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Responses of two wheat varieties to sulphur addition and diagnosis of sulphur deficiency

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Abstract

Sulphur deficiency has become increasingly widespread in wheat in the U.K. Growth, nutrient content and biochemical responses to S and N supply of a breadmaking wheat variety (Hereward) and a non-breadmaking variety (Riband) were investigated in a pot experiment. Shoot dry matter (DM) at stem extension (Zadok's GS 37) and at maturity was increased markedly by S. Grain production of the Riband variety was more susceptible to the imbalance of N to S than the Hereward variety. At GS 37, the concentrations of total S and sulphate-S of shoots, chlorophyll meter readings and the concentrations of glutathione of the uppermost fully expanded leaves were increased significantly by increasing S supply, whereas the concentrations of nitrate and amides were decreased by S. The greatest relative changes in response to S supply were those of the glutathione and asparagine concentrations. Riband also showed greater response to S than Hereward. Critical values of various diagnostic indices at GS 37 were derived from the relationships between DM yield and different indices. The two varieties showed similar diagnostic curves except that for the ratio of total N to total S (N:S) in shoots. Either total S or sulphate-S can be used alone as a good indicator of deficiency, and with values of 1500 and 190 mg kg^{-1} DM in shoots for the two indices respectively. There was also a well defined relationship between DM yield and the glutathione concentration, with a critical value of 240 nmol g^{-1} FW. There were no advantages of using % of total S as sulphate-S. Shoot N:S ratio was found to be less accurate in predicting S deficiency than total S or sulphate-S. For prognostic purposes, a much higher S status at GS 37 was required to ensure no losses of DM yield due to S deficiency at maturity.

Introduction

It has been increasingly realised in recent years that sulphur (S) can be a major nutrient limitation to growth of crops, particularly in many areas of western Europe, where the inputs of S from atmospheric deposition have decreased considerably during the last two decades (Whelpdale, 1992). For example, total emissions of sulphur dioxide in the UK have decreased by more than 50% since 1970 and are expected to decrease much further in the next decade (Department of Environment, 1995). These changes, although beneficial to air quality and protection of natural ecosystems, have resulted in widespread S deficiency in arable crops (McGrath and Zhao, 1995, 1996).

Symptoms of S deficiency in cereals are not easily identifiable under field conditions, because they can be confused with those of N deficiency. Yield losses can also occur in crops with marginal S deficiency showing no visual symptoms. Reliable diagnosis of S deficiency is therefore needed to avoid yield losses and to ensure efficient use of S fertilisers. It is generally recognised that plant tissue analysis offers a better tool than soil testing for the prediction of S deficiency (Syers et al., 1987). Several diagnostic indices have been proposed, but there is no agreement as to which index gives the best result. For example, Rasmussen et al. (1977) found that the N:S ratio was better than the total S concentration of the vegetative issue of wheat, whereas Freney et al. (1978) and Spencer and Freney (1980) advocated the index of sulphate-S as a percent-

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age of total S. Scaife and Burns (1986) criticised the use of sulphate-S as a percentage of total S on the grounds that the numerator (sulphate-S) is the major variable in the denominator, thus decreasing the sensitivity of the index, and its determination involves twice as much analytical work as the measurement of total S or sulphate alone. They pointed out that sulphate-S by itself was the most satisfactory index because of its sensitivity to the changes of S supply.

Some recent studies have shown a rapid accumulation of amides in roots in response to the interruption of S supply (Bell et al., 1995; Karmoker et al., 1991). Glutathione also responds markedly to S supply (Macnicol and Randall, 1987), and has been found to act as a signal to regulate sulphate uptake and transport (Herschbach and Rennenberg, 1994). Analysis of these compounds may provide an early indication of S deficiency in plants.

Since S deficiency in cereals is a very recent development in the UK and other European countries, little research has been done on their S requirement and the use of diagnostic indices. The objectives of the study were to investigate responses of growth, nutrient and biochemical indicators of a breadmaking and a non-breadmaking wheat to the addition of S under controlled conditions and to compare various diagnostic indices for the prediction of S deficiency.

Materials and methods

Pot experiment

A sandy loam (top 20 cm) of the Cottenham series was collected from Woburn farm, Bedfordshire, and was air-dried and passed through a 5 mm sieve. The soil contained 8.9 mg g^{-1} organic C, 1.12 mg g^{-1} total N, 0.18 mg g^{-1} total S and 2.1 mg kg^{-1} extractable $SO_4 - S$.

Two varieties of winter wheat (*Triticum aestivum*) were used. Hereward is a hard breadmaking variety, and Riband is a soft wheat variety used mainly for feed. Seeds were sown in a plastic tray, covered with small amount of vermiculite and sprayed with deionised water. After germination, seedlings were vernalised in a growth cabinet for 5 weeks at 6.5 °C, 8 h day length with 125 μEinstein m^{-2} s^{-1} light intensity.

