentration of proteolytic enzymes in the grain due to fusarium head blight infections causes dough properties to weaken substantially, leading to unsatisfactory baking performance. Therefore, in our study, the lack of baking quality responses to S fertilization at Erickson (Table 4.1), even though dough extensibility increased and  $R_{\max}$  declined, was probably due to fusarium head blight damage.

#### **4.4.9. Dough Quality on the Mixograph**

The mixograph is a dough-mixing instrument that records a number of dough mixing characteristics for the evaluation of dough strength (Kunerth and D'apponia 1985). Mixograph peak height is largely a function of grain protein content and dough strength (Lukow 1991), with strong dough producing larger mixograph peak heights than weaker dough. As a result, N fertilization had a greater effect on mixograph peak height than S fertilization in both years. Athabasca was the only site where S fertilization increased mixograph peak height (Table 4.1). Therefore, our observations are similar to those of Moss et al. (1981) who found no responses in mixograph peak height to S fertilization. Mixograph peak height was significantly increased by increasing the rate of N fertilization from 26 to 100 kg N ha<sup>-1</sup> at Athabasca, Erickson, Archerwill, and Rosebank (Table 4.1). In addition, at Athabasca, the interaction between the N and S treatments was also significant; the increase in peak height due to N fertilization was much

larger in the presence of applied S fertilizer than where no S fertilizer was applied (Appendix Table E.22).

Mixograph peak time measures dough strength (Shuey 1975). Over the two growing seasons, mixograph peak time was reduced by S fertilization at the two marginal S, yield responsive sites and at the marginal S, yield unresponsive site at Archerwill (Table 4.1). At Erickson, the other marginal S, yield unresponsive site, where the soil N fertility was very low, even though the overall effect of the S treatment was not significant, the interaction between the N and S treatments was highly significant; for wheat grown under the high N treatment only, S fertilization significantly reduced mixograph peak time (Table 4.2). In addition, at Archerwill where the soil N fertility was also low, due to the significant interaction between the N and S treatments, the reduction in mixograph peak time was due primarily to S fertilization at the high N rate (Table 4.2). Moss et al. (1981) also found that S fertilization significantly reduced mixograph peak time. Increasing the N fertilization rate from 26 to 100 kg ha<sup>-1</sup> had no significant effect on mixograph peak time at any site in 1999 and 2000 (Table 4.1).

Stronger, tougher dough also tends to require more work than weaker dough to reach mixograph peak consistency. Sulphur fertilization significantly reduced work input to peak at four of seven sites over the two growing seasons, including all of the marginal S sites (Table 4.1). However, at Erickson and Archerwill, where the soil N fertility was low, S fertilization had the greatest impact on work input to peak at the high rate of N fertilization (Table 4.2).

Increasing the rate of N fertilization from 26 to 100 kg N ha<sup>-1</sup> increased work input to peak at four of six sites where grain quality and the effect of N were measured, including all of the marginal S sites (Table 4.1).



The overall impact of S fertilization on total work input was significant at one of seven sites over the two growing seasons. At Athabasca, S fertilization significantly increased total work input (Table 4.1). At Archerwill, S fertilization increased total work input only where the high rate of N fertilizer was applied (Appendix Table E.26). Increasing the rate of N fertilization significantly increased total work input at four of six sites where grain quality and the effect of N were examined (Table 4.1).

Responses of mixograph peak width to S fertilization were not consistent. Sulphur fertilization significantly reduced mixograph peak width at Erickson and increased peak width at Athabasca (Table 4.1). At Archerwill, S fertilization increased mixograph peak width, but only at the high rate of N fertilization (Appendix Table E.24). No other significant responses to S fertilization were observed. Increasing the rate of N from 26 to 100 kg ha<sup>-1</sup> significantly increased mixograph peak width at Erickson and Archerwill (Table 4.1).

Finally, as was observed on the extensigraph, the mixograph parameters of peak time and work input to peak demonstrated that dough from Erickson was weaker than dough from the other marginal S locations (Appendix Tables E.23 and E.25). Again, demonstrating that the fusarium head blight damage at this location is probably responsible for the lack of breadmaking quality responses to S fertilization.

#### **4.4.10. Dough Quality on the Farinograph**

The farinograph is a dough-mixing instrument that records a number of dough mixing characteristics for the evaluation of dough strength and stability (Preston and Kilborn 1990). There were no consistent responses in any of the farinograph parameters to either S or N fertilization over the two growing seasons. For example, S fertilization significantly affected

farinograph absorption at only two of seven sites, increasing absorption slightly at Athabasca and reducing it slightly in Archerwill (Table 4.1). Increasing the rate of N fertilization from 26 to 100 kg ha<sup>-1</sup> significantly increased farinograph absorption at two of six sites over the two growing seasons, Erickson and Archerwill.

Mixing tolerance index, dough stability, and time to dough breakdown all provide some indication of dough's overall resistance to over-mixing and are estimates of dough stability (Shuey 1990). Sulphur fertilization did not have a large or consistent impact on these parameters. For example, S fertilization significantly increased mixing tolerance index (reduced dough stability) at two of seven sites, Melfort and Archerwill (Table 4.1). Sulphur fertilization significantly reduced dough stability at Melfort (Table 4.1), and nearly significantly at Archerwill (Appendix Table E.28). The effects of S fertilization on dough stability at Athabasca and Rosebank were also nearly significant ( $P = 0.054$  and  $0.053$ , respectively); however S fertilization increased dough stability at these two sites (Appendix Table E.28). Time to dough breakdown was significantly increased by S fertilization at Athabasca only (Table 4.1).

Increasing the rate of N fertilization from 26 to 100 kg ha<sup>-1</sup> also had no major impact on mixing tolerance index, dough stability, or time to dough breakdown. Nitrogen fertilization reduced mixing tolerance index at five of six sites where grain quality and the effect of N were measured; however, the reduction was significant at only Brandon South (Table 4.1). The same five sites where mixing tolerance index declined with the increased N fertilization rate demonstrated improvements in dough stability, with the improvement being significant only at Brandon South and Archerwill (Table 4.1). Furthermore, at these two sites, time to breakdown was also significantly increased due to increasing the rate of N fertilization (Table 4.1).

Dough development time is a function of the amount of water required for the dough to reach the 500 BU line on the farinograph. This parameter is strongly affected by the protein or N content of grain, and tends to increase with rising grain N concentrations and increasing dough strength (Ayoub et al. 1994, Dexter et al. 1994, Pechanek et al. 1997, Shuey 1990). In our study, S fertilization significantly increased dough development time at Athabasca, Melfort, and Archerwill (Table 4.1). Furthermore, increasing the rate of N fertilization from 26 to 100 kg N ha<sup>-1</sup> significantly increased dough development time at only two of six sites where grain quality and the effect of N were measured, Archerwill and Rosebank.

#### **4.4.11. Protein Fractionation**

Flour protein fractionation provides an indication of the compositional make up of flour. The soluble glutenin fraction is comprised primarily of LMW glutenin subunits as well as a small amount of HMW glutenin subunits and residual gliadins (Suchy 2002). According to Zhao et al. (1999c), the LMW glutenin subunits contain 2 to 3 mol % of cysteine; therefore, this fraction is considered to be composed of S-rich soluble glutenin subunits. Sulphur fertilization significantly increased the concentration of soluble glutenin in flour at all marginal S sites (Tables 4.1 and 4.2). At Athabasca and Melfort, where the N fertility was moderate to high, S fertilization significantly increased the concentration of soluble glutenin in flour at both rates of N fertilization (Table 4.2). However, at Erickson and Archerwill, where the N fertility was low, the increase in concentration of soluble glutenin in flour was only significant when S fertilizer was applied in the presence of the high rate of N fertilization (Table 4.2).

Increasing the rate of N fertilization from 26 to 100 kg ha<sup>-1</sup> significantly decreased the concentration of soluble glutenin in flour at Archerwill (Table 4.1). In addition, the interaction

between the N and S fertilization treatments was also significant because the high rate of N fertilization reduced the concentration of soluble glutenin substantially more in the absence of S fertilization than it did in the presence of S fertilization (Appendix Table E.32).

Over the two growing seasons, N or S fertilization had no consistent impact on the concentration of insoluble glutenin in flour. Sulphur fertilization significantly increased the concentration of insoluble glutenin in flour at Athabasca only (Table 4.1). At Athabasca, increasing the rate of N fertilization from 26 to 100 kg ha<sup>-1</sup> significantly reduced the concentration of insoluble glutenin in flour. At no other location were the overall effects of the fertilization treatments significant except Melfort, where the interaction between the N and S treatments was significant and S fertilization significantly reduced the concentration of insoluble glutenin in the flour when the rate of N fertilization was low (Appendix Table E.33).

Insoluble glutenin is comprised of both LMW and HMW glutenin subunits that are soluble only in the presence of a reducing agent (Suchy 2002). A balance between HMW glutenin subunits and LMW glutenin subunits is important in maintaining the integrity of the protein and is desirable for the purposes of breadmaking (Castle and Randall 1987, MacRitchie and Gupta 1993, Wrigley et al. 1980, 1984). According to Zhao et al. (1999c), the HMW glutenin subunits contain moderate concentrations of cysteine; therefore, the HMW glutenin fraction is not considered to be S-rich. In our study, the ratio of insoluble glutenin to soluble glutenin provides an estimate of the ratio of HMW to LMW glutenin subunits. Flour containing low ratios of insoluble to soluble glutenin usually tend to be less strong and more extensible than flour containing high ratios of insoluble to soluble glutenin (Suchy 2002). Over the two growing seasons, S fertilization significantly reduced the ratio of insoluble to soluble glutenin in flour at three of seven sites, including the marginal S, yield responsive sites of Athabasca and Melfort

and one marginal S, yield unresponsive site, Archerwill (Table 4.1). At Erickson, where the soil N fertility was low, S fertilization reduced the ratio of insoluble to soluble glutenin only at the high rate of N fertilization (Table 4.2). At Archerwill, where the soil N fertility was also low, S fertilization resulted in a larger reduction in the ratio of insoluble to soluble glutenin at the higher rate of N fertilization (Table 4.2). At Melfort, the response to S fertilization was also greatest when the high rate of N fertilizer was applied even though the soil contained very high NO<sub>3</sub>-N concentrations (Table 4.2). At Rosebank, there is also an indication that S fertilization may have reduced the insoluble to soluble glutenin ratio at the low rate of N fertilization; however, at the high N rate, the opposite trend was seen where the application of S fertilizer slightly increased the ratio (Appendix Table E.34). In addition, at Brandon North, S fertilization appeared to cause a small, but statistically significant increase in the ratio of insoluble to soluble glutenin in flour (Table 4.1).

Increasing the rate of N fertilization from 26 to 100 kg N ha<sup>-1</sup> did not have a large impact on the ratio of insoluble to soluble glutenin in flour. The only location where the increased rate of N fertilization significantly increased the ratio was at Archerwill (Table 4.1). The reason for this is that the application of N significantly reduced the concentration of soluble glutenin in the flour, thus increasing the overall balance between the insoluble and soluble glutenin fractions.

The monomeric protein fraction is composed of the gliadins ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\omega$ -gliadins), albumins, and globulins (Suchy 2002). According to Zhao et al. (1999c), the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins are considered to be S-rich and the  $\omega$ -gliadins are considered to be S-poor. Sulphur fertilization significantly reduced the concentration of monomeric proteins in flour at all of the marginal S sites (Tables 4.1 and 4.2). At Erickson and Archerwill, where the soil N fertility was

low, S fertilization had the greatest effect on the monomeric protein fraction when 100 kg N ha<sup>-1</sup> was applied (Table 4.2).

A number of researchers (Fullington et al. 1987, Moss et al. 1981, Wrigley et al. 1980, 1984) observed that S deficiency in wheat resulted in increases in the relative proportion of HMW glutenin subunits and S-poor,  $\omega$ -gliadins and concurrent decreases in the relative proportions of S-rich groups including LMW glutenin subunits and  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins. In addition, the increased synthesis of  $\omega$ -gliadins appeared to be the most noticeable change in response to S deficiency. Therefore, the negative relationship observed between S fertilization and the proportion of monomeric protein in flour, in our study, may be due to S fertilization reducing the production of  $\omega$ -gliadins in the flour (increasing the proportion of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins in flour). However, further fractionation would be required to determine if this is, in fact, true.

Increasing the rate of N fertilization significantly increased the concentration of monomeric protein in flour at five of six sites where grain quality and the effect of N were measured. The increase was significant only at Archerwill (Table 4.1), especially when N fertilizer was applied in the absence of S fertilization (Appendix Table E.35). This observation may support the earlier hypothesis that the fertilization responses of the monomeric fraction may be driven by the response of the  $\omega$ -gliadin fraction to the supplies of S and N; however, as mentioned earlier further fractionation of the monomeric protein would be necessary to confirm this.

The residue protein fraction is the leftover fraction during the fractionation process. The only significant effect of fertilization on residue protein was at Athabasca where this fraction was

significantly reduced by S fertilization and increased by N fertilization (Table 4.1). No other significant responses were observed.

#### **4.4.12. Prediction of Breadmaking Quality Responses to S Fertilization Using Grain Nutrition and Soil Test SO<sub>4</sub>-S**

At the marginal S sites, concentrations of soil SO<sub>4</sub>-S were < 40 kg ha<sup>-1</sup>, average grain S concentrations were ≤ 0.17 % S, and grain N:S ratios were > 17:1 for the high N, zero S treatment (Table 4.2). At all four of these sites, S fertilization increased the S concentration and reduced the N:S ratio of grain. At three of the four sites, S fertilization in the presence of the high N fertilization rate increased loaf height, loaf volume, and oven spring (Table 4.2). Sulphur fertilization in the presence of the high N fertilization rate reduced the viscoelastic ratio and increased dough extensibility at all four sites and reduced R<sub>max</sub> where the N fertilization rate was high at three of four sites. Application of S fertilizer in the presence of the high N fertilization rate also reduced mixograph peak time and work input to peak (Table 4.2). Finally, at all four marginal sites, S fertilization in the presence of the high N fertilization rate reduced the concentration of monomeric protein in flour as well as the ratio of insoluble to soluble glutenin in flour and increased the concentration of soluble glutenin in flour.

At the sites where the soil S fertility was adequate, concentrations of soil SO<sub>4</sub>-S were > 40 kg ha<sup>-1</sup>, average grain S concentrations were ≥ 0.17 % S, and grain N:S ratios were < 17:1 for the high N, zero S treatment. At these sites, no breadmaking and dough quality responses and few flour protein responses to S fertilization were observed.

Therefore, in our study, CWRS wheat that contained an S concentration ≤ 0.17 % S and an N:S ratio > 17:1 responded to S fertilization and was regarded as deficient in S for maximum

breadmaking quality. However, the critical values for optimum wheat quality vary with the intended use. The N:S ratio threshold in our study for predicting grain quality responses to S fertilization is the same as the N:S ratio threshold developed by Randall et al. (1981) for Australian wheat varieties, where grain was considered to be deficient in S for yield when it contained an N:S ratio  $> 17:1$ . However, these researchers also noted that grain was only deficient in S if it also contained  $< 0.12\%$  S. The critical S concentration of  $0.17\%$  S observed in our study was greater than that observed in the Australian study, probably because CWRS wheat generally contains significantly greater grain N concentrations than Australian wheat varieties. Therefore, more S is required to balance the ratio between N and S in the grain.

The combination of these criteria avoids the limitation associated with using the N:S ratio, alone, as an indicator of grain S nutrition, where the surplus of one nutrient may falsely indicate a deficiency of the other nutrient or where the deficiency of one nutrient may falsely indicate the sufficiency of the other nutrient (Finck 1970, Schnug and Haneklaus 1998). Furthermore, the combination avoids the problem of relying on grain S concentration, alone, which provides no indication of the balance, or lack of, between N and S in the grain. For example, even though grain from Brandon N contained  $0.17\%$  S and could have been regarded as deficient for maximum quality based on S concentration alone, the ratio of N to S in grain was  $< 17:1$ ; therefore, the S nutrition of the grain was adequate in relation to N and no breadmaking responses to S fertilization were observed. In Australia, Randall et al. (1981) made similar conclusions, finding that that low concentrations of S in grain were associated with inadequate supplies of S or N and could not, by themselves, be used as a diagnostic index of S responsiveness for grain yield.



#### 4.4.13. Prediction of Breadmaking Quality Responses to S Fertilization Using Plant Tissue Samples Collected at the 50 % Heading Stage

Similar to the observations for the critical grain thresholds for breadmaking quality, at the four marginal S sites where grain quality responses to S fertilization were frequent, the average S concentration of whole plant tissue samples collected at 50 % heading was  $< 0.15\%$  S and the average N:S ratio was  $> 17:1$  for the high N, zero S treatment (Table 4.2). Spencer and Freney (1980) obtained critical values in whole plant shoots at stem elongation for grain yield of  $1.5\text{ mg g}^{-1}$  for total S, 11 % for percent of total S as sulphate-S, and 19:1 for N:S ratio. Westfall et al. (1990) found critical values of total S in whole wheat plants for grain yield to be  $2.2\text{ mg g}^{-1}$  at plant tillering,  $1.9\text{ mg g}^{-1}$  at stem elongation, and  $1.5\text{ mg g}^{-1}$  at booting.

At three of the seven sites, where supplies of soil S were always adequate for grain yield and usually adequate for grain quality, S concentrations of whole plant tissue samples collected at 50 % heading were  $\geq 0.15\%$  S and average N:S ratios were  $< 17:1$  for the high N, zero S treatment (Table 4.2).

Therefore, in our study, quality responses to S fertilization were consistently predicted by the concentration of S and the ratio of N to S in plant tissue samples collected at the 50 % heading stage. Again, these criteria used together avoid the problems associated with using each value alone.

Zhao et al. (1999c) pointed out that wheat requires correction of S deficiency prior to or at the second node stage to fully recover and produce maximum yields. Therefore, more research is required to determine if S fertilization on S deficient wheat after the 50 % heading stage improves the S nutrition and overall breadmaking quality of the CWRS wheat.

#### 4.5. Summary and Conclusions

Sulphur fertilization increased grain yield of CWRS wheat at only two of twelve sites in 1999 and 2000, but increased the S concentration in grain at six of twelve sites. At these six sites, the inherent soil  $\text{SO}_4\text{-S}$  concentration was  $< 40 \text{ kg ha}^{-1}$  and would be regarded as marginally sufficient for wheat yield. At the six remaining sites where the concentration of  $\text{SO}_4\text{-S}$  in the soil was  $> 40 \text{ kg ha}^{-1}$ , S fertilization did not increase grain S concentration. Sulphur fertilization also reduced the N to S ratio in grain at eight of the twelve sites in the two growing seasons, including all six sites where the S concentration in grain was increased by S fertilization.

Of the seven sites used in grain quality analyses, grain yield was significantly increased by the application of  $20 \text{ kg S ha}^{-1}$  at only two sites. At the marginal S sites of Melfort and Athabasca, where concentrations of soil  $\text{SO}_4\text{-S}$  were  $< 40 \text{ kg ha}^{-1}$ , grain yields were increased by 142 and  $515 \text{ kg ha}^{-1}$ , respectively. At the marginal S sites of Erickson and Archerwill, where concentrations of soil  $\text{SO}_4\text{-S}$  were also  $< 40 \text{ kg ha}^{-1}$ , no yield response to S fertilization were observed.

At all four marginal S sites, plants collected at 50 % heading contained  $< 0.15 \%$  S and an N:S ratio  $> 17:1$  and grain harvested at maturity contained  $\leq 0.17 \%$  S and an N:S ratio  $> 17:1$  for the high N, zero S treatment. At all of these marginal S sites, S fertilization significantly increased the concentration of S in grain and reduced the N:S ratio in grain, leading to improvements in breadmaking and dough rheological quality. For the breadmaking analyses, S fertilization increased loaf height and oven spring at three of the four sites. There were also improvements in loaf volume at two of the four sites when S fertilizer was applied in

combination with 26 or 100 kg N ha<sup>-1</sup> and at one more site, only where 100 kg N ha<sup>-1</sup> was applied. For the dough rheological analyses, S fertilization significantly increased dough extensibility at all four marginal S sites and reduced R<sub>max</sub> and mixograph peak time at three of four sites; mixograph peak time was significantly reduced by S fertilization at the other site only in the presence of 100 kg N ha<sup>-1</sup>. Furthermore, S fertilization reduced the viscoelastic ratio and mixograph work input to peak at all four marginal S sites. The improvements in dough quality and baking performance were probably a result of compositional changes in the protein associated with the application of S fertilizer. Sulphur fertilization increased the proportion of soluble glutenin in flour and reduced the ratio of insoluble to soluble glutenin in the flour for both rates of N fertilization at three of four sites, but only at the high N fertilization rate at the other site.

At the three sites where soil SO<sub>4</sub>-S concentrations were > 40 kg ha<sup>-1</sup> and the S concentration and N:S ratio of plant tissue samples collected at 50 % heading was > 0.15 % S and < 17:1, respectively, S fertilization had little impact on grain yield, grain nutrition, breadmaking quality, dough quality, or flour protein composition.

At the sites where the native N fertility of the soil was low, high rates of N fertilization were required to generate grain quality responses to S fertilization. This observation confirms results of previous studies where adding S fertilizer under high N fertility conditions improved the balance between N and S, leading to enhanced dough quality and breadmaking performance of wheat (Wooding et al. 2000, Wrigley et al. 1984, Byers et al. 1987, Zhao et al. 1999a, and Schnug et al. 1993).

In conclusion, CWRS wheat grain containing ≤ 0.17 % S and an N:S ratio > 17:1 should be regarded as deficient in S for maximum grain quality and will frequently respond to S

fertilization. These thresholds are only accurate when used in combination to avoid the limitations associated with using each individually. Furthermore, these thresholds are of greatest value for the commercial processors of CWRS wheat because an indication of inadequate supplies of S in grain at maturity does not provide the producer with the opportunity to correct the S deficiency with the application of S fertilizer.

For the producer, S fertilization will frequently improve the overall breadmaking quality of CWRS wheat where tissue samples collected at 50 % heading contain  $< 0.15\%$  S and N:S ratios  $> 17:1$ . Again, these thresholds should be used together to avoid the limitations associated with each individually. However, more research is required to determine if S fertilization this late in the growing season improves the S nutrition and breadmaking quality of grain. If S fertilization at this point makes no difference, a tissue test at this point in the growing season would also generate little value to the producer.

Where the soil contains  $< 40 \text{ kg SO}_4\text{-S ha}^{-1}$ , S fertilizer will frequently improve the breadmaking quality of CWRS wheat, even where no grain yield response to S fertilization is observed. The beneficial effects of S fertilization on the breadmaking quality of CWRS wheat were due to decreased dough strength and increased dough extensibility resulting from the increased synthesis of soluble glutenin and a reduction in the ratio of insoluble to soluble glutenin in the flour.

Finally, in the Canadian wheat industry, the price of CWRS wheat is currently determined by grade and grain N concentration (grain N concentration  $\times 5.7 =$  protein concentration), only. In other words, producers receive quality premiums for adding N fertilizer, but not for adding S fertilizer. This policy ignores the quality improvements from S fertilization and increases the potential for imbalances of N to S in grain. However, until S is measured and

rewarded as an important factor for determining the overall breadmaking quality of CWRs wheat, producers should apply S fertilizer only if they expect a grain yield response.

## 5. CRITERIA FOR THE PREDICTION OF THE SULPHUR STATUS OF CANADA WESTERN RED SPRING WHEAT GROWN IN WESTERN CANADA

### 5.1. Abstract

Canada Western Red Spring wheat (*Triticum aestivum* L. cv. AC Barrie) was grown at twelve different locations over two years across western Canada to examine the impact of S fertilization on the yield and quality of wheat. Soil and plant tissue tests were also evaluated for their ability to predict grain S concentration, grain N:S ratio, and total S accumulation in the plant. The S concentration of whole plant samples collected at the 50 % heading stage was poorly correlated to the S concentration in grain. The S concentration of whole plant samples collected at the 4 – 6 leaf stage was not correlated to the S concentration in grain. However, grain N:S ratio correlated well with the ratio of N to S in the plant tissue samples collected at the 50 % heading stage.

In the absence of S fertilization, soil  $\text{SO}_4\text{-S}$  concentration to 60 cm was moderately correlated with the S concentration in grain and with total S accumulation in the plant. However, when two additional sources of S, including fertilizer S and estimated mineralizable soil organic S, were included in multiple regression analysis for the prediction of grain S concentration and total S accumulation in the plant, the relationships were weak. Finally, when the soil N:S ratio, calculated with the soil  $\text{NO}_3\text{-N}$  and  $\text{SO}_4\text{-S}$  values, was plotted against grain N:S ratio, for the low N, zero S treatment, there was a modest correlation. When the fertilizer treatments were added to the soil  $\text{NO}_3\text{-N}$  and  $\text{SO}_4\text{-S}$  concentrations in the calculation of the soil N:S ratio, the

correlation improved but was not strong. Therefore, the plant tissue and soil tests did not accurately predict the S nutrition of the wheat.

## 5.2. Introduction

In western Canada, deficiencies of soil S are becoming more prominent; however, tools that accurately predict S deficiencies are limited. Anderson (1966), in a greenhouse study, demonstrated that adsorbed  $\text{SO}_4\text{-S}$  was not a significant source of S in Prairie soils and that soils with low concentrations of water-soluble  $\text{SO}_4\text{-S}$  were unlikely to mineralize appreciable quantities of S. Therefore, this researcher observed a relatively strong correlation ( $r = 0.75$ ) between plant accumulation of S and water soluble  $\text{SO}_4\text{-S}$  in the solum and concluded that the measurement of water soluble  $\text{SO}_4\text{-S}$  was a satisfactory measure of the S supply of the soil. More recently, Bailey (1987) concluded that it is difficult to satisfactorily correlate soil  $\text{SO}_4\text{-S}$  concentrations with crop yield and plant S accumulation in the field due to the different rates of net mineralization of organic S during the growing season, the heterogeneous distribution of soil  $\text{SO}_4\text{-S}$ , and unequal plant root distribution and activity in the soil. This researcher also noted that the chemistry of S in the soil is influenced by a host of processes that make it very difficult to predict, with accuracy, the quantity of S available to the crop through the growing season. Therefore, Bailey (1985, 1987) suggested that the ratio of total N to total S in soil might serve as a useful measure of the S status of the soil and indicate plant responses to S fertilizer since this ratio can be a more stable and predictable value than soil  $\text{SO}_4\text{-S}$  alone. Janzen and Bettany (1984), working with rape in a growth chamber, also reported that the available N:available S ratio of a soil might be a good predictor of crop response to S. In this study, maximum

assimilation of both N and S into the rapeseed occurred when the ratio of (soil  $\text{NO}_3\text{-N}$  plus fertilizer N) : (soil  $\text{SO}_4\text{-S}$  plus fertilizer S) was approximately 7.

Plant tissue analysis is another means of diagnosing S deficiency in plants. Several diagnostic indices have been proposed. One promising method for the prediction of grain and plant yield responses to S fertilization is the determination of the proportion of total S as sulphate-S in the plant (Freney et al. 1987, Spencer and Freney 1980). Spencer and Freney (1980) noted that this index was the least affected by the age of the plant. However, in later work, Scaife and Burns (1986) concluded that the sulphate / total S index has two fundamental disadvantages as compared to using sulphate-S or total S alone as indices. First, the numerator (sulphate-S) is the major variable in the denominator, so the ratio is likely to be less sensitive than either of the measurements alone. The second disadvantage of this ratio as a means of predicting the S status of a plant is that it requires twice as much analytical work as either measurement alone.

In a pot experiment, Zhao et al. (1996) found that total S concentration of the uppermost leaf at stem elongation was a good indicator of S deficiency in wheat because it was closely related to relative dry matter yield at stem elongation (Zadoks GS 37). Spencer and Freney (1980), in a field study, found total S concentration of whole plants to be correlated with final grain yield for wheat. However, these researchers also noted that the critical total S value for wheat yield was strongly influenced by growth stage and time of sampling.

The use of N:S ratio in plant tissue has also been examined for its value in predicting the S status of plants. Spencer and Freney (1980) found that the ratio of total N to total S in total above ground plant tissue provided a good indication of the S status in relation to grain yield. However, the major drawback of using the N:S ratio to measure the S status of a plant is that the



same N:S ratio can be obtained at totally different concentration levels in the tissue (Schnug and Haneklaus 1998). Therefore, surplus of one element may falsely indicate a deficiency of the other element (Finck 1970). In addition, Spencer and Freney (1980) also noted that the N:S ratio of above ground plant matter was affected by the age of plant at sampling.

