Project Report No. 369

August 2005

Price: £3.50

Sulphur requirement of malting barley: effects on yield and quality and diagnosis of sulphur deficiency

by

F. J. Zhao[1], S. Fortune[1], V. L. Barbosa[1], S. P. McGrath[1], R. Stobart[2], M. Self[2], P. E. Bilsborrow[3], E. J. Booth[4], A. Brown[5] and P. Robson[6]

[1]Agriculture and Environment Division, Rothamsted Research, Harpenden, Herts AL5 2JQ
[2]The Arable Group, Eastern Region, The Old Rectory, Wymondham NR18 9DB
[3]School of Agriculture Food and Rural Development, University of Newcastle upon Tyne, NE1 7RU
[4]SAC, Craibstone, Aberdeen AB21 9YA
[5]Bairds Malt Limited, Arbroath, Angus DD11 2NJ, U.K.
[6]Muntons plc, Dunnikier Maltings, Kirkcaldy KY1 2EF

This is the final report of a 24 month project which started in April 2003. The work was funded by a contract of £196,070 from HGCA (Project No. 2516).

Contents

Abstract

Eight field experiments were conducted on four sites in 2003 and 2004 to investigate the effects of sulphur (S) application on yield and malting quality of barley. Winter barley (cv Pearl) was grown at two sites in East Anglia (Docking) and southeast of England (Woburn). Spring barley (cv Optic) was grown at two sites in Scotland (Corsekelly, Aberdeenshire) and County Durham (Bishop Middleham). Sulphur was applied as gypsum at 0, 10, 20 and 40 kg S/ha, in combination with two rates of N, which differed between sites. In addition, two additional treatments tested application of S at a later timing. Leaf tissues were sampled at tillering and stem extension and determined for a number of diagnostic indicators. A selected number of grain samples were analysed for malting quality.

Significant yield responses to S additions were obtained in five out of the eight experiments conducted at four different sites in two seasons, with yield increases ranging from 0.2 to 1.2 t/ha. Depending on the experimental site, the rate of S addition to achieve the maximum yield varied between 10 and 20 kg S/ha. This project showed that S should be applied to winter barley between mid-March to mid-April. The S-responsive experiments were associated with a soil extractable S in the range of 2.8 – 4.1 mg/kg, whereas the non-responsive trials had soil extractable S in the range of 6.4 – 14 mg/kg.

None of the leaf tissue indicators predicted yield responses with 100% accuracy at either tillering or stem extension. Leaf N:S ratio and sulphate-S concentration, measured at stem extension, appeared to be quite reliable at distinguishing the two most responsive sites from the rest. Retrospectively, grain N:S ratio at 17:1 also separated the two most responsive sites from the other sites.

Sulphur had both positive and negative influences on grain and malting quality at the two S-deficient sites. Sulphur application significantly increased malt diastatic power, α-amylase activity, friability and homogeneity, indicating an improved endosperm modification during malting. Sulphur applications also significantly decreased β-glucan concentration in wort, which is beneficial for beer filtration. At the two S-deficient sites, S application significantly increased the concentration of dimethylsulphide (DMS) precursor in the wort, which is expected to have an impact on the flavour of beer. When N supply was limiting, S applications decreased grain N concentration due to a dilution effect as a result of increased grain yield. However, no significant effect was observed when N was not limiting. The main negative effect of S applications was decreased grain size, with increased proportion of small grain (<2.25 mm) and decreased thousand grain weight, although this effect was not observed in every S-responsive trial. Increasing N rate also produced similar effects. As expected, at sites that were not S-deficient or were marginally deficient, S applications had little effect on grain or malting quality parameters.

Summary

Objectives

The objectives of this project were:
1) to investigate yield responses of winter and spring malting barley to S addition at different sites in the UK;
2) to establish optimal rate and timing of S addition for malting barley;
3) to establish diagnostic guidelines for predicting sulphur deficiency in malting barley; and
4) to evaluate the effects of S on grain size, protein concentration and malting quality of spring and winter malting barley.

Methods

Field trials were set up at four sites during the 2002-03 and 2003-04 growing seasons, referred to as 2003 and 2004, respectively. Winter barley (cv Pearl) was grown at Woburn (Bedfordshire) and Docking (Norfolk), and spring barley (cv Optic) at Corsekelly (Aberdeenshire) and Bishop Middleham (County Durham). The main experimental design included factorial combinations of four S levels: 0, 10, 20 and 40 kg S/ha, and two levels of N. In addition, two extra treatments were included to compare the effect of the timing of S application on crop yield and quality parameters. The two additional S timing treatments (both 20 kg S/ha) were applied approximately 4 weeks or 8 weeks after the timing used for the NxS factorial treatments. All of the treatments were replicated four-fold in a randomised block design. Grain yield and grain size were determined at maturity.

Prior to fertiliser application, topsoil (0-20 cm) samples were taken from each site. Available soil S was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) after extraction with 0.016 M KH_2PO_4. Other basic soil properties were also analysed. Crop samples were collected from each plot at the tillering and stem extension stages and were used to obtain diagnostic values for S deficiency in malting barley. Fifty leaves (the youngest, fully expanded leaves) were sampled from each plot. Total S concentration of leaf or grain samples was determined by ICP-AES after digestion with a mixture of HNO_3 and $HClO_4$ and total N by a combustion method (LECO CNS 2000). Sulphate-S and malate concentrations of leaf samples were determined by ion chromatography after the hot water extraction of plant material. The malate:sulphate ratios were calculated by dividing the peak area of malate by the peak area of sulphate (Blake-Kalff et al., 2000).

Twenty-four and 20 grain samples from the 2003 and 2004 trials, respectively, were selected for micro-malting tests, based mainly on grain N concentration (<1.8%). The samples selected in 2003

included those from the S0 and S20 plots, both with the low N rate, at Woburn, Corsekelly and Bishop Middleham. In 2004, samples were selected from the S0 and S20 plots with the low N rate at Docking, and from the S0/N110 and S20/N150 (two different timings of S application: either 23 March or 4 May) plots at Woburn. The tests were performed in kind by Bairds Malt Ltd and Muntons plc in 2003 and by Bairds Malt Ltd in 2004. The standard test procedure recommended by the Institute of Brewing (IoB, 2000) was followed.

Key results and conclusions

Yield response to S

Of the eight experiments conducted in the two seasons, five showed significant yield responses to S additions. Winter barley at Woburn and Docking were highly responsive in both seasons, whereas spring barley at Corsekelly was marginally responsive in 2003 and Bishop Middleham was not responsive in either season. At both Woburn and Docking, yield was increased by S by approximately 0.4 and 1.2 t/ha in 2003 and 2004, respectively, representing on average 8 and 23% increase over the nil S treatment. The 2004 season was clearly more responsive than the previous season. At Corsekelly, yield was increased by S by a moderate 0.22 t/ha in 2003 (4.7%), and this was achieved only from the higher doses of S application (20 and 40 kg/ha). In general, responses to S tended to be larger with a higher rate of N application, although the interactions between N and S were not statistically significant.

The optimal dose of S varied between different sites. For Woburn, 10 kg/ha of S was sufficient in both seasons, whereas 20 kg/ha was required for Docking and Corsekelly. Sulphur was applied as gypsum in this study. Other fertilisers in the form of sulphate are expected to give a similar efficacy. In terms of timing, the optimal window of S application appears to be between mid-March to mid-April for winter barley. An application of S in May did not fully prevent yield losses due to S deficiency in three out of the four experiments at Woburn and Docking in the two seasons.

The five S-responsive experiments shared one thing in common, i.e. low concentrations of soil extractable S (2.8 – 4.1 mg/kg). In contrast, the three S non-responsive trials had soil extractable S in the range of 6.4 – 14 mg/kg. The results from this series of trials suggest that soil analysis does provide useful information of available S, with a threshold value of around 4 – 5 mg/kg. This threshold is for soils sampled in spring and the S in the extracts analysed by inductively-coupled plasma atomic emission spectroscopy. It should be emphasised that only a limited number of sites (four) were used in this study, although a different field was used in each season.

Tissue analysis for diagnosis of S deficiency

Leaves were sampled at the tillering and stem extension – flag leaf stages and analysed for total S, sulphate-S, N:S ratio and malate:sulphate ratio. These diagnostic indicators were compared for their accuracy in predicting yield responses to S. None of the indicators predicted yield responses at 100% accuracy. Leaf N:S ratio and sulphate-S concentration, measured at the stem extension – flag leaf stage, appeared to be quite reliable at distinguishing the two most responsive sites (Woburn and Docking) from the others (Corsekelly and Bishop Middleham), although the threshold value for N:S ratio should probably be lowered to 16:1 from the more commonly used value of 17:1. However, these two indicators were not reliable at the tillering stage when the crop was still very small. With the threshold value of 1.5, leaf malate:sulphate ratio tended to over-predict S deficient sites.

Grain N:S ratios in barley were above 17:1 at Woburn and Docking in both seasons, and considerably lower than this value at the other two sites. Based on the limited number of trials in this project, the ratio does appear to separate S-deficient sites from those which are S-sufficient or only marginally deficient.

Grain and malting quality

Applications of S significantly decreased thousand grain weight (TGW) and specific weight, and increased the proportion of small grain (<2.25 mm) at Woburn in the 2003 trial. Similar effects were observed at Docking in the 2004 trial, although the effect on TGW was not significant. Sulphur application had no significant effect on these grain measurements in all other trials, including the yield responsive site at Woburn in 2004. Increasing N rate also tended to decrease TGW and increase the proportion of small grain.

At Woburn, S applications significantly decreased grain N concentration. This effect was observed at both N levels in 2004, but only at the low N level in 2003 when grain N was brought down from over 1.8% to well below this threshold value for malting. Sulphur applications had no significant effect on grain N concentration at the other three sites. The S effect observed at Woburn was probably due to a dilution of N in grain as a result of increased yield. The dilution effect would occur when N supply was limiting, e.g. at Woburn.

At Docking and Woburn, the two S-responsive sites, S applications significantly influenced a number of malting quality parameters On the positive side, S applications clearly improved endosperm modification during malting, resulting in a higher malt friability and homogeneity, and a lower β-glucan content in the wort. The effect on wort β-glucan content was one of the most noticeable; grain from the +20 kg S/ha treatment produced malt with 30 – 75% lower β-glucan content than those from the nil S treatment. High contents of β-glucan are undesirable because of the adverse effect on beer filtration rate, and thus the brewing house performance. The improved modification in the +S samples was probably a result of higher enzyme activities. Diastatic power was increased by S by 45 – 60%. The activity of α-amylase was also increased by S, significantly in two of the three sample sets from Woburn and Docking. Another noticeable effect of S was an increased concentration of DMS (dimethylsulphide) precursor (S-methylmethionine, or SMM). DMS is a major flavour attribute of some lager-type beers. At Woburn and Docking, the concentration of DMS precursor in the malt samples from the 20 kg S/ha treatment was 2.5 – 3.7 greater than that from the nil S treatment. These results indicate that S application to S-deficient malting barley crop could have a significant impact on the flavour of beer. Whether this effect is beneficial will depend on the type of beer; some lager beers require certain levels of DMS levels (30 – 60 μg/L) for their flavour characteristic, whereas in other lager beers DMS is maintained as low as possible.

Implications

Sulphur deficiency can result in a considerable yield loss in barley. Even at the current depressed grain price, the cost to yield benefit ratio is favourable to the use of S fertiliser under S deficiency conditions, particularly for the more deficient sites like Woburn and Docking. To reduce the cost of fertiliser, barley growers could opt for an application rate of 10 kg S/ha, even though this rate may not achieve the maximum yield at some sites.

Sulphur deficiency, if left untreated, could also influence a number of malting quality parameters adversely, particularly enzyme activity and endosperm modification. In some cases, S application produced the benefit of reduced grain protein content, but a negative effect of decreased grain size. Sulphur application could have a significant impact on beer flavour through its effect on DMS precursor.

