



# Article Effect of Different Row Spacing and Sowing Density on Selected Photosynthesis Indices, Yield, and Quality of White Lupine Seeds

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Abstract: The use of appropriate plant spacing in the canopy is an important and cost-free agrotechnical factor for increasing seed yield. Proper row spacing and sowing density are important in maintaining adequate plant light, ensuring good physiological processes, and influencing the nutritional status of plants. As a consequence, this leads to better plant productivity while maintaining economic profitability. A four-year field experiment with white lupine was conducted in 2016–2019 at the Experimental Station for Cultivation Assessment in Przecław in southeastern Poland. The factors of the experiment were different row spacing (15 and 30 cm) and sowing density (60, 75, and 90 pcs  $m^{-2}$ ). Row spacing and sowing density had no significant effect on the yield obtained, the number of pods per plant, and the weight of 1000 seeds of white lupine. There was also no effect of experimental factors on nodulation and chlorophyll fluorescence parameters (Fv/Fm, Fv/F0, PI, and RC/R). The use of wider row spacing (30 cm) and the lowest sowing density (60 pcs m<sup>-2</sup>) resulted in the highest chlorophyll content. For the LAI index, the highest value was found at a row spacing of 15 cm. However, lower plant density per unit area had an effect on increasing the number of seeds per pod and seed weight per plant, which was associated with better conditions for plant growth. It was shown that greater competition between lupine plants in the canopy increased the height of the first pod set without affecting their height.

Keywords: Lupinus albus L.; planting pattern; yield; photosynthesis efficiency; nodules; protein

# 1. Introduction

The sustainable development of agriculture is currently at the center of debate on the agricultural policy of the European Union. Crop diversification is believed to promote sustainable development. Legumes are an important source of nitrogen, protein, and an alternative crop for rehabilitating degraded land [1–3]. These plants can play a key role in reducing the deficit of high-protein feeds and contribute to the reduction in greenhouse gas emissions and nitrogen fertilizers. In addition, they affect the protection of soil fertility and biodiversity [4,5]. Due to their symbiosis with papillary bacteria, legumes play a key role in crop rotations, leaving a good stand for the successor plant [6,7].

White lupine (*Lupinus albus* L.) has a particularly favorable role in crop rotations due to its high biomass production and high nitrogen (N) uptake capacity [3,6,7]. Lucas et al. [8] believe that the cultivation of white lupine could be an alternative crop to soybean cultivation in Europe. Lupine, in addition, improves soil structure [3] and can be used for cultivation in various agricultural systems [9]. White lupin is grown for seed, ruminant feed, green manure, and for human nutrition [10,11]. Erbas et al. [12] showed that white lupin grown in Turkey is an excellent food commodity with high nutritional value. The seeds can be used in human nutrition and treatment. The possibility of using



Citation: Tobiasz-Salach, R.; Jańczak-Pieniążek, M.; Augustyńska-Prejsnar, A. Effect of Different Row Spacing and Sowing Density on Selected Photosynthesis Indices, Yield, and Quality of White Lupine Seeds. *Agriculture* **2023**, *13*, 1845. https://doi.org/10.3390/ agriculture13091845

Academic Editor: Chengfang Li

Received: 31 August 2023 Revised: 15 September 2023 Accepted: 19 September 2023 Published: 21 September 2023



**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). lupin as a functional food was discovered. The protein content with desirable amino acids, fatty acids with a favorable ratio of omega-6 to omega-3 acids, fiber, oligosaccharides, and antioxidants makes white lupin an excellent ingredient for many healthy diets in human nutrition [11]. Currently, the use of white lupin seed is limited by the small scale of its production. In the EU, there is a decline in the area of legume cultivation, despite the beneficial effects of their cultivation, especially increasing biodiversity, reducing emissions (up to 50% of  $N_2O$ ), as well as positive effects on the soil, successor plants in the rotation, and economic benefits [13]. The reasons for the reduction in legume acreage are large yield differences and the cost of cultivation [14,15]. The yield of legumes is the result of the interaction of agricultural practices and environmental factors [16]. Compared to other legumes, lupin shows narrower adaptation to soil type, and on suitable soils, its cultivation can achieve higher crude protein yields than peas or soybeans due to its unique seed protein composition [17]. However, lupin has lower yields compared to other legumes [18]. Agronomic strategies such as choosing the optimal row spacing and sowing density of legumes, including lupine, are crucial to increasing crop productivity [19]. Inter-row width and plant sowing density are among the main factors determining legume seed yield and chemical composition [20–22]. Optimal plant density is important because it determines not only the course of plant vegetation, growth, yield, and seed quality but also affects the ability to compete with weeds and absorb light in the process of photosynthesis. In Brazil, the optimal density of white lupin is 25 plants per 1 m<sup>2</sup> [1]; in the Czech Republic, it is 75 plants per 1 m<sup>2</sup> [19]; and in Poland, it is 70–80 plants per m<sup>2</sup> [23–25] or 50–60 pcs per m<sup>-2</sup> [26].

Chlorophyll a fluorescence parameters are important indicators of the state of the photosynthetic apparatus indicating changes in its functioning. Chlorophyll *a* fluorescence measurements are used to determine the limitations of the photosynthetic apparatus, particularly in response to various stress factors [27]. The method is used to assess the effects of stresses on plants at a point in time where visual changes in leaves are not yet apparent [28]. By analyzing the parameters of chlorophyll fluorescence and its elevated content in leaves, Allakhverdiev and Murata [29] determined the effect of environmental stresses on the course of photosynthesis in plants. These stresses can be caused by a lack of optimal spacing and plant density. Too wide or narrow row spacing causes changes in the reflectance of wavelengths (400–700 nm), which disrupts metabolic processes in leaves by altering chlorophyll concentration. Different sowing density affects the structure of the plant canopy. The mutual overlap of plants caused by the increase in sowing density reduces the light reaching individual parts of the plants, which causes a decrease in the photosynthetic rate [30]. Increasing sowing density causes the shading of plants mutually and decreases in fluorescence parameters such as Fv/Fm and Fv/F0. Such relationships have been demonstrated in peas [31,32] and soybeans [33].

Therefore, undertaking a study to determine the effect of row spacing and sowing density on selected physiological parameters, yield, and yield structure elements of white lupin cv. Butan is fully justified, especially since the response of new lupin cultivars to the factors studied varies. The research hypothesis was accepted, which assumes that the cultivation of white lupin with higher plant density and narrow row spacing will have a beneficial effect on its yield.

