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Article

Potential of Sunflower-Legume Intercropping: A Way Forward in Sustainable Production of Sunflower in Temperate Climatic Conditions

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Abstract: Changing climate conditions coupled with the transformations of cultivation practices and land use in sole crop-based sunflower production may significantly decline yield stability of this oilseed crop. Given that sunflower takes the third place in the world oilseed market, with 45 million tons per year, and in the fourth place in vegetable oil production, it is necessary to adapt production technologies toward sustainable agriculture. Considering that, the goal of the research was to analyze and beneficial sustainable production technology of sunflower in intercropping systems. A four-year trial was conducted in Serbia's agroecological rain-fed conditions (45°34'23.2"N 19°86'18.9"E) using a split-plot design. Two oil types and one confectionary sunflower hybrid were intercropped with common vetch, red clover and alfalfa. Analyses showed that intercropping of sunflower with common vetch resulted in the decrease in almost all sunflower trait values. Also, sunflower × alfalfa intercropping provided to be the most appropriate. The yield of NS Gricko and Rimi PR were statistically on the same level with sole cropping, while alfalfa biomass had better results when intercropped with NS Gricko as compared to sole cropping. Concerning the general belief that yields are more stable in intercropping than in sole crop, further research in this respect is needed, in addition to the research of time and method of sowing.

Keywords: sunflower, intercropping, legumes, sustainable production

1. Introduction

Changing climate conditions coupled with the transformations in cultivation practices and land use in sole crop-based sunflower production may significantly decline yield stability of this important oilseed crop. More than 80% of this crop is mainly grown in just 10 world countries, primarily in Ukraine, Russia and Argentina, and two thirds of the world's production is concentrated in Europe, a small part of the world with favorable but extremely changing climate conditions. Given the sunflower global significance, it is very important to theist the methods of sustainable sunflower production in the era of population growth and accelerated climate change characterized by higher average temperatures, extreme climatic hazards, reduced water availability and water logging, etc. [1]. Despite permanent genetic yield potential improvement over the years, most of the mentioned sunflower production countries have experienced major yield gaps, between 1.1 and 2.4 t/ha at a national level [2]. Thus, modification and improvement of cropping practices for the changing environment will have a major role in the achievement of sustainable sunflower production and global food security [3].

Diversity at every level, from genetic to ecosystem, contributes to the ability of cropping systems to overcome and adapt to the forthcoming changes. According to EU Biodiversity Strategy for 2030 biodiversity is fundamental in preserving the EU and world food safety [4]. Along with the food safety, biodiversity supports healthy and nutritious diets, enhances the efficiency of agroecosystems, and boosts sector resilience to the changing environmental conditions, climate risks and socioeconomic challenges. In addition to the EU Biodiversity Strategy for 2030, Farm to Fork Strategy and the new Common Agricultural Policy (CAP) are also encouraging eco-schemes and sustainable systems.

Many alternative agricultural practices can be applied in order to adapt crop production to climate change and variability. Although it is a centuries-old farming system, intercropping can be used as the 21st century agriculture solution to improve resource use efficiency and yield stability under the conditions of changing precipitation sums and adverse temperatures. Some of the reasons for including intercropping are: increased yields of crops by a better utilization of soil and rainfall resources in a given area of land, better disease and insect control, better distribution of labour and saleable produce over a calendar year, stabilization of annual yields to a greater extent than by sole-cropping, more effective control of erosion, decrease or elimination of the need for commercial fertilizers, reduced risk of crop failure, and many other [5-10].

In some countries, intercropping has a widespread usage; so over 40% of maize is intercropped in the Dominican Republic and 50% in Jamaica, 60% of beans is intercropped in Brazil, while 50% of the area in Zimbabwe is planted with mixed crops [11]. In Latin America, small-scale farmers grow between 70–90% of beans with maize, potatoes and other crops, whereas maize is intercropped on 60% of the maize-growing areas [12]. However, in Europe, intercropping disappeared from many systems and nowadays perseveres mostly in agroforestry, orchards or production of cereals and legumes. It has also been rarely found in the research on production technologies, probably because of its complexity. Intercropping is therefore rare in industrial agriculture, intensive systems, yet it is expanding in organic [13].

Research on intercropping has shown that it is best when one of the species is from the *Fabaceae* family. Species from this family are "expected" to be able to create a large amount of aboveground mass, have a strong root system with high absorption power, and be moderate in nutrition requests e.g. adaptable to poorer soils, adaptable to shaded conditions, with high weed suppression and ability to fix atmospheric nitrogen [14-16]. Driven by photosynthesis, biological nitrogen fixation by legumes is a significant ecosystem service, which can partially meet the nitrogen needs of other crops in the system, and improve overall productivity [17]. According to the Food and Agriculture Organization of the United Nations (FAO) present global use of N fertilizer is assessed at around 110 Tg of N/year [18]. The N2 fixed by grain legumes is assessed at around 22 Tg N and by forage legumes around 20 Tg N/year, which is low given the fact that, prior to the mass introduction of N fertilizers, commonly 25–50% of agricultural land was cropped on a legume base using cover crops and animal manure [19].

Given that sunflower with 45 million tons per year takes the third place in the world oilseed market, representing a little less than 10% of the global oilseeds production, and the fourth place in vegetable oils production, also with a little less than 10% [20], it is necessary to intensify production technologies in the direction of sustainable agriculture favorable for agroecosystems. In favor of the above mentioned are the facts that sunflower is a relatively adaptable crop, grown in different, mostly dry areas, and that Helianthus genus is very large and diverse (39 perennial and 14 annual species) [21], which is confirmed by herbicide resistant genes found in the sunflower wild population [22].

With all of the above-mentioned in mind, the goal of this research is to analyze and recommend sustainable production technology of sunflower in intercropping systems. The research considered sunflower as the main, cash crop, and investigated different legumes as complementary crops, with living mulch as the soil cover. In order to achieve the stated advantages of this production method, the research focused on defining the most suitable legumes for intercropping with sunflower. The aim was to determine how legumes affect sunflower production, as well as affecting qualitative, physiological and morphological traits of sunflower, but also how this system affects biannual legumes as crops that remain for exploration in the second year after the sunflower harvest (in the first year).

2. Materials and Methods

2.1. Experimental Design

For the purpose of our study a four-year trial was conducted from 2017 to 2020 at the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia at Rimski Šančevi (45°34’23.2"N 19°86’18.9"E). A rain-fed experiment was established using a split-plot design in four repetitions. Three hybrids of sunflower, two oil types (Rimi PR and Dukat) and one confectionary type (NS Gricko), were intercropped with forage legumes (*Vicia sativa* L., Novi Beograd variety; *Trifolium pratense* L., Una variety; *Medicago sativa* L., Banat VS variety), whereas sole cropping of sunflower and legumes was used as the control treatment. The implemented cultivation practices the same as the production conditions of the Republic of Serbia, by using plowing at 27-30 cm soil depth in autumn. Grain sorghum was used as preceding crop to ensure a neutral impact on the main crop and legumes as complementary crop. Pre-sowing seedbed preparation and cultivation was done by System Kompactor at optimal soil moisture. Sunflower Hybrids were sown as the main crop in the first half of April, while legumes, as a supplementary crop, were sown a day before. Sunflower was sown with Wintersteiger PSP Single Disk seeder in six rows, with a row spacing of 70 cm and a 25 cm spacing of plants in a row. Legumes were sown with Amazone 08-30 Super seed drill (seeder). The sowing rate of red clover and alfalfa was 18 kg/ha and the common vetch 120 kg/ha, the same as in biomass production. The size of the single plot was 9×3.5 m, with 1.5 m spacing between treatments, i.e. legume, and repetitions. In the autumn before winter ploughing, basic macronutrients were supplied to the soil in the form of double NP (11:52) fertilizer MAP with high phosphorus content and sufficient amounts of nitrogen, in the amount of 220 kg/ha. Before sowing, the soil was rolled to provoke the initial emergence of weeds, which were then destroyed by interrow cultivation. After the emergence of crops, hand weeding was applied in the row area.

