

# EFFECT OF NITROGEN AND RHIZOBIUM INOCULATION ON YIELD AND BIOLOGICAL N<sub>2</sub> FIXATION OF BLUE LUPINS (LUPINUS ANGUSTIFOLIUS L.)

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## ABSTRACT

The effects of N supply and *Rhizobium* inoculation on biological N<sub>2</sub> fixation and yield of blue lupins were investigated in pot experiments with a mixture of sand and soil as substrate and <sup>15</sup>N labelled fertiliser. This seems important, as 90% of lupin-growing area in Germany consist of blue lupins because their anthracnosis tolerance and the knowledge about N<sub>2</sub>-fixing capacity of German blue lupin strains is inadequate. <sup>15</sup>N was used to differ between fertiliser-born N and the N derived from dinitrogen fixation.

*Rhizobium* inoculation increased the symbiotic biological N<sub>2</sub> fixation of lupins in absence of mineral nitrogen. In presence of mineral N the effect of *Rhizobium* inoculation was not significant. *L. angustifolius* fixed considerable amounts of dinitrogen also after flowering. For getting high yield of blue lupins, a *Rhizobium* inoculation is essential.

Mineral N supply at sowing increased dry matter production, but decreased partly the biological N<sub>2</sub> fixation of *Rhizobium* inoculated blue lupins. The total N amounts were increased by N supply at sowing. Nevertheless, nitrogen fertilisation of blue lupins at sowing is not essential if the soil could supply the plant N demand the for early growth stages.

At flowering mineral N supply affected only slightly the dry matter production of *L. angustifolius* and reduced the biological N<sub>2</sub> fixation after flowering in inoculated plants. Therefore, a late N supply to blue lupins is not recommended.

The N dynamics, that means the relationship between biological N<sub>2</sub> fixation, supply of combined N, and plant yields was similar to yellow lupins and contrary to white lupins. The biological N<sub>2</sub> fixation of *L. angustifolius* appears relatively tolerant to mineral N supply.

Further studies are necessary to quantify the N<sub>2</sub> fixation with native *Rhizobium* strains and to specify the proportion of different N origin in lupin plants.

## KEYWORDS

*Lupinus angustifolius*, dinitrogen fixation, nitrogen fertilisation, *Rhizobium* inoculation

## INTRODUCTION

*Lupinus angustifolius* L. is an interesting crop in agricultural land use systems (Cowling *et al.* 1998, Hanelt, 1960, Römer, 2007, Stelzner and Merbach, 1978). Due to their biological N<sub>2</sub> fixing ability, their high phosphate-mobilising efficiency (Dinkelaker *et al.* 1989) and their deep root system support lupins the soil fertility (Römer, 2007, Stelzner and Merbach, 1978).

Otherwise their seeds due to their high protein and low starch contents compared to other endemic grain legumes are good animal food intermixtures (Merbach *et al.* 1980, Merbach, 1983, 1985, Meixner *et al.* 1982/83, Römer, 2007). During the last years the growing area of lupins amounted between 33 000 and 45 000 ha in Germany (Römer, 2007).

A third advantage of lupin growth is the low labor intensity during April to June, the months with highest fertilisation and pesticide treatments in cereal production in Germany.

Unlike white (*L. albus*) and yellow lupins (*L. luteus*), the blue lupins (*Lupinus angustifolius*) is tolerant against the lupins illness *Anthracnosis*. Therefore, more than 90% of the lupins growing area are tilled with blue lupins in Germany. Additionally, blue lupins are more lime tolerant than yellow lupins and contain low amounts of antinutritive substances like tannine, lectine and protease inhibitors.

Compared to the white and yellow lupins, the knowledge about biological N<sub>2</sub> fixing ability and the nutrient efficiency of blue lupins at German growth conditions are low (Merbach and Schilling, 1980, Merbach *et al.* 1983, Schulze *et al.* 1999). In practice fertilisation instructions are easily adopted from the other lupins. To realise optimal yields of blue lupins a detailed knowledge about the biological N<sub>2</sub> fixing ability (duration, amount and affectability) is essential, since N acts as a basic nutrient of seed proteins and therefore affects the yield amount and quality.