In Chapter 3, we showed that the S nutrition of wheat, measured as grain S concentration or N:S ratio, was strongly related to the breadmaking performance of wheat. Low concentrations of S and high ratios of N to S in grain were associated with reduced dough extensibility and increased dough strength. As a result, grain containing low concentrations of S and high N:S ratios produced bread loaves that were smaller and of poorer quality than grain containing high concentrations of S and low N:S ratios. In Chapter 4, we demonstrated that S fertilization improved the breadmaking quality of CWRS wheat where soil  $\text{SO}_4\text{-S}$  concentrations were  $< 40 \text{ kg ha}^{-1}$  and where the midseason tissue collected at 50 % heading contained  $< 0.15 \%$  S and an N:S ratio  $> 17:1$ . Also, the quality of grain containing an S concentration  $\leq 0.17 \%$  S and an N:S ratio  $> 17:1$  responded to S fertilization. However, the critical values for wheat quality parameters vary with the intended use. Therefore, an ideal crop nutrition program would provide grain with a specific concentration of S or a specific N:S ratio. As a result, the objective of this portion of the study was to evaluate soil and plant tissue tests for their ability in predicting the S concentration and N:S ratio in grain as well as S accumulation in the plant.

A number of soil tests were evaluated for their predictive value, including the measurement of water-soluble soil  $\text{SO}_4\text{-S}$  and  $\text{NO}_3\text{-N}$  as well as estimating the potentially mineralizable S and N from a phosphate-borate extract. Whole plant tissue samples taken at early and late stages of the growing season were also evaluated for their value in predicting grain S concentration and N:S ratio.

## **5.3. Methods and Materials**

### **5.3.1. Field Experiments**

The materials and methods used in the field experiments are described in Chapter 3 (section 3.3.). However, in addition to measuring grain yields, straw yields were determined by collecting the straw behind the combine or by harvesting two one-meter rows (except at Athabasca in 1999 and Archerwill in 2000 where no straw yields were collected). The straw yields were adjusted to dry matter basis, based on the moisture content of grain. Total above ground dry matter yield was calculated by the addition of the grain and straw yields, except at Archerwill in 2000 where, at plant maturity, entire plants from two one-meter rows were collected for determining total above ground dry matter yield.

### **5.3.2. Soil Sampling and Analysis**

Soil sampling and analyses were conducted as outlined in Chapter 3 (section 3.3.). In addition, mineralizable N was estimated on all surface samples (0 – 15 cm) using a modification of the phosphate-borate method developed by Gianello and Bremner (1986b). Five grams of soil were used instead of four grams and 50 mL of phosphate-borate buffer solution was used instead of 40 mL. Furthermore, native exchangeable  $\text{NH}_4\text{-N}$  was not measured in a cold KCl extract and subtracted from the phosphate-borate extractable N values because in other studies in western Canada, subtraction of exchangeable  $\text{NH}_4\text{-N}$  resulted in weaker correlations between phosphate-borate extractable N and mineralizable organic N (Jalil et al. 1996). Initial concentrations of mineralizable N were reported on a part-per-million basis and were then converted to a  $\text{kg ha}^{-1}$  basis assuming all soils had a bulk density of  $1.33 \text{ g cm}^3$ . Estimated mineralizable S was

calculated by dividing the mineralizable N values determined in the phosphate-borate extraction by 8.3. This value of 8.3 was adopted from the work of Bailey (1985) who found the average N:S ratio of soil organic matter in Prairie Canadian soils to be 8.3:1.

### **5.3.3. Grain and Straw Analysis**

Sub-samples of grain and straw from each plot were ground with a Wiley Mill to pass a 2 mm sieve and analyzed for total N and S by combustion using a Leco CNS Analyzer (Leco Corporation 1996). The moisture content of the ground samples was also determined and the N and S concentrations were converted to dry matter basis. Grain N:S ratio was calculated from the N and S concentrations.

### **5.3.4. Data Analysis**

To evaluate the predictive value of the plant tissue tests, linear, polynomial, and logarithmic regression equations were determined for each predictive variable, including plant tissue S concentration, N concentration, and N:S ratio at 50 % heading (Feekes 10.3 stage) and 4 – 6 leaf stages (Feekes stages 1.4 to 1.6), with grain S concentration, N concentration, and N:S ratio as the response variables. To evaluate the predictive value of the soil SO<sub>4</sub>-S test, linear and polynomial regression equations were determined with grain S concentration and total S accumulation in the plant as the response variables. Soil SO<sub>4</sub>-S, NO<sub>3</sub>-N, and fertilizer S and N were also used in correlation analysis to determine how they predicted the N to S ratio in grain. Multiple regression analysis was used to determine the predictability of grain S concentration and total S accumulation using three potential sources of S (fertilizer S, soil SO<sub>4</sub>-S, and estimated mineralizable S). In the regression analysis for total S accumulation, replicate one

from Erickson in 1999 was eliminated due to extensive deer damage and all treatments from Athabasca in 1999 were excluded because no straw yields were determined. In all of the regression analyses using the soil data, Archerwill in 2000 was not included because soil samples were not collected on a plot-by-plot basis, only one composite sample was collected for the entire site. The JmpIn statistical package was used for all analyses.

## **5.4. Results and Discussion**

### **5.4.1. Plant Tissue Analysis**

Table 5.1 summarizes the regression equations and the coefficients of determination ( $R^2$ ) for the predictive relationships between the plant tissue nutrient analyses conducted on whole plant tissue samples collected at 50 % heading stage and the 4 - 6 leaf stage with the nutrient concentrations in mature grain. The regression equations and corresponding coefficients of determination provide evidence that the prediction of the S and N concentrations in grain is difficult using early and late season plant tissue analysis. A logarithmic model best described the relationship between the S concentration of plant tissue at 50 % heading and grain S concentration (Table 5.1 and Figure 5.1). However, although the overall model was significant, it explained only 27 percent of the variation in grain S concentration. The weak relationship observed between grain S concentration and S concentration in the whole plant at the 50 % heading is probably due to variability in plant uptake and biological dilution of S during the time between the two stages. Other researchers have generally focused on the relationships between midseason tissue S concentration and grain yield or total dry matter yield, rather than grain S concentration. For example, Zhao et al. (1996) found that total tissue S in the upper leaf of

Table 5.1. Equations for the prediction of grain and whole plant nutrition using plant tissue samples collected at the 50 % heading and 4 - 6 leaf stages

Plant Tissue at 50 % Heading Stage Versus Grain					
Response Variable (y)	Predictor Variable (x)	Regression Equation	R <sup>2</sup>	P <sub>r&gt;F</sub>	Number of Samples
S Concentration in Grain	S Concentration in Tissue	$y = 0.256 + 0.039\text{Ln}(x)$	0.27	0.0001**	191
N Concentration in Grain	N Concentration in Tissue	$y = 2.601 + 0.199(x)$	0.099	0.0001**	191
N:S Ratio in Grain	N:S Ratio in Tissue	$y = 9.370 + 0.537(x)$	0.64	0.0001**	191
Plant Tissue at 4 - 6 Leaf Stage Versus Grain					
Response Variable (y)	Predictor Variable (x)	Regression Equation	R <sup>2</sup>	P <sub>r&gt;F</sub>	Number of Samples
S Concentration in Grain	S Concentration in Tissue	$y = 0.223 - 0.109(x)$	0.05	0.12	48
N Concentration in Grain	N Concentration in Tissue	$y = 1.085 + 0.296(x)$	0.12	0.017*	48
N:S Ratio in Grain	N:S Ratio in Tissue	$y = 12.050 + 0.198(x)$	0.17	0.0037**	48
Plant Tissue at 4 - 6 Leaf Stage Versus Plant Tissue at 50 % Heading Stage					
Response Variable (y)	Predictor Variable (x)	Regression Equation	R <sup>2</sup>	P <sub>r&gt;F</sub>	Number of Samples
S Concentration at 50 % Heading	S Concentration at 4 - 6 Leaf	$y = 0.067 + 0.313(x) - 5.149(x - 0.336)^2$	0.21	0.006**	48
N Concentration at 50 % Heading	N Concentration at 4 - 6 Leaf	$y = 1.209 + 0.163(x) - 1.152(x)^2$	0.16	0.02*	48
N:S ratio at 50 % Heading	N:S Ratio at 4 - 6 Leaf	$y = 6.516 + 0.319(x)$	0.14	0.008**	48

\*, \*\* Significantly greater than 0 at P<0.05 and P<0.01, respectively

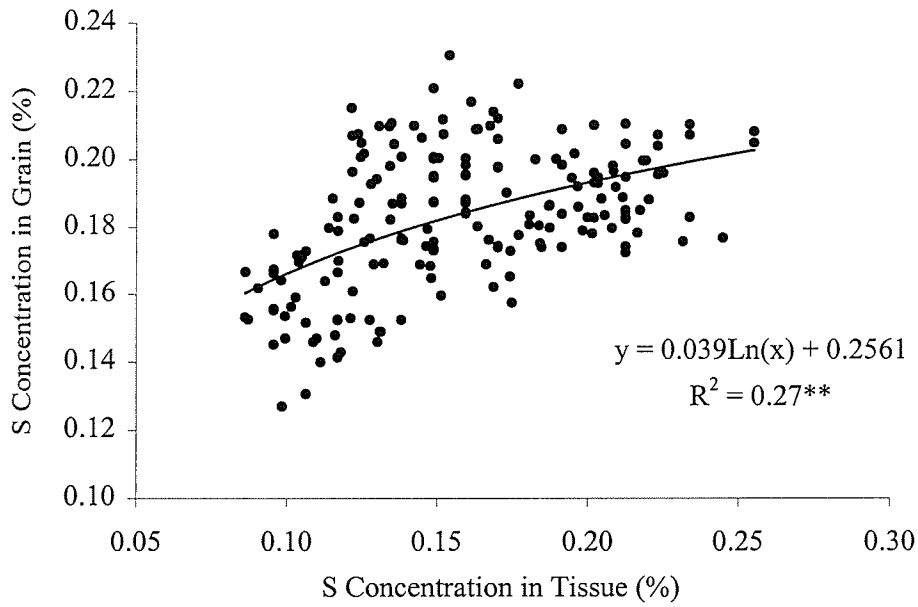


Figure 5.1. Relationship between plant tissue S concentration at 50 % heading and grain S concentration

\*\* Significantly greater than 0 at P<0.01

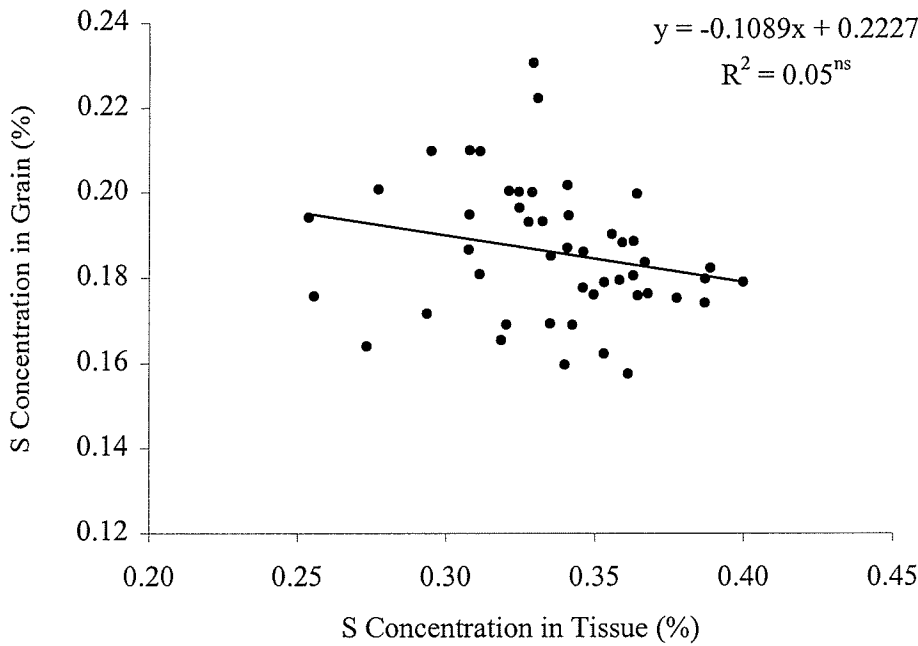


Figure 5.2. Relationship between plant tissue S concentration at the 4 - 6 leaf stage and grain S concentration

<sup>ns</sup> Not significantly greater than 0

wheat sampled at stem elongation (Zadoks GS 37) was a good indicator of S deficiency in wheat because it was closely related to relative dry matter yield at stem elongation. Spencer and Freney (1980), in a field study, found that total S concentration of whole plants was also correlated with final grain yield for wheat.

The predictability of grain S concentration with the plant tissue samples was even weaker for the earlier tissue sampling period. When the S concentration of the 4 – 6 leaf tissue samples was used, the linear model best explained the relationship. However, the overall regression model was insignificant and negative (Table 5.1 and Figure 5.2) indicating that the early season tissue sample was not useful for predicting the absolute concentration of S in the grain.

Overall, the concentration of S in whole plant tissue samples declined from the early to late sampling period as is demonstrated by the regression equation for the relationship between early and late season tissue S concentrations (Table 5.1). The concentration of S in the plant is higher at an early stage and as the plant grows and matures, the S concentration in plant tissue is probably diluted by the production of plant biomass. This observation is similar to the findings of Spencer and Freney (1980) who noted that the critical total S value for predicting wheat yield responses to S fertilizer was strongly influenced by growth stage and varied considerably with time of sampling. In their study, the critical S concentration in early season tissue was much greater than in late season tissue. In Chapter 4, we saw that midseason plant tissue samples at 50 % heading indicated that wheat quality responded to S fertilization if it contained < 0.15 % S and an N:S ratio > 17:1. Therefore, plant tissue samples must be consistently collected at this precise growth stage to provide an accurate indication of the S status of wheat.

Grain N concentration was also difficult to predict using the early and late season tissue samples. For example, the N concentration of the plant tissue at the 4 – 6 leaf stage explained

only 12 % of the variation in grain N concentration (Table 5.1). Furthermore, the N concentration of the samples taken at 50 % heading explained only 10 % of the variation in grain N concentration (Table 5.1).

Grain N:S ratio was more accurately predicted by the tissue samples than absolute concentrations of grain S and N. According to the regression analysis, the N:S ratio of plants at 50 % heading explained 64 % of the variation in grain N:S ratio (Table 5.1 and Figure 5.3). However, when the plant tissue samples were collected earlier in the growing season, at the 4 – 6 leaf stage, the predictability of grain N:S ratio declined dramatically (Table 5.1 and Figure 5.4). The strong relationship between N:S ratio of the 50 % heading samples and N:S ratio of grain indicates that even though the absolute concentrations of N and S in the plant changed during the period between 50 % heading and maturity, due to a similar degree of biological dilution for each nutrient the N:S ratio remained relatively stable and provided a good estimate of N:S ratio in the grain.

According to the regression equation for the relationship between the N:S ratio of the early and late season tissue samples, the N:S ratios of the tissue samples collected at the 50 % heading stage were lower than the N:S ratios of the samples collected at the 4 – 6 leaf stage (Table 5.1). Again, this indicates that the timing of plant tissue sampling is critical in maintaining the accuracy of the midseason tissue samples in predicting S responses in wheat.

Zhao et al. (1999c) concluded that reliable diagnosis of S deficiency in wheat tends to be most accurate towards the end or at the end of vegetative growth. These researchers noted that plant tissue sampling at earlier stages usually provides less accurate predictive value. The same is true in our study where the tissue samples collected at the 50 % heading stage predicted grain N:S ratio (and grain S concentration) more accurately than the tissue samples collected at the 4 –



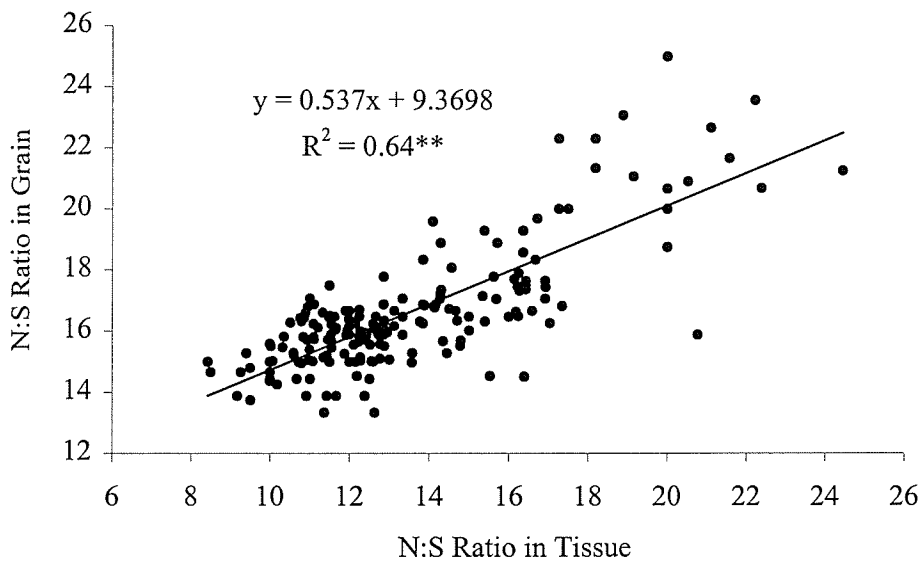


Figure 5.3. Relationship between plant tissue N:S ratio at 50 % heading and grain N:S ratio

\*\* Significantly greater than 0 at  $P < 0.01$

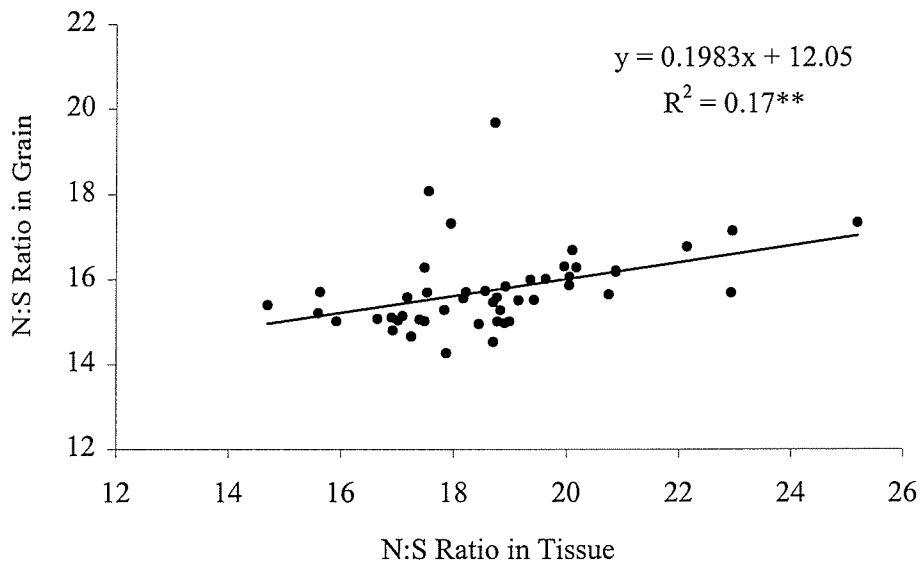


Figure 5.4. Relationship between plant tissue N:S ratio at the 4 - 6 leaf stage and grain N:S ratio

\*\* Significantly greater than 0 at  $P < 0.01$

6 leaf stage. The poor predictability of the S nutrition of grain early in the growing season is problematic because S deficiencies need to be corrected prior to the end of the vegetative stage to ensure that yield loss does not occur (Zhao et al. 1999c). However, more research is required to determine if S fertilization of wheat after the 50 % heading stage improves the overall breadmaking quality of the grain.

#### 5.4.2. Soil Analysis

In western Canada, the measurement of water-soluble  $\text{SO}_4\text{-S}$  to a depth of 60 cm is the standard procedure for estimating the S status of agricultural soils. A quadratic regression model better described the relationship between grain S concentration and soil  $\text{SO}_4\text{-S}$  than the linear regression model, but accounted for only 37 % of the variation in grain S concentration (Figure 5.5).

The previous regression equations for the relationship between soil  $\text{SO}_4\text{-S}$  and grain S concentration demonstrate the challenge of predicting the concentration of S in grain based on the concentration of  $\text{SO}_4\text{-S}$  in the soil. However, in our experiment there were two additional sources of S contributing to the S nutrition of the plant, fertilizer S and organic S that mineralized during the growing season. The mineralizable fraction of organic S was estimated through the determination of mineralizable N using the phosphate-borate procedure developed by Gianello and Bremner (1986b) and dividing the mineralizable N value ( $\text{kg ha}^{-1}$ ) by a factor of 8.3, the average ratio of N to S in soil organic matter in Prairie Canadian soils as determined by Bailey (1985).

The three sources of S contributing to the S nutrition of the wheat were used in a multiple regression equation for predicting grain S concentration (Equation 5.1). The coefficient of

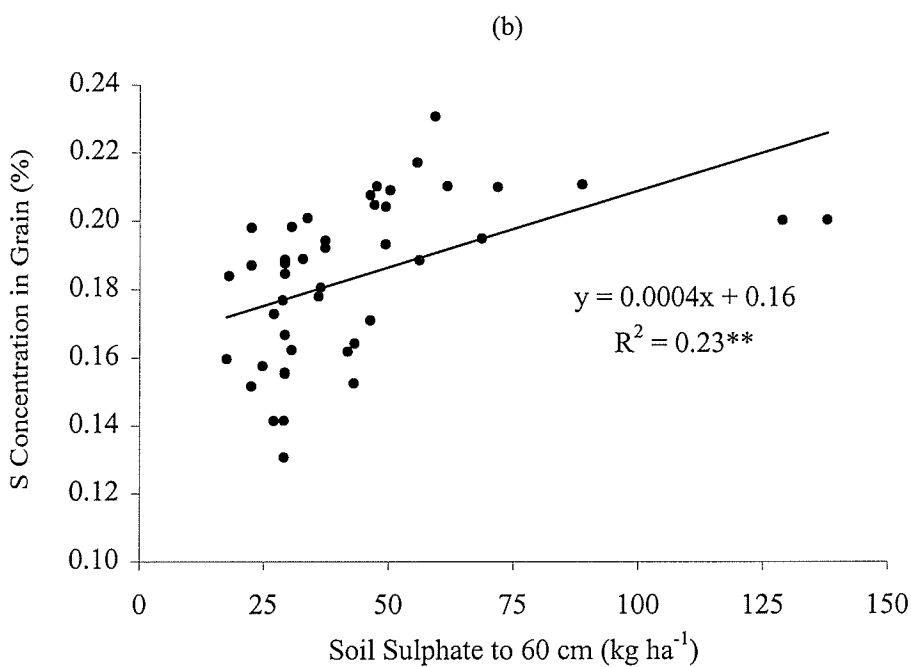
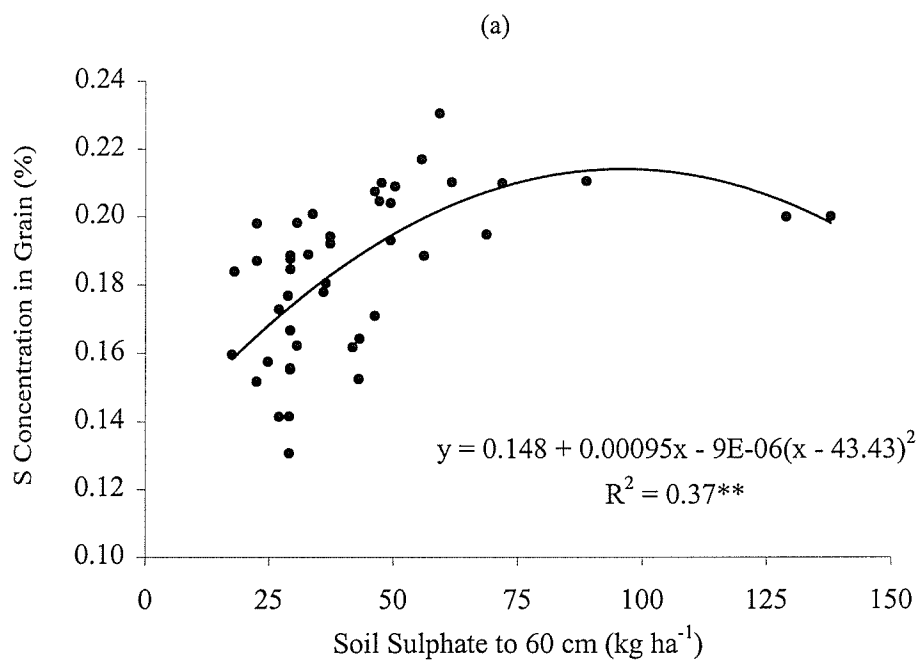


Figure 5.5. Quadratic (a) and linear (b) relationships between S concentration in grain and the amount of  $\text{SO}_4\text{-S}$  in the 0 - 60 cm depth of soil (for 0 S and 100  $\text{kg N ha}^{-1}$  treatments, only)

\*\* Significantly greater than 0 at  $P < 0.01$

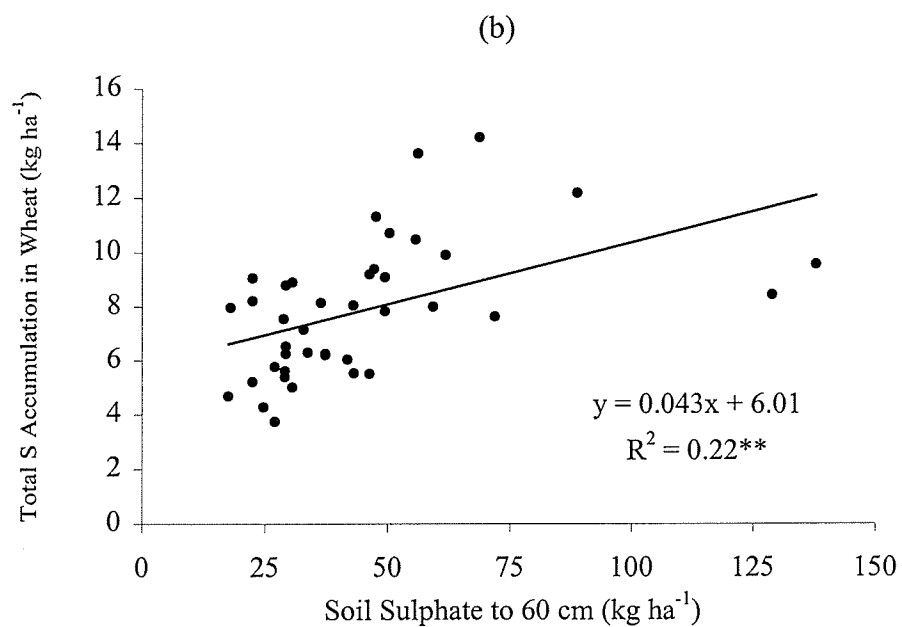
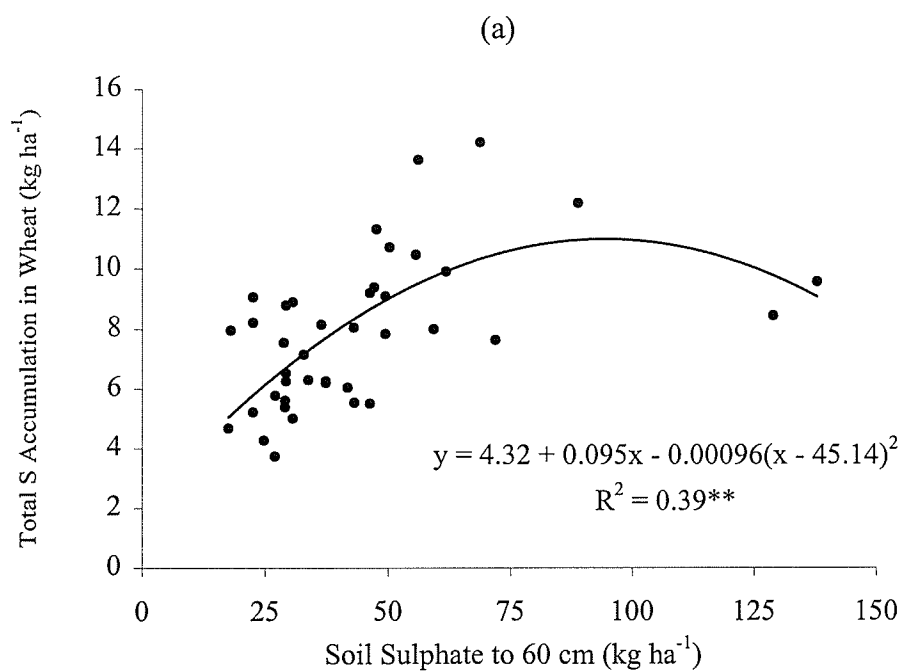


Figure 5.6. Quadratic (a) and linear (b) relationships between total S accumulation in wheat and the amount of  $\text{SO}_4\text{-S}$  in the 0 - 60 cm depth of soil (for 0 S and 100  $\text{kg N ha}^{-1}$  treatments, only)

\*\* Significantly greater than 0 at  $P < 0.01$

determination for the overall equation was only 0.19, indicating that the multiple regression equation did not accurately predict the concentration of S in the grain. Furthermore, the regression coefficients indicated that mineralizable organic S was contributing much more to the S nutrition of the plant than the SO<sub>4</sub>-fertilizer and that the soil SO<sub>4</sub>-S reserve did not contribute significantly to the S nutrition of the plant. However, SO<sub>4</sub>-S from fertilizer and soil is highly soluble and available to plants. Therefore, the validity of the coefficients in the equation is questionable.

