# Effects of starter nitrogen on early growth and nodulation of lentil (*Lens culinaris* Medik.)

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## Abstract

Effects of additional nitrogen (N) on early growth and nodulation of *Lens culinaris* Medik. (lentil) were examined under glasshouse and field conditions. Under glasshouse conditions, N effects on lentil, *Vicia faba* L. cv.Puma (field bean) and *Phaseolus vulgaris* L. cv. Sanilac (navy bean) were compared.

For lentil cvs. Olympic and Titore under glasshouse conditions, leaf area, and shoot and root fresh weight (f.wt.) and dry weight (d.wt.), when nodules were first visible, increased with increased applied nitrate ($NO_3^-$) concentration to 5.0 mol/m$^3$ then changed little with additional $NO_3^-$ thereafter. Increases were substantial in all cases. For example, the increase in leaf area with additional $NO_3^-$ was > 100% for both cvs. Additional $NO_3^-$ (10 mol/m$^3$) had similar effects on navy bean as on lentil. Field bean showed little response to additional $NO_3^-$. Three weeks after nodules were first visible, nodule d.wt of all species decreased with additional $NO_3^-$ (10 mol/m$^3$). This decrease was greater for lentil cv. Olympic (92%) and navy bean (100%) than for field bean (60%).

The field experiment was autumn sown (23 May) and used cv. Olympic only. Leaf area and shoot and root d.wt at the onset of nodulation were lower in the field than in the glasshouse and were not affected by additional N (calcium ammonium nitrate) up to 100 kg/ha. Eighty five days after sowing, shoot d.wt in the field was similar to that at the onset of nodulation in the glasshouse. At this time, leaf area and shoot d.wt were respectively, 80 and 33% greater with additional N (100 kg/ha). Nodule d.wt was 60% lower with additional N.

***Additional key words:*** Phaseolus vulgaris *L., navy bean,* Vicia faba *L., field bean, nitrate*

## Introduction

In most agricultural soils, low nitrogen (N) availability is the major nutrient factor limiting growth and yield of many crops. Nitrogen is the most commonly used fertilizer world wide and for a range of crops, the economic return from its use is usually substantial (Wibberley, 1989; Hay and Walker, 1989). The primary effect of adding N is to increase crop photosynthesis and hence yield (Andrews *et al.*, 1991). Nitrogen can affect photosynthetic rate per unit leaf area by, for example, altering concentrations of photosynthetic pigments or enzymes (Dejong and Doyle, 1985; Lawlor *et al.*, 1987a,b; Olesinki *et al.*, 1989). However, often the main effect of additional N on photosynthesis is due to changes in the total leaf area and hence light absorption (Metivier and Dale, 1977; Novoa and Loomis, 1981; Radin, 1983; Andrews *et al.*, 1985, 1991; Sinclair and Horie, 1989 ). Plant species differ in their response to additional N. For example, many legume species can obtain a substantial amount of their N requirement via symbiotic N fixation and in comparison with cereals, their response to additional N is generally lower (Saxena, 1981). For grain legumes prior to nodulation, plant N requirements are met from seed reserves and soil N. Effects of additional N on grain legumes prior to nodulation are greatly dependent on species. For example, additional N at sowing often results in a substantial increase in growth of navy bean prior to nodulation but usually has little effect on field bean (Sprent and Thomas, 1984; Andrews *et al.*, 1992 ). On the negative side, additional N was found to cause greater inhibition of nodulation with navy bean than with field bean (Andrews *et al.*, 1992). There have been few studies of N effects on lentils. Since the crop is of increasing importance in Canterbury, information on the effect of soil fertility on lentil growth may prove of benefit to Canterbury farmers. The objectives of the present study were to determine the effects of additional N on early growth and nodulation of lentil.

## Materials and Methods

### Source of plant material

Seeds of *Lens culinaris* Medik. (lentil) cvs. Olympic (mean seed weight=67 mg) and Titore (34 mg) were obtained from Challenge Seeds, Christchurch, New Zealand. Seeds of *Phaseolus vulgaris* L. cv. Sanilac, (navy bean, 110 mg) and *Vicia faba* L. cv. Puma, (field bean, 409 mg) were obtained from Rogers Brothers, Christchurch, New Zealand and Plant Breeding Institute, Trumpington Lane, Cambridge, UK, respectively.

