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Weed community in soybean responses to agricultural management systems

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Abstract: Weed infestation is a major cause of poor yield of soybean (*Glycine max* (L.) Merr.), therefore, proper weed management represents one of the most important and expensive step in soybean production. Field experiments were established in northeastern part of Croatia, in Vukovar-Syrmia county from 2014 to 2016, arranged in a split-plot design with four replications. Two different studies were conducted: study 1 to determine the weed interference, weed biomass accumulation, yield and yield components of soybean growing in three different row spacing (25, 50 and 70 cm) and study 2 to simulate risk analysis by building models of possible results of achieving profit as a result of weed control. Weed community in soybean during the study period comprised of 34 dicot and grass species of varied perennation. Compositional differences in weed community tend to be affected most by year (humid – arid environment), followed by row spacings. There was no differences in weed biomass accumulation with reduction in row spacing from 70 to 50 and 25 cm. Dominant weed species *Amaranthus retroflexus*, *Ambrosia artemisiifolia*, *Chenopodium album*, *Datura stramonium*, *Setaria viridis*, and *Sorghum halepense* formed the main biomass and were spread over all row spacings. There was significant influence of row spacing, duration of weed interference, and year on soybean yield and yield components. Weed infestation until second trifoliate (V2) stage had no detrimental effect on soybean yield regardless the row spacing. Number of pods per plants significantly decrease at the same, V2 stage in 25 and 50 cm rows, but in 70 cm wide soybean rows this process started later, at four unfolded trifoliate leaves (V4 stage). A 1000 kernel weight was less sensitive to weed infestation and significantly decreased at full flowering (R2 stage) in 25 and 70 cm rows, while in 50 cm rows in V4 stage. Probability distribution of achieving a profit showed the best results for soybean growing in 70 cm rows, with preemergence herbicide application and 2 inter-row cultivation.

Keywords: soybean (*Glycine max* (L.) Merr.); row spacing; weed community; biomass accumulation; yield, economic return

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1. Introduction

Soybean (*Glycine max* (L.) Merr.) is an important oilseed and protein crop on a global scale [1,2,3]. Production of this crop has increased, particularly in the past several decades, when farming practices have changed dramatically, especially in the methods used to

control weed species. Before the introduction of herbicides, mechanical and cultural methods were the only option for weed control. Soil-incorporated and preemergence herbicides began to replace tillage and cultivation practices, and after the 1960's became the dominant weed control method, followed with mechanical cultivation until soybean canopies closed and shaded the weeds [4]. The development of postemergence herbicides after 1980's allowed farmers to control weeds in-season, and became a predominant weed control treatments which included a single or multiple postemergence herbicide applications with less reliance on soil-applied herbicide [5].

Moreover, the availability of postemergence herbicide gave farmers an alternative weed control tool allowing them to plant soybean in narrow row spacing, and eliminate the need for cultivation [6]. Traditionally, soybean has been planted in 70 cm rows follows by late-season cultivation [7], however, with rows as narrow as 25 cm cultivation becomes impossible. Soybean planted in narrow rows closes canopy more quickly, providing better competition against weeds. Weed emergence following herbicide application decreases, since solar radiation that stimulates weed seed germination and weed development is intercepted by the crop canopy [8,9].

Besides, increasing soybean seeding rate for narrow-row soybean is 20-45% greater than for wide-row soybean [10,11], however research results [12] demonstrated no difference in weed-free soybean yield, at low, moderate, and high population in 38 and 76 cm rows.

Soybean yield has increased over the past 35 years due the crop improvement through plant breeding [13] and due to significantly changed production systems such as diverse crop rotations, reduced tillage, precision seeding, optimal fertilization rate etc. Besides, introduction of glyphosate (Roundup Ready®) and glufosinate (Liberty Link®) resistant soybean in 1996 and 2009, respectively, resulted in a shift in practices towards postemergence herbicide application [14,15]. Transgenic, herbicide-tolerant varieties of soybean have had a very rapid adoption rate because of reduced weed control costs and increased yields compared to conventional varieties [16]. Moreover, they represent a revolutionary solution in weed management, since they allowed farmers the opportunity and flexibility to manage a broad spectrum of weeds without crop injury or crop rotation restrictions.

Although herbicides have revolutionized weed management, replacing manual labor and mechanical weed control, unfortunately, they face some challenges like safety, environmental issues and development of herbicide resistant weeds, particularly in countries growing genetically modified (GM) soybean varieties [17].