Equation 5.1. Regression equation for the relationship between grain S concentration and the three sources of soil and fertilizer S (for high N treatments only)

$$y = 0.172^{**} + 5.4E-6(x_1)^{ns} + 0.00062(x_2)^{**} + 0.001(x_3)^{*} \quad (R^2 = 0.19)$$

where: y = grain S concentration (%)

x<sub>1</sub> = soil SO<sub>4</sub>-S (kg ha<sup>-1</sup>)

x<sub>2</sub> = fertilizer S (kg ha<sup>-1</sup>)

x<sub>3</sub> = estimated mineralizable S (kg ha<sup>-1</sup>)

\* significant at P < 0.05

\*\* significant at P < 0.01

<sup>ns</sup> – not significant

Differences in environmental conditions between the sites is one potential explanation for the poor relationship observed between grain S concentration and the concentration of soil SO<sub>4</sub>-S alone as well as in combination with fertilizer S and estimated mineralizable S in the soil. Similar to the accumulation of N in the grain, the accumulation of S in the grain may be strongly affected by biological dilution factors caused by environmental conditions under which the wheat is grown, masking any relationship between soil S concentrations and grain S concentration. For example, Grant et al. (1991) demonstrated that the N concentration in the grain of barley was diluted with the accumulation of grain biomass. In their study, as the moisture level of the soil increased, protein concentration of barley grain decreased while protein yield and total N uptake increased (grain yield increased). In our study, there is some evidence

that the same may be true for grain S concentration in wheat; however, the evidence is not consistent. For example, Athabasca in 1999, Melfort in 1999, and Brandon South in 1999 had similar concentrations of soil  $\text{SO}_4\text{-S}$ , but due to the lack of moisture, Athabasca produced the lowest grain yields. However, grain S concentrations at Athabasca were less than grain S concentrations at Brandon South and greater than grain S concentrations at Melfort, both of which had significantly larger grain yields.

In order to examine whether dilution of grain S concentration due to differences in biomass accumulation between sites was responsible for the poor predictability of grain S concentration, total S accumulation in the plant ( $\text{kg ha}^{-1}$ ) was also determined for each plot at each site. Linear and quadratic regression models were used to examine the relationship between total S accumulation in the plant and soil  $\text{SO}_4\text{-S}$  concentration for the high N treatment that did not include S fertilizer (Figure 5.6). The linear regression model, although significant, accounted for only 22 percent of the variability in total S accumulation. The quadratic model accounted for 39 percent of the variability in total S accumulation. This coefficient of determination was slightly lower than that observed by Anderson (1966), who found a relatively good relationship between water-soluble  $\text{SO}_4\text{-S}$  and total S accumulation ( $R^2 = 0.56$ ) using a linear regression model. However, Anderson's observations were based on a controlled, greenhouse experiment where variability in environmental conditions would not have played a role. Furthermore, the coefficient of determination for the prediction of total S accumulation was only slightly larger than the coefficient of determination for the prediction of grain S concentration. Therefore, dilution of grain S concentration due to differences in biomass accumulation between sites was not the major reason for the poor predictability of grain S concentration using the soil  $\text{SO}_4\text{-S}$  concentration to 60 cm. The poor predictability of grain S concentration, as well as total S

accumulation, in the field was probably due to a number of fundamental problems associated with measuring the water-soluble soil  $\text{SO}_4\text{-S}$  fraction. These include the variability in net mineralization of organic S during the growing season, the heterogeneous spatial distribution of soil  $\text{SO}_4\text{-S}$  due to varying gypsum deposits with soil depth and location within the landscape, and unequal plant root distribution and activity throughout the total rooting depth of soil (Bailey 1987). Furthermore, concentrations of soil  $\text{SO}_4\text{-S}$  also fluctuate temporally due to changes in the balance between inputs of S from the atmosphere and losses due to leaching, plant uptake,  $\text{SO}_4\text{-S}$  adsorption, and microbial immobilization (Eriksen et al. 1998, Tabatabai 1982). Therefore, the measurement of soil  $\text{SO}_4\text{-S}$  does not provide a good indication of the S that is available to a crop throughout the entire growing season.

The three sources of S contributing to the S nutrition of the wheat, including soil  $\text{SO}_4\text{-S}$ , fertilizer S, and estimated mineralizable S, were used in a multiple regression analysis to develop an equation for the prediction of total S accumulation in wheat (Equation 5.2). The coefficient of determination for the overall equation was only 0.23, indicating that this approach was not reliable for predicting total S accumulation in the plant. Furthermore, the regression coefficient for estimated mineralizable S was much larger than the regression coefficients for fertilizer S and soil  $\text{SO}_4\text{-S}$ , implying that the estimated mineralizable S source was contributing the majority of S to the plant. However, the  $\text{SO}_4\text{-S}$  from fertilizer and soil would probably contribute substantially to the plant due to its high solubility and relatively high availability.

Due to the difficulties and limitations of using water-soluble  $\text{SO}_4\text{-S}$  as an index of S availability, Bailey (1985, 1987) suggested that the ratio of total N to total S in soil might serve as a more useful indicator of the S status of the soil and expected responses to S fertilizer since it may be a more stable and predictable value than soil  $\text{SO}_4\text{-S}$  alone. Janzen and Bettany (1984),

working in a growth chamber with rape and soils from Saskatchewan, also reported that the ratio of available N to available S in soil may be a good predictor of crop response to S fertilizer.

Equation 5.2. Regression equation for the relationship between total S accumulation and the three sources of soil and fertilizer S (for high N treatments only)

$$y = 6.35 + 0.0013(x_1)^* + 0.062(x_2)^* + 0.14(x_3)^{**} \quad (R^2 = 0.23)$$

where:  $y$  = total S accumulation (kg ha<sup>-1</sup>)  
 $x_1$  = soil SO<sub>4</sub>-S (kg ha<sup>-1</sup>)  
 $x_2$  = fertilizer S (kg ha<sup>-1</sup>)  
 $x_3$  = estimated mineralizable S (kg ha<sup>-1</sup>)  
\* significant at  $P < 0.05$   
\*\* significant at  $P < 0.01$   
<sup>ns</sup> – not significant

In our study, available N to available S ratios were calculated using the soil NO<sub>3</sub>-N and soil SO<sub>4</sub>-S values alone, as well as using these values plus the fertilizer treatment sources of N and S (potentially mineralizable N and S were not used). These soil N:S ratios were then used in simple linear regression analysis to evaluate how well they predicted the N to S ratio in grain. When the soil N:S ratio, calculated with the soil NO<sub>3</sub>-N and SO<sub>4</sub>-S values, was plotted against grain N:S ratio, for the low N, zero S treatment, there was a modest correlation (Figure 5.7). Furthermore, when all of the fertilizer treatments were included in the calculation of soil N:S ratio, the relationship was improved (Figure 5.8). These observations confirm that the N:S ratio of the grain and soil are probably more stable than the absolute concentration of N or S in grain or the NO<sub>3</sub>-N or SO<sub>4</sub>-S concentrations in the soil. However, caution must be used when using N:S ratio as a predictive measure. As pointed out by Schnug and Haneklaus (1998) in reference to plant tissue tests, the major drawback of using the N:S ratio to measure the S status of a plant is that the same N:S ratio can be obtained at excessive, deficient, and sufficient concentrations of



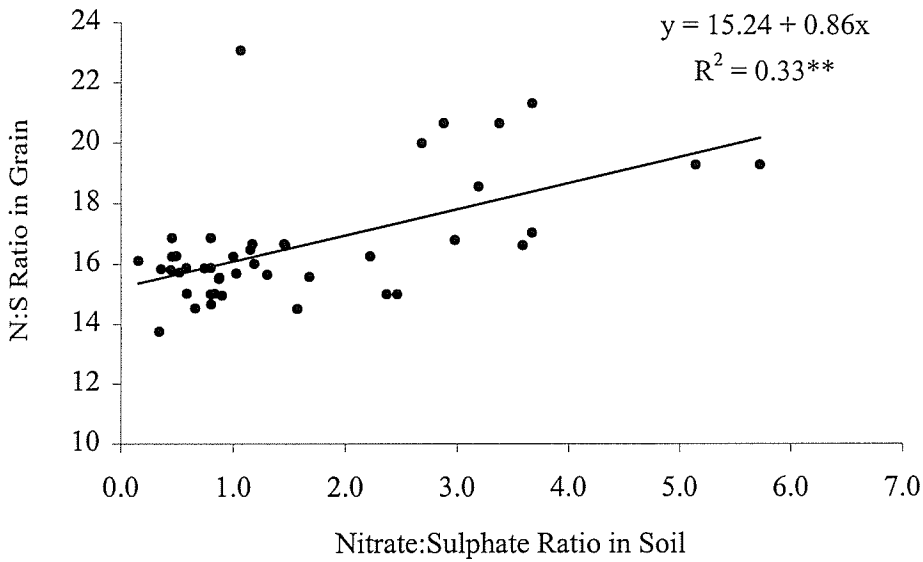


Figure 5.7. Relationship between nitrate:sulphate ratio in soil and N:S ratio in grain (for 0 S and 26 kg N ha<sup>-1</sup> treatments, only)

\*\* Significantly greater than 0 at P<0.01

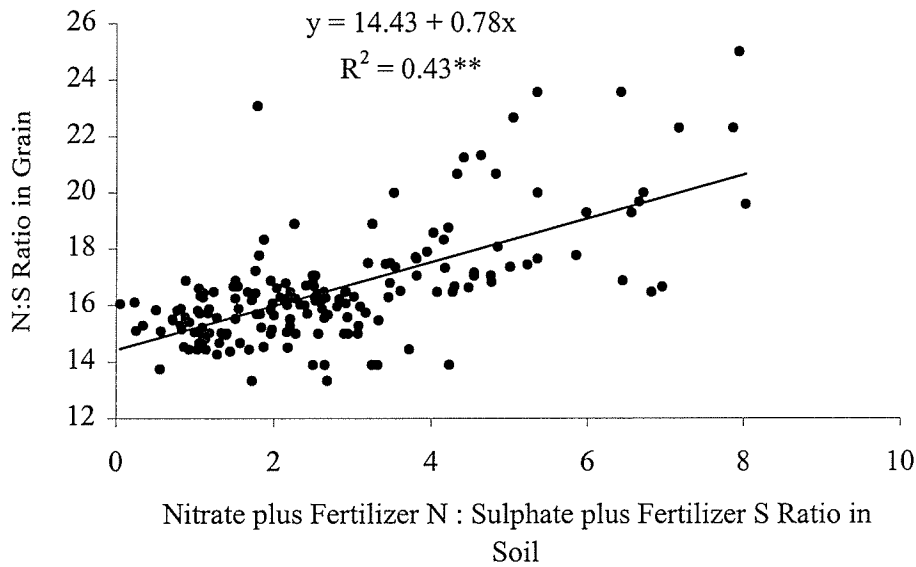


Figure 5.8. Relationship between nitrate plus fertilizer N : sulphate plus fertilizer S in soil and N:S ratio in grain (for all fertilizer treatments)

\*\* Significantly greater than 0 at P<0.01

both nutrients in the tissue. The same problem would apply to the soil where a surplus of one nutrient may falsely indicate a deficiency of the other nutrient and a deficiency of one nutrient may falsely indicate that the other nutrient is in sufficient supply.

### **5.5. Summary and Conclusions**

Due to the strong relationships observed between grain S concentration and grain N:S ratio and breadmaking quality of CWRS wheat in Chapter 3, a tool that accurately predicts the S nutrition of the grain would be valuable. However, our study demonstrated that the S nutrition of wheat grain, measured as grain S concentration, N:S ratio, and total S accumulation is difficult to predict precisely using plant tissue and soil tests. The S concentration of whole plant samples collected at 50 % heading was poorly correlated to the S concentration in grain. Furthermore, the S concentration of whole plants collected at the 4 – 6 leaf stage was not significantly correlated with grain S concentration. Grain N:S ratio was closely correlated to the ratio of N to S in the midseason tissue samples collected at 50 % heading.

The soil tests did not predict the S nutrition of wheat with great accuracy. In the absence of S fertilization, soil  $\text{SO}_4\text{-S}$  was moderately correlated with grain S concentration and total S accumulation in the plant.

When the two additional sources of S, including fertilizer S and estimated mineralizable S, were included in multiple regression analysis for the prediction of grain S concentration, the relationship was weak. When the three sources of S were used in multiple regression analysis for the prediction of total S accumulation in the plant a weak relationship was also observed.

The soil N:S ratio, calculated with the soil  $\text{NO}_3\text{-N}$  and  $\text{SO}_4\text{-S}$ , was moderately correlated with grain N:S ratio. Furthermore, when the fertilizer treatments were included in the calculation of the soil N:S ratio, the relationship improved. These observations suggest that the N:S ratio of the grain and soil are probably more stable than the absolute N or S concentrations in grain or the  $\text{NO}_3$  or  $\text{SO}_4\text{-S}$  concentrations in the soil.

In conclusion, the plant tissue and soil analyses did not accurately predict the concentration of S in wheat grain and total S accumulation in wheat. The N:S ratio of plant tissue selected at 50 % heading provided the best indication of N:S ratio in grain because the ratio of N to S is more stable than the absolute concentrations of each nutrient between the two stages. Estimation of the mineralizable fraction of soil organic S in the phosphate borate extraction for mineralizable N did not provide a good indication of S that may become available to the crop throughout the growing season and did not improve the predictability of grain S nutrition. More research is required to develop a tool that accurately predicts the S nutrition of wheat and to measure the mineralization of organic S throughout the growing season and its contribution to the S nutrition of wheat. However, until a better soil test is developed, the measurement of soil  $\text{SO}_4\text{-S}$  to 60 cm will provide a crude estimate of plant available S. Nitrogen to sulphur ratios of grain and soil also appear to be more stable than the absolute concentrations of N or S in the plant and soil. Therefore, the N:S ratio of the soil provides a weak indication of the N to S ratio expected in grain at maturity.

## 6. General Discussion

In our study, correlation analysis demonstrated and confirmed that the S nutrition of grain, expressed as grain S concentration or the ratio of N to S, is an important contributor to the breadmaking quality of CWRS wheat grown in western Canada. For example, grain S concentration was strongly and positively correlated to loaf volume and loaf height, while grain N:S ratio was negatively correlated with these baking parameters. These observations are consistent with similar findings for European and Australian wheat varieties (Haneklaus et al. 1992, Moss et al. 1981, Schnug et al 1993, Zhao et al. 1999a, 1999b).

Zhao et al. (1999b) concluded that the beneficial effects of improved S nutrition on breadmaking quality of wheat were associated with decreased dough strength and increased dough extensibility. The same is true in our study where grain S concentration was positively correlated with dough extensibility and negatively correlated with  $R_{\max}$ . A negative correlation was also observed between grain S concentration and mixograph peak time and work input to peak providing additional evidence that the dough was becoming weaker with rising concentrations of S in grain and confirms the earlier observations made by Moss et al. (1981). In Australia, Wrigley et al. (1984) also demonstrated on the extensigraph that flour containing a low ratio of N to S exhibited high dough extensibility and moderate dough strength; whereas, flour containing a high ratio of N to S exhibited poor dough extensibility and high dough strength. Our study confirmed these observations because grain N:S ratio was positively correlated with  $R_{\max}$  and negatively correlated with dough extensibility. The increase in dough strength with

rising grain N:S ratios was further demonstrated by the positive correlation between grain N:S ratio and mixograph work input to peak.

Researchers have shown that S deficiency in wheat increases the relative proportions of HMW glutenin subunits and S-poor  $\omega$ -gliadins. At the same time, there is a decrease in the relative proportions of LMW glutenin subunits,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -gliadins, albumins, and globulins which are S-rich (Castle and Randall 1987, Fullington et al. 1987, MacRitchie and Gupta 1993, Wrigley et al. 1980, 1984). Consistent with these studies, our study demonstrated that the improvement in dough quality and baking properties with rising concentrations of S in the grain and declining grain N:S ratios was probably due to changes in the composition of protein in the flour. The soluble glutenin content of flour protein, composed primarily of S-rich, LMW glutenin subunits as well as a small amount of HMW glutenin subunits and residual gliadins (Suchy 2002), was positively correlated with grain S concentration and negatively correlated with grain N:S ratio. Grain S concentration was also negatively correlated with the ratio of insoluble to soluble glutenin in flour while grain N:S ratio was positively correlated with this ratio. The insoluble glutenin fraction is comprised of both LMW and HMW glutenin subunits (Suchy 2002); therefore, the insoluble to soluble glutenin ratio provides an estimate of the ratio of HMW to LMW glutenin subunits. As a result, it appears that increasing concentrations of S in grain and declining N:S ratios were associated with more balanced synthesis of LMW, S-rich glutenin subunits and HMW glutenin subunits.

So, our study confirmed the observations of many previous studies conducted on European, New Zealand, and Australian wheat varieties, that the S nutrition of grain is an important factor affecting the overall breadmaking quality of CWRS wheat grown in western Canada. However, for the commercial processors of CWRS wheat, the question remains: “what

are the critical grain S concentration and N:S ratio thresholds at which CWRS wheat should be considered deficient in S for breadmaking quality?" In our study, S fertilization improved the breadmaking quality, dough quality, and flour protein composition when grain contained  $\leq 0.17$  % S and an N:S ratio  $> 17:1$ . These criteria should be used in combination to avoid the problem associated with using the N:S ratio alone, as an indicator of grain S nutrition, where the surplus of one nutrient may falsely indicate a deficiency of the other nutrient or where the deficiency of one nutrient may falsely indicate the sufficiency of the other nutrient (Finck 1970, Schnug and Haneklaus 1998). Furthermore, if the grain S concentration alone, is used as an indicator of grain S nutrition, it provides no indication of the balance, or lack of, between N and S in the grain.

The N:S ratio threshold in our study for predicting grain quality responses to S fertilization is the same as the N:S ratio threshold developed by Randall et al. (1981) for Australian wheat varieties, where grain was considered to be deficient in S for yield when it contained an N:S ratio  $> 17:1$ . These researchers also noted that grain was only deficient in S if it also contained  $< 0.12$  % S. The critical S concentration of 0.17 % S observed in our study was significantly greater than that observed in the Australian study because CWRS wheat generally contains significantly greater grain N concentrations than Australian wheat varieties. Therefore, more S is required to balance the ratio between N and S in the grain.

For the western Canadian grain producer, evidence that grain is deficient in S at maturity is of little value because no corrective measures can be taken at this point. A soil or plant tissue test that accurately predicts the S concentration and N:S ratio in grain at maturity would be more valuable because, if a S deficiency is identified prior to seeding or during the earlier stages of plant growth, it would allow the producer to correct the deficiency through the application of S

fertilizer. However, in our study, the S concentration of whole plant tissue samples collected at the 4 – 6 leaf stage and the 50 % heading stage did not predict the S concentration in grain with great accuracy. Grain N:S ratio was more easily predicted by the ratio of N to S in the plant tissue samples collected at the 50 % heading stage, indicating that this variable was more stable than S concentration alone.

The soil tests evaluated in our study were also limited in their ability to predict the S nutrition of grain. In the absence of S fertilization, the soil  $\text{SO}_4\text{-S}$  concentration to 60 cm was moderately correlated with the S concentration in grain. However when two additional sources of S, including fertilizer S and estimated mineralizable soil organic S, were included in multiple regression analysis for the prediction of grain S concentration the relationship was weak. Differences in environmental conditions between sites were thought to be the reason for the poor relationship observed between the S sources and grain S concentration. Similar to the accumulation of N in the grain, the accumulation of S in the grain may be strongly affected by biological dilution factors caused by environmental conditions under which the wheat is grown, masking any relationship between the sources of S and grain S concentration. However, in the absence of S fertilization, soil  $\text{SO}_4\text{-S}$  concentration to 60 cm was only moderately correlated with total S accumulation in the plant, which accounted for some of the dilution of grain S concentration. Furthermore, when the two additional sources of S were included in multiple regression analysis for the prediction total S accumulation in the plant, the relationship remained weak. Therefore, the poor predictability of grain S concentration and total S accumulation in the field was also probably due to a number of soil and environmental factors. These include the variability in net mineralization of organic S during the growing season, the heterogeneous spatial distribution of soil  $\text{SO}_4\text{-S}$  due to varying concentrations of gypsum with depth and at

different landscape positions, and unequal plant root distribution and activity throughout the rooting depth of soil (Bailey 1987). Furthermore, concentrations of soil  $\text{SO}_4\text{-S}$  also fluctuate temporally due to changes in the balance between inputs of S from the atmosphere and fertilizer additions and losses due to leaching, plant uptake,  $\text{SO}_4\text{-S}$  adsorption, and microbial immobilization (Eriksen et al. 1998, Tabatabai 1982). As a result, the measurement of the amount of S made available to the crop throughout the entire growing season is very difficult (Bailey 1985, 1987).

From a more practical viewpoint, a plant tissue or soil test that would accurately predict breadmaking quality responses to S fertilization would be valuable to the producer. In our study, seven sites were used in the breadmaking quality analyses, four of which contained  $< 40 \text{ kg SO}_4\text{-S ha}^{-1}$  and were considered to contain marginal S concentrations for the production of CWRS wheat. Sulphur fertilization increased grain yield at two of the four marginal S sites, which both contained significantly more  $\text{SO}_4\text{-S}$  than  $11 \text{ kg ha}^{-1}$  which was previously found to be sufficient for the production of cereals (Anderson 1966, Hamm 1969). Also, at these marginal S sites, the S concentration and N:S ratio of plant tissue samples collected at 50 % heading was  $< 0.15 \%$  S and  $> 17:1$ , respectively, for the treatment receiving  $100 \text{ kg N fertilizer ha}^{-1}$  only. At all four marginal S sites, S fertilization increased the concentration of S and reduced the N:S ratio in grain. As a result, S fertilization increased loaf volume at two of the four sites when in combination with 26 or  $100 \text{ kg N ha}^{-1}$ , and at one more site, for the  $100 \text{ kg N ha}^{-1}$  treatment, only. These observations confirmed the observations made much earlier in western Canada (Newton et al. 1959) and more recently in Australia and Europe where S fertilization also improved loaf volume (Byers et al. 1987, Moss et al. 1981, Haneklaus et al. 1992, Schnug et al. 1993, Zhao et al. 1999a, 1999b). Furthermore, similar to other studies (Moss et al. 1981, 1983,



Wooding et al. 2000, Zhao et al. 1999a, 1999b) the improvements in baking quality were due to a decrease in dough strength and an increase in dough extensibility in response to S fertilization as was observed on the mixograph and extensigraph. Sulphur fertilization also increased the proportion of S-rich, soluble glutenin in flour and reduced the concentration of monomeric protein and the ratio of insoluble to soluble glutenin in the flour at three of four sites; S fertilization in the presence of 100 kg N ha<sup>-1</sup> only, increased the proportion of soluble glutenin in flour and reduced the concentration of monomeric protein and the ratio of insoluble to soluble glutenin in the flour at the other site. At the three sites used for quality analyses where soil SO<sub>4</sub>-S concentrations were > 40 kg ha<sup>-1</sup> and adequate for CWRs wheat yield, S fertilization had little impact on grain yield, grain nutrition, breadmaking quality, dough quality, or flour protein composition. Therefore, quality and yield responses should not be expected where there are fully adequate concentrations of soil SO<sub>4</sub>-S present. Furthermore, where plant tissue samples contain a S concentration > 0.15 % S and an N:S ratio < 17:1, no response to S fertilization should be expected.

Zhao et al. (1999c) noted that wheat requires correction of S deficiency prior to or at the second node stage to fully recover and produce maximum yields. However, these authors make no reference to breadmaking quality and the critical stage at which S deficiencies need to be corrected. Therefore, more research is required to determine if S fertilization of wheat, determined to be deficient in S after the 50 % heading stage, improves the overall breadmaking quality of the grain. If S fertilization does not improve grain quality when applied this late in the growing season, diagnosis of S deficiencies at such a late stage would be of no value. Furthermore, different S fertilizer sources and application methods should be evaluated to see if one is more effective than the other.

Finally, our study also demonstrated that S fertilization improved the breadmaking quality of CWRS wheat in some cases where the soil contained sufficient S for grain yield. At the four marginal S sites where grain quality was improved by S fertilization, only two sites demonstrated positive yield responses to S fertilization. Therefore, these observations are similar to the observations of Zhao et al. (1999a, 1999b) who also observed that breadmaking quality responses to S fertilization were more common than grain yield responses.

## 7. SUMMARY AND CONCLUSIONS

The first objective of our study was to investigate the relationship between grain S concentration, grain N concentration, and grain N:S ratio and grain yield and breadmaking quality of CWRS wheat. Both grain S concentration and N:S ratio were poor predictors of grain yield. However, our study confirmed the observations from Europe and Australia: grain S concentration and N:S ratio are important factors affecting the breadmaking quality of CWRS wheat grown in western Canada. Grain containing low concentrations of S or high ratios of N to S produced dough that was tough (strong) and inextensible and ultimately produced bread loaves of poor quality. The poor baking performance of grain containing low S concentrations was demonstrated by the strong, positive correlations between grain S concentration and loaf volume, loaf height, and oven spring and the negative correlations between grain N:S ratio and loaf volume, loaf height, and oven spring. The associated loss of dough extensibility as grain S concentration declined and N:S ratio increased was demonstrated by the strong, positive correlation between grain S concentration and dough extensibility and the negative correlation between grain N:S ratio and dough extensibility. The deterioration in dough extensibility with declining grain S concentrations and rising grain N:S ratios was compounded by an increase in dough strength, as was demonstrated by the negative correlation between grain S concentration and  $R_{\max}$  and the positive correlation between grain N:S ratio and  $R_{\max}$ . The increased strength of dough produced from grain containing low concentrations of S was further demonstrated by the negative partial correlations between grain S concentration and mixograph peak time and

work input to peak. Both mixograph peak time and work input to peak declined with increasing grain S concentrations because the dough was apparently becoming weaker and less resistant to the mixing process. In addition, as the ratio of N to S in grain increased, work input to peak also increased.

The improvements in dough and baking properties with rising concentrations of S in the grain and declining grain N:S ratios was probably due to changes in the composition of protein in the flour. These compositional changes probably increased the concentration of cysteine residues available for the production of disulphide and other types of bonds, produced dough that was more extensible and pliable, and ultimately produced bread loaves of better quality. The soluble glutenin content in flour protein increased with grain S concentration and decreased with grain N:S ratio. The associated rise in proportion of soluble glutenin with rising grain S concentrations and declining ratios of N to S in grain lead to the negative correlation observed between grain S concentration and the ratio of insoluble to soluble glutenin in flour and the positive correlation between grain N:S ratio and the ratio of insoluble to soluble glutenin in flour. Therefore, it appears that rising concentrations of S in grain and declining N:S ratios resulted in more balanced synthesis of LMW and HMW glutenin subunits.

The concentration of N in grain was poorly correlated with loaf volume, probably, in part, because most grain samples in our experiment contained sufficient N. In addition, for a number of quality parameters, including loaf height, dough extensibility, and  $R_{max}$ , rising concentrations of N in grain were associated with the deterioration of grain quality.

Breadmaking quality, dough quality, and flour protein composition improvements due to S fertilization were consistently observed when grain contained  $\leq 0.17\%$  S and an N:S ratio  $> 17:1$ . Grain containing S concentrations  $\geq 0.17\%$  S and N:S ratios  $< 17:1$  was regarded to

contain sufficient S for maximum breadmaking quality because S fertilization had little impact on grain quality when grain met these thresholds.

Therefore, the first conclusion of our study is that the S nutrition of grain, measured as total S concentration in grain and grain N:S ratio should be considered, in addition to the concentration of N in grain, in the quality evaluation of CWRS wheat grown in western Canada. The second conclusion of our study, which is most applicable to hard red spring wheat processors, is that CWRS grain containing  $\leq 0.17$  % S and an N:S  $> 17:1$  should be regarded as deficient in S for maximum breadmaking quality.

The second objective of our study was to investigate the impact of S fertilization on the grain yield and breadmaking quality characteristics of CWRS wheat. Within this objective, we wanted to examine whether the breadmaking quality of CWRS wheat was improved by the application of S fertilizer in the absence of a yield response. Of the seven sites used for quality analysis, the S fertility was marginal for the production of hard red spring wheat at four sites. At these marginal S sites, where the average concentration of soil  $\text{SO}_4\text{-S}$  was  $< 40 \text{ kg ha}^{-1}$ , S fertilization significantly increased grain yield at only two sites but increased the concentration of S in grain and reduced the N:S ratio in grain leading to improvements in breadmaking and dough rheological quality at all four marginal S sites. Sulphur fertilization increased loaf height, and oven spring at three of the four sites. There were also improvements in loaf volume at two of the four sites when S fertilizer was applied in combination with 26 or 100  $\text{kg N ha}^{-1}$ , and at one more site, but for the 100  $\text{kg N ha}^{-1}$  treatment, only. Sulphur fertilization significantly increased dough extensibility at all four sites and reduced  $R_{\text{max}}$  and mixograph peak time at three of four sites. Mixograph peak time was significantly reduced by S fertilization at the other site only in the presence of 100  $\text{kg N ha}^{-1}$ . Furthermore, S fertilization reduced the viscoelastic ratio

and mixograph work input to peak at all four marginal S sites. Sulphur fertilization increased the proportion of soluble glutenin in flour and reduced the ratio of insoluble to soluble glutenin in the flour at three of four marginal S sites; at the other marginal S site, S fertilization in the presence of 100 kg N ha<sup>-1</sup> only, increased the proportion of soluble glutenin in flour and reduced the ratio of insoluble to soluble glutenin in the flour. At the two marginal S sites where the soil N fertility was low, high rates of N fertilization amplified the grain quality responses to S fertilization. Therefore, the third conclusion of our study is that S fertilization is especially effective for improving the breadmaking quality of CWRS wheat under conditions of high N fertility and marginal S fertility. In addition, quality responses to S fertilization are more frequent than yield responses and soils that contain sufficient S for maximum grain yield may not contain sufficient S for maximum grain quality.