2.2. Plant Biometric Assessment

2.2.1. Yield and yield related traits in sunflower

During the four-year period, the yield and yield components of sunflower were analyzed, as well as the quality components. The sample size for the field measurement was 10 random plants per repetition, and for laboratory measurement 20 plants per repetition. Two internal rows (excluding the first and last plant), i.e. every other plant, were used for the analysis of sunflower yield and yield components. The weight of 1000 seeds (g) was measured on a random sample of absolutely clean and air-dried seed by measuring 2 x 200 seeds and converted to 1000 seeds. Seed yield per head (g) was measured after manually harvesting the plot. The obtained yield per sunflower head was converted into t/ha relative to counted number of plants per plots. The analysis of oil content in the grain was carried out non-destructively on pure air-dried seed samples, in a nuclear magnetic resonance (NMR) analyzer [23] and expressed in %. The oil yield, obtained as a product of grain yield and grain oil content, was expressed in kg/ha. Samples for determining the oil content were formed according to the ISO 9001 procedure.

In addition to seed an oil yield and yield component analysis, plant height (cm) and head diameter (cm) were also analyzed, measuring 10 plants in each hybrid in four repetitions. The total leaf area of sunflower per plant (cm^2) was measured using LI3100 area meter (LI-COR) at the BBCH stage 55 (bud stage) and 63 (flowering stage). The height of sunflower plants was measured by a graduated bar, at the BBCH stage 55 and 63. The diameter of the head was measured at the flowering stage using a millimeter strip.

2.2.2. Biomass and biomass related traits in legumes

During the four-year period, plant biomass and crop height were measured for common vetch, red clover and alfalfa in the year of the establishment. The number of shoots per plant was also measured in red clover and alfalfa. All measurements results were collected during the flowering phase of the legumes. The sample size for crop height and number of shoots per plant was 10 plants per repetition at internal rows (excluding the first and last plants). Plant biomass was taken from 50 square cm wooden frame. Crop height was measured by a graduated bar, and the number of shoots were measured manually.

2.2.3. Land equivalent ratio (LER)

The efficiency of intercropping was assessed by Land equivalent ratio (LER) [24] defined as the relative land area required for sole crops to produce the same yields as intercrops, at the same management level. Considering that the selected combination of crops included sunflower as an annual and red clover and alfalfa as perennial species, and that they manifested their full potential after the first year, we modified LER and created the LERmax index. Therefore, the land that would be used for three years in sole cropping (one year of sunflower and two years of legumes) was used for two years in the intercropping system. LERmax indicated the yield of sunflower in the first and fresh legume biomass in the second year and was calculated as follows:

$$\mathrm{LERmax} = \mathrm{L(a)} + \mathrm{L(b)} = \frac{\mathrm{Y(a)}}{\mathrm{S(a)}} + \frac{\mathrm{Y(b)}}{\mathrm{S(b)}} \quad (1)$$

where L (a) and L (b) are partial LER indexes for sunflower in the first year and legumes in the second
year from the trial establishment, Y (a) and Y (b) - yields of species A and B in the intercropping, S (a) and S (b) - yields of species A and B in sole cropping.

If LERmax value is equal to 1, it would mean that it does not matter whether the crop is grown insole cropping or in intercropping, because the productivity is the same. If LERmax value is for example 1.1 or 1.3, intercropping stands out by 10% namely 30% or more precisely, 10% i.e. 30%more land area is needed in sole cropping to gain the same yield obtained as in intercropping.

2.3. Field site description

2.3.1. Location and weather conditions

Field trial area is located in the southern part of the Pannonian plane, where the climate is moderate continental with seasonal variation in temperature and precipitation. Based on the long-term data (1970–2020), the mean annual temperature was 11.7°C, annual precipitation sum 642.8 mm, the mean temperature for the growing period (April–October) 17.6°C and the precipitation sum for the growing period was 431.7 mm. The mean monthly temperature and monthly precipitation data for the vegetation period from 2017 to 2020 were taken from the Main Meteorological Station - Rimski Šančevi, the Republic Hydrometeorological Service of Serbia, and showed at the Walter climate diagram (Figure 1). In 2017, precipitation level was 82.9 mm in May, and 12.0 mm and 17.4 mm in July and August, respectively with extremely high temperatures in these two months. The average temperature during the growing season was 19°C. In 2018, May had about 20 mm less precipitation than in 2017, but June was extremely humid with 163.2 mm of precipitation, which is about 100 mm more than in 2017. July precipitation was also above the 50-year average (81.2 mm). The average daily temperature during the 2018 growing season was 19.8°C. The beginning of the growing period in April was extremely warm with an average daily temperature of 17.2°C. In 2019, the highest sum of precipitation was recorded in May, namely 147.6 mm, whereas July was dry with just 21.00 mm of rain. Regarding the temperatures, this year was similar to the long-term averages, with May being about 3 degrees colder than average and June about 3 degrees warmer. In 2020, April and October were very dry with just 14.0 mm and 5.0 mm of rain, respectively. Regarding the

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temperatures, 2020 was similar to the long-term averages. The average monthly temperatures in the four-year period of the experiment varied considerably compared to the 50-year average, and it can be concluded that the weather conditions in recent years have been quite variable and unpredictable.