The present study shows first results of the effect of biological N<sub>2</sub> fixing activity, *Rhizobium* inoculation as well as mineral N fertilisation on the yield of blue lupins at German conditions.

## MATERIAL AND METHODS

### a) Experimental setup and treatments

Blue (*Lupinus angustifolius* L., var. Boruta), were grown in pots containing a mixture of 5 kg quartz sand and 1 kg soil. The soil contained 0.76 g K (double lactate soluble) and 0.087 g soluble and exchangeable N per kg soil. Substrate was mixed and seeds were inoculated with *Rhizobium lupini* (peat inocula, HStick, Radicin).

N fertilisation was varied according to the treatments shown in Table 1. The N fertilisation (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>

(10 at-% <sup>15</sup>N<sub>exc.</sub>) was applied at sowing and at flowering time. Plants were harvested at flowering and at maturity.

### b) Measuring and methods

After harvesting the plants were divided into root, shoot and pods, dried until weight kept constant and powdery milled. Total N was analysed using a C/N analyser (Elementar, Hanau). The <sup>15</sup>N-abundance was measured using the NOI 7-emission spectrophotometer (Fischer, Leipzig).

The calculations of the nitrogen derived from the air based on these assumptions could be partly incorrect since the plant N uptake derived from the soil was not measured and higher mineral N supply has shown to inhibit biological N<sub>2</sub> fixation in other lupins species (Merbach *et al.* 1980)

### c) Calculation of the N origin in plants

The source of nitrogen in plants was calculated as follows:

$$\frac{\text{Plant dry matter} \times \text{N content (\%)}}{100}$$

$$(1) \text{ Total-N} =$$

$$100$$

$$(2) \text{ Fertiliser-N}^{(1)} = \text{mg } ^{15}\text{N}_{\text{exc.}} \text{ within the plant} \times 10$$

$$(3) \text{ Seed-N}^{(2)} = 27 \text{ mg (measured)}$$

$$(4) \text{ Soil derived N}^{(3)} = \frac{86.8 \text{ mg (measured)} \times ^{15}\text{N} - \text{fertiliser N uptake (\%)}}{100}$$

$$(5) \text{ Biologically fixed N}_2 = \text{Total N} - (\text{fertiliser derived N} + \text{seed N} + \text{N deposition}^{(4)} + \text{soil derived N})$$

<sup>1)</sup> <sup>15</sup>N-enrichment in the fertiliser applied = 10 at% <sup>15</sup>N<sub>exc.</sub>

<sup>2)</sup> Assumption: a total N-consumption of the plant out of these sources.

<sup>3)</sup> Assumption: uptake percental analog to the <sup>15</sup>N- fertiliser uptake, which amounted of 30.3–40.2% and 37.0–51.5% of the applied <sup>15</sup>N-fertiliser at flowering and maturity, respectively.

<sup>4)</sup> N-Deposition: 10.3 kg N ha<sup>-1</sup> (Immissionsbericht 2006, Landesamt für Umweltschutz Sachsen-Anhalt), that equals to 3.7 mg N per pot and 6.2 mg N per pot at flowering (after 6 weeks growth) and at maturity (after 10 weeks growth), respectively, related to the pot total surface area.

## RESULTS

### a) *Rhizobium* inoculation effect on N supply and yield

Plant dry matter yield is shown in Figure 1. At both flowering and maturity harvest times *Rhizobium* inoculation increased the yield of blue lupins when the plants were not fertilised with mineral N. After mineral N fertilisation a *Rhizobium* induced yield increase was not detectable.