Many countries in European Union, including Croatia, restrict or prohibit the cultivation of GM crops, and active ingredients of some extremely and highly hazardous pesticides in their territory due to its cumulative long-term effects on human health and environment [18]. Farmers are, therefore, encouraged to grow non-GM soya in crop rotations, using good agricultural practice in soil and nutrient management, and the best practice in weed management. To implement all above mentioned initiatives into a common practice in Croatia, farmers need to be informed of benefits and risk of implementing all required sustainability criteria [1,19].

Therefore, the main objective of this research were: (i) to describe how weed community in soybean respond to the above mentioned agronomic practices, and analyze if differences in floristic composition exist between crop at different row spacing; (ii) to evaluate weed biomass production with competitiveness of soybean; (iii) to determine if soybean yield and its attributes will increase and weed yield decrease as row spacing was decreased; (iv) to simulate risk analysis by building models of possible results of economic return in soybean growing in 25, 50 and 70 cm rows.

2. Materials and Methods

2.1. Site description and experimental set up

The field experiments were conducted on semi-clay type of soil near city of Vukovar (45°21′N 18°59′E), at the family farm "Zeleno polje" in Vukovar-Syrmia county situated in northeastern part of the Republic of Croatia. This is an open and flat region with agriculture as a main economic sector where soybean production is important. Climatically, this region experiences a warm to moderate dry lowland climate with an average yearly temperature 11.4°C, and average yearly rainfall of 699 mm with highest spring regime in June.

Soybean cultivar IKA, maturity group 0-I (Agricultural Institute Osijek) was sown to achieve population of 500 000 plants/ha^{-1} to a soil depth of 4 cm on 7th May 2014 (after sunflower), on 26th April 2015 (after sugar beet), and on 1st May 2016 (after barley). Cropping practice, typical for the local practice of soybean production in this region consisted of primary tillage in autumn, followed by the spring ploughing and harrowing [20]. Fertilizer was applied as follows: 300 kg ha^{-1} (NPK 7:20:30) in fall during the primary tillage, and 350 kg/ha^{-1} (NPK 15:15:15) at sowing to achieve 74 kg ha^{-1} N, 113 kg ha^{-1} P_2O_5 and 143 kg ha^{-1} K_2O. There was no irrigation applied, and rainfall ranged from 293.8, 357.1 to 595.4 in 2015, 2016 and 2014 respectively (Table 1).

Table 1. Total monthly rainfall, average temperature for the growing season of April through September in 2014, 2015 and 2016, and their 30-year averages in Vukovar.

Months	Temperature (°C)				Rainfall (mm)			
	2014	2015	2016	30-yr average	2014	2015	2016	30-yr average
April	13.6	12.8	14.3	13.1	56.9	18.1	4.6	49.3
May	16.6	18.5	17.2	17.9	157.5	100.4	31.0	65.1
June	20.8	21.4	21.8	21.2	58.7	24.3	105.6	106.0
July	22.3	24.6	23.3	23.0	118.6	12.6	112.9	49.8
August	21.2	24.0	21.1	22.5	84.0	78.6	65.1	59.1
September	17.3	18.7	18.7	17.3	119.7	59.8	37.9	60.6

All treatments were arranged in a split-plot design with four replications. Two different studies were conducted during the experiment: study 1 to determine weed interference, weed biomass accumulation and yield and yield attributes in soybean, and study 2 to simulate risk analysis by building models of possible results of achieving a profit as a result of weed control.

In Study 1 the three main plots included different soybean row spacings: 25 cm, 50 cm, and 70 cm. Sub-plots consisted of eleven weed removal timings, where weeds were allowed to grow until crop reached: V2 (second trifoliate), V4 (fourth trifoliate), R1 (beginning bloom, first flower), R2 (full flower), R3 (beginning pod), R4 (full pod), R5 (beginning seed), R6 (full seed), R7 (beginning maturity). Weedy and weed-free control treatments were also included. Weeds were removed by hand pulling and hoeing. Plot size was 2.4 x 3.5 m. Plots were separated by 0.5 m and blocks by 1.5 m unplanted distances.