Detailed Technical Report

1. Introduction

National sulphur (S) emissions have been decreasing more rapidly than expected over the last few years (McGrath et al., 2002). As a result of reduced S inputs from atmosphere, coupled with wet winters over the last few years, there is clear sign that S deficiency in cereals has increased in the major cereal growing areas in the UK (Zhao et al., 2002).

Most of the previous work on S nutrition has focused on oilseed rape and winter wheat. Results from these projects showed the extent and scale of yield responses to S applications (Withers et al., 1995b; McGrath et al., 1999). For winter wheat, S deficiency can also adversely affect breadmaking quality (Zhao et al., 1999a; Zhao et al., 1999b). Malting barley is often grown on light soils to achieve <1.8% total N in grain, which is required for malting purposes. Light textured soils are most at risk of S deficiency (McGrath and Zhao, 1995), and barley crops may therefore be susceptible to S deficiency (Withers et al., 1995a). However, there is very limited information available on the S requirement and yield response of malting barley. Sulphur deficiency has been shown to affect the composition of proteins in barley grain (Shewry et al., 1983). However, there is little knowledge as to the potential influence of S on barley malting quality. The lack of information on the effects of sulphur on barley yield and quality has been highlighted in an HGCA review (Chalmers et al., 1999).

To avoid yield losses due to S deficiency, it is important that the S status of a crop can be predicted from soil information or diagnosed in the early phase of growth. A range of tissue diagnostic indicators have been examined for oilseed rape and wheat (Withers et al., 1997; Blake-Kalff et al., 2000). A recent project showed that the ratio of malate to sulphate in leaves appears to be a reliable indicator of the crop S status for oilseed rape and wheat (Blake-Kalff et al., 2004). Again, little information is available for malting barley.

The objectives of this project were:

1) to investigate yield responses of winter and spring malting barley to S addition at different sites in the UK;

2) to establish optimal rate and timing of S addition for malting barley;

3) to establish diagnostic guidelines for predicting sulphur deficiency in malting barley; and

4) to evaluate the effects of S on grain size, protein concentration and malting quality of spring and winter malting barley.

2. Materials and Methods

2.1. Field trials

Field trials were set up at four sites during the 2002-03 and 2003-04 growing seasons (referred to as 2003 and 2004, respectively). Winter barley (cv Pearl) was grown at Woburn (Bedfordshire) and Docking (Norfolk), and spring barley (cv Optic) at Corsekelly (Aberdeenshire) and Bishop Middleham (County Durham). Both varieties are recommended malting barley varieties. The trials at Woburn, Docking, Corsekelly and Bishop Middleham were managed by Rothamsted Research, The Arable Group (TAG), Scottish Agricultural College (SAC) and Newcastle University, respectively.

The main experimental design included factorial combinations of four S levels: 0, 10, 20 and 40 kg S/ha, and two levels of N. The levels of N differed between sites and were based on normal practical applications at each site (Table 1). The four rates of S application were designed to provide a growth response curve at each site and help evaluate S fertilisation strategies. Sulphur was applied as gypsum and N as ammonium nitrate, either in mid March for winter barley or before sowing for spring barley (Table 1). In addition, two extra treatments were included to compare the effect of the timing of S application on crop yield. The two S timing treatments, both with an addition of 20 kg S/ha, were applied approximately 4 or 8 weeks after the first S was applied in the main N×S factorial treatments. The exact timing of the two late S applications was also dictated by weather. For these two extra S treatments, N was applied at the N2 level at each site. All of the treatments were replicated four-fold in a randomised block design, giving 40 plots in total at each site. A foliar Cu spray (0.5 l/ha of Coptrel 500 containing 33% Cu w/w) was applied at Docking, due to the low concentration of EDTA extractable Cu in the soil (<2.5mg Cu/kg), in order to prevent the possibility of Cu deficiency.

At crop maturity, grain yields were determined using a plot combine harvester. Grain moisture content was determined, and yields were recorded on a 85% dry matter basis. Grain samples were air-dried prior to micro-malting tests and physical analysis (specific weight, thousand grain weights, grain size distribution).

2.2. Sampling and analysis

Prior to fertiliser application, topsoil (0-20 cm) samples were taken from each site. Fresh soils were analysed for mineral N and air dried soils (<2 mm sieved) for available S, Cu, P and K, soil total N, organic C, $CaCO_3$ and pH. Available soil S was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) after extraction with 0.016 M KH_2PO_4 and Cu by ICP-AES after extraction with 0.05 M NaEDTA (pH 7.0).

Crop samples were collected from each plot at the tillering and stem extension – flag leaf stages (Table 2) and were used to obtain diagnostic values for S deficiency in malting barley. Fifty leaves (the youngest, fully expanded leaves) were sampled from each plot, oven dried (80^{o}C), and ground to a fine powder for analysis. Total S concentration of leaf or grain samples was determined by ICP-AES after digestion with a mixture of HNO_3 and $HClO_3$ and total N by a combustion method (LECO CNS 2000). Sulphate-S and malate concentrations of leaf samples were determined by ion chromatography (Dionex DX500) after the hot water extraction of plant material (100 mg plant:20 ml H_2O, shaken in a water bath at 80ºC, for 2 hrs). The malate:sulphate ratios were calculated by dividing the peak area of malate by the peak area of sulphate (Blake-Kalff et al., 2000).

Table 1 Rates and dates of fertiliser applications

Site	Season	Sowing date	Sulphur (kg/ha)	Application date	Nitrogen (kg/ha)	Application Date
Corsekelly, (SAC)	2003	7/4/03	0, 10, 20, 40 Late S (4 weeks) Late S (8 weeks)	09/4/03 09/5/03 3/6/03	70, 120	29/4/03
	2004	17/4/04	0, 10, 20, 40 Late S (4 weeks) Late S (8 weeks)	21/4/04 27/5/04 30/6/04	70, 120	13/5/04
Bishop Middleham (Newcastle University)	2003	21/3/03	0, 10, 20, 40 Late S (4 weeks) Late S (8 weeks)	11/4/03 9/05/03 29/5/03	80, 120	40 kg 11/04 rest 25/04
	2004	14/4/04	0, 10, 20, 40 Late S (4 weeks) Late S (8 weeks)	20/5/04 15/6/04 25/6/04	70, 120	70 kg seedbed rest 20/5
Docking (TAG)	2003	23/09/02	0, 10, 20, 40 Late S (4 weeks) Late S (8 weeks)	20/3/03 23/4/03 27/5/03	110, 150 (applied as a 2 way split	40 kg/ha/N on 13/03/03 70 or 110 kg/ha/N on 26/03/03
	2004	22/09/03	0, 10, 20, 40 Late S (4 weeks) Late S (8 weeks)	19/3/04 16/4/04 12/5/04	110, 150 (applied as a 2 way split)	40 kg/ha/N on 18/03/04 70 or 110 kg/ha/N on 30/03/04
Woburn (RRes)	2003	27/9/02	0, 10, 20, 40 Late S (4 weeks) Late S (8 weeks)	17/3/03 15/4/03 16/5/03	110, 150	24/3/03
	2004	06/10/03	0, 10, 20, 40 Late S (4 weeks) Late S (8 weeks)	23/3/04 04/5/04 19/5/04	110, 150	18/3/04

Table 2: Dates of leaf sampling and the corresponding growth stage of barley

Site	Season	Tillering	Stem Extension
Corsekelly (SAC)	2003 2004	16/5/03 GS 21 27/5/04	04/6/03 GS 30-31 23/6/04 GS 31-32
Bishop Middleham (Newcastle University)	2003 2004	29/5/03 GS 25 Not sampled.	20/6/03 GS 55 15/6/04 GS 55-57
Docking (TAG)	2003 2004	20/3/03 GS 24-27 02/03/04 GS 23-25	06/5/03 GS 47 12/05/04 GS 39-49
Woburn (RRes)	2003 2004	03/4/03 GS 25-27 1/4/04 GS 25-27	01/5/03 GS 34-35 26/4/04 GS 32-33

2.3. Micro-malting tests

Twenty-four and 20 grain samples from the 2003 and 2004 trials, respectively, were selected for micro-malting tests, based mainly on grain N concentration (<1.8%). The samples selected in 2003 included those from the S0 and S20 plots, both with the low N rate, at Woburn, Corsekelly and Bishop Middleham. None of the samples from Docking were selected because of the high grain N concentration. In 2004, samples were selected from the S0 and S20 plots with the low N rate at Docking, and from the S0/N110 and S20/N150 (two different timings of S application: either 23 March or 4 May) plots at Woburn. The Woburn samples were chosen because they had similar N concentrations. Samples from Corsekelly and Bishop Middleham were not selected because of the lack of a yield response at the two sites.

Malting quality tests were performed in kind by Bairds Malt Ltd and Muntons plc in 2003 and by Bairds Malt Ltd in 2004. The tests were carried out on the grain retained on the 2.2 mm screen in the Bairds laboratory, and on all grain in the Muntons laboratory. Germinative energy was tested with 4 ml water, water sensitivity with 8 ml water and germinative capacity with hydrogen peroxide (IoB, 2000). The following conditions were used by the Bairds laboratory for malting: steeping cycle 10 h wet – 12 h dry – 8 h wet – 12 h dry – 5h wet, germination temperature 16^{o}C for 4.5 days, kiln temperature 70^{o}C for 20 h. The conditions employed in the Muntons laboratory were: steeping cycle 8 h wet – 20 h dry – 16 h wet, germination temperature 15^{o}C for 4 days, kiln temperature 70^{o}C for 24 h. Giberellic acid was not used in either laboratory during the steeping cycle. A range of analyses were performed, including hot water extract (coarse grind), total and soluble N concentrations in malt, diastatic power, α-amylase activity, friability, homogeneity, unboiled fermentability, wort β-glucan concentration and free amino N. The standard test procedure recommended by the Institute of Brewing (IoB, 2000) was followed. The concentrations of DMS precursor (SMM) and glycosidic nitrile in the malt samples produced from the grain samples of the Corsekelly and Bishop Middleham in 2003, and of Woburn and Docking in 2004, were also determined. Malt SMM was measured indirectly by measuring the amount of DMS liberated from the SMM following caustic/heat degradation. Total DMS was measured by treating the ground malt with sodium hydroxide and boiling in a sealed vial. This chemically degrades SMM to release DMS. The liberated DMS was determined by headspace chromatography following incubation at 50°C for 30 minutes. Free malt DMS was measured by mixing ground malt with water in a vial sealed with a septum. The vial was incubated at 35°C for 30 minutes to volatilise any DMS, but avoiding conversion of precursor. DMS in the headspace samples was analysed using a gas chromatograph with a flamephotometric detector (FPD). The level of DMS liberated from the precursor SMM was calculated by subtracting the free DMS from the Total DMS.

2.4. Data analysis

Analysis of variance (ANOVA) was performed to test the significance of the effects of N and S and the N×S interactions. The *F* probabilities are given. In addition, the two late S treatments were compared to the early S and nil S treatments. If ANOVA indicated a significant ($p<0.05$) difference between these four treatments, the least significant difference (LSD, $p<0.05$) was used to compare treatment means. Statistical analyses were carried out using Genstat for Windows Version 8 (VSN International, Hemel Hempstead).

3. Results

3.1. Soil properties

Selected soil properties are shown in Table 3. The soils at Bishop Middleham and Corsekelly, which were used for the spring barley trials, had a much higher organic C and total N contents than those at the winter barley trial sites (Woburn and Docking). Extractable S concentrations were low for Woburn and Docking, as well as Corsekelly in 2003. The Corsekelly site in 2004 had high concentrations of mineral N and extractable S, and both showed large variations between the four experimental blocks. The level of extractable S at Bishop Middleham (> 6 mg/kg) is also considered to be sufficient for cereal crops (Withers et al., 1997). All soils contained sufficient available P for crop growth. Exchangeable K ranged from medium to high. EDTA extractable Cu clearly showed that the Docking soil was potentially deficient in Cu supply. For this reason, foliar Cu was applied to the crop at Docking.