### Glasshouse experiments

In experiment 1, seeds of lentil cvs. Olympic and Titore were inoculated with nodule extract obtained from mature plants and germinated on moist paper towels (distilled water) in the dark at room temperature. Seedlings with a shoot length of about 20 mm were transferred to 100 mm diameter, 200 mm tall pots (1 plant per pot ) containing a perlite/vermiculite (1:1) mixture soaked in basal nutrient solution (Andrews *et al.*, 1989) containing the appropriate nitrate ($NO_3^-$) concentration. Seven different applied $NO_3^-$ concentrations ranging from 0 to 20 mol/m$^3$ were used. Nitrate was added as $KNO_3$ and potassium was maintained at 23.6 mol/m$^3$ by the addition of $K_2SO_4$ as necessary. The rooting medium was flushed every 2 days with the appropriate nutrient solution. Nodule extract was added to all pots with the nutrient solution 2 and 7 days after planting (DAP). Plants were grown in a glasshouse under natural spring daylight. The photoperiod was approximately 12 hours and the temperature ranged from 15-30°C although it was rarely above 25°C. Plants were sampled every 3 days commencing 7 DAP and roots were examined for nodules. Plants were harvested 18 DAP when nodules were visible. Leaf area for all plants was determined with a Licor model L1 3100, area meter. Plants were divided into root, shoot and cotyledon for fresh weight (f.wt) determination. Plant material was oven dried at 70°C to constant weight.

In experiment 2, seeds of lentil cv. Olympic, navy bean and field bean, were inoculated and germinated as in experiment 1. Plants were grown under the same conditions as experiment 1 and were supplied 0, 1 or 10 mol/m$^3$ $NO_3^-$. At first harvest, when nodules were visible (19, 20 and 21 DAP for lentil, navy bean and field bean, respectively), leaf area and shoot and root f.wt and dry weight (d.wt) were determined as in experiment 1. At second harvest, which was 21 days after nodules were first visible, nodule d.wt was determined.

### Field experiment

The trial site was a Templeton silt loam at the Lincoln University Henley block. The previous crop was annual ryegrass (*Lolium multiflorum* Lam.) The paddock was prepared using standard farm practice. The design was a randomized complete block factorial with 3 levels of N (0, 50, and 100 kg/ha) and 4 replicates. Plot size was 21 m$^2$.

Seeds of lentil cv. Olympic were sown on 23 May, 1991 using an Oyjord cone seeder at a rate equivalent to 150 plants/m$^2$. Calcium ammonium nitrate (28%N) was applied once on 25 May 1991. Weeds were controlled by pre-emergence application of cyanazine at 1.1 kg a.i./ha. Nitrogen did not affect plant population throughout the experiment. Six plants were sampled randomly from each plot when nodules were first visible which was 50 days after sowing (DAS). Plants were also sampled 85 and 148 DAS. Measurements taken at 50 and 85 DAS were as in glasshouse experiments 1 and 2 respectively. Leaf area and shoot d.wt were determined 148 DAS.

### Data Analysis

All data were subjected to analysis of variance. All effects discussed have F values with probability $p < 0.01$.

## Results and Discussion

The objectives of the present study were to determine the effects of additional N on early growth and nodulation of lentil. For both cvs. Olympic and Titore under glasshouse conditions, leaf area and shoot and root f.wt and d.wt, when nodules were first visible, increased with increased applied $NO_3^-$ concentration to 5.0 mol/m$^3$ then changed little with additional $NO_3^-$ (Fig. 1.). Increases were substantial in all cases. For example, the increase in leaf area with additional $NO_3^-$ to 5.0 mol/m$^3$ was >100% for both cvs. (Fig. 1A). For both cvs., cotyledons contributed little (<10%) to total plant f.wt or d.wt at harvest: values were not affected by $NO_3^-$ (Fig. 1 B,C).

Previously, effects of additional N on early growth of grain legumes have been shown to be dependent on species. Additional N at sowing usually has a much greater effect on members of the *Phaseoleae* (sub-tropical origin) such as navy bean, *Glycine max* (L.) Merr. (soybean) and *Vigna unguiculata* (L.) Walp. (cowpea) than on the temperate species, field bean, *Pisum sativum* L. (pea) and *Lupinus albus* L. (white lupin) (Sprent and Thomas, 1984; Andrews *et al.*, 1992). Data obtained for navy bean and field bean in the present study are in agreement with previous findings (Table 1.).