In Study 2 as input for the simulation model, yield losses due to weeds in soybean were estimated from plots where row spacing was the main plot and different weed control treatments were subplots with four replications of each treatment. Six various weed control options were evaluated: i) pre-emergence application of metribuzin +flufenacet (2 kg/ha^{-1}) in 25 cm soybean; ii) pre-emergence application in 50 cm soybean; iii) pre-emergence application in 50 cm soybean and cultivation at first trifoliate; iv) pre-emergence application in 70 cm soybean; v) pre-emergence application in 70 cm soybean followed by cultivation at first trifoliate; vi) pre-emergence application in 70 cm soybean followed by cultivation at first trifoliate and at R1 stage.

2.2. Data assessment and statistical analysis

To characterize weed community in soybean, density of each species was counted in all weedy control plots at the R2 stage (16th July, 4th July and 6th July in 2014, 2015 and 2016 respectively) from sixteen 0.5 by 0.5 quadrats per each soybean row spacing. To overcome non-uniform weed distribution a relative abundance was calculated [21]. This synthetic importance value includes density and frequency components and was calculated by plot for each weed species as follows: (relative density + relative frequency)/2. Variation in species composition were analyzed with Canonical Correspondence Analysis (CCA) using CANOCO 5 [22]. Statistical significance of fitting CCA axes was tested using a global permutation test (Monte-Carlo test) of the species data at 1000 iterations. Forward selection of explanatory variables was tested with Monte-Carlo permutations, and was also used in determining statistical significance for each explanatory variable singly (simple effect) and in order for additionally explained variance (conditional effect).

Weed biomass accumulation were estimated at each soybean growth stage (described in Study 1) by harvesting all weeds within each of four 1m² randomly located within each treatment plot. Weeds were clipped at the soil surface and dried at 70⁰C to constant moisture content. Weed dry weight was converted to a g m² basis. The relationship between treatments and the weed biomass accumulation was described by using PROC REG in SAS, version 9.4 [23]. To determine the type of relationship, a Schumacher's model [24] was fitted to the weed-infested treatment and weed biomass accumulation.

$$Y=e^{a+b/x}$$

where: Y is the weed dry weight (g m^{-2}), e is a constant, a is the maximum weed biomass, b is the asymptote of curve and x is the duration of weed infested period express in growing degree days (GDD). GDD was used as explanatory variable in the regression analysis, and for that purpose, temperature was converted to GDD by using following equation [25]:

$$GDD=\sum [\ \{(T_{max} - T_{min})/2\} - T_{base}\ ]$$

where T_{max} and T_{min} are daily maximum and minimum air temperatures (⁰C) and T_{base} is the base threshold temperature, which was set to 10⁰C.

Crop yield and yield components (number of pods per plants and 1000 kernel weight) were recorded from each plot by hand harvesting of the two middle rows (on 1st October, 30th September and 3rd October in 2014, 2015 and 2016 respectively), shelled and adjusted to 11% moisture. A mixed model (PROC MIXED in SAS) was used to evaluate the interference of weeds in soybean, planted in 25, 50 and 70 cm row on soybean yield and yield components. The analysis was a three factors (row spacing, weed interference, year) repeated measures ANOVA model, with year as a repeated measure. Significance was assumed at $P < 0.05$.

A Monte-Carlo simulation model was constructed to forecast the distribution of the difference in profit based on data from the study 2. For that purpose, data for seeds, fertilizers and herbicide prices were obtained from the local seed and agrichemical dealers. Prices for the fuel, services and soybean market price were obtained from the TISUP [26]. Weed management costs were the sum of herbicide costs and cost of application. Gross margin for the treatments were determined, and represented the difference between gross receipt (product of crop yield and assumed market price) and production costs (cost of seed, fertilizers, fuel, services and weed management costs).

By using a Monte-Carlo simulation model [27] inputs are not simply mean values of estimated parameters, but variability of these estimates by using their simulated distributions. Calculations were done by using a Risk Solver® platform in Excel.

3. Results

3.1. Weed community characteristics

Weed community present in this study was comprised of 34 grass and dicot weed species of varied perennation (Table 2). They belong to 30 genera and 19 families, with *Asteraceae* (7 weed species) and *Poaceae* (with 4 weed species) as leading families. The weed

community composed of twenty-six species in 2014, eighteen species in 2015 and fifteen species in 2016. The dominant weeds during this study were *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Chenopodium album* L., *Datura stramonium* L., *Setaria viridis* (L.)PB., and *Sorghum halepense* (L.)Pers.

Table 2. Floristic composition in 25, 50 and 70 cm soybean crop rows during the experiment.