### 2. Materials and Methods

## 2.1. Experimental Design

A two-factor experiment with white lupine cv. Butan (HR Smolice, Poland) was conducted in Poland, Przecław, ( $50^{\circ}$  110' N,  $21^{\circ}$  290' E) at the Experimental Station for Cultivar Assessment in 2016–2019.

Experimental factors:

- I. Row spacing: 15 and 30 cm;
- II. Sowing density: 60, 75, and 90 plants per m<sup>2</sup>.

The experiment was established using the split-plot method in four repetitions. There were 24 plots with an area of 19.5 m<sup>2</sup> (13.0 m  $\times$  1.5 m). The developmental stages of

lupin plants are given on the BBCH scale (Biologische Bundesanstalt, Bundessortenamtund CHemische Industrie) according to [34].

In the years of research, the forecrop of white lupine was winter wheat (2016–2018) and sugar beet (2019). The soil mineral fertilization was applied before sowing. The phosphorus, potassium, and nitrogen were used in the amount of 40 kg ha<sup>-1</sup>, 55 kg ha<sup>-1</sup>, and 30 kg ha<sup>-1</sup>, respectively. Seeds were sown at a depth of 3–4 cm. The list of conducted agrotechnical treatments is shown in Table 1.

 Table 1. Agrotechnical treatments during the vegetation of white lupine.

Agrotechnical	Years of Research					
Treatments	2016	2017	2018	2019		
Sowing date	31 March	29 March	09 April	22 March		
Herbicide	1 April Afalon dyspersyjny 450 SC (linuron) 1.25 dm <sup>3</sup> ha <sup>-1</sup>	30.03. Afalon dyspersyjny 450 SC (linuron) 1 dm <sup>3</sup> ha <sup>-1</sup>	9 April Boxer 800 EC (prosulfocarb) 4 dm <sup>3</sup> ha <sup>-1</sup>	1 April Boxer 800 EC (prosulfocarb) 4 dm <sup>3</sup> ha <sup>-1</sup>		
Insecticide	23 June Proteus 110 OD (tiachlopryd, deltamatryna) 0.75 dm <sup>3</sup> ha <sup>-1</sup> 31 May Mospilan 20 SP (acetampityt) 0.2 kg·ha <sup>-1</sup>	5 June; 19 June Mospilan 20 SP (acetampityt) 0.2 kg·ha <sup>-1</sup>	23 May Mospilan 20 SP (acetampityt) 0.2 kg·ha <sup>-1</sup>	28 May Mospilan 20 SP (acetampityt) 0.2 kg·ha <sup>-1</sup>		
Fungicide	16 May, 30 May Gwarant 500 (chloralonil) SC 2 dm <sup>3</sup> ha <sup>-1</sup>	23 May Gwarant 500 (chloralonil) SC 2 dm <sup>3</sup> ha <sup>-1</sup>	23 May Gwarant 500 (chloralonil) SC 2 $dm^3 ha^{-1}$	19 May Gwarant 500 (chloralonil) SC 2 dm <sup>3</sup> ha <sup>-1</sup>		
Harvest date	11 August	09 August	09 August	05 August		

#### 2.2. Soil Conditions

The experiment was set up on clay loam, which was classified according to the FAO WRB [35] as Fluvic Cambisol (*CMfv*). In 2017 and 2019, the soil pH was neutral; in 2016 and 2018 it was slightly acidic; and it was neutral in 2017 and 2019. The phosphorus content was characterized as high in 2016, 2017, and 2018, and very high in 2019. The potassium content was determined to be average. In 2016, 2017, and 2019, the magnesium content was characterized as very high, and in 2018 as high. In the research years 2016, 2017, and 2019, the content of Fe, Mn, and Cu was average. The Zn content in 2016, 2017, and 2019 was considered average, while in 2018, it was low (Table 2).

Table 2. Soil composition before white lupine sowing.

In one di on to	Years of Research					
ingreatents —	2016	2017	2018	2019		
pH <sub>KCl</sub>	6.26	6.90	6.04	7.01		
Humus content (%)	0.996	1.16	1.10	1.19		
Content (mg kg <sup>-1</sup> in 0–60 cm)						
Р	156.4	156.2	151.0	226.3		
K	189.7	191.4	150.1	173.0		
Mg	241.5	238.1	104.3	179.1		
Fe	1855.3	2784.3	1045.0	2059.3		
Zn	13.1	13.54	11.90	12.5		
Mn	192.6	371.40	114.00	319.9		
Cu	8.53	10.43	3.98	8.23		

# 2.3. Weather Conditions

The weather conditions were noted at the Experimental Station for Cultivar Assessment in Przecław (Table 3). In the years of the study, a large variation in terms of the

amount of precipitation and lower temperature variations was found. The most favorable hydrothermal conditions were recorded in May 2017 and 2019. They significantly exceeded the average multiannual rainfall for that month. Less favorable conditions were in June and July. However, rainfall in May offset the effects of drought in those months. Good soil moisture in July (108.3 mm) in 2018 with optimal thermal conditions contributed to the best yield of seeds. Analyzing the hydrothermal conditions from the four years of research, they did not differ significantly from the long-term average for that period. The exception was 2019, when slightly higher precipitation (10.6 mm higher) and air temperature were found (Table 3).

Month	2016	2017	2018	2019	1956–2015	2016	2017	2018	2019
Precipitation (mm)			Deviation	from the long-term r	nean precipi	tation (%)			
March	38.9	15.6	40.9	24.3	35.5	9.6	-56.2	15.2	-31.9
April	54.7	78.3	15.7	62.1	48.1	13.7	62.8	-67.4	29.1
May	41.5	111.9	68.8	182	39.2	5.5	285.4	75.5	464
June	23.8	41.6	47.4	19.2	79.3	-69.9	-47.6	-40.2	-75.8
July	151.6	44.4	108.3	45.1	101.6	49.2	-56.3	6.2	-55.9
August	68.1	84	97.4	82.1	71.3	-4.5	15.1	36.6	15.2
March–August	63.10	62.63	63.08	69.13	62.50	0.95	0.2	0.92	10.6
	Avera	ige air temp	erature (°C	2)		Deviation from the long-term mean temperature (°C)			
March	4.9	4.9	2.4	3.2	2.6	2.3	2.3	-0.2	2.3
April	9.8	6.8	12.2	10.4	8.8	1.0	-2	3.4	1.6
May	14.0	12.5	15.4	13.6	14.2	-0.2	-1.7	1.3	-0.6
June	18.6	17.4	16.9	21.5	17.5	1.1	-0.1	-0.6	4.0
July	18.9	17.9	18.5	18.7	19.4	-0.5	-1.5	-0.9	-0.7
August	17.6	18.2	18.5	20.3	18.1	-0.5	0.1	0.4	2.20
March–July	14.0	13.0	13.2	14.6	13.4	0.6	-0.6	-0.2	1.2

Table 3. Weather conditions during the vegetative period of plants in the years of research.

