

## **EFFECT OF INOCULATION OF SEEDS WITH NITRAGINA ON DEVELOPMENT AND YIELD OF BLUE LUPINE (*Lupinus angustifolius* L.)**

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**Abstract.** The response of two cultivars of blue lupine – Zeus and Kalif – to the bacterial inoculant Nitragina was estimated in this study. Field experiment was carried out in 2009-2011. It was located at the Experimental Station of the Faculty of Biology and Agriculture in Krasne, owned by the University of Rzeszów. In comparison to the control, application of Nitragina results in prolongation of the budding and flowering stages, as well as a delay in reaching the full maturity of plants. After inoculation of seeds with nodule bacteria the number of pods per plant and TSW increased significantly, whereas the number of seeds per pod did not change. The use of Nitragina resulted in a significant growth in seed yield by  $0.3 \text{ t}\cdot\text{ha}^{-1}$ , i.e. 13%, as compared with the control. In 2009 cultivar Zeus gave significantly higher yield than cultivar Kalif. In following years of the study the studied cultivars yielded on a statistically similar level. Cultivar Kalif, in comparison with Zeus, was characterized by a longer growing period and significantly smaller number of pods per plant. Total protein content in seed dry matter increased significantly under the influence of bacterial inoculant in relation to seeds obtained from not inoculated plants.

**Keywords:** bacterial inoculant – Nitragina, blue lupine cultivars, nodule bacteria, plant protein, yield structure components

### **INTRODUCTION**

Lupines have a considerable nutritive value resulting from a large content of protein with rather good biological value. Their growing in crop rotation is justified both from natural and economic point of view [Księżak and Podleśny 2005, Faligowska and Szukała 2007, Krawczyk *et al.* 2010]. One of the most essential processes that occur during the growth of lupines, determining the height and quality of yield, is entering into effective symbiosis with nodule bacteria. In this way the plants will be provided with atmospheric nitrogen, which allows reduction in the use of mineral nitrogen to the

start rate [Faligowska and Szukała 2010, Pudełko 2010]. Inoculation of seeds with *Nitragina* is usually abandoned in the case when lupine often occurs in crop rotation. It happens, however, that the number of symbiotic bacteria in soil is small and their lines are different and not very virulent. In soil also bacteriophages occur which destroy beneficial bacteria [Borucki 1998, Martyniuk *et al.* 2005, Martyniuk and Oroń 2007, Martyniuk and Oroń 2008, Sujkowska 2009]. The use of *Nitragina* allows limiting the effect of those unfavorable phenomena. Bacterial inoculant contains live symbiotic bacteria suitable for the given plant species. Therefore it is important for the sake of research to determine the effects of *Nitragina* application in the lupine cultivation technology. This results from the fact that economic importance of lupines is growing systematically, both in pure stands and in mixtures [Galek *et al.* 2006, Podleśny 2009, Podleśny and Podleśna 2010].

The aim of this study was to assess the response of blue lupine plants on the bacterial inoculant *Nitragina*. In the research hypothesis it was assumed that the inoculant would have a favorable effect on seed yield, as well as on the protein content in seeds.

## MATERIAL AND METHODS

In 2009-2011 a strict two-factorial field experiment with blue lupine was carried out. It was located at the Experimental Station at Krasne owned by the Faculty of Biology and Agriculture of the University of Rzeszów (50°03' N; 22°06' E). The first factor was inoculation of seeds: *Nitragina*, without *Nitragina*, the other, the cultivars: Kalif and Zeus.

The experiment was conducted in the randomized split-plot design in four replications.

The soil in which the experiment was conducted was classified as the good wheat complex of soil quality class IIIa, with acid or slightly acid pH value. The content of available macro- and microelements was moderate, and only that of magnesium was low. Analysis of soil was conducted in the laboratory of the Regional Chemical and Agricultural Station in Rzeszów.