Weed species	Common name	Bayer code	Functional groups*		Row spacing**		
			MF	LF	25 cm	50 cm	70 cm
Abutilon theophrasti Med.	velvetleaf	ABUTH	D	A	0.05	-	0.05
Amaranthus retroflexus L.	redroot pigweed	AMARE	D	A	0.18	0.12	0.23
Ambrosia artemisiifolia L.	common ragweed	AMBEL	D	A	0.35	0.05	0.02
Artemisia vulgaris L.	mugwort	ARTVU	D	P	0.05	0.01	0.01
Calystegia sepium (L.)R.Br.	hedge bindweed	CAGSE	D	P	0.02	0.02	0.03
Chenopodium album L.	common lambsquarters	CHEAL	D	A	2.02	2.08	1.97
Chenopodium hybridum L.	mapleleaf goosefoot	CHEHG	D	A	0.12	0.02	0.02
Convolvulus arvensis L.	field bindweed	CONAR	D	P	0.03	0.05	0.07
Datura stramonium L.	jimsonweed	DATST	D	A	0.22	0.12	0.07
Daucus carota L.	wild carrot	DAUCA	D	B	0.05	0.05	0.01
Erigeron annuus (L.)Pers.	annual fleabane	ERIAN	D	A	0.05	0.01	-
Erigeron canadensis L.	horseweed	ERICA	D	A	0.05	0.05	-
Euphorbia helioscopia L.	sun spurge	EPHHE	D	A	-	-	0.01
Glechoma hederacea L.	ground ivy	GLEHE	D	P	0.01	-	-
Helianthus annuus L.	sunflower	HELAN	D	A	0.04	0.01	0.03
Hordeum murinum L.	mouse barley	HORMU	M	A	-	0.01	0.02
Lactuca serriola L.	prickly lettuce	LACSE	D	A	-	-	0.01
Lathyrus pratensis L.	meadow peawine	LTHPR	D	P	0.05	0.01	-
Matricaria chamomilla L.	wild chamomille	MATCH	D	A	-	0.05	-
Oxalis corniculata L.	creeping woodsorrel	OXACO	D	P	0.05	-	0.01
Papaver rhoeas L.	corn poppy	PAPRH	D	A	-	0.01	-
Plantago major L.	broadleaf plantain	PLAMA	D	P	0.05	0.05	-
Robinia pseudoaccacia L.	black locust	ROBPS	D	P	-	-	0.01
Rorippa sylvestris (L.)Bess.	yellow fieldcress	RORSY	D	P	-	0.01	0.01
Rumex crispus L.	curly dock	RUMCR	D	P	0.05	-	-
Setaria verticillata (L.)PB.	Bristly foxtail	SETVE	M	A	-	-	0.02
Setaria viridis (L.)PB.	green foxtail	SETVI	M	A	0.13	0.23	0.24
Solanum nigrum L.emend. Mill.	black nightshade	SOLNI	D	A	0.04	0.05	0.07
Sonchus arvensis L.	perennial sowthistle	SONAR	D	P	0.01	0.02	0.01
Sonchus oleraceus L.	annual sowthistle	SONOL	D	A	-	0.01	-
Sorhgum halepense (L.)Pers.	johnsongrass	SORHA	M	P	1.54	1.59	1.61
Urtica dioica L.	stinging nettle	URTDI	D	P	-	0.01	-
Veronica persica Poir.	Persian speedweel	VERPE	D	A	0.09	-	0.01
Xanthium strumarium L.	common cocklebur	XANSI	D	A	0.04	-	0.01

*Functional groups: MF = morphotype: D = dycotyledoneae; M = monocotyledoneae; LC = life cycle: A = annual; B = bi-annual; P = perennial

**relative abundance data of weed species in soybean growing in 25, 50, and 70 cm rows

Weed relative abundance data over all study years is presented in Table 2 to give an overview of community structure and the overall effects of the various row spacing in soybean. However, in the analysis of the comprehensive data using CCA, specific weed species responses to the explored external variables proved to be statistically significant

(Figure 1). A Monte-Carlo permutation test showed both the first and all CCA axes together to be statistically significant (test of significance of first canonical axis: eigenvalue = 0.1667; F-ratio = 30.2908; P<0.001; test of significance of all canonical axes: eigenvalue = 0.3195; F-ratio = 12.6323; P<0.001).

