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# Rate of nitrogen and sulphur fertilizers on yield, yield components and seed quality of oilseed rape (*Brassica napus* L.)

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**Abstract**

Two experiments were carried out to investigate the influences of nitrogen and sulphur fertilizers on the morphology, reproductive structures and seed quality of new cultivars of oilseed rape (*Brassica napus*). Yield and yield component responses were not consistent between years and cultivars. For the crop sown in 1988, application of nitrogen at 240 kg/ha increased total dry matter production and combine seed yield. Seed yield increased mainly due to greater number of pods on the terminal raceme and heavier seed weight whereas number of seeds per pod was not affected. Sulphur fertilizer application did not significantly increase plant dry matter production, number of seeds per pod or individual seed weight.

Considerable variation occurred between cultivars and years in seed glucosinolate concentrations with the application of nitrogen and sulphur fertilizers. Nitrogen top dressings caused significant increases in seed glucosinolate concentration in the droughty year of 1989 but not in 1990. Similar significant increases also occurred with sulphur application in 1990. Seed protein content generally increased with increasing rate of nitrogen application, with concomitant decrease in oil content. Sulphur application did not influence oil and protein contents.

*Keywords*: Fertilizer; Glucosinolate; Nitrogen; Oilseed rape; Sulphur

## 1. Introduction

Two factors that severely limit development of oilseed rape as a competitive protein and oil break crop are its high contents of long-chain fatty acids, especially erucic acid, and glucosinolates that remain in rapeseed cake after oil extraction. Glucosinolates are sulphur-containing compounds that occur predominantly in *Brassica* species (Schnug and Haneklaus, 1988). These substances (or more correctly the resulting by-products of their breakdown during oil extraction) lowered rapeseed cake palatability and produced a range of nutritional disorders in farm livestock (Vermorel et al., 1986). They are said to be linked with the availability of sulphur during plant growth (Schnug, 1987). In recent years, breeders have been successful in reducing the glucosinolate content from more than 100 $\mu$mol/g seed to less than 30 $\mu$mol/g thus producing "double-zero" or "double-low" (low erucic acid, low glucosinolate) cultivars.

The introduction of double-low cultivars is an important step towards improving the quality of the seed. Little is known, however, of effects of husbandry practices such as nitrogen (Ramans, 1989) and sulphur fertilizer application on glucosinolate concentrations in these cultivars. Sulphur deficiency has become a yield-

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limiting factor for rapeseed production due to decreased inputs of sulphur-containing fertilizers, and a reduction in sulphur dioxide levels in the atmosphere. About one third of British farm land is currently at high risk of sulphur deficiency (McGrath and Zhao, 1995). This study was undertaken to investigate the effects of these practices on yield and seed quality.

## 2. Materials and methods

The experiments were sited on Woodfield at the Wye College Farm (53°14′N, 1°46′W), which consists of a gleyed calcareous soil. The available sulphur content in soil was 2.0 g/m$^3$ with nitrogen index of zero (low nitrogen status; Halley and Soffe, 1988) and pH 8.0. The sites were ploughed after the removal of the previous winter wheat crop and a basal dressing of 60 kg/ha of each P and K applied.

Oilseed rape cvs. Ariana, Cobra, Libravo and Susana (Experiment 1), and Ariana and Susana (Experiment 2), were drilled in 15.5 cm rows in September using a seed rate of 9.0 kg/ha. The 9-kg sowing rate corresponded to about 175 seeds m$^{-2}$. Each plot in Experiment 1 measured 12 × 1.83 m whilst Experiment 2 employed plots 24.0 × 1.83 m.

These cultivars were chosen because they were the first commercial double lows recommended by the National Institute of Agricultural Botany (NIAB) for use in UK agriculture. The changeover from single-low to double-low cultivars was actively encouraged by revised EEC rapeseed subsidy and marketing regulations imposed during 1989. The new regulations stated that economic support would only be paid for cultivars that consistently provided seed with a glucosinolate level below 20 μmol/g. Only three double lows (Ariana, Cobra, and Libravo) were recommended one year before the 1989 EEC deadline. Many growers were concerned that new cultivars that had been rapidly introduced in order to meet the new EEC glucosinolate regulations had not been fully assessed under field conditions.

The experiments were factorial, laid out in randomised block designs with four replications. Both experiments had two levels of nitrogen (120 and 240 kg/ha). The treatments in Experiment 2 were combinations of the two levels of nitrogen with sulphur at three levels (0, 40 and 80 kg/ha). Nitrogen and sulphur levels are referred to as N1 and N2, and S1, S2 and S3, respectively. The nitrogen (as $NH_4NO_3$) and sulphur (as $K_2SO_4$) fertilizers were broadcast manually in late winter at the beginning of the second growing season. Further experimental details are given in Table 1. At the end of the 4- to 5-week flowering period a 0.5 × 0.5-m quadrat area was removed from each plot. The number of plants was recorded, and a random sub-sample of 10 plants was removed in order to assess the number of reproductive branches and pods per plant. All plants from each quadrat were dried in a forced air oven at 80°C for 48 h.

At final harvest, an area measuring 1.83 × 10.0 m in each plot was individually direct-combined and the seed yield weighed. Sub-samples of 2 kg were further cleaned to estimate the percentage chaff content and 500 g of this cleaned seed were dried at 80°C for 48 h to establish moisture content. Yield data were then corrected for chaff content, and adjusted to 9% moisture content. Weight per seed was determined from a dry,

Table 1
Experimental details

| | Experiment 1 | Experiment 2 |
|---|---|---|
| *Drilling date* | 6-9-88 | 13-9-89 |
| *Cultivars* | Ariana, Susana, Cobra, Libravo | Ariana and Susana |
| *Crop protection* | | |
| Pesticide, pre-emergence | Draza (metaldehyde) slug pellets, 5 kg/ha | same |
| Insecticide to control flea beetle | Gamma col (Gamma HCH) 0.35 l/ha | same |
| Herbicide for grass weed control | Fusilade 1.5 l/ha | same |
| Herbicide, broad leaved weed control | Basagran 2.5 l/ha | Kerb 50W (Propyzamide) 1.4 kg/ha |
| Fungicide | Sportak (Prochlaraz) 1.25 l/ha | same |
| Insecticide | Fastac 0.19 l/ha at mid flowering | same |
| *Harvest date* | 21-7-89 | 23-7-90 |

10-g sub-sample. Seed protein and oil percentages were measured using the Technicon 400 Auto Infralyser. Glucosinolate concentrations were measured by the X-ray fluorescence method (Schnug and Haneklaus, 1988). Data were analyzed using the Genstat statistical package (Kempson, 1988). The least significant difference test at the 5% level was used to compare treatment means.

## 3. Results and discussion

### 3.1. General response

In Experiment 1, nitrogen fertilizer application increased the overall mean dry matter yield per plant and per unit area but not in Experiment 2 (Tables 2 and 3). Cultivar responses to nitrogen and sulphur applications were not significantly different in either experiment.

Table 2
Influence of nitrogen application on plant characteristics in Experiment 1

| N rate | Cultivar | | | | Mean | LSD (5%) | CV% |
|---|---|---|---|---|---|---|---|
| | Ariana | Cobra | Libravo | Susana | | | |
| Final stand (plant/m$^2$) | | | | | | | |
| | 100 | 87 | 90 | 96 | | | |
| Dry matter yield/plant (g) | | | | | | | |
| N1 | 18.1 | 18.6 | 18.2 | 18.8 | 18.8 | | |
| N2 | 20.6 | 22.6 | 22.9 | 20.7 | 21.7 | 3.03 | 11.3 |
| Reproductive branches/plant (assessed on 26 May 1989) | | | | | | | |
| N1 | 5.3 | 5.4 | 5.3 | 5.4 | 5.4 | | |
| N2 | 5.6 | 5.6 | 5.8 | 5.7 | 5.7 | 0.21 | 5.3 |
| Pods/plant (assessed on 26 May 1989) | | | | | | | |
| N1 | 126 | 124 | 133 | 129 | 128 | | |
| N2 | 130 | 131 | 143 | 142 | 137 | 8.1 | 8.9 |
| Seeds/pod (final harvest) | | | | | | | |
| N1 | 16.3 | 17.8 | 14.3 | 14.7 | 15.8 | | |
| N2 | 17.1 | 17.1 | 14.6 | 14.4 | 15.8 | 2.62 | 11.5 |
| Weight per 1000 seed (g) | | | | | | | |
| N1 | 4.4 | 4.6 | 4.5 | 4.5 | 4.5 | | |
| N2 | 4.6 | 4.7 | 4.6 | 4.5 | 4.6 | 0.36 | 5.4 |
| Combine seed yield (t/ha) | | | | | | | |
| N1 | 3.23 | 3.18 | 3.05 | 3.06 | 3.13 | | |
| N2 | 3.31 | 3.38 | 3.35 | 3.43 | 3.37 | 0.20 | 12.3 |
| Seed protein content (% DM) | | | | | | | |
| N1 | 20.9 | 20.6 | 20.8 | 20.8 | 20.8 | | |
| N2 | 21.1 | 21.2 | 21.5 | 21.4 | 21.3 | 0.41 | 1.8 |
| Seed oil content (% DM) | | | | | | | |
| N1 | 41.2 | 41.8 | 41.7 | 41.3 | 41.5 | | |
| N2 | 40.3 | 40.6 | 41.0 | 40.6 | 40.6 | 0.81 | 2.0 |
| Seed glucosinolate content (μmol/g seed) | | | | | | | |
| N1 | 22.1 | 12.1 | 10.0 | 13.4 | 14.4 | | |
| N2 | 24.8 | 18.8 | 20.7 | 13.8 | 19.5 | 4.04 | 22.9 |

The time course of dry matter production, as measured in Experiment 2, is illustrated in Fig. 1. The plants averaged about 4.0 g each at 191 days after drilling (DAD) in late March but they increased rapidly in a linear manner thereafter to a maximum in early July (294 DAD). Dry matter yield per plant was increased but not always significantly by nitrogen applications. Sulphur application did not influence dry matter yield.

### 3.2. Yield components and combine yield

In both experiments, nitrogen application increased mean number of pods per plant. Cultivar differences ($P<0.05$) were also found. In Experiment 2, the number of pods per plant at final harvest was significantly ($P<0.001$) increased by sulphur application but due to small changes in other yield components, the effect on yield was nonsignificant.

The pattern of pod distribution within the canopy for the treatments in these trials was similar at all harvest dates. The terminal raceme carried the largest numbers of pods. With the exception of the first uppermost branch, which had slightly fewer pods than basal branch two, mean numbers declined with increasing canopy depth.

Although mean seed weight for this species appears to be relatively constant under different environmental conditions (Scarisbrick et al., 1981), some important differences were observed in the present experiments. For example, seed weight of the combine seed samples was increased by nitrogen in Experiment 2, but not in Experiment 1. As plants in N2-nitrogen plots tended to remain greener longer than those in N1, an extended period of assimilation for seed filling may have occurred before senescence curtailed development. Scott et al. (1973) found that leaf area duration of oilseed rape was closely related to LAI (leaf area index) and that LAI was greatly increased by nitrogen.

The differences in seed weight appear to be related to a shorter period (33 days versus 40 days) of seed filling and drought in Experiment 1. Warm mean temperatures in June and July 1989, 19.8°C and 23.8°C, respectively, in Experiment 1 contrasted with corresponding values of 18.3°C and 21.8°C recorded in

Table 3
Influence of nitrogen and sulphur application on plant characteristics in Experiment 2

| Cultivar | Nitrogen rate N1 | N2 | Mean | LSD (%) | Rate of sulphur S1 | S2 | S3 | Mean | LSD (5%) |
|---|---|---|---|---|---|---|---|---|---|
| Dry matter yield/plant (g) | | | | | | | | | |
| Ariana | 22.2 | 22.9 | 22.6 | | 20.4 | 22.1 | 24.1 | 22.3 | |
| Susana | 21.2 | 22.5 | 21.9 | 3.05 | 21.5 | 22.6 | 22.9 | 22.3 | 3.7 |
| Reproductive branches/plant (assessed on 30 May 1990) | | | | | | | | | |
| Ariana | 5.8 | 5.8 | 5.8 | | 5.6 | 5.7 | 6.1 | 5.8 | |
| Susana | 5.6 | 5.8 | 5.7 | 0.23 | 5.6 | 5.7 | 5.9 | 5.7 | 0.28 |
| Pods/plant (assessed on 30 May 1990) | | | | | | | | | |
| Ariana | 131 | 136 | 134 | | 128 | 134 | 140 | 134 | |
| Susana | 138 | 142 | 140 | 4.4 | 132 | 140 | 149 | 140 | 5.4 |
| Seed/pod (final harvest) | | | | | | | | | |
| Ariana | 15.2 | 15.7 | 15.4 | | 15.8 | 14.7 | 15.9 | 15.5 | |
| Susana | 15.1 | 14.5 | 14.8 | 0.79 | 14.9 | 14.9 | 14.7 | 14.8 | 0.96 |
| Weight per 1000 seed (g) | | | | | | | | | |
| Ariana | 5.16 | 5.27 | 5.22 | | 5.16 | 5.24 | 5.25 | 5.21 | |
| Susana | 5.05 | 5.34 | 5.20 | 0.10 | 5.14 | 5.16 | 5.31 | 5.20 | 0.18 |
| Combine seed yield (t/ha) | | | | | | | | | |
| Ariana | 3.68 | 4.01 | 3.85 | | 3.82 | 3.83 | 3.88 | 3.84 | |
| Susana | 3.86 | 4.06 | 3.96 | 0.20 | 3.88 | 3.89 | 4.12 | 3.96 | 0.25 |
| Seed protein (% DM) | | | | | | | | | |
| Ariana | 21.6 | 22.0 | 21.8 | | 21.5 | 21.9 | 21.9 | 21.8 | |
| Susana | 21.9 | 22.5 | 22.2 | 0.24 | 22.1 | 22.2 | 22.4 | 22.2 | 0.39 |
| Seed oil content (% DM) | | | | | | | | | |
| Ariana | 42.4 | 41.7 | 42.1 | | 41.9 | 42.1 | 42.1 | 42.0 | |
| Susana | 42.5 | 42.3 | 42.4 | 0.45 | 42.2 | 42.2 | 42.6 | 42.4 | 0.56 |
| Seed glucosinolate content (μmol/g seed) | | | | | | | | | |
| Ariana | 10.2 | 10.4 | 10.3 | | 8.7 | 10.2 | 11.9 | 10.3 | |
| Susana | 9.6 | 10.3 | 10.0 | 0.91 | 9.1 | 9.4 | 11.6 | 10.0 | 1.11 |

The fertilizer treatments did not influence plant population. Final plant stands for Ariana and Susana were 97 plants and 94 plants/m$^2$ respectively.

Experiment 2. These differences, which occurred during the seed filling period, may account for the limitation on seed growth.

Combine seed yields increased with increasing nitrogen availability through an effect on the number of fertile primary branches and pods. Allen and Morgan (1972) and Yau and Thurling (1987) demonstrated a similar effect of nitrogen on spring cultivars. Combine seed yields in Experiment 1 were smaller than in Experiment 2 because of drought and warm mean temperatures. Low soil moisture during May and June may have hampered N uptake, or reduced photosynthetic activity of leaves and pods, or reduced mobilisation of assimilates.

Commercial rapeseed producers know that a vast amount of seed is lost during the period of pod development because, following harvest and autumn rains, sites are quickly covered by volunteer seedling plants. Seed losses from oilseed rape as a result of shedding can leave up to 10 000 seeds/m$^2$ on the soil surface (Lutman, 1993). The extent of seed loss can also be demonstrated by calculating a 'yield potential' based on sampled yield components. For example, in Experiment 1 the yield of Ariana based on 100 plants/m$^2$ $\times$ 126 pods/plant $\times$ 16.3 seeds/pod and a mean seed weight of 0.0044 g is 9.04 t/ha, whilst the final seed yield obtained using the plot combine harvester was only 3.23 t/ha (Table 2).

It is important to attempt to explain this large discrepancy which has been shown to occur in many rapeseed husbandry trials (Addo-Quaye, 1985; Smith, 1987). The most important source of error in the yield component calculation is number of pods per plant, because these data were collected not at final harvest in July, but at the end of the flowering periods when all immature pods were still undamaged. As rapeseed pods are initiated on floral racemes over a period of 4 to 5 weeks, they vary in their respective maturities. A percentage of seed is shed as the oldest, lowermost pods naturally dehisce. Additional seed losses are caused by three insect pests that invade the canopy at the beginning (pollen beetle, *Meligethes* spp.), middle (weevil, *Ceutorhynchus assimilis*) and end (pod midge, *Dasineura brassicae*) of the flowering period. In Experiments 1 and 2, these pests were contained by an insecticide applied at mid-flowering during the first week of May (Table 1). Finally, as the crop matures, small birds (usually finches and sparrows) and thunderstorms cause additional pod damage and result in further losses of seed.

Because pod damage rapidly increases during the ripening period it is not practical to remove quadrat samples prior to final harvest in order to assess numbers of pods per plant. At maturity it is difficult and tedious to distinguish between damaged and undamaged pods, and as plant subsamples are brittle, the surviving pods shatter easily when removed to the crop laboratory for detailed study.

For a crop of oilseed rape to achieve its yield potential, all immature pods must contribute to final yield. For physiological and environmental reasons this is never likely to occur (Addo-Quaye et al., 1986), and

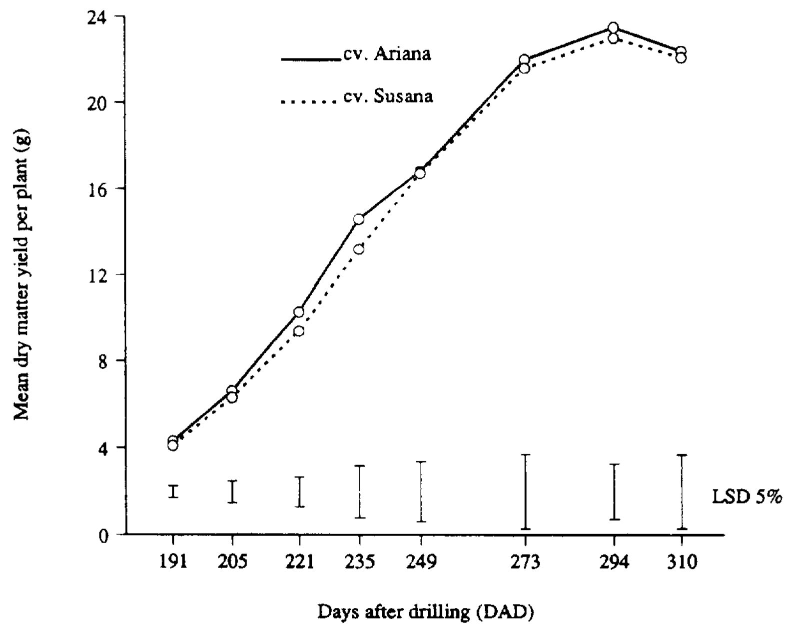


Fig. 1. Time course of dry matter production.

most growers accept there is little they can do to minimise seed shedding during the final ripening period. It is important to note however, that the mean harvested yields achieved in Experiments 1 (3.25 t/ha) and Experiment 2 (3.90 t/ha) were slightly higher than the average 1995 UK yield of 2.85 t/ha. Average yields have not increased greatly during the past decade, and annual within-farm yields are extremely variable. The absence of yield improvement is mainly associated with pod and seed losses during ripening.

### 3.3. Seed composition

Nitrogen application affected seed protein and oil content inversely. Plants at N1 had less protein content than at N2 with a concomitant increase in oil content (Table 2 and Table 3). There were significant ($P<0.05$) differences between cultivars in seed protein but not oil content with rate of sulphur application in Experiment 2. The reverse of the rank of protein content was not exactly the same as the rank for oil content with sulphur application. A negative correlation between protein and oil content was also seen in older cultivars (Grami and Stefansson, 1977).

Seed glucosinolate content responded variably to rate of N fertilizer application. It increased significantly with N application in Experiment 1 but not in Experiment 2. These results agree with those reported by Augustinussen et al. (1984) for older cultivars. As N fertilization can stimulate sulphur uptake (Rasmussen et al., 1975), it seems likely that this accounts for the increased S content of the seed, resulting in higher glucosinolate contents. In contrast, reduced glucosinolate content with increased N application has been reported by Josefsson (1970). Josefsson attributed this lower glucosinolate content to an increase in protein production, reducing the availability of carbohydrates, so that glucose might be a limiting factor for glucosinolate synthesis. Holmes (1980) associated the reduced glucosinolate content with a decrease in S content, and assumed it to be a simple dilution effect resulting from increased dry matter production. Results of Experiment 2, with no significant effect of nitrogen fertilizer application on glucosinolate content, are in agreement with this findings of Ramans (1989).

The importance of an adequate supply of S for glucosinolate synthesis has been well documented (Schnug, 1987; Mailer, 1989) and is supported by the results of Experiment 2. The present trials revealed the influence of N and S fertilization on seed quality of new cultivars of oilseed rape.

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