In detail the *Rhizobium* inoculation and mineral N fertilisation effects on the plant root, shoot and pod dry matter production are shown in Figure 2. Primarily, *Rhizobium lupini* inoculation increased the dry matter production of the pods irrespective of mineral N fertilisation. However, the non inoculated control plants also showed a relatively high yield, probably due to high soil mineral N content or

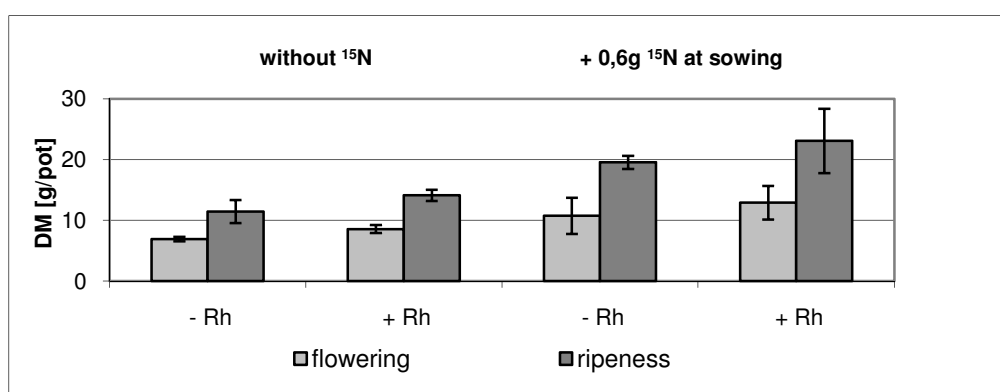
*Rhizobium* wild populations. N supply at sowing increased both seed and vegetative plant parts in inoculated and non inoculated treatments (Fig. 2, middle column pair). At flowering mineral N fertilisation showed only minimal yield effects.

### b) *Rhizobium* inoculation and N fertilisation effects on the symbiotic biological N<sub>2</sub> fixation and total N uptake of blue lupins

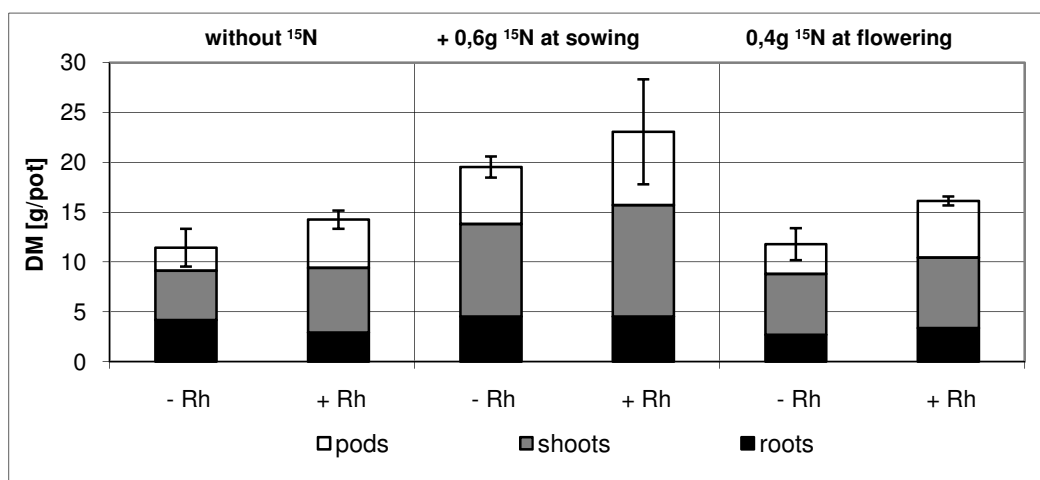
Figure 3 demonstrates that even without *Rhizobium* inoculation a clear biological N<sub>2</sub> fixation was detectable, presumably due to a root infection by native *Rhizobium* strains derived from the soil, which similarly was detected at white and yellow lupins earlier (Merbach, 1997). Furthermore, the biologically fixed nitrogen was clearly increased

**Table 1.** Inoculation and fertilisation treatments of the pot experiment (5 kg quartz sand + 1 kg soil per pot). Fertilisation: N as  $(^{15}\text{NH}_4)_2\text{SO}_4$  (10 at-%  $^{15}\text{N}_{\text{exc}}$ ), 1 g K as  $\text{K}_2\text{SO}_4$  (at sowing), 3 replicates per treatment, 4 plants/pot.

