Effects of nitrogen (N) and sulphur (S) on canola (Brassica napus L.) vegetative and reproductive growth under controlled conditions

Wonder Ngezimana* and Gert Andries Agenbag

Department of Agronomy, University of Stellenbosch, P/Bag X1, Matieland, 7602, South Africa.

Accepted 10 October, 2013

Canola is becoming a major source of vegetable oil with increasing demand in South Africa, yet low yields are presently experienced in production areas of the Western Cape Province. Crop response to fertiliser applications in field trials under rainfed conditions are often poor, because of a large number of growth factors that may limit growth and yield. To determine how canola responds to nitrogen (N) with no (low) and high sulphur (S) rates under ideal conditions in a controlled environment, a 5 x 2 factorial experiment, with N (0, 40, 80, 120 and 160 kg ha\(^{-1}\)) and S (0 and 40 kg ha\(^{-1}\)) fertilisation rates, was conducted. Plants were irrigated with a nutrient solution which contained all nutrients, but with very low N and S contents. Nitrogen application significantly increased leaf area, hence dry mass accumulation and ultimately flowering and pod formation, but high N and S application levels during early growth stages may have a negative effect on growth. Significant interaction between N and S were shown, however the positive effects of S were more pronounced in the reproductive phases. In this experiment, conducted under controlled temperature and watering conditions, but short winter daylight lengths, yield components of canola as measured by the number of flowers and pods at 91 DAP tended to reach a peak at application rates of 120 kg N ha\(^{-1}\) and 40 kg S ha\(^{-1}\).

Key words: Canola, nitrogen, sulphur, vegetative and reproductive growth.

INTRODUCTION

The challenge of increasing crop yield per hectare to satisfy the needs of an ever increasing human population cannot be ignored. The supply of oil and protein is becoming scarce especially in developing countries (Ahmad et al., 2006) and South Africa currently relies on imports for its domestic plant oil and protein needs. Canola is becoming a major source of vegetable oil in the world (Kandil and Gad, 2012), but is still a relatively new crop in South Africa and considerable research is still needed for best production practices to optimize yield. Amongst many agronomic factors responsible for low yields, imbalanced and injudicious use of fertilisers also limits crop production (Sattar et al., 2011). Several Western Cape farmers (Glennes, 2007) reported yields barely reaching 1.5 tonnes ha\(^{-1}\). These low yields were mainly caused by poor soil management (Hardy et al., 2004). Soil fertility management (especially N and S application) may for this reason be one of the best options to increase canola yields in the Western Cape production area.

Generally, fertilisation with N has previously shown effectiveness in increasing yields of canola (Allen and Morgan, 1972; Yau and Thurling, 1987; Ahmad et al., 2006; Gan et al., 2008; El-Nakhlawy and Bakhashwain, 2009).

*Corresponding author. E-mail: wonderjohnngezimana@yahoo.com.
Table 1. Chemical analysis of the sandy soil at planting and critical nutrient levels for canola.

<table>
<thead>
<tr>
<th>Unit</th>
<th>pH</th>
<th>Resistance</th>
<th>Ca</th>
<th>Mg</th>
<th>T-value</th>
<th>Na</th>
<th>K</th>
<th>P</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>B</th>
<th>S</th>
<th>Ca</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil level</td>
<td>6.7</td>
<td>3520</td>
<td>0.97</td>
<td>0.15</td>
<td>1.21</td>
<td>8.0</td>
<td>17</td>
<td>30</td>
<td>0.12</td>
<td>0.23</td>
<td>3.60</td>
<td>0.01</td>
<td>5.8</td>
<td>0.04</td>
<td>0</td>
</tr>
<tr>
<td>Critical levels</td>
<td>&lt;5²</td>
<td>-</td>
<td>&lt;1.0¹</td>
<td>&lt;0.4¹</td>
<td>-</td>
<td>&gt;250²</td>
<td>&lt;80²</td>
<td>&lt;36²</td>
<td>&lt;0.3¹</td>
<td>&lt;0.5¹</td>
<td>&lt;5.0¹</td>
<td>&lt;0.2¹</td>
<td>&lt;6¹</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*a* shows the critical soil nutrient levels at which the nutrient became deficient or where the growth of canola plants will be negatively affected (Canola production manual, undated; Peverill et al., 1998).

Franzen (1997) showed that sulphur is one of the most important soil factors to be considered when growing canola and S deficiency will reduce yield potential (Pouzet, 1995; Fismes et al., 2000; Abdallah et al., 2010). Adequate supply of N encourages leaf development, assisting in retaining leaves in active photosynthesis, hence facilitating development of flowers and pods (Weiss, 1983).

Sulphur concentration in canola plants varies between 1 and 16 g kg⁻¹ dry mass, depending on the external supply (Balint and Rengel, 2009). Sulphur is a constituent of certain amino acids needed for protein synthesis in canola. Besides the formation of proteins during growth and development of canola, S can also increase seed yield and improving oil content (Zhao et al., 1993; Jan et al., 2011). Sulphur deficiencies will greatly reduce N uptake hence the application of S needs to be balanced with N for optimum yields (Ceccoti, 1996; Fismes et al., 2000; Brennan and Bolland, 2008). Results of canola field experiments conducted in various localities in the Western Cape province from 2009 to 2011 (Ngezimana, 2012) showed that yields tend to reach a plateau with 15 kg S per ha⁻¹, but did not reach a plateau with nitrogen rates as high as 120 kg ha⁻¹. Crop response in field trials with rainfed conditions, however, may often be less accurate, because a large number of growth factors cannot be controlled (Lobell et al., 2009).

According to Abdallah et al. (2010), appearance of S deficiency is fairly recent in agriculture and research on crop S nutrition still lags far behind that on other major nutrients such as N, even though the effect of one nutrient tends to be more dependent on the other (Sattar et al., 2011). Hence, the aim of this study was to determine the response to high nitrogen application rates with no (low) and high S rates under ideal conditions in an environmental controlled glasshouse.

**MATERIALS AND METHODS**

Pot experiments were conducted at the Welgevallen Experimental Station of Stellenbosch University, during the 2011 winter season (21 May to 20 August) in a temperature controlled glasshouse with a 20°C day and 15°C night temperature. The coarse sandy soil used for the experiment was chemically analyzed and showed low contents for almost all nutrients, as compared to the amounts required for optimum growth and yield of canola (Table 1).

Plastic pots of 17.5 cm length, 15 cm width and 35 cm height (surface area of 0.02625 m²) were filled with 6 000 g of air dried soil. Mid-season maturing canola cultivar Hyola 61 was planted on 20 May 2011. Five application rates of N (0, 40, 80, 120 and 160 kg ha⁻¹) and 2 rates of S (0 and 40 kg ha⁻¹) were applied to the pots to have a 5 x 2 factorial design. Plants were fertilized with a balanced nutrient solution which contained all nutrients but with low N and S, by means of the irrigation system (Table 2). In the fertigation system, micronutrients were added as Microplex mix to give Fe (1.68 ppm), Mn (0.4 ppm), Zn (0.2 ppm), Cu (0.03 ppm), B (0.5 ppm) and Mo (0.05 ppm). The concentrations of the other nutrients in the solution were K (205 ppm), Ca (138 ppm), Mg (37 ppm), N (28 ppm), P (71 ppm), S (16 ppm) and Cl (107 ppm). In order to apply magnesium (magnesium sulphate and magnesium nitrate), nitrogen and sulphur were inevitable also applied, but the levels of N and S were about 20% of that in a standard solution (Steiner, 1984) and it was assumed that such levels should result in deficiencies.

Nitrogen was applied as Limestone Ammonium Nitrate (LAN) with 28% N while S was applied as gypsum (CaSO₄·2H₂O) with 16% S. Although Ca was also applied it is not likely that it would have an effect due to sufficient Ca levels in the nutrient solution. The amounts of gypsum and LAN were calculated by using the pot surface area to supply the required levels of S and N respectively. Both nutrients were added in splits of a quarter of the prescribed treatment at planting, 28 DAP (Days after planting), 49 DAP and 70 DAP hence for N; 0.09, 0.19, 0.28 and 0.38 g of LAN were applied and for S; 0.16 g of gypsum at each application time. Treatment combinations were allotted randomly to pots in each replication. Water was added to wet the soil to field capacity a day before planting. Up to crop emergence, the pots were kept moist using a light overhead irrigation, thereafter water was applied through drippers. Irrigation frequency was determined by solar radiation and the amount per irrigation event was adjusted for different growth stages, to make sure that no water stress or leaching of nutrients occurred. Ten seeds were sown in each pot. After emergence, seedlings were hand thinned with the first thinning to 4 plants per pot done at 14 DAP, and the second thinning to maintain a uniform stand of 2 plants per pot, done at 21 DAP. The experiment was laid out in completely randomized design (CRD). The experiment was replicated four times to facilitate destructive samplings at 28 DAP, 49 DAP, 70 DAP and at flowering (about 91 DAP). Because of the split application of N and S, plants sampled at 28, 49 and 70 DAP have at that stage not received their fully allotted rates of N and S (Table 3).

The replicated trials were harvested through destructive sampling for both leaf area and dry mass at 28 DAP, 49 DAP, 70 DAP and at flowering (91 DAP). Leaf area per plant was measured using a leaf area meter (Licor, Model 3100, LICOR Ltd., Lincoln, NE). Thereafter total above ground plant samples were oven dried to determine dry mass. Experiment was terminated at 91 DAP because plants became too tall and tended to lodge. During the final sampling at flowering (91 DAP), the total number of flowers and pods per plant were counted as an indication of the yield potential (Ahmadi and Bahrani, 2009).

Data recorded was analyzed statistically, using analysis of variance (ANOVA) with nitrogen and sulphur rates considered as fixed factors (Statisticia, 2012). Main and interaction effects were
Table 2. Nutrient solution with low N and S applied by fertigation to canola in pot trial.

<table>
<thead>
<tr>
<th>Nutrient solution with minimum N and S</th>
<th>EC=1.5 mS cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macronutrient in 900l</td>
<td></td>
</tr>
<tr>
<td>Ca chelat</td>
<td>446 ml</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>313 g</td>
</tr>
<tr>
<td>KCl</td>
<td>224 g</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>123 g</td>
</tr>
<tr>
<td>Mg(NO₃)₂·6H₂O</td>
<td>256 g</td>
</tr>
<tr>
<td>Micronutrients in 900l</td>
<td></td>
</tr>
<tr>
<td>Microplex</td>
<td>18 g</td>
</tr>
</tbody>
</table>

Table 3. Accumulative quantities of N and S received at different sampling times.

<table>
<thead>
<tr>
<th>Sampling time (DAP)</th>
<th>N rate (kg ha⁻¹)</th>
<th>S rate (kg ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>49</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>70</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>91</td>
<td>0</td>
<td>40</td>
</tr>
</tbody>
</table>

RESULTS

Nitrogen application rates significantly affected leaf area per plant at 28, 49 and 70 DAP, dry mass at all sampling dates as well as the number of flowers and pods at 91 DAP. Leaf area per plant and dry mass per plant at 49 DAP, as well as the number of pods (91 DAP) were affected by sulphur. Leaf area and dry mass per plant at 49 DAP as well as number of pods at 91 DAP showed a response due to the interaction between sulphur and nitrogen application rates.

Sampling at 49 DAP

Sampling at 49 DAP showed a significant interaction between nitrogen and sulphur rates with regard to leaf area and dry mass per plant. Increased N application rates resulted in an almost linear increase in both leaf area (Figure 1b) and dry mass per plant (Figure 2b) at high S applications (40 kg S ha⁻¹ rate) with no plateau even at 80 kg N ha⁻¹ (160 kg N ha⁻¹ rate). With low S application rates (0 kg S ha⁻¹); both leaf area per plant (Figure 1b) and dry mass per plant (Figure 2b) reached a plateau at 40 kg N ha⁻¹ (80 kg N ha⁻¹ rate).

Sampling at 70 DAP

At this sampling stage, plants had previously received 0, 30, 60, 90 and 120 kg N ha⁻¹ respectively and either 0 or 30 kg S ha⁻¹. Leaf area and dry mass per plant were significantly affected by N. Vegetative growth as measured by leaf area (Figure 1c) and dry mass (Figure 2c) increased with increasing N rates with no plateau even at 120 kg N ha⁻¹ (160 kg N ha⁻¹ level), illustrating the high nitrogen requirements during peak vegetative growth stages. Addition of 30 kg S ha⁻¹ (40 kg S ha⁻¹ level)
Figure 1. Effect of N and S fertilisation rates on leaf area of canola plants at (a) 28, (b) 49, (c) 70 and (d) 91 DAP.
tended to improve leaf area response to N at higher (90 and 120 kg ha\(^{-1}\)) rates (Figure 1c) but this effect was not significant even though the plants were growing very rapidly during this bolting stage.

**Sampling at 91 DAP**

At the last sampling time, plants had received all allotted N (0, 40, 80, 120 and 160 kg N ha\(^{-1}\) rate) and S (0 and 40 kg S ha\(^{-1}\)). Plant dry mass was significantly affected by N application rate, but not by sulphur. Significant increases in dry mass due to increasing N application rates were therefore similar for both S application rates (Figure 2d). Leaf area response to N at 91 DAP (Figure 1d) was much less than at earlier stages as senescing progressed (leaf area reached a plateau) (Figure 1d), whilst dry mass per plant still showed a significant response to N applications.

**Figure 2.** Effect of N and S fertilisation rates on dry mass (DM) of canola plants at (a) 28, (b) 49, (c) 70 and (d) 91 DAP.
Figure 3. Effect of N and S fertilisation rates on total number of (a) flowers and (b) pods per plant at 91 DAP.

DISCUSSION

These results showed that canola do not need much N and S during the early growth stages and higher application rates at planting may even suppress vegetative growth. N application in canola can be delayed at least until four leaf stage, without any compromise of dry matter yield. Cheema et al. (2010) also recommended split application of nitrogen with more application at branching to flowering period than at sowing. Under field conditions, such split application should be depended on other cultural practices (Malhi and Gill, 2002; Grant et al., 2002) as they influence the effectiveness of applied fertiliser. Canola plants need high amounts of both N and S during the late vegetative (rosette) stage, as there is rapidly expanding of leaf area and chlorophyll synthesis, which requires both S and N (McGrath and Zhao, 1996). During this early development, leaves represent a major store of nutrients which can be remobilized from old to younger leaves specifically for N (Malagoli et al., 2005). The leaves appearing during the rosette stage play a crucial role for seed filling and contribute to the maintain-
Canola leaf growth is almost complete at the stage when plants start to flower. When the crop is flowering and podding, assimilates are remobilized from senescing leaves for reproductive growth (Malagoli et al., 2005). A delay in the onset of flowering at higher N application rates as shown by Brandt et al. (2007) favours luxurious vegetative growth and is not necessarily an indication of the final grain yield.

It is clear that S had a larger effect on reproductive growth than on vegetative (leaf growth). McGrath and Zhao (1996) reported that the effects of S were more pronounced during reproductive stages of canola. During vegetative growth, S is involved in photosynthesis and, deficiencies decreases chlorophyll content and cause leaves to turn yellow showing inter-veinal chlorosis (Pouzet, 1995). Poor responses to S applications at this sampling stage in spite of deficient S contents in the soil used in this experiment (5.8 mg kg⁻¹), may be ascribed to the unavoidable addition of S when magnesium sulphate was added. Although, the amount of S added this way was very low and about 20% of that in a standard solution (Steiner, 1984), continuous application with the irrigation seemed to be enough to prevent significant S responses.

Increased availability of N enhances vegetative growth of canola plants (Cheema et al., 2001; Ahmad et al., 2006; Yasari and Patwardhan, 2006). This N which accumulates in early growth stages becomes useful in reproductive stages (Hocking and Strapper, 2001; Malagoli et al., 2005), with S essential in protein synthesis (Fismes et al., 2000; Jan et al., 2002). In addition, naturally occurring compounds called glucosinolates are also synthesized from S (McGrath and Zhao, 1996) and they mostly accumulate in canola reproductive tissues (Bellostas et al., 2007; Bushan et al., 2013). This makes canola to be particularly sensitive to S deficiency, which reduces both seed yield (Kandil and Gad, 2012) and quality (De Pascale et al., 2008). The results also clearly indicate that S requirement and metabolism in canola plants are closely related to N nutrition (Sattar et al., 2011), and N metabolism is also strongly affected by the S (Mirzashahi et al., 2010). Fismes et al. (2000) have shown using field-grown oilseed rape that S deficiency can reduce nitrogen use efficiency (NUE: ratio of harvested N to N fertilization) and that N deficiency can also reduce sulphur use efficiency (SUE).

Conclusions

In this study, canola grown in a temperature controlled glasshouse and irrigated with a balanced nutrient solution which contained very low levels of N and S, responded positively to N and S applications splitted between planting, 28, 49 and 70 days after planting (DAP). High rates of N and S applied at planting however tend to have a negative effect on leaf area and dry matter production. High application rates of N and S to canola grown in dry areas, under rainfed conditions, should therefore be avoided. At later growth stages vegetative growth as measured by leaf area and dry mass did not reach a plateau even at N rates of 120 to 160 kg of N ha⁻¹, suggesting that such rates of N may be needed under high rainfall conditions or where canola is grown under irrigation. More research dealing with split applications of N and S on canola may however be needed.

REFERENCES

Canola Production Manual. Undated pamphlet sponsored by the PNS and THRIP.
Fismes J, Vong PC, Guckert A, Frossard E (2000). Influence of sulphur...


Statistica (2012). StatSoft, Inc. USA.