#### 2.4. Biometric Measurements

In the full maturity stage (BBCH 92), 20 plants were randomly selected for yield structure analysis. The height of the plant, the height of the first pod, the number of pods per plant, the number of seeds per plant, the weight of 1000 seeds, and the weight of seeds per plant were measured. The weight of seeds from the plant and the weight of 1000 seeds were determined with an accuracy of 0.1 g. The yield from the plot was converted to the yield from 1 ha at 15% humidity.

#### 2.5. Physiological Measurements

Physiological measurements on white lupine leaves were taken in the morning, and twice during the growing season in the development stages at the beginning of flowering (BBCH 59) and the end of flowering (BBCH 69). The results are given as the average of the measurements in two phases.

#### 2.5.1. Relative Chlorophyll Content

Chlorophyll content measurements were made using the SPAD 502 apparatus (Konica-Minolta, Tokyo, Japan). SPAD measurements were performed on 20 randomly selected plants. Chlorophyll *a* fluorescence measurements were performed according to Jańczak-Pieniążek et al. [33], using a portable fluorometer (Pocket PEA, Hansatech Instruments, King's Lynn, Norfolk, UK). Measurements were conducted on fully developed leaves on 4 randomly selected plants. Leaf clips were placed on the upper part of the leaf blade for a 30 min adaptation to the darkness of the leaves [36]. The following parameters were analyzed in the experiment: maximum quantum yield of photosystem II (PSII) (Fv/Fm), maximum quantum yield of photochemistry (Fv/F0), the efficiency index (PI), and total number of active reaction centers for absorption (RC/ABS).

#### 2.5.3. Leaf Area Index

The LAI (leaf area index) value was determined in 4 repetitions, one measurement over the canopy and four measurements on the canopy. The measurements were performed four times in each plot. LAI measurements were performed with the LAI 2000 apparatus (LI-COR, Lincoln, NE, USA).

#### 2.6. Analytical Methods

Protein yield was determined as the product of seed content (%) and seed yield per area unit (t·ha<sup>-1</sup>). The protein content of white lupine was determined by near-infrared spectroscopy (NIRS) using an MPA FT NIR spectrometer (Bruker, Billerica, MA, USA). The measurement was performed in triplicate.

#### 2.7. Determination of the Number of Nodules

At the beginning of pod development (BBCH 71), lupine plants were dug up (10 plants per plot). After rinsing the roots with water, nodules were plucked from the roots manually, and then their number was determined separately for the main root and lateral roots.

#### 2.8. Data Analysis Methods

The obtained test results were subjected to the analysis of variance (ANOVA). The significance of differences between treatments was verified using Tukey's test at a significance level of  $p \le 0.05$ . Pearson correlations at  $p \le 0.05$  between the main parameters were also determined. Statistical analysis was performed using the program TIBCO Statistica 13.3.0 statistical software (TIBCO Software Inc., Palo Alto, CA, USA).

#### 3. Results and Discussion

#### 3.1. White Lupine Seed Yield and Its Components

The average seed yield of white lupin was 4.2 t ha<sup>-1</sup> and was not dependent on row spacing and sowing density. There were only differences in yield between the years of the study (Table 4). Similar results were obtained by Bhardway et al. [37]. However, other authors have shown a significant effect of wider interrows [38] on white lupin yield. Lopez-Bellido et al. [39] showed the effect not only of row spacing but also of plant density per 1  $m^2$ . The analysis of plant yield structure in the study showed that there was no significant effect of row spacing and sowing density on the number of pods per plant and the weight of 1000 seeds. However, a wider row spacing (30 cm) compared to a narrower one (15 cm) had a positive effect on the number and weight of seeds per plant. In addition, the highest plant density per 1 m<sup>2</sup> (90 pods m<sup>-2</sup>) significantly reduced seed weight per plant compared to lower densities (75 and 60 pods  $m^{-2}$ ) by 13.5% and 9.5%, respectively. In studies by Borowska et al. [23], Podleśny [26], and Prusiński and Borowska [40], higher plant density reduced the number of pods per plant. However, Pospišil and Pospišil [41] found no significant effect of higher plant density on white lupin yield but showed that an increase in plant density above 60 plants per m<sup>2</sup> significantly reduced the number of pods, seeds per pod, and seed weight per plant. Prusiński and Borkowska [40] indicated that the number of pods per plant and per  $1 \text{ m}^2$  had the greatest impact on white lupin yield. Koetz et al. [42] showed an increase in seed yield (18%) when the inter-row spacing was

increased from 25 to 50 cm. However, increasing the row spacing from 25 to 75 resulted in a decrease in seed yield by 29% and a reduction in the number of plants per 1  $m^2$ . Other researchers did not demonstrate these relationships in their studies [26,37,39,41]. However, they indicated that plant density per 1 m<sup>2</sup> influenced yield structure traits and yield of white lupin plants. They observed that an increase in plant density per 1 m<sup>2</sup> resulted in a decrease in the number of pods per plant, while the number and weight of seeds per pod did not change. The yield of white lupin depended on the weather conditions during the growing season and was stable during the years of the study (Table 4). The exception was in 2018 when the highest yield  $(5.1 \text{ t ha}^{-1})$  was obtained with high rainfall (especially in July). In contrast, structural traits were variable across the study years. The number of pods and seeds per plant was the highest in 2019, while the weight of 1000 seeds was the lowest. The low weight of 1000 seeds was to the result of drought in June and July (-75.8 mm and -55.9 mm of precipitation compared to the long-term average) and high air temperatures. Many authors indicated the sensitivity of legumes to the lack of water during the growing season [19,23,24]. Drought periods have a negative impact on the morphological traits of legumes, in particular, the elements affecting the yield (number and weight of pods per plant), which may consequently lead to a decrease in yield. The greatest demand for water in these plants occurs during the flowering and seed-setting phases. However, the most critical period for the productivity of these plants falls on the seed setting phase, which determines the number and weight of seeds in the pod [43,44]. Annicchiarico et al. [14] found a decrease in white lupin seed yields (79%) due to extreme drought. Drought and increased air temperature accelerate plant flowering and shorten vegetation by increasing evapotranspiration. Annicchiarico et al. [14] and Reckling et al. [15] indicated that different moisture conditions during the growing season of white lupin plants are the cause of low crop yields. Improving the moisture conditions will increase the cultivation of legumes and increase the fixation of free nitrogen in the soil.