Ten seedlings were then transplanted into each plastic pot, containing an equivalent of 1.5 kg oven-dried soil. The treatments consisted of the combinations of 2 wheat varieties, 2 N levels (300 and 500 mg pot^{-1}) and 6 S levels (0, 2.5, 5, 10, 25 and 50 mg pot^{-1}). Sulphur was applied as K_2SO_4 and N as NH_4NO_3. All of the S treatments, 200 mg pot^{-1} of N and other basal nutrients were mixed thoroughly with soil before transplanting. The basal nutrients included 100 mg P (KH_2PO_4), 248 mg K (K_2SO_4, KH_2PO_4 or KCl), 50 mg Mg ($MgCl_2.6H_2O$), 10 mg Mn ($MnCl_2.4H_2O$), 1 mg Cu ($CuCl_2.2H_2O$), 2 mg Zn ($ZnCl_2$) and 1 mg B (H_3BO_3). The soil moisture content was maintained at 60–70% of the water holding capacity using deionised water. For the N_{300} treatments, the remaining 100 mg N was applied 51 days after transplanting. For the N_{500} treatments, the remaining 300 mg N was applied in three equal portions 31, 51 and 71 days after transplanting. All treatments were replicated four times and arranged randomly on benches inside a greenhouse. The growing conditions were : 14h/ 10h day/night, 16 °C/12 °C day/night temperatures, natural light supplemented with 1 kW SON-T lamps to maintain a minimum light intensity of 250 μEinstein $m^{-2}s^{-1}$.

Two plants were harvested from each pot at the stem extension stage (50 days after transplanting; flag leaf just visible, Zadok's GS 37). Fresh weight was measured, then the plants were rinsed with deionised water. The uppermost fully expanded leaves were cut, weighed and frozen in liquid nitrogen. The other plant parts were weighed fresh, then dried at 80 °C for 16 h before the dry weight was measured. Total dry weight was calculated from the total fresh weight and the dry matter (DM) percentage of the plant parts without the uppermost fully expanded leaves. Since total DM per pot was small, all replicates of the same treatment were bulked for chemical analyses.

The final harvest was done at maturity, 143 and 150 days after transplanting for Riband and Hereward, respectively. The plants were cut at the soil surface, separated into grain and straw components, dried at 80 °C for 16 h, and the dry weights determined. The dried plant materials were ground to pass through a 0.5 mm sieve for chemical analyses. For the final harvest, replicates were analysed separately.

Measurements

The chlorophyll content of the uppermost fully expanded leaves was measured at stem extension using a chlorophyll meter (Minolta SPAD 502).

The uppermost fully expanded leaves were used in the determination of free amino acids and thiols. The frozen leaf tissues were ground in a pestle and mortar with liquid nitrogen, and the free amino acids

were extracted by mixing 0.5 g of ground sample with 5 mL of ethanol:chloroform:water (12:5:3). The mixture was left to stand overnight at −20 °C then shaken and filtered through Whatman No. 1 paper. An aliquot of 4 mL of the filtrate was mixed with 1 mL chloroform and 1.5 mL water, and centrifuged to separate the two phases. A 2 mL aliquot of the upper phase was evaporated to dryness at room temperature under vacuum. The dried residue was re-dissolved in 2 mL of deionised water and filtered through a 0.22 μm membrane (Acrodisc, Gelman Sciences). The filtrate was further diluted with deionised water by 1000 times. The total amino acids were determined by injecting 50 μL into a Dionex amino acid analyser, using sodium buffer eluent (17.2 m*M* sodium hydroxide and 4.4 m*M* sodium borate) detected by reaction with ninhydrin. Nor-leucine was used to prepare the calibration curve.

The method of Newton et al. (1981) was used for the determination of thiols. Each ground leaf sample (0.10 g) was extracted with 1.5 mL of 0.1 *M* HCl and 0.10 g acid-washed polyvinylpolypyrrolidone at room temperature for 2 h. The mixture was then centrifuged and 0.5 mL of the supernatant filtered through a 0.22 μm membrane. A 0.10 mL aliquot of the thiol extract was brought to pH 8.7 ± 0.1 with the addition of 0.15 mL of 0.25 *M* CHES (2-[N- cyclohexylamino]ethanesulphonic acid, pH 9.3) buffer and 0.07 mL of 6 m*M* DTT (dithiothreitol) and allowed to stand at room temperature for 1 h. To derivatize the thiol compounds, 0.03 mL mono-bromobimane (4.2 mg mL^{-1} in methyl cyanide) was added to the above mixture, which was then kept in the dark for 15 min at room temperature. The derivatization was halted by adding 0.15 mL of 40 m*M* methanesulphonic acid. The sample was centrifuged to remove precipitated saccharides before HPLC analysis. An Alltech Econosphere C18 5 μm column (250 mm × 4.6 mm) was used. Separation was carried out by isocratic elution with 14% (v/v) methanol in 0.25% (v/v) acetic acid (pH 3.5) at a flow rate of 0.8 mL min^{-1}. The HPLC system was calibrated against a derivatized glutathione (GSH) and cysteine (Cys) standard mixture. The thiol concentration present was directly proportional to the elution peak height.

Dried ground plant materials were extracted with deionised water and the nitrate and sulphate concentrations were determined using ion chromatography (Dionex 2000i/sp fitted with AS9C separation column). Total N was determined by the Kjeldahl method. For the determination of total S, plant materials were digested with $HClO_4 - HNO_3$, and the S concentration measured by inductively coupled plasma atomic emission spectroscopy (Fisons ARL, Maxim III) using the 182.037 nm wavelength (Zhao et al., 1994). All chemical analyses were performed in duplicate.

