THE EFFECT OF SITE, FOLIAR SULPHUR AND NITROGEN APPLICATION ON GLUCOSINOLATE CONTENT AND YIELD OF OILSEED RAPE (*Brassica Napus* L.)

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INTRODUCTION

Sulphur requirement by oilseed rape compared to other crops is high; an oilseed rape crop removes 20-30 kg S ha^{-1} in the seed whereas a cereal crop removes 5-15 kg S ha^{-1} (Klessa and Sinclair, 1989). A general decline in atmospheric pollution has led to a sharp reduction in atmospheric deposition of sulphur in the UK and a change in fertilizer use to those that contain negligible amounts of sulphur has occurred. As a result, sulphur supply has been reduced and sulphur deficiency has been observed more frequently in Scotland and in the UK.

A survey of 125 farms in northern Scotland found that 46% of soils tested were low in sulphate (Scott and Munro, 1979). The amount of sulphur deposited from the atmosphere in north-east Scotland is the lowest in the UK at less than 15 kg S ha^{-1} $year^{-1}$, with under half of this in the summer growing period (Martin, 1980). Trials have shown substantial yield responses to sulphur fertilizer application on oilseed rape in north-east Scotland (Anon, 1984; 1985) and elsewhere, eg. Nuttall *et al.*(1987); Schnug (1991a). Sulphur application for oilseed rape, where the soil sulphur status is low (under 6 mg $litre^{-1}$) or moderate (6-11 mg $litre^{-1}$) is recommended by The Scottish Agricultural College. However, increasing the amount of sulphur which is available to the plant has also been shown to raise the glucosinolate levels of the seeds produced (Josefsson, 1970; Wetter *et al.*, 1970, Schnug, 1989).

With pressure from the European Commission (EC) to produce rapeseed of a low glucosinolate content, it is very important to establish the effect of environmental factors such as sulphur nutrition on yield and glucosinolate content and to attempt to establish the optimum level of sulphur fertilization required. The occurrence in Northern Scotland of soils which produce sulphur deficiency and soils which provide adequate amounts of sulphur make it an ideal place to compare sulphur applications on sulphur deficient and non-deficient sites. The trials reported also include the effects of combinations of different nitrogen and sulphur application rates.

EXPERIMENTAL

The choice of both sites was determined by previous observations of whether sulphur deficiency had or had not been noted in the past. Soil sulphur status, determined by the amount of extractable sulphate in the soil, was also considered. The low sulphur site chosen for this trial had a content of 3.8 mg $litre^{-1}$ and the high sulphur site had a content of 14.4 mg $litre^{-1}$ extractable sulphate at sowing. The 2 sites in this trial were situated in adjacent fields in Morayshire, Scotland and were about 200 m from each other so that they would be exposed to the same climate and a similar amount of atmospheric sulphur deposition.

Three varieties of winter oilseed rape (Brassica napus L.) chosen for their range in glucosinolate content were grown. These were Rafal, a high glucosinolate variety, and 2 low glucosinolate varieties: Cobra and Tapidor. The trials were sown on 5 September 1989 at a seed rate of 6 kg ha^{-1}. Seedbed fertilizer was applied at 0N 65P 65K kg ha^{-1}. Weeds, diseases and pests were controlled using standard husbandry practices. The trials were desiccated with glyphosate at 720 g ai ha^{-1} on 25 July and combine harvested on 10 August.

Two nitrogen treatments of 150 kg and 250 kg ha^{-1} ammonium nitrate were applied; half of each treatment was applied on 23 February and half on 6 April. Sulphur was applied as elemental sulphur (Thiovit - 80% sulphur) at 10, 20, 40 and 80 kg ha^{-1} of Thiovit on 27 March. A control treatment of no sulphur was included. Plots giving a 20 m x 2.13 m area at harvest were used. The plots were randomised for sulphur treatments within blocks which were themselves randomised for variety and nitrogen treatment. There were 3 replicates.

The plots at both sites were visually assessed for sulphur deficiency throughout the growing season. Tissue samples, consisting of the uppermost, fully differentiated 2-3 leaves of plants from plots which received no sulphur were taken on 10 April. These samples were finely ground after drying and then diluted with wax 1:4 whereby the two components were exactly homogenised prior to determination of total sulphur content by X-Ray Fluorescence as described by Schnug (1984). At harvest, yields were recorded and seed samples from each plot were dried at less than 60°C. A 20 g subsample was ground in a coffee mill for 30 s, the material homogenised and then ground for a further 7 s. X-Ray Fluorescence (XRF) analysis was then carried out by the method outlined by Schnug and Haneklaus (1988). The repeatability of the XRF method was estimated to be 1.5 uM g^{-1}.