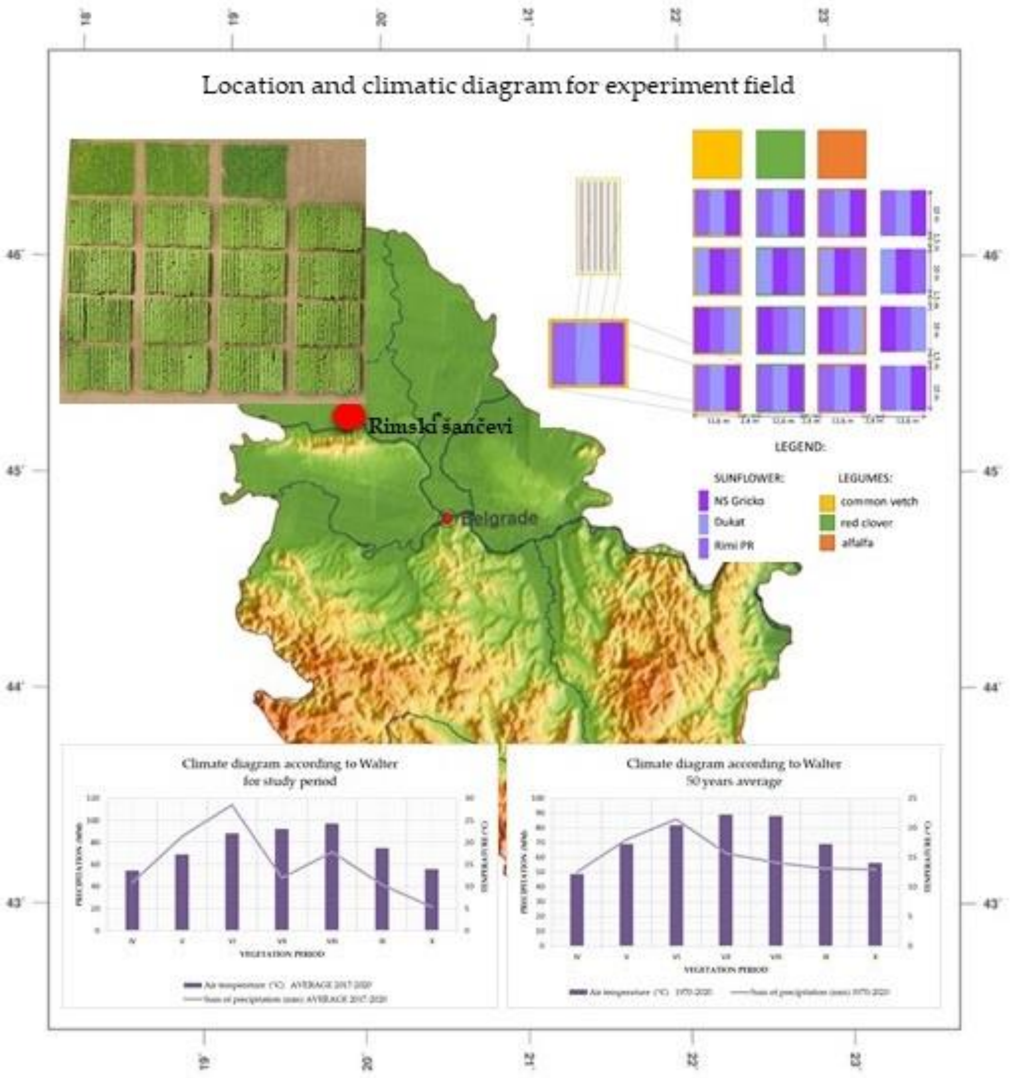

Figure 1. Location of the trial and climatic conditions for the study period with long-term averages

2.3.2. Soil sampling and characteristics

Composite samples were taken each year at the beginning of the vegetative season in three replicates and mixed to obtain the representative soil samples at depth of 10-30 cm. Determination of active acidity (pH in H_2O) was done in suspension (10g: 25cm^3) of soil with water, potentiometrically by pH meter; determination of potential acidity (pH in KCl) was done in suspension (10g: 25cm^3) of soil with potassium chloride, potentiometrically by pHmeter. Calcium carbonate (CaCO3) was determined volumetrically, using a Scheibler calcimeter. Organic matter content was determined by oxidation of organic matter (Tyurin method). The content of total nitrogen (CNS elemental analysis of total combustion of the sample) was determined by an automatic method, using the CHNS analyzer. Readily available phosphorus (extraction with ammonium lactate) was determined by the AL method (spectrophotometrically); readily available potassium (extraction with ammonium lactate) – by AL method (flame-photometrically). Determination of mechanical composition of soil (sieving-sedimentation) was done by pipette method, preparation of samples with Na-pyrophosphate according to Thyn. Analyses were performed in the Laboratory for Soil and Agroecology of the Institute of Field and Vegetable Crops, Novi Sad.

According to the FAO/WRB classification [25] the soil in the experimental plots is slightly carbonated loamic chernozem – Calcic Gleyic Chernozem (CH-cc.gl-lo). Before the trial establishment, the analysis showed that pH in KCl was 7.05 and in H_2O 7.94; organic matter was 3.13%; CaCO3 1.06%; N total 0.236%; AL-K_2O 42.3 mg/100 g; AL-P_2O_5 9.33 mg/100 g. Based on the results of soil analyzes (Table 1.), it can be concluded that the pH reaction of the soil in KCl is neutral, while in water neutral to alkaline; The soil can be characterized as slightly carbonate. It is moderately provided with humus and total nitrogen, poorly to moderately provided with radialy available phosphorus, as well as with potassium. The mechanical composition represents the content of all mechanical fractions, expressed as a percentage of the dry sample mass. Table 1 shows values of above-mentioned soil characteristics before the trial establishment.

Table 1. Characteristics of the soil before the establishment of the trial

Soil chemical properties						
pH		$CaCO_3$	OM %	total N	AL-P_2O_5	AL-K_2O
in KCl	in H_2O	%		%	mg/100 g	mg/100 g
7.05	7.94	1.06	3.13	0.236	9.33	42.30

Soli mechanical composition				
rough sand (2-0.2 mm) %	fine sand (0.2-0.02 mm) %	silt (0.02-0.002 mm) %	clay (<0.002 mm) %	texture class (Tommerup)
0.82	38.12	31.30	30.31	loamy clay

2.4 Statistical Analyses

The linear mixed model (LMM) was fitted to every sunflower trait in according to the model for split plot design. Three different legumes and sunflower sole cropping as control were used as main plot, and sunflower hybrids were used as a sub-plot. All terms related to the main plot and sub-plot were treated as the fixed effects in the model (including various interaction effects), whereas the corresponding errors related to the fixed effects in the model as the random terms. For every particular trait, two independent models were fitted: (i) model which assumes the homogeneous residual variances across the years and (ii) model which assumes the heterogeneous residual variances across the years. The selection among two competitive models was made in according to the Bayesian Information Criterion (BIC). The model with lowest BIC value was selected for further discussion regarding the statistical inference of fixed model terms and least-squares comparisons. LMM procedure was done in SAS [26]. A linear relationship between sunflower variables (traits) was calculated by Pearson correlation coefficients in SPSS [27]. Analysis of variance (ANOVA), with additional post hoc tests, was used in SPSS [27] to evaluate how type of cropping influenced the analyzed legume traits.

3. Results

3.1 Yield and yield related traits in sunflower

Yield is consider as most important indices of sunflower growing, however to reveal the potential of intercropping it is required to accesses various morphological properties. Two different Models were used based on AIC (Akaike's Information Criteria) and BIC (Bayesian Information Criteria) statistics as penalized-likelihood criteria. Head diameter, oil content, plant height in bud phase, plant height in flowering, total leaf area per plant in flowering and thousand seed weight more suitable model (Model 1) is with the homogeneous residual error variances across the years, while for number of full seeds per head, seed yield, oil yield, seed yield per head, and total leaf area per plant in bud phase more suitable model (Model 2) is with the heterogeneous residual error variances across the years (Table 2.).

Table 2. Bayesian Information Criteriastatistics for Model 1 and 2.

Trait	Model 1	Model 2
Plant height/bud phase (PHB)	707.4	718.6
Plant heigh/flower phase (PHF)	717.8	732.7
Head diameter (HD)	380.5	393.8
Total leaf area/bud phase (TLAB)	1549.3	1525.5
Total leaf area/flower phase (TLAF)	1579.5	1594.5
Number of full seeds per head (NFSH)	1304.9	1286.3
Thousand seed weight (TSW)	670.7	681.2
Seed yield per head (SYH)	782.2	781.1
Seed yield (SY)	168.2	163.5
Oil content (OC)	389.5	401.8
Oil yield (OY)	14.5	11.7

Table 3 shows LMM analyses for all sunflower traits and indicates high statistical significance for different types of cropping (legumes and sole cropping), hybrids, and types of cropping ×hybrid interaction so additional tests were performed.