Statistics

Genstat 5 software (Genstat 5 Committee, 1993) was used for analysis of variance and curve fitting. Mitscherlich, $Y = \alpha - \beta \times Exp(-\gamma X)$, or logistic, $Y = \alpha + \gamma/(1 + Exp(-\beta(X - \mu)))$, curves were fitted to the yield responses of the two varieties and two N levels separately. The mean relative DM yield of each treatment was calculated as a percentage of the total DM of the S_{50} treatment with the same variety and N level. Linear or Mitscherlich models were fitted to the relationships between various diagnostic indices and relative DM yield. The critical values were taken as the values of independent variables corresponding to 90% relative DM yield on the fitted curves.

Results

Responses at stem extension

Figure 1 shows the responses of the variety Riband to S addition at GS 37. The response patterns of Hereward were similar and are not shown in this Figure. Shoot DM was increased significantly by S additions up to 25 mg pot^{-1} at both N levels (Fig. 1a). Chlorophyll meter readings of the uppermost fully expanded leaves showed patterns similar to the DM responses (Fig. 1b). Between the 0 and 50 mg S pot^{-1} treatments, total S concentrations in Riband shoots increased by 2.5 times with N_{300}, and by 4 times with N_{500}. Hereward had slightly higher S concentrations at S_0 but lower concentrations at S_{50}, indicating a smaller response of total S concentration to the S addition in this variety (data not shown). In comparison, the sulphate-S concentration of shoots showed much greater relative increases, which were 8.3–8.9 fold in Riband (Fig. 1c) and 4.9–5.3 fold in Hereward. Depending on the S treatments, shoot sulphate-S accounted for between 10 and 40% of the total S. The ratio of total N to total S (N:S) in shoots was higher at N_{500} than at N_{300}, and decreased sharply with increasing S level up to 25 mg pot^{-1} (Fig. 1d). Nitrate accumulated in the N_{300} treatments at S_0 and the N_{500} treatments with $S_0 - S_{10}$ (Fig. 1e). A large accumulation of free amides was also evident, particularly asparagine. Increasing the S level

