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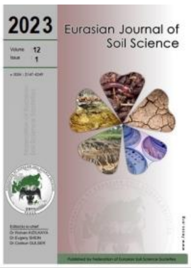

Assessing the biomass yield and nitrogen fixation of Lupinus angustifolius varieties as green manure in Jalisco, Mexico

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Abstract

Limited information is available in Mexico regarding the use of *Lupinus angustifolius* L. as a green manure. This study aimed to assess the effectiveness of six *Lupinus angustifolius* varieties as green manure in terms of above-ground biomass production, expressed as dry matter (DM), and total nitrogen (N) accumulation at successive harvest dates. Additionally, the study aimed to estimate N_2 fixation 110 days after sowing (DAS). The varieties Haags Blaue, Boregine, Borlu, Probor, Sonate, and Boruta were sown during the winter season of 2018-2019 using a randomized block factorial design. The N difference method was employed to estimate N_2 fixation, with wheat serving as the reference crop. Data on above-ground biomass production, N concentration, and total N accumulation were recorded at different harvest times: 80, 95, and 110 DAS. The biomass yield of all varieties significantly increased from the first to the last harvest, with the highest yield observed at the final harvest (ranging from 7,632 to 10,200 kg ha^{-1}). The highest total N accumulation from biomass was recorded at the last harvest. On average, the Borlu, Boregine, Haags Blaue, and Boruta varieties accumulated 195.4 kg ha^{-1} of total N (ranging from 195.6 to 221.2 kg ha^{-1}). The proportion of N derived from the atmosphere (%Ndfa) through N_2 fixation averaged 80.09% (ranging from 72% to 93%), resulting in an average N fixation of 160 kg ha^{-1} (ranging from 106 to 185 kg ha^{-1}) in above-ground biomass. All six varieties demonstrated potential as green manure, considering their above-ground biomass production, total N accumulation, and ability to fix N_2.

Keywords: Dry matter, harvest date, legumes, lupins, N_2 fixation, Rhizobia.

Introduction

Leguminous green manure crops have been widely reported to play an important role in managing soil health and recently received greater attention for improving soil fertility and agricultural sustainability (Meena et al., 2018). In favorable conditions, legumes can produce up to 8-10 t ha^{-1} of dry matter (DM) as manure crops, adding up to 300 kg ha^{-1} of nitrogen (N) to soil (Talgre et al., 2012). Green manures may also provide other benefits, such as a nutrient source for future crops, conservation of soil water, and control of plant pests, pathogens, and weeds (McSorley, 1999; Ross et al., 2001).

The genus *Lupinus* belongs to the family Leguminosae and is well known in several parts of the world for its economic value as a food source, fodder, and green manure due to its ability to obtain atmospheric N through biological nitrogen fixation (BNF) (Carranca et al., 2013). In such a scenario, the BNF process is considered an important route of N entry to agricultural and forestry systems (Ridley et al., 2004). Compared to other legumes, lupin species are considered to be highly efficient at fixing atmospheric N (Unkovich et al., 2010). The genus *Lupinus* includes 200 to 500 species, but only *L. albus*, *L. angustifolius*, *L. luteus,* and *L. mutabilis*) are

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cultivated for different purposes (Gladstones, 1974; Drummond et al., 2012). *L angustifolius* first appeared in an agricultural context as a spring-sown green manure and forage crop in the acidic sands of northern Europe in the nineteenth century (Hondelmann, 1984; Kurlovich, 2002). The cultivar "Lupi" was bred especially for use as green manure in Estonia (Bender and Tamm, 2014).

The incorporation of plants of these species in soils as green manure and their beneficial effects have been reported in different countries (Hanly and Gregg, 2004; Fowler et al., 2013). Although these species are not yet economically important in Mexico, some recent research in narrow-Leafed lupin (*L. angustifolius*), a winter legume, has been carried out in Jalisco Mexico, to determine their potential as winter grain crops (Lara-Rivera et al., 2017). However, no information is available on the potential of lupin to provide green manure in terms of biomass yield and N accumulation throughout the growing season. In this regard, Wivstad and Naetterlund (2008) reported that an important aspect of management associated with green manuring is choosing the development stage of the plant at which a green manure crop is incorporated into the soil. Therefore, the objective of this research was to determine the value of six *L. angustifolius* varieties as green manure in terms of aboveground biomass production and N accumulation at successive harvest dates. Additionally, this research aimed to estimate N fixation in these varieties 110 days after sowing (DAS)

Material and Methods

Plant material

Seeds of the Haags Blaue, Boregine, Borlu, Probor, Sonate, and Boruta varieties were used in this experiment.