Table 3. Test of fixed effects for LMM analyses for sunflower traits analyzed in sunflower legumes intercropping

Source of variation	PHB	PHF	HD	TLAB	TLAF	NFSH	TSW	SYH	SY	OC	OY
year	693.91**	61.99**	2.83*	61.15**	66.56**	73.39**	41.15**	5.56*	6.07*	111.45**	15.55**
type of cropping	100.21**	103.82**	64.13**	137.15**	100.82**	95.44**	1.75^{ns}	111.39**	116.77**	5.73*	103.43**
hybrid	873.88**	1828.98**	173.77**	572.70**	624.12**	510.37**	4426.96**	336.86**	311.99**	1478.75**	43.03**
year×type of cropping	23.47**	20.10**	10.15	2.60*	1.73^{ns}	4.62**	1.36^{ns}	5.57**	5.07**	4.07*	5.96**
year×hybrid	253.97**	68.87**	22.76**	173.15**	67.43**	13.99**	6.02**	9.56**	10.81**	23.01**	12.26**
type of cropping×hybrid	5.37**	5.10**	4.85*	0.79^{ns}	2.51*	6.98**	0.67^{ns}	1.59^{ns}	2.16^{ns}	2.48*	1.21^{ns}
year×type of cropping×hybrid	6.65**	6.59**	1.29^{ns}	1.11^{ns}	1.69^{ns}	1.72^{ns}	1.63^{ns}	2.48*	2.37*	1.55^{ns}	1.87*

PHB - Plant height/bud phase, PHF - Plant heigh/flower phase, HD - Head diameter, TLAB - Total leaf area/bud phase, TLAF - Total leaf area/flower phase, NFSH - Number of full seeds per head, TSW - Thousand seed weight, SYH - Seed yield per head, SY - Seed yield, OC - Oil content, OY - Oil yield; * and ** indicated significant differences were found at p = 0.05 and 0.001, respectively; ^{ns} - not significant; values from the models selected on the basis of the table Table 2.

Additional tests in Table 4 show a clear interaction between intercrops and control. Analyzed combinations showed high variability in sunflower **head diameter** (HD), which is to be expected considering that three hybrids of different purposes were tested (short vegetation oil hybrid, Clearfield oil hybrid, confectionary hybrid). NS Gricko×common vetch had the smallest statistically significant HD (16.50 cm), and the highest when intercropped with alfalfa (19.68), but not significant compared to control (18.98 cm). Hybrid Dukat had the same trend; Dukat × common vetch 18.71 cm, Dukat in sole cropping 21.91 cm. Hybrid Rimi PR had the smallest HD (16.77 cm) when intercropped with common vetch but significant only compared to control (18.55 cm); Rimi PR in red clover alfalfa and control were on the same level of significance. **Oil content** (OC) was not significantly different in any legume regarding all tree hybrids. It was insignificantly the highest in sole crop of sunflower, and usually the lowest in intercropping sunflower hybrids with common vetch. **Plant height** of sunflower was analyzed in bud (PHB) and flower phase (PHF), and clear growth progression was observed in all three hybrids. In hybrid NS Gricko PHB and PHF were significantly the lowest when intercropped with common vetch (143.84 and 205.66 cm, respectively), and significantly highest in sole cropping (169.01 and 231.50, re-

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spectively). No significant difference was observed between sole cropping and intercropping with red clover. In hybrid Dukat, PHB and PHF were also significantly the lowest when intercropped with common vetch (125.50 and 155.25, respectively), but no statistically significant differences between intercropping with red clover, alfalfa and sole cropping, although PHB and PHF were the highest in sole cropping (136.11 and 167.98, respectively). Hybrid Rimi PR had the same trend as Dukat regarding PHB and PHF. The only statistical significance was observed between Rimi PR × common vetch and all other treatments. As one of the most important traits **seed yield** (SY) varied more in terms of hybrids than treatments. Hybrid NS Gricko had the lowest SY statistically significant when intercropped with common vetch (3.59 t/ha), and the highest, but not significant compared to the other two legumes in sole cropping (4.93 t/ha). Hybrid Dukat had the similar trend as NS Gricko regarding SY, where significantly lower SY was observed in intercropping with common vetch (2.31 t/ha) compared to the other three treatments, but SY in sole cropping was significantly higher than in intercropping (3.55 t/ha). Clearfield hybrid Rimi PR had SY (2.77 t/ha) intercropped with common vetch, which is significantly less than in other treatments. The same hybrid intercropped with alfalfa had SY 3.47 t/ha which is not significantly less than the sole crop (4.05 t/ha), and not significantly more compared to Rimi PR × red clover (3.65 t/ha). **Total leaf area in flowering** (TLAF) in NS Gricko was statistically the lowest when intercropped with common vetch (3070.79 cm^2) and the highest in sole cropping (5024.42 cm^2). The same trait measured for hybrid Dukat was statistically different in all treatments; the lowest in intercropping with common vetch and the highest in sole cropping (4661.62, 6634.81, respectively). Performances regarding TLAF in Rimi PR hybrid were the same as with the NS Gricko. Different treatments had no significant effect on one **thousand seed weight** (TSW) in any of the all three hybrids. This trait was significantly higher for NS Gricko in all treatments compared the other two hybrids.

Table 4. Least-square means difference for sunflower traits analyzed by selected model in sunflower legume intercropping

intercrop	trait	NS Gricko	Dukat	Rimi PR
common vetch	HD (cm)	16.50^{Aa}	18.71^{Ba}	16.77^{Aa}
	OC (%)	36.34^{Aa}	46.88^{Ba}	41.96^{Ca}
	PHB (cm)	143.84^{Aa}	125.50^{Ba}	161.30^{Ca}
	PHF(cm)	205.66^{Aa}	155.25^{Ba}	202.30^{Aa}
	SY (t/ha)	3.59^{Aa}	2.31^{Ba}	2.77^{Ca}
	TLAF (cm^2)	3070.79^{Aa}	4661.62^{Ba}	6574.56^{Ca}
	TSW (g)	119.26^{Aa}	53.01^{Ba}	54.22^{Ba}
	NFSH (number)	775^{Aa}	1106^{Ba}	1269^{Ca}
	OY (kg/ha)	1.30^{Aa}	1.07^{Ba}	1.17^{ABa}
	SYH (g)	90.22^{Aa}	57.84^{Ba}	69.34^{Ca}
	TLAB(cm^2)	3127.81^{Aa}	5135.06^{Ba}	5845.54^{Ca}
red clover	HD (cm)	19.23^{Ab}	21.60^{Bb}	17.75^{Cab}
	OC (%)	36.64^{Aa}	47.03^{Ba}	41.49^{Ca}
	PHB (cm)	163.63^{Abc}	134.84^{Bb}	179.72^{Cb}
	PHF(cm)	225.53^{Abc}	165.71^{Bb}	222.41^{Ab}
	SY (t/ha)	4.58^{Ab}	2.91^{Bb}	3.65^{Cb}
	TLAF (cm^2)	4560.81^{Ab}	5407.54^{Bb}	7709.15^{Cb}
	TSW (g)	120.99^{Aa}	51.52^{Ba}	55.17^{Ba}
	NFSH (number)	940^{Ab}	1370^{Bb}	1700^{Cb}
	OY (kg/ha)	1.68^{Ab}	1.37^{Bb}	1.51^{Ab}
	SYH (g)	114.46^{Abc}	72.81^{Bb}	91.16^{Cb}
	TLAB(cm^2)	4299.48^{Ab}	6129.38^{Bb}	6983.31^{Cb}
alfalfa	HD (cm)	19.68^{Ab}	21.82^{Bb}	17.83^{Cab}
	OC (%)	36.38^{Aa}	47.26^{Ba}	42.09^{Ca}
	PHB (cm)	160.49^{Ab}	135.99^{Bb}	177.61^{Cb}
	PHF(cm)	220.01^{Ab}	165.51^{Bb}	221.34^{Ab}