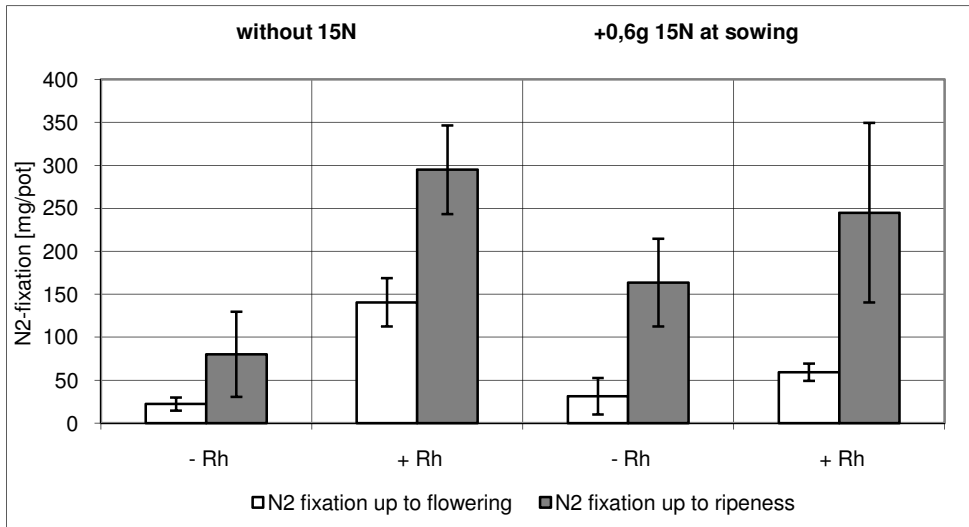
Treatment numbers	<i>Rhizobium</i> inoculation	Fertilisation per pot	Harvest time
1a	without	without $^{15}\text{N}$	Flowering
1b	with	without $^{15}\text{N}$	
2a	without	0.6 g $^{15}\text{N}$ at sowing time	Maturity
2b	with	0.6 g $^{15}\text{N}$ at sowing time	
3a	without	without $^{15}\text{N}$	Maturity
3b	with	without $^{15}\text{N}$	
4a	without	0.6 g $^{15}\text{N}$ at sowing time	Maturity
4b	with	0.6 g $^{15}\text{N}$ at sowing time	
5a	without	0.4 g $^{15}\text{N}$ flowering time	Maturity
5b	with	0.4 g $^{15}\text{N}$ flowering time	



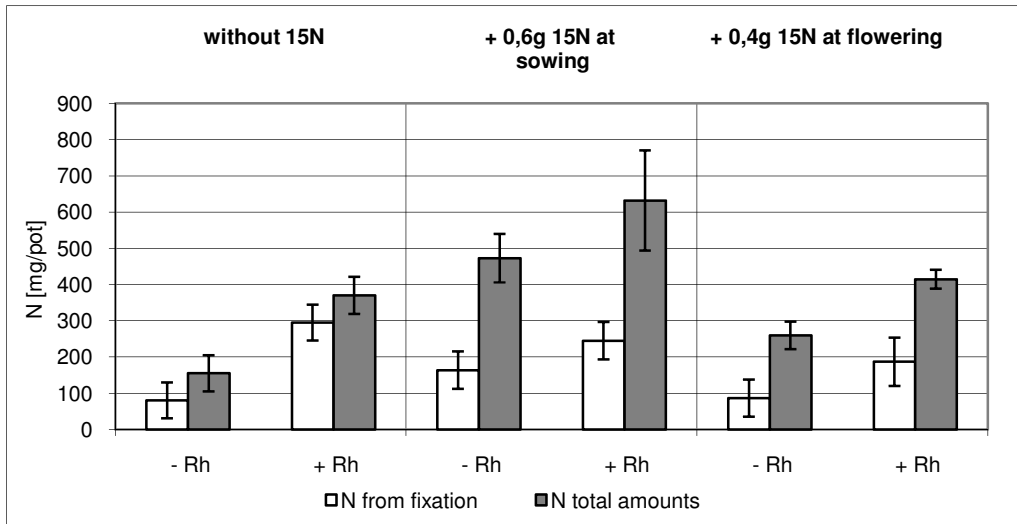
**Fig. 1.** Blue lupins dry matter (DM) yield at flowering and maturity after inoculation with *Rhizobium lupini* and with and without mineral N fertilisation  $(^{15}\text{NH}_4)_2\text{SO}_4$ , 10 at-%  $^{15}\text{N}_{\text{exc}}$ .