Site description

The field experiment was conducted during the winter season of 2018–2019 in the experimental agricultural station at the University of Guadalajara in Zapopan, Jalisco, Mexico (20° 44' 47" N and 103° 30' 43" W) at an altitude of 1523 m.a.s.l. Climatic characteristics of this site were previously reported by Zapata et al. (2019). The experiment was established on loamy sandy soil, Regosol, according to the FAO/UNESCO classification. The chemical and physical properties of the soil were analyzed (Table 1).

Table 1. Basic chemical characteristics of the soil used in the experiment.

Depth (cm)	pH (KCl)	Organic matter (%)	N (%)	P (mg kg^{-1})	K (cmol kg^{-1})	Mineral components of soil (%)			Soil Texture
						Sand	Clay	Silt	
0-30	5.13	1.81	0.12	68.53	0.84	56	16	28	Sandy loam

Treatments and experimental design

On 7 November 2018, all six *L. angustifolius* varieties were planted following complete disking and plowing of the field in an experimental randomized block design with five replicates. Individual plots were 6.0 m long and four rows (75 cm between rows) wide in each replication. Lupin seeds were planted at a rate of 20 per meter of row with a target plant population of 120 000 plants ha^{-1}. Table 2 shows the treatments and characteristics of the lupin cultivars used in the experiment. The experimental design was a randomized block factorial design (factor A is the variety, and factor B is the time of harvest of the green manure biomass). At the time of sowing, no inoculum was added with the seed, but inspections showed that all plants were well nodulated during the growing season. A 2–m strip of spring wheat (*Triticum aestivum* cv Salamanca) was planted adjacent to each variety plot to serve as a non–N–fixing reference plant, with a target plant population of 320 000 plants ha^{-1}.

Table 2. Treatments used in the experiment and characteristics of the varieties.

Treatments	Variety	Type	Origin
Lupin cultivars	Haags Blaue	Early	Germany
	Boregine	Medium early	Germany
	Borlu	Medium early	Germany
	Probor	Medium early	Germany
	Sonate	Late	Germany
	Boruta	Early	Germany
Wheat	Salamanca	Medium early	México
Harvest dates: 80, 95, and 110* days after sowing			

*Assessment of N_2 fixation

Agronomic practices

Approximately 30 days after sowing, the plants in the four rows in each plot were thinned to 10 plants m^{-1}. The two rows located in the central part were then used for plant sampling during the experiment. After seeding, drip irrigation was performed at field capacity; this was repeated every 15 days but suspended 10 days before the last measurement. The weeds were removed manually during cultivation. Fertilizer was not applied, and pesticide application was not necessary because of the low incidence of pests and diseases. The meteorological data for the experimental area during the growing season and harvest date sowing are provided in Table 3.

Table 3. Harvest dates, temperature (T), and precipitation data during crop growth.

Month	Harvest date	Days after sowing	T max (°C)	T min (°C)	T mean (°C)	Rainfall (mm)
November	Seeds sown	----	23.40	9.30	16.35	0.80
December	----	----	17.60	5.40	11.50	5.00
January	1st (01–2019)	80	15.60	5.70	10.65	1.20
February	2nd (15–2019)	95	20.60	8.80	14.70	0.00
March	3rd (01–2019)	110	26.20	10.30	18.20	0.00