Blue lupine seeds were sown at the earliest possible times: 16.04.2009, 13.04.2010 and 05.04.2011. Seeds were dressed with Sarfun T 450 FS. The bacterial inoculant came from the Processing-Service-Trade Plant BIOFOOD s.c. – Wałcz. *Nitragina* was applied on seeds directly before sowing according to the instructions of the producer. The seeding rate for both cultivars was 110 pcs·m<sup>-2</sup>. Qualified seed came from the Plant Breeding Smolice, branch Przebędowo. The previous crop each year was winter wheat. The area of plot for sowing was 15 m<sup>2</sup> and for harvest 12 m<sup>2</sup>. Fertilization with phosphorus (triple superphosphate 46%) and potassium (potash salt 60%) was applied under autumn plowing at rates: 26.2 kg·ha<sup>-1</sup> P and 66.4 kg·ha<sup>-1</sup> K. Starter fertilization with mineral nitrogen was not applied. The preparation Afalon Dyspersyjny 450 SC in a dose of 1.5 dm<sup>3</sup>·ha<sup>-1</sup> was used to eliminate weeds and the preparation Gwarant500 SC in a dose of 2 dm<sup>3</sup>·ha<sup>-1</sup> to control diseases.

Plant density per 1 m<sup>2</sup> was calculated in the emergence stage and before harvest. Dates of individual developmental stages during the growing period were recorded: full emergences, the beginning of budding and flowering and full maturity. To assess lodging at the technical maturity stage, 9° scale was used. Biometric measurements were made on 20 plants randomly collected from each plot.

Seed harvest with a combine harvester was conducted on: 20.08.2009, 24.08.2010 and 19.08.2011. Desiccation was not applied. Seed yield was adjusted to 15% humidity. Total protein content in seeds was determined with the Kjeldahl method.

Humidity-thermal conditions (Table 1) were given according to Agrometeorological Bulletins IMiGW in Warsaw, based on recordings of the Meteorological Station at Jasionka near Rzeszów.

Table 1. Mean daily air temperature and total rainfalls in 2009-2011 according to the Meteorological Station in Rzeszów

Tabela 1. Średnia dobową temperatura powietrza i sumy opadów w latach 2009-2011 według Stacji Meteorologicznej w Rzeszowie

Rainfall – Opady, mm				Temperature – Temperatura, °C			
2009	2010	2011	1986-2008	2009	2010	2011	1986-2008
April – Kwiecień							
3.7	49.9	50.0	50.6	11.1	8.9	10.3	8.7
May – Maj							
102.6	177.0	49.2	80.8	13.8	14.3	13.9	13.9
June – Czerwiec							
146.4	126.1	88.5	82.0	16.6	17.9	18.1	17.0
June – Lipiec							
98.0	200.2	233.7	88.2	20.7	20.8	18.6	19.0
August – Sierpień							
21.8	98.6	28.6	68.8	19.4	19.5	19.0	18.2

The course of the weather conditions in 2009 and 2011 was favorable for plant growth and development. In the period from April to August, the total rainfall amounted to 372.5 mm and 450.0 mm, respectively. In the same period of 2010, in turn, heavy rainfalls occurred amounting to 651.8 mm. They prolonged the plant growth and seed drying.

The results of the study were analyzed statistically using the analysis of variance. Significance of differences between the values of characters were tested based on confidence half-intervals, at the significance level  $P = 0.05$ . Calculations were made using the ANALWAR-5FR program.

## RESULTS AND DISCUSSION

The number of plants after emergences amounted on average to 106.5 pcs·m<sup>-2</sup>, whereas before harvest 96.3 pcs·m<sup>-2</sup>. The studied factors did not differentiated significantly plant density (Table 2). Krawczyk *et al.* [2010] report that for branching (traditional) forms of lupine about 20% smaller seeding rate is recommended, as compared with epigonal (determinate) forms. The author also proved that diversified density of lupine plants has an effect on the weed infestation of crops [Krawczyk 2009].

The application of Nitragina did not have a significant effect on plant lodging. The average degree of lodging for the cultivar Kalif was 7.5, and for the cultivar Zeus – 7.7. Similar results concerning lodging of the studied cultivars are given by COBORU [2010].

Table 2. Plant density and degree of lodging (means in years)  
Tabela 2. Obsada i stopień wylegania roślin (średnia z lat)

Seed inoculation Szczepienie nasion	Cultivar Odmiana	Liczba roślin – Number of plants		Lodging Wyleganie 1-9°
		after emergence, pcs. · m <sup>-2</sup> po wschodach, szt. · m <sup>-2</sup>	before harvest, pcs. · m <sup>-2</sup> przed zbiorem, szt. · m <sup>-2</sup>	
Control Kontrola	Kalif Zeus	108 104	97 94	7.6 7.8
Nitragina	Kalif	108	98	7.4
	Zeus	106	96	7.5
LSD <sub>0,05</sub> – NIR <sub>0,05</sub> for – dla:				
inoculation – szczepienia		ni – ns	ni – ns	ni – ns
cultivars – odmian		ni – ns	ni – ns	ni – ns
interaction – interakcji:				
inoculation x cultivars szczepienia x odmiany		ni – ns	ni – ns	ni – ns

ni – ns – non-significant differences – różnice nieistotne

Plant emergences were recorded on average after 22 days from the date of sowing. In the study by Podleśny [2009], the period from sowing to emergences was differentiated in years and ranged from 8 to 19 days and according to the author, this was affected mainly by the weather conditions.