The third and final objective of our study was to evaluate agronomic tools (e.g. soil tests and plant tissue tests) that would aid western Canadian producers in predicting the S nutrition of grain and quality responses to S fertilization. Soil test SO<sub>4</sub>-S measured to a depth of 60 cm predicted responses to S fertilizer reasonably well. As already discussed, at the four sites where concentrations of soil SO<sub>4</sub>-S were < 40 kg ha<sup>-1</sup>, quality responses to S fertilization were consistently observed. At the three remaining sites, where soil SO<sub>4</sub>-S concentrations were > 40 kg ha<sup>-1</sup> and were regarded as adequate for hard red spring wheat production, S fertilization had little impact on grain yield, grain nutrition, flour nutrition, breadmaking quality, dough rheology, or flour protein composition. Plant tissue samples collected at 50 % heading from the marginal S sites contained < 0.15 % S and N:S ratios > 17:1 and also consistently predicted breadmaking quality, dough quality, and flour protein responses to S fertilization. Therefore, S fertilization of CWRS wheat where concentrations of soil SO<sub>4</sub>-S are < 40 kg ha<sup>-1</sup> or where plant tissue samples

collected at 50 % heading contain  $< 0.15$  % S and N:S ratios  $> 17:1$  will frequently improve the breadmaking quality of CWRS wheat. However, in the Canadian wheat industry, the price of CWRS wheat is currently determined by grade and grain N concentration (grain N concentration  $\times 5.7 =$  protein concentration) only. In other words, producers receive quality premiums for adding N fertilizer, but no S fertilizer. This policy ignores the quality improvements from S fertilization and increases the potential for imbalances of N to S in grain. Until S is measured and rewarded as an important factor for determining the overall breadmaking quality of CWRS wheat, producers should apply S fertilizer only if they expect a grain yield response.

Although quality responses were accurately predicted with the soil and plant tissue analyses, our study demonstrated that the S nutrition of wheat grain, measured as grain S concentration and total S accumulation is difficult to predict using plant tissue and soil tests. For the plant tissue tests, the S concentration of whole plant samples collected at 50 % heading and the 4 – 6 leaf stage was poorly correlated to the S concentration in grain. Grain N:S ratio was more closely correlated to the ratio of N to S in the midseason tissue samples collected at 50 % heading. For the soil tests, in the absence of S fertilization, soil  $\text{SO}_4\text{-S}$  was moderately correlated with grain S concentration and total S accumulation in the plant. When the two additional sources of soil S, including fertilizer S and estimated mineralizable organic S, were included in multiple regression analysis for the prediction of grain S concentration and total S accumulation in the plant, the relationships were weak. The soil N:S ratio, calculated with the soil  $\text{NO}_3\text{-N}$  and  $\text{SO}_4\text{-S}$  concentrations, was moderately correlated with grain N:S ratio. Furthermore, when the fertilizer treatments were included in the calculation of the soil N:S ratio, the relationship improved.

Therefore, the final conclusion of our study is that more research is required to develop a tool that accurately predicts the S nutrition of wheat and to measure the mineralization of organic S throughout the growing season and its contribution to the S nutrition of wheat. However, until a better soil test is developed, the measurement of soil SO<sub>4</sub>-S to 60 cm will provide a crude estimate of plant available S. Nitrogen to sulphur ratios of grain and soil also appear to be more stable than the absolute concentrations of N or S in the plant and soil. Therefore, the N:S ratio of the soil provides an indication of the N to S ratio in grain at maturity

Although our study generated several important observations and conclusions, a number of questions have been left unanswered and require further research. These include:

1. Will other CWRS wheat varieties respond to S fertilization, in a similar fashion, as what was observed for AC Barrie wheat?
2. Are the grain S concentration and N:S ratio thresholds for predicting quality responses to S fertilization for AC Barrie wheat applicable to other CWRS wheat varieties?
3. Is there sufficient carryover of S from one year to the next to allow producers who are applying S fertilizer to canola to maintain the breadmaking quality of their wheat?
4. Can a quick infrared test, similar to the evaluation of wheat for grain N content, be developed for the evaluation of CWRS wheat grain for S status?
5. Will S fertilizer improve the quality of S deficient wheat if applied after the 50 % heading stage? If S fertilization does not improve grain quality when applied this late in the growing season, diagnosis of S deficiencies at such a late stage would be no value to the producer.
6. How effective are different S fertilizer sources and application methods for improving the quality of CWRS wheat? For example, are midseason or late season applications reasonably effective or should all S fertilizer be applied prior to seeding or early in the growing season?



## 8. CONTRIBUTION TO KNOWLEDGE

Our research has confirmed that the S nutrition of grain, expressed as grain S concentration and N:S ratio, is an important factor contributing to the overall breadmaking performance of CWRS wheat in western Canada. Most research up to present has been conducted in Europe, Australia, and New Zealand. In addition, for the western Canadian producer, very little research up to this point has focused on the impact of S fertilization on quality of CWRS wheat. The only research conducted in western Canada was from the early to middle part of the 20<sup>th</sup> century where it was found the S fertilization increased loaf volume in plots where low S fertility severely limited grain yields (Newton et al. 1959). However, no other western Canadian research has demonstrated that S fertilization improves grain quality in the absence of grain yield responses. Our research has demonstrated that soils containing < 40 kg SO<sub>4</sub>-S in the top 60 cm of soil are to be regarded as marginal for the production of CWRS wheat because grain quality will frequently be improved by S fertilization. However, in the Canadian wheat industry, the price of CWRS wheat is currently determined by grade and grain N concentration (grain N concentration x 5.7 = protein concentration) only. In other words, producers receive quality premiums for adding N fertilizer, but no S fertilizer. This policy ignores the quality improvements from S fertilization and increases the potential for imbalances of N to S in grain. However, until S is measured and rewarded as an important factor for determining the overall breadmaking quality of CWRS wheat, producers should apply S fertilizer only if they expect a grain yield response.

Canada Western Red Spring wheat grain containing  $\leq 0.17$  % S and an N:S ratio  $> 17:1$  was regarded as deficient in S for maximum grain quality. These thresholds are the first of their kind in western Canada and are of value to the processors of CWRS wheat because they will allow for evaluation of grain quality with respect to its S status. Therefore, processors can select wheat that meets their desired quality. Furthermore, the Canadian wheat industry should consider using the S nutrition of grain as an evaluative tool and provide producers with an incentive, such as a price premium, to apply S fertilizer. This would help guarantee that CWRS wheat is not limited by S deficiencies and remains the highest quality wheat in the world. In addition, for grain companies offering identity preserved programs, wheat could be priced to urge producers to apply S fertilizer so the wheat produced is of high quality.

Our research also demonstrated that plant tissue sampling at 50 % heading was valuable in predicting quality responses to S fertilization. However, further research is required to determine if S fertilization to S deficient CWRS wheat at this stage will improve grain quality. Other researchers have shown that S fertilization at this stage is too late to correct S deficiencies related to grain yield (Zhao et al. 1999c).

Finally, most previous research regarding the value of plant tissue and soil tests has focused on the relationship between plant and soil S concentrations or N:S ratios and grain yield, total plant yield, or S accumulation in the plant. Very little research has focused on the predictability of grain S nutrition expressed as grain S concentration or N:S ratio. Our research has demonstrated that these estimates of grain S nutrition are difficult to predict using soil and plant tissue tests. Therefore, more research is required to develop a more accurate method of predicting the S nutrition of grain.

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## 10. APPENDICES

### 10.1. Appendix A - Materials and Methods for Soil Analysis

#### A.1. Sulphate

Soil  $\text{SO}_4\text{-S}$  was extracted using the method described by McKeague (1978), with several modifications. A 15-g sample of ground soil was shaken for 30 minutes in 30 mL of 0.001 M  $\text{CaCl}_2$  extraction solution. Immediately after shaking, the extract was filtered through Whatman #40 filter paper. The  $\text{SO}_4\text{-S}$  was then determined in the extract using the automated methylthymol blue method as described by Greenberg et al. (1992) and illustrated in the Technicon Autoanalyzer II Continuous Flow Instrument Manual (1975).

#### A.2. Nitrate

Soil  $\text{NO}_3\text{-N}$  was extracted using the method described by McKeague (1978), with several modifications. A 15-g sample of ground soil was shaken for 30 minutes in 30 mL of 0.001 M  $\text{CaCl}_2$  extraction solution. Immediately after shaking, the extract was filtered through Whatman #40 filter paper. The  $\text{NO}_3\text{-N}$  was then determined in the extract using the automated cadmium reduction method as described by Greenberg et al. (1992) and illustrated in the Technicon Autoanalyzer II Continuous Flow Instrument Manual (1975).

### A.3. Mineralizeable Nitrogen and Sulphur

Mineralizeable-N was estimated using a modified version of the phosphate-borate method developed by Gianello and Bremner (1986b). Five mL of boric acid-indicator solution was added to a 100-mL Erlenmeyer receiver flask and placed under the condenser of a Kjel Tech 1030 steam distillation apparatus so that the end of the condenser was approximately 4 cm above the surface of the boric acid-indicator solution. A 5-g sample of ground soil was transferred into a distillation flask containing 50 mL of pH 11.2 phosphate-borate buffer solution. The distillation flask was immediately attached to the Kjel Tech 1030 distillation apparatus and the soil solution was distilled for approximately 8 minutes or until approximately 50 mL of distillate was captured. Mineralizeable-N was determined in the distillate by titration with 0.005 N H<sub>2</sub>SO<sub>4</sub> and calculated using the following calculation:

$$\text{Mineralizeable N (ppm)} = \frac{(\text{mL to titrate distillate} - \text{mL to titrate blank}) \times 0.01 \times 14000}{\text{weight of sample}}$$

Estimated mineralizeable S was calculated by dividing the mineralizeable N values determined in the phosphate-borate extraction by 8.3. This value of 8.3 was adopted from the work of Bailey (1985) who found the average N:S ratio of soil organic matter in Prairie Canadian soils to be 8.3:1.

### A.5. Phosphorous

Soil PO<sub>4</sub>-P was extracted using a modification of the Bray Method described by McKeague (1978). The extraction solution contains 1 N ammonium acetate, 0.005 N acetic acid, and 0.015 N ammonium fluoride. Twenty-five mL of this extraction solution was mixed with 2.5 g of ground soil and was shaken for 30 minutes. The extract was then filtered through

Whatman #40 filter paper. The PO<sub>4</sub>-P was determined in the extract using the stannous chloride method as outlined by Greenberg et al. (1992).

#### A.6. Potassium

Soil K was extracted using a modification of the Bray Method described by McKeague (1978). The extraction solution contains 1 N ammonium acetate, 0.005 N acetic acid, and 0.015 N ammonium fluoride. Twenty-five mL of this extraction solution was mixed with 2.5 g of ground soil and was shaken for 30 minutes. The extract was then filtered through Whatman #40 filter paper. The K was determined in the extract using the flame photometric method as outlined by Greenberg et al. (1992).

#### A.7. Copper, Manganese, Zinc, and Iron

A 20-g ground soil sample was shaken with 40 mL DTPA extracting solution (Lindsay and Norvell 1978) for 2 hours. The extract was filtered using Whatman #40 filter paper. Cu, Mn, Zn, and Fe were then determined in the extract using the inductively coupled plasma method (Greenberg et al. 1992).

#### A.8. Soil pH

The pH of soil samples was determined on a 2:1 water to soil ratio using a pH meter.

#### A.9. Soil Organic Matter

Soil organic matter was determined using a modified Walkley-Black procedure (McKeague 1978). A 0.5-g sample of ground soil was digested with 10 mL of 1 N potassium

dichromate and 20 mL of sulphuric acid for 45 minutes. After allowing for the digestion time, 5 mL of concentrated phosphoric acid was added and the digest was brought up to a volume of 250 mL with distilled water. Two mL of indicator solution was added and the organic matter was determined by back-titration using a 0.5 N ferrous sulphate solution.

## 10.2. Appendix B

### Materials and Methods for Grain Quality Analysis

#### B.1. Farinograph (Approved Method 54-21, AACC, 2000)

Farinograph tests, using a 10-g Brabender Farinograph (Brabender Instruments Inc., South Hackensack, NJ. U.S.A.), were performed on each flour sample using the constant flour weight method (Approved Method 54-21, AACC, 2000). The procedure involves adding 10 g of flour (14 % m.b.) to a temperature regulated ( $30 \pm 0.1^{\circ}\text{C}$ ) farinograph bowl. Water is added to the mixing bowl in increments while observing the farinograph curve approach the 500-BU line. If the curve moves past the 500-BU line, water is added until the curve moves down and centres on the 500-BU line (if the curve peaks below the 500 BU line, too much water has been added; therefore, the water added is readjusted and the test is rerun). Once the curve plateaus at the 500-BU line, the amount of water added is recorded as the titration value. The farinograph water absorption (%) (FAB) is then calculated using the equation  $\text{FAB} = 10 (10 - \text{wt of flour} + \text{titration value})$ .

Using the farinograph curve (Figure B.1.1), a number of dough mixing characteristics are measured. Dough development time is the time (minutes) from the onset of mixing to the point at which the dough reaches maximum consistency (500 BU). Dough stability is the time (minutes) between the point at which the top line of the curve first intercepts the 500-BU line and the point at which the top line of the curve drops below the 500-BU line. Time to breakdown is the time (minutes) from the onset of mixing to the time at which the dough consistency has decreased by 30 BU from the maximum consistency. Mixing tolerance index is

the difference (BU) between the top of the curve at peak (500 BU) and the top of the curve 5 minutes after peak.

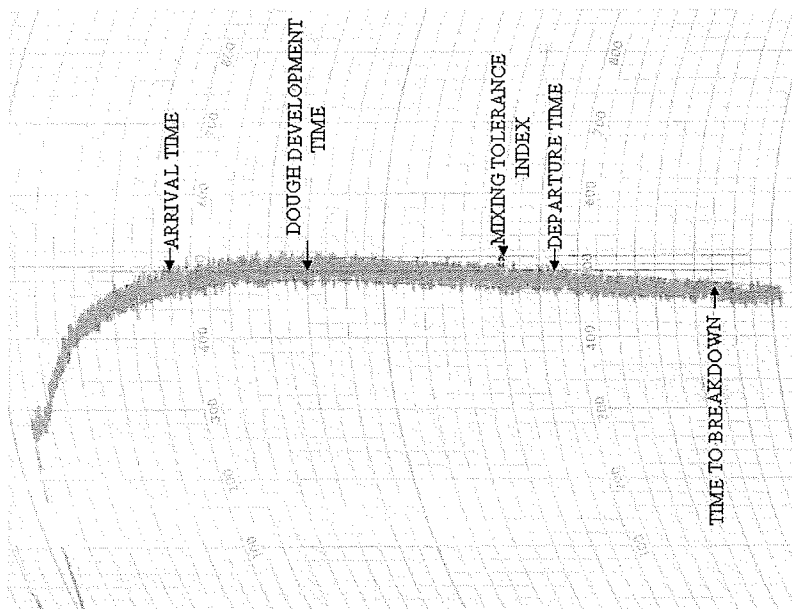


Figure B.1.1. A typical farinograph curve

## B.2. Mixograph

Mixograph tests were conducted with a 2-g Micro-mixograph (National Mfg., TMCO, Lincoln, NE., U.S.A) using a modification of the method developed by Pon et al. (1989). The procedure involves adding 2 g ( $\pm 0.001$  g) of flour (14 % m.b.) to a temperature-regulated water-jacketed mixograph bowl. The calculated amount of water (based on a fixed water absorption of 62 %) is then added to the bowl with a syringe. The mixograph is run at a temperature of  $25 \pm 0.1^\circ\text{C}$ .

During the mixing process, a computer-generated mixograph curve is created. A typical mixograph curve is shown in Figure B.2.1. A number of important dough mixing characteristics are calculated with the mixograph. Mixograph development time, or peak time, is the time

(minutes) to the maximum height of the mixograph curve. Mixograph peak height is the height of the curve at peak time (% torque). Computerized data collection has also made it possible to measure other curve parameters like energy to peak (% torque minute<sup>-1</sup>), total energy (% torque minute<sup>-1</sup>), and band-width at peak (% torque).

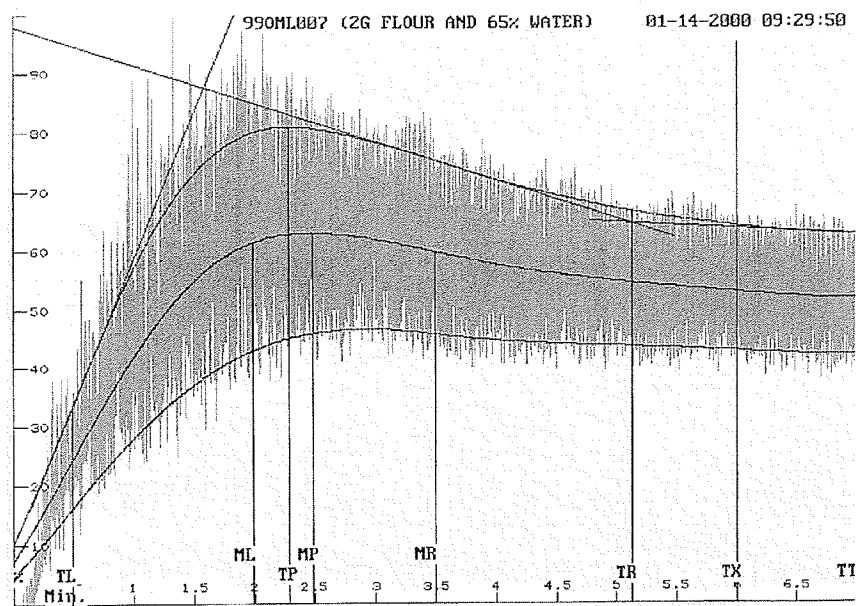
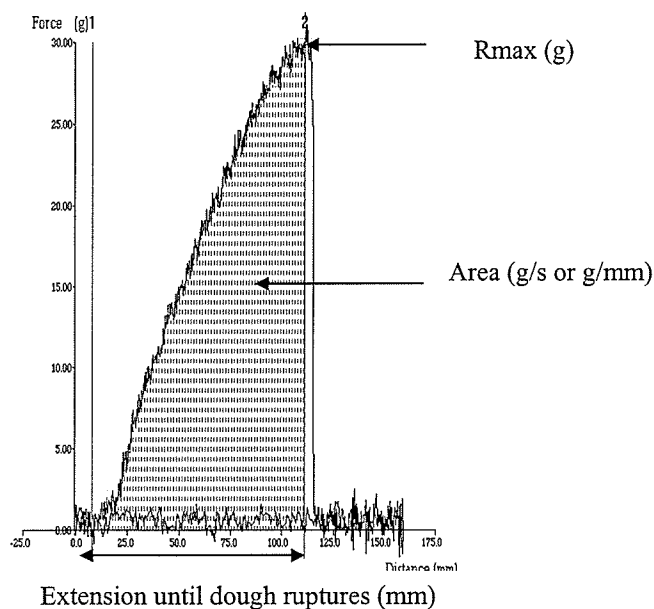


Figure B.2.1. A typical mixograph curve

### B.3. Extensigraph

A 2-g Micro-mixograph (National Mfg., TCMCO, Lincoln, NE., U.S.A) and Texture Analyzer (TA.XT2, Texture Technologies Corp., Scarsdale, NY., U.S.A. / Stable Micro Systems, Godalming, Surrey, U.K.) were used to evaluate maximum dough resistance ( $R_{max}$ ), dough extensibility (Ext), and extensigraph peak area according to the method of Suchy et al. (1999). The procedure involves mixing a 2-g flour sample (14% m.b.) with 0.29 g of 0.16 g/mL salt solution and FAB + 6 % distilled water (minus 0.25 mL used for the salt solution) on a 2-g micro-mixograph. The micro-mixograph is run twice for each sample. The first 10-minute run

is to determine the mixograph development time from the midline analysis. The second run is stopped at this pre-determined peak time when the dough reaches its maximum development. The dough is then placed over a Teflon based dough press designed to distribute the dough over 3 or 4 channels to yield an equivalent number of dough strips of uniform geometry. The dough strips are left to rest for 40 minutes at 25°C. The individual strips are then placed on a Kieffer rig dough holder and tested on the TA-XT2 Texture Analyzer. At a hook speed of 3.3 mm/second, the hook stretches the dough strip positioned across the dough holder, until the piece of dough tears. During this stretching process, a computer-generated extensigram is created (Figure B.3.1). With the extensigram, several extensigraph parameters are determined. First, maximum dough resistance to extension ( $R_{max}$ ), is the maximum height of the extensigraph curve (g), and is a measure of dough strength. Second, area under the curve is the area (g/mm) above the baseline bordered by the curve and is also a measure of dough strength. Third, dough extensibility (Ext) measures the ability of a dough piece to stretch without breaking and is defined by the total length of the curve (mm). Finally, the viscoelastic ratio, which is the ratio of the maximum resistance to stretching ( $R_{max}$ ) to dough extensibility (Ext), is calculated.



<u>TA-XT2 settings</u>	
Test Mode:	Measure Force in Tension
Pre Test Speed:	2mm/s
Test Speed:	3.3 mm/s
Post Test Speed:	10 mm/s
Distance:	160 mm
Force:	40g
Time:	0.09 s
Trigger Force:	2g

Figure B.3.1. A typical extensigram curve



#### B.4. Protein Fractionation (Suchy et al. 2002)

The flour protein extraction protocol relies on a sequential solubility of the flour protein in various concentrations of 1-propanol (Suchy et al. 2002). As in the fractionation scheme (Figure B.4.1), three identical samples with known flour nitrogen content and identical mass (100 mg at 14% mb) were extracted simultaneously three times with 1.0 mL of 7.5 % 1-propanol and 0.3 M NaI at 25°C in 1.5 mL microcentrifuge tubes. The extraction was carried out in a temperature-controlled shaker (Thermomixer 5436, Eppendorf) with additional rapid vortexing (10 s at max setting using a Genie-2, Fisher Sci.) at the beginning and end of the extraction step. Tubes were centrifuged at 10,000 x g (stage 1, 2 and 3). Insoluble residues were retained and actual fractions (supernatants) were used to monitor quality of extraction by SDS-PAGE. In the second stage only the residues from flour 2 and 3 were further extracted three times with the 50 % 1-propanol at 25°C. After that step, the supernatant was discarded and residue from flour 3 was extracted three times with 40 % 1-propanol and 0.2 % dithiothreitol (DTT) at 60°C. The insoluble residues from step 1, 2 and 3 were dried for 16 hours at 75°C using a solid bed heater.

Nitrogen content was determined on the insoluble residue from stage 1, 2 and 3, as identified in Figure B.4.1. Combustion nitrogen analysis was performed using the LECO FP-528 nitrogen analyzer (LECO Corp., St. Joseph, MI) according to the manufacturer recommendations. The four principal flour protein solubility groups were obtained by the difference: monomeric protein (MP, flour nitrogen content minus nitrogen content of insoluble residue stage 1), soluble glutenin, (SG, nitrogen content of insoluble residue stage 1 minus nitrogen content of insoluble residue stage 2), insoluble glutenin (IG, nitrogen content of insoluble residue stage 2 minus nitrogen content of insoluble residue stage 3), residue protein

(RP, nitrogen content of insoluble residue after stage 3). The flour protein solubility fractions were expressed as a percentage of nitrogen over total flour nitrogen at 14 % m.b.

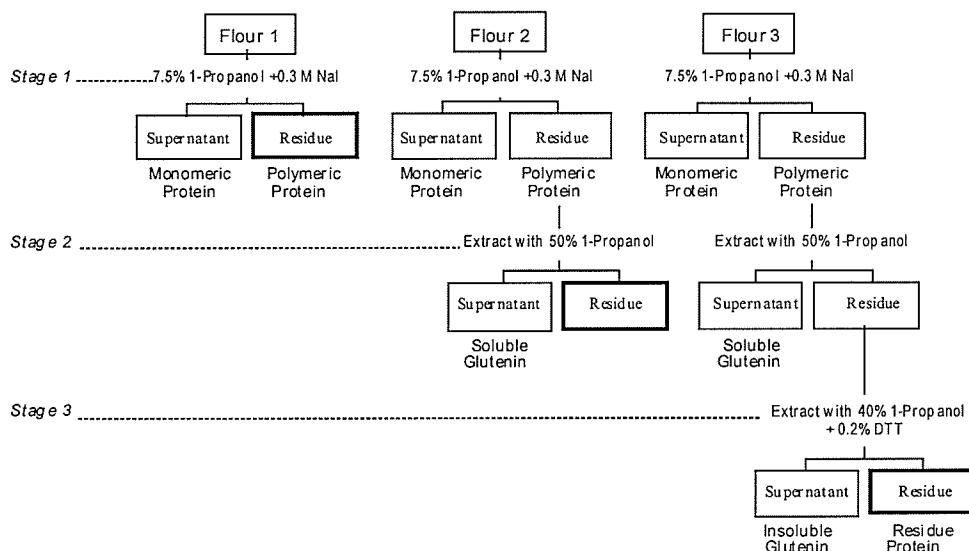


Figure B.4.1. Wheat protein fractionation scheme (Box frame in bold indicates the fraction analyzed for nitrogen content) (Courtesy Suchy et al. 2002).

## B.5. Bake Parameters

### B.5.1. Baking Procedure and Sample Performance

The optimized long-fermentation bake test (Approved Method 10-10B, AACC, 2000) is used to evaluate the baking quality of flour samples. The following ingredients are used: 100 g flour (14 % m.b.), salt (1.5 %), yeast (1.0 %), sugar (6.0 %), malt syrup (0.2 %), ammonium phosphate (0.1 %), ascorbic acid (20 p.p.m.), whey (4.0 %), shortening (3.0 %), and water (FAB – 3 %). The ingredients are mixed according to the procedure specifications. After mixing, the dough is left to ferment for 105 minutes, after which it is punched for the first time. The dough is then left to ferment for another 50 minutes, after which it is punched again. After a further 25 minutes of fermentation, the dough is molded and placed into a baking pan. The samples are

proofed (allowed to rise) for the time it takes the control samples to reach a height of 95 mm. At this time, the height of the unbaked loaf is determined and recorded as proof height (mm). The loaf is then baked for 25 minutes at 400°F. When finished, the loaf is removed and loaf height (mm) is recorded. Oven spring (mm) is determined as the difference in loaf height between the beginning of bake (proof height) and the end of bake (loaf height). The loaf is then cooled for 30 minutes, after which loaf volume is measured by rapeseed displacement in a loaf volumeter.

### B.5.2. Crumb Firmness (Approved Method 74-19, AACC, 2000)

Crumb firmness is recorded the day after baking. Four slices of the bread are taken from the middle of the loaf. Two of them are stacked for each test: each two-slice are compressed at two different spots. The probe used is the TA-4 1.5” (38 mm) acrylic probe with a slight chamfer. Bread crumb firmness is determined at 25 % (f1) and 40 % (f2) compression (Figure B.5.1.).