Data collection and nitrogen analysis

Sequential aboveground biomass samples were collected from a 1.0–m–long row (0.75 cm spacing) throughout the growing season. A chronological period was used because the varieties had different phenologies and it was not possible to make their flowering times coincide. The first sampling was done on 1 January, the second on 15 February, and the last on 15 March when plants were still immature to minimize the loss of leaves due to senescence. We used pruning scissors to cut plants approximately 3–5 cm above the ground level. The harvested material was washed with water and placed on absorbent paper for 30 min. The plants were dried in a forced air oven at 70 °C until constant weight (48 h). After registering the dry weights, we ground each whole plant in a mill for N analysis. The Kjeldahl method was used to quantify the percent N in each sample (whole plant). The total N content per plant was calculated using the following equation:

$$\text{N content (g plant}^{-1}\text{)} = [\text{DM (g plant}^{-1}\text{)} \times \%\ \text{N in whole plant}^{-1}] / 100$$

N_2 fixation

Symbiotic N_2 fixation in all six varieties of lupin was estimated 110 days after sowing. The N difference between fixing and non-fixing crops was used to quantify N_2 fixation (Hardarson et al., 1984; Evans et al., 1987). Reference plants (wheat) were cut in the third sampling and the amount of symbiotically fixed N was calculated according to the following equation:

$$\text{Fixed N} = \text{total N (fixing crop)} - \text{total N (non–fixing crop)}$$

Statistical analysis

To analyze the data, the statistical software Statgraphics Centurion, version XVII (Statgraphics Technologies Inc., USA) was used. Analysis of variance (ANOVA) was carried out on the parameters evaluated for the treatments. Means were compared using the Tukey test at $p \leq 0.05$.

Results and Discussion

Above-ground biomass yield

The effect of *L. angustifolius* varieties and harvest date on the above-ground biomass yield was significant ($p<0.05$). Variety × harvest date interactions were also observed (data not shown). Above-ground biomass yields expressed as the Kg ha^{-1} of DM for all six varieties at different harvest dates are presented in Table 4. As expected, we measured a significant increase in biomass DM yield for all varieties from the first to the last harvest date. In the first sampling, the values ranged from 1867 Kg ha^{-1} for the Sonate variety to 2964 Kg ha^{-1} for the Borlu variety. At the second harvest date, the yield varied from 3616 for Boruta to 5662 Kg ha^{-1} for Haags Blaue, whereas at the last harvest date, the yield ranged from 7632 Kg ha^{-1} for the Sonate variety to 10 200 Kg ha^{-1} for the Borlu variety (average 7864 Kg ha^{-1}).

Table 4. Change in dry matter yield of *L. angustifolius* varieties at successive harvest dates.

Days after sowing	Aboveground biomass yield (kg ha^{-1})					
	Haags Blaue	Boregine	Borlu	Probor	Sonate	Boruta
80	2412c	2765b	2964a	3098a	1949d	1867d
95	5662a	4116c	4887b	5465a	4082c	3616d
110	8568c	9132b	10200a	7956c	7632d	9096b

Values in the same row with different letters were significantly different ($p<0.05$) according to Tukey test.

Our results revealed that, in general, aboveground DM progressively increased in all six varieties with increasing maturity, which is considered a natural phenomenon in favorable conditions new tissues and organ are formed with the progression of maturation. A similar trend has already been reported in other species of legumes with potential as green manures and forage (Matos et al., 2008; Odhiambo, 2010; Perdigão et al., 2012; Prusiński, 2014; Solati et al., 2017; Dhamala et al., 2017; Zapata et al., 2019; Hernández et al., 2022). On the other hand, the aboveground biomass yields measured in this study were in the range of those reported by other authors, who reported values up to 8000-10 000 Kg ha^{-1} of DM in different legumes under favorable conditions (Dubrovskis et al., 2011; Talgre et al., 2012; Dhamala et al., 2017). In particular, the above-ground biomass yield of *Lupinus* species under different climatic and soil conditions reported in the reference varies; for example, in Portugal, *Lupinus luteus* harvested 5 months after sowing has a yield of 4900 to 4930 Kg ha^{-1} in the first year of cultivation and from 5330 to 6370 Kg ha^{-1} in the second year of cultivation (Perdigão et al. 2012). In Poland, the DM yields of this species ranges from 5820 to 6190 Kg ha^{-1} (Pietrzykowski et al., 2017). In New Zealand, the above-ground DM yield of the *L. angustifolius* variety "Fest" harvested at 150 days reached 4850 Kg ha^{-1} (Fowler et al., 2004). In another study in New Zealand with *L. angustifolius* sampled 6 months just before being incorporated into the soil, the above-ground biomass ranged from 7400 to 8200 Kg ha^{-1} (Hanly and Greeg, 2004). The progressive and gradual accumulation of above-ground DM as the maturation period increased indicate that all six *L. angustifolius* varieties tested as green manure showed good adaptation to sandy and acidic soils and the moderately cool winter temperatures in the central region of Jalisco, Mexico (Figure 1). This can also be attributed to the plants having access to adequate amounts of moisture, nutrients, temperature, and sunlight.