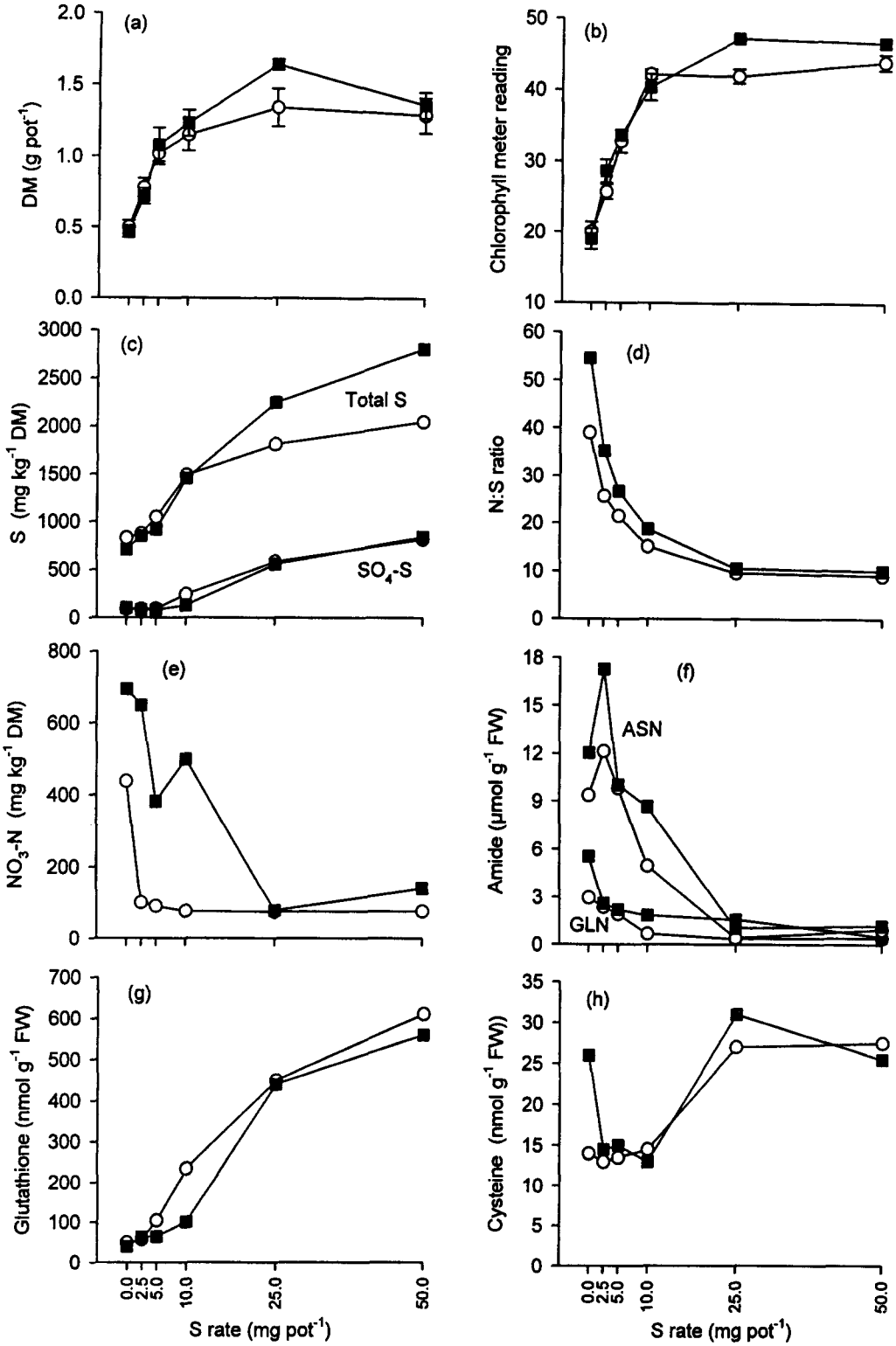

Figure 1. Responses of Riband to S and N additions at GS 37: (**a**) shoot DM, (**b**) chlorophyll meter reading of the uppermost fully expanded leaves, (**c**) concentrations of total S and sulphate-S in shoots, (**d**) shoot N:S ratio, (**e**) shoot nitrate-N concentration, (**f**) concentrations of asparagine and glutamine in uppermost fully expanded leaves, (**g**) concentration of glutathione in uppermost fully expanded leaves, and (**h**) concentration of cysteine in uppermost fully expanded leaves. Symbols: ○ 300 mg N pot^{-1} (N_{300}), ■ 500 mg N pot^{-1} (N_{500}) Vertical bars represent ±SE of the means of treatment replicates.

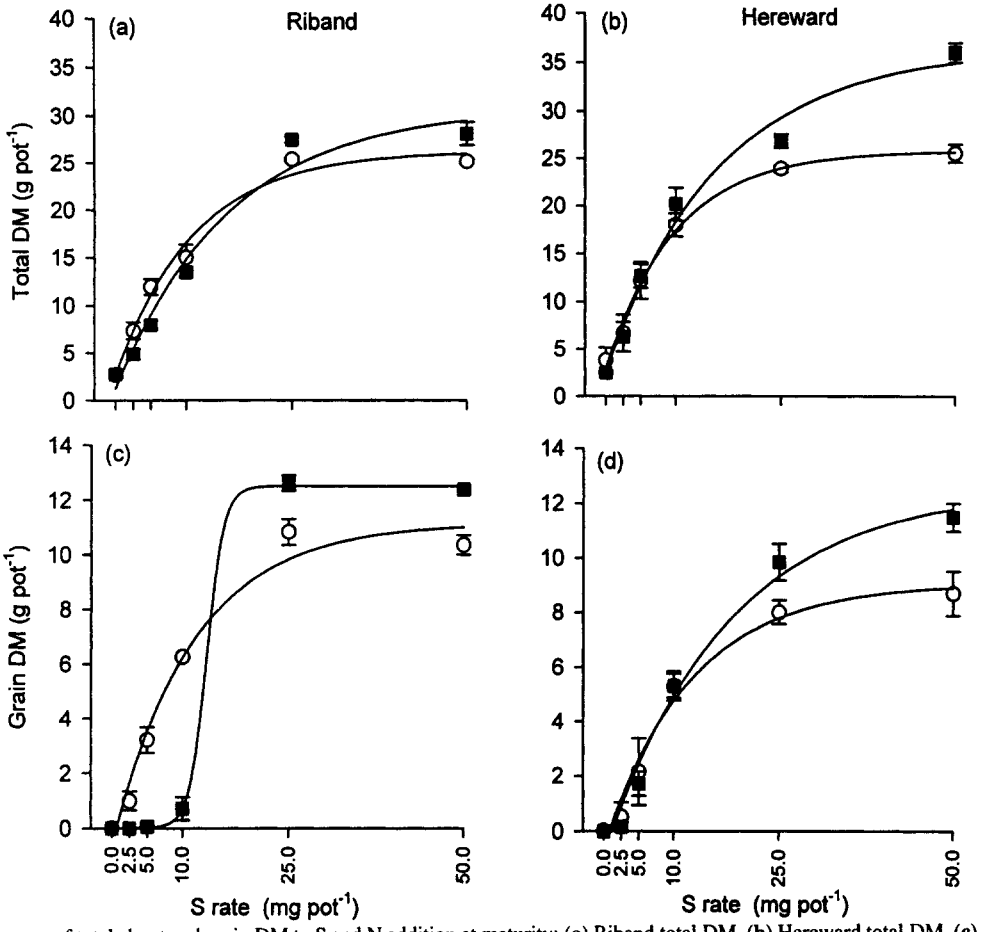

Figure 2. Responses of total shoot and grain DM to S and N addition at maturity: (a) Riband total DM, (b) Hereward total DM, (c) Riband grain DM, and (d) Hereward grain DM. Symbols: ○ N_{300}, ■ N_{500} Vertical bars represent ±SE of the means of treatment replicates. Lines are the best fits of Mitscherlish or logistic models.

from 0 to 50 mg pot^{-1} decreased the concentrations of asparagine and glutamine in the uppermost expanded leaves by approximately 10 and 7 times, respectively. The S addition had much smaller, and often inconsistent, effects on the concentrations of other free amino acids (data not shown). The concentration of the S containing tripeptide, glutathione, increased markedly, with increasing S levels (Fig. 1g). This response was greater in Riband than in Hereward: addition of 50 mg S pot^{-1} increased the concentration of glutathione in the uppermost expanded leaves by 12–14 times in Riband and 5- 6.5 times in Hereward. The concentration of free cysteine was much smaller than that of glutathione, and showed small and rather inconsistent responses to S (Fig. 1h).