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	SY (t/ha)	4.54^{Ab}	2.83^{Bb}	3.74^{Cbc}
	TLAF (cm^2)	4217.81^{Ab}	5890.04^{Bc}	7596.71^{Cb}
	TSW (g)	121.87^{Aa}	53.27^{Ba}	53.3^{Ba}
	NFSH (number)	924^{Ab}	1378^{Bb}	1683^{Cb}
	OY (kg/ha)	1.64^{Ab}	1.34^{Bb}	1.57^{Abc}
	SYH (g)	112.59^{Ab}	71.38^{Bb}	93.53^{Cb}
	TLAB(cm^2)	4248.19^{Ab}	6289.92^{Bb}	6802.96^{Bb}
	HD (cm)	18.98^{Ab}	21.91^{Bb}	18.55^{Ab}
	OC (%)	36.70^{Aa}	47.51^{Ba}	42.51^{Ca}
	PHB (cm)	169.01^{Ac}	136.11^{Bb}	179.68^{Cb}
control	PHF(cm)	231.50^{Ac}	167.98^{Bb}	223.33^{Cb}
	SY (t/ha)	4.93^{Ab}	3.55^{Cc}	4.05^{Cc}
	TLAF (cm^2)	5024.42^{Ac}	6634.81^{Bd}	8264.15^{Cc}
	TSW (g)	123.51^{Aa}	53.50^{Ba}	55.42^{Ba}
	NFSH (number)	997^{Ab}	1618^{Bc}	1824^{Cb}
	OY (kg/ha)	1.81^{Ab}	1.64^{Ac}	1.73^{Ac}
	SYH (g)	123.14^{Ac}	85.00^{Bc}	100.64^{Cb}
	TLAB(cm^2)	5062.58^{Ac}	7123.08^{Bc}	7541.52^{Bc}

HD - Head diameter, OC - Oil content, PHB - Plant height/bud phase, PHF - Plant heigh/flower phase, SY - Seed yield, TLAF - Total leaf area/flower phase, TSW - Thousand seed weight, NFSH - Number of full seeds per head, OY - Oil yield, SYH - Seed yield per head, TLAB - Total leaf area/bud phase; capital letters in superscript denote differences between hybrids; Small letters in superscript denote differences between legumes;

Number of full seeds per head (NFSH) is the first trait better described by Model 2. Intercropping of all three of these hybrids with common vetch significantly reduced NFSH (775, 1106 and 1269, respectively) compared to other treatments. Hybrids NS Gricko and Rimi PR had the highest NFSH in sole cropping, but not significantly higher than red clover and alfalfa intercrops (997 and 1824, respectively). Hybrid Dukat had significantly higher NFSH in sole cropping (1618) than in all three intercrops. The trait that results from seed yield and oil content in hybrid is oil yield (OY) and, while the OC is under low influence of environmental conditions, it is a fact that OY performs the same as SY. As in SY, intercropping of all three hybrids with common vetch significantly reduced OY to 1.30, 1.07, and 1.17 t/ha, respectively, compared to other treatments. Hybrid NS Gricko had the highest OY in sole cropping (1.81 t/ha) but not significantly compared to red clover and alfalfa intercropping (1.68 and 1.64 t/ha). Dukat had significantly higher OY in sole cropping than in other treatments (1.64 t/ha), whereas Rimi PR had the best performance regarding OY in sole cropping and intercropping with alfalfa (1.73 and 1.57, respectively). Seed yield per head (SYH) can closely explain the influence of legumes on sunflower hybrids. Intercropping of legumes with NS Gricko and Dukat significantly lowered SYH, except in NS Gricko × red clover combination. Hybrid Rimi PR showed different performances in intercropping. Even though SYH was the highest in sole cropping (100.64 gr), it was not statistically higher than in intercropping with red clover and alfalfa (91.16 and 93.53 gr, respectively). Total leaf area in bud phase (TLAB) performed the same in all three hybrids. Intercropping of sunflower with common vetch resulted in the lowest values, while sole cropping had statistically the highest values of TLAB. The interaction of all three hybrids with red clover and alfalfa was at the same level of significance.

The correlation of sunflower traits and different intercrops were visualized by the heat maps in order to elucidate in a simple and understandable way (Figure 2). Colored dots represent significant (positive or negative) correlations with a significance level from 95% to 99.9%. Significant positive correlation was found between SY and SYH in all three types of intercropping and control. Additionally, SYH and SY were positively correlated with OY to a greater extent in all three types of intercrops compared to control. TLAF and TLAB were also positively correlated but to a greater extent in sunflower × common vetch interaction compared to other intercrops and control. PHB and PHF positive correlation

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was most pronounced in control (sunflower sole cropping). PHF and OY were in a significant positive correlation in sunflower × red clover and alfalfa intercrops, but not in sunflower × common vetch and control. HD and OY are significant positive correlatated only in sunflower × alfalfa intercrop. TSW and OY are in significant positive correlation in all three types of intercrops but not in control. FSH and SYH showed significant positive correlation with PHB in sunflower × common vetch intercrops. OY and OC are in a significant negative correlation in sunflower × red clover and alfalfa intercrops but not in control and sunflower × common vetch intercrops. HD is not in significant negative correlation with PHB and PHF only in sunflower × common vetch intercrops. TSW is in significant negative correlation with TLAB, TLAF and FSH in all three types of intercropping and control. TSW, SYH and SY are also in a significant negative correlation with OC but the least pronounced correlation was found in sunflower × common vetch intercrops.