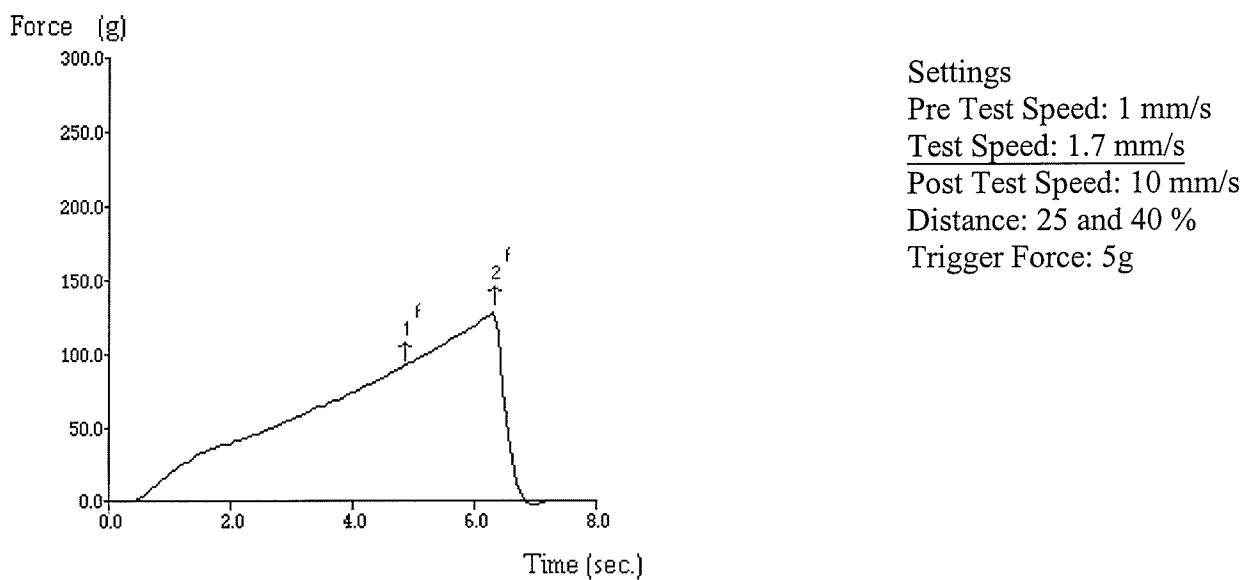


Figure B.5.1. A typical loaf compression curve

### B.5.3. Crumb Evaluation

Objective evaluation of the bread crumb is done by a computerized image analysis system using the American Institute of Baking Crumb Scan software, where the images of two slices of fresh cut bread are scanned and analyzed for cell size and shape and reported as crumb fineness and crumb elongation according to Wesley et al. (1999).

### B.6. SDS Sedimentation Volume

SDS Sedimentation tests are conducted on 2.5 g samples of flour according to the method of Kovacs (1985).

### B.7. Milling

Grain samples are tempered to 16.5 % moisture content and are milled to flour using a Buhler laboratory mill. During the milling process, flour yield is determined with the following calculation:

$$\text{Flour Yield} = (\text{flour out of mill} / \text{total recovered product out of mill}) \times 100 \%$$

### B.8. Rapid Visco Analyzer

The rapid visco analyzer (RVA) test is done in duplicate using the Approved Method (AACC Method 76-21, 2000).

### 10.3. Appendix C

#### Materials and Methods for Grain, Flour, and Plant Tissue Nutrient Analysis

##### C.1. Total Sulphur in Grain, Flour, and Plant Tissue

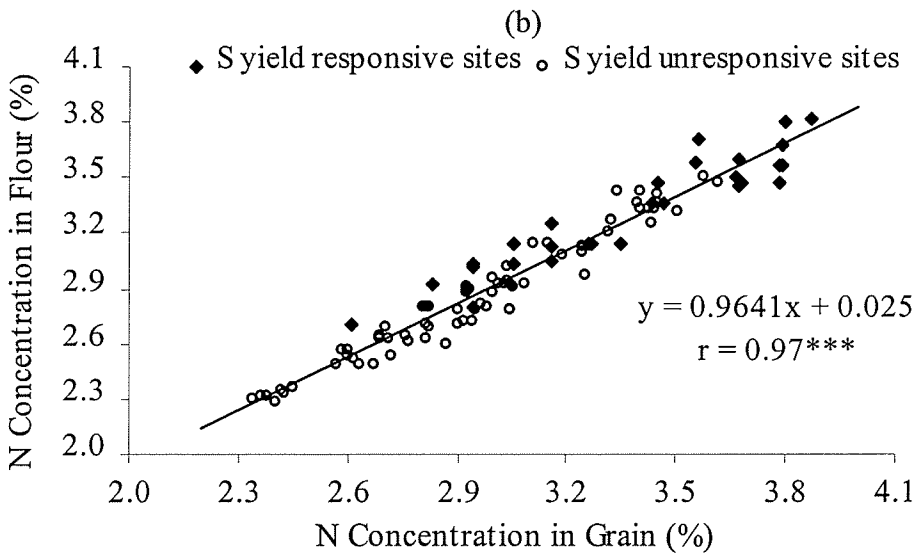
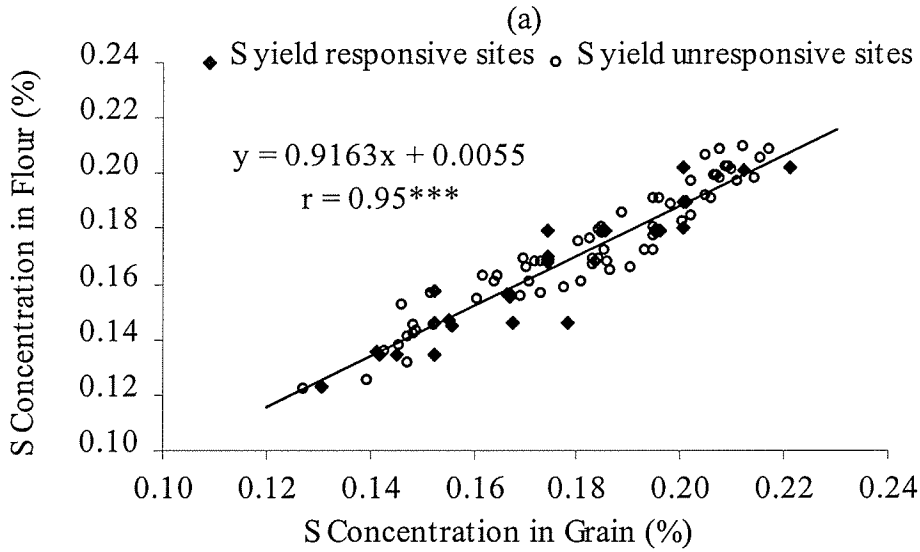
The sulphur measurement is made by infrared (IR) radiation detection. A 0.2 g sample of dried plant tissue (grain, flour, or leaf) is inserted into a LECO CNS 2000 Elemental Analyzer (Leco Corporation, 1996) and is converted to SO<sub>2</sub> by combustion, with pure oxygen. The SO<sub>2</sub> gas absorbs IR radiation and the amount of energy absorbed is proportional to the level of SO<sub>2</sub> present. The output from the IR cell for sulphur is fed to a preamplifier, then fed to an analog, then to a digital converter. The output, a digital signal, is then fed to a computer, where it is processed and reported as % sulphur.

##### C.2. Total Nitrogen in Grain, Flour, and Plant Tissue

Total nitrogen measurements are made by thermal conductivity (TC) detection. A 0.2 g sample of dried plant tissue (grain, flour, or leaf) is inserted into a LECO CNS 2000 Elemental Analyzer (Leco Corporation, 1996) and is converted to NO<sub>2</sub> gas. The TC cell has the ability to detect the difference in the thermo conductivity of gases. The cell consists of two pairs of matched filaments used in four legs of a wheatstone bridge. The lower thermal conductivity of NO<sub>2</sub> gas causes an imbalance in the wheatstone bridge proportional to the concentration of nitrogen present. The output from the TC cell for nitrogen is fed to a preamplifier, then fed to an analog, then to a digital converter. The output, a digital signal, is then fed to a computer, where it is processed and reported as % nitrogen.

## 10.4. Appendix D

### Grain Quality Correlations



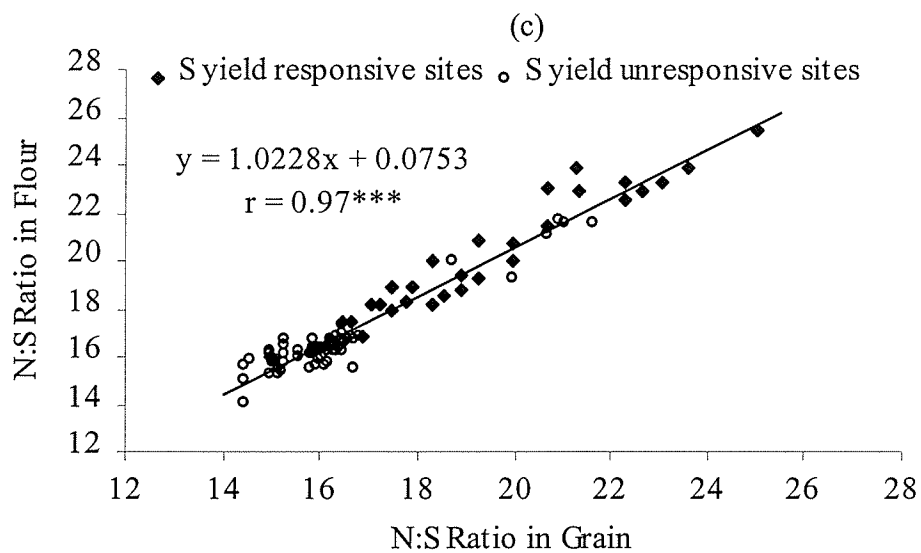


Figure D.1. Relationship between (a) concentration of S in grain and concentration of S in flour, (b) concentration of N in grain and concentration of N in flour, and (c) N:S ratio in grain and N:S ratio in flour

\*\*\* Significantly greater than 0 at  $P < 0.001$

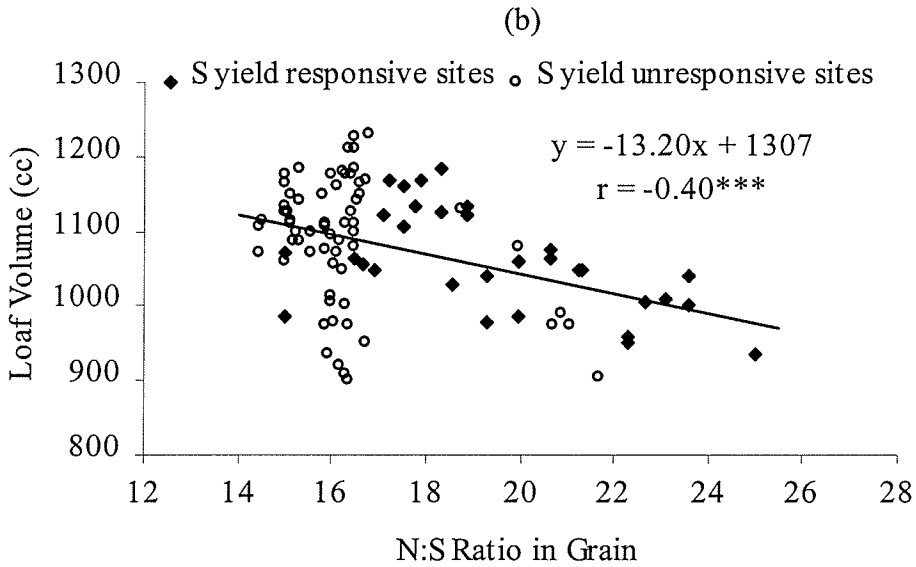
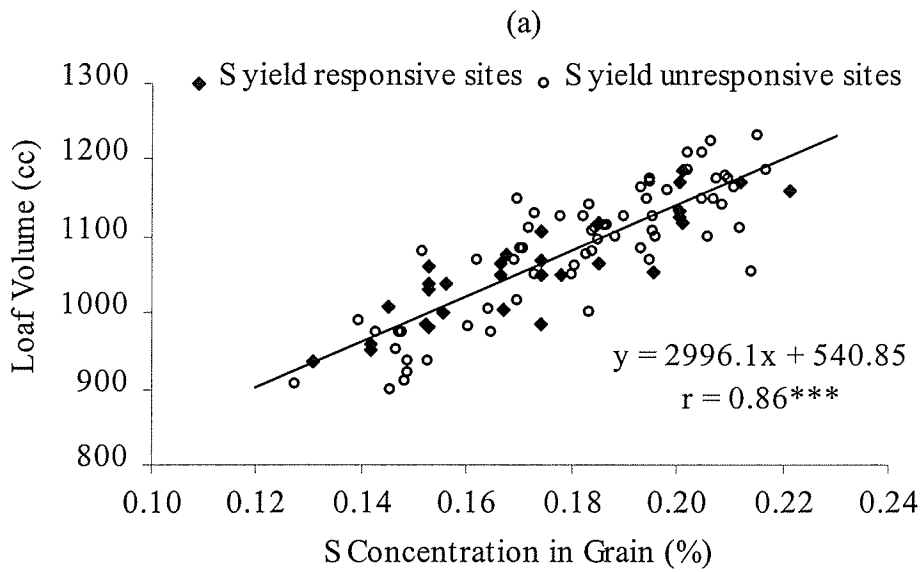


Figure D.2. Relationship between (a) S concentration in grain and loaf volume and

(b) N:S ratio in grain and loaf volume

\*\*\* Significantly greater than 0 at  $P < 0.001$



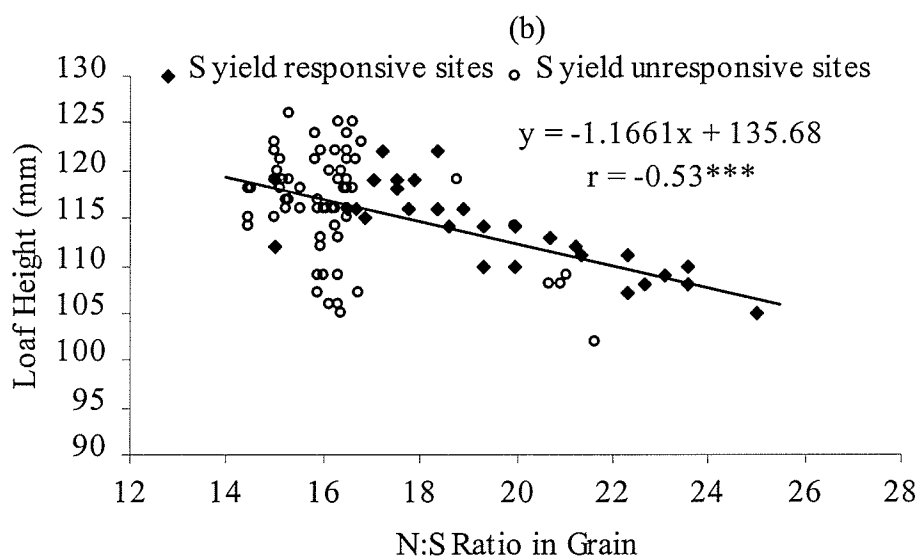
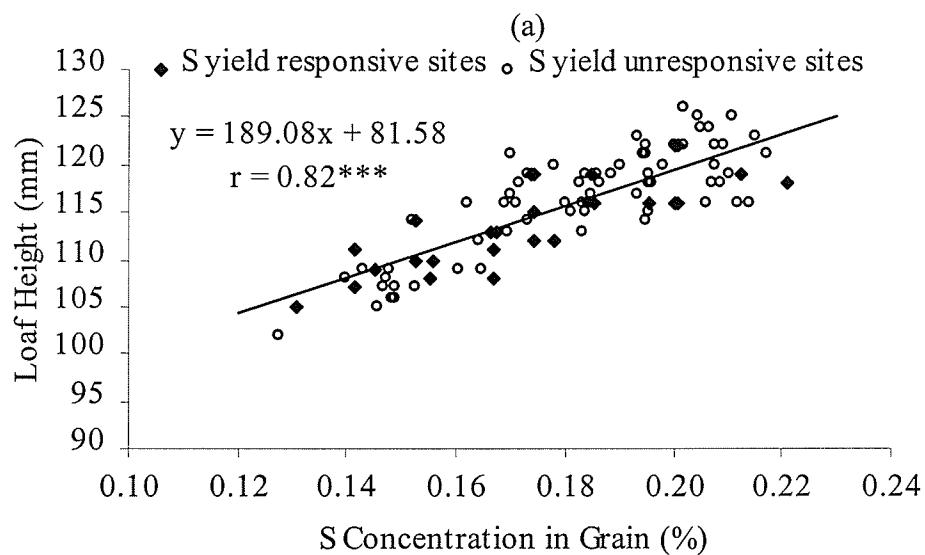


Figure D.3. Relationship between (a) S concentration in grain and loaf height and (b) N:S ratio in grain and loaf height

\*\*\* Significantly greater than 0 at  $P < 0.001$

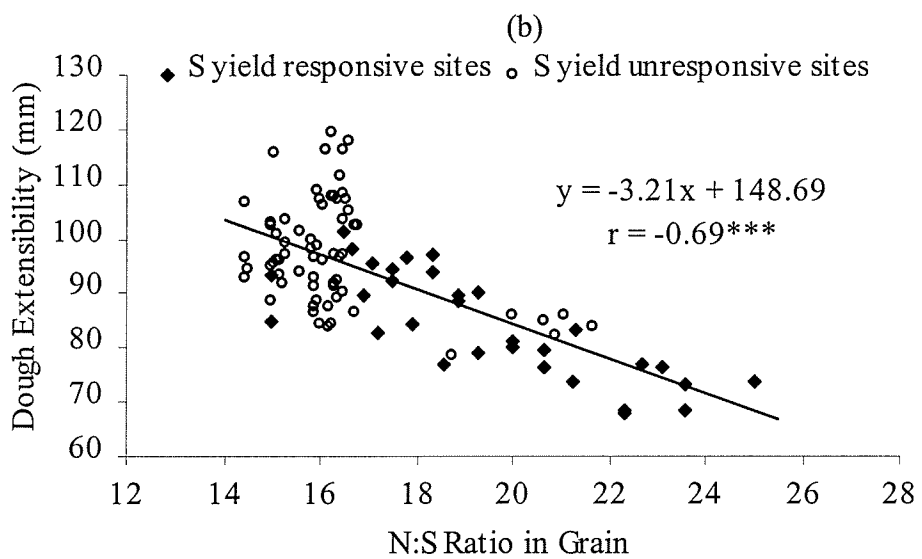
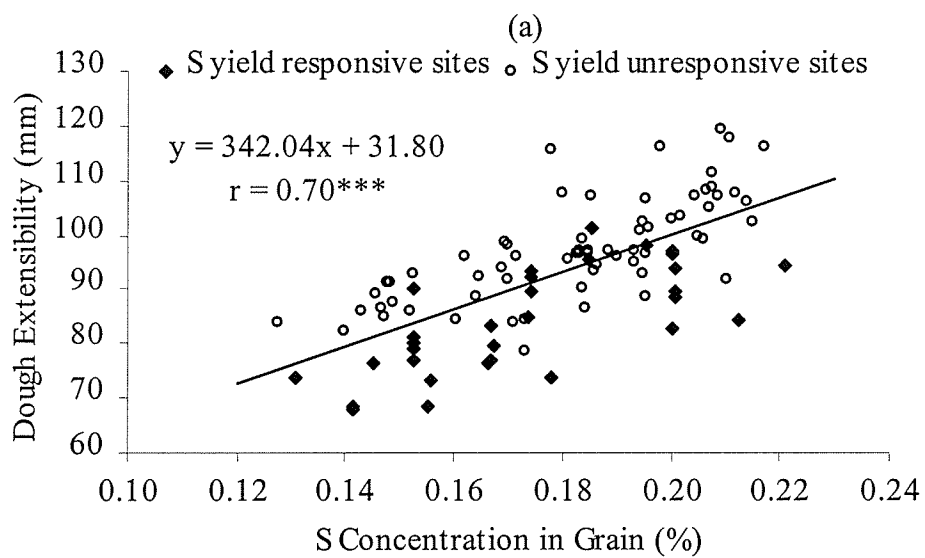


Figure D.4. Relationship between (a) S concentration in grain and dough extensibility and (b) N:S ratio in grain and dough extensibility

\*\*\* Significantly greater than 0 and  $P < 0.001$

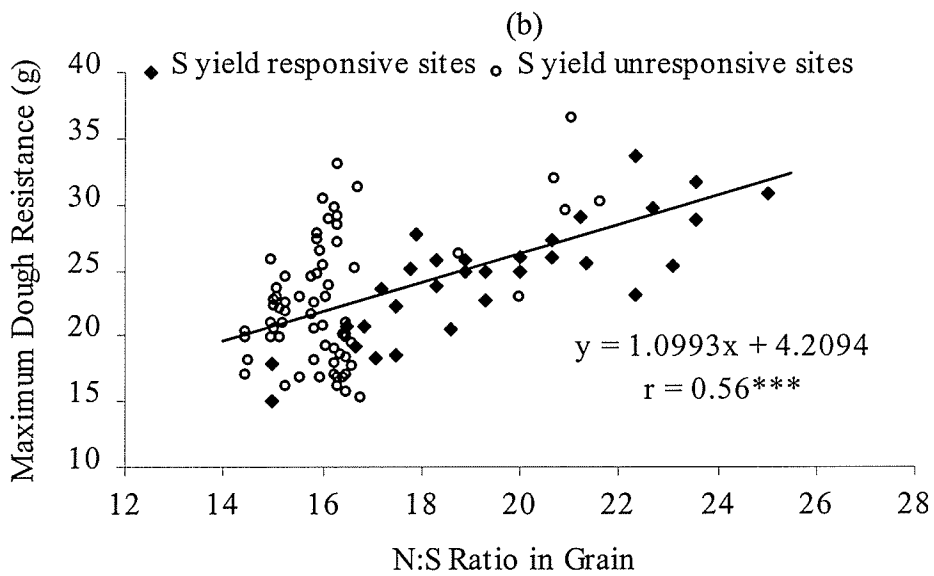
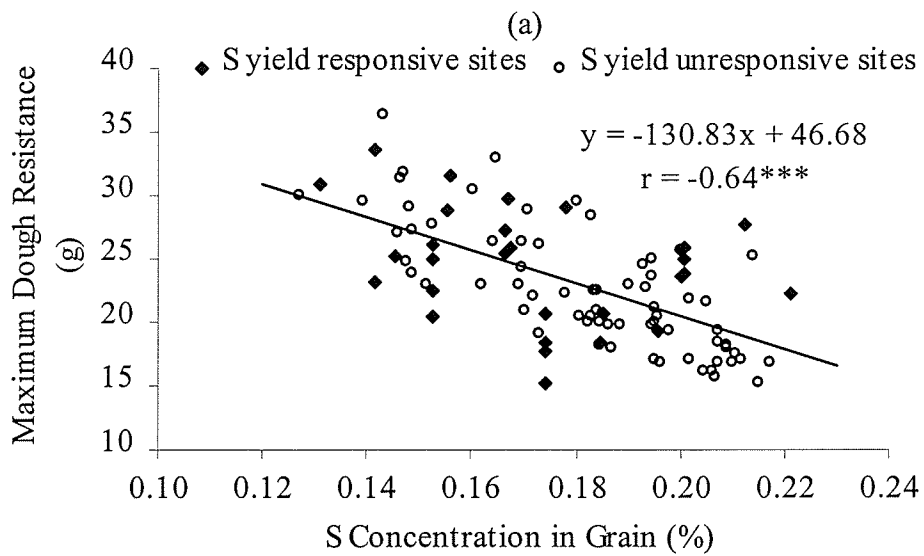


Figure D.5. Relationship between (a) S concentration in grain and maximum dough resistance and (b) N:S ratio in grain and maximum dough resistance

\*\*\* Significantly greater than 0 at  $P < 0.001$

## **10.5. Appendix E**

### **Analysis of Variance, LSDs, and Contrasts for the Effects of S and N Fertilization on Yield and Breadmaking Quality Measurements**

Table E.1. Effect of nitrogen and sulphur fertilization on grain yield<sup>†</sup> (kg/ha) in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	1038	2182	1772	3488	2831	1644	2369	2060	1651	1969	2030	2607
20	26	1107	2372	1976	3392	3280	1613	2315	1781	1526	1917	1822	2717
0	100	978	2342	2479	2730	3013	2088	2202	2098	2326	2162	2173	2590
20	100	1192	2450	2360	2583	3645	2086	1881	2123	2486	2376	1923	2570
Group Means													
0		1008	2262	2126	3109	2922	1866	2285	2079	1989	2065	2101	2599
20		1150	2411	2168	2987	3437	1849	2098	1952	2006	2147	1872	2643
LSD (P=0.05)		124	ns	ns	ns	440	ns	ns	ns	ns	ns	ns	ns
	26	1072	2277	1874	3440	3056	1629	2342	1921	1589	1943	1926	2662
	100	1085	2396	2419	2656	3284	2087	2041	2110	2407	2269	2048	2580
LSD (P=0.05)		ns	ns	391	206	ns	221	ns	186	242	224	ns	ns
ANOVA													
	df	Pr>F											
Sulphur (S)	1	0.029*	0.49	0.80	0.21	0.014*	0.87	0.21	0.16	0.87	0.43	0.15	0.58
Nitrogen (N)	1	0.82	0.58	0.014*	0.0001**	0.12	0.001**	0.057	0.047*	0.0001**	0.009**	0.42	0.31
S*N	1	0.22	0.85	0.35	0.78	0.44	0.88	0.36	0.099	0.22	0.21	0.89	0.43
Contrasts													
0 S vs 20 S at 26 N		0.39	0.53	0.40	0.48	0.12	0.82	0.79	0.040*	0.43	0.72	0.33	0.34
0 S vs 20 S at 100 N		0.022*	0.72	0.62	0.28	0.029*	0.99	0.14	0.84	0.32	0.16	0.25	0.86
C.V. (%)		10.13	17.67	12.91	5.99	11.66	10.52	12.60	8.17	10.69	9.42	14.46	5.90

<sup>†</sup> Grain yields reported on dry matter basis, except for Kelvington

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.2. Effect of nitrogen and sulphur fertilization on sulphur concentration in grain (%)<sup>†</sup> in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	0.165	0.185	0.183	0.178	0.150	0.147	0.178	0.207	0.167	0.173	0.177	0.176
20	26	0.200	0.190	0.193	0.185	0.175	0.153	0.176	0.206	0.164	0.177	0.182	0.187
0	100	0.168	0.193	0.170	0.205	0.140	0.139	0.189	0.211	0.172	0.165	0.209	0.197
20	100	0.208	0.198	0.195	0.210	0.183	0.174	0.196	0.211	0.174	0.184	0.211	0.195
Group Means													
0		0.166	0.189	0.176	0.191	0.145	0.143	0.184	0.209	0.169	0.169	0.193	0.186
20		0.204	0.194	0.194	0.198	0.179	0.164	0.186	0.208	0.169	0.181	0.196	0.191
LSD (P=0.05)		0.0071	0.0042	0.015	ns	0.013	0.0077	ns	ns	ns	0.0083	ns	ns
	26	0.183	0.188	0.188	0.181	0.163	0.150	0.177	0.206	0.165	0.175	0.180	0.181
	100	0.188	0.195	0.183	0.208	0.159	0.157	0.193	0.211	0.173	0.175	0.210	0.196
LSD (P=0.05)		ns	0.0042	ns	0.011	ns	ns	0.006	ns	ns	ns	0.009	0.007
ANOVA													
	df	Pr>F											
Sulphur (S)	1	0.0001**	0.025*	0.026*	0.23	0.0003**	0.0002**	0.39	0.87	0.97	0.011*	0.42	0.17
Nitrogen (N)	1	0.14	0.003**	0.47	0.0004**	0.92	0.074	0.0005**	0.066	0.056	1.00	0.0001**	0.002**
S*N	1	0.44	1.00	0.28	0.8	0.14	0.0022**	0.15	0.96	0.50	0.081	0.70	0.065
Contrasts													
0 S vs 20 S at 26 N		0.0001**	0.09	0.31	0.31	0.012*	0.23	0.64	0.88	0.61	0.41	0.40	0.032*
0 S vs 20 S at 100 N		0.0001**	0.09	0.025*	0.49	0.0008**	0.0001**	0.11	0.94	0.65	0.005**	0.76	0.68
C.V. (%)		3.37	1.95	7.09	5.02	6.74	4.45	3.14	2.12	3.97	4.22	4.11	3.47

<sup>†</sup> S concentrations reported on dry matter basis

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.3. Effect of nitrogen and sulphur fertilization on nitrogen concentration in grain (%)<sup>†</sup> in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	3.45	3.04	2.89	2.65	2.94	2.40	2.88	3.41	2.69	2.68	2.79	2.63
20	26	3.62	3.08	2.82	2.70	2.83	2.44	2.79	3.36	2.58	2.63	2.79	2.83
0	100	3.72	3.34	3.09	2.91	3.16	2.93	3.35	3.44	2.77	2.94	3.53	3.08
20	100	3.78	3.25	3.01	2.86	3.12	2.83	3.36	3.42	2.72	2.85	3.41	3.02
Group Means													
0		3.59	3.19	2.99	2.78	3.05	2.67	3.12	3.42	2.73	2.81	3.16	2.86
20		3.70	3.17	2.92	2.78	2.95	2.63	3.08	3.39	2.65	2.74	3.10	2.92
LSD (P=0.05)		0.074	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	26	3.54	3.06	2.86	2.67	2.89	2.42	2.84	3.38	2.63	2.65	2.79	2.73
	100	3.75	3.30	3.05	2.88	3.14	2.88	3.35	3.43	2.75	2.90	3.47	3.05
LSD (P=0.05)		0.074	0.045	0.074	0.12	0.22	0.12	0.14	ns	ns	0.14	0.10	0.14
ANOVA													
	df	Pr>F											
Sulphur (S)	1	0.0067**	0.34	0.051	0.93	0.48	0.56	0.55	0.46	0.21	0.27	0.24	0.29
Nitrogen (N)	1	0.0001**	0.0001**	0.0002**	0.0029**	0.026*	0.0001**	0.0001**	0.32	0.10	0.0028**	0.0001**	0.0005**
S*N	1	0.13	0.0092**	0.97	0.36	0.69	0.18	0.47	0.72	0.68	0.83	0.22	0.07
Contrasts													
0 S vs 20 S at 26 N		0.0053**	0.14	0.14	0.56	0.42	0.57	0.35	0.44	0.24	0.51	0.98	0.051
0 S vs 20 S at 100 N		0.22	0.014*	0.15	0.47	0.83	0.18	0.93	0.78	0.54	0.35	0.1	0.52
C.V. (%)		1.78	1.24	2.21	3.74	6.03	3.89	4.06	2.68	4.57	4.29	2.84	4.16