Responses at maturity

Large increases of total shoot DM and grain DM were obtained in both varieties in response to the S addition (Fig. 2). There were also significant *($p<0.01$)* interactions between N and S, whereby the responses to S were greater with N_{500} than with N_{300}. All response curves to the S were fitted satisfactorily by the Mitscherlich equation, except that for the grain DM of the Riband N_{500} treatments, which showed near zero grain production up to S_{10}, followed by an abrupt increase at S_{25} (Fig. 2c). For this response curve a logistic equation was fitted, although there was uncertainty about the inflexion point in the curve due to lack of data around the steep central part. In general, Hereward was more responsive to the increase of N than Riband. For both varieties, S had a strong positive effect on the harvest index (grain/total shoot DM).

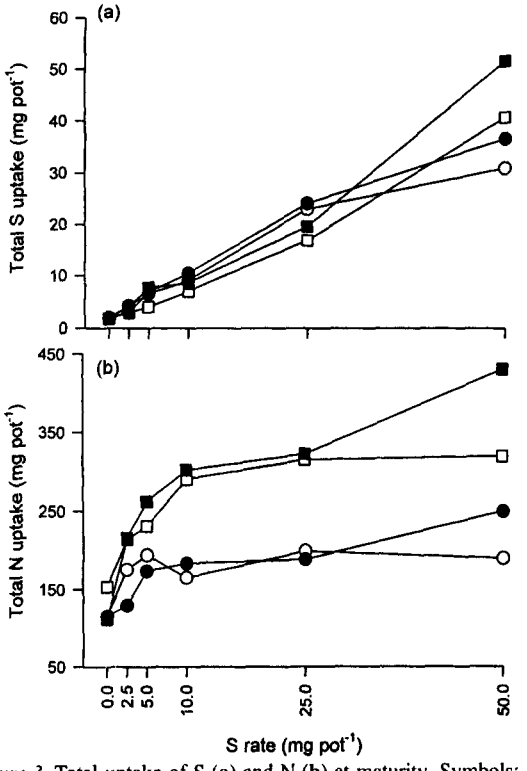

Figure 3. Total uptake of S (a) and N (b) at maturity. Symbols: ○ Riband $N_{3(0)}$, □ Riband $N_{5(0)}$, ● Hereward $N_{3(0)}$, ■ Hereward $N_{5(0)}$

Sulphur uptake increased linearly with increasing S levels in both varieties (Fig. 3a), and Hereward had significantly *($p<0.01$)* higher uptake of S than Riband. Nitrogen uptake increased with increasing S addition up to S_{10} in Riband, but continued to increase up to S_{50} Hereward (Fig. 3b). Averaged across those treatments where grain was produced, Riband had 61.4 and 42.7% of the total N and S in the plant located in grain, respectively. These were significantly higher than the percentages for Hereward (51.2 and 38.0% for N and S, respectively). However, there were no significant differences between the two varieties in the N:S ratio in the grain.

Diagnosis of S deficiency

Relative DM yields at GS 37 were plotted against various indices measured at the same growth stage to derive critical values for diagnosis of S deficiency (i.e. the immediate effect of S on dry matter; Fig. 4 and Table 1). The N levels used did not influence the shape of the regression curves significantly, and the data for the two N levels were combined for the model fitting. It is also apparent that the two varieties had similar relationships between the relative DM yields and various diagnostic indices, except those for the N:S ratio in the above ground shoot tissue. The concentrations of the total S, sulphate-S and glutathione showed a well defined non-linear relationship with the relative DM yields. The transition of data points between deficiency and sufficiency was sharper with sulphate-S and glutathione than with total S (Fig. 4). A pronounced Piper-Steenbjerg effect (Marschner, 1995) occurred in the relationship between relative DM yields and the % of the total S as sulphate-S (Fig. 4c). For example, a value of 13% may indicate either severe deficiency of S or adequate S status. The sulphate-S and glutathione concentrations also showed a small Piper-Steenbjerg effect. For this reason, the data points falling into this range were not included in the fitted curves shown. The relative DM yields showed a linear pattern in relation to shoot N:S ratio and the chlorophyll meter reading, and thus lacked a clear transition from deficiency to sufficiency.

Table 1. Critical values at GS 37 for the diagnosis and prognosis of S deficiency of wheat

Indices	Diagnosis	Prognosis
Total S (mg kg^{-1} DM)	1496	2026
Sulphate-S (mg kg^{-1} DM)	190	504
% S as sulphate-S	16.7	25.1
Glutathione (nmol g^{-1} FW)	237	383
N:S ratio Riband	16.9	8.3
N:S ratio Hereward	14.2	8.3
Chlorophyll meter reading	40.0	44.9

To derive critical values for prognostic purposes (i.e. predicting the effects of S deficiency on final yield), the data of various indices measured at GS 37 were plotted against the relative total DM yields at maturity (Fig. 5). In general, the patterns were similar to those for the diagnosis (Fig. 4), but the critical values for total S, sulphate-S, % S as sulphate-S, glutathione concentration and chlorophyll meter reading increased considerably, and those for the N:S ratio decreased (Table 1).