Table 3. Selected soil properties at the four experimental sites

Season	Site	pH	Organic C (%)	Total N (%)	Mineral N (mg/kg)	Extractable S (mg/kg)	Olsen P (mg/kg)	Exchangeable K (mg/kg)	Extractable Cu (mg/kg)
2003	Woburn	7.3	1.05	0.096	0.9	3.4	56.9	126	5.0
	Docking	8.1	0.73	0.073	1.3	4.1	44.8	87	1.4
	Bishop Middleham	8.0	2.99	0.223	5.5	6.4	29.2	112	6.9
	Corsekelly	ND[a]	3.47	ND	ND	3.2	19.8	81	2.6
2004	Woburn	6.5	0.96	0.084	1.9	2.8	35.8	168	2.0
	Docking	7.7	0.77	0.075	4.7	3.7	36.4	70	1.3
	Bishop Middleham	6.7	3.21	0.195	9.0	6.5	65.2	73	4.9
	Corsekelly	5.8	3.42	0.233	22.9 (8.7 -53.1)[b]	14.0 (5.7 - 31.1)[b]	20.2	87	3.7

[a] ND, not determined.
[b] Values in parentheses are the range for the soil samples collected from the 4 separate blocks.

3.2. Yield responses

3.2.1. Yield responses to S and N in 2003

Grain yields of winter barley were relatively low at Woburn and Docking in the 2003 season, averaging 5.2 and 5.6 kg/ha, respectively. Sulphur applications significantly increased grain yields at both of these sites ($p<0.01$) (Figure 1). At Woburn, yields were almost identical for the two N levels applied. Grain yields were increased by 6.4-12.1% by different S treatments, with a mean of 8.6%. The lowest rate of S (10 kg S/ha) was sufficient for grain yield, and there was no significant interactions between N and S. At Docking, yield increases in response to S applications varied from 4.9 to 13.1%, with a mean of 7.8%, and the highest yields were obtained with an application of 20 kg S/ha. The response to S was also larger at 150 kg N/ha than at 110 kg N/ha, although the interactions between N and S were not statistically significant. The higher N treatment produced a larger yield, but the difference between the two N rates was not significant.

At Corsekelly, applications of 20 and 40kg S/ha increased grain yields of spring barley by 4.7% ($p<0.01$) and the lower rate of S (10 kg S/ha) was insufficient to increase grain yields (Figure 1). Increasing the N rate from 70 to 120 kg/ha did not increase yield significantly. No significant effects of S application on grain yield were observed at Bishop Middleham. The yields at this site appeared to be limited by N rather than S (Figure 1); yields were 20% higher at 120 kg N/ha than at 70 kg N/ha. No interactions between S and N were observed at any of these two sites with spring barley.

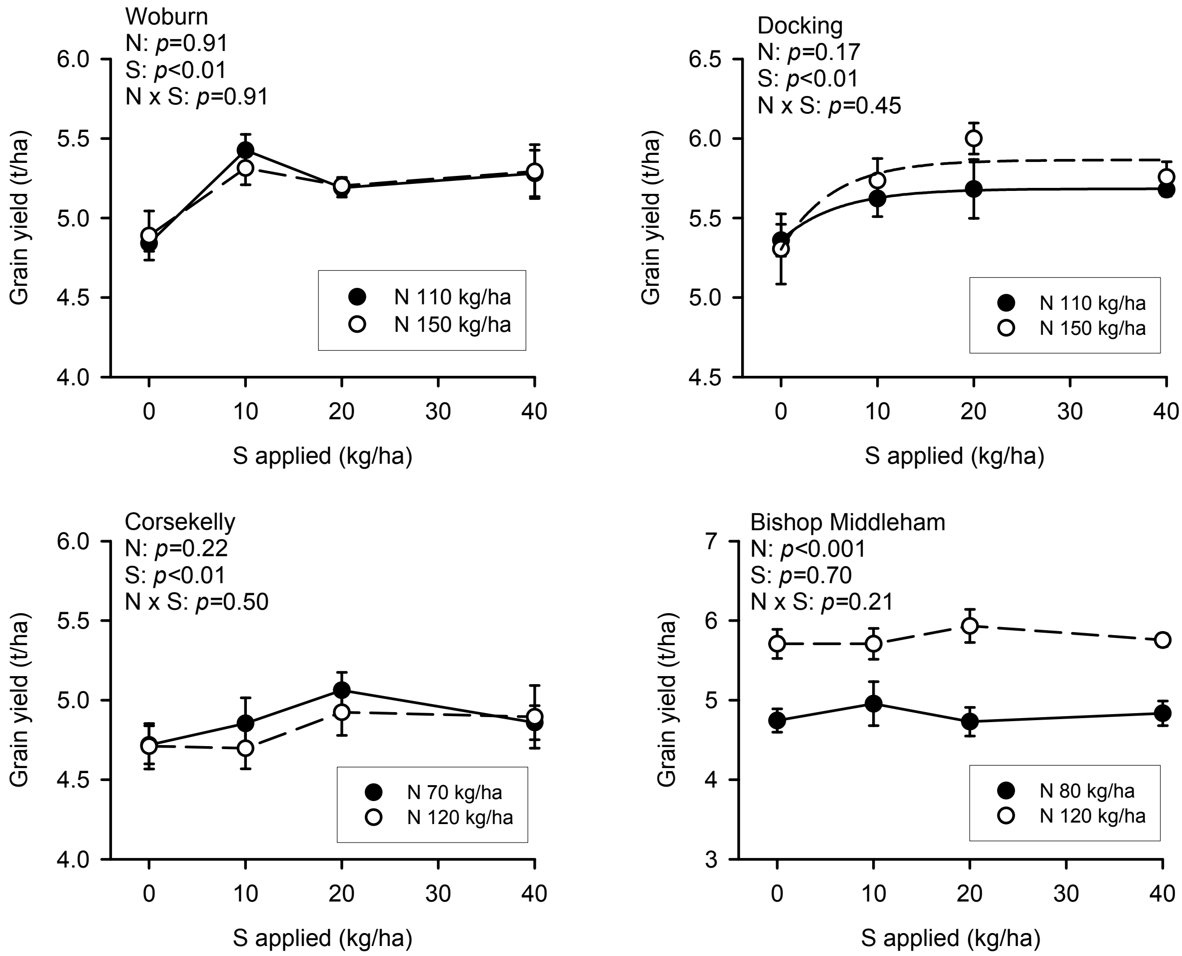

Figure 1: Yield responses to S and N applications of winter (Woburn and Docking) and spring (Corsekelly and Bishop Middleham) barley in 2003. Grain yield are based on 85% dry matter.

3.2.2. Effects of S timing on grain yield in 2003

At Woburn, timings of S application from mid March to mid May did not have a significant impact on yields (Figure 2). In contrast, at Docking, the timing of S application was important ($p<0.05$); the plots receiving S applications in March or April had significantly larger yields than the plots receiving S in late May (27/5/03) (Figure 2). The latest S application was obviously too late to benefit grain yields and similar yields were produced by the control and the latest S treatment.

For the two spring barley sites, differences in yield between the nil S control and S timing treatments were small and not significant.

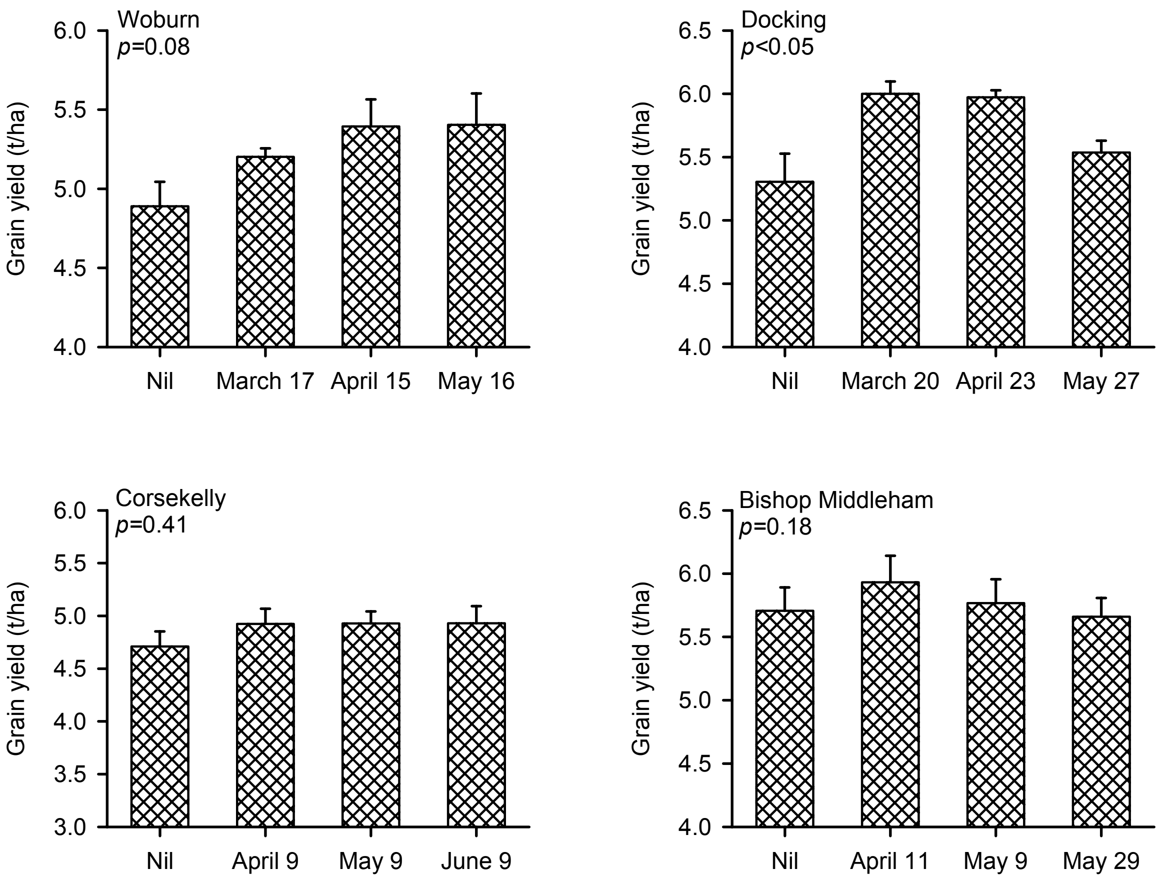

Figure 2: Effects of timing of S application on yields of winter (Woburn and Docking) and spring (Corsekelly and Bishop Middleham) barley in 2003. Sulphur was applied at 20 kg/ha. Grain yields are based on 85% dry matter.

3.2.3. Yield response to S and N in 2004

Yields of winter barley in the 2004 trials were higher than those in the previous year. Applications of S produced a large response at both Woburn and Docking (Figure 3). When averaged across all three rates of S , yield responses were 22.4 and 23.0% (p<0.001), corresponding to yield increases of 1.25 and 1.21 t/ha, at Woburn and Docking, respectively. Similar to the previous season, 10 kg S/ha was sufficient at Woburn, but higher rates of S (20-40 kg/ha) were required to achieve the maximum yield at Docking. Increasing N rate from 110 to 150 kg/ha increased yield significantly only at Woburn. There were no significant interactions between N and S on grain yield.

No significant responses to S were obtained in the trials with spring barley at both Corsekelly and Bishop Middleham in the 2004 season (Figure 3). The higher N rate produced significantly larger yield at both sites, with the effect being more noticeable at Corsekelly than at Bishop Middleham.