**Fig. 2.** Blue lupins root, shoot and pods dry matter (DM) yield at maturity with and without inoculation with *Rhizobium lupini* and with or without mineral N fertilisation  $(^{15}\text{NH}_4)_2\text{SO}_4$ , 10 at-%  $^{15}\text{N}_{\text{exc}}$ .



**Fig. 3.** N<sub>2</sub> fixation of blue lupins with and without *Rhizobium* inoculation and without and with mineral N fertilisation (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 10 at-% <sup>15</sup>N<sub>exc.</sub>) at flowering and maturity.



**Fig. 4.** Total N and N derived from dinitrogen fixation in blue lupins with and without *Rhizobium* inoculation and without and with mineral N fertilisation (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 10 at-% <sup>15</sup>N<sub>exc.</sub>), analysed at maturity.

between flowering and maturity that means during the generative phase of plant growth, which is similar to the N<sub>2</sub> fixing properties of the yellow lupins and field beans (*Vicia faba* L.) (Merbach and Schilling, 1980). Consequentially, the N<sub>2</sub> fixing activity was increased after *Rhizobium* inoculation without mineral N fertilisation mainly after flowering (Fig. 3). As expected, the mineral N fertilisation at sowing inhibited the biological N<sub>2</sub> fixation. However, due to a high variability between pot replicates these data are not significant. Former results obtained with other legumes are partly verified (Merbach and Schilling, 1980). The 'apparent' increase of the N<sub>2</sub> fixing activity in the non inoculated control after mineral N fertilisation at sowing could be due to a N fertiliser induced higher uptake of unlabelled soil N.

Figure 4 shows which effect *Rhizobium* inoculation and mineral N fertilisation have on the total N yield and the proportion of biologically fixed N<sub>2</sub> at blue lupins. Generally *Rhizobium* inoculation increased both the total N and the biological fixed N<sub>2</sub> of blue lupins irrespective of mineral N fertilisation. The not-inoculated variants fixed dinitrogen also. That means the blue lupins is at least partly tolerant for mineral N.

The total N uptake of blue lupins fertilised with mineral N at sowing time was increased after *Rhizobium* inoculation (Fig. 4). However, the proportion of biological fixed N<sub>2</sub> amounted up to 80% in the treatment without mineral N fertilisation and only 39% in the treatment with mineral N fertilisation applied at sowing time.

Apparently, mineral N application inhibits the proportion of biological N<sub>2</sub> fixation at the total plant N uptake. Instead of N<sub>2</sub> fixation a higher amount of soil or fertiliser N was incorporated and finally the total N uptake of the plant was significantly increased. Nevertheless, a mineral N fertilisation is not recommended for soils containing a sufficient amount of available mineral N at sowing time since the replicated data are highly variable.

A mineral N fertilisation at flowering (Fig. 4, right columns) reduced the biological N<sub>2</sub> fixation compared to the control plants with a reduced proportion of fixed N<sub>2</sub> at the total N uptake in the inoculated treatment. Therefore, the total N uptake was not increased in the inoculated treatment compared to the treatment without N fertilisation. Again, a mineral N fertilisation could not be recommended at flowering time. In their N dynamics the blue lupins reacts similar to the yellow lupins and contrary to the white lupins (Merbach and Schilling, 1980). However, the biological N<sub>2</sub> fixing activity seems to be quite robust against mineral N. Final conclusions could not be drawn until the N derived from the soil has been quantified exactly within the lupins plant samples.

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