<sup>†</sup> N concentrations reported on dry matter basis

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.4. Effect of nitrogen and sulphur fertilization on N:S ratio in grain 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	21.44	16.67	15.75	14.61	19.29	16.32	16.12	16.50	16.12	15.54	15.77	15.02
20	26	18.06	16.48	14.65	14.49	15.99	15.99	15.82	16.31	15.68	14.83	15.34	15.10
0	100	22.77	17.54	17.92	14.03	22.41	21.08	17.77	16.31	16.17	17.85	16.90	15.62
20	100	18.15	16.62	15.43	13.75	16.87	16.21	17.12	16.24	15.65	15.50	16.19	15.50
Group Means													
0		22.10	17.10	16.84	14.32	20.85	18.70	16.94	16.40	16.15	16.69	16.33	15.32
20		18.10	16.55	15.04	14.12	16.37	16.10	16.47	16.27	15.67	15.17	15.77	15.30
LSD (P=0.05)		1.00	0.45	1.17	ns	1.71	0.28	ns	ns	0.28	0.92	0.36	ns
	26	19.75	16.57	15.20	14.55	17.64	16.16	15.97	16.40	15.90	15.18	15.55	15.06
	100	20.46	17.08	16.67	13.89	20.03	18.65	17.44	16.28	15.91	16.67	16.55	15.56
LSD (P=0.05)		ns	0.45	1.17	0.38	1.71	0.28	0.68	ns	ns	0.92	0.36	ns
ANOVA													
	df	Pr>F											
Sulphur (S)	1	0.0001**	0.021*	0.0069**	0.27	0.0004**	0.0001**	0.15	0.064	0.0034**	0.0044**	0.0057**	0.93
Nitrogen (N)	1	0.13	0.032*	0.019*	0.0035**	0.032*	0.0001**	0.0008**	0.072	0.93	0.0051**	0.0001**	0.083
S*N	1	0.18	0.10	0.21	0.65	0.16	0.0001**	0.58	0.35	0.76	0.072	0.39	0.71
Contrasts													
0 S vs 20 S at 26 N		0.0003**	0.51	0.17	0.62	0.012*	0.094	0.50*	0.056	0.031*	0.25	0.089	0.84
0 S vs 20 S at 100 N		0.0001**	0.010**	0.0076**	0.28	0.001**	0.0001**	0.16	0.45	0.015*	0.0027**	0.011*	0.75
C.V. (%)		4.20	2.37	6.48	2.35	7.63	1.43	3.58	0.76	1.53	5.08	1.96	3.38

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively



Table E.5. Effect of nitrogen and sulphur fertilization on sulphur concentration in flour (%)<sup>†</sup> in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	0.150	0.175	0.150	0.197	-	0.143	0.160
20	26	0.193	0.180	0.173	0.199	-	0.147	0.166
0	100	0.150	0.168	0.135	0.203	0.167	0.129	0.178
20	100	0.193	0.185	0.180	0.205	0.17	0.169	0.176
Group Means								
0		0.150	0.171	0.143	0.199	0.167	0.136	0.169
20		0.193	0.183	0.176	0.202	0.170	0.158	0.171
LSD (P=0.05)		0.0094	0.0081	0.01	0.0022	ns	0.003	ns
	26	0.171	0.178	0.161	0.198	-	0.145	0.164
	100	0.171	0.176	0.154	0.204	-	0.149	0.177
LSD (P=0.05)		ns	ns	ns	0.0022	-	0.003	0.007
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.012*	0.0001**	0.041*	0.57	0.0001**	0.46
Nitrogen (N)	1	1.00	0.73	0.60	0.0002**	-	0.015*	0.004**
S*N	1	1.00	0.11	0.026*	0.90	-	0.0001**	0.26
Contrasts								
0 S vs 20 S at 26 N		0.0001**	0.35	0.0063**	0.14	-	0.077	0.20
0 S vs 20 S at 100 N		0.0001**	0.007**	0.0001**	0.11	0.57	0.0001**	0.76
C.V. (%)		4.87	4.02	5.48	0.99	3.26	1.80	3.02

<sup>†</sup> S concentrations reported on dry matter basis

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.6. Effect of nitrogen and sulphur fertilization on nitrogen concentration in flour (%)<sup>†</sup> in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	3.36	2.85	2.92	3.29	-	2.33	2.55
20	26	3.56	2.75	2.90	3.30	-	2.35	2.61
0	100	3.50	3.00	3.09	3.35	2.70	2.77	2.91
20	100	3.73	2.96	3.14	3.37	2.66	2.75	2.82
Group Means								
0		3.43	2.93	3.00	3.32	2.70	2.55	2.73
20		3.64	2.85	3.00	3.33	2.66	2.55	2.70
LSD (P=0.05)		0.10	ns	ns	ns	ns	ns	ns
	26	3.46	2.80	2.91	3.29	-	2.34	2.58
	100	3.61	2.98	3.11	3.36	-	2.76	2.86
	LSD (P=0.05)	0.10	0.10	0.18	0.06	-	0.07	0.13
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0012**	0.16	0.74	0.60	0.61	0.93	0.83
Nitrogen (N)	1	0.0072**	0.003**	0.023*	0.021*	-	0.0001**	0.003**
S*N	1	0.75	0.52	0.54	0.95	-	0.56	0.22
Contrasts								
0 S vs 20 S at 26 N		0.013*	0.15	0.84	0.74	-	0.63	0.45
0 S vs 20 S at 100 N		0.0063**	0.56	0.53	0.68	0.61	0.72	0.30
C.V. (%)		2.54	3.21	4.96	1.46	4.23	2.42	3.59

<sup>†</sup> N concentrations reported on dry matter basis

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.7. Effect of nitrogen and sulphur fertilization on N:S ratio in flour in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	22.68	16.46	19.67	16.66	-	16.31	15.91
20	26	18.41	15.36	16.88	16.55	-	16.03	15.72
0	100	23.60	18.22	23.01	16.54	16.11	21.52	16.36
20	100	19.30	16.07	17.50	16.42	15.64	16.27	16.05
Group Means								
0		23.14	17.34	21.34	16.60	16.11	18.92	16.14
20		18.85	15.71	17.14	16.48	15.64	16.15	15.83
LSD (P=0.05)		0.66	1.22	1.61	ns	ns	0.44	ns
	26	20.54	15.91	18.28	16.61	-	16.17	15.80
	100	21.45	17.14	20.65	16.48	-	18.89	16.17
	LSD (P=0.05)	0.66	1.22	1.61	ns	-	0.44	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.014*	0.0003**	0.22	0.055	0.0001**	0.13
Nitrogen (N)	1	0.013*	0.048*	0.028*	0.18	-	0.0001**	0.079
S*N	1	0.95	0.36	0.77	0.97	-	0.0001**	0.55
Contrasts								
0 S vs 20 S at 26 N		0.0001**	0.18	0.019*	0.39	-	0.34	0.47
0 S vs 20 S at 100 N		0.0001**	0.020*	0.0006**	0.36	0.055	0.0001**	0.15
C.V. (%)		2.80	6.52	6.94	1.07	1.37	2.23	1.80

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.8. Effect of nitrogen and sulphur fertilization on flour yield (%) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	68.80	71.65	71.98	68.40	-	71.18	71.47
20	26	70.63	73.23	73.35	68.25	-	70.85	72.78
0	100	68.33	72.15	72.65	68.60	69.68	70.95	71.77
20	100	69.65	72.05	73.57	68.70	69.95	71.68	71.73
Group Means								
0		68.56	71.90	72.31	68.50	69.68	71.06	71.62
20		70.14	72.64	73.44	68.48	69.95	71.26	72.33
LSD (P=0.05)		1.32	ns	1.00	ns	ns	ns	ns
26		69.71	72.44	72.66	68.33	-	71.01	72.21
100		68.99	72.10	73.04	68.65	-	71.31	71.75
LSD (P=0.05)		ns	ns	ns	ns	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.024*	0.18	0.018*	0.94	0.667	0.709	0.499
Nitrogen (N)	1	0.25	0.52	0.21	0.342	-	0.577	0.71
S*N	1	0.68	0.13	0.88	0.709	-	0.338	0.476
Contrasts								
0 S vs 20 S at 26 N		0.054	0.055	0.049*	0.751	0.667	0.668	0.335
0 S vs 20 S at 100 N		0.14	0.89	0.095	0.832	-	0.349	0.978
C.V. (%)		1.68	1.40	1.15	0.95	1.17	1.46	1.95

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.9. Effect of nitrogen and sulphur fertilization on SDS sedimentation volume (mL) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	78.50	85.75	72.25	71.25	-	76.25	74.17
20	26	86.50	83.50	74.50	70.63	-	76.25	74.88
0	100	75.75	82.75	72.75	73.13	69.25	71.38	79.17
20	100	85.25	86.25	78.33	72.63	70.75	82.88	77.67
Group Means								
0		77.13	84.25	72.50	72.19	69.25	73.81	76.67
20		85.88	84.88	76.14	71.63	70.75	79.56	76.07
LSD (P=0.05)		1.92	ns	3.64	ns	ns	1.82	ns
	26	82.50	84.63	73.38	70.94	-	76.25	74.57
	100	80.50	84.50	75.14	72.88	-	77.13	78.42
LSD (P=0.05)		1.92	ns	ns	ns	-	ns	2.72
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.44	0.03*	0.57	0.605	0.0001**	0.783
Nitrogen (N)	1	0.043*	0.88	0.15	0.073	-	0.305	0.016*
S*N	1	0.40	0.0048**	0.23	0.949	-	0.0001**	0.351
Contrasts								
0 S vs 20 S at 26 N		0.0001**	0.07	0.34	0.654	0.605	1.00	0.628
0 S vs 20 S at 100 N		0.0001**	0.011*	0.029*	0.72	-	0.0001**	0.394
C.V. (%)		2.09	1.83	4.25	2.65	5.27	2.10	2.62

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.10. Effect of nitrogen and sulphur fertilization on loaf height (mm) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	111.50	116.30	112.00	122.50	-	106.80	116.30
20	26	119.00	116.50	116.50	122.80	-	107.30	120.00
0	100	109.50	116.00	109.30	120.00	115.80	106.80	120.00
20	100	117.30	118.30	116.70	118.50	118.50	112.80	121.70
Group Means								
0		110.50	116.10	110.60	121.30	115.80	106.80	118.20
20		118.10	117.40	116.60	120.60	118.50	110.00	120.70
LSD (P=0.05)		2.84	ns	3.26	ns	ns	2.41	ns
26		115.30	116.40	114.30	122.60	-	107.00	118.40
100		113.40	117.10	112.40	119.30	-	109.80	120.80
LSD (P=0.05)		ns	ns	ns	3.27	-	2.41	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0002**	0.36	0.002**	0.68	0.20	0.014*	0.11
Nitrogen (N)	1	0.17	0.58	0.6	0.044*	-	0.030*	0.11
S*N	1	0.92	0.46	0.21	0.56	-	0.030*	0.50
Contrasts								
0 S vs 20 S at 26 N		0.0022**	0.89	0.048*	0.91	-	0.75	0.11
0 S vs 20 S at 100 N		0.0018**	0.25	0.0042**	0.48	0.2	0.003**	0.43
C.V. (%)		2.19	2.22	2.41	2.39	1.99	1.96	2.03

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.11. Effect of nitrogen and sulphur fertilization on loaf volume (cc) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	1050.00	1088.80	1008.80	1191.30	-	933.80	1081.70
20	26	1156.30	1105.00	1056.30	1183.80	-	942.50	1128.80
0	100	1023.80	1100.00	976.30	1168.80	1065.00	961.30	1130.00
20	100	1143.80	1121.30	1075.00	1130.00	1117.50	1010.00	1148.30
Group Means								
0		1036.90	1094.40	992.50	1180.00	1065.00	947.50	1105.80
20		1150.00	1113.10	1064.30	1156.90	1117.50	976.30	1137.10
LSD (P=0.05)		29.24	ns	44.07	ns	ns	ns	ns
	26	1103.10	1096.90	1032.50	1187.50	-	938.10	1108.60
	100	1083.80	1110.60	1018.60	1149.40	-	985.60	1139.20
LSD (P=0.05)		ns	ns	ns	ns	-	29.60	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.27	0.0029**	0.21	0.12	0.055	0.076
Nitrogen (N)	1	0.17	0.41	0.91	0.054	-	0.005**	0.076
S*N	1	0.61	0.88	0.11	0.39	-	0.16	0.37
Contrasts								
0 S vs 20 S at 26 N		0.0003**	0.49	0.11	0.77	-	0.65	0.071
0 S vs 20 S at 100 N		0.0001**	0.37	0.0037**	0.15	0.12	0.027*	0.44
C.V. (%)		2.36	2.88	3.60	2.94	3.14	2.72	2.40

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.12. Effect of nitrogen and sulphur fertilization on oven spring (mm) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	9.00	15.25	9.50	20.75	-	17.75	22.67
20	26	17.50	15.75	15.75	22.00	-	19.75	26.25
0	100	6.25	13.25	7.25	19.75	23.00	14.50	25.00
20	100	14.50	16.00	13.33	21.00	23.50	22.25	28.33
Group Means								
0		7.63	14.25	8.38	20.25	23.00	16.13	23.83
20		16.00	15.88	14.71	21.50	23.50	21.00	27.14
LSD (P=0.05)		2.68	ns	3.70	ns	ns	1.34	ns
26		13.25	15.50	12.63	21.38	-	18.75	24.71
100		10.38	14.63	9.86	20.38	-	18.38	26.67
LSD (P=0.05)		2.68	ns	ns	ns	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.27	0.0048**	0.32	0.77	0.0001**	0.14
Nitrogen (N)	1	0.038*	0.54	0.22	0.42	-	0.54	0.22
S*N	1	0.92	0.44	0.97	1.00	-	0.0009**	0.93
Contrasts								
0 S vs 20 S at 26 N		0.0007**	0.80	0.021*	0.48	-	0.040*	0.29
0 S vs 20 S at 100 N		0.0008**	0.19	0.030*	0.48	0.77	0.0001**	0.25
C.V. (%)		20.07	18.39	27.36	11.43	9.46	6.36	12.48

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively



Table E.13. Effect of nitrogen and sulphur fertilization on crumb elongation score in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	1.49	1.51	1.53	1.43	-	1.56	1.53
20	26	1.46	1.50	1.48	1.51	-	1.53	1.50
0	100	1.47	1.49	1.51	1.49	1.50	1.53	1.45
20	100	1.42	1.44	1.49	1.51	1.53	1.53	1.44
Group Means								
0		1.48	1.50	1.52	1.46	1.50	1.54	1.49
20		1.44	1.47	1.49	1.51	1.53	1.53	1.48
LSD (P=0.05)		0.037	ns	ns	ns	ns	ns	ns
	26	1.47	1.51	1.51	1.47	-	1.54	1.51
	100	1.44	1.46	1.50	1.50	-	1.53	1.44
	LSD (P=0.05)	ns	ns	ns	ns	-	ns	0.04
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.040*	0.36	0.11	0.12	0.19	0.55	0.45
Nitrogen (N)	1	0.11	0.24	0.68	0.26	-	0.55	0.008**
S*N	1	0.82	0.52	0.65	0.26	-	0.62	0.67
Contrasts								
0 S vs 20 S at 26 N		0.16	0.84	0.13	0.07	-	0.44	0.40
0 S vs 20 S at 100 N		0.095	0.28	0.4	0.74	0.19	0.94	0.81
C.V. (%)		2.21	4.57	2.79	3.47	1.80	3.14	2.15

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.14. Effect of nitrogen and sulphur fertilization on crumb fineness score in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	868.00	939.00	888.00	888.25	-	956.50	907.33
20	26	860.25	907.50	871.75	904.00	-	926.25	902.25
0	100	818.00	905.50	864.75	902.50	925.50	924.75	898.00
20	100	842.75	889.75	889.33	882.00	887.25	936.00	902.67
Group Means								
0		843.00	922.25	876.38	895.38	925.50	940.63	902.67
20		851.50	898.63	879.29	893.00	887.25	931.13	902.43
LSD (P=0.05)		ns	ns	ns	ns	ns	ns	ns
	26	864.13	923.25	879.88	896.13	-	941.38	904.43
	100	830.38	897.63	875.29	892.25	-	930.38	900.33
	LSD (P=0.05)	23.51	ns	ns	ns	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.43	0.16	0.98	0.90	0.41	0.68	0.88
Nitrogen (N)	1	0.010**	0.13	0.58	0.84	-	0.63	0.75
S*N	1	0.15	0.62	0.19	0.36	-	0.37	0.98
Contrasts								
0 S vs 20 S at 26 N		0.61	0.18	0.33	0.57	-	0.36	0.93
0 S vs 20 S at 100 N		0.13	0.49	0.36	0.46	0.41	0.73	0.90
C.V. (%)		2.45	3.37	2.52	4.21	6.21	4.69	4.97

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.15. Effect of nitrogen and sulphur fertilization on 25 % loaf compression (g) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	81.17	81.71	72.18	46.61	-	125.80	91.32
20	26	74.41	85.46	74.86	50.76	-	129.50	83.61
0	100	81.11	75.88	72.90	45.71	72.85	102.98	81.07
20	100	74.16	68.27	70.12	57.54	69.00	110.31	78.31
Group Means								
0		81.14	78.80	72.54	46.16	72.85	114.39	86.19
20		74.28	76.86	72.83	54.15	69.00	119.91	81.34
LSD (P=0.05)		4.4	ns	ns	ns	ns	ns	ns
	26	77.79	83.59	73.52	48.68	-	127.65	86.91
	100	77.63	72.07	71.71	51.63	-	106.65	79.69
	LSD (P=0.05)	ns	4.83	ns	ns	-	11.58	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0065**	0.39	0.96	0.16	0.75	0.31	0.69
Nitrogen (N)	1	0.94	0.0004**	0.47	0.59	-	0.0027**	0.28
S*N	1	0.96	0.026*	0.32	0.48	-	0.73	0.94
Contrasts								
0 S vs 20 S at 26 N		0.036*	0.25	0.43	0.59	-	0.62	0.74
0 S vs 20 S at 100 N		0.033*	0.032*	0.51	0.14	0.75	0.34	0.82
C.V. (%)		5.01	5.49	6.21	20.71	22.34	8.74	16.79

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.16. Effect of nitrogen and sulphur fertilization on 40 % loaf compression (g) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	105.63	107.25	96.26	65.08	-	166.74	124.40
20	26	97.82	111.59	99.63	72.65	-	171.37	116.57
0	100	104.66	98.58	96.28	65.68	103.21	137.56	111.55
20	100	96.26	92.61	92.51	83.22	98.27	146.46	109.85
Group Means								
0		105.14	102.91	96.27	65.38	103.21	152.15	117.97
20		97.04	102.10	96.57	77.93	98.27	158.91	113.69
LSD (P=0.05)		5.86	ns	ns	ns	ns	ns	ns
	26	101.73	109.42	97.94	68.87	-	169.05	119.92
	100	100.46	95.60	94.66	74.45	-	142.01	110.70
LSD (P=0.05)		ns	6.12	ns	ns	-	15.26	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.012*	0.77	0.98	0.097	0.76	0.34	0.77
Nitrogen (N)	1	0.64	0.0006**	0.31	0.43	-	0.0031**	0.28
S*N	1	0.91	0.089	0.30	0.48	-	0.76	0.90
Contrasts								
0 S vs 20 S at 26 N		0.062	0.29	0.43	0.45	-	0.64	0.77
0 S vs 20 S at 100 N		0.048*	0.15	0.49	0.10	0.76	0.38	0.91
C.V. (%)		5.12	5.28	5.91	18.91	20.51	8.67	14.66

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.17. Effect of nitrogen and sulphur fertilization on proof height (mm) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	102.00	101.00	102.50	101.75	-	89.00	93.67
20	26	101.50	100.75	100.75	100.75	-	87.50	93.75
0	100	103.25	102.75	102.00	100.25	92.75	92.25	95.00
20	100	102.75	102.25	103.33	97.50	95.00	90.50	93.33
Group Means								
0		102.63	101.88	102.25	101.00	92.75	90.63	94.33
20		102.13	101.50	101.86	99.13	95.00	89.00	93.57
LSD (P=0.05)		ns	ns	ns	ns	ns	ns	ns
	26	101.75	100.88	101.63	101.25	-	88.25	93.71
	100	103.00	102.50	102.57	98.88	-	91.38	94.17
LSD (P=0.05)		ns	ns	ns	ns	-	1.71	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.52	0.69	0.9	0.18	0.32	0.06	0.72
Nitrogen (N)	1	0.13	0.11	0.32	0.098	-	0.0025**	0.90
S*N	1	1.00	0.89	0.19	0.51	-	0.87	0.41
Contrasts								
0 S vs 20 S at 26 N		0.65	0.85	0.36	0.60	-	0.19	0.73
0 S vs 20 S at 100 N		0.65	0.71	0.32	0.17	0.32	0.14	0.41
C.V. (%)		1.47	1.80	2.47	2.57	2.84	1.68	2.43

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.18. Effect of nitrogen and sulphur fertilization on dough extensibility (mm) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	78.91	89.03	81.83	110.07	-	89.49	94.62
20	26	90.51	98.81	92.98	105.00	-	88.11	101.44
0	100	73.15	87.90	72.63	105.91	91.47	84.24	100.89
20	100	91.37	99.92	94.47	111.12	95.86	98.91	101.70
Group Means								
0		76.03	88.46	77.23	108.00	91.47	86.86	97.76
20		90.94	99.36	93.62	108.06	95.86	93.51	101.55
LSD (P=0.05)		5.70	7.03	7.81	ns	ns	4.69	ns
	26	84.71	93.92	87.40	107.53	-	88.80	98.52
	100	82.26	93.91	81.99	108.51	-	91.57	101.30
LSD (P=0.05)		ns	ns	ns	ns	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0002**	0.0067**	0.0013**	0.99	0.21	0.010**	0.31
Nitrogen (N)	1	0.36	1.00	0.32	0.82	-	0.21	0.60
S*N	1	0.22	0.73	0.14	0.25	-	0.0038**	0.39
Contrasts								
0 S vs 20 S at 26 N		0.0099**	0.053	0.043*	0.41	-	0.65	0.20
0 S vs 20 S at 100 N		0.0006**	0.023*	0.0024**	0.40	0.21	0.0007**	0.9
C.V. (%)		6.04	6.62	7.71	7.69	4.22	4.60	7.37

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.19. Effect of nitrogen and sulphur fertilization on maximum dough resistance (g) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	26.02	20.77	23.24	17.30	-	28.02	20.51
20	26	24.61	19.93	18.04	18.19	-	27.35	22.16
0	100	29.85	22.50	28.43	17.16	24.50	31.95	25.07
20	100	25.19	17.78	19.45	19.70	21.79	29.40	21.81
Group Means								
0		27.93	21.64	25.83	17.23	24.50	29.98	22.79
20		24.90	18.85	18.65	18.95	21.79	28.38	22.01
LSD (P=0.05)		1.60	2.64	2.84	ns	ns	ns	ns
	26	25.31	20.35	20.64	17.75	-	27.69	21.46
	100	27.52	20.14	24.58	18.43	-	30.68	23.44
LSD (P=0.05)		1.60	ns	2.84	ns	-	2.74	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0021**	0.041*	0.0004**	0.085	0.28	0.22	0.41
Nitrogen (N)	1	0.012*	0.86	0.036*	0.46	-	0.036*	0.08
S*N	1	0.048*	0.13	0.14	0.38	-	0.46	0.07
Contrasts								
0 S vs 20 S at 26 N		0.19	0.62	0.015*	0.49	-	0.71	0.39
0 S vs 20 S at 100 N		0.0012**	0.018*	0.0011**	0.074	0.28	0.17	0.072
C.V. (%)		5.37	11.54	10.58	9.80	12.68	8.32	8.17

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.20. Effect of nitrogen and sulphur fertilization on viscoelastic ratio in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	0.33	0.24	0.29	0.16	-	0.32	0.22
20	26	0.28	0.21	0.19	0.17	-	0.31	0.22
0	100	0.41	0.27	0.40	0.16	0.28	0.38	0.25
20	100	0.29	0.18	0.21	0.18	0.23	0.30	0.22
Group Means								
0		0.37	0.25	0.34	0.16	0.28	0.35	0.23
20		0.28	0.19	0.20	0.18	0.23	0.31	0.22
LSD (P=0.05)		0.033	0.041	0.047	ns	ns	0.04	ns
	26	0.30	0.22	0.24	0.17	-	0.31	0.22
	100	0.35	0.22	0.32	0.17	-	0.34	0.23
LSD (P=0.05)		0.033	ns	0.047	ns	-	ns	ns
ANOVA								
	df				Pr>F			
Sulphur (S)	1	0.0001**	0.013*	0.0001**	0.22	0.23	0.040*	0.23
Nitrogen (N)	1	0.011*	0.84	0.018*	0.66	-	0.17	0.28
S*N	1	0.046*	0.18	0.041*	0.91	-	0.064	0.37
Contrasts								
0 S vs 20 S at 26 N		0.021*	0.27	0.0094**	0.33	-	0.84	0.8
0 S vs 20 S at 100 N		0.0002**	0.011*	0.0002**	0.41	0.23	0.011*	0.15
C.V. (%)		8.92	16.36	14.29	14.33	17.96	10.88	10.38

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively



Table E.21. Effect of nitrogen and sulphur fertilization on extensigraph peak area ( $\text{g mm}^{-1}$ ) in 1999 and 2000

Treatment		Site						
S Applied ( $\text{kg ha}^{-1}$ )	N Applied ( $\text{kg ha}^{-1}$ )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	333.53	304.57	306.23	1016.32	-	1342.41	978.16
20	26	368.35	322.63	283.15	1027.07	-	1268.51	1120.50
0	100	360.22	327.39	338.68	951.44	1187.72	1417.94	1326.90
20	100	383.10	298.07	305.67	1227.04	1123.28	1569.41	1126.11
Group Means								
0		346.88	315.98	322.45	983.88	1187.72	1380.17	1152.53
20		375.73	310.35	292.80	1127.05	1123.28	1418.96	1122.90
LSD (P=0.05)		27.31	ns	ns	110.37	ns	ns	ns
26		350.94	313.60	294.69	1021.69	-	1305.46	1059.49
100		371.66	312.73	324.53	1089.24	-	1493.68	1226.51
LSD (P=0.05)		ns	ns	ns	ns	-	112.72	140.64
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.041*	0.75	0.21	0.017*	0.55	0.46	0.59
Nitrogen (N)	1	0.12	0.96	0.22	0.20	-	0.004**	0.02*
S*N	1	0.63	0.19	0.81	0.02*	-	0.05*	0.03*
Contrasts								
0 S vs 20 S at 26 N		0.072	0.47	0.43	0.88	-	0.32	0.17
0 S vs 20 S at 100 N		0.21	0.25	0.30	0.003**	0.55	0.06	0.055
C.V. (%)		6.68	10.72	12.60	9.25	11.75	7.12	9.09

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.22. Effect of nitrogen and sulphur fertilization on mixograph peak height (% torque) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	61.94	55.15	56.21	57.50	-	40.13	40.02
20	26	68.41	52.21	56.53	59.46	-	37.73	42.18
0	100	61.99	59.05	58.29	59.16	50.41	46.74	49.08
20	100	74.20	58.00	62.40	58.81	49.30	46.90	47.05
Group Means								
0		61.96	57.10	57.25	58.33	50.41	43.43	44.55
20		71.31	55.11	59.04	59.14	49.30	42.31	44.26
LSD (P=0.05)		1.22	ns	ns	ns	ns	ns	ns
	26	65.18	53.68	56.37	58.48	-	38.93	41.25
	100	68.09	58.53	60.05	58.99	-	46.82	48.07
LSD (P=0.05)		1.22	2.68	ns	ns	-	2.33	4.66
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.13	0.21	0.56	0.72	0.31	1.00
Nitrogen (N)	1	0.0004**	0.0027**	0.053	0.71	-	0.0001**	0.012*
S*N	1	0.0005**	0.45	0.26	0.41	-	0.25	0.34
Contrasts								
0 S vs 20 S at 26 N		0.0001**	0.11	0.91	0.33	-	0.13	0.49
0 S vs 20 S at 100 N		0.0001**	0.55	0.12	0.86	0.72	0.91	0.49
C.V. (%)		1.61	4.22	6.39	4.54	8.01	4.81	7.70