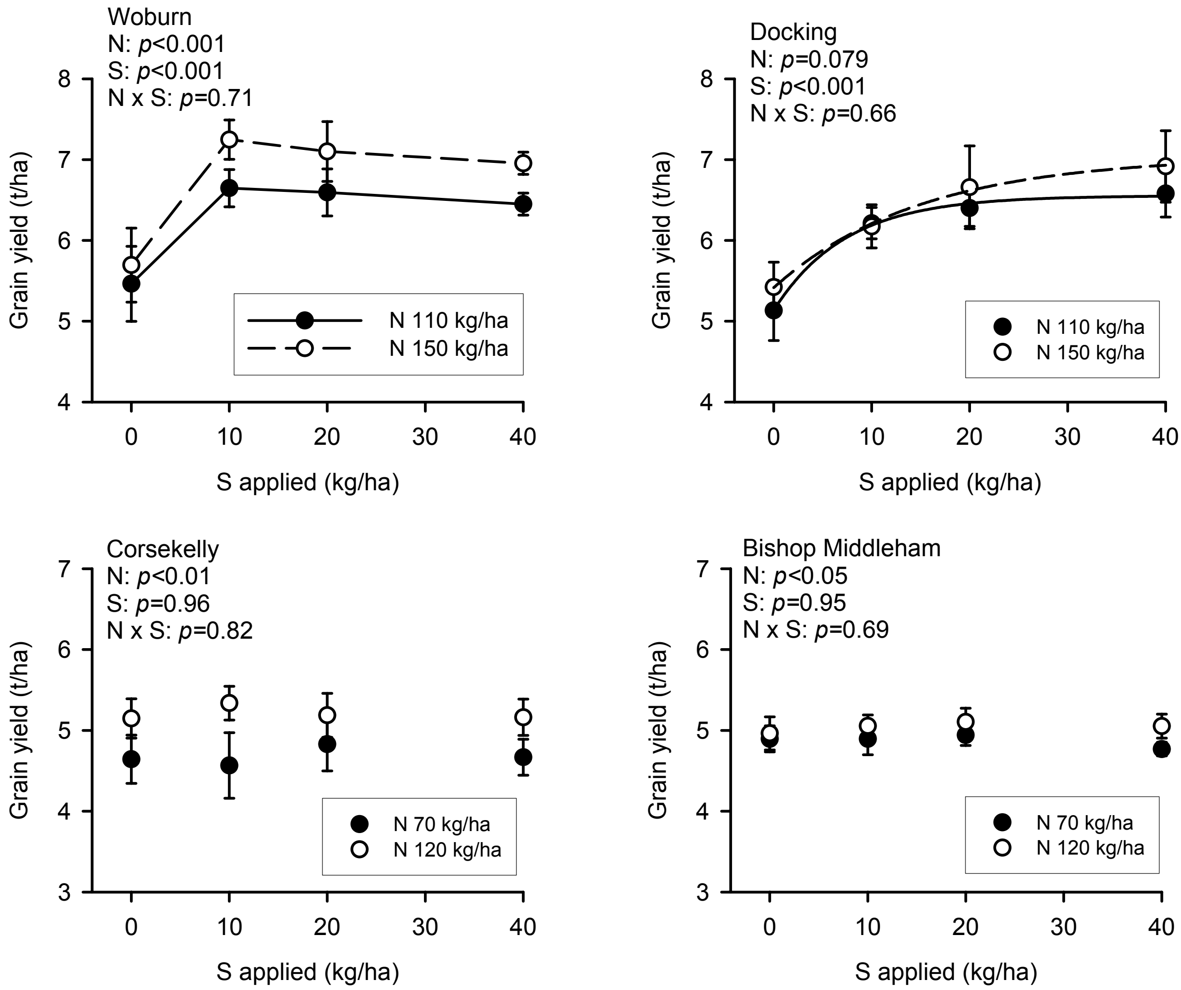

Figure 3: Yield responses to S and N applications of winter (Woburn and Docking) and spring (Corsekelly and Bishop Middleham) barley in 2004. Grain yields are based on 85% dry matter.

3.2.4. Effects of S timing on grain yield in 2004

There were significant differences between the four treatments (Nil S and 20 kg S/ha applied at three different timings) at both Woburn and Docking (Figure 4). At Woburn, the largest yield was obtained with the March application, whereas the applications in May produced significantly smaller yields. At Docking, application of S in mid March produced the largest yield, followed by the late applications in mid-April and mid May, although the yield difference between March and April applications was small.

For spring barley, both the Corsekelly and Bishop Middleham sites were not responsive to S applications, and there were little differences between the S timing treatments and the nil S control (Figure 4).

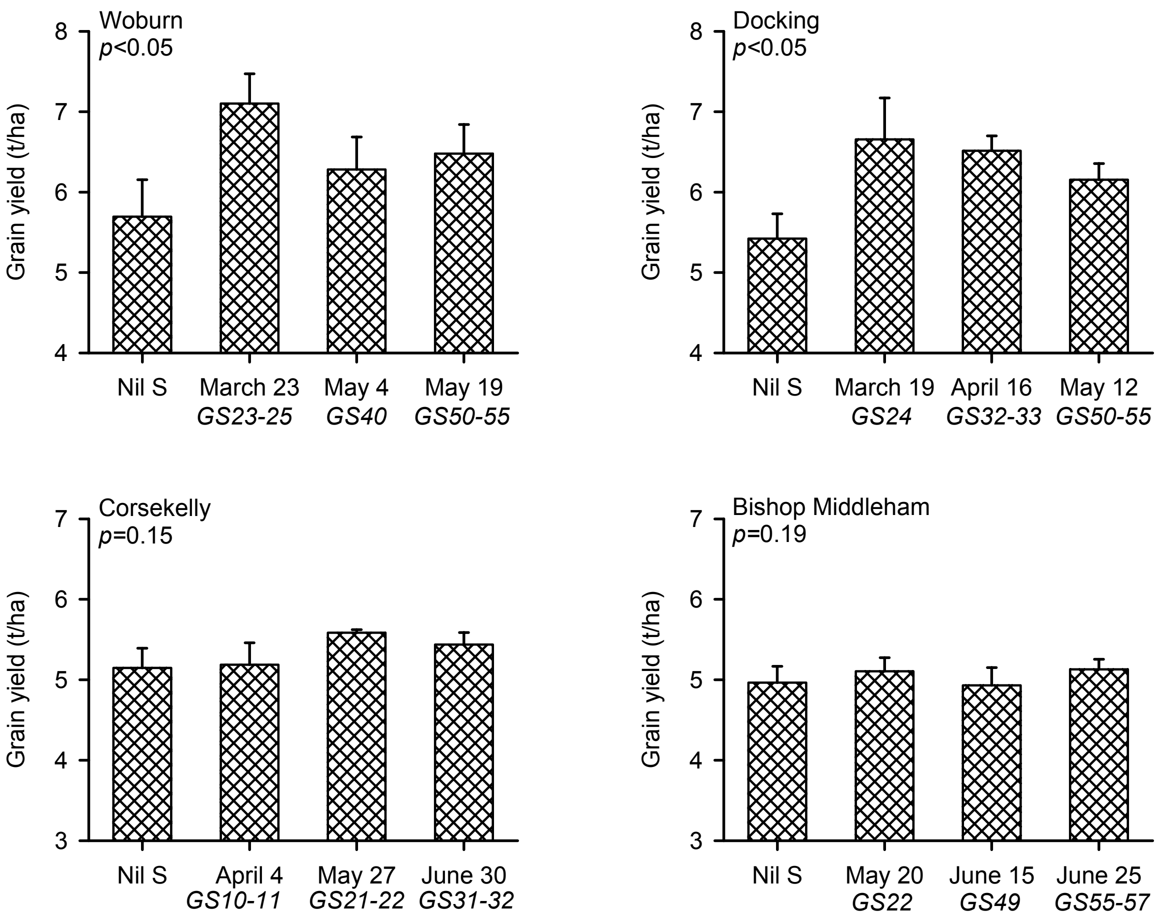

Figure 4: Effects of timing of S application on yields of winter (Woburn and Docking) and spring (Corsekelly and Bishop Middleham) barley in 2004. Sulphur was applied at 20 kg/ha. Grain yield are based on 85% dry matter.

3.3. Grain N and S concentrations and N:S ratio

3.3.1. Experiments in 2003

Grain N and S concentrations and N:S ratios are presented in Tables 4-7 for Woburn, Docking, Corsekelly and Bishop Middleham, respectively.

Grain N concentration ranged from 1.25 to 2.49%, on a dry weight basis, across the different sites and treatments in the 2003 season. The N and protein content of grain is important for malting purposes. Only barley with less than 1.8 % total N in grain is used for malting in the UK. This threshold value was exceeded in all samples from Docking, as well as those of the high N treatments at Woburn and Corsekelly. In contrast, grain N concentrations were very low (<1.5%) in both N treatments at Bishop Midddleham. At all four sites, increasing N application rate increased grain N concentration significantly (P<0.001). The S treatment had no significant effect on grain N concentration, except at Woburn where a significant NxS interaction was observed. This interaction was associated with a substantial decrease in grain N concentration in response to S additions at the low N treatment, but not at the high N treatment (Table 4). Sulphur additions brought grain N% from above 1.8% down to around 1.7% in the 110 kg N/ha treatment, an effect that is beneficial for malting purposes. This effect was probably caused by a dilution as a result of increased yield. However, the dilution effect was not apparent when N supply was high (150 kg N/ha). At all sites, the timing of S application had no significant effect on grain N concentration.

Grain S concentrations were significantly (p<0.001) increased by both N and S applications at Woburn and Docking (Tables 4-5). The effect of S was greater with the higher N than with the lower N treatment, showing a significant NxS interaction at both sites. At these two sites, S application in March, April or May increased grain S concentration to a similar extent. In contrast to the two winter barley trials, S applications had no significant effect on grain S concentration of spring barley at Corsekelly and Bishop Middleham (Tables 6-7). This lack of an effect may be because the Corsekelly site was only marginally deficient in S and the Bishop Middleham was not deficient at all. At both sites, increasing N rate increased grain S concentration significantly.

Grain N:S ratio has been suggested to indicate crop S status for wheat, with a ratio of greater than 17 being generally associated with S deficiency (Randall et al., 1981). However, trial results for winter wheat in the UK showed that grain N:S ratio alone could not satisfactorily distinguish yield responsive sites (Withers et al., 1997; McGrath et al., 1999). Little information is available for barley. In the field experiments in 2004, grain N:S ratio was 17 or above in the nil S plots at the two yield responsive sites at Woburn and Docking (Tables 4-5). Applications of S significantly decreased the grain N:S ratio, whereas increasing N rate increased the ratio. Compared to these two S responsive sites, grain N:S ratios were much lower, and well below 17, for the nil S plots at the S-marginal site (Corsekelly) and the S-sufficient site (Bishop Middleham) (Tables 6-7). Both S and N treatments also had significant, but opposite, effects on grain N:S ratio at Corsekelly and Bishop Middleham, although the S effect was considerably smaller than that found at the two responsive winter barley sites.

Table 4. Effects of N and S applications and timing of S application on grain N and S concentrations, and grain N:S ratio (Woburn, 2003).

N applied (kg/ha)	S applied (kg/ha)	Grain N (%)	Grain S (mg/g)	Grain N:S ratio
110	0	1.87	0.92	20.3
110	10	1.72	1.36	12.7
110	20	1.70	1.43	11.9
110	40	1.68	1.44	11.7
150	0	2.10	0.94	22.5
150	10	2.14	1.49	14.3
150	20	2.10	1.61	13.1
150	40	2.15	1.65	13.0
ANOVA F prob.	*N*	*<0.001*	*<0.001*	*<0.001*
	S	*0.083*	*<0.001*	*<0.001*
	N x S	*0.015*	*0.003*	*0.513*
	Timing of S			
	Nil	2.10	0.94	22.5
	Mar-17	2.10	1.61	13.1
	Apr-15	2.09	1.56	13.4
	May-16	2.11	1.61	13.1
	ANOVA F Prob.	*0.979*	*<0.001*	*<0.001*
	LSD	*n/a*	*0.071*	*1.28*

Table 5. Effects of N and S applications and timing of S application on grain N and S concentrations, and grain N:S ratio (Docking, 2003).