The concentrations of S in grain and N:S ratio have also been used to retrospectively diagnose S deficiency in wheat (Randall et al., 1981). Figure 6 shows that the N:S ratio of 17, as proposed by (Randall et al., 1981), gave a fairly reasonable separation between sufficiency and deficiency. In a total of 59 grain samples obtained from the pot experiment, 49 would be diagnosed correctly according to this critical value. The proposed

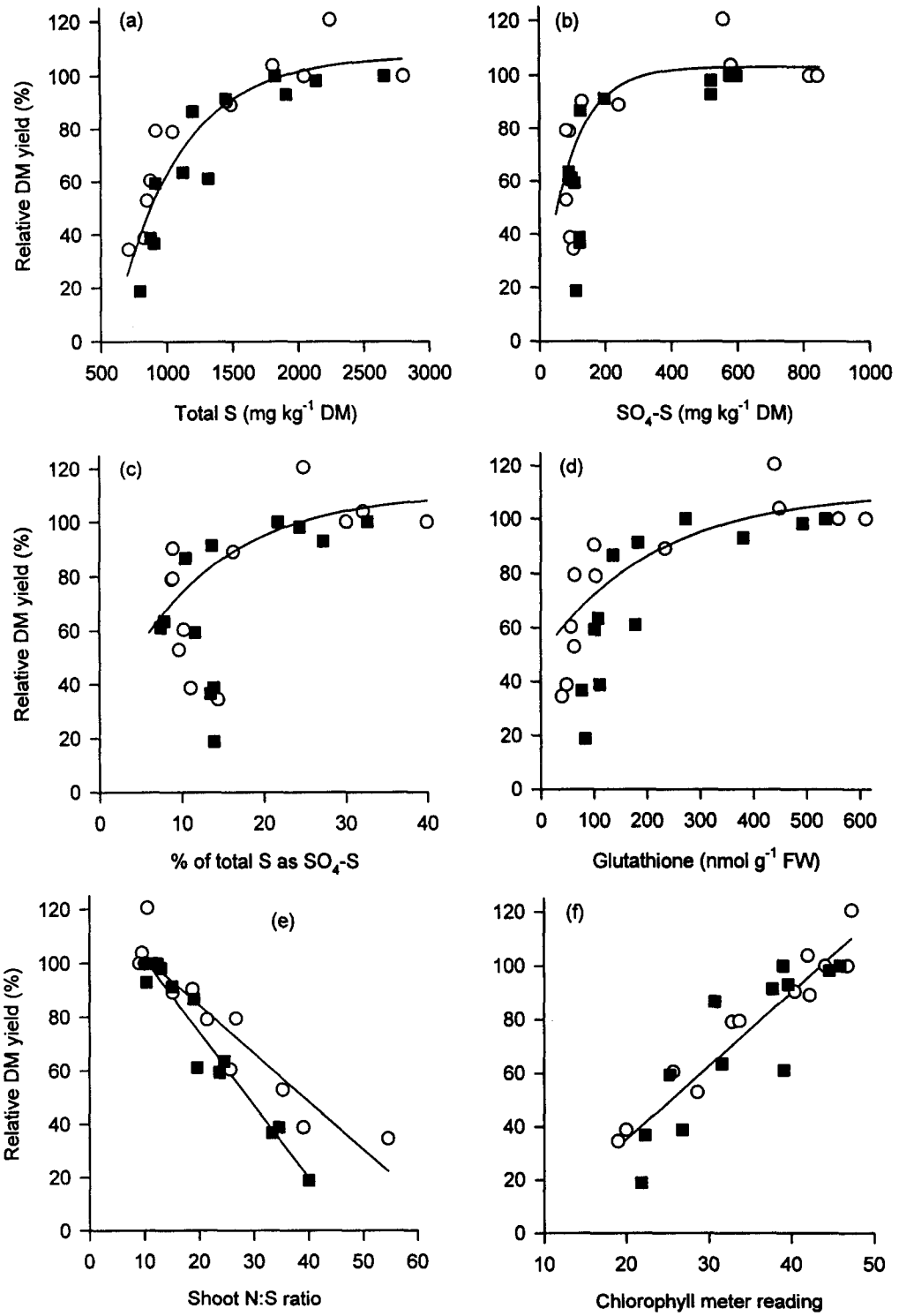

Figure 4. Relationships between relative DM yield and various indices at GS 3 7 : (**a**) total S concentration of shoot, (**b**) sulphate-S concentration of shoot, (**c**)% of total S as sulphate-S, (**d**) glutathione concentration of uppermost fully expanded leaves, (**e**) shoot N:S ratio, and (**f**) chlorophyll meter reading of uppermost fully expanded leaves. Symbols: ○ Riband, ■ Hereward. Lines are Mitscherlish curves for (a)-(d) and linear regression model for (e)-(f). In (b)-(d) where a Piper-Steenbjerg effect occurred, the data points with relative DM less than 40% were not used in the curve fitting.