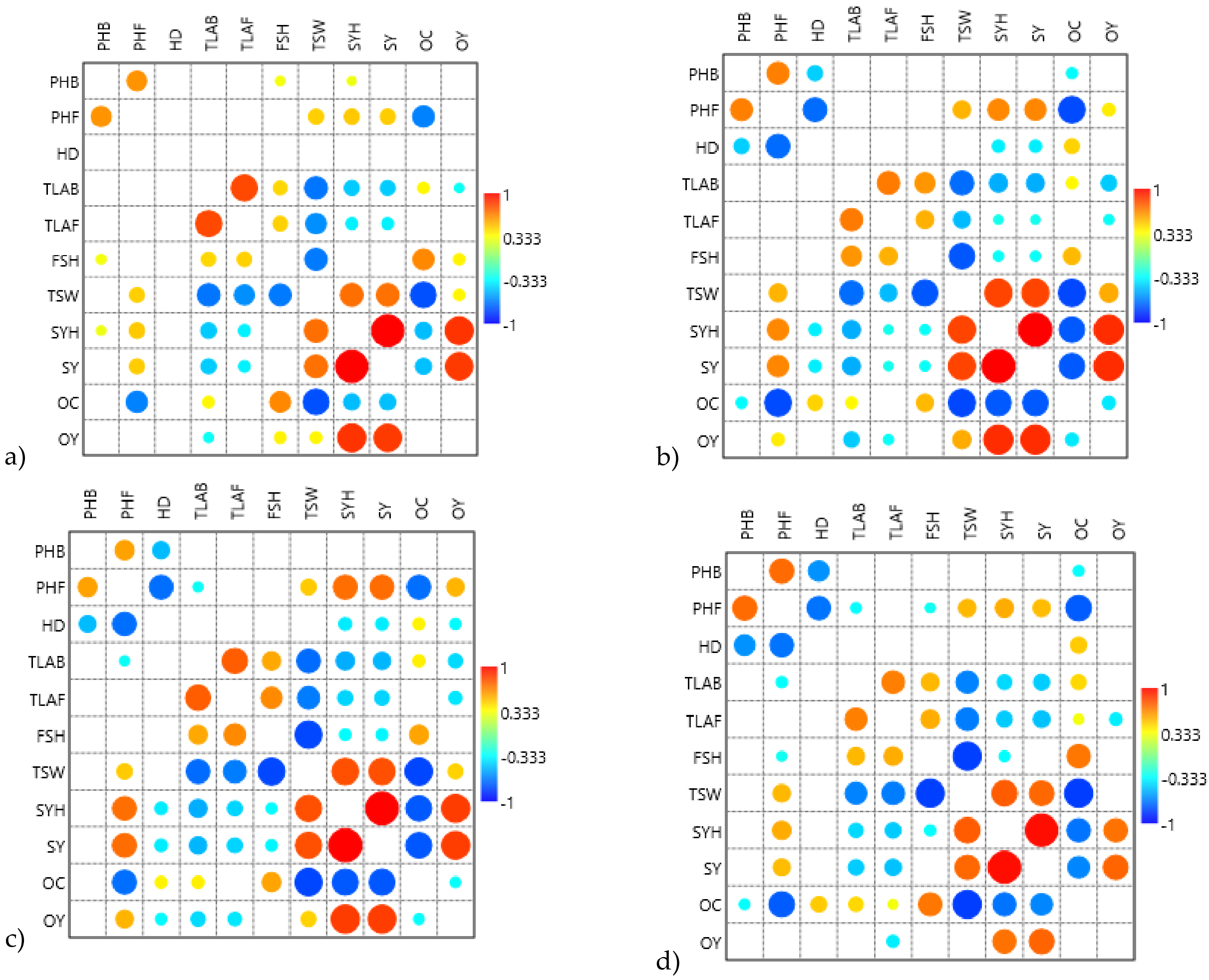

Figure 2. Heat map of sunflower traits correlations; a) sunflower × common vetch intercrop; b) sunflower × red clover intercrop; c) sunflower × alfalfa intercrop; d) sunflower sole cropping; HD-head diameter; OC-oil content; PHB-plant height in bud phase; PHF-plant height in flowering; SY-seed yield; TLAF-total leaf area per plant in flowering; TSW-thousand seed weight; NFSH-number of full seeds per head; OY-oil yield; SYH-seed yield per head; TLAB-total leaf area per plant in bud phase

3.2 Biomass and biomass related traits in legumes

In order to establish how type of cropping, as the main effect, influences common vetch, red clover and alfalfa, ANOVA was performed with the main effect as factor. Table 5 shows high statistical significance for the influence of different types of cropping (sunflower hybrids and sole cropping) on all traits analyzed in common vetch.

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Table 5. ANOVA analyses of effects for legumes in sunflower-legumes intercropping compared to sole crop of legumes

	Source of variation	F
	common vetch	
Biomass	Year	0.445NS
	type of cropping	18.887**
	year × type of cropping	0.403NS
Crop height	Year	11.918**
	type of cropping	57.503**
	year × type of cropping	4.716**
	red clover	
Biomass	Year	17.635**
	type of cropping	12.811**
	year × type of cropping	1.463
Crop height	Year	21.090**
	type of cropping	74.529**
	year × type of cropping	5.722**
Shoots per plant	Year	0.094
	type of cropping	29.005**
	year ×type of cropping	0.506
	Alfalfa	
Biomass	Year	65.717**
	type of cropping	24.093**
	year × type of cropping	0.336
Crop height	year	38.219**
	type of cropping	86.470**
	year × type of cropping	0.892
Shoots per plant	year	5.573*
	type of cropping	51.096**
	year ×type of cropping	1.772

F – value of F-test; * and ** indicated significant differences were found at p = 0.05 and 0.001, respectively

Considering that statistical significances were found, additional tests were performed in order to estimate how each of the hybrids affected legume traits. As shown in Table 6, the biomass of the common vetch and red clover was at the same level of significance in sole crops and in interaction with Rimi PR hybrid. Regarding biomass of alfalfa, interaction with NS Gricko was at the same level of significance in sole cropping. Legume crop height and number of shoots per plant were significantly lowered by all three hybrids.

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Table 6. Analyses of legume biomass, crop height and number shoots per plant in sunflower legumes intercropping compared to sole crop of legumes

type of cropping	trait	common vetch	red clover	alfalfa
NS Gricko	biomass	245.69[a]	321.01[a]	280.62[bc]
	crop height	45.0[a]	31.5[a]	45.0[a]
	shoots per plant	/	6[a]	2[a]
Dukat	biomass	269.56[a]	306.48[a]	245.95[ab]
	crop height	54.0[b]	41.0[b]	60.0[b]
	shoots per plant	/	6[a]	2[a]
Rimi PR	biomass	297.42[ab]	335.09[ab]	223.07[a]
	crop height	50.5[ab]	35.5[ab]	50.0[ab]
	shoots per plant	/	6[a]	2[a]
sole crop	biomass	370.51[b]	394.77[b]	328.80[c]
	crop height	64.0[c]	53.5[c]	71.5[c]
	shoots per plant	/	8[b]	4[b]

letters in superscript denote differences between different intercrops within each legume; significance level 95%

3.3 Indicators of intercropping competition and efficiency

This study showed that LERmax for both sunflower × red clover and sunflower × alfalfa intercropping's regarding all three hybrids was greater than 1 (Figure 3), which indicate that there is an advantage of utilizing land resources by this type of intercropping systems relative to the corresponding sole crops. The higher average LERmax values were obtained in hybrids NS Gricko and Rimi PR intercropped with red clover (1.6) resulting in a positive impact on land and a clear yield advantage. More precisely, 60% more land area is needed in sole cropping to gain the same yield obtained as in NS Gricko and Rimi PR × red clover intercropping. The lowest average LERmax value were obtained in Dukat × alfalfa intercropping (1.4), indicating that 40% more area is needed to obtain the same yields in sole cropping. By comparing the years of study the highest LERmax values were achieved in 2019/2020.