N applied (kg/ha)	**S applied (kg/ha)**	**Grain N (%)**	**Grain S (mg/g)**	**Grain N:S ratio**
110	0	2.11	1.25	17.0
110	10	2.09	1.54	13.5
110	20	2.08	1.62	12.9
110	40	2.08	1.64	12.7
150	0	2.40	1.26	19.1
150	10	2.45	1.67	14.7
150	20	2.39	1.72	13.9
150	40	2.49	1.86	13.4
ANOVA F prob.	*N*	*<0.001*	*<0.001*	*<0.001*
	S	*0.564*	*<0.001*	*<0.001*
	N x S	*0.391*	*<0.001*	*0.055*
	Timing of S			
	Nil	2.40	1.26	19.1
	Mar-20	2.39	1.72	13.9
	Apr-23	2.36	1.75	13.5
	May-27	2.45	1.74	14.0
	ANOVA F Prob.	*0.191*	*<0.001*	*<0.001*
	LSD	*n/a*	*0.087*	*1.01*

Table 6. Effects of N and S applications and timing of S application on grain N and S concentrations, and grain N:S ratio (Corsekelly, 2003).

N applied (kg/ha)	S applied (kg/ha)	Grain N (%)	Grain S (mg/g)	Grain N:S ratio
70	0	1.61	1.20	13.4
70	10	1.54	1.20	12.9
70	20	1.50	1.19	12.6
70	40	1.52	1.22	12.5
120	0	1.97	1.34	14.7
120	10	2.06	1.45	14.2
120	20	1.99	1.41	14.1
120	40	1.95	1.41	13.8
ANOVA F prob.	*N*	*<0.001*	*<0.001*	*<0.001*
	S	*0.543*	*0.303*	*0.004*
	N x S	*0.499*	*0.313*	*0.971*
	Timing of S			
	Nil	1.97	1.34	14.7
	Apr-09	1.99	1.41	14.1
	May-09	2.01	1.45	13.9
	Jun-09	1.94	1.41	13.8
	ANOVA F Prob.	*0.791*	*0.148*	*0.071*
	LSD	*n/a*	*n/a*	*n/a*

Table 7. Effects of N and S applications and timing of S application on grain N and S concentrations, and grain N:S ratio (Bishop Middleham, 2003).

N applied (kg/ha)	S applied (kg/ha)	Grain N (%)	Grain S (mg/g)	Grain N:S ratio
80	0	1.33	1.10	12.1
80	10	1.29	1.12	11.5
80	20	1.25	1.11	11.3
80	40	1.30	1.17	11.4
120	0	1.41	1.14	12.4
120	10	1.46	1.17	12.5
120	20	1.43	1.18	12.1
120	40	1.42	1.16	12.0
ANOVA F prob.	*N*	*<0.001*	*0.005*	*<0.001*
	S	*0.678*	*0.212*	*0.011*
	N x S	*0.285*	*0.318*	*0.197*
	Timing of S			
	Nil	1.41	1.14	12.4
	May-20	1.43	1.18	12.1
	June-15	1.40	1.17	12.0
	June-25	1.45	1.19	12.2
	ANOVA F Prob.	*0.617*	*0.387*	*0.441*
	LSD	*n/a*	*n/a*	*n/a*

3.3.2. Experiments in 2004

Grain N concentrations of <1.8% were obtained in the samples from all treatments at Woburn (Table 8) and Corsekelly (Table 10), as well as the low N treatment at Bishop Middleham (Table 11). At Docking (Table 9), grain N concentrations were >1.8% in all samples except the N110/S20 treatment. Similar to the 2003 season, S applications decreased grain N concentration significantly only at Woburn, whereas increasing N rate increased it significantly at all sites.

The effects of S and N treatments on grain S concentration were similar to those observed in the previous season. At the two S responsive sites (Woburn and Docking), grain S concentration was increased significantly by S additions. Timing of S application also had a significant effect; delayed S application resulted in a higher S concentration in grain than the application in March (Tables 8-9). At the two S non-responsive sites (Corsekelly and Bishop Middleham), S additions had no significant effect on grain S concentration (Tables 10-11). At all sites, a higher rate of N application increased grain S concentration significantly.

Grain N:S ratios were 17.6 – 19.9 and 19.8 – 22.2 in the nil S treatments at Woburn and Docking, respectively, consistent with the large yield responses to S at these two sites. The ratio was markedly reduced by S applications. In contrast, grain N:S ratios were in the range of 13 – 16 at Corsekelly and Bishop Middleham, and S applications had no significant effect.

Table 8. Effects of N and S applications and timing of S application on grain N and S concentrations, and grain N:S ratio (Woburn, 2004).

N applied (kg/ha)	S applied (kg/ha)	Grain N (%)	Grain S (mg/g)	Grain N:S ratio
110	0	1.61	0.93	17.6
110	10	1.41	1.19	11.9
110	20	1.43	1.28	11.2
110	40	1.50	1.30	11.5
150	0	1.78	0.90	19.9
150	10	1.65	1.27	13.0
150	20	1.63	1.33	12.2
150	40	1.64	1.40	11.8
ANOVA F prob.	*N*	*<0.001*	*0.001*	*0.031*
	S	*0.015*	*<0.001*	*<0.001*
	N x S	*0.804*	*0.021*	*0.557*
	Timing of S			
	Nil	1.78	0.90	19.86
	Mar-23	1.63	1.33	12.25
	May-04	1.78	1.51	11.80
	May-19	1.75	1.40	11.09
	ANOVA F Prob.	*0.313*	*<0.001*	*<0.001*
	LSD	*n/a*	*0.090*	*3.100*

Table 9. Effects of N and S applications and timing of S application on grain N and S concentrations, and grain N:S ratio (Docking, 2004).

N applied (kg/ha)	**S applied (kg/ha)**	**Grain N (%)**	**Grain S (mg/g)**	**Grain N:S ratio**
110	0	1.85	0.93	19.8
110	10	1.87	1.41	13.2
110	20	1.76	1.36	12.9
110	40	1.83	1.45	12.6
150	0	2.08	0.94	22.2
150	10	2.12	1.44	14.8
150	20	2.02	1.41	14.3
150	40	2.10	1.52	13.8
ANOVA F prob.	*N*	*<0.001*	*0.383*	*0.002*
	S	*0.675*	*<0.001*	*<0.001*
	N x S	*0.998*	*0.974*	*0.797*
	Timing of S			
	Nil	2.08	0.94	22.2
	Mar-19	2.02	1.41	14.3
	Apr-16	2.13	1.59	13.4
	May-12	2.18	1.58	13.8
	ANOVA F Prob.	*0.452*	*<0.001*	*<0.001*
	LSD	*n/a*	*0.12*	*2.24*

Table 10. Effects of N and S applications and timing of S application on grain N and S concentrations, and grain N:S ratio (Corsekelly, 2004).

N applied (kg/ha)	S applied (kg/ha)	Grain N (%)	Grain S (mg/g)	Grain N:S ratio
70	0	1.38	1.00	13.7
70	10	1.35	0.97	13.9
70	20	1.40	1.06	13.2
70	40	1.34	1.03	13.1
120	0	1.69	1.05	16.3
120	10	1.65	1.17	14.1
120	20	1.67	1.15	14.5
120	40	1.66	1.09	15.2
ANOVA F prob.	*N*	*<0.001*	*0.001*	*<0.001*
	S	*0.615*	*0.279*	*0.201*
	N x S	*0.864*	*0.222*	*0.183*
	Timing of S			
	Nil	1.69	1.05	16.3
	Apr-04	1.67	1.15	14.5
	May-27	1.64	1.15	14.4
	Jun-30	1.59	1.20	13.2
	ANOVA F Prob.	*0.269*	*0.350*	*0.107*
	LSD	*n/a*	*n/a*	*n/a*

Table 11. Effects of N and S applications and timing of S application on grain N and S concentrations, and grain N:S ratio (Bishop Middleham, 2004).

N applied (kg/ha)	**S applied (kg/ha)**	**Grain N (%)**	**Grain S (mg/g)**	**Grain N:S ratio**
70	0	1.77	1.23	14.4
70	10	1.75	1.23	14.2
70	20	1.79	1.22	14.6
70	40	1.77	1.27	13.9
120	0	1.96	1.31	15.0
120	10	2.02	1.35	14.9
120	20	1.95	1.26	15.4
120	40	1.99	1.33	14.9
ANOVA F prob.	*N*	*<0.001*	*<0.001*	*<0.001*
	S	*0.937*	*0.158*	*0.145*
	N x S	*0.279*	*0.435*	*0.864*
	Timing of S			
	Nil	1.96	1.31	15.0
	May-20	1.95	1.26	15.4
	Jun-15	2.00	1.35	14.8
	Jun-25	1.97	1.36	14.5
	ANOVA F prob.	*0.844*	*0.320*	*0.491*
	LSD	*n/a*	*n/a*	*n/a*

3.4. Grain size

Grain size was not determined on the samples from Docking in 2003, because the samples contained rather high levels of impurity (stones and chaff etc). Thousand grain weight (TGW), specific weight and grain size distribution varied between sites and seasons (Tables 12 and 13). Applications of S significantly decreased TGW and specific weight, and increased the proportion of small grain (<2.25 mm) at Woburn in the 2003 trial (Table 12). Similar effects were observed at Docking in the 2004 trial, although the effect on TGW was not significant (Table 13). Sulphur applications had no significant effects on these grain measurements in all other trials that were determined, including the yield responsive site at Woburn in 2004. The two N rates also produced significant differences in some of the trials. Increasing N rate resulted in a significant decrease in TGW in four of the seven trials tested, and a significant increase in the proportion of small grain (<2.25 mm) in five of the seven trials tested. Grain specific weight was significantly increased by N in two trials (Bishop Middleham in 2003 and Woburn in 2004), and significantly decreased by N in one trial (Woburn in 2003), although overall these effects were small.

Table 12. Effects of S and N on thousand grain weight (TGW), % of small grain and specific weight of barley grain in 2003.

Site	N (kg/ha)	S (kg/ha)	TGW (g)	Small grain (1-2.25mm, %)	Specific weight (kg/hl)
Woburn	110	0	40.1	2.7	67.9
	110	10	35.5	7.0	65.0
	110	20	34.3	7.4	64.5
	110	40	35.7	7.5	64.7
	150	0	39.7	3.2	67.8
	150	10	33.0	12.3	62.3
	150	20	32.3	12.6	63.3
	150	40	32.2	10.6	63.1
	ANOVA *F* prob.	N	0.002	<0.001	0.003
		S	<0.001	<0.001	<0.001
		NxS	0.344	0.084	0.226
Corsekelly	70	0	38.6	5.0	70.3
	70	10	38.4	5.4	70.5
	70	20	38.3	5.3	70.5
	70	40	38.3	5.4	70.6
	120	0	36.2	9.7	71.0
	120	10	35.9	10.9	70.3
	120	20	36.3	10.0	70.9
	120	40	37.6	8.9	70.5
	ANOVA *F* prob.	N	<0.001	<0.001	0.314
		S	0.629	0.407	0.779
		NxS	0.507	0.500	0.372
Bishop Middleham	80	0	44.6	1.1	67.9
	80	10	43.7	1.1	68.5
	80	20	43.5	1.1	67.8
	80	40	43.9	1.1	67.8
	120	0	43.8	1.3	69.0
	120	10	44.3	1.3	69.2
	120	20	44.3	1.2	68.9
	120	40	44.3	1.3	69.0
	ANOVA *F* prob.	N	0.322	0.012	<0.001
		S	0.911	0.619	0.256
		NxS	0.176	0.857	0.631

Table 13. Effects of S and N on thousand grain weight (TGW), % of small grain and specific weight of barley grain in 2004.