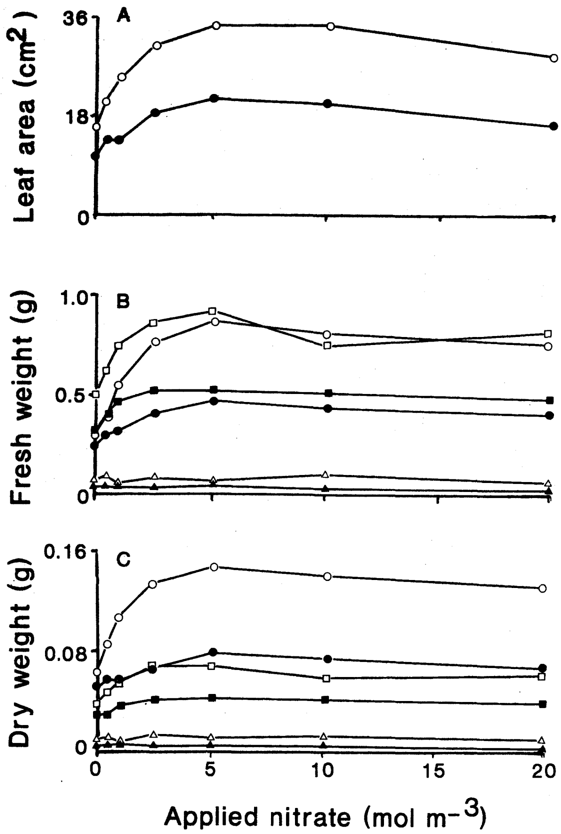


**Figure 1. Effects of different concentrations of applied nitrate on leaf area (A) and fresh weight (B) and dry weight (C) of shoot (O,●), root (□,■) and cotyledon (△,▲) of lentil cvs. Olympic (open symbols) and Titore (closed symbols).**

For example, additional $NO_3^-$ caused a 133% increase in leaf area of navy bean but had little effect on leaf area of' field bean. The magnitude of the increase in leaf area and shoot f.wt and d.wt was similar for lentil and navy bean. Thus it appears that with regard to N effects on early growth, lentil is different from other temperate grain legumes studied. Differences between grain legume species with regard to N effects on early growth have been related to pattern of germination (epigeal or hypogeal), area/d.wt of the first foliage leaves and whether the main site of $NO_3^-$ assimilation is the root or shoot (Sprent and Thomas, 1984). More recent work indicates that for field bean and navy bean at least, differences in response to $NO_3^-$ are primarily due to differences in $NO_3^-$ uptake (Andrews *et al.*, 1992). In experiments 1 and 2 here, there is no doubt that uptake of $NO_3^-$ by lentil was substantial in view of the magnitude of its growth response to additional $NO_3^-$ (Fig. 1, Table 1). Substantial $NO_3^-$ uptake by legumes is usually associated with decreased nodule weight (Andrews *et al.*, 1992). In the present study, additional $NO_3^-$ caused a decrease in nodule d.wt of all species, but this decrease was greater for lentil cv. Olympic (92%) and navy bean (100%) than for field bean (60%) (Table 1). Greater inhibition of nodulation with lentil and navy bean than with field bean is likely to be related to greater $NO_3^-$ uptake (Andrews *et al.*, 1992).

In the field, $NO_3^-$ concentration in the interstitial water of non-fertilized agricultural soil in temperate regions is likely to be 1 mol/m³ or more after cultivation and hence during seedling development (Barber, 1984; Haynes *et al.*, 1986; Wild, 1988). Thus response of lentil to additional N is unlikely to be as great in the field as in the glasshouse with a 0 N treatment. The field experiment described here was autumn sown. During the first three months of the experiment, monthly mean values for daily air and soil temperatures ranged from 4.4-8.8°C and 1.6-6.0°C respectively (Table 2). At such temperatures, germination, seedling growth and nodulation of lentil are slow (McKenzie, 1987) and nodules were not visible until 50 DAS. At this time, leaf area and shoot and root d.wt were not affected by additional N (Table 3). This differs from findings in the glasshouse (Table 1). Leaf area and shoot and root d.wt at the onset of nodulation were much lower in the field than in the glasshouse (Tables 1, 3) and it is likely that the main factor limiting growth in the field was low temperature and not N. At second harvest in the field (85 DAS), shoot d.wt was similar to that at the onset of nodulation in the glasshouse (Tables 1,3). At this time, leaf area and shoot d.wt were respectively 80 and 33% greater with additional N (100 kg/ha). Nodule d.wt was 60% lower with additional N. Thus, findings in the field and glasshouse are consistent. At the third harvest (180 DAS), leaf area and shoot d.wt increased from 93 to 145 cm² and 680 to 1082 mg per plant respectively with additional N from 0 to 100 kg/ha, indicating that N effects on early growth were maintained in the longer term. Effects of N on yield remain to be determined.