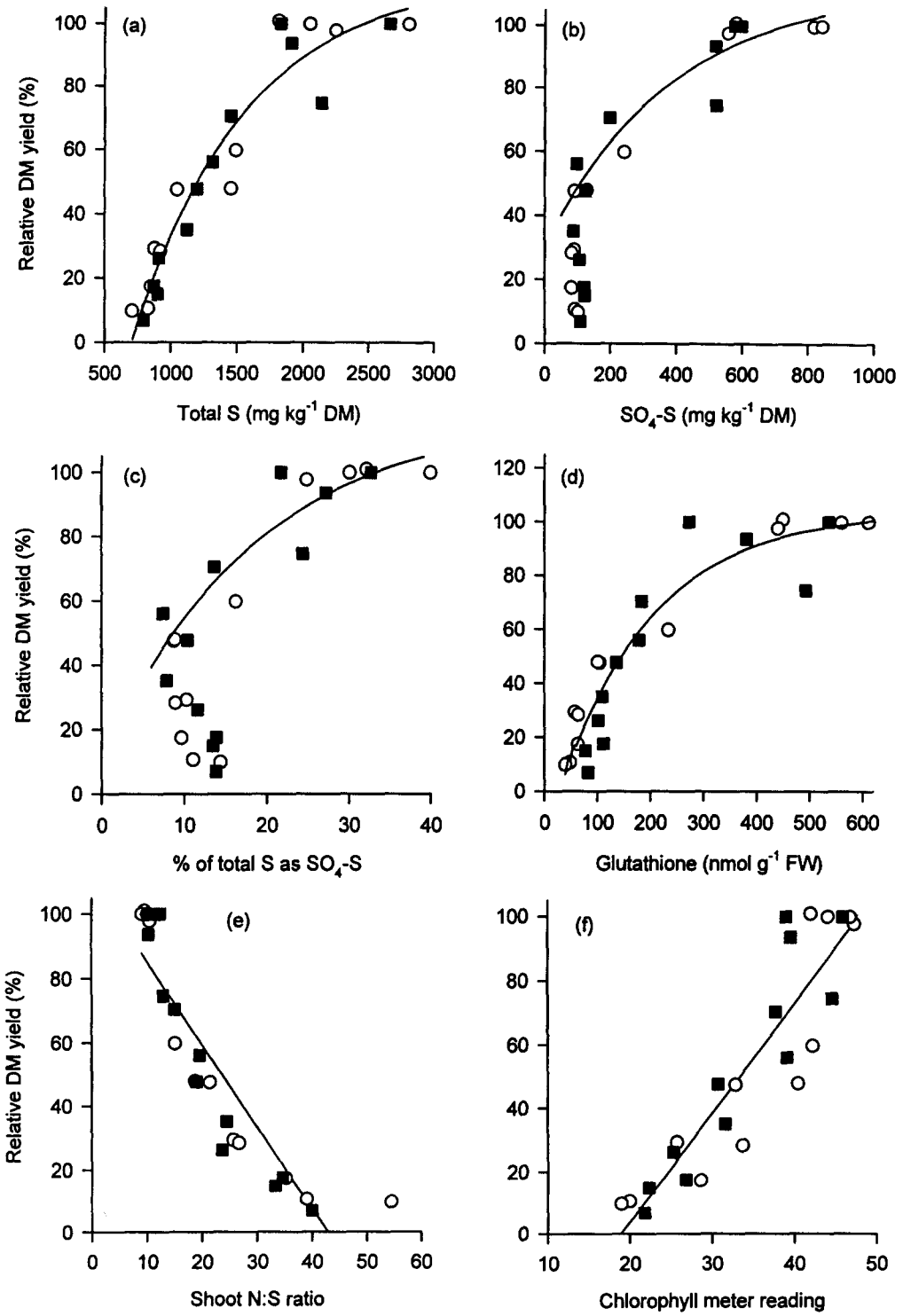

Figure 5. Relationships between relative DM yield at maturity and various indices measured at GS 37: (**a**) total S concentration of shoot, (**b**) sulphate-S concentration of shoot, (**c**) % of total S as sulphate-S, (**d**) glutathione concentration of uppermost fully expanded leaves, (**e**) shoot N:S ratio, and (**f**) chlorophyll meter reading of uppermost fully expanded leaves. Symbols: ○ Riband, ■ Hereward. Lines are Mitscherlish curves for (a)-(d) and linear regression model for (e)-(f). In (b)-(c) where a Piper-Steenbjerg effect occurred, the data points with relative DM less than 40% were not used in the curve fitting.

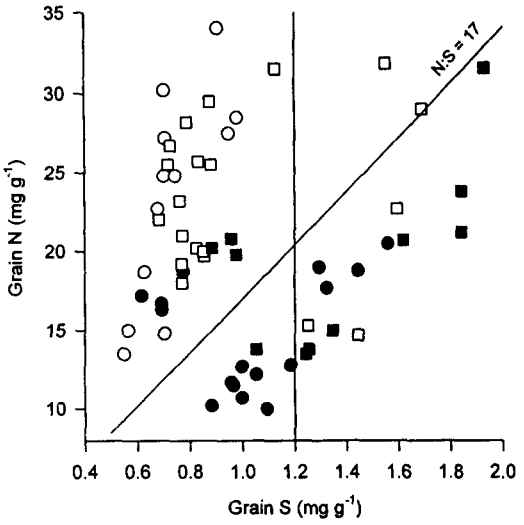

Figure 6. Relationship between grain N and S concentration. Open symbols are for S deficient plants (relative grain DM yield <90%) and closed symbols for S sufficient plants (relative grain DM yield>90%). ○● Riband, □■ Hereward.

critical value of 1.2 mg g^{-1} of total S in grain was less useful (Fig. 6).