Site	N (kg/ha)	S (kg/ha)	TGW (g)	Small grain (1-2.25mm, %)	Specific weight (kg/hl)
Woburn	110	0	45.2	1.8	69.0
	110	10	45.0	1.3	69.1
	110	20	44.6	1.5	68.9
	110	40	44.4	1.5	68.9
	150	0	44.4	1.7	69.1
	150	10	45.7	1.5	69.6
	150	20	44.6	1.8	69.2
	150	40	45.1	1.6	69.3
	ANOVA *F* prob.	N	0.560	0.220	0.043
		S	0.311	0.438	0.379
		NxS	0.030	0.881	0.904
Docking	110	0	39.3	3.3	66.2
	110	10	35.8	10.7	64.0
	110	20	38.9	6.5	65.1
	110	40	38.9	8.8	64.9
	150	0	40.1	2.6	66.9
	150	10	35.7	16.0	63.8
	150	20	37.2	12.8	64.1
	150	40	38.0	9.7	65.3
	ANOVA *F* prob.	N	0.647	0.125	0.984
		S	0.085	0.006	0.004
		NxS	0.842	0.488	0.627
Corsekelly	70	0	35.0	7.7	64.4
	70	10	35.2	6.1	64.2
	70	20	35.3	6.4	64.0
	70	40	35.2	5.8	64.3
	120	0	33.8	10.0	63.8
	120	10	33.7	10.6	63.8
	120	20	33.6	10.1	64.1
	120	40	34.2	12.4	63.4
	ANOVA *F* prob.	N	<0.001	<0.001	0.187
		S	0.883	0.767	0.967
		NxS	0.819	0.158	0.791

Table 13 (continued)

Site	N (kg/ha)	S (kg/ha)	TGW (g)	Small grain (1-2.25mm, %)	Specific weight (kg/hl)
Bishop Middleham	70	0	39.0	3.8	64.8
	70	10	39.3	3.5	65.1
	70	20	38.5	4.0	63.6
	70	40	38.9	3.4	64.6
	120	0	37.3	4.6	64.6
	120	10	36.9	4.7	63.5
	120	20	38.0	4.3	64.5
	120	40	37.4	4.6	64.8
	ANOVA *F* prob.	N	0.004	<0.001	0.645
		S	0.997	0.705	0.530
		NxS	0.573	0.036	0.129

3.5. Malting quality

Malting quality data are shown in Tables 14 and 15 for the 2003 and 2004 trials, respectively. A number of malting quality parameters showed significant responses to S at the yield responsive sites at Woburn in both seasons and at Docking in 2004. Samples from Docking in 2003 were not suitable for malting due to high concentrations of N in the grain. At Woburn in 2003, S application at 20 kg/ha significantly increased germinative energy (tested with 4 ml water), soluble N ratio in wort, diastatic power, α-amylase activity and friability of malt, but significantly decreased malt total N concentration, the percentage of whole corns, wort β-glucan content and free amino N concentration in wort (Table 14). The grain samples showed a high water sensitivity (tested with 8 ml water), but this was not affected significantly by S. Other parameters not influenced significantly by S included coarse extract and predicted spirit yield. The concentrations of the DMS (dimethylsulphide) precursor and glycosidic nitrile were not determined for the Woburn samples from the 2003 trial.

At Woburn in 2004, S application significantly decreased wort colour and wort β-glucan content, but significantly increased diastatic power, friability and homogeneity, as well as the concentration of the DMS precursor in wort (Table 15). There was little difference between the two different timings of S application. At Docking in 2004, S application significantly decreased wort β-glucan content, but significantly increased germinative energy (4 ml water test), diastatic power, friability, homogeneity and the concentration of the DMS precursor in wort (Table 15). At both Woburn and Docking, coarse extract and the predicted spirit yield were not significantly influenced by the S treatment. At both sites, coarse extracts were generally low (<76%), probably as a result of rather high grain N concentrations.

For the trial at Woburn in 2004, the S0 samples were from the low N treatment, while the S20 samples from the high N treatment. Thus, the N treatment became a confounding factor. However, because the concentrations of N in grain and malt were similar between the three treatments selected for the micro-malting tests (Tables 8 and 15), the observed effects were most likely due to S rather than N. Also, the observed effects were generally consistent with those seen in the trials at Woburn in 2003 and at Docking in 2004.

For spring barley at Corsekelly and Bishop Middleham in 2003 (Table 14), S application had no significant effect on all quality parameters, except a small but significant decrease in wort free amino N (Corsekelly) and unboiled fermentability (Bishop Middleham). The general lack of a response to S at these two sites is consistent with them being sufficient or only marginally deficient in S.

Table 14. Effects of S on barley malting quality in the trials at Woburn, Corsekelly and Bishop Middleham in 2003

	Woburn [a] (cv Pearl)			Corsekelly b (cv Optic)			Bishop Middleham [b] (cv Optic)		
	S0	S20	Sig.[c]	S0	S20	Sig.	S0	S20	Sig.
Germinative energy (4ml, %)	94.0	96.8	*	97.8	99.3	ns	98.7	98.2	ns
Germinativ energy (8ml, %)	50.5	72.0	ns	ND [d]	ND		ND	ND	
Germination capacity (peroxide, %)	ND	ND		99.3	99.5	ns	99.8	99.0	ns
IoB Coarse extract (% DW)	77.6	77.8	ns	77.7	78.3	ns	78.7	78.9	ns
Wort colour (degree EBC)	ND	ND		2.7	3.0	ns	2.7	2.8	ns
Malt total N (%)	1.80	1.63	*	1.61	1.46	ns	1.42	1.41	ns
Wort soluble N (% DW)	0.58	0.58	ns	0.60	0.58	ns	0.54	0.56	ns
Soluble N ratio (%)	32.2	35.4	*	37.8	39.5	ns	38.3	39.5	ns
Diastatic power (Degree IoB, DW)	81	124	***	98	91	ns	86	88	ns
α-Amylase (DU)	32	37	**	50	53	ns	55	54	ns
Friability (%)	81.4	93.9	*	84.5	92.0	ns	97.0	97.3	ns
Homogeneity (%)	95.7	99.9	ns	98.25	99.5	ns	99.5	99.0	ns
Whole corns (%)	0.7	0.1	*	0.8	0.2	ns	0.2	0.2	ns
Unboiled fermentability (%)	88.9	88.7	ns	87.3	87.2	ns	87.9	87.7	*
Predicted spirit yield (l/t, DW)	418	419	ns	411	415	ns	419	419	ns
IoB Wort β-glucan (mg/l)	223	57	***	39	40	ns	45	42	ns
IoB Wort free amino N (mg/l)	131	108	*	136	130	**	120	127	ns
DMS precursor (mg/kg)	ND	ND		7.9	6.2	ns	6.4	6.8	ns
Glycosidic nitrile (ppb extract)	ND	ND		219	239	ns	253.3	257.3	ns

[a] Tested by Muntons; [b] Tested by Bairds. [c] Significance: ns, not significant; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. [d] ND, not determined.

Table 15. Effects of S on barley malting quality in the trials at Woburn and Docking in 2004

	Woburn (cv Pearl) [a]				Docking (cv Pearl) [a]		
	S0[a]	S20[a]	S20 late Application [b]	Sig.[b]	S0	S20	Sig.[c]
Germination energy (4ml, %)	98.3	99.0	98.3	ns	96.0	98.0	*
Germination energy (8ml, %)	41.0	47.0	50.5	ns	50.3	59.0	ns
Germination capacity (peroxide, %)	98.3	99.1	99.1	ns	97.8	98.9	ns
IoB Coarse extract (% DW)	75.9	75.8	75.3	ns	73.9	74.8	ns
Wort colour (degree EBC)	3.3	3.0	2.9	*	3.3	3.0	ns
Malt total N (%)	1.71	1.73	1.84	ns	1.88	1.76	ns
Wort soluble N (% DW)	0.57	0.61	0.58	ns	0.66	0.65	ns
Soluble N ratio (%)	33.1	34.9	31.5	ns	35.1	37.2	ns
Diastatic power (Degree IoB, DW)	78	125	129	***	82	119	***
α-Amylase (DU)	39.3	40.8	43.3	ns	39.5	45.5	**
Friability (%)	75.5	84.0	80.3	*	66.0	83.3	**
Homogeneity (%)	94.8	98.3	97.3	*	92.0	97.5	*
Whole corns (%)	1.4	0.4	0.8	ns	2.5	1.3	ns
Unboiled fermentability (%)	86.8	86.8	87.2	ns	86.7	87.3	ns
Predicted spirit yield (l/t DW)	399	399	398	ns	388	396	ns
IoB Wort β-glucan (mg/l)	222	160	158	**	160	85	**
IoB Wort free amino N (mg/l)	139	135	125	ns	142	132	ns
DMS precursor (mg/kg)	2.2	8.1	8.1	**	2.9	7.3	**
Glycosidic nitrile (ppb extract)	264	337	318	ns	262	343	ns

[a] Tested by Bairds.

[b] The S0 samples were from the N110 treatment and the S20 samples from the N150 treatment.

[c] Significance: ns, not significant; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

3.6. Leaf tissue analysis

Yield response data showed that Woburn and Docking were deficient in S, and Bishop Middleham not deficient, in both seasons. Corsekelly was marginally deficient only in the 2003 season and not deficient in 2004. Leaf tissues were sampled at the tillering and stem extension – flag leaf stages to evaluate whether tissue analysis can be used to diagnose S deficiency in barley. Tissue analyses included total S and sulphate-S concentrations, N:S ratio and malate:sulphate ratio.

3.6.1. Tissue analysis data for the nil S treatments

Data for the nil S treatments are shown in Tables 16 and 17 for the tillering and stem extension – flag leaf stages, respectively. The usual critical values for cereals (Withers et al., 1997; Blake-Kalff et al., 2000) are also included for comparison. Bishop Middleham was not sampled at the tillering stage in 2004, whereas leaf samples from all nil S plots of the two N rates at Docking were bulked into a single sample.

At the tillering stage, leaf total S concentration with a critical value of 2.5 mg/g correctly identified Woburn in both seasons, and Docking in 2003, as S-deficient sites (Table 16). However, this indicator misdiagnosed Docking in 2004 and Corsekelly in 2003 as S-sufficient, when both were either severely or marginally deficient in S. When leaf sulphate-S concentration with a critical value of 0.25 mg/g was used, only Woburn in 2004 was identified as S-deficient. Leaf N:S ratios were low at all four sites in 2003, and therefore did not identify any of the S-deficient sites. In 2004, leaf N:S ratio correctly identified Woburn as being S-deficient, but missed the other S-deficient site at Docking. With the malate:sulphate ratio, Woburn and Docking were diagnosed as S-deficient in both seasons. However, this ratio over-predicted S deficiency, identifying Bishop Middleham (the high N level) in 2003 and Corsekelly in 2004 as S-deficient when they did not show yield responses. In contrast, the marginally deficient site at Corsekelly in 2003 was not identified with the malate:sulphate ratio.

At the stem extension – flag leaf stage (Table 17), leaf total S concentration correctly identified both S-deficient sites in 2004, but failed to identify the S-deficient (Docking) or marginally deficient (Corsekelly) sites in 2003. Leaf sulphate-S concentration predicted S-deficient sites reasonably well in both seasons, with the exception of Woburn at the high N rate in 2004 and the marginally deficient site at Corsekelly in 2003. The samples from Woburn and Docking in both seasons showed N:S ratios approaching or exceeding 17:1, although this indicator also did not identify the marginally deficient site at Corsekelly in 2003. The malate:sulphate ratio identified all S responsive sites in both seasons. However, this ratio also incorrectly identified Bishop Middleham (high N treatment) as S-deficient.