Row Spacing (R)	Planting Density (D)	Yield (t∙ha <sup>-1</sup> )	Number of Pods per Plant (pcs.)	Number of Seeds per Plant (pcs.)	Seed Weight per Plant (g)	Weight of 1000 Seeds (g)
	60	$3.9~\mathrm{a}\pm1.06$	$9.5~\mathrm{a}\pm4.9$	$27.5~\mathrm{ab}\pm12.0$	$7.22\ bc\pm 2.34$	$285~a\pm51.8$
15	75	$4.1~\mathrm{a}\pm1.01$	$8.6~a\pm4.2$	$25.0 \text{ a} \pm 10.3$	$7.00\ bc\pm2.26$	$288~\mathrm{a}\pm49.0$
	90	$4.4~\mathrm{a}\pm0.72$	$8.5 \text{ a} \pm 3.3$	$25.8~ab\pm8.1$	$6.13~\mathrm{a}\pm2.02$	$286~a\pm56.9$
	60	$4.1~\mathrm{a}\pm0.92$	$9.4~\mathrm{a}\pm3.2$	$29.8b\pm15.8$	$7.63~\mathrm{c}\pm3.89$	$283~a\pm58.8$
30	75	$4.2~\mathrm{a}\pm0.76$	$8.9~\mathrm{a}\pm4.2$	$28.1~\text{ab}\pm13.1$	$7.18~\mathrm{bc}\pm3.21$	$281~\mathrm{a}\pm57.7$
	90	$4.4~\mathrm{a}\pm0.91$	$8.5~\mathrm{a}\pm4.7$	$27.2~\text{ab}\pm10.6$	$6.70~ab\pm3.26$	$288~\mathrm{a}\pm54.3$
15		$4.1~\mathrm{a}\pm0.94$	$8.8~\mathrm{a}\pm4.1$	$26.1 \text{ a} \pm 10.1$	$6.80~\mathrm{a}\pm2.21$	$286~\mathrm{a}\pm51.5$
30		$4.3~\mathrm{a}\pm0.86$	$8.9~\mathrm{a}\pm5.2$	$28.4 \text{ b} \pm 13.1$	$7.20~b\pm3.42$	$284~a\pm55.8$
	60	$4.0~\mathrm{a}\pm0.98$	$9.5~a\pm5.6$	$28.6~\mathrm{a}\pm13.9$	$7.42~b\pm3.17$	$284~a\pm54.5$
	75	$4.1~\mathrm{a}\pm0.88$	$8.7~\mathrm{a}\pm4.1$	$26.5 \text{ a} \pm 11.7$	$7.09~b\pm2.74$	$285~\mathrm{a}\pm52.8$
	90	$4.4~\mathrm{a}\pm0.81$	$8.5~a\pm4.0$	$26.5 \text{ a} \pm 9.3$	$6.42~a\pm2.69$	$287~\mathrm{a}\pm54.7$
Ye	ear (Y)					
	2016	$3.8 \text{ a} \pm 0.96$	$4.3~\mathrm{a}\pm1.4$	$16.7~a\pm3.8$	$4.1~\mathrm{a}\pm0.34$	$324\ c\pm 18.2$
	2017	$4.0~\mathrm{a}\pm0.49$	$5.5~\mathrm{a}\pm1.1$	$17.5~\mathrm{a}\pm2.9$	$6.0b\pm1.10$	$342~d\pm7.4$
	2018	$5.1 \ b \pm 0.45$	$11.3b\pm2.7$	$36.3 \text{ b} \pm 5.0$	$7.8~\mathrm{c}\pm0.76$	$260 \text{ b} \pm 17.1$
	2019	$4.0~\mathrm{a}\pm0.97$	$14.4~\mathrm{c}\pm2.6$	$38.4b\pm9.3$	$10.1~\text{d}\pm2.37$	$215~\mathrm{a}\pm1.06$
N	Mean	$4.2\pm0.90$	$8.9\pm4.7$	$27.2 \pm 11.7$	$7.00\pm2.87$	$285\pm\!20.5$
	R	ns	ns	**	*	ns
	D	ns	ns	ns	***	ns
	Y	***	***	***	***	***

**Table 4.** White lupin yield and structural yield components depend on row spacing and plant density (mean 2016–2019).

Row Spacing (R)	Planting Density (D)	Yield (t∙ha <sup>-1</sup> )	Number of Pods per Plant (pcs.)	Number of Seeds per Plant (pcs.)	Seed Weight per Plant (g)	Weight of 1000 Seeds (g)
I	$R \times D$	ns	ns	ns	ns	ns
]	$R \times Y$	***	***	***	***	ns
I	$D \times Y$	ns	***	***	*	ns
R	$\times$ D $\times$ Y	ns	ns	ns	ns	ns

Table 4. Cont.

Experimental results have been presented as mean values  $\pm$  standard deviation. Values in the columns marked with different letters are significantly different (followed by Tukey's HSD test, p = 0.05). \*, \*\*, \*\*\*, and ns mean  $\leq 0.05$ ,  $\leq 0.01$ , <0.001, and not significant, respectively.

#### 3.2. Protein Yield and Protein Content

The average protein yield was 1488 kg $\cdot$ ha<sup>-1</sup>. It was differentiated in the years of the research and did not depend on row spacing and sowing density (Table 5). The protein content in lupine seeds was on average 35.5%. This value was higher at sowing densities 75 and 90 pcs.  $m^{-2}$  compared to 60 pcs.  $m^{-2}$  by 1.42% and 0.85%, respectively. The row spacing did not affect the protein content of white lupin seeds, which is consistent with the results obtained by Prusiński [45]. The cultivation of lupine in 2018 resulted in the highest protein yield (1829 kg·ha<sup>-1</sup>) in relation to 2016 (by 26.05%), 2017 (by 32.54%), and 2019 (by 41.34%). The highest protein content (38.5%) was obtained in 2016, while the lowest was in 2019 (32,3%). The difference in protein content and yield in lupin seeds depends on the temperature, sum, and distribution of precipitation during the growing season, which was found in yellow lupin [10], but also in peas [40] and other *Fabaceae* species [46,47]. In a study conducted by Prusinski and Borowska [40] on peas, weather conditions also had an effect on seed protein content. These authors indicated that although rainfall deficit stress has a strong negative effect on seed dry matter, resulting in a decrease in yield, it affects the increase in seed protein content. This relationship was confirmed in our research, which showed the highest protein content in lupin seeds in 2016, in which a significant rainfall deficit was found during the pod-setting period.