The present study indicated that the bacterial inoculant affected the prolongation of the following stages: budding and flowering and full maturity, in relation to the control (Table 3). The effect of different cultivation practices on the duration of individual developmental phases was also indicated by Podleśny [2009]. The cultivar Kalif, in comparison with Zeus, was characterized by a longer growing period, which is similar to the results of experiments carried out by COBORU [2010].

Table 3. Length of developmental stages in days from sowing date (means in years)  
Tabela 3. Długość faz rozwojowych w dniach od daty siewu (średnia z lat)

Seed inoculation Szczepienie nasion	Cultivar Odmiana	Emergence Wschody	Budding Pąkowanie	Flowering Kwitnienie	Full maturity Dojrzałość pełna
Control Kontrola	Kalif Zeus	22 22	49 47	56 53	115 112
Nitragina	Kalif	22	52	60	118
	Zeus	22	48	55	116
Means for factors – Średnie dla czynników					
Control – Kontrola		22	48.0	54.5	113.5
Nitragina		22	50.0	57.5	117.0
Kalif		22	50.5	58.0	116.5
Zeus		22	47.5	54.0	114.0

The obtained results of the study allowed showing the significant effect of the bacterial inoculant on increasing the number of pods per plant and TSW. Nevertheless, the number of seeds per pod did not depend on the experimental factors (Table 4). Faligowska and Szukała [2010] observed a favorable effect of inoculation of seeds with

Nitragina on the studied functional parameters of legume plants. These first of all concerned yield structure components, i.e. the number of seeds and their weight.

Table 4. Yield elements and total protein content in seeds (means in years)  
Tabela 4. Komponenty plonu i zawartość białka ogólnego w nasionach (średnie z lat)

Seed inoculation Szczepienie nasion	Cultivar Odmiana	Number of pods per plant Liczba strąków na roślinie	Number of seeds per pod Liczba nasion w strąku	Thousand seed weight Masa tysiąca nasion g	Protein Białko %
Control Kontrola	Kalif Zeus	4.4 5.0	3.6 3.5	143.0 140.0	28.5 29.0
Mean for control Średnia dla kontroli		4.7	3.6	141.5	28.8
Nitragina	Kalif Zeus	4.8 5.6	3.7 3.5	153.0 152.0	29.5 32.4
Mean for Nitragina Średnia dla Nitraginy		5.2	3.6	152.5	31.0
Mean for cultivar Średnia dla odmian	Kalif Zeus	4.6 5.3	3.7 3.5	148.0 146.0	29.0 30.7
Mean – Średnia		4.95	3.6	147.0	29.9
LSD <sub>0.05</sub> – NIR <sub>0.05</sub> for – dla:					
inoculation – szczepienia		0.47	ni – ns	10.78	1.98
cultivars – odmian		0.64	ni – ns	ni – ns	ni – ns
interaction – interakcji:					
inoculation x cultivars szczepienia x odmiany		ni – ns	ni – ns	ni – ns	ni – ns

ni – ns – non-significant differences – różnice nieistotne

The cultivar Kalif, in comparison with Zeus, was characterized by a significantly smaller number of pods per plant. The other yield structure components and the protein content in seeds were not diversified in respect of cultivars. According to Galek *et al.* [2006], there is a significant variability of yield structure components in individual lines and cultivars of blue lupine. This however refers to differentiation of traditional cultivars in relation to the determinate ones.

Results of the study showed a significant effect of Nitragina on increasing content of total protein in seeds in relation to seeds obtained from the control plots. Wiatr *et al.* [2007] report that the content of total protein in blue lupine seeds is subject to genetic variability and the effects of the habitat. The extreme values of protein content in their study ranged from 29.4 to 34.2%.