Table 16. Total S and SO_4-S concentrations, N:S and M:S ratios in leaves from the nil S plots at the tillering stage

Season	Site	N rate (kg/ha)	Total S (mg/g)	SO4-S (mg/g)	N:S ratio	M:S ratio
2003	Corsekelly	70	4.7	0.91	13.2	1.1
		120	4.8	1.06	13.9	0.9
	Bishop Middleham	80	3.9	0.77	12.3	1.1
		120	4.0	0.56	13.6	**2.5**
	Docking	Bulked	**2.0**	0.32	13.3	**1.8**
	Woburn	110	**1.9**	0.34	12.8	**6.5**
		150	**2.0**	0.31	12.3	**6.9**
2004	Corsekelly	70	3.1	0.53	12.9	**2.3**
		120	3.1	0.56	12.9	**1.9**
	Bishop Middleham	Not sampled				
	Docking	Bulked	2.9	0.40	13.0	**1.7**
	Woburn	110	**2.3**	**0.03**	**25.9**	**93.6**
		150	**2.5**	**0.12**	**25.6**	**12.6**
Critical value		*n/a*	2.5	0.25	>17	>1.5

Values in bold indicate that the samples were S-deficient according to the critical values used.

Table 17. Total S and SO_4-S concentrations, N:S and M:S ratios in leaves from the nil S plots at the stem extension – flag leaf stage

Season	Site	N rate (kg/ha)	Total S (mg/g)	SO4-S (mg/g)	N:S ratio	M:S ratio
2003	Corsekelly	70	3.2	0.42	12.8	1.5
		120	3.3	0.27	13.5	**2.7**
	Bishop Middleham	80	2.9	0.80	10.2	1.4
		120	2.8	0.51	12.4	**2.9**
	Docking	110	3.0	**0.24**	16.6	**9.3**
		150	2.9	**0.19**	**17.6**	**12.7**
	Woburn	110	**2.3**	**0.07**	**17.0**	**20.9**
		150	**2.5**	**0.11**	**17.3**	**18.1**
2004	Corsekelly	70	2.9	0.51	13.5	**1.9**
		120	3.0	1.11	15.2	0.5
	Bishop Middleham	70	4.6	1.52	8.3	0.6
		120	5.3	2.22	8.2	0.4
	Docking	110	**1.8**	**0.09**	16.7	**30.4**
		150	**2.0**	**0.17**	**17.8**	**20.5**
	Woburn	110	**1.5**	**0.19**	**22.1**	**29.6**
		150	**1.5**	0.36	**26.1**	**7.2**
Critical value		*n/a*	2.5	0.25	>17	>1.5

Values in bold indicate that the samples were S-deficient according to the critical values used.

3.6.2. Responses in leaf S concentration to S treatments

The effect of S additions on leaf S concentration indicates the extent of fertiliser S being utilised by the crop. Increasing N rate tended to increase leaf S concentration. However, there were no significant interactions between N and S treatments on leaf S concentration. For this reason, means of the two N rates at each site are presented in Figure 5. It is clear that S additions increased leaf S concentration at the stem extension or flag leaf stage more markedly at Woburn and Docking than at Corsekelly and Bishop Middleham. For example, an application of 20 kg S/ha increased leaf S concentration by 70, 63, 9 and 22% at Woburn, Docking, Corsekelly and Bishop Middleham, respectively, in 2003. The corresponding increases were 118, 107, 32 and 1% in 2004. Little of the applied S was taken up by the crop at Bishop Middleham in 2004. There may be three reasons for these results. Firstly, S was applied later for the spring barley trials than for the winter barley trials (Table 1), thus allowing shorter time for S being taken up by spring barley than by winter barley. Second, it is also likely that S may have been applied to spring barley during a dry period, which means that the fertiliser may remain on the soil surface. Finally, S-starved plants such as those at Woburn and Docking tend to take up more S when the nutrient is provided.

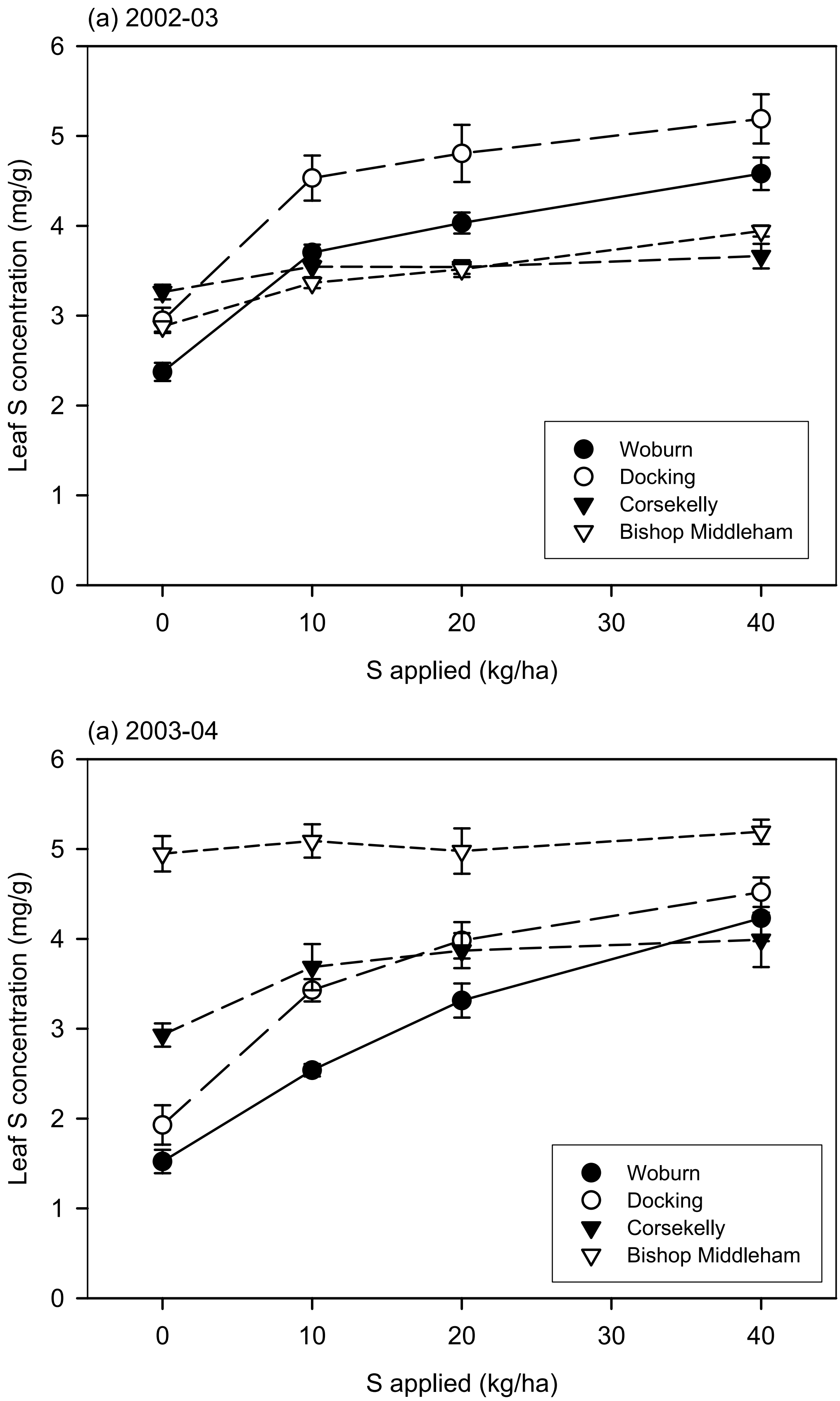

Figure 5. Effect of S additions on leaf S concentration at the stem extension or flag leaf stage in 2003 (a) and 2004 (b). Data are means of the two N rates at each site.

4. Discussion

4.1. Yield response to S and prediction of S deficiency

Of the eight experiments conducted in the two seasons, five showed significant yield responses to S additions. Woburn and Docking were highly responsive in both seasons, whereas Corsekelly was marginally deficient in 2003 and Bishop Middleham was not responsive in either season. At both Woburn and Docking, yield was increased by S by approximately 0.4 and 1.2 t/ha in 2003 and 2004, respectively. The 2004 season was clearly more responsive than the previous season, probably because of a larger rainfall during the growth season in 2004. At Corsekelly, yield was increased by S by a moderate 0.22 t/ha in 2003, and this was achieved only from the higher doses of S application (20 and 40 kg/ha). In general, responses to S tended to be larger with a higher rate of N application, although the interactions between N and S were not statistically significant.

The dose of S required to produce the maximum yield varied between different sites. For Woburn, 10 kg/ha of S was sufficient in both seasons, whereas 20 kg/ha was required for Docking and Corsekelly. Sulphur was applied as gypsum in this study. Other fertilisers in the form of sulphate are expected to give a similar efficacy. In terms of timing, the optimal window of S application appears to be between mid-March to mid-April for winter barley. An application of S in May did not fully prevent yield losses due to S deficiency in three out of the four experiments at Woburn and Docking in the two seasons. For spring barley, different timings of S application produced no conclusive results, because only one out of the four trials showed a moderate yield response. However, it is reasonable to suggest that S should be applied at sowing or an early growth stage for spring barley.

At a grain price of £70/t, yield responses of 0.2 – 1.2 t/ha increase output by £14 – 84/ha. The cost of S fertiliser is not well known, but is likely to be similar to that of N, i.e. about 40 pence per kg S. The cost of S fertiliser applied at 10 – 20 kg/ha would be £4 – 8/ha. Therefore, the cost to benefit ratio is favourable to the use of S fertiliser under S deficiency conditions, particularly for the more deficient sites like Woburn and Docking. To reduce the cost of fertiliser, it may be justifiable to apply only 10 kg S/ha because of the diminished return for any extra S.

Both Woburn and Docking are light sandy loam soils, containing low organic matter (organic C around 1% or below, Table 3). In contrast, the soils at Corsekelly and Bishop Middleham had relatively high organic matter contents (>3% organic C). The five S-responsive experiments shared one thing in common, i.e. low concentrations of soil extractable S (2.8 – 4.1 mg/kg). In contrast, the three S non-responsive trials had soil extractable S in the range of 6.4 – 14 mg/kg. The results from this series of trials suggest that soil analysis did provide useful information concerning available S, with a threshold value of around 4 – 5 mg/kg. Timing of soil sampling and method of S analysis influence the threshold value (McGrath et al., 1996). This threshold is for soils sampled in spring and the S in the extracts analysed by inductively-coupled plasma atomic emission spectroscopy. It should be emphasised that only a limited number of sites (four) were used in this study, although different fields were used in different seasons. Previous work showed that soil analysis could not satisfactorily distinguish yield responsive from non-responsive sites for cereals (Withers *et al.*, 1997).

A possible contributing factor to the greater responsiveness at Docking and Woburn is that winter barley was grown, compared to the spring crops grown at the other two sites. Autumn sown crops may be more responsive to S than spring sown ones, as was observed for oilseed rape crops (Chalmers et al., 1998). However, no firm conclusion can be drawn here because winter and spring barley were not grown at the same sites.

Leaves were sampled at the tillering and stem extension or flag leaf stages and analysed for total S, sulphate-S, N:S ratio and malate:sulphate ratio. These diagnostic indicators were compared for their accuracy in predicting yield responses to S. None of the indicators predicted yield responses at 100% accuracy. Leaf N:S ratio and sulphate-S concentration, measured at stem extension or the flag leaf stage, appeared to be quite reliable at distinguishing the two most responsive sites from the rest, although the threshold value for N:S ratio should probably be lowered to 16:1 from the more commonly used value of 17:1. However, these two indicators were not reliable at the tillering stage when crop was still very small. With the threshold value of 1.5, leaf malate:sulphate ratio tended to over-predict S deficient sites. However, it is not obvious whether the threshold value can be increased to avoid over-prediction yet without missing the real S-deficient sites. By definition, diagnosis provides information of the nutritional status at the time of sampling. Using diagnostic data to predict final outcome in grain yield is not straight forward, because this relies on the assumption that deficiency is persistent rather than growth phase-dependent. Obviously, the assumption is not true in all cases; some crops may overcome the initial deficiency by exploring the S supply from subsoil, whereas other crops may run into S deficiency during a later phase of crop growth because available S is being depleted. Also, an S-deficient crop may not show a significant yield response to S addition, if other factors (e.g. drought) were in fact limiting yield.