<b>Table 5.</b> Effect of different row spacing and sowing density on protein yield $(kg \cdot ha^{-1})$ as	nd protein
content (%) of seeds.	

Row Spacing (R)	Planting Density (D)	Protein Yield (kg·ha <sup>-1</sup> )	Protein Content (%)
	60	1393 a $\pm$ 416	$35.1 \text{ a} \pm 2.6$
15	75	1441 a $\pm$ 402	$35.4$ ba $\pm$ 2.8
	90	1578 a $\pm$ 310	$35.9~b\pm2.6$
	60	$1450~\mathrm{a}\pm329$	$35.3~\mathrm{a}\pm2.0$
30	75	1525 a $\pm$ 294	$35.9b\pm2.3$
	90	1543 a $\pm$ 271	$35.2 \text{ a} \pm 2.4$
15		1471 a $\pm$ 379	$35.6~\mathrm{a}\pm2.6$
30		$1506~\mathrm{a}\pm295$	$35.5~a\pm2.2$
	60	1422 a $\pm$ 370	$35.2~\mathrm{a}\pm2.3$
	75	1483 a $\pm$ 349	$35.7b\pm2.5$
	90	$1560~\mathrm{a}\pm287$	$35.5 \text{ b} \pm 2.4$
Yea	ar (Y)		
	2016	$1451~\mathrm{a}\pm369$	$38.5~d\pm0.5$
	2017	$1380~\mathrm{a}\pm164$	$34.9~b\pm0.8$
	2018	$1829~\mathrm{b}\pm175$	$36.2 c \pm 1.4$
	2019	$1294~\mathrm{a}\pm324$	$32.3~\mathrm{a}\pm0.5$

Row Spacing (R)	Planting Density (D)	Protein Yield (kg∙ha <sup>-1</sup> )	Protein Content (%)
Mean		$1488\pm338$	$35.5\pm2.4$
R		ns	ns
D		ns	**
Y		***	***
R  imes D		ns	***
$R \times Y$		***	***
$D \times Y$		ns	***
$R \times D \times T$	Y	ns	***

Table 5. Cont.

Experimental results have been presented as mean values  $\pm$  standard deviation. Values in the columns marked with different letters are significantly different (followed by Tukey's HSD test, *p* = 0.05). \*\*, \*\*\*, and ns mean  $\leq$ 0.01, <0.001, and not significant, respectively.

#### 3.3. Morphophysical Features of Plants

An appropriate value of LAI allows for the capture of radiation and conversion of light energy into chemical energy, resulting in high seed yield [48]. White lupin plants at narrower row spacing achieved a 12.0% higher LAI value, while plant density did not differentiate the value (Table 6). In drier years, the LAI value was the lowest at 1.5. The decrease in the LAI value could be due to the water deficit observed at the seed-filling stage in 2017. This year was characterized by the least favorable weather conditions (rainfall deficits in June and July) [49]. A study conducted by Cordeiro and Echer [48] confirmed this relationship, as years with less favorable weather conditions in the critical phase for plant growth, a lower value for this indicator was obtained. Chlorophyll is the most important pigment in plants. Chlorophyll content plays an important role in determining the photosynthetic process and affecting chlorophyll fluorescence. Soil Plant Analysis Development (SPAD) provides one of the diagnostic tools used to measure the nutritional status of crop plants. The average SPAD value was 56.1, which was higher (by 2.52%) at wider row spacing (30 cm) compared to a narrower one (15 cm). Lower plant density (60 pcs.  $m^{-2}$ ) resulted in a significant increase in the value of this indicator compared to higher density (75 and 90 pcs/m<sup>2</sup>) by 1.97 and 2.89%, respectively. Studies conducted by Hussain et al. [50], Fritschi and Ray [51], and Jańczak-Pienążek [33] on soybean plants also showed a similar relationship. Such authors obtained higher chlorophyll content under conditions of higher sunlight compared to the shading occurring at higher sowing density. The average plant height was 62.8 cm and did not differ significantly with varying row spacing and sowing density. The highest-set first pod was obtained via plants at a sowing density of 90 pcs.  $m^{-2}$  (41.9 cm). According to Gong et al. [52], the elongation of the stem and the increase in the height of the seating of the first pod may be a strategy of legumes against avoiding shading caused by increased plant density per unit area. Increases in plant height and first pod set height in soybeans caused by increased plant density were obtained in the studies of Sobko et al. [53] and Jańczak-Pieniążek et al. [33].

Weather conditions modified the morphophysiological traits of white lupin plants. The effect of weather factors in the years of the study on the value of LAI and SPAD in white lupin was also shown by Prusinski [45].