The results of the study confirmed that a bacterial inoculant significantly increases the seed yield (Table 5). Difference in relation to not inoculated plants – on average for the years of the study – was 0.3 t·ha<sup>-1</sup>, i.e. 13%. Favorable effect of Nitragina on the results of legume cultivation was also found by Truchliński *et al.* [2002], Faligowska and Szukała [2010] and Pytlarz-Kozicka [2010]. The cultivars Zeus and Kalif gave yields on the statistically identical level, except for 2009, where the cultivar Zeus gave significantly better yields than the cultivar Kalif. High instability and fluctuations of lupine yields in particular years is also reported by Podleśny [2007], Prusiński [2007], and Podleśny *et al.* [2010].

Table 5. Seed yield, t·ha<sup>-1</sup>  
Tabela 5. Plon nasion, t·ha<sup>-1</sup>

Seed inoculation Szczepienie nasion	Cultivar Odmiana	Year – Rok			Mean in years Średnia z lat
		2009	2010	2011	
Control Kontrola	Kalif Zeus	1.95 3.02	1.88 2.25	2.32 2.38	2.05 2.55
Mean for control – Średnia dla kontroli		2.49	2.07	2.35	2.30
Nitragina	Kalif	2.45	2.12	2.48	2.35
	Zeus	3.38	2.75	2.42	2.85
Mean for Nitragina – Średnia dla Nitraginy		2.92	2.44	2.45	2.60
Mean for cultivars Średnia dla odmian	Kalif	2.20	2.00	2.40	2.20
	Zeus	3.20	2.50	2.40	2.70
Mean – Średnia		2.70	2.25	2.40	2.45
LSD <sub>0.05</sub> – NIR <sub>0.05</sub> for – dla:					
inoculation – szczepienia		0.33	0.29	0.09	0.25
cultivars – odmian		0.85	ni – ns	ni – ns	ni – ns
interaction – interakcji:					
inoculation x cultivar szczepienia x odmiany		ni – ns	ni – ns	ni – ns	ni – ns

ni – ns – non-significant differences – różnice nieistotne

## CONCLUSIONS

1. Application of the bacterial inoculant Nitragina resulted in prolongation of the budding and flowering stages, as well as in a delay of the full maturity of blue lupine plants.

2. Nitragina had an effect on a significant increase in the number of pods per plant, 1000 seed weight, seed yield and on increasing the total protein content in blue lupine seeds.

3. Cultivar Kalif was characterized by a longer period of growing and a significantly smaller number of pods per plant, in comparison with cultivar Zeus.

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## WPLYW INOKULACJI NASION SIEWNYCH NITRAGINĄ NA PLONOWANIE ŁUBINU WĄSKOLISTNEGO (*Lupinus angustifolius* L.)

**Streszczenie.** W badaniach oceniono reakcję dwóch odmian łubinu wąskolistnego – Zeus i Kalif na szczepionkę bakteryjną Nitraginę. Doświadczenie polowe przeprowadzono w latach 2009-2011. Zlokalizowane ono zostało w Stacji Doświadczalnej Wydziału Biologiczno-Rolniczego w Krasnem, należącej do Uniwersytetu Rzeszowskiego. W porównaniu z obiektem kontrolnym zastosowanie Nitraginy spowodowało wydłużenie fazy pąkowania, kwitnienia oraz opóźnienie osiągnięcia dojrzałości pełnej roślin. Po inokulacji nasion siewnych bakteriami brodawkowymi istotnie wzrosła liczba strąków na roślinie i MTN, zaś liczba nasion w strąku nie uległa zmianie. Efektem użycia Nitraginy był istotny wzrost plonu nasion o  $0,3 \text{ t} \cdot \text{ha}^{-1}$ , tj. 13% w odniesieniu do kontroli. W 2009 r. odmiana Zeus plonowała istotnie wyżej niż Kalif. W kolejnych latach badane odmiany plonowały na statystycznie jednakowym poziomie. Odmiana Kalif, w porównaniu z Zeus, odznaczała się dłuższym okresem wegetacji i istotnie mniejszą liczbą strąków na roślinie. Zawartość białka ogólnego w suchej masie nasion istotnie wzrosła pod wpływem szczepionki bakteryjnej w stosunku do nasion uzyskanych z roślin nie szczepionych.

**Słowa kluczowe:** bakterie brodawkowe, białko roślinne, elementy struktury plonu, odmiany łubinu wąskolistnego, szczepionka bakteryjna Nitragina

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