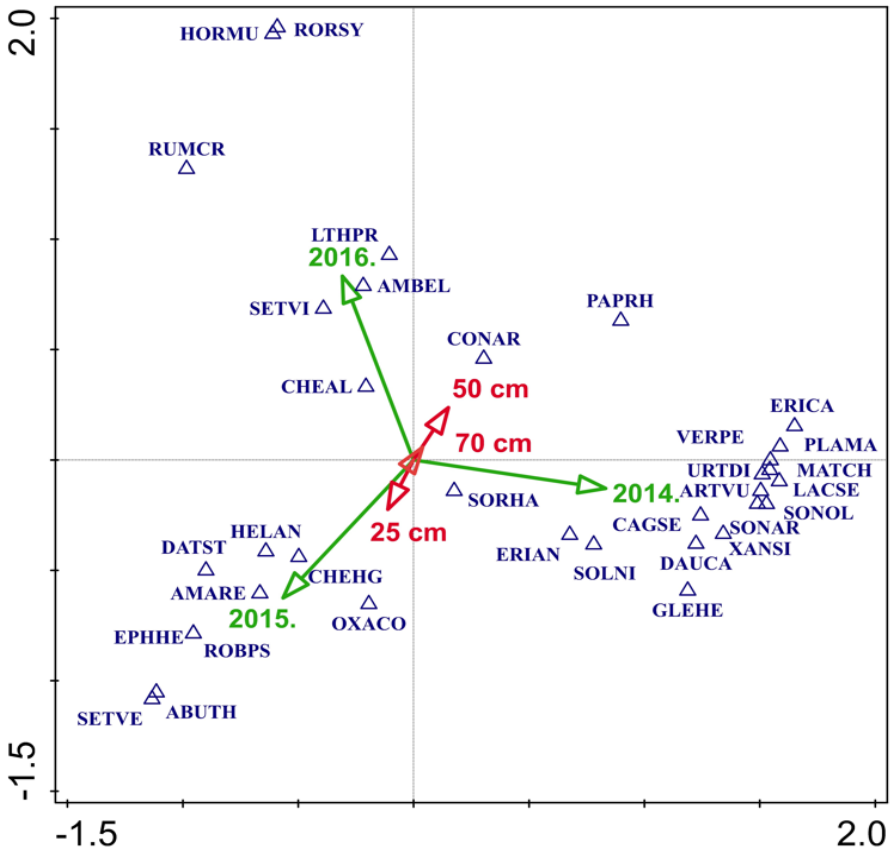


Figure 1. Ordination of species, row spacing and years on Axis 1 and Axis 2 of a Canonical Correspondence Analysis (CCA) with scaling based on inter-species distances. For acronyms for species (Bayer code) see Table 1.

The first CCA axis captured 52.9 % of variation in the species composition, and was explained more by the seasonal aspect, than management factors. Biplot score for 2015 and 2016 have the longer vector length and were in opposite orientation in the ordination than for 2014 (Figure 1). During the growing seasons 2015 and 2016 (April-September) the amount of rainfall (293.8 mm and 357.1 mm for 2015 and 2016 respectively) was significantly lower, than in 2014 (595,4 mm). Less strong association were observed for crop rows. Soybean planting in narrow rows (25 cm) were located in the same orientation space as very arid 2015, compared to wider row spacing with less seasonal influence.

Weed relative abundance indicated that species such as *Abutilon theophrasti*, *Setaria verticilata*, *Euphorbia helioscopia*, *Amaranthus retroflexus*, *Datura stramonium* are associated with drier environment in soybean crop growing in 25 cm rows (Table 3).

The second axis explains further 29.4% of the species composition variability, and species *Rumex crispus*, *Rorippa sylvestris* and *Hordeum murinum* appeared only in 2016 in wider crop rows (Table 3).

Table 3. Results from the canonical correspondence analysis. Species rank along the main gradients are presented, and only species with the highest fit are selected.