Figure 1. *Lupinus angustifolius* varieties cultivated in Zapopan, Jalisco, México

The results indicate that the accumulation of DM could continue for a few more days, but there is a risk that the quality of the biomass used as a green manure decreases in terms of chemical composition (decreased N content and increased lignin concentration in the tissues). Delaying the harvest date and subsequent incorporation of green manure into the soil may affect its decomposition due to greater lignification of plant tissues. Although the lignin content was not quantified in the *L. angustifolius* varieties tested during the growing season in the present study, the results of other studies have shown that lignin concentrations on legume and non-legume species can consistently increase with successive harvest dates (Abiven et al., 2011; Markovi et al., 2012). On the other hand, the differences in DM accumulation between varieties can be explained in terms of varietal differences, as well maturation time (early and late maturation). Under the environmental conditions of the experimental site for the current study, the late maturing varieties (Boregine, Borlu, and Boruta) showed potential for producing more biomass than the early maturing varieties (Haags Blaue, Sonate, and Probor). In addition, inspections at each harvest date showed that all plants were well-nodulated during the growing season; therefore, the increase in aboveground biomass from the first to the third sampling date is probably due to increased N fixation by the six lupin varieties.

Nitrogen concentration and total accumulation in above-ground biomass

Significant differences in the N concentration and total N accumulation in the aboveground biomass were observed among the varieties at each sampling date (p<0.05). The N concentration expressed percentage decreased significantly in all six varieties across the growing season (Table 5). From the first to the third harvest date, the N concentration (as the average of six varieties) was 2.95%, 2.62%, and 2.30%, respectively. Among all six varieties, Probor accumulated significantly higher N concentrations at the first harvest date (3.6%), whereas the highest N concentration was registered at the second and third harvest date with the Haags Blaue variety (3.1% and 2.6%, respectively).

Table 5. Nitrogen (N) concentration and total accumulation in six *Lupinus angustifolius* varieties at different harvest dates.

Variety	N concentration (%) Days after sowing			Total N accumulation (kg ha^{-1}) Days after sowing		
	80	95	110	80	95	110
Haags Blaue	3.0b	3.1a	2.6a	74.5b	179.2a	193.7b
Boregine	3.0b	2.4b	2.4a	90.4a	102.6c	197.5b
Borlu	2.4b	2.0c	2.1a	49.9c	100.9c	212.2a
Probor	3.6a	2.7b	2.3a	58.5c	152.4b	166.5c
Sonate	2.8b	2.5b	1.9b	44.6c	105.3c	126.1d
Boruta	2.6c	2.6b	2.2a	49.6c	97.0c	195.6b
Mean	2.95	2.62	2.30	61.25	122.9	181.9

Values in the same column with different letters were significantly different (p<0.05) according to Tukey test.