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.23. Effect of nitrogen and sulphur fertilization on mixograph peak time (minutes) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	3.77	2.44	3.27	2.92	-	4.05	4.21
20	26	2.82	2.53	2.71	2.83	-	3.83	4.12
0	100	3.97	2.57	3.79	2.78	3.87	4.67	3.75
20	100	2.89	2.35	2.45	2.84	4.00	3.29	4.30
Group Means								
0		3.87	2.50	3.53	2.85	3.87	4.36	3.98
20		2.86	2.44	2.60	2.84	4.00	3.56	4.20
LSD (P=0.05)		0.29	ns	0.20	ns	ns	0.31	ns
	26	3.29	2.48	2.99	2.88	-	3.94	4.16
	100	3.43	2.46	3.22	2.81	-	3.98	4.03
LSD (P=0.05)		ns	ns	ns	ns	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.19	0.0001**	0.88	0.29	0.0002**	0.50
Nitrogen (N)	1	0.33	0.67	0.27	0.46	-	0.78	0.71
S*N	1	0.63	0.007**	0.0018**	0.4	-	0.002**	0.25
Contrasts								
0 S vs 20 S at 26 N		0.0006**	0.18	0.0018**	0.48	-	0.28	0.71
0 S vs 20 S at 100 N		0.0002**	0.007**	0.0001**	0.62	0.29	0.0001**	0.21
C.V. (%)		7.69	3.66	5.54	6.07	3.81	6.89	11.69

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.24. Effect of nitrogen and sulphur fertilization on mixograph peak width (% torque) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	37.08	34.75	34.04	31.20	-	25.86	26.10
20	26	40.70	32.08	32.30	31.34	-	25.55	25.66
0	100	37.18	36.76	34.16	32.91	29.06	27.64	29.30
20	100	42.79	34.48	34.90	32.56	28.19	30.56	27.18
Group Means								
0		37.13	35.76	34.10	32.06	29.06	26.75	27.70
20		41.74	33.28	33.41	31.95	28.19	28.06	26.30
LSD (P=0.05)		1.46	2.10	ns	ns	ns	ns	ns
	26	38.89	33.41	33.17	31.27	-	25.71	25.85
	100	39.98	35.62	34.48	32.74	-	29.10	28.24
LSD (P=0.05)		ns	2.10	ns	ns	-	1.51	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.026*	0.82	0.89	0.48	0.083	0.40
Nitrogen (N)	1	0.12	0.042*	0.17	0.077	-	0.0007**	0.16
S*N	1	0.16	0.84	0.20	0.75	-	0.039*	0.65
Contrasts								
0 S vs 20 S at 26 N		0.0033**	0.073	0.26	0.90	-	0.75	0.77
0 S vs 20 S at 100 N		0.0002**	0.12	0.46	0.74	0.48	0.013*	0.37
C.V. (%)		3.28	5.39	5.98	4.60	5.36	4.88	9.82

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.25. Effect of nitrogen and sulphur fertilization on work input to peak (% torque minute<sup>-1</sup>) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	164.34	95.25	129.93	117.34	-	113.16	120.62
20	26	134.41	94.55	104.28	119.15	-	100.15	123.49
0	100	173.86	108.16	158.71	117.33	139.34	146.26	131.35
20	100	149.31	97.61	104.37	119.71	146.13	102.06	145.40
Group Means								
0		169.10	101.71	144.32	117.33	139.34	129.71	125.98
20		141.86	96.08	104.31	119.43	146.13	101.11	132.88
LSD (P=0.05)		12.17	5.42	14.05	ns	ns	9.38	ns
	26	149.38	94.90	117.10	118.24	-	106.66	122.26
	100	161.59	102.89	135.42	118.52	-	124.16	138.38
LSD (P=0.05)		12.17	5.42	14.05	ns	-	9.38	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0007**	0.043*	0.0002**	0.63	0.14	0.0001**	0.36
Nitrogen (N)	1	0.049*	0.0087**	0.048*	0.95	-	0.002**	0.058
S*N	1	0.63	0.07	0.050*	0.95	-	0.005**	0.41
Contrasts								
0 S vs 20 S at 26 N		0.0034**	0.84	0.015*	0.77	-	0.054	0.95
0 S vs 20 S at 100 N		0.010**	0.012*	0.0004**	0.70	0.14	0.0001*	0.23
C.V. (%)		6.92	4.84	9.37	7.19	3.33	7.18	9.95

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.26. Effect of nitrogen and sulphur fertilization on mixograph total work input (% torque minute<sup>-1</sup>) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	299.94	281.01	277.25	331.14	-	190.39	229.63
20	26	339.96	264.64	275.61	342.33	-	180.44	241.49
0	100	298.90	296.90	283.00	311.05	292.86	207.33	283.80
20	100	365.01	293.00	305.60	341.21	289.19	224.86	267.00
Group Means								
0		299.42	288.96	280.13	321.09	292.86	198.86	256.72
20		352.49	278.82	288.46	341.77	289.19	202.65	252.42
LSD (P=0.05)		8.88	ns	ns	ns	ns	ns	ns
	26	319.95	272.83	276.43	336.73	-	185.41	236.41
	100	331.96	294.95	292.69	326.13	-	216.09	275.40
LSD (P=0.05)		8.88	12.21	ns	ns	-	12.21	30.42
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.093	0.19	0.21	0.83	0.50	0.84
Nitrogen (N)	1	0.014*	0.0027**	0.056	0.51	-	0.0003**	0.021*
S*N	1	0.0089**	0.28	0.15	0.55	-	0.031*	0.32
Contrasts								
0 S vs 20 S at 26 N		0.0001**	0.061	0.9	0.62	0.83	0.23	0.56
0 S vs 20 S at 100 N		0.0001**	0.62	0.076	0.2	-	0.047*	0.39
C.V. (%)		2.41	3.80	6.08	9.23	7.70	5.38	8.78

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.27. Effect of nitrogen and sulphur fertilization on time to dough breakdown (minutes) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	14.26	10.08	11.99	13.03	-	12.20	15.00
20	26	18.51	9.70	10.69	14.95	-	12.58	18.18
0	100	14.49	11.46	12.08	15.93	16.65	15.63	15.75
20	100	18.90	10.55	11.27	17.15	14.70	14.78	17.40
Group Means								
0		14.38	10.77	12.03	14.69	16.65	13.91	15.30
20		18.71	10.13	10.94	16.05	14.70	13.68	17.92
LSD (P=0.05)		1.42	ns	ns	ns	ns	ns	ns
	26	16.39	9.89	11.34	14.13	-	12.39	16.81
	100	16.69	11.01	11.73	16.54	-	15.20	16.58
	LSD (P=0.05)	ns	ns	ns	2.11	-	0.88	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.24	0.068	0.12	0.56	0.56	0.45
Nitrogen (N)	1	0.64	0.055	0.27	0.023*	-	0.0001**	0.74
S*N	1	0.90	0.61	0.36	0.67	-	0.15	0.73
Contrasts								
0 S vs 20 S at 26 N		0.001**	0.61	0.051	0.18	-	0.51	0.37
0 S vs 20 S at 100 N		0.0008**	0.24	0.47	0.36	0.56	0.16	0.79
C.V. (%)		7.57	9.73	6.97	11.47	19.54	5.65	19.25

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.28. Effect of nitrogen and sulphur fertilization on dough stability (minutes) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	8.99	5.89	7.85	5.55	-	7.48	9.90
20	26	9.84	5.84	5.74	6.68	-	7.08	12.30
0	100	9.25	6.69	8.60	7.53	9.38	9.73	8.77
20	100	10.36	6.04	5.95	8.13	9.23	8.45	11.40
Group Means								
0		9.12	6.29	8.23	6.54	9.38	8.60	9.33
20		10.10	5.94	5.83	7.40	9.23	7.76	11.91
LSD (P=0.05)		ns	ns	0.69	ns	ns	ns	ns
	26	9.41	5.86	6.79	6.11	-	7.28	11.27
	100	9.81	6.36	7.46	7.83	-	9.09	10.08
	LSD (P=0.05)	ns	ns	ns	1.35	-	0.96	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.053	0.22	0.0001**	0.18	0.90	0.079	0.054
Nitrogen (N)	1	0.40	0.09	0.14	0.018*	-	0.002**	0.35
S*N	1	0.77	0.28	0.43	0.67	-	0.33	0.98
Contrasts								
0 S vs 20 S at 26 N		0.21	0.90	0.0009**	0.21	-	0.52	0.15
0 S vs 20 S at 100 N		0.11	0.12	0.0004**	0.49	0.90	0.062	0.14
C.V. (%)		9.19	8.62	8.14	17.07	16.62	10.36	17.58

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively



Table E.29. Effect of nitrogen and sulphur fertilization on mixing tolerance index (BU) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	15.00	27.50	18.75	31.25	-	21.25	16.67
20	26	15.00	27.50	26.25	22.00	-	25.00	12.50
0	100	12.50	23.75	15.00	18.75	13.00	16.25	16.00
20	100	12.50	26.25	30.00	19.50	20.50	22.50	14.33
Group Means								
0		13.75	25.63	16.88	25.00	13.00	18.75	16.33
20		13.75	26.88	27.86	20.75	20.50	23.75	13.29
LSD (P=0.05)		ns	ns	4.72	ns	ns	4.22	ns
	26	15.00	27.50	22.50	26.63	-	23.13	14.29
	100	12.50	25.00	21.43	19.13	-	19.38	15.17
LSD (P=0.05)		ns	ns	ns	6.57	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	1.00	0.37	0.0006**	0.18	0.14	0.025*	0.19
Nitrogen (N)	1	0.14	0.09	1.00	0.030*	-	0.075	0.93
S*N	1	1.00	0.37	0.11	0.12	-	0.52	0.64
Contrasts								
0 S vs 20 S at 26 N		1.00	1.00	0.028*	0.051	-	0.19	0.21
0 S vs 20 S at 100 N		1.00	0.21	0.0013**	0.86	0.14	0.042*	0.51
C.V. (%)		22.68	10.04	17.97	25.4	32.06	17.54	20.01

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.30. Effect of nitrogen and sulphur fertilization on farinograph absorption (%) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	66.90	64.53	65.33	65.48	-	57.23	59.33
20	26	67.98	63.88	64.85	64.63	-	56.70	58.23
0	100	67.23	65.18	65.03	64.85	60.88	58.80	59.63
20	100	68.63	64.78	65.70	62.58	61.15	58.05	60.03
Group Means								
0		67.06	64.85	65.18	65.16	60.88	58.01	59.48
20		68.30	64.33	65.21	63.60	61.15	57.38	59.00
LSD (P=0.05)		0.49	ns	ns	ns	ns	0.50	ns
	26	67.44	64.20	65.09	65.05	-	56.96	58.70
	100	67.93	64.98	65.31	63.71	-	58.43	59.83
	LSD (P=0.05)	ns	0.71	ns	ns	-	0.50	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0003**	0.13	0.6	0.23	0.71	0.018*	0.70
Nitrogen (N)	1	0.051	0.037*	0.26	0.30	-	0.0001**	0.25
S*N	1	0.47	0.70	0.047*	0.57	-	0.62	0.40
Contrasts								
0 S vs 20 S at 26 N		0.0065**	0.18	0.22	0.63	-	0.13	0.39
0 S vs 20 S at 100 N		0.0013**	0.39	0.087	0.22	0.71	0.039*	0.74
C.V. (%)		0.64	0.98	0.77	3.79	1.51	0.76	2.38

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.31. Effect of nitrogen and sulphur fertilization on dough development time (minutes) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	5.20	4.64	5.11	7.80	-	4.80	4.80
20	26	8.03	4.13	5.29	7.50	-	5.43	7.10
0	100	4.93	5.19	4.88	8.00	6.48	6.33	7.50
20	100	8.36	4.85	5.22	7.95	8.08	7.53	8.77
Group Means								
0		5.06	4.91	4.99	7.90	6.48	5.56	6.15
20		8.19	4.49	5.26	7.73	8.08	6.48	7.81
LSD (P=0.05)		0.74	ns	0.27	ns	ns	0.62	ns
	26	6.61	4.38	5.20	7.65	-	5.11	6.11
	100	6.64	5.02	5.02	7.98	-	6.93	8.13
LSD (P=0.05)		ns	ns	ns	ns	-	0.62	2.02
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.25	0.044*	0.62	0.28	0.009**	0.10
Nitrogen (N)	1	0.93	0.10	0.97	0.36	-	0.0001**	0.044*
S*N	1	0.37	0.81	0.21	0.72	-	0.32	0.63
Contrasts								
0 S vs 20 S at 26 N		0.0002**	0.32	0.47	0.55	-	0.14	0.13
0 S vs 20 S at 100 N		0.0001**	0.51	0.035*	0.92	0.28	0.013*	0.35
C.V. (%)		9.89	14.8	6.31	8.68	23.9	9.08	21.74

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.32. Effect of nitrogen and sulphur fertilization on the concentration (%) of soluble glutenin in flour in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	5.40	9.88	5.70	9.05	-	7.68	8.00
20	26	9.63	10.25	8.15	8.63	-	7.98	8.90
0	100	5.35	8.35	4.10	9.40	9.55	4.53	8.43
20	100	9.23	10.58	8.83	9.18	8.50	8.00	8.00
Group Means								
0		5.38	9.11	4.90	9.23	9.55	6.10	8.22
20		9.43	10.41	8.44	8.90	8.50	7.99	8.51
LSD (P=0.05)		0.51	ns	1.38	ns	ns	0.62	ns
	26	7.51	10.06	6.93	8.84	-	7.83	8.51
	100	7.29	9.46	6.13	9.29	-	6.26	8.22
LSD (P=0.05)		ns	ns	ns	ns	-	0.62	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.054	0.0003**	0.44	0.11	0.0001**	0.48
Nitrogen (N)	1	0.34	0.33	0.62	0.29	-	0.0003**	0.48
S*N	1	0.46	0.15	0.068	0.81	-	0.0002**	0.056
Contrasts								
0 S vs 20 S at 26 N		0.0001**	0.66	0.017*	0.47	-	0.46	0.07
0 S vs 20 S at 100 N		0.0001**	0.025*	0.0005**	0.70	0.11	0.0001**	0.3
C.V. (%)		6.08	12.03	17.63	8.84	7.31	7.72	5.54

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.33. Effect of nitrogen and sulphur fertilization on the concentration (%) of insoluble glutenin in flour in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	26.73	26.55	27.33	24.08	-	27.70	27.10
20	26	28.18	27.00	25.63	23.48	-	27.18	26.85
0	100	25.95	26.00	26.00	23.38	25.05	26.68	26.80
20	100	26.53	26.73	27.17	23.38	25.68	26.45	27.03
Group Means								
0		26.34	26.28	26.66	23.73	25.05	27.19	26.95
20		27.35	26.86	26.29	23.43	25.68	26.81	26.93
LSD (P=0.05)		0.66	ns	ns	ns	ns	ns	ns
	26	27.45	26.78	26.48	23.78	-	27.44	26.96
	100	26.24	26.36	26.50	23.38	-	26.56	26.92
LSD (P=0.05)		0.66	ns	ns	ns	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.007**	0.28	0.62	0.48	0.24	0.56	0.93
Nitrogen (N)	1	0.002**	0.44	0.81	0.35	-	0.20	0.80
S*N	1	0.17	0.79	0.017*	0.48	-	0.82	0.61
Contrasts								
0 S vs 20 S at 26 N		0.0063**	0.55	0.031*	0.33	-	0.56	0.76
0 S vs 20 S at 100 N		0.19	0.34	0.13	1.00	0.24	0.80	0.67
C.V. (%)		2.16	3.83	3.46	3.47	2.39	4.63	2.40

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.34. Effect of nitrogen and sulphur fertilization on the ratio of insoluble to soluble glutenin in flour in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg/ha)	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	4.99	2.72	4.92	2.68	-	3.61	3.41
20	26	2.94	2.66	3.17	2.72	-	3.42	3.02
0	100	4.91	3.20	6.96	2.49	2.64	5.97	3.19
20	100	2.89	2.53	3.16	2.57	3.02	3.32	3.39
Group Means								
0		4.95	2.96	5.94	2.59	2.64	4.79	3.30
20		2.91	2.59	3.16	2.64	3.02	3.37	3.18
LSD (P=0.05)		0.39	ns	1.90	ns	0.32	0.62	ns
	26	3.96	2.69	4.04	2.70	-	3.52	3.19
	100	3.90	2.86	5.33	2.53	-	4.64	3.29
LSD (P=0.05)		ns	ns	ns	ns	-	0.62	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.12	0.0081**	0.62	0.031*	0.0006**	0.35
Nitrogen (N)	1	0.71	0.41	0.34	0.17	-	0.0027**	0.53
S*N	1	0.93	0.18	0.19	0.91	-	0.0015**	0.013*
Contrasts								
0 S vs 20 S at 26 N		0.0001**	0.84	0.16	0.79	-	0.65	0.020*
0 S vs 20 S at 100 N		0.0001**	0.05*	0.011*	0.66	0.031*	0.0001**	0.13
C.V. (%)		8.68	15.04	34.32	8.67	4.96	13.49	4.27

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.35. Effect of nitrogen and sulphur fertilization on the concentration (%) of monomeric protein in flour in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	58.08	54.05	57.68	53.38	-	57.10	55.23
20	26	53.53	53.85	57.00	59.10	-	57.30	55.68
0	100	57.98	56.30	60.23	57.78	57.10	60.23	55.67
20	100	54.65	53.58	55.33	58.70	57.20	57.28	56.37
Group Means								
0		58.03	55.18	58.95	58.08	57.10	58.66	55.45
20		54.09	53.71	56.29	58.90	57.20	57.28	55.97
LSD (P=0.05)		0.88	ns	1.16	ns	ns	1.13	ns
	26	55.80	53.95	57.34	58.74	-	57.20	55.49
	100	56.31	54.94	58.13	58.24	-	58.75	56.02
LSD (P=0.05)		ns	ns	ns	ns	-	1.13	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.0001**	0.062	0.0004**	0.42	0.87	0.022*	0.50
Nitrogen (N)	1	0.22	0.18	0.71	0.62	-	0.013*	0.35
S*N	1	0.15	0.10	0.002**	0.92	-	0.012*	0.73
Contrasts								
0 S vs 20 S at 26 N		0.0001**	0.84	0.35	0.61	-	0.78	0.81
0 S vs 20 S at 100 N		0.0002**	0.02*	0.0001**	0.52	0.87	0.002**	0.48
C.V. (%)		1.39	2.52	1.68	3.33	1.43	1.72	2.02

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table E.36. Effect of nitrogen and sulphur fertilization on the concentration (%) of residue protein in flour in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	9.78	9.53	9.30	8.88	-	8.18	9.67
20	26	8.65	8.88	9.25	8.75	-	8.95	8.58
0	100	10.68	9.33	9.65	8.55	9.45	8.55	9.10
20	100	9.63	9.13	8.70	8.78	8.60	8.53	8.57
Group Means								
0		10.23	9.43	9.48	8.71	9.45	8.36	9.38
20		9.14	9.00	9.01	8.76	8.60	8.74	8.57
LSD (P=0.05)		0.57	ns	ns	ns	ns	ns	ns
	26	9.21	9.20	9.28	8.81	-	8.56	9.04
	100	10.15	9.23	9.24	8.66	-	8.54	8.83
LSD (P=0.05)		0.57	ns	ns	ns	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.002**	0.35	0.14	0.86	0.19	0.54	0.18
Nitrogen (N)	1	0.005**	0.96	0.97	0.59	-	0.97	0.45
S*N	1	0.88	0.62	0.19	0.53	-	0.51	0.71
Contrasts								
0 S vs 20 S at 26 N		0.011*	0.32	0.89	0.75	-	0.37	0.23
0 S vs 20 S at 100 N		0.016*	0.75	0.073	0.57	0.19	0.98	0.46
C.V. (%)		5.19	9.38	5.17	6.20	7.90	13.63	9.22

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively



## 10.6. Appendix F

### Regression Equations for the Three Sources of N for the Prediction of Grain N Concentration and N Accumulation in the Plant

Equation F.1. Regression equation for the relationship between grain N concentration and the three sources of soil and fertilizer N (for all treatments)

$$y = 2.62 + 0.0013(x_1)^* + 0.0037(x_2)^{**} + 0.0013(x_3)^* \quad (R^2 = 0.20)$$

where: y = grain N concentration (%)  
x<sub>1</sub> = soil NO<sub>3</sub>-N (kg ha<sup>-1</sup>)  
x<sub>2</sub> = fertilizer N (kg ha<sup>-1</sup>)  
x<sub>3</sub> = mineralizable N in PB extract(kg ha<sup>-1</sup>)  
\* significant at P < 0.05  
\*\* significant at P < 0.01

Equation F.2. Regression equation for the relationship between total N accumulation and the three sources of soil and fertilizer N (for all treatments and excluding rep 1 from Erickson in 1999)

$$y = 39.69 + 0.32(x_1)^{**} + 0.30(x_2)^{**} + 0.20(x_3)^{**} \quad (R^2 = 0.53)$$

where: y = total N accumulation (kg ha<sup>-1</sup>)  
x<sub>1</sub> = soil NO<sub>3</sub>-N (kg ha<sup>-1</sup>)  
x<sub>2</sub> = fertilizer N (kg ha<sup>-1</sup>)  
x<sub>3</sub> = mineralizable N in PB extract(kg ha<sup>-1</sup>)  
\*\* significant at P < 0.01

## **10.7. Appendix G**

### **Analysis of Variance, LSDs, and Contrasts for the Effects of S and N Fertilization on Agronomic Measurements**

Table G.1. Effect of nitrogen and sulphur fertilization on S accumulation<sup>†</sup> in grain (kg ha<sup>-1</sup>) in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	1.68	4.00	3.25	6.33	4.32	2.41	4.23	4.25	2.83	3.41	3.62	4.60
20	26	2.20	4.46	3.78	6.31	5.82	2.48	4.09	3.67	2.56	3.39	3.32	5.08
0	100	1.60	4.44	4.32	5.68	4.29	2.90	4.18	4.42	4.01	3.59	4.51	5.11
20	100	2.45	4.79	4.53	5.37	6.71	3.64	3.69	4.48	4.32	4.38	4.04	5.00
Group Means													
0		1.64	4.22	3.79	6.00	4.30	2.65	4.20	4.33	3.42	3.50	4.06	4.85
20		2.33	4.62	4.16	5.84	6.20	3.06	3.89	4.07	3.44	3.89	3.68	5.04
LSD (P=0.05)		0.32	ns	ns	ns	0.84	0.25	ns	ns	ns	ns	ns	ns
	26	1.94	4.23	3.51	6.32	5.07	2.44	4.16	3.96	2.69	3.40	3.47	4.84
	100	2.03	4.61	4.43	5.52	5.33	3.27	3.93	4.45	4.17	3.99	4.27	5.06
LSD (P=0.05)		ns	ns	0.89	0.61	ns	0.25	ns	0.41	0.50	0.54	0.65	ns
ANOVA													
	df	Pr>F											
Sulphur (S)	1	0.0009**	0.35	0.35	0.56	0.0005**	0.005**	0.32	0.18	0.94	0.14	0.21	0.28
Nitrogen (N)	1	0.55	0.37	0.046*	0.16*	0.17	0.0001**	0.47	0.023*	0.0001**	0.036*	0.02*	0.22
S*N	1	0.28	0.88	0.67	0.62	0.15	0.013*	0.57	0.11	0.22	0.13	0.77	0.11
Contrasts													
0 S vs 20 S at 26 N		0.028*	0.46	0.34	0.95	0.17*	0.7	0.75	0.048*	0.4	0.97	0.48	0.066
0 S vs 20 S at 100 N		0.0021**	0.57	0.71	0.45	0.0012**	0.001**	0.27	0.83	0.35	0.045*	0.28	0.66
C.V. (%)		14.28	18.76	15.85	9.11	13.5	7.7	14.55	8.55	12.89	12.9	14.89	6.63

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

<sup>†</sup> accumulation reported on dry matter basis, except for Kelvington

Table G.2. Effect of nitrogen and sulphur fertilization on N accumulation<sup>†</sup> in grain (kg ha<sup>-1</sup>) in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	35.75	66.76	51.04	92.28	83.24	39.55	67.99	70.04	46.01	52.81	57.09	68.88
20	26	39.98	73.44	55.70	91.26	94.11	39.51	64.46	59.84	40.44	50.40	50.96	76.70
0	100	36.40	77.79	75.24	79.51	94.93	61.11	73.65	71.99	64.94	63.50	76.26	79.79
20	100	44.90	79.45	70.16	73.72	113.22	58.95	62.97	72.63	67.86	67.82	65.38	77.50
Group Means													
0		36.08	72.27	63.14	85.90	89.08	50.33	70.82	71.01	55.48	58.16	66.68	74.34
20		32.44	76.45	62.93	82.49	102.30	49.23	63.71	66.23	54.15	59.11	58.17	77.10
LSD (P=0.05)		4.72	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	26	37.86	70.10	53.37	91.77	88.68	39.53	66.23	64.94	43.23	51.61	54.03	72.79
	100	40.65	78.62	72.70	76.61	102.77	60.03	68.31	72.61	66.40	65.66	70.82	78.64
LSD (P=0.05)		ns	ns	11.85	8.27	ns	4.67	ns	6.83	8.04	8.29	11.09	4.60
ANOVA													
	df	Pr>F											
Sulphur (S)	1	0.014*	0.56	0.97	0.38	0.044*	0.61	0.19	0.15	0.72	0.8	0.12	0.21
Nitrogen (N)	1	0.21	0.25	0.0072**	0.0025**	0.037*	0.0001**	0.69	0.037*	0.0001**	0.004**	0.0076**	0.018*
S*N	1	0.33	0.73	0.35	0.53	0.42	0.62	0.5	0.11	0.26	0.38	0.64	0.035*
Contrasts													
0 S vs 20 S at 26 N		0.19	0.51	0.52	0.85	0.28	0.99	0.63	0.04*	0.3	0.65	0.4	0.024*
0 S vs 20 S at 100 N		0.018*	0.87	0.49	0.29	0.061	0.48	0.17	0.88	0.58	0.43	0.15	0.45
C.V. (%)		10.64	18.72	13.31	8.68	14.01	8.29	14.99	8.8	12.97	12.51	15.71	5.37

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

<sup>†</sup> accumulation reported on dry matter basis, except for Kelvington

Table G.3. Effect of nitrogen and sulphur fertilization on S accumulation<sup>†</sup> in total plant (kg ha<sup>-1</sup>) in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	-	5.65	5.32	9.83	5.62	3.39	6.57	8.70	4.70	5.10	5.22	7.81
20	26	-	6.82	6.60	10.12	10.36	4.02	6.83	7.30	4.04	5.34	4.87	8.02
0	100	-	7.53	7.33	10.15	6.22	4.14	7.46	10.44	7.69	5.54	7.06	10.02
20	100	-	8.58	11.12	6.69	12.35	6.29	7.04	12.31	8.23	7.19	7.86	10.55
Group Means													
0		-	6.59	6.33	9.99	5.92	3.77	7.02	9.57	6.20	5.32	6.14	8.92
20		-	7.70	8.86	9.90	11.21	5.16	6.93	9.80	6.13	6.26	6.36	9.28
LSD (P=0.05)		-	0.98	1.83	ns	2.68	ns	ns	ns	ns	0.83	ns	ns
	26	-	6.23	5.96	9.98	7.99	3.71	6.70	8.00	4.37	5.22	5.04	7.92
	100	-	8.05	9.23	9.92	8.85	5.22	7.25	11.37	7.96	6.36	7.46	10.28
LSD (P=0.05)		-	0.98	1.83	ns	ns	ns	ns	1.62	0.99	0.83	0.62	1.94
ANOVA													
	df		Pr>F										
Sulphur (S)	1	-	0.032*	0.015*	0.89	0.0014**	0.098	0.78	0.75	0.89	0.029*	0.43	0.68
Nitrogen (N)	1	-	0.0022**	0.0047**	0.92	0.24	0.075	0.094	0.0011**	0.0001**	0.012*	0.0001**	0.022*
S*N	1	-	0.91	ns	0.55	0.47	0.34	0.27	0.049*	0.2	0.085	0.064	0.86
Contrasts													
0 S vs 20 S at 26 N		-	0.09	0.27	0.75	0.018*	0.57	0.55	0.2	0.31	0.65	0.38	0.87
0 S vs 20 S at 100 N		-	0.12	0.012*	0.61	0.0056**	0.073	0.33	0.1	0.41	0.011*	0.07	0.68
C.V. (%)		-	12.12	17.07	12.48	26.81	33.64	8.44	14.82	14.19	12.61	8.73	18.87