Axes	1	2	3	4	Total inertia
Eigenvalues	0.281	0.156	0.048	0.031	0.638
Species-environment correlations	0.886	0.768	0.471	0.363	
Cumulative percentage variance of species data	4.5	6.9	7.7	8.2	
Cumulative percentage of fitted response data	52.9	82.3	91.3	97.1	
Species scores					
Setaria verticilata	-1.0733	-0.8828	-0.1365	-0.2181	
Abutilon theoprasti	-1.0474	-0.8551	-0.2683	-0.2266	
Datura stramonium	-0.8350	-0.3858	0.1359	0,1291	
Euphorbia helioscopia	-0.8230	-0.6156	-1.4103	-0.3001	
Rumex crispus	-0.6159	1.3443	1.4399	-0.7333	
Amaranthus retroflexus	-0.6090	-0.5122	-0.2105	-0.0380	
Rorippa sylvestris	-0.2140	1.882	-0.3548	0.3462	
Hordeum murinum	-0.2000	1.8908	-0.4969	0.0005	
Artemisia vulgaris	1.5051	-0.4150	-0.1942	-0.2818	
Sonchus arvensis	1.5140	-0.3584	-0.1470	0.0054	
Erigeron canadensis	1.5193	-0.2797	-0.0325	0.3959	
Sonchus oleraceus	1.5426	-0.1207	0.1412	1.8013	
Matricaria chamomilla	1.5473	-0.3507	-0.3541	0.1809	
Urtica dioica	1.5871	-0.1756	-0.3080	0.7085	
Lactuca serriola	1.6170	-0.2126	-0.6150	-0.0275	
Biplot scores of explanatory variables					
2014	0.8699	-0.2630	0.1169	0.0018	
2015	-0.6750	-0.5689	-0.2497	0.1253	
2016	-0.1876	0.8949	0.1478	-0.1328	
25 cm	-0.1488	-0.2211	0.5515	-0.4038	
50 cm	0.1099	0.1971	-0.0081	0.9630	
70 cm	0.1050	0.0769	-0.7920	-0.4137	

The results from forward selection of explanatory variables indicate that most variance in the species data, when examined singly (simple effects) was explained by weather conditions in very humid 2014 and very arid 2015, and moderate 2016 (Table 4). Next, the variation explained by environmental variables in order of their inclusion in the model, i.e. conditional effect, indicated that only year 2014 and 2015 explain significant portions of the variation in the data, since the additional variance each variable explained at the time it was included.

Table 4. Results of forward selection of explanatory variables.

Simple effects				Conditional effects			
Variable	Explains %	Pseudo-F	P	Variable	Explains %	Pseudo-F	P
2014	8.4	29.5	0.001	2014	8.4	29.5	0.001
2015	7.1	25.5	0.001	2015	5.8	21.8	0.001
2016	6.0	20.4	0.001	70 cm	1.0	3.9	0.001
25 cm	1.1	3.5	0.001	50 cm	0.9	3.6	0.001
50 cm	0.9	3.1	0.001	25 cm	0.4	1.5	0.001
70 cm	0.9	2.8	0.003				

3.2. Weed biomass accumulation

Weed biomass accumulation increased in each row spacings as the duration of weed infested period increase (Figure 2). The highest total dry weed biomass (3329. 2 g m^{-2}) was recorded in 2016 in soybean growing in 70 cm rows, following the 2015 (3082.4 g m^{-2}) in soybean growing in 25 cm rows and 2014 (2521.7 g m^{-2}) in soybean growing in 50 cm rows.

There was no reduction in weed biomass accumulation with reduction in row spacing from 70 to 50 and 25 cm. Dominant weed species *Amaranthus retroflexus*, *Ambrosia artemisiifolia*, *Chenopodium album*, *Datura stramonium*, *Setaria viridis*, and *Sorghum halepense* germinate and emerge with soybeans and formed the main biomass and were spread over all row spacings (Table 2).