The N concentrations found in the tested varieties were well within the reported values in the literature for similar types of green manures and forage crops at different locations and conditions. For example, in New Zealand, *L. angustifolius* variety "Fest" harvested 150 DAS recorded an N concentration of 3.39%, whereas in South Africa, *L. angustifolius* (variety name was not mentioned) at 3.5 months, just before incorporation into the soil, had an N concentration of 3.0% (Van Antwerpent et al., 2002; Fowler et al., 2004). However, lower N concentrations (1.4% and 1.5%) than those found in this experiment were reported in another study carried out at two sites in New Zealand (Hanly and Greeg, 2004). A decrease in N concentration with successive harvest dates was reported in other studies with different species and conditions, possibly due to the dilution effect of greater above-ground biomass. Similarly, a decreasing trend in N content has been reported for different forage crops and green manures in one season of growth as the plant matured beyond flowering (Odhiambo 2010; Dhamala et al., 2017; Zapata et al., 2019; Hernández et al., 2022). According to Müller et al. (1988), the biomass production of a green manure is important when incorporating it into the soil, but it is necessary to also consider its concentration of N, as this is an important chemical characteristic that governs the rate of decomposition and mineralization into the soil. The pattern in DM accumulation of green manures with increasing maturity is important for proper timing of incorporation into the soil; however, even though delaying the harvest will increase the DM yield of the crop significantly, the quality will probably decrease (Odhiambo and Bomke, 2001). In general, the quality of a green manure in terms of chemical composition varies a great deal due to a number of factors, such as species, stage of growth, soil conditions, fertilizer application, availability of water, and climatic conditions.

In the current research, the amount of total N that can return to the soil through the biomass produced varied greatly among the six varieties evaluated and the harvest dates, with a tendency to increase the total yield of N accumulated in the biomass throughout the growth period. The highest total N yields were found in the biomass produced at the last harvest date, with an average of 181 Kg ha^{-1} (range 126.2–212.1 Kg ha^{-1}). Borlu was the variety that exhibited the highest amount of total N accumulation, followed by Boregine, Borlua, and Boruta varieties. As expected, the lowest total N accumulation was recorded at the first harvest date (range 44.6–90.4 Kg ha^{-1}). This general tendency of increased total N yield in the biomass of the *L. angustifolius* varieties tested during the growth period is consistent with the literature for different legume species used as forage and green manure (Wivstad, 1999; Odhiambo and Bomke, 2001; Unkovich et al., 2010; Bhardwaj et al., 2010). Previous research in different environmental and growth conditions has shown a close association between total N accumulated and biomass yield throughout the growth period (Carranca et al., 2013; Zapata; et al., 2019). Although we did not observe a decrease in the N content in the harvested biomass as the sampling dates increased in this study, different studies with species of the genus *Lupinus* and other legumes have observed that the N content in the biomass can decrease during the growth cycle (from vegetative to pod-filling phases). In the current research and considering that the N concentration decline and C/N ratio probably increase as the plant matures, it is convenient to cut and incorporate the green manure at the last harvest date tested.

Atmospheric nitrogen fixation

The lupin varieties evaluated in this study were able to fix atmospheric N in symbiosis with the native soil bacteria 110 days after planting and under the climatic conditions of Zapopan, Jalisco (Mexico). The genus *Lupinus* has been known for some time to be nodulated by *Bradyrhizobum* sp. in the soil (Jordan, 1982). Observations during the growing season (all sampling dates) showed that plants were well nodulated. However, significant ($p<0.05$) differences were observed in the amount of N fixed and percentage of N derived from the atmosphere (Ndfa) between lupin genotypes (Figure 2).