**Table 1. Effects of different concentrations of applied nitrate ($NO_3^-$) on growth of three legume species. Shoot, root and cotyledon (cot) measurements were taken when nodules were first visible; nodule (nod) dry weight was determined 3 weeks after nodules were first visible.**

| Species | Applied $NO_3^-$ (mol/m³) | Leaf area (cm²) | Fresh weight (mg) | | Dry weight (mg) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Shoot | Root | Shoot | Root | Cot | Nod |
| Lentil | 0 | 11.1 | 274 | 534 | 59 | 41 | - | 26 |
| | 1 | 15.3 | 348 | 676 | 73 | 52 | - | 16 |
| | 10 | 25.0 | 580 | 856 | 119 | 74 | - | 2 |
| | SEM | 1.7 | 32 | 66 | 6 | 4 | - | 2 |
| Navy bean | 0 | 32.5 | 1283 | 1881 | 194 | 115 | - | 154 |
| | 1 | 46.3 | 1680 | 2308 | 200 | 123 | - | 131 |
| | 10 | 75.7 | 3408 | 3362 | 289 | 160 | - | 0 |
| | SEM | 3.1 | 126 | 174 | 13 | 9 | - | 8 |
| Field bean | 0 | 33.0 | 1479 | 2982 | 137 | 166 | 81 | 168 |
| | 1 | 39.1 | 1772 | 2978 | 164 | 153 | 87 | 140 |
| | 10 | 38.1 | 1858 | 1903 | 161 | 109 | 127 | 67 |
| | SEM | 2.1 | 152 | 178 | 10 | 7 | 20 | 11 |

**Table 2. Monthly mean values for mean daily temperatures, monthly rainfall and daily solar radiation during the period of study in 1991 compared with long term means (values in parentheses).**

| Month | Temperatures (°C) | | | Rainfall (mm) | Solar Rad (MJ/m²/d) |
|---|---|---|---|---|---|
| | Air | Grass | Soil | | |
| May | 8.8 (8.7) | 1.1 (-0.9) | 6.0 (6.9) | 13.6 (71) | -[1] (7.3) |
| June | 5.5 (6.2) | -0.9 (-1.6) | 2.8 (4.5) | 105.5 (61) | -[1] (5.5) |
| July | 4.4 (5.7) | -2.5 (-1.3) | 1.6 (4.0) | 39.8 (68) | -[1] (6.3) |
| August | 8.7 (6.7) | 1.1 (-0.6) | 3.6 (5.2) | 12.7 (62) | -[1] (9.6) |

[1] data not available

**Table 3. Effects of different rates of applied nitrogen (N) on leaf area and shoot, root and nodule (nod) dry weight of autumn sown (23 May) lentil.**

| Applied N (kg/ha) | Harvest date: 12 July | | | | 16 August | | | |
|---|---|---|---|---|---|---|---|---|
| | Leaf area (cm²) | Dry weight (mg) | | | Leaf area (cm²) | Dry weight (mg) | | |
| | | Shoot | Root | Nod | | Shoot | Root | Nod |
| 0 | 1.74 | 19 | 6 | - | 3.04 | 45 | 11 | 5 |
| 50 | 1.78 | 19 | 6 | - | 3.74 | 50 | 11 | 2 |
| 100 | 1.76 | 19 | 6 | - | 5.48 | 60 | 12 | 2 |
| SEM | 0.24 | 2 | 1 | - | 0.13 | 3 | 1 | 1 |

## Conclusions

1. Under glasshouse and field conditions, additional N can cause a substantial increase in early growth of lentil.
2. Increased growth of lentil with additional N is associated with decreased nodule d.wt.

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