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

<sup>†</sup> accumulation reported on dry matter basis, except for Kelvington

Table G.4. Effect of nitrogen and sulphur fertilization on N accumulation<sup>†</sup> in total plant (kg ha<sup>-1</sup>) in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	-	83.28	65.46	121.66	101.97	45.79	88.50	101.72	57.67	67.85	68.73	90.42
20	26	-	92.93	69.59	121.64	121.12	50.05	86.76	84.91	48.29	63.28	61.80	93.76
0	100	-	117.80	110.17	116.70	126.01	73.87	107.04	112.98	91.34	84.41	101.46	115.56
20	100	-	112.95	105.93	107.61	143.66	75.12	97.35	120.18	95.25	85.46	93.94	109.52
Group Means													
0		-	100.54	87.82	119.18	113.99	59.83	97.77	107.35	74.50	76.13	85.09	102.99
20		-	102.94	87.76	114.63	130.78	62.59	92.06	102.54	71.77	74.37	77.87	101.64
LSD (P=0.05)		-	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	26	-	88.10	67.52	121.65	111.55	47.92	87.63	93.31	52.98	65.56	65.27	92.09
	100	-	115.37	108.05	112.16	133.57	74.49	102.19	116.58	93.30	84.94	97.70	112.54
	LSD (P=0.05)	-	12.87	14.57	ns	ns	25.42	12.14	15.92	8.58	13.17	7.70	17.73
ANOVA													
	df		Pr>F										
Sulphur (S)	1	-	0.68	0.99	0.41	0.072	0.81	0.32	0.51	0.49	0.77	0.063	0.87
Nitrogen (N)	1	-	0.001**	0.0005**	0.11	0.034*	0.04*	0.024*	0.0091**	0.0001**	0.0088**	0.0001**	0.028*
S*N	1	-	0.23	0.51	0.41	0.84	0.90	0.49	0.12	0.11	0.64	0.93	0.56
Contrasts													
0 S vs 20 S at 26 N		-	0.26	0.64	1	0.2	0.79	0.82	0.13	0.11	0.59	0.18	0.77
0 S vs 20 S at 100 N		-	0.56	0.63	0.26	0.16	0.94	0.23	0.49	0.49	0.9	0.15	0.6
C.V. (%)		-	11.18	11.75	9.08	15.99	36.72	11.31	13.41	10.37	15.47	8.36	15.32

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

<sup>†</sup> accumulation reported on dry matter basis, except for Kelvington

Table G.5. Effect of nitrogen and sulphur fertilization on straw yield<sup>†</sup> (kg ha<sup>-1</sup>) in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	-	2899.18	3070.62	4102.12	3161.69	-	3797.90	4758.80	2430.92	3449.40	2622.45	4343.52
20	26	-	3172.85	3123.99	4234.29	4097.44	-	3885.79	3845.01	2027.05	3578.85	2342.44	3823.78
0	100	-	4045.05	4857.39	3564.21	4165.47	-	4093.09	5985.35	4726.10	4046.44	3405.75	5120.69
20	100	-	4398.83	5891.88	3782.88	4234.11	-	3521.04	6171.66	5286.58	4582.04	3828.66	6008.88
Group Means													
0		-	3472.10	3694.00	3833.20	3663.60	-	394535.00	5372.10	3578.50	3747.90	3014.10	4732.10
20		-	3785.80	4507.90	4008.60	4156.00	-	3703.40	5008.30	3656.80	4080.40	3085.50	4916.30
LSD (P=0.05)		-	ns	ns	ns	ns	-	ns	ns	ns	ns	ns	ns
	26	-	3036.00	3097.30	4168.20	3629.60	-	3741.80	4301.90	2229.00	3514.10	2482.40	4083.60
	100	-	4221.90	5374.60	3673.50	4194.90	-	3807.10	6078.50	5006.30	4314.20	3617.20	5564.80
LSD (P=0.05)		-	461.13	882.57	465.87	ns	-	ns	762.08	528.36	401.73	266.24	ns
ANOVA													
	df		Pr>F										
Sulphur (S)	1	-	0.16	0.18	0.42	0.35	-	0.054	0.31	0.75	0.094	0.56	0.8
Nitrogen (N)	1	-	0.0003**	0.0007**	0.040*	0.3	-	0.76	0.0005**	0.0001**	0.0015**	0.0001**	0.07
S*N	1	-	0.85	0.22	0.84	0.56	-	0.015*	0.14	0.069	0.28	0.015*	0.35
Contrasts													
0 S vs 20 S at 26 N		-	0.37	0.92	0.66	0.27	-	0.58	0.087	0.25	0.62	0.13	0.62
0 S vs 20 S at 100 N		-	0.25	0.089	0.47	0.8	-	0.005**	0.7	0.12	0.062	0.032*	0.41
C.V. (%)		-	11.23	14.75	10.5	28.55	-	5.73	12.98	12.91	9.07	7.72	29.84

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

<sup>†</sup> straw yields adjusted to dry matter basis based on grain moisture content, except for Kelvington

Table G.6. Effect of nitrogen and sulphur fertilization on S accumulation<sup>†</sup> in straw (kg ha<sup>-1</sup>) in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	-	1.65	2.07	3.50	1.30	-	2.35	4.46	1.88	1.70	1.60	3.22
20	26	-	2.36	2.82	3.82	4.54	-	2.74	3.64	1.49	1.95	1.55	2.94
0	100	-	3.08	3.01	4.48	1.93	-	3.28	6.02	3.68	1.94	2.55	4.91
20	100	-	3.80	6.59	4.32	5.63	-	3.34	7.83	3.91	2.81	3.82	5.54
Group Means													
0		-	2.37	2.54	3.99	1.61	-	2.81	5.24	2.78	1.82	2.07	4.06
20		-	3.08	4.71	4.07	5.00	-	3.04	5.73	2.70	2.38	2.69	4.24
LSD (P=0.05)		-	0.68	1.01	ns	2.17	-	ns	ns	ns	0.44	ns	ns
	26	-	2.00	2.44	3.66	2.92	-	2.54	4.05	1.68	1.82	1.57	3.08
	100	-	3.44	4.80	4.40	3.51	-	3.31	6.92	3.79	2.38	3.19	5.23
LSD (P=0.05)		-	0.68	1.01	ns	ns	-	0.36	1.47	1.01	0.44	0.96	2.05
ANOVA													
	df		Pr>F										
Sulphur (S)	1	-	0.041*	0.0019**	0.86	0.0058**	-	0.2	0.47	0.86	0.018*	0.18	0.85
Nitrogen (N)	1	-	0.001**	0.0012**	0.13	0.34	-	0.001**	0.0016**	0.0011**	0.018*	0.0042**	0.042*
S*N	1	-	0.98	0.014*	0.6	0.73	-	0.33	0.074	0.51	0.14	0.15	0.63
Contrasts													
0 S vs 20 S at 26 N		-	0.13	0.24	0.62	0.036*	-	0.12	0.4	0.55	0.39	0.94	0.84
0 S vs 20 S at 100 N		-	0.12	0.0009**	0.8	0.025*	-	0.81	0.08	0.73	0.011*	0.063	0.63
C.V. (%)		-	21.98	19.72	21.78	56.95	-	10.96	23.68	32.57	18.37	35.76	43.6

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

<sup>†</sup> accumulation reported on dry matter basis, except for Kelvington



Table G.7. Effect of nitrogen and sulphur fertilization on N accumulation<sup>†</sup> in straw (kg ha<sup>-1</sup>) in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	-	16.52	14.42	39.38	18.73	-	20.51	31.68	11.65	15.03	11.64	21.53
20	26	-	19.50	13.89	30.39	27.01	-	22.29	25.06	7.85	12.88	10.84	17.07
0	100	-	40.00	34.94	37.19	31.08	-	33.39	40.99	26.40	20.90	25.20	35.77
20	100	-	33.50	35.77	33.89	30.44	-	34.39	47.55	27.40	17.64	28.56	32.02
Group Means													
0		-	28.27	24.68	33.28	24.90	-	26.95	36.34	19.03	17.97	18.42	28.65
20		-	26.50	24.83	32.14	28.50	-	28.34	36.31	17.62	15.26	19.70	24.55
LSD (P=0.05)		-	ns	ns	ns	ns	-	ns	ns	ns	ns	ns	ns
	26	-	18.01	14.16	29.88	22.87	-	21.40	28.37	9.75	13.96	11.24	19.30
	100	-	36.75	35.35	35.54	30.81	-	33.89	44.27	26.90	19.27	26.88	33.90
	LSD (P=0.05)	-	11.38	4.79	5.56	ns	-	5.07	12.72	5.39	ns	6.64	ns
ANOVA													
	df		Pr>F										
Sulphur (S)	1	-	0.73	0.94	0.65	0.38	-	0.55	1	0.57	0.4	0.67	0.59
Nitrogen (N)	1	-	0.0047**	0.0001**	0.047*	0.11	-	0.0003**	0.020*	0.0001**	0.11	0.0005**	0.079
S*N	1	-	0.37	0.74	0.4	0.46	-	0.87	0.27	0.34	0.86	0.5	0.96
Contrasts													
0 S vs 20 S at 26 N		-	0.69	0.86	0.78	0.24	-	0.59	0.43	0.29	0.63	0.85	0.68
0 S vs 20 S at 100 N		-	0.38	0.77	0.37	0.92	-	0.76	0.43	0.77	0.47	0.44	0.73
C.V. (%)		-	36.75	13.71	15.03	34.37	-	16.22	30.97	26.03	36.57	30.79	55.39

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

<sup>†</sup> accumulation reported on dry matter basis, except for Kelvington

Table G.8. Effect of nitrogen and sulphur fertilization on N concentration<sup>†</sup> in midseason tissue (50 % heading) (%) in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	1.94	1.99	1.81	2.13	1.99	1.84	2.85	1.46	1.13	1.72	1.39	2.13
20	26	2.02	2.05	1.67	2.00	2.05	1.54	2.74	1.46	1.09	1.73	1.33	2.33
0	100	2.16	2.45	2.42	2.58	2.13	2.22	3.43	1.74	1.30	2.55	2.00	2.63
20	100	2.55	2.26	2.39	2.69	2.38	2.13	3.56	1.80	1.23	2.48	1.93	2.82
Group Means													
0		2.05	2.22	2.12	2.35	2.06	2.03	3.14	1.60	1.21	2.13	1.69	2.38
20		2.29	2.15	2.03	2.34	2.19	1.83	3.15	1.63	1.16	2.10	1.63	2.57
LSD (P=0.05)		0.17	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	26	1.98	2.02	1.74	2.06	2.02	1.69	2.79	1.46	1.11	1.72	1.36	2.23
	100	2.35	2.35	2.41	2.63	2.23	2.17	3.49	1.77	1.26	2.51	1.96	2.72
LSD (P=0.05)		0.17	0.12	0.18	0.21	ns	0.25	0.31	0.17	0.14	0.13	0.12	0.25
ANOVA													
	df	Pr>F											
Sulphur (S)	1	0.010**	0.23	0.34	0.9	0.23	0.11	0.95	0.7	0.38	0.65	0.29	0.11
Nitrogen (N)	1	0.0007**	0.0001**	0.0001**	0.0002**	0.1	0.0019**	0.0007**	0.0027**	0.031*	0.0001**	0.0001**	0.0014**
S*N	1	0.059	0.047*	0.52	0.23	0.38	0.39	0.42	0.74	0.77	0.47	0.94	0.94
Contrasts													
0 S vs 20 S at 26 N		0.46	0.5	0.26	0.34	0.78	0.09	0.59	0.97	0.67	0.84	0.48	0.22
0 S vs 20 S at 100 N		0.0041**	0.032*	0.82	0.44	0.17	0.55	0.54	0.61	0.41	0.41	0.42	0.25
C.V. (%)		6.79	4.78	7.84	7.94	12.87	11.54	8.8	9.45	10.22	5.5	5.6	8.75

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

<sup>†</sup> concentrations reported on dry matter basis

Table G.9. Effect of nitrogen and sulphur fertilization on S concentration<sup>†</sup> in midseason tissue (50 % heading) (%) in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	0.105	0.143	0.145	0.205	0.125	0.116	0.175	0.129	0.097	0.142	0.116	0.177
20	26	0.148	0.158	0.163	0.220	0.185	0.126	0.186	0.132	0.107	0.179	0.125	0.186
0	100	0.100	0.150	0.143	0.210	0.120	0.107	0.216	0.148	0.102	0.170	0.135	0.195
20	100	0.158	0.175	0.198	0.235	0.207	0.170	0.216	0.159	0.112	0.216	0.157	0.208
Group Means													
0		0.102	0.146	0.144	0.208	0.123	0.112	0.195	0.138	0.099	0.156	0.126	0.186
20		0.153	0.166	0.180	0.228	0.194	0.148	0.200	0.146	0.109	0.197	0.141	0.197
LSD (P=0.05)		0.007	0.010	0.019	0.016	0.021	0.017	ns	ns	0.008	0.016	0.009	0.011
	26	0.126	0.150	0.154	0.213	0.155	0.121	0.180	0.131	0.102	0.160	0.121	0.181
	100	0.129	0.163	0.170	0.223	0.157	0.138	0.216	0.153	0.107	0.193	0.146	0.201
LSD (P=0.05)		ns	0.010	ns	ns	ns	ns	0.023	0.015	ns	0.016	0.009	0.011
ANOVA													
	df	Pr>F											
Sulphur (S)	1	0.0001**	0.0016**	0.0016**	0.019*	0.0001**	0.001**	0.59	0.31	0.022*	0.0003**	0.003**	0.040*
Nitrogen (N)	1	0.41	0.021*	0.078	0.19	0.25	0.052	0.007**	0.0078**	0.19	0.0014**	0.0001**	0.0019**
S*N	1	0.029*	0.29	0.048*	0.49	0.11	0.0069**	0.59	0.53	0.95	0.55	0.11	0.58
Contrasts													
0 S vs 20 S at 26 N		0.0001**	0.042*	0.16	0.16	0.0011**	0.38	0.45	0.78	0.077	0.0056**	0.14	0.23
0 S vs 20 S at 100 N		0.0001**	0.0034**	0.001**	0.032*	0.0001**	0.0002**	1	0.25	0.091	0.0014**	0.0028**	0.065
C.V. (%)		4.53	5.74	10.1	6.41	10.1	11.73	10.15	9.35	6.8	8.14	5.79	4.84

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

<sup>†</sup> concentrations reported on dry matter basis

Table G.10. Effect of nitrogen and sulphur fertilization on N:S ratio in midseason tissue (50 % heading) (%) in 1999 and 2000

Treatment		Site											
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999					2000						
		Athabasca	Brandon South	Erickson	Kelvington	Melfort	Archerwill	Athabasca	Brandon South	Brandon North	Erickson	Glenboro	Rosebank
Treatment Means													
0	26	19.27	14.14	12.59	10.25	16.34	15.94	16.33	11.28	11.59	12.17	11.99	12.01
20	26	13.82	13.05	10.32	9.02	10.93	12.21	14.79	11.07	10.15	9.73	10.65	12.58
0	100	22.50	16.48	17.75	12.13	18.24	20.91	16.01	11.85	12.74	15.11	14.80	13.55
20	100	16.27	12.89	12.00	11.34	11.34	12.55	16.49	11.31	10.97	11.51	12.30	13.53
Group Means													
0		20.88	15.31	15.17	11.19	17.29	18.43	16.17	11.57	12.17	13.64	13.39	12.78
20		15.04	12.97	11.16	10.18	11.10	12.38	15.64	11.19	10.56	10.62	11.47	13.05
LSD (P=0.05)		1.08	0.78	1.61	0.76	0.97	2.08	ns	ns	0.66	1.08	0.52	ns
	26	16.55	13.59	11.45	9.64	13.64	14.08	15.56	11.17	10.87	10.95	11.32	12.30
	100	19.38	14.68	14.87	11.74	15.28	16.73	16.25	11.58	11.85	13.31	13.55	13.54
LSD (P=0.05)		1.08	0.78	1.61	0.76	0.97	2.08	ns	ns	0.66	1.08	0.52	1.11
ANOVA													
	df	Pr>F											
Sulphur (S)	1	0.0001**	0.0001**	0.0003**	0.015*	0.0001**	0.0001**	0.14	0.075	0.0004**	0.0001**	0.0001**	0.59
Nitrogen (N)	1	0.0002**	0.011*	0.001**	0.0001**	0.034*	0.018*	0.063	0.056	0.0079**	0.0008**	0.0001**	0.033*
S*N	1	0.43	0.0054**	0.037*	0.52	0.096	0.033*	0.013*	0.39	0.59	0.26	0.033*	0.57
Contrasts													
0 S vs 20 S at 26 N		0.0001**	0.051	0.051	0.029*	0.0001**	0.019*	0.0085**	0.45	0.0064**	0.0057**	0.0027**	0.44
0 S vs 20 S at 100 N		0.0001**	0.0001**	0.0003**	0.13	0.0001**	0.0001**	0.32	0.069	0.0019**	0.0005**	0.0001**	0.98
C.V. (%)		5.31	4.85	10.84	6.26	5.62	11.93	4.09	3.29	5.1	7.87	3.71	7.66

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table G.11. Effect of nitrogen and sulphur fertilization on S concentration<sup>†</sup> in early season tissue (4 - 6 leaf stage) (%) in 1999 and 2000

Treatment				
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	2000		
		Erickson	Glenboro	Rosebank
Treatment Means				
0	26	0.349	0.287	0.315
20	26	0.381	0.368	0.347
0	100	0.354	0.289	0.322
20	100	0.364	0.318	0.343
Group Means				
0		0.352	0.288	0.318
20		0.372	0.343	0.345
LSD (P=0.05)		0.012	0.014	0.0088
26		0.365	0.327	0.331
100		0.359	0.303	0.333
LSD (P=0.05)		ns	0.014	ns
ANOVA				
	df			
Sulphur (S)	1	0.0038**	0.0001**	0.0001**
Nitrogen (N)	1	0.3	0.0042**	0.66
S*N	1	0.062	0.0027**	0.16
Contrasts				
0 S vs 20 S at 26 N		0.0022*	0.0001**	0.0002**
0 S vs 20 S at 100 N		0.25	0.01**	0.0044**
C.V. (%)		2.95	4	2.34

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

<sup>†</sup> concentrations reported on dry matter basis

Table G.12. Effect of nitrogen and sulphur fertilization on N concentration<sup>†</sup> in early season tissue (4 - 6 leaf stage) (%) in 1999 and 2000

Treatment				
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	2000		
		Erickson	Glenboro	Rosebank
Treatment Means				
0	26	6.53	5.89	5.82
20	26	6.58	5.68	5.87
0	100	6.35	6.47	6.22
20	100	6.62	6.51	6.31
Group Means				
0		6.44	6.18	6.02
20		6.60	6.09	6.09
LSD (P=0.05)		0.15	ns	ns
26		6.56	5.78	5.85
100		6.48	6.49	6.27
LSD (P=0.05)		ns	0.15	0.12
ANOVA				
	df			
Sulphur (S)	1	0.033*	0.22	0.18
Nitrogen (N)	1	0.29	0.0001**	0.0001**
S*N	1	0.12	0.077	0.68
Contrasts				
0 S vs 20 S at 26 N		0.58	0.044*	0.49
0 S vs 20 S at 100 N		0.015*	0.64	0.22
C.V. (%)		1.98	2.09	1.69

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

<sup>†</sup> concentrations reported on dry matter basis

Table G.13. Effect of nitrogen and sulphur fertilization on N:S ratio in early season tissue (4 - 6 leaf stage) (%) in 1999 and 2000

Treatment				
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	2000		
		Erickson	Glenboro	Rosebank
<b>Treatment Means</b>				
0	26	18.75	20.66	18.52
20	26	17.31	15.47	16.92
0	100	17.93	22.56	19.31
20	100	18.22	20.49	18.43
<b>Group Means</b>				
0		18.34	21.61	18.92
20		17.76	17.98	17.67
LSD (P=0.05)		0.52	1.23	0.57
26		18.03	18.06	17.72
100		18.07	21.53	18.87
LSD (P=0.05)		ns	1.23	0.57
<b>ANOVA</b>				
	df			
Sulphur (S)	1	0.033*	0.0001**	0.0008**
Nitrogen (N)	1	0.85	0.0001**	0.0014**
S*N	1	0.004**	0.019*	0.19
<b>Contrasts</b>				
0 S vs 20 S at 26 N		0.0016**	0.0001**	0.0015**
0 S vs 20 S at 100 N		0.39	0.025*	0.036*
C.V. (%)		2.54	5.51	2.78

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

## **10.8. Appendix H**

### **Analysis of Variance, LSDs, and Contrasts for the Effects of S and N Fertilization on Rapid Visco Analyzer Measurements in 1999 and 2000**



Table H.1. Effect of nitrogen and sulphur fertilization on RVA peak viscosity (RVU) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	189.13	145.75	288.50	233.33	-	248.88	250.33
20	26	185.13	152.06	229.88	225.00	-	250.00	248.25
0	100	198.25	143.92	242.38	228.46	236.46	229.75	238.06
20	100	187.50	150.63	228.67	234.25	233.63	235.88	244.67
Group Means								
0		193.69	144.83	240.44	230.90	236.46	239.31	244.20
20		186.31	151.34	229.36	229.63	233.63	242.94	246.71
LSD (P=0.05)		ns	ns	6.25	ns	ns	ns	ns
	26	187.13	148.91	234.19	229.17	-	249.44	249.13
	100	192.88	147.17	236.50	231.35	-	232.81	241.36
LSD (P=0.05)		ns	ns	ns	ns	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.10	0.24	0.0027**	0.62	0.73	0.77	0.66
Nitrogen (N)	1	0.18	0.76	0.80	0.40	-	0.20	0.08
S*N	1	0.41	0.97	0.28	0.020*	-	0.84	0.19
Contrasts								
0 S vs 20 S at 26 N		0.49	0.41	0.048*	0.042*	-	0.95	0.49
0 S vs 20 S at 100 N		0.09	0.39	0.0064**	0.13	0.73	0.73	0.22
C.V. (%)		4.15	7.03	2.22	2.16	4.54	10.06	2.39

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table H.2. Effect of nitrogen and sulphur fertilization on RVA final viscosity (RVU) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	253.88	156.13	283.25	270.29	-	279.13	284.00
20	26	241.88	170.94	277.13	264.88	-	282.75	281.75
0	100	261.38	154.21	289.25	260.13	272.38	262.63	271.56
20	100	245.88	164.04	278.83	264.38	271.00	278.50	278.67
Group Means								
0		257.63	155.17	286.25	265.21	272.38	270.88	277.78
20		243.88	167.49	277.86	264.63	271.00	280.63	280.43
LSD (P=0.05)		9.76	ns	5.48	ns	ns	ns	ns
	26	247.88	163.53	280.19	267.58	-	280.94	282.71
	100	253.63	159.13	284.79	262.25	-	270.56	275.11
LSD (P=0.05)		ns	ns	ns	ns	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.011*	0.11	0.0081**	0.82	0.79	0.53	0.79
Nitrogen (N)	1	0.22	0.54	0.17	0.07	-	0.50	0.17
S*N	1	0.69	0.73	0.36	0.09	-	0.69	0.20
Contrasts								
0 S vs 20 S at 26 N		0.08	0.16	0.10	0.17	-	0.87	0.44
0 S vs 20 S at 100 N		0.032*	0.34	0.017*	0.27	0.79	0.47	0.27
C.V. (%)		3.44	8.56	1.63	1.92	2.41	10.77	2.54

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table H.3. Effect of nitrogen and sulphur fertilization on RVA peak time (minutes) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	6.09	5.85	6.23	6.38	-	6.43	6.15
20	26	6.11	5.93	6.31	6.30	-	6.47	6.24
0	100	6.06	5.83	6.26	6.32	6.40	6.33	6.26
20	100	6.07	5.96	6.30	6.30	6.32	6.51	6.24
Group Means								
0		6.08	5.84	6.25	6.35	6.40	6.38	6.21
20		6.09	5.94	6.31	6.30	6.32	6.49	6.24
LSD (P=0.05)		ns	ns	ns	ns	ns	ns	ns
	26	6.10	5.89	6.27	6.34	-	6.45	6.20
	100	6.07	5.89	6.27	6.31	-	6.42	6.25
LSD (P=0.05)		ns	ns	ns	ns	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.73	0.09	0.13	0.29	0.22	0.07	0.38
Nitrogen (N)	1	0.35	0.91	0.72	0.52	-	0.60	0.38
S*N	1	0.97	0.69	0.79	0.60	-	0.20	0.19
Contrasts								
0 S vs 20 S at 26 N		0.78	0.31	0.19	0.27	-	0.66	0.14
0 S vs 20 S at 100 N		0.83	0.14	0.38	0.69	0.22	0.040*	0.72
C.V. (%)		1.03	1.80	1.17	1.37	1.23	1.69	1.23

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table H.4. Effect of nitrogen and sulphur fertilization on RVA trough time (minutes) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	8.09	8.13	8.14	8.33	-	8.31	8.18
20	26	8.09	8.15	8.20	8.32	-	8.30	8.24
0	100	8.07	8.24	8.21	8.30	8.35	8.19	8.17
20	100	8.07	8.19	8.10	8.21	8.37	8.34	8.25
Group Means								
0		8.08	8.19	8.17	8.32	8.35	8.25	8.18
20		8.08	8.17	8.15	8.26	8.37	8.32	8.25
LSD (P=0.05)		ns	ns	ns	0.05	ns	ns	ns
	26	8.09	8.14	8.17	8.32	-	8.30	8.22
	100	8.07	8.21	8.16	8.26	-	8.26	8.21
LSD (P=0.05)		ns	ns	ns	0.05	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.95	0.78	0.56	0.043*	0.88	0.27	0.18
Nitrogen (N)	1	0.32	0.35	0.68	0.012*	-	0.52	0.85
S*N	1	0.77	0.64	0.13	0.16	-	0.26	0.95
Contrasts								
0 S vs 20 S at 26 N		0.87	0.89	0.45	0.58	-	0.98	0.34
0 S vs 20 S at 100 N		0.80	0.60	0.16	0.022*	0.88	0.13	0.31
C.V. (%)		0.50	1.83	1.26	0.53	1.24	1.54	1.06

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table H.5. Effect of nitrogen and sulphur fertilization on RVA trough viscosity (RVU) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	129.63	74.63	161.25	158.63	-	165.25	158.83
20	26	126.63	83.38	159.50	152.00	-	169.00	161.75
0	100	132.75	74.04	165.25	149.71	162.04	154.88	152.61
20	100	128.50	81.00	159.67	153.25	159.50	164.63	158.00
Group Means								
0		131.19	74.33	163.25	154.17	162.04	160.06	155.72
20		127.56	82.19	159.57	152.63	159.50	166.81	160.14
LSD (P=0.05)		ns	ns	ns	ns	ns	ns	ns
	26	128.13	79.00	160.38	155.31	-	167.13	160.50
	100	130.63	77.52	162.86	151.48	-	159.75	155.31
LSD (P=0.05)		ns	ns	ns	3.41	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.29	0.14	0.17	0.33	0.52	0.45	0.33
Nitrogen (N)	1	0.46	0.77	0.46	0.032*	-	0.41	0.24
S*N	1	0.85	0.86	0.44	0.008**	-	0.74	0.62
Contrasts								
0 S vs 20 S at 26 N		0.53	0.23	0.62	0.013*	-	0.77	0.72
0 S vs 20 S at 100 N		0.37	0.33	0.16	0.13	0.52	0.44	0.31
C.V. (%)		4.97	12.31	2.96	1.97	3.07	10.53	3.75

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively

Table H.6. Effect of nitrogen and sulphur fertilization on RVA difference (peak viscosity - trough viscosity) in 1999 and 2000

Treatment		Site						
S Applied (kg ha <sup>-1</sup> )	N Applied (kg ha <sup>-1</sup> )	1999			2000			
		Athabasca	Erickson	Melfort	Brandon South	Brandon North	Archerwill	Rosebank
Treatment Means								
0	26	-59.50	-71.13	-77.50	-74.71	-	-83.63	-91.50
20	26	-58.50	-68.69	-70.38	-73.00	-	-81.00	-86.50
0	100	-65.50	-69.88	-77.13	-78.75	-74.42	-74.88	-85.44
20	100	-59.00	-69.63	-69.00	-81.00	-74.13	-71.25	-86.67
Group Means								
0		-62.50	-70.50	-77.31	-76.73	-74.42	-79.25	-88.47
20		-58.75	-69.16	-69.79	-77.00	-74.13	-76.13	-86.57
LSD (P=0.05)		3.41	ns	4.10	ns	ns	ns	ns
	26	-59.00	-69.91	-73.94	-73.85	-	-82.31	-88.64
	100	-62.25	-69.75	-73.64	-79.88	-	-73.06	-86.06
	LSD (P=0.05)	ns	ns	ns	3.31	-	ns	ns
ANOVA								
	df	Pr>F						
Sulphur (S)	1	0.035*	0.29	0.002**	0.86	0.95	0.58	0.38
Nitrogen (N)	1	0.06	0.90	0.47	0.003**	-	0.13	0.25
S*N	1	0.10	0.38	0.60	0.21	-	0.93	0.18
Contrasts								
0 S vs 20 S at 26 N		0.65	0.18	0.019*	0.43	-	0.74	0.14
0 S vs 20 S at 100 N		0.014*	0.88	0.0094**	0.31	0.95	0.65	0.71
C.V. (%)		4.97	3.39	4.65	3.80	8.23	14.09	4.32

\*, \*\* Significant at the 0.05 and 0.01 levels, respectively