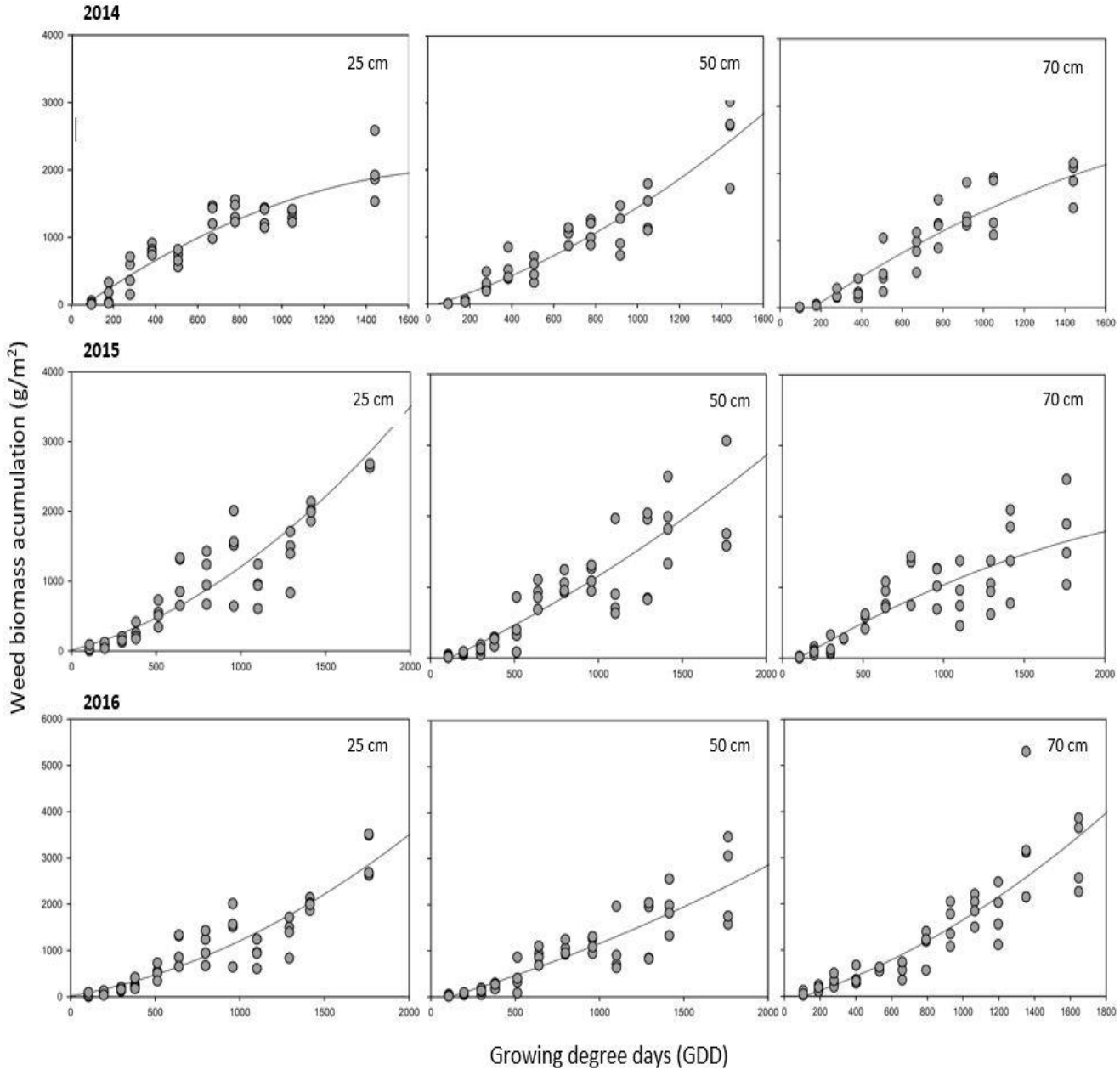


Figure 2. The effect of increasing duration of weed interference on weed dry weight accumulation in soybean growing in 25, 50, and 70 cm rows. Dots indicate observed data. Parameter values for response curves calculated using Schumacher's model are presented in Table 5.

Table 5. Parameter values for response curves based on Schumacher's model: $Y=e^{a+b/x}$ (values in parentheses are standard errors of parameters).

Year	a	b	R^2
25 cm soybean row spacing			
2014	6.1 (0.086)	-427 (44.52)	0.73
2015	4.3 (0.114)	-271 (53.78)	0.83
2016	3.8 (0.113)	-229 (40.26)	0.88
50 cm soybean row spacing			
2014	4.4 (0.072)	-254 (45.63)	0.85
2015	7.8 (0.062)	-271 (53.78)	0.89
2016	6.1 (0.002)	-292 (50.33)	0.81
70 cm soybean row spacing			
2014	7.9 (0.088)	-302 (54.12)	0.78
2015	6.9 (0.039)	-298 (54.77)	0.76
2016	9.9 (0.009)	-325 (73.78)	0.79

3.3. Weed community interference on soybean yield and yield components

Weed competition with soybean can cause significant reductions in yield potential (Table 6). Soybean yield, number of pods per plant and 1000 kernel weight were significantly influenced by row spacings (P<0.001). Duration of weed interference affected soybean yield and its components (P<0.001). Significant year differences (P<0.001) were