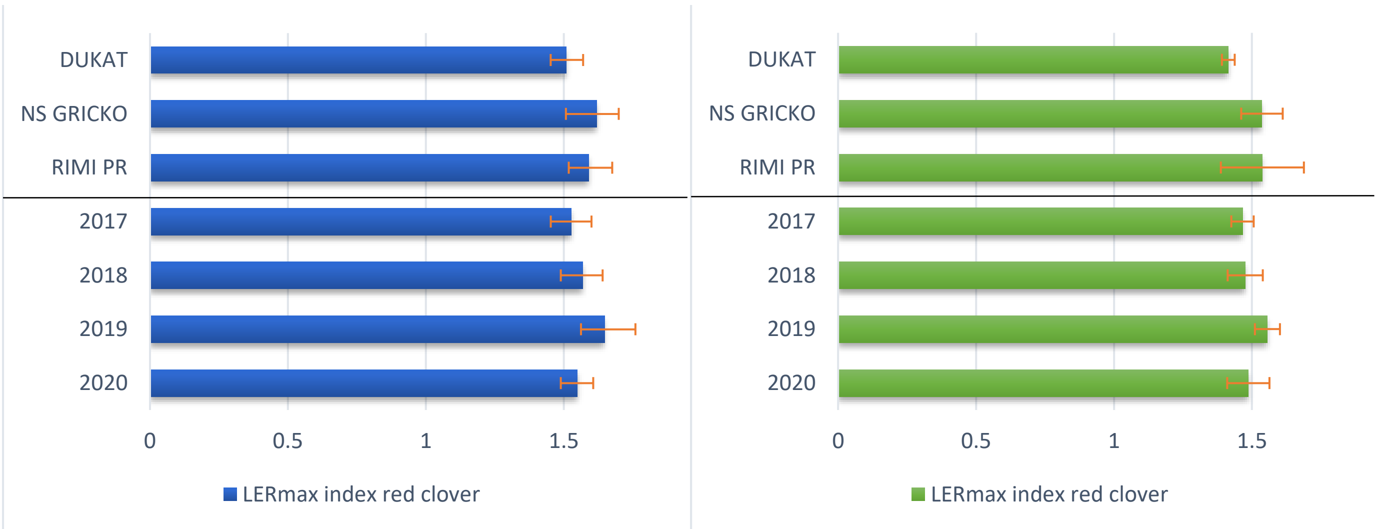

Figure 3. Adjusted land equivalent ratio (LERmax) for sunflower × red clover and sunflower × alfalfa intercropping

4. Discussion

Anthropogenic systems have a specific dynamic as a result of intensive land use. Concerning an increasing loss of livestock in our region [28], and the resulting loss of manure, it is very important to maintain and improve soil characteristics, which directly affect crop yields, taking into account the use of nature resources. Therefore, in addition to sustainability of the system, it is very important to achieve an adequate yield of sunflower as the most important agronomic trait. Sunflower seed yield has genetics potential of up to 6 tons per hectare. However, in production conditions this is very rarely achieved [2]. More importantly, the yield varies greatly from year to year, so it has become very important to achieve stability over the years to face adverse climate conditions. As mentioned, the accelerated climate change characterized by extreme climatic hazards expose sunflower, as spring-sown and usually rain-fed crop, to the direct effect of heat stress and different drought scenarios which can result in harsh yield losses and oil content decrease [29,30]. The above-mentioned claims indicate that the conditions during the growing season were very variable and unpredictable which was confirmed by four different vegetation seasons in which the experiment was performed. Figure 4. shows the yield data from our trial compared to the data from multi-environment trials for hybrid performance evaluation. In all four years, the yields of sunflower intercropped with red clover and alfalfa were similar to the averages from multi-environment trials.

From the producer's perspective, sunflower, as a cash crop in this system, should yield near its potential when planted at its optimum plant density which is the case in our research. Figure 4. shows that it is possible that sunflower legumes intercropping system give almost the same yield as the average yield in our region. Considering that perennial legumes give a more significant forage yield from the second year of establishment, while in the first year of our trial they served as soil cover e.g., living mulch. After the sunflower harvest in the first year, we have already established a legume crop on the same plot, which will reach its full potential in the next vegetation period.

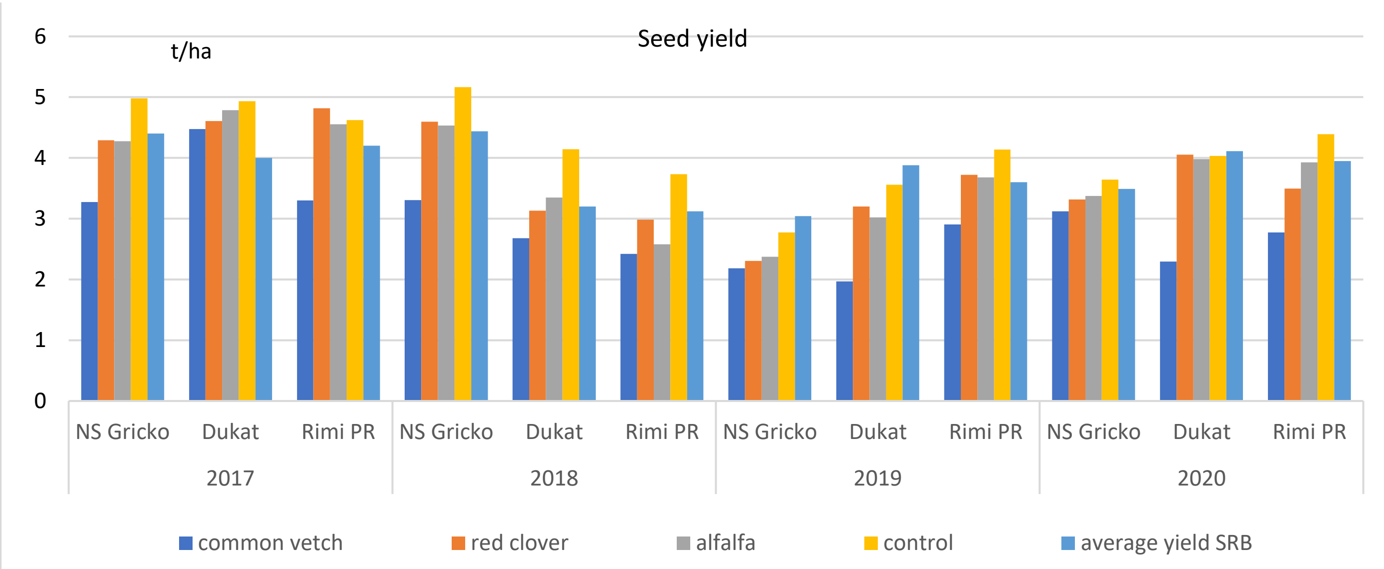

Figure 4. Sunflower seed yield in intercrops compared to average yield in multi-environment trials in Republic of Serbia agroecological conditions (average yield SRB-unpublished data from IFVCNS multi-environment trials)