Row Spacing (R)	Planting Density (D)	ΙΔΙ	SPAD	Plant Height (cm)	1st Pod Height (cm)
Kow Spacing (K)	f lanting Density (D)	$2.7 \text{ sh} \perp 0.99$	51AD		28.4 a + 0.19
		$2.7 \text{ ab} \pm 0.00$	36.4 D ± 4.36	$60.3 \text{ a} \pm 16.3$	30.4 a ± 9.10
15	75	$2.6 a \pm 0.93$	54.6 a ± 2.71	$60.8 a \pm 17.3$	$39.0 a \pm 9.06$
	90	$2.9~\mathrm{c}\pm0.62$	55.2 a $\pm$ 3.42	$61.4~\mathrm{a}\pm16.1$	$41.2~\text{ab}\pm10.2$
	60	$2.3~\mathrm{a}\pm0.87$	57.6 abc $\pm$ 3.87	$61.2~\mathrm{a}\pm18.5$	$38.8 \text{ a} \pm 8.94$
30	75	$2.6 \text{ ab} \pm 0.86$	57.2 cd $\pm$ 4.00	$61.6~\mathrm{a}\pm17.3$	$39.3~ab\pm8.09$
	90	$2.5~ab\pm0.73$	55.7 abc $\pm$ 3.36	$62.5~\mathrm{a}\pm17.3$	$42.6\ b\pm 9.68$
15		$2.8~b\pm0.83$	$55.4~\mathrm{a}\pm3.65$	$60.9 \ \mathrm{a} \pm 17.3$	$39.6~a\pm9.45$
30		$2.5~a\pm0.82$	$56.8 \text{ b} \pm 4.12$	$61.9~\mathrm{a}\pm16.5$	$40.2~\mathrm{a}\pm8.90$
	60	$2.5~\mathrm{a}\pm0.89$	$57.0~b\pm4.68$	$61.1~\mathrm{a}\pm18.2$	$38.6~\mathrm{a}\pm8.92$
	75	$2.6~\mathrm{a}\pm0.88$	55.9 a $\pm$ 3.61	$61.2 \text{ a} \pm 16.7$	$39.2 \text{ a} \pm 8.45$
	90	$2.7~\mathrm{a}\pm0.70$	$55.4~\mathrm{a}\pm3.35$	$61.9~\mathrm{a}\pm16.1$	$41.9~b\pm9.95$
Ye	ar (Y)				
	2016	$3.6~\text{d}\pm0.34$	54.4 a $\pm$ 1.91	$78.6~\mathrm{c}\pm2.5$	$57.7~b\pm3.94$
	2017	$1.5~\mathrm{a}\pm0.28$	54.2 a $\pm$ 1.44	$53.2~\mathrm{a}\pm3.0$	$30.7~\mathrm{a}\pm2.12$
	2018	$2.9~\mathrm{c}\pm0.33$	53.9 a $\pm$ 1.47	$75.3b\pm4.7$	$53.6~b\pm3.81$
	2019	$2.5b\pm0.45$	$61.9~b\pm2.96$	$58.7~\mathrm{a}\pm3.7$	$37.7~\mathrm{a}\pm3.94$
N	lean	$2.78\pm0.83$	$56.1\pm3.93$	$62.8\pm16.8$	$41.2\pm9.15$
R		***	***	ns	ns
D		*	***	ns	***
Y		***	***	***	***
$R \times D$		*	*	ns	ns
$R \times Y$		*	ns	*	ns
$D \times Y$		*	**	*	ns
R  imes D  imes Y		ns	ns	ns	ns

**Table 6.** Effect of row spacing and sowing density on selected morphophysical traits of white lupin plants.

Experimental results have been presented as mean values  $\pm$  standard deviation. Values in the columns marked with different letters are significantly different (followed by Tukey's HSD test, p = 0.05). \*, \*\*, \*\*\*, and ns mean  $\leq 0.05$ ,  $\leq 0.01$ , <0.001, and not significant, respectively. LAI—Leaf Area Index, SPAD—Soil Plant Analysis Development.

#### 3.4. Chlorophyll Fluorescence Parameters

Chlorophyll *a* fluorescence is a good indicator of photosynthetic activity, light energy conversion efficiency, and excitation energy transfer [54]. Therefore, chlorophyll a fluorescence measurements can be used to study the effects of environmental stresses, e.g., caused by water deficit or low and high temperatures [28]. This allows us to understand the mechanisms occurring during photosynthesis [54]. In addition, chlorophyll fluorescence measurements can be a helpful tool to assess the response of the plant photosynthetic apparatus under environmental conditions and predict its yield [55]. Light availability has a significant impact on the physiological processes taking place in plants. This is important, especially under conditions of varying plant density in the canopy. The mutual proximity of plants at too high a sowing density can result in reduced PAR availability, which causes changes in the quality of light reaching the plant. The average values of selected chlorophyll fluorescence indicators were 0.82 (Fv/Fm), 4.70 (Fv/F0), 20.2 (PI), and 5.07 (RC/ABS) (Table 7). The conducted studies showed no influence of experimental factors on the differentiation of these parameters. Research conducted by Jańczak-Pieniazek et al. [33] on soybean plants and by Tobiasz-Salach et al. [32] on pea plants demonstrated the opposite relationship. It was found that sowing density and row spacing had a differentiating effect on the obtained chlorophyll fluorescence parameters. These authors showed that increasing the density of plants per unit area causes mutual shading of plants, which reduces the availability of light, reduces photosynthesis, and

causes a decrease in the value of chlorophyll fluorescence parameters. Similar relationships have been demonstrated in the conducted studies. This may indicate a lower susceptibility of the lupine photosynthetic apparatus to changes in plant density in the canopy compared to peas and soybeans. Only the influence of weather conditions in the study years was found. High values of chlorophyll fluorescence parameters were found in 2016. The lowest values of the Fv/Fm parameter (0.81) were shown in 2018 and 2019, Fv/F0 in 2019 (4.13), and PI and RC/ABS in 2017 (18.3 and 4.48) and 2018 (16.5 and 4.51). In many plants, stress caused by insufficient rainfall induces a disruption of photosynthesis caused by changes in the ultrastructure of organelles and the concentration of pigments (pigments), metabolites, and enzymes that are involved in stomatal regulation [56]. Studies conducted on lupin and peas by Juzoń et al. [57] have shown variability in the species studied for the adaptation of the photosynthetic apparatus to drought conditions. This variability is also noticeable not only between species but also within species.

Table 7. Effect of row spacing and sowing density on selected physiological parameters of white lupin.