Grain N:S ratio in wheat has also been used to indicate whether the crop was deficient in S (Randall *et al.*, 1981). The results from previous trials with wheat and barley in the UK were mixed (Withers et al., 1997; McGrath et al., 1999). Grain N:S ratios in barley were above 17:1 at Woburn and Docking in both seasons, and considerably lower than this value at the other two sites. Based on the limited number of trials in this project, the ratio does appear to separate S-deficient sites from those that are S-sufficient or marginally deficient. The ratio was influenced by both N and S treatments. However, the N effect was not large enough to invalidate its usefulness. Also, its use is retrospective and only useful for decisions concerning fertiliser use on following crops.

4.2. Effect of S on grain and malting quality

Sulphur applications produced little significant effect on grain and malting quality at Corsekelly and Bishop Middleham. This is not surprising because the two sites were not or were only marginally deficient in S, and S applications did not significantly increase grain S concentration.

In contrast, S applications significantly influenced a number of grain and malting quality parameters at Docking and Woburn, the two S-responsive sites. On the positive side, S applications clearly improved endosperm modification during malting, resulting in a higher malt friability and homogeneity, and a lower β-glucan content in the wort. The effect on wort β-glucan content was one of the most noticeable; grain from the +20 kg S/ha treatment produced malt with 30 – 75% lower β-glucan content than those from the nil S treatment. High contents of β-glucan are undesirable because of the adverse effect on beer filtration rate, and thus the brewing house performance (Palmer, 1989; Fox et al., 2003). For malting purposes, the level of β-glucan in wort (derived using the IoB extraction method) should be <120 mg/L. This value was exceeded in the samples from the nil S plots at Woburn in both seasons and at Docking in 2004, and S applications brought wort β-glucan content to well below 120 mg/L in two of these three experiments. The improved modification in the +S samples was probably a result of higher enzyme activities. Diastatic power was increased by S by 45 – 60%. The activity of α-amylase was also increased by S, significantly in two of the three sample sets from Woburn and Docking.

Another noticeable effect of S was increased concentrations of the DMS precursor (S-methylmethionine, or SMM). SMM is produced from methylation of methionine during germination, part of which is degraded during kilning. The SMM remaining in the malt breaks down to DMS during brewing; the latter is a major flavour attribute of some lager-type beers (Bamforth and Barclay, 1993). Some lager beers require certain levels of DMS levels (30 – 60 μg/L) for their flavour characteristic, whereas in other lager beers DMS is maintained at as low level as possible. At Woburn and Docking, the concentration of DMS precursor in the malt samples from the 20 kg S/ha treatment was 2.5 – 3.7 times greater than that of the nil S treatment. These results indicate that S application to S-deficient malting barley crop could have a significant impact on the flavour of beer, depending on the type of beer and brewer.

At Woburn, S applications significantly decreased grain N concentration and, thus, protein content. This effect was observed at both N levels in 2004, but only at the low N level in 2003. Sulphur applications had no significant effect on grain N concentration at Docking. These results suggest that the S effect was probably due to a dilution of N in grain as a result of increased yield. The dilution effect would occur at sites where the N supply was limiting, e.g. at Woburn. At Docking, N supply was not liming, as can be seen from a general lack of yield response to N levels and also high grain N concentrations in both seasons. The decreased grain N and protein content in response to S is beneficial for malting quality, and may lead to a higher premium. High protein content is undesirable because of the strong inverse correlation between protein and carbohydrate contents; thus a high protein content leads to a low malt extract (Bishop, 1930).

However, S applications also produced some negative effects on malting quality. The most noticeable were decreased grain size and decreased thousand grain weight (TGW), although it is worth stressing that these effects were not observed in all of the S-responsive trials. Increasing N rate also produced similar effects on grain size and TGW. At the same protein content, a lower TGW would lead to a lower malt extract, because smaller grain contain less carbohydrate (Bishop, 1948). Bishop (1948) established that malt extract could be predicted from grain N concentration and TGW. When the data for Woburn and Docking in 2004 were combined, which were of the same barley cultivar and the micro-malting tests were performed by the same laboratory, the following regression equation was obtained:

Coarse extract = 76.7 - 4.4 x Grain N% + 0.15 x TGW (n=20, $R^2_{adjusted}$=0.82, p<0.001)

Thus, the effects of S on both grain N concentration and TGW appear to have cancelled each other out in terms of influencing the malt extract. This would explain the lack of a significant effect of the S treatment on malt extract and predicted spirit yield in experiments such as Woburn in 2003.

5. Conclusions

5.1. Significant yield responses to S additions were obtained in five out of the eight experiments conducted at four different sites in two seasons, with yield increases ranging from 0.2 to 1.2 t/ha.

5.2. Depending on the experimental site, the rate of S addition to achieve the maximum yield varied between 10 and 20 kg S/ha. Sulphur should be applied to winter barley between mid-March to mid-April.

5.3. The S-responsive experiments were associated with a soil extractable S in the range of 2.8 – 4.1 mg/kg, whereas the non-responsive trials had soil extractable S in the range of 6.4 – 14 mg/kg.

5.4. None of the leaf tissue indicators could predict yield responses with 100% accuracy at either tillering or the stem extension – flag leaf stage. Leaf N:S ratio and sulphate-S concentration, measured at the stem extension – flag leaf stage, appeared to be quite reliable at distinguishing the two most responsive sites from the rest. Grain N:S ratio at 17:1 also separated the two most responsive sites from the other sites.

5.5. At the two S-deficient sites, S application significantly increased malt diastatic power, α-amylase activity, friability and homogeneity, indicating an improved endosperm modification during malting. Sulphur applications also significantly decreased β-glucan concentration in the wort.

5.6. At the two S-deficient sites, S application significantly increased the concentration of the DMS precursor in the wort, which is expected to have an impact on the flavour of beer, depending on the type of beer and brewer.

5.6. When the N supply was limiting, S applications decreased grain N concentration due to a dilution effect as a result of increased grain yield. However, no significant effect was observed when N was not limiting.

5.7. Sulphur applications tended to decrease grain size, with increased proportion of small grain (<2.25 mm) and decreased thousand grain weight. Increasing N rate also produced similar effects.

5.8. At S non-deficient or marginally deficient sites, as measured by yield responsiveness, S applications had little effect on grain or malting quality parameters.

6. Acknowledgements

We thank Adrian Crosland, Ruth Skilton, Xiaoyun Zhou and Phil Cundill at Rothamsted Research for assistance in analysis. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council.

7. References

Bamforth, C.W., Barclay, A.H.P., 1993. Malting technology and the uses of malt. In: MacGregor, A.W., Bhatty, R.S. (Eds.), Barley: Chemistry and Technology. American Association of Cereal Chemists, St. Paul, Minnesota, USA, pp. 297-354.

Bishop, L.R., 1930. The nitrogen content and quality of barley. Journal of Institute of Brewing 36, 352-369.

Bishop, L.R., 1948. The prediction of extract. IV. The adjustment of prediction to give true extract in malt. Journal of Institute of Brewing 54, 330-333.

Blake-Kalff, M.M.A., Hawkesford, M.J., Zhao, F.J., McGrath, S.P., 2000. Diagnosing sulfur deficiency in field-grown oilseed rape (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). Plant and Soil 225, 95-107.

Blake-Kalff, M.M.A., Zhao, F.J., McGrath, S.P., Withers, P.J.A., 2004. Development of the malate:sulphate ratio test for sulphur deficiency in winter wheat and oilseed rape. HGCA Project Report No. 327. Home-Grown Cereals Authority, London.

Chalmers, A.G., Sinclair, A.H., Carver, M., 1999. Nutrients other than nitrogen, phosphorus and potassium for cerelas. HGCA Research Review 41. Home-Grown Cereals Authority, London.

Chalmers, A.G., Fisher, N., Hartley, A.R., Walker, K.C., Zhao, F.J., McGrath, S.P., 1998. Amount and form of sulphur fertiliser required to prevent sulphur deficiency in spring oilseed rape. HGCA Oilseeds Project Report: OS34. Home-Grown Cereals Authority, London.

Fox, G.P., Panozzo, J.F., Li, C.D., Lance, R.C.M., Inkerman, P.A., Henry, R.J., 2003. Molecular basis of barley quality. Australian Journal of Agricultural Research 54, 1081-1101.

IoB, 2000. Institute of Brewing Methods of Analysis, Volume 1 Analytical. 1997 Issue updated 2000.

McGrath, S.P., Zhao, F.J., 1995. A risk assessment of sulfur deficiency in cereals using soil and atmospheric deposition data. Soil Use and Management 11, 110-114.

McGrath, S.P., Zhao, F.J., Withers, P.J.A., 1996. Development of sulphur deficiency in crops and its treatment. Proceedings No. 379. The Fertiliser Society, Peterborough.

McGrath, S.P., Zhao, F.J., Blake-Kalff, M.M.A., 2002. History and outlook for sulphur fertilisers in Europe. Proceedings No. 497. International Fertiliser Society, York, U.K.

McGrath, S.P., Zhao, F.J., Withers, P.J.A., Evans, E.J., Monaghan, J.M., Salmon, S.E., Shewry, P.R., 1999. Yield and breadmaking quality responses of winter wheat to sulphur fertiliser. HGCA Project Report No. 197. Home-Grown Cereals Authority, London, pp. 85.

Palmer, G.H., 1989. Cereals in malting and brewing. In: Palmer, G.H. (Ed.), Cereal Science and Technology. Aberdeen University Press, Aberdeen, pp. 61-242.

Randall, P.J., Spencer, K., Freney, J.R., 1981. Sulfur and nitrogen fertilizer effects on wheat.1. Concentrations of sulfur and nitrogen and the nitrogen to sulfur ratio in grain, in relation to the yield response. Australian Journal of Agricultural Research 32, 203-212.

Shewry, P.R., Franklin, J., Parmar, S., Smith, S.J., Miflin, B.J., 1983. The effects of sulphur starvation on the amino acid and protein compositions of barley grain. Journal of Cereal Science 1, 21-31.

Withers, P.J.A., Tytherleigh, A.R.J., Odonnell, F.M., 1995a. Effect of sulphur fertilizers on the grain yield and sulphur content of cereals. Journal of Agricultural Science 125, 317-324.

Withers, P.J.A., Evans, E.J., Bilsborrow, P.E., Milford, G.F.J., McGrath, S.P., Zhao, F., Walker, K.C., 1995b. Development and prediction of sulphur deficiency in winter oilseed rape. Home-Grown Cereals Authority, London.

Withers, P.J.A., Chalmers, A.G., Hodgson, I.H., Kane, R., McGrath, S.P., Zhao, F.J., Sinclair, A.H., Evans, E.J., Bilsborrow, P.E., 1997. Diagnosis and prediction of sulphur deficiency in winter wheat and barley. Project Report No. 142. Home Grown Cereal Authority, London.

Zhao, F.J., McGrath, S.P., Blake-Kalff, M.M.A., Link, A., Tucker, M., 2002. Crop responses to sulphur fertilisation in Europe. Proceedings No. 504. International Fertiliser Society, York.

Zhao, F.J., Salmon, S.E., Withers, P.J.A., Evans, E.J., Monaghan, J.M., Shewry, P.R., McGrath, S.P., 1999a. Responses of breadmaking quality to sulphur in three wheat varieties. Journal of the Science of Food and Agriculture 79, 1865-1874.

Zhao, F.J., Salmon, S.E., Withers, P.J.A., Monaghan, J.M., Evans, E.J., Shewry, P.R., McGrath, S.P., 1999b. Variation in the breadmaking quality and rheological properties of wheat in relation to sulphur nutrition under field conditions. Journal of Cereal Science 30, 19-31.