4.1 Yield and yield related traits in sunflower

Morphological traits as head diameter, plant height and total leaf area in flowering and bud phase acted almost the same. Significant decrease in head diameter was observed in intercropping sunflower with common vetch in all three hybrids, and intercropping sunflower with red clover and alfalfa did not decrease this trait. Head diameter is influenced by genotype and environmental conditions that occur during flowering and fertilization. Previous study in the same environment conducted by Mrđa (2015) [31], points out that management practices that results in favorable number of plants per unit area, precipitation arrangement and nutrients are significant for head diameter. Considering that yield is positively correlated with head diameter [32], it is necessary to choose for intercropping sunflowers hybrids with biggest head diameter is the highest, especially in the confectionary sunflower production. Growth dynamic and final height can interact with subsidiary legume crops. In addition to the fact that plant height is a genetically determined trait [33] it is also affected by the environment. In terms of plant height, hybrids from 1.5 m to 2 m are the most acceptable for hybrid production [34,35]. Also, lower hybrids have the same yield potential as standard height hybrids but better adaptability to different environmental conditions [34, 36-38]. All three hybrids in our study were significantly lowest when intercropped with common vetch which could be considered as good, but this combination also significantly reduced other agronomically important traits in sunflower. This could be explained with the fact that initial growth of vetch is somehow faster compared to alfalfa and red clover. Intercropping of hybrid NS Gricko with alfalfa significantly lowered plant height compared to sole cropping but important traits as yield were not reduced, so we can conclude that this combination can result in a crop less susceptible to lodging and easier to combine, which is especially important for confectionary hybrids that have robust plant architecture. Plant height in other combinations was the same as in sole cropping. The importance of the total leaf area in the development of seed yield results from the basic function of leaves, i.e. photosynthesis, producing more than 90% of organic matter. This trait is very variable and depends on the genotype, as well as mineral nutrition, humidity, plant light, density of the assembly, sowing date, etc. [39]. In his work, Balalić (2009) [40] stated that the application of various cultivation practices, which may include intercropping as a way of production, can affect the size of the leaf area more than the yield or other characteristics of sunflower. The total leaf area in our study was significantly lowered in all intercrops compared to control as the seed yield in interaction with common vetch in all three hybrids, but also in Rimi PR× red clover interaction. The assumption is that intercropping of sunflower with legumes can slow down its growth to a certain extent, but only until the moment of complete root development (budding). In the later stages, there should be no competition for soil moisture. Interaction of all three hybrids with red clover and alfalfa was on the same level of significance, so we conclude that there is no difference in the influence of these two species on the leaf area.

During the combination selection it is very important to pay attention to agronomically important traits of sunflower as a cash crop in the first year. Considering that, the oil content is lower in dry than in humid years, especially if the lack of moisture occurs in the period from flowering to maturation [41] which determines the oil content [42], the microclimate created by legumes can be very important. The four-year period of the experiment was very different in terms of the weather conditions, but the oil content remained unchanged in all combinations as well as in sole cropping. Seed yield is the main objective of the sunflower crop and it is related to many factors such as sowing density, one thousand seed weight, the number of full seeds per head, head diameter, as well as cultivation practices during the vegetation period, so any decrease or increase in any of the above-mentioned factors will affects the yield. In our study, seed yield was affected by intercropping of all hybrids with common vetch, as well as the other two legumes in interaction with Dukat. Given that Dukat is a hybrid of shorter vegetation, this was probably due to inter and intraspecific competition for light, moisture, space and nutrients in the first part of the vegetation, period which could not be compensated due to the short period of vegetation. NS Gricko and Rimi PR were not significantly affected by intercropping with alfalfa. Also, NS Gricko, as confectionary hybrid and the one with the most robust plant architecture, have good competition ability so intercropping with red clover did not significantly decrease its yield. Olowe et Adebimpe (2009) [43] also stated that average yields of the sunflower and soybean intercrops were similar to those of the monocrop probably because of enhanced productivity of individual plants under intercropping conditions. Kandel et all (1997) [44] stated that hairy vetch sown at the same time as sunflower reduced number of full seeds per head compared with the control which is in accordance with our study. Since number of full seeds per head is in direct correlation with yield [45] it was expected that this trait as well as the yield of hybrid Dukat were influenced by interactions with common vetch. Thousand seeds weight belongs to the major yield component, and breeding for its increase leads to direct seed yield increase [45]. The fact that the interaction with legumes did not affect this trait is very important both for oil and for confectionary hybrids. Significant positive correlation was found between SY and SYH which was to be expected having in mind that SYH directly affects final yield per hectare.

4.2 Biomass and biomass related traits in legumes

In intercropping systems legumes tend to be beneficial for main crops because they bring high amounts of nitrogen into the soil and show positive effects on yield [46]. It is therefore highly likely that they contributed to the yields of sunflowers in our systems. Since red clover and alfalfa are perennial species, the characteristics of their traits are also important. Biomass, as the main outcome in the second year, is formed on the basis of performance from the year of establishment. Biomass of the common vetch and red clover was at the same level of significance in sole crops and in interaction with Rimi PR hybrid, as well as the biomass of alfalfa in interaction with NS Gricko, which tells us that the biomass in the year of exploitation for fodder will be satisfactory, as established by other authors [47].

4.3 Land equivalent ratio (LERmax)

In the present additive intercropping model, sunflower and legumes were both intercropped at 100% of the optimal plant densities, resulting in a total of 200%. It was, therefore obvious that the pure stands of these crops, sown at the optimal (100%) plant density for each crop, were significantly more productive compared to all the evaluated intercropping treatments, in terms of seed yields and biomass. These results are in agreement with the findings of Kandel et al. (1997) [44] who has reported higher yields for sole over intercropped sunflower and hairy vetch. However, the analysis of land use efficiency and yield gain revealed that the LERmax values for both sunflower × red clover and sunflower × alfalfa were higher than one, which indicated the advantage of intercropping over the sole cropping of both crops. The maximum LER value (1.6) was obtained when hybrids NS Gricko and Rimi PR were intercropped with red clover indicating 60% yield gain over sole cropping. The achieved yield gain in terms of high LERmax values could be attributed to the complementarily in utilization of the above- and below-ground resources and farming inputs between the intercrop component crops. It can be said that this intercropping model increased the overall resource use efficiency during the part of the growing season which was occupied by both crops.

5. Conclusions

Sunflower and legumes intercropping has not been extensively analysed and this combination is rarely used in practice. Therefore our study is an attempt to enhance sunflower cropping management encompassing sustainable practices complemented with legume utilization. Based on the obtained results in intercropping systems, sunflower × alfalfa intercropping is the most appropriate one. Having in mind that thousand seeds weight is one of the most important confectionery sunflower traits, the fact that interaction with legumes did not affect this trait is very important. For oil hybrids, in addition to the seed yield, oil content and yield are very important traits. Oil content for all hybrids remained unchanged in all combinations as in sole crops. The yield of NS Gricko and Rimi PR are statistically on the same level with sole cropping and alfalfa biomass gives the best results when intercropped with NS Gricko (compared to sole cropping). Also, yields of sunflower intercropped with red clover and alfalfa were similar to averages from multi-environment trials, which indicates that this a system has great potential for transfer into practice . Longer-term effects of the system can also be extremely important (though not considered further in this particular paper) especially in view of the general belief that intercropping yields are more stable, so further research in this direction is needed. In addition, research focused on the time and method of sowing will be the second phase of the experiment.

Author Contributions: Conceptualization, B.B. and S.Š.; methodology, S.Š and N.H.; software, B.B. and D.S.; validation, B.B., S.Š and N.H.; formal analysis, B.B., N.Ć. and M.R.; investigation, B.B.; resources, S.Š. and N.H.; data curation, D.S.; writing—original draft preparation, B.B.; writing—

review and editing, S.Š, N.H. and M.R.; visualization, B.B. and N.Ć.; supervision, S.Š. and N.H.; All authors have read and agreed to the published version of the manuscript."

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