Row Spacing (R)	Planting Density (D)	F <sub>v</sub> /F <sub>m</sub>	Fv/F0	PI	RC/ABS
	60	$0.85~\mathrm{a}\pm0.02$	$4.71~\mathrm{a}\pm0.87$	$21.0~\mathrm{a}\pm4.91$	$4.98~\mathrm{a}\pm0.91$
15	75	$0.83~\mathrm{a}\pm0.02$	$4.80~\mathrm{a}\pm0.39$	$20.7~\mathrm{a}\pm4.94$	$5.10~\mathrm{a}\pm1.04$
-	90	$0.85~\mathrm{a}\pm0.02$	$4.76~\mathrm{a}\pm0.74$	$20.3~\mathrm{a}\pm5.04$	$5.05~\mathrm{a}\pm0.79$
	60	$0.83~\mathrm{a}\pm0.02$	$4.56~\mathrm{a}\pm0.55$	19.3 a $\pm$ 5.17	$5.19~\mathrm{a}\pm0.97$
30	75	$0.83~\mathrm{a}\pm0.01$	$4.79~\mathrm{a}\pm0.36$	$21.4~\mathrm{a}\pm5.91$	$5.24~\mathrm{a}\pm1.20$
	90	$0.84~\mathrm{a}\pm0.05$	$4.54~\mathrm{a}\pm0.64$	$18.8~\mathrm{a}\pm4.21$	$4.85~a\pm0.82$
15		$0.82~\mathrm{a}\pm0.03$	$4.76~\mathrm{a}\pm0.68$	$20.7~\mathrm{a}\pm4.86$	$5.04~\mathrm{a}\pm0.90$
30		$0.82~\mathrm{a}\pm0.03$	$4.63~\mathrm{a}\pm0.53$	$19.8~\mathrm{a}\pm5.16$	$5.09~\mathrm{a}\pm1.00$
	60	$0.82~\mathrm{a}\pm0.02$	$4.64~\mathrm{a}\pm0.72$	$20.1~\mathrm{a}\pm5.04$	$5.08~\mathrm{a}\pm0.93$
	75	$0.82~\mathrm{a}\pm0.02$	$4.80~\mathrm{a}\pm0.37$	$21.0~\mathrm{a}\pm5.37$	$5.17~\mathrm{a}\pm1.11$
	90	$0.82~\mathrm{a}\pm0.04$	$4.65~\mathrm{a}\pm0.69$	$19.6~\mathrm{a}\pm4.63$	$4.95~\mathrm{a}\pm0.80$
Y	ear (Y)				
	2016	$0.84\ b\pm 0.01$	$5.29~d\pm0.34$	$23.6b\pm3.43$	$5.33~\mathrm{c}\pm0.51$
	2017	$0.83~b\pm0.01$	$4.88~\mathrm{c}\pm0.21$	$18.3~\mathrm{a}\pm2.16$	$4.48~\mathrm{a}\pm0.41$
	2018	$0.81~\mathrm{a}\pm0.04$	$4.47~b\pm0.38$	$16.5 \text{ a} \pm 3.12$	$4.51~\mathrm{a}\pm0.49$
	2019	$0.81~\mathrm{a}\pm0.02$	$4.13~\mathrm{a}\pm0.68$	$22.6b\pm6.44$	$5.96~d\pm1.21$
Mean		$0.82\pm0.03$	$4.70\pm0.61$	$20.2\pm5.01$	$5.07\pm0.95$
R		ns	ns	ns	ns
D		ns	ns	ns	ns
Y		***	***	***	***
$R \times D$		ns	ns	ns	**
$R \times Y$		ns	ns	ns	*
$D \times Y$		ns	*	*	ns
$R \times D \times Y$		ns	ns	ns	ns

Experimental results have been presented as mean values  $\pm$  standard deviation. Values in the columns marked with different letters are significantly different (followed by Tukey's HSD test, p = 0.05). \*, \*\*, \*\*\*, and ns mean  $\leq 0.05, \leq 0.01, < 0.001$ , and not significant, respectively. Fv/Fm—maximum quantum yield of photosystem II (PSII), Fv/F0—maximum quantum yield of primary photochemistry, PI—efficiency index, RC/ABS—total number of active reaction centers for absorption.

#### 3.5. Number of Nodules on the Main Root and Lateral Roots

Nitrogen is one of the most important plant nutrients, and biological nitrogen fixation is an important source of this element in agricultural crops [58]. Symbiotic systems that are N<sub>2</sub>-fixing systems can play a significant role in improving the fertility and productivity of soils with low nitrogen content [59]. Both row spacing and sowing density did not affect the number of nodules on the main root and lateral roots. Only a statistically significant interaction between the factors of the experiment on the number of nodules in lupine was noted (Table 8). Cultivation of lupine using a row spacing of 15 cm and a sowing density of 60 pcs.  $m^{-2}$  resulted in a higher number of nodules compared to a row spacing of 30 cm and a density of 75 pcs.  $m^{-2}$  by 85.59% on the main root, and 123,10% on the lateral roots, respectively. The highest number of nodules on lateral roots was found at a wider spacing and density of 90 pcs.  $m^{-2}$  (9.58 pcs.), while the lowest number was found at 15 cm spacing and a density of 90 pcs.  $m^{-2}$  (4.20 pcs.) and 30 cm row spacing and density of 75 pcs.  $m^{-2}$ (3.81 pcs.). In a study conducted by Jańczak-Pieniążek et al. [33], the effect of sowing density on soybean nodulation was noted, while no effect of row spacing was found. However, these authors showed a reduction in the number of nodules as a result of using a higher sowing density. A significant effect of test years on lupine nodulation was shown. The highest number of nodules was shown in 2017 on the main root (10.38 pcs.) and in 2018 on lateral roots (16.33 pcs.). According to Zahran [56], environmental conditions are factors that limit the growth and activity of N<sub>2</sub>-fixing plants. The N<sub>2</sub> fixation process in the *Rhizobium*legume symbiosis is strongly related. According to Kunert et al. [60], water deficiency, in addition to a decrease in yield levels, may lead to a reduction in the number of root nodules and the associated inhibition of N<sub>2</sub> fixation. However, Bordeleau and Prevost [61] indicated that N nodulation and fixation are observed in a wide temperature range, with optimal temperatures between 20 and 30 °C. Low temperatures reduce nodulation and nitrogen fixation in legumes. Therefore, under environmental stresses, including rainfall deficiency during the growing season, there may be an incomplete ability of plants to fix nitrogen. One of the direct responses of plants to water stress is morphological changes that appear at low water potential, which ultimately leads to a reduction in infection and nodulation of legumes [16].

Row Spacing (R)	Planting Density (D)	Number of Nodules per Plant in Main Root (pcs.)	Number of Nodules per Plant in Lateral Roots (pcs.)
	60	$5.03 c \pm 5.63$	$8.50~{ m bc}\pm9.45$
15	75	$3.17~\mathrm{abc}\pm4.51$	$7.66~\mathrm{abc}\pm8.76$
	90	$2.96~\mathrm{ab}\pm3.36$	$4.20 \text{ a} \pm 2.88$
	60	$3.23~\mathrm{abc}\pm4.43$	$5.26~\mathrm{ab}\pm3.19$
30	75	$2.77~\mathrm{a}\pm3.36$	3.81 a ± 3.31
	90	$4.87~\mathrm{bc}\pm6.54$	$9.58 c \pm 13.16$
15		$3.72~\mathrm{a}\pm4.56$	$6.79 a \pm 7.64$
30		$3.62~\mathrm{a}\pm4.90$	$6.22 \text{ a} \pm 8.20$
	60	$4.13~\mathrm{a}\pm5.04$	$6.88 \text{ a} \pm 7.09$
	75	$2.97~\mathrm{a}\pm3.89$	$5.74 \text{ a} \pm 6.77$
	90	$3.92~\mathrm{a}\pm5.18$	$6.89 a \pm 9.71$
Year	: (Y)		
	2016	$1.02~\mathrm{a}\pm0.52$	$4.01~\mathrm{a}\pm2.63$

Table 8. Effect of row spacing and sowing density on nodulation of white lupin.