Discussion

It appeared that the non-breadmaking variety Riband was more responsive to S than the breadmaking variety Hereward, whereas Hereward showed greater responses to increasing N supply. Grain production of Riband was also more susceptible to the imbalance of N and S supply. The two varieties differ significantly in grain protein content and the spectrum of storage proteins (Payne et al., 1987). Hereward had higher concentrations of N and S in grain than Riband, but the N:S ratios were similar (Zhao et al., 1995). Whether the different responses to S and N of Hereward and Riband are characteristic of the two types of varieties requires further investigation.

Apart from the shoot N:S ratio, the two varieties had similar patterns of diagnostic curves and thus similar critical values of diagnostic indices. Both total S and sulphate-S concentrations of shoots can be used alone as a good indicator of the current S status of wheat at GS 37. The critical values of about 1500 mg kg^{-1} DM total S and 190 mg kg^{-1} DM sulphate-S obtained in this study are identical to those reported by Spencer and Freney (1980) in Australia for field-grown wheat at the early jointing stage, but lower than a critical value of 2280 mg kg^{-1} DM total S (for 90% relative yield) reported by Haneklaus et al. (1995) who used the boundary line technique to derive diagnostic criteria. The transition zone from sufficiency to deficiency was narrower with sulphate-S than with total S. Against this advantage is the larger variability in the measurement of sulphate-S than total S (Pinkerton and Randall, 1995).

There appears to be no advantage of using % of total S as sulphate-S over either total S or sulphate-S alone. Besides, it has the disadvantage of requiring two analyses and also suffers the most severe Piper-Steenbjerg effect. The argument for the use of this index is that the critical value does not change with plant age (Freney et al., 1978). A recent study showed that this was not the case for three legumes and one grass (Pinkerton and Randall, 1995). As pointed out by Scaife and Burns (1986), it is unreasonable to expect any indices to have a constant critical value at different growth stages. This is because nutrient requirement is more closely related to the growth rate than the DM yield at the time of measurement, and the growth rate will be different at different stages. Sampling at a precise growth stage is required to overcome the problem of varying critical values for either total S or sulphate-S. As was found with field grown winter oilseed rape (McGrath and Zhao, 1996), shoot N:S ratio appeared to relate to relative DM yield in a linear fashion, lacking a sharp transition from sufficiency to deficiency as the N:S ratio increases. The critical values of 14–17 obtained here are in line with those reported elsewhere (Freney et al., 1978; Rasmussen et al., 1977; Spencer and Freney, 1980). However, the critical values derived from a linear relationship inherently lack accuracy.

Bell et al. (1995) and Karmoker et al. (1991) showed that the accumulation of amides in roots was the earliest response to the withdrawal of S supply. Large accumulations of asparagine and glutamine were also found in the leaves of S deficient plants in this study. It was not possible to tell if these changes occurred before others in the present study, because plants in the low S treatments were already very deficient in S by GS 37. There are other environmental stresses which can result in the accumulation of amides (Rabe, 1990). The usefulness of amide concentration as an early indication of S deficiency needs to be investigated further.

The concentration of glutathione in leaves showed the greatest response to the S supply. This contrasted with relatively small changes in the concentration of free cysteine. The ratio of glutathione to cysteine was

3–4 in the treatments without S addition, but increased to about 20 in the treatments with the highest S addition. This suggests that the concentration of cysteine in the soluble pool is under much tighter control than that of glutathione. Glutathione is the major form of organic S in the soluble fraction of plants and the predominant form of reduced S in long distance transport (Rennenberg, 1995). Some recent evidence also suggests that glutathione produced in the leaves may act as a signal to control sulphate uptake and transport in the roots (Herschbach and Rennenberg, 1994). The results from this study show the potential of using the concentration of glutathione in leaves as an indicator of S status of wheat. A critical value of about 240 nmol g^{-1} FW in uppermost fully expanded leaves was obtained at GS 37. Because glutathione plays important roles in stress physiology of plants, and its biosynthesis and degradation can be influenced by factors other than S nutrition, the diagnostic usefulness of glutathione needs to be examined further under different conditions.

The chlorophyll meter is being increasingly used in the recommendation of N fertiliser use (Fox et al., 1994; Peltonen et al., 1995). However, a low chlorophyll meter reading can also be due to S deficiency, as is shown clearly in the present study. The responses of chlorophyll meter readings to the addition of S were very similar to those of DM yield. Use of a chlorophyll meter to diagnose S deficiency is probably impractical on its own because of the complication of N nutrition. On the other hand, since S deficient areas are increasing (McGrath and Zhao, 1995), diagnosis of crop N status using chlorophyll meter can run into great difficulty without a clear means of assessing crop S status.

For prognostic purposes, a much higher S status at GS 37 was required to ensure no losses of DM yield due to S shortage at maturity. This is probably characteristic of pot experiments (Freney et al., 1978), and does not reflect field conditions where roots can explore much larger soil volume. Prognostic critical values obtained in pot experiments are probably less relevant to the field conditions than diagnostic ones.

Grain N:S ratio seems to indicate the grain DM yield reasonably well. An N:S ratio of 17 separates about 80% of the S responsive samples, but of course has no prognostic value for that crop.

Pot experiments have been widely used to study plant responses to nutrient supply and to derive critical values for diagnosis of nutrient deficiency. Using this technique, a wide range of nutrient supply can be imposed, and variations in growing conditions other than the supply of the nutrient studied can be minimised. These factors are difficult to control in field experiments. It must be emphasised, however, that the critical values obtained from pot experiments need to be validated in field trials.

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