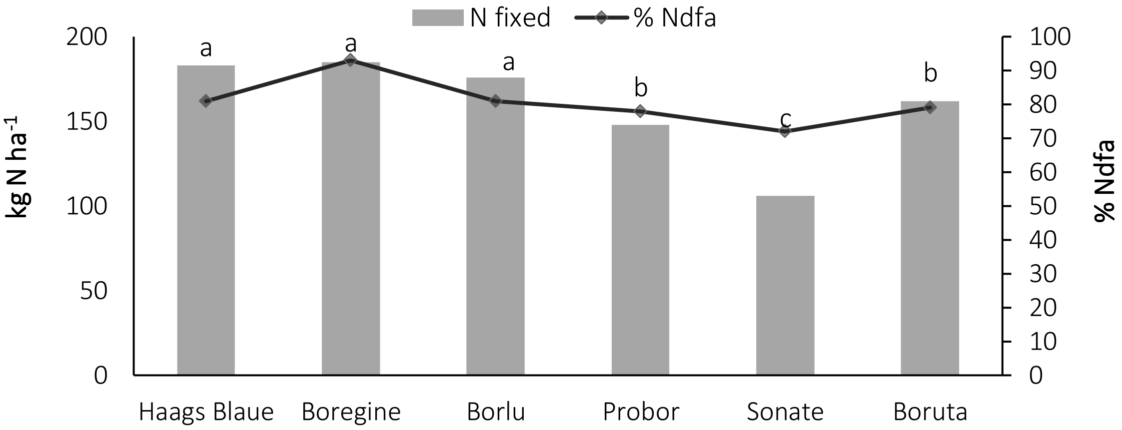

Figure 2. Amount of nitrogen (N) fixed and proportion of N derived from the atmosphere (Ndfa)

The Ndaf averaged 80.9% (range 72.1–93.2%), with a corresponding N_2 fixation input in aboveground biomass of 162 Kg ha^{-1} (range 106–185 Kg ha^{-1}). Boregine, Haags Blaue, and Borlu varieties showed a better response in fixing ability with 185, 183, and 176 Kg N ha^{-1} (Figure 2). The highest percentage of Ndfa 110 days after sowing was achieved with Haags Blaue, Boregine, Borlu, and Boruta varieties (up to 84%). Sonate and Probor varieties were found to have the worst adaptive ability in regard to the biomass yield and N fixation, but the average N_2 fixation values found in this study are generally within the ranges reported by other researchers for *L. angustifolius* varieties. For example, in one of the first studies to measure N fixation in *L. angustifolius,* Pálmason et al. (1992) reported 117 DAS and at a mean temperature below 10°C in Iceland that lupin derived an average of 92% or 195 Kg N ha^{-1}. Later, in an Andisol soil of southern Chile, Barrientos et al. (2002) reported that *L. angustifolius* cv. Gungurru fixed 197 Kg N ha^{-1} 80 days after planting, which was equivalent to 91% Ndfa. However, in some cases, our values for fixed N_2 were higher than those reported by other studies, with the exception of the Sonate variety. Kelstrup et al. (1996) reported that the total amount of N derived from N_2 fixation was 119 Kg ha^{-1} for the Feste variety harvested in New Zealand when plants were judged to be physiologically mature. In comparison to other studies, our results were slightly lower. In Iceland, for example, Pálmason et al. (2004) reported that *L. angustifolius* cv Uniharvest reached symbiotic N yields as high as 185 to 212 Kg ha^{-1} at seeding rates of 200 Kg ha^{-1}. On the other hand, Denton et al. (2017) reported maximum symbiotic N fixation values in Australia of 225 Kg ha^{-1} N. These differences may be due to the conditions established before and during the experiments, such as the evaluated varieties, inoculant application, climatic and soil conditions, sampling dates, and methods used to evaluate N_2 fixation, among others. On the other hand, the present study revealed variation between genotypes in the ability to fix N_2. These and other results obtained with different legumes and using different methods showed that genetic variability exists between genotypes in regard to their ability to support N_2 fixation (Hardarson et al., 1984; Zimmer et al., 2016; Akter et al., 2018; Diatta et al., 2020). These results should be useful to growers and researchers interested in selecting management practices that optimize N fixation and increase the aboveground biomass yield of *L angustifolius* used as green manures.

Conclusion

All *L. angustifolius* varieties showed good adaptation to acidic soils and moderately cool winter temperatures in central Jalisco, Mexico, with a rapid accumulation of DM (up to 8700 Kg ha^{-1} on average) and little damage from pests or disease. The N concentration in the plants decreases with maturity from 2.95 to 2.30 %, but the total N accumulated in the aboveground biomass of *L. angustifolius* varieties increased significantly from the first to the last harvest. The varieties with major potential as green manure for agricultural soils in Zapopan, Jalisco, were Haags Blaue, Boregine, Borlu, and Boruta in terms of aboveground biomass production, total N accumulated and ability to fix N_2. At 110 days after sowing, Haags Blaue and Boregine were the varieties with the highest ability to fix atmospheric N, with 180 kg ha^{-1} on average.

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