Row Spacing (R)	Planting Density (D)	Number of Nodules per Plant in Main Root (pcs.)	Number of Nodules per Plant in Lateral Roots (pcs.)
	2017	$10.38 \text{ b} \pm 4.38$	$1.95~\mathrm{a}\pm0.78$
	2018	$2.46~{ m a}\pm 2.69$	$16.33 \text{ b} \pm 10.31$
	2019	$0.82~\mathrm{a}\pm0.56$	$3.72 \text{ a} \pm 2.50$
Mean		$3.67\pm4.70$	$6.50\pm7.88$
R		ns	ns
D		ns	ns
Y		***	***
$R \times D$		*	***
$R \times Y$		ns	ns
$D \times Y$		ns	ns
$R \times D \times Y$		ns	***

Table 8. Cont.

Experimental results have been presented as mean values  $\pm$  standard deviation. Values in the columns marked with different letters are significantly different (followed by Tukey's HSD test, p = 0.05). \*, \*\*\*, and ns mean  $\leq 0.05$ , <0.001, and not significant, respectively.

# 3.6. Correlations between Features

In the experiment, the yield of white lupine seeds was positively correlated with the number of pods per plant (r = 0.430), the number of seeds per plant (r = 0.527), the weight of seeds per plant (r = 0.311), the plant height (r = 0.345), the first pod height (r = 0.697), protein yield (r = 0.958), LAI (r = 0.419), and the number of nodules per plant on lateral roots (r = 0.463) (Table 9). The number of pods and seeds per plant are the main components of the yield structure, so they may influence the obtained yield value [23,40]. A negative correlation of seed yield was found with the weight of 1000 seeds (r = -0.527) and the number of nodules per plant on the main root (r = -0.376).

Variables 1		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.000															
2	-0.527	1.000														
3	0.430	-0.705	1.000													
4	0.527	-0.796	0.892	1.000												
5	0.311	-0.590	0.865	0.863	1.000											
6	0.345	-0.035	-0.464	-0.293	-0.606	1.000										
7	0.697	-0.771	0.350	0.497	0.196	0.481	1.000									
8	0.958	-0.436	0.284	0.399	0.148	0.495	0.686	1.000								
9	0.419	-0.251	-0.059	0.041	-0.288	0.624	0.448	0.517	1.000							
10	-0.022	-0.208	0.621	0.531	0.618	-0.706	-0.187	-0.153	-0.175	1.000						
11	-0.061	0.458	-0.596	-0.460	-0.524	0.439	-0.169	0.042	0.185	-0.451	1.000					
12	-0.091	0.394	-0.443	-0.330	-0.359	0.205	-0.225	-0.002	0.086	-0.177	0.668	1.000				
13	0.152	-0.070	0.346	0.396	0.369	-0.344	-0.141	0.069	0.099	0.607	0.224	0.148	1.000			
14	0.050	0.095	0.070	0.142	0.114	-0.184	-0.196	0.032	0.215	0.390	0.433	0.403	0.775	1.000		
15	-0.376	0.419	-0.340	-0.363	-0.105	-0.168	-0.365	-0.385	-0.635	-0.265	0.090	0.043	-0.347	-0.311	1.000	
16	0.463	-0.547	0.197	0.324	0.154	0.386	0.707	0.425	0.276	-0.260	-0.161	-0.338	-0.275	-0.269	-0.112	1.000

Table 9. Pearson Correlation Coefficient (r).

1. seed yield; 2. weight of 1000 seeds; 3. number of pods per plant; 4. number of seeds per plant; 5. seed weight per plant; 6. plant height; 7. 1st. pod height; 8. protein yield; 9. LAI; 10. SPAD; 11. Fv/F0; 12. Fv/Fm; 13. RC/ABS; 14. PI; 15. number of nodules per plant (main root); 16. number of nodules per plant (lateral roots).

A strong positive correlation was indicated between the number of seeds per plant and the number of pods per plant (r = 0.892) and between the weight of seeds per plant, the number of pods per plant (r = 0.865) and the number of seeds per plant (r = 0.863).

A similar relationship was obtained in the research of Georgieva et al. [62] on white lupine. These authors found a positive correlation between the number of pods per plant with the number and weight of seeds per plant and the first pod height. A highly positive correlation was also demonstrated between the number of seeds per plant and the weight of seeds per plant. Similar to their own research, these authors showed a negative correlation between the weight of 1000 seeds and the height of the plant as well as the number of pods and seeds per plant.

## 4. Conclusions

In this study, it was shown that different row spacing and sowing density have no significant effect on the yield obtained, the number of pods per plant, and the weight of 1000 seeds of white lupin. The effect of the experiment factors on nodulation and chlorophyll fluorescence parameters (Fv/Fm, Fv/F0, PI, and RC/R) was also not shown. However, lower plant density per unit area had an effect on increasing the number of seeds per pod and seed weight per plant, which was associated with better conditions for plant growth. Wider row spacing (30 cm) and lowest sowing density (60 pcs. m<sup>-2</sup>) resulted in the highest chlorophyll content. For the LAI index, the highest value was found at a row spacing of 15 cm. The study also showed that greater competition between lupine plants in the canopy resulted in an increase in the height of the setting of the first pod. The results obtained prove that a lower density of lupine plants per unit area is a more profitable variant because it allows obtaining a yield at a similar level to variants in which a higher plant density was used, which is associated with a lower cost of seed purchase. This knowledge is valuable and can be useful for farmers in lupine cultivation, especially on low-income farms.

**Author Contributions:** Conceptualization, R.T.-S. and M.J.-P.; methodology, R.T.-S. and M.J.-P.; formal analysis, A.A.-P.; investigation, R.T.-S. and M.J.-P.; writing—original draft preparation, R.T.-S.; M.J.-P., and A.A.-P.; writing—review and editing, R.T.-S. and M.J.-P. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was made possible by a grant from the Polish Ministry of Agriculture and Rural Development; Project: Improving domestic sources of plant protein, their production, trading and use in animal feed, project No. HOR 3.3/2016–2020.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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