RESULTS

The tissue samples, taken on 10 April, revealed that leaves from plants of all 3 varieties at the low sulphur site contained considerably less total sulphur than those from the high sulphur site (Table 1). Sulphur deficiency symptoms were observed at the low sulphur site for all varieties from the beginning of stem extension in March becoming progressively more severe until harvest.

Site had the most influential effect on seed glucosinolate content of all 3 varieties at harvest (Table 2 and 3). Rafal was affected more than the low glucosinolate varieties; at the non sulphur deficient site, the mean glucosinolate contents were 69, 15 and 8 uM g^{-1} seed for Rafal, Cobra and Tapidor respectively, while for the sulphur deficient site the corresponding values were 23, 6 and 5 uM g^{-1} seed. Site also had a large influence on yield (Tables 2 and 3); the mean yield for all varieties at the non deficient site was 4.8 t ha^{-1} compared to 2.7 t ha^{-1} at the deficient site.

At the low sulphur site (Table 2), the glucosinolate content in the seeds of the 3 varieties rose (significantly only at the 10% level) in response to the sulphur treatments. Rafal showed the greatest response and the glucosinolate content of the other varieties rose slightly with the application of sulphur. Application of 250 kg nitrogen compared to 150 kg depressed the glucosinolate content of Rafal and (non-significantly) Cobra.

The application of foliar sulphur had a very large significant effect of raising the yield at the low sulphur site. Irrespective of nitrogen treatment, Cobra gave a greater response than Rafal, and Tapidor had the largest response of the 3 varieties. Where no foliar sulphur was applied, the yield from plots of all varieties which had 250 kg ha^{-1} nitrogen was considerably lower than plots with 150 kg ha^{-1} nitrogen. A larger response to sulphur application was associated with the high nitrogen application than the low nitrogen application and the difference between yields for the 2 nitrogen treatments was less for higher sulphur applications. Combined with the 150 kg ha^{-1} nitrogen, there was a yield response to additional sulphur up to the 32 kg ha^{-1} treatment. For the 250 kg nitrogen treatment, there continued to be a response to the highest sulphur treatment of 64 kg ha^{-1}.

The application of foliar sulphur at the non-deficient site (Table 3) resulted in a slight, non significant increase in glucosinolate content for Rafal and Tapidor. There was no significant response in yield to the application of sulphur at this site.

The amount of nitrogen applied at the non deficient site did not significantly affect the glucosinolate content. At this site, the yield was greater with the high nitrogen treatment for Rafal (significantly) and for Tapidor (non-significantly).

DISCUSSION

The greater yield response of the low glucosinolate varieties, particularly Tapidor, to sulphur application would confirm the findings of Schnug (1989) that lower glucosinolate varieties are more susceptible to sulphur deficiency. It would support the hypothesis that such varieties afford less capacity for sulphur storage in the form of glucosinolates which can be broken down in times of sulphur deficiency. These findings would stress the importance of correct sulphur nutrition for low glucosinolate varieties, particularly the new varieties which have even lower glucosinolate contents.

The relative amounts of sulphur and nitrogen can also have an important influence on yield. Work by Aulakh et al. (1980) with mustard and Janzen and Bettany (1984) with rapeseed showed that there was a significant interaction between nitrogen and sulphur applications and that the maximum yield was only obtained when sulphur and nitrogen applications were balanced. In contrast, Nutall et al. (1987) in field trials on rapeseed in north-east Saskatchewan, did not find a significant nitrogen, sulphur interaction. Data from the low sulphur site in the present study would indicate that the relative availability of sulphur and nitrogen in the field is important. The results would correspond with work by Scott (1985) on grass and cereals in northern Scotland, who found that high nitrogen inputs decreased yields when sulphur availability was low. This underlines the importance of correct diagnosis of sulphur deficiency symptoms in the field, as if it were mistaken for nitrogen deficiency and additional nitrogen was applied, a greater yield penalty would result than if the deficiency remained undiagnosed.

The small rise in glucosinolate levels of the low glucosinolate varieties in the experiment in response to sulphur treatment compared with the much larger response of Rafal at the low sulphur site has important implications as the pressure to produce low glucosinolate rapeseed at optimum yields increases. On the high sulphur site, certain sulphur applications raised the glucosinolate level of Cobra very close to the 20 uM limit proposed by the EC; this would stress the importance of assessing the sulphur requirement of the crop for yield before application. It has been suggested that there is a dilution effect of higher yields to produce lower glucosinolate levels; the present work would not support this. There have been conflicting reports on the effect of nitrogen on glucosinolate content (Trzenby, 1970; Herrmann, 1976). The present study would indicate that glucosinolate content may be depressed by large nitrogen applications but in this case only on sulphur deficient soils.

It was noteworthy that "site" had the largest effect on yield - applying large amounts of sulphur on the sulphur deficient site did not increase yield of the individual varieties to the level achieved on the site with adequate soil sulphur. However "site" summarises a number of individual factors, of which sulphur seems to be a major component (Wetter et al., 1970; Schnug, 1989). It is suggested that the difference in the effect of sulphur fertilizer on the 2 sites may be due to a decreased transport of the fertilizer in the soil rather than the result of the different sulphate status of the soils (see also Schnug 1991b). The effect of sulphur might also be influenced by the form of the sulphur fertilizer and method of application. As plant uptake of elemental sulphur is mainly via the root after the sulphur has been washed off the leaves, uptake may have been reduced due to the dry period which followed application in this trial. In the soil, elemental sulphur is oxidised to sulphate by microorganisms, and in soils which are not frequently treated with reduced sulphur compounds the activity of the microorganisms is low. Until the population of microorganisms has risen there may be a time lag before sulphur is available to the plant (Schnug and Eckhardt, 1981). However, the results do show that the applications of elemental sulphur on the sulphur deficient site, through improving the sulphur status of the plant, increase yield. Further work is needed on the topics discussed, especially to investigate site effects and nitrogen sulphur fertilizer interactions, on yield and glucosinolate content of oilseed rape.

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TABLES

Table 1. Total sulphur concentration (parts per million) and [visual sulphur deficiency symptoms (1 = plants green and healthy, 9 = plants with extreme sulphur deficiency symptoms)]

Variety	Rafal	Cobra	Tapidor
Low sulphur site	2.57 [5.6]	2.49 [6.5]	2.11 [4.2]
High sulphur site	4.46 [1.1]	4.82 [1.1]	4.66 [1.0]
SE (internal mean) 0.269		SE (site mean) 0.190	SE (variety mean) 0.155)

TABLE 2. Low Sulphur Site

Total glucosinolate concentration (uM g^{-1} seed)

Nitrogen (kg ha^{-1})	150			250		
Variety	Rafal	Cobra	Tapidor	Rafal	Cobra	Tapidor
Sulphur (kg ha^{-1})						
0	25.4	6.6	4.0	7.3	4.1	3.9
8	27.0	6.2	3.9	5.5	3.4	4.2
16	27.9	6.8	4.1	7.1	4.5	3.5
32	38.0	7.9	4.9	18.2	7.6	5.2
64	46.0	9.6	4.9	29.8	7.9	7.6
SE (internal mean)	7.22	SE (S mean) 1.32		SE (N mean) 5.10		

Yield (t ha^{-1})

Nitrogen (kg ha^{-1})	150			250		
Variety	Rafal	Cobra	Tapidor	Rafal	Cobra	Tapidor
Sulphur (kg ha^{-1})						
0	2.36	2.27	1.18	1.05	1.12	0.82
8	3.41	2.76	2.23	1.90	2.41	1.69
16	3.53	3.13	2.83	2.56	2.56	2.04
32	3.95	3.79	3.50	3.17	3.19	3.13
64	3.77	3.78	3.40	3.40	3.93	3.50
SE (internal mean)	0.370	SE (S mean) 0.320		SE (N mean) 0.261		

TABLE 3. High Sulphur Site

Total glucosinolate concentration (uM g^{-1} seed)

Nitrogen (kg ha^{-1})	150			250		
Variety	Rafal	Cobra	Tapidor	Rafal	Cobra	Tapidor
Sulphur (kg ha^{-1})						
0	66.2	14.2	5.2	69.0	14.3	6.9
8	69.3	14.8	5.5	62.1	19.6	6.9
16	71.6	11.0	9.4	70.8	16.3	7.0
32	68.4	14.8	8.0	69.6	16.6	7.6
64	75.0	13.4	8.7	69.5	18.6	10.5
SE (internal mean)	4.10	SE (S mean) 2.98		SE (N mean)		2.90

Yield (t ha^{-1})

Nitrogen (kg ha^{-1})	150			250		
Variety	Rafal	Cobra	Tapidor	Rafal	Cobra	Tapidor
Sulphur (kg ha^{-1})						
0	3.91	4.66	4.80	5.00	5.01	4.52
8	4.75	4.64	4.60	5.47	5.03	4.75
16	4.39	4.83	4.84	4.76	4.86	5.09
32	4.68	4.85	4.61	5.50	4.70	5.02
64	3.99	4.64	4.79	5.11	4.32	5.08
SE (internal mean)	0.258	SE (S mean) 0.109		SE (N mean) 0.182		