

# BORAGE (*Borago officinalis* L.) RESPONSE TO N, P, K, AND S FERTILIZATION IN SOUTH CENTRAL CHILE

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## ABSTRACT

Borage (*Borago officinalis* L.) is an oilseed with a high gamma-linolenic acid (GLA) content in its seed. The objective of this study was to determine the response of borage seed yield, oil content, and fatty acid composition to N, P, K, and S fertilizer treatments. Three experiments were conducted in Osorno (40°22' S, 73°04' W; 72 m.a.s.l.), Chile. The first experiment was conducted during the 2005-2006 growing season, with four N rates (0, 100, 200, and 300 kg N ha<sup>-1</sup>), three P rates (0, 60, and 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and two K rates (0 and 150 kg K<sub>2</sub>O ha<sup>-1</sup>). The second experiment was conducted in 2006-2007 and evaluated only the effect of four N rates (0, 50, 100 and 150 kg N ha<sup>-1</sup>) was evaluated because there was no response to P and K in the first experiment. The third experiment was conducted in the 2005-2006 and 2006-2007 and evaluated only two rates of S, 0 and 40 kg S ha<sup>-1</sup> were evaluated. Seed yield was not affected by N, P, K, or their interactions in any of the experiments. As N rates increased GLA content increased. Mean seed yield increased to 98 kg ha<sup>-1</sup> when applying of 40 kg S ha<sup>-1</sup>. Results indicate that borage has a higher response to S applications than N. Further research is needed to determine the interactions between N and S applications, given that the experiments were conducted on soils with high levels of P and K levels.

**Key words:** seed yield, gamma-linolenic acid.

## INTRODUCTION

Borage (*Borago officinalis* L., Boraginaceae) is an herbaceous annual plant (Janick *et al.*, 1989). Current interest in this crop is for its seed which contains a high content of gamma-linolenic acid (GLA) (all- *cis* 6,9,12-octadecatrienoic acid) in the oil. This acid (GLA) is a precursor of the prostaglandin PGE1 in the human body (Coupland, 2008), which is vital in many body functions, such as antithrombotic inhibitory effects on aggregation of platelets, lowering blood pressure, and inhibiting cholesterol formation (Coupland, 2008). Potential medical uses of GLA include treating atopic eczema to decrease disease symptoms (Horrobin, 2000) and reducing side effects of diabetes, such as vascular damage, altered platelet function, and arteriosclerosis (Coupland, 2008). Commercial seed sources of GLA

include evening primrose (*Oenothera biennis* L.) and some *Ribes* species. Oil content of borage seeds fluctuates between 300 and 380 g kg<sup>-1</sup> of which 20% to 23% is GLA. Oil and GLA content are higher in borage seed compared to those from evening primrose and *Ribes* spp. (Deng *et al.*, 2001).

Main producers of borage seed are Canada, England, United States, and Chile (Nicholls, 1996). Chile had 2000 ha of borage under contract each year in 2004, 2005, and 2006 becoming an interesting crop alternative for the area. However, contracts were ended in 2007 due to reduction of international prices and cheaper sources of oils containing GLA. The market for borage oil, as for evening primrose oil, fluctuates dramatically with some years of over-supply and others of low production. One of the reasons for this is that the major borage producer is Canada, where growers can produce seed at the lowest cost, but where there is a high risk for crop failure due to early frosts. Also, evening primrose oil from China, another source of GLA, flooded the North American market in 2001 reducing the prices for GLA-containing oils (El Hafid *et al.*, 2002a). Therefore the quantity of borage seed marketed each year is variable, fluctuating between 500 and 2000 t worldwide. Borage seed price

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fluctuates between US\$2.5 to 4 kg<sup>-1</sup> of seed depending on supply and seed quality. Seeds higher than 24% GLA are easier to market during years of oversupply. Borage oil price fluctuates between of US\$30 to 35 kg<sup>-1</sup> in the market place (Lindemann and Merolli, 2006).

Borage is grown usually at higher latitudes to increase GLA content. Most processing companies require a minimum 22% GLA, which is not easy to obtain at latitudes lower than 38°. Southern Chile has climatic and soil conditions favorable for borage seed production (Berti *et al.*, 2002). Borage grows in a broad range of climates and soil pH from 4.3 to 8.5 (Janick *et al.*, 1989); however to obtain a high quality seed oil, the seed development must occur with temperatures below 25 °C.

Borage grows rapidly and it is ready to harvest in about 75 d after sowing. Nutrient requirements for borage are not clear. Previous studies indicate no response to N up to 80 kg N ha<sup>-1</sup> in Alberta, Canada, although the lack of response was attributed to high initial soil N content (El Hafid *et al.*, 2002a; 2002b). However, in Egypt, sequential application or foliar N, as urea, increased seed yield and decreased oil content (Refaat *et al.*, 2000). In Australia, borage is fertilized with 500 kg ha<sup>-1</sup> of a (13:14:13) N:P:K fertilizer (Laurence, 2004). There are no references on borage requirements of P and S. However, oilseeds, such as canola (*Brassica napus* L.), require 23 to 34 kg ha<sup>-1</sup> of applied S when soil test S are low to medium (Berglund *et al.*, 2007). The total S uptake for canola is 60 kg S ha<sup>-1</sup> (Jackson, 2000).

The objective of this study was to determine the response of borage seed yield, oil content and fatty acid composition to N, P, K, and S fertility treatments in southern Chile.

## MATERIALS AND METHODS

Experiments were conducted in Osorno (40°22' S, 73°04' W; 72 m.a.s.l.), Los Lagos Region, Chile, in the 2005-2006 and 2006-2007 growing seasons. The experiments were conducted under dryland conditions and no-till. The previous crop was wheat (*Triticum aestivum* L.). Soil at Osorno is classified as Osorno series (ashy, mesic Typic Haploxerand), and slightly hilly. Climate is classified as cold Mediterranean with rainfall from 1200 to 1500 mm. Average monthly temperature and rainfall at Osorno in the 2005-2006 and 2006-2007 growing seasons, and soil analysis from both seasons are presented on Table 1.

### Experiment 1. Interaction N, P, and K effect

In the 2005-2006 growing season, the experimental design was a randomized complete block (RCB) with a factorial arrangement (4 x 3 x 2), with four rates of N (0, 100, 200, and 300 kg N ha<sup>-1</sup>), three rates of P (0, 60, and

**Table 1. Mean monthly temperature, monthly rainfall, and soil analysis of two environments. Osorno 2005-2006 and 2006-2007.**

	Osorno	
	2005/2006	2006/2007
	Monthly rainfall (mm)	
September	56.70	106.40
October	28.80	144.20
November	85.10	41.90
December	30.70	100.70
January	89.20	18.90
	Monthly mean temperature (°C)	
September	9.10	8.90
October	10.60	10.30
November	13.20	12.30
December	14.70	14.70
January	15.50	16.20
	Soil analysis	
pH (in water)	4.20	5.03
Organic matter, %	7.60	5.79
N-NO <sub>3</sub> , mg kg <sup>-1</sup>	9.30	10.00
P Olsen, mg kg <sup>-1</sup>	27.70	18.70
K, mg kg <sup>-1</sup>	200.90	262.60

120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and two rates of K (0 and 150 kg K<sub>2</sub>O ha<sup>-1</sup>) and four replications. The N was applied as urea (46-0-0). Phosphate was applied as triple superphosphate (0-46-0), and K as KCl (0-0-60). Experimental units consisted of six rows, 5 m long and spaced 17.5 cm apart. The experiment was sowed on 31 September 2005 with a seeding rate of 8 kg ha<sup>-1</sup>. Glyphosate (N-(phosphonomethyl) glycine; 2 L ha<sup>-1</sup>) was applied 2 d after seeding. At seeding all fertilizers were applied and incorporated in seeding furrows at 2 cm depth, N was not partialized. Plots were hand weeded. The two-center rows of each plot were swathed and 7 d later threshed with a stationary plot combine (according to method developed by Simpson (1993b). Harvest date was 3 January 2006.

### Experiment 2. Nitrogen fertility

In the 2006-2007 growing season, only the effect of N was evaluated because there was no response to P and K on most of the characters evaluated the first season. The experiment had four N rates: 0, 50, 100, and 150 kg N ha<sup>-1</sup>. The design was a RCB with four replicates and experimental units consisted of six rows, 5 m long and spaced 20 cm apart. Each N rate was split-in-half and hand-broadcasted in two stages: leaf stage (1.2) and rosette stage (2.0) (Simpson, 1993a). Nitrogen fertilizer used was urea. No other fertilizers were applied since the

soil analysis indicated soil P and K levels were high. The experiment was planted on 22 August 2006 with a seeding rate of 8 kg ha<sup>-1</sup>. Glyphosate (2 L ha<sup>-1</sup>) was applied before seeding. Thereafter plots were hand weeded. The two-center rows of each plot were swathed by hand and 7 d later threshed with a stationary plot combine. Harvest date was 5 January 2007.

### Experiment 3. Sulphur fertility

The S fertilizer experiment was conducted in the 2005-2006 and 2006-2007 growing seasons at Osorno. The experimental design was a RCB with two rates 0 and 40 kg S ha<sup>-1</sup> and with different split-application treatments and four replications. Treatments were: control treatment (T1), 25 kg S ha<sup>-1</sup> applied at seeding and 15 kg ha<sup>-1</sup> applied at stage 2.0 (T2), 15 kg S ha<sup>-1</sup> at seeding and 25 kg ha<sup>-1</sup> at stage 2.0 (T3), 40 kg S ha<sup>-1</sup> at seeding (T4), and 40 kg S ha<sup>-1</sup> at stage 2.0 (T5). Planting was done no-till with a preplant application of glyphosate (2 L ha<sup>-1</sup>) for weed control. Seeding dates were 22 September 2005 and 22 August 2006. Seeding rate was 8 kg ha<sup>-1</sup> and experimental units had six rows of 5 m long and spaced 20 cm apart.

Harvest was conducted on 3 January 2006 and 5 January 2007 when the first four fruits on main stem started to shed the seed, stage 5(4.0) (Simpson, 1993a; Berti *et al.*, 1998).

In all experiments, plant height was measured at harvest for five plants in each plot. Seed yield was obtained from a 2 m section of both center rows of each plot. Test weight was calculated by determining the weight of 40 mL of seed from a clean seed sample and 1000-seed weight was calculated by counting 250 seeds of each experimental unit.

A sample of 0.1 g of dried ground plant tissue was analyzed by the Kjeldahl procedure to determine total seed N. Seed N evaluations were only conducted for Experiment 1 for the N treatments 0, 100, 200, and 300 kg N ha<sup>-1</sup>, and three replications, and for Experiment 3 samples from 2005-2006 for 0 and 40 kg S ha<sup>-1</sup>, treatments T1 through T5, and three replications.

Seed oil content was determined on 40 mL of clean dried seeds with a Nuclear Magnetic Resonance (NMR) Analyzer (Newport 4000, Oxford Institute Limited, Oxford, England), at the Department of Plant Sciences, North Dakota State University. Oil content was expressed on a 0% moisture basis. This is the standard procedure for determining oil content of oilseeds (Robertson and Morrison, 1979). Oil yield was calculated multiplying seed yield by oil content.

Fatty acids analysis for Experiment 1 was done only for the samples with 0, 100, 200 and 300 kg N ha<sup>-1</sup>, and three replicates. Fatty acid analysis was conducted at the Northern Crops Science Research Laboratory-USDA,

Fargo, North Dakota. Seed samples (10 whole borage seeds per sample) were ground with a glass hex wrench on a 2 mL vial. Hexane chloroform sodium methoxide solution (HCSM, 0.5 M, 1 mL) was added to a vial (2 mL). The samples were derivatized by vortexing. Aliquots of the sample (200 µL) were diluted with HCSM (200 µL) in separate vials and injected on to the gas chromatographer (Hewlett-Packard 5890 Series II, Palo Alto, California, USA), equipped with a flame-ionization detector and an autosampler/injector. Analyses were conducted on a DB-23 30 m x 0.25 mm column with a 0.25 µm film (J&W Scientific, Folsom, California, USA). Analysis was conducted as follows: column flow 1.9 mL min<sup>-1</sup> with helium head pressure of 200 kPa; injector split flow at 50-100 mL min<sup>-1</sup>; column oven temperature at 190 °C for 5 min followed by a ramp to 220 °C at 10 °C min<sup>-1</sup> with a 1 min hold ending with a ramp to 240 °C at 20 °C min<sup>-1</sup> with a 5 min hold; and injector and detector temperatures set at 230 and 250 °C, respectively. Standard curves of methyl-caprate, methyl stearate, and methyl oleate from Nu-check 21A and 411 provided standards for calculating equivalent chain length (ECL) values, which were used to make FAME assignments. Each sample was run with two replicates.

Fatty acid composition analysis for 2006-2007 Experiments 2 and 3 was performed at the Facultad de Ingeniería Agrícola, Universidad de Concepción, Chillán, Chile. Gas chromatography (GC) of fatty acid methyl esters (FAME) was performed with a gas chromatograph (Varian 3900, Palo Alto, California, USA) equipped with a flame ionization detector (FID). Analyses were conducted on CP-WAX 52 CB, 30 m x 0.25 mm column with an external diameter of 0.39 mm and the size of filling particle of 0.25 µm. Analysis was conducted with a set temperature of 120 to 240 °C, in three stages 120 °C for 3 min, increasing temperature in 3 °C min<sup>-1</sup> until 210 °C, maintaining that temperature for 55 min, finally temperatures were increased in 15 °C min<sup>-1</sup> until 240 °C for 65 min. The temperature detector was set at 300 °C. The temperature injector was set at 200 °C, with a flow of 1 mL min<sup>-1</sup>. Standard curves of methyl oleate, methyl linoleate, and methyl linolenate were used to confirm response factors for the GC FID that matched those previously reported by Ackman (2002).

### Statistical analysis

Statistical analysis was conducted using standard procedures for a RCBD with a factorial arrangement for Experiment 1, and RCBD for Experiment 2 (Steel and Torrie, 1980). N, P, K, and S effects were considered fixed for all analysis. For Experiment 3, each location-year combination was defined as an 'environment' and was considered a random effect in the statistical analysis.

Residual mean squares were compared for homogeneity among environments for each trait. If homogeneous, then a combined ANOVA was performed across environments. Means separation was performed by applying *F*-protected Least Significant Differences (LSD) comparisons at  $P \leq 0.05$  level of significance. The estimated variance of pairwise mean differences and the corresponding degrees of freedom were calculated to estimate the correct LSD values for comparison of significant. Combined analysis for Experiment 3 was analyzed as a RCBD for only two treatments 0 and 40 kg S ha<sup>-1</sup> and as a RCBD with five treatments, including different split-applications. Linear and quadratic regression models were evaluated for each variable. The regression models and all parameter estimates were significant at  $P \leq 0.05$ . SAS System was used to process the data (SAS Institute, 2005).

## RESULTS AND DISCUSSION

### Experiment 1. Interaction of N, P, and K effect

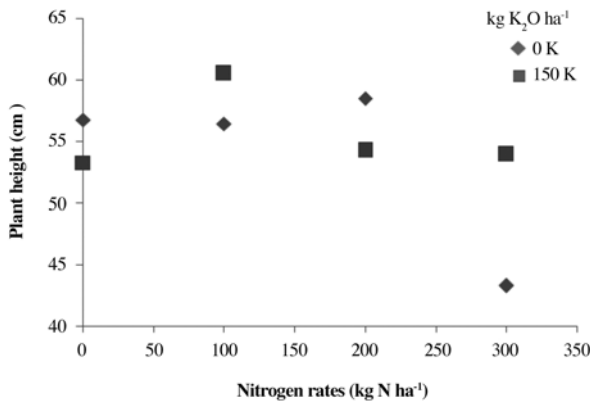
**Plant height.** The ANOVA indicated that plant height was affected by N, P, and the interaction between N and K (Table 2). The interaction between N and K was significant because when 300 kg N ha<sup>-1</sup> and 0 kg K<sub>2</sub>O ha<sup>-1</sup> were applied the plant height was greatly reduced (Figure 1); however, this reduction was not significant when 150 kg K<sub>2</sub>O were added with 0 K added. This occurred because plants branched more when more N was available and this reduced plant height, only when K availability was reduced. Potassium plays an important role in plant turgescence; therefore is more likely to grow in height with higher N if K is available.

**Seed yield.** Seed yield was not affected by N, P, K, (or their interactions) (Table 2). The high soil P and K level explain the lack of response of borage to these nutrients. Other reports indicated also that N fertility levels did not affect seed yield on an experiment conducted at Alberta, Canada, although in this study the non-response to N was explained by the high initial nitrate content in the soils (El-Hafid *et al.*, 2002a; 2002b). Soil nitrate content was 9.3 mg kg<sup>-1</sup> for this experiment, classified as a low to medium level (Soil Department, University of Concepción), which would not explain the non-response to N observed. Seed yields obtained in this study are similar to those obtained by other researchers (El-Hafid *et al.*, 2002a; 2002b). The crop did not have other limitations for growth and development such as water stress, pH or management. Although soil pH is low there was not a concern for high Al which was tested but analysis is not available to report it. Nitrogen fertility experiments conducted in same experimental site and in same or different growing season with other crops such as flax (*Linum usitatissimum* L.),

Table 2. Sources of variation, mean squares, and significance for plant height, seed yield, 1000-seed weight, seed N, oil content, and fatty acid composition for four levels of N, three levels of P, and two levels of K and their interactions. Osorno, Chile, 2005-2006.

SOV	df	Plant height	Seed yield	df	1000-seed weight	Test weight	Seed N	Oil content	Oil yield	Palmitic acid	Oleic acid	Linoleic acid	GLA
Rep	3	513	42 405	2	7.1	4.1	2.40	16.5	3766*	3.05	5.07	0.038	3.51
N	3	399**	21 172	3	57.6*	158.9*	0.29	3.5	1507	0.41	0.55	0.024	0.21
P	2	221*	13 764	2	17.6	17.3	-	0.3	1097	-	-	-	-
K	1	176	24 247	1	1.4	4.2	-	0.4	1816	-	-	-	-
N x P	6	23	12 554	6	15.0	59.7*	-	1.0	957	-	-	-	-
N x K	3	223*	313	3	7.9	36.0*	-	0.7	726	-	-	-	-
P x K	2	118	5 158	2	44.4*	40.9*	-	0.7	24	-	-	-	-
N x P x K	6	65	20 322	6	8.3	9.9	-	0.8	1452	-	-	-	-
Error	72	52	10 440	48	7.6	9.0	0.36	1.8	912	0.10	1.38	0.388	1.08
CV, %		156	50		18.5	8.1	4.06	4.4	48	9.20	6.7	1.7	4.6

\*\* $P \leq 0.01$ ; \* $P \leq 0.05$ ; SOV: sources of variation; df: degrees of freedom; GLA: gamma-linolenic acid.



**Figure 1. Interaction response of N and K in plant height in borage fertility experiment in 2005-2006 growing season.**

canola (*Brassica napus* L.), camelina (*Camelina sativa* L.), and mustard (*Brassica juncea* L.) all had a very clear response to increasing N rates. This indicates that there were no other soil limitations to the lack of response, but the nature of borage growth and development. It is hard to determine borage seed yield response to N since the crop shatters heavily. Most of grain yield in borage is shattered which increases the difficulty to measure grain yield response to N. The plant also has an indeterminate growth habit and seed shatter of 75% is common (Berti *et al.*, 2002).

**1000-seed weight, test weight and seed N.** The 1000-seed weight was affected by N fertility levels, and by the interactions between P x K and the test weight was affected by N fertility levels and the N x K, P x K, and N x P interactions (Table 2). Seed weight decreased as N fertility rates were increased from 16.4 g with 0 kg N ha<sup>-1</sup> to 12.3 g with 300 kg N ha<sup>-1</sup> (Table 3). Seed N, and therefore, seed protein content did not increase significantly on this study although there was an increasing trend as N rate increased (Table 2). The slightly higher seed N for the treatment with 200 kg N ha<sup>-1</sup> may explain the lower 1000-seed weight and test weight at higher N rates. Generally,

a higher N fertility increases seed protein content. Protein utilizes less space on the seed than starch, reducing the seed weight and test weight (Otterson *et al.*, 2007). A reduction on thousand-kernel weight as N fertility increased has been reported for spring wheat (Otterson *et al.*, 2007). The interaction between P and K for 1000-seed weight occurred because the seed weight increased from 13.7 g to 17.3 g 1000 seeds<sup>-1</sup> when the P fertilizer was increased from 60 to 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> without K fertilizer added, on the other hand, when the same P rates were used and 150 kg K<sub>2</sub>O ha<sup>-1</sup> as added there was not a significant increase in seed weight indicating P is more important than K for seed dry matter accumulation. Both significant interactions, N x P and N x K indicate that highest test weight was observed when rates used were the highest, 42.8 kg hL<sup>-1</sup> (300:120 N:P kg ha<sup>-1</sup>) and 39.8 (300:150 N:K kg ha<sup>-1</sup>). The interaction between P x K indicate that lowest test weight was observed with 0 P<sub>2</sub>O<sub>5</sub> kg ha<sup>-1</sup> and 150 kg K<sub>2</sub>O ha<sup>-1</sup>.

**Oil content and oil yield.** Oil content and yield was not affected by N, P, K, or their interactions (Table 2). Also, El-Hafid (2002b) did not find effect of N rates on oil and GLA content. In sunflower (*Helianthus annuus* L.), excessive N fertilization favors vegetative growth and reduces seed oil content (Scheiner *et al.*, 2002), effect that was not observed in this experiment. Oil content in borage grown in southern Chile fluctuates between 300 and 330 g kg<sup>-1</sup> and this character depends mainly on harvest stage, temperature during seed development, and seed maturity. Oil content increases when the majority of the seed harvested are at or past physiological maturity (Berti *et al.*, 1998).

**Fatty acid composition.** Nitrogen levels did not have an effect on any of the fatty acids analyzed for this experiment (Table 2). Average GLA content was 22.5%. The only other research that has evaluated GLA content for different N rates was El-Hafid *et al.* (2002b), who did not find significant differences for GLA content. Previous studies in Chile and in North Dakota, USA,

**Table 3. Effect of four N rates on plant height, seed yield, 1000-seed weight, and test weight averaged across three P rates (0, 100, and 200 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and two K rates (0 and 150 kg K<sub>2</sub>O ha<sup>-1</sup>). Osorno, Chile, 2005-2006.**

Nitrogen rates	Plant height	Seed yield	1000-seed weight	Test weight	Oil content	Oil yield	GLA
kg ha <sup>-1</sup>	cm	kg ha <sup>-1</sup>	g	kg hL <sup>-1</sup>	g kg <sup>-1</sup>	kg ha <sup>-1</sup>	%
0	55	243	16.4	39.3	312	72	22.4
100	58	224	15.7	39.0	306	65	22.5
200	54	224	15.2	32.9	306	64	23.0
300	48	274	12.3	37.6	302	50	22.5
P > F value	0.0002	0.118	0.0003	<0.0001	0.1427	0.1907	-

GLA: gamma-linolenic acid.

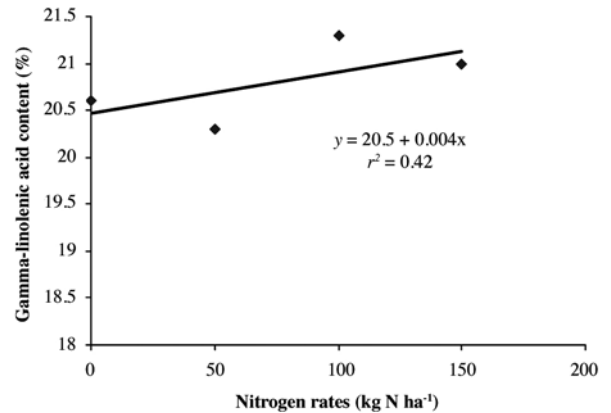
have evaluated the factors that influence GLA content and fatty acid composition in borage seeds (Berti *et al.*, 2002). Air temperature during seed development and seed maturity at harvest are the key factors to the fatty acid composition of borage seed oil. Berti *et al.* (2002) reported GLA content to fluctuate from 18.4% to 26.4% in the northern most and warmer location evaluated, Chillan to Puerto Varas in Southern Chile. The differences in GLA content were associated to cooler temperatures during seed development.

### Experiment 2. Nitrogen fertility

**Seed yield.** This experiment also showed a non significant response of seed yield to N fertility levels as in Experiment 1 (Tables 2 and 4). The results were similar to results in Experiment 1, which may be explained for the high shattering of the crop that difficult the ability of measuring differences. As explained for Experiment 1 soil pH, water stress, or other soil limiting condition do not explain the lack of response in this study. The rainfall for this season was much higher than the one before, this may have caused more N to leach in deeper layers of the soil and not be available for borages shallow-root system.

### Oil content, yield and fatty acid composition

Nitrogen fertility levels did not have an effect on oil content, oil yield, palmitic acid, stearic acid, oleic acid, and linoleic acid (data not shown) (Table 4). The only significant response was observed for GLA (Figure 2). As N rates were increased GLA increased; however, the difference between the control treatment and the highest rate was only 0.6 percentage points. Seed oil GLA contents were lower than required by industry (22%). Probably, this was due to high temperatures during seed development. El-Hafid *et al.* (2002b) did not find significant differences for seed oil GLA with increasing N fertility rates. However, delaying harvest had a significant effect on seed oil GLA due to lower temperatures during seed development. Presumably, a higher N availability allowed the crop to grow vegetative longer, then at



**Figure 2. Response of gamma-linolenic acid content in borage seeds to nitrogen fertility in the 2006-2007 growing season ( $r^2 = 0.42$  significant at  $P < 0.05$ ,  $df = 14$ ).**

harvest the plants from the higher N treatments were at an earlier stage of maturity (5:(1.0)) than the other treatments at probably (5:(4.0)) or more (Berti *et al.*, 2002). Previous research indicates that the first mature seeds from the plants (stage (5:1:0)) are known to have higher GLA content (Berti *et al.*, 1998; 2002). This indicates that the positive effect of N on GLA content may be indirect effect and not an effect of the N itself.

### Experiment 3. Effect of sulfur fertilizer

**Seed yield.** The effect of S application was significant for seed yield when analyzed without split-application effects (Table 5). Mean seed yield increased 98 kg ha<sup>-1</sup> when 40 kg S ha<sup>-1</sup> were applied. There were no significant differences among treatments when all S was applied at seeding or part of it was split at seeding and the rest applied at the rosette stage (Table 6). There is no reported information on S requirements for borage. As a reference, S uptake of canola is 60 kg ha<sup>-1</sup> and its deficiency decreases seed yield (Jackson, 2000). Canola and wheat are commonly fertilized with 40 kg S ha<sup>-1</sup> for soils with low and medium levels in southern Chile where this study was conducted.

**Table 4. Sources of variation, mean squares, and significance for seed yield, oil yield, oil content, 1000-seed weight and test weight for four N levels (0, 50, 150, and 200 kg N ha<sup>-1</sup>). Osorno, Chile, 2006-2007.**

SOV	Seed yield	Oil yield	Oil content	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	GLA
Rep	23 743	4428	0.61	0.02	0.1	1.33	0.02	0.2
N	2 467	609	0.85	0.00064	0.2	0.62	0.14	0.59*
Error	4 205	279	1.19	0.014	0.21	0.22	0.11	0.19
CV, %	20	16	3.70	1.15	9.2	2.9	0.8	2.1

Significant at  $P \leq 0.06$ ; GLA: gamma-linolenic acid.

**Table 5. Sources of variation, mean squares, and significance for seed yield, oil yield, oil content, fatty acids composition and seed N for two S levels averaged across two environments. Osorno, Chile, 2005-2006 and 2006-2007.**

SOV	df	Seed yield	df	Oil yield	Oil content	df	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	GLA	Seed N
Env	1	51 980	1	2511	17.59	1	0.14	38.82	2.052	0.467	1.189	-
Rep (Env)	6	10 212	4	1069	0.43	2	0.02	0.18	0.757	0.027	0.198	0.717
S	1	34 918*	1	2427	6.59	1	0.009	0.01	0.193	0.022	0.118	0.019
S x Env	1	2 363	1	1373	0.36	1	0.0012	0.04	0.065	0.095	0.497	-
Error	26	7 162	19	610	0.90	11	0.043	0.099	0.105	0.249	0.131	3.45
CV, (%)		22.7		22.1	3.10		1.97	8.2	1.97	1.33	1.74	12.7

\* Significant at  $P \leq 0.05$ ; GLA: Gamma-linolenic acid.

**Table 6. Sources of variation, mean squares, and significance for seed yield, oil content, oil yield, fatty acid composition and seed N for five treatments of sulfur application averaged across two environments. Osorno, Chile, 2005-2006 and 2006-2007.**

SOV	df	Seed yield	df	Oil yield	Oil content	df	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	GLA	Seed N
Env	1	53 964	1	4340	22.8	1	0.011	36.9	2.00	0.033	0.38	-
Rep (Env)	6	10 212	4	1069	0.4	2	0.016	0.17	0.75	0.027	0.09	0.71
Treatment	4	10 512	4	708	2.0	4	0.088	0.01	0.1	0.465	0.06	3.54
T x Env	2	10 931	2	1391	1.1	2	0.022	0.02	0.02	0.190	0.42	-
Error	22	7 269	15	672	0.87	7	0.017	0.13	0.13	0.103	0.14	2.98
CV, %		22.9		23.2	3.1		1.25	9.5	2.18	0.86	1.79	11.9

\*Significant at  $P \leq 0.05$ ; GLA: gamma-linolenic acid.

**1000-seed weight and test weight and seed N.** Sulfur rates and the interaction with environment were not significant ( $P \leq 0.05$ ) for 1000-seed weight and test weight (Tables 5 and 7). Sulfur rates did not have an effect on seed N for the S treatments in the 2005-2006 season (Table 5); therefore mean 1000 seed-weight, test weight, and seed N data is not shown.

**Seed oil content, yield, and oil composition.** Sulfur rates and the interaction with environment were not significant for seed oil content, oil yield, palmitic acid, stearic acid, oleic acid, and linoleic acid (Tables 5 and 7). Sulfur application increased slightly the GLA content. Sulfur application of 22 kg ha<sup>-1</sup> increased seed oil content in canola, in sites testing low for S (Jackson, 2000) but this response was not observed in this study.

## CONCLUSIONS

Seed yield was not affected by N, P, K, or their interactions in the conditions this study was conducted. Both 1000-

seed weight and test weight were affected by N fertility levels and some of the interactions between N x K, N x P, and P x K. Seed weight decreased as N fertility rates were increased. As N rates increased GLA content increased significantly by 0.6%. The effect of S application was significant for seed yield. Mean seed yield increased 98 kg ha<sup>-1</sup> when 40 kg ha<sup>-1</sup> of S was applied. There were no yield differences for the split-applications of S treatments. Results indicate that borage has a response to sulfur applications more than N for the soil studied; further research is needed to determine the interactions between N and S applications.

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**Table 7. Effect of sulfur rates and time of sulfur application on mean seed yield, oil yield, seed oil content, palmitic acid, stearic acid, oleic acid, linoleic acid, and gamma-linolenic acid (GLA) averaged across two environments. Osorno, Chile, 2005-2006 and 2006-2007.**

	Sulfur rates		Split-application of S rates (kg ha <sup>-1</sup> ) at 1,2 leaf stage/rosette stage			
	0	40	40/0	25/15	15/25	0/40
Seed yield, kg ha <sup>-1</sup>	306b	404a	451	406	430	368
Seed oil content, g kg <sup>-1</sup>	304	298	306	297	298	298
Oil yield, kg ha <sup>-1</sup>	93	121	129	120	131	114
Palmitic acid, %	10.5	10.5	10.4	10.7	9.9	10.6
Stearic acid, %*	4.4	3.4	1.2	4.1	3.9	1.1
Oleic acid, %	16.5	16.5	17.1	16.5	16.6	16.4
Linoleic acid, %	37.5	37.2	37.6	37.4	35.9	37.4
GLA, %	20.5b	22.1a <sup>1</sup>	21.7	21.0	21.2	21.0
Seed N, %	2.76	2.77	2.83	2.89	2.42	2.95

<sup>1</sup>Only significant difference was observed for GLA content ( $P \leq 0.06$ ).

## RESUMEN

### Respuesta de borraja (*Borago officinalis* L.) a la fertilización con N, P, K, y S en el Centro Sur de Chile.

La borraja (*Borago officinalis* L.) es una oleaginosa con alto contenido de ácido gamma-linolénico (GLA) en su semilla. El objetivo de este estudio fue determinar la respuesta en rendimiento de semillas, contenido y composición del aceite de borraja, a la fertilización con N, P, K y S. Tres experimentos fueron conducidos en Osorno (40°22' S, 73°04' O; 72 m.s.n.m.), Chile. El primer experimento fue conducido en la temporada 2005-2006, con cuatro dosis de N (0, 100, 200 y 300 kg N ha<sup>-1</sup>), tres dosis de P (0, 60 y 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) y dos dosis de K (0 y 150 kg K<sub>2</sub>O ha<sup>-1</sup>). El segundo experimento fue conducido en el 2006-2007, cuando sólo se evaluaron cuatro dosis de N (0, 50, 100, y 150 kg N ha<sup>-1</sup>) ya que en la primera temporada no se observó respuesta a P y K. El tercer experimento se realizó en 2005-2006 y 2006-2007 y se evaluaron dos dosis de S, 0 y 40 kg S ha<sup>-1</sup>. El rendimiento de semillas no fue afectado por la dosis de N, P, K o la interacción entre ellos en ninguno de los experimentos. A medida que aumentó la dosis de N se observó un aumento en el contenido de GLA. La fertilización con 40 kg S ha<sup>-1</sup> aumentó en promedio el rendimiento de semillas en 98 kg ha<sup>-1</sup>. Los resultados indican que la borraja tiene una mejor respuesta a S que a N en las condiciones evaluadas de suelos con alto nivel de P y K; sin embargo, se requiere de un estudio en mayor profundidad para determinar el efecto de las interacciones entre N y S.

**Palabras clave:** producción de semilla, ácido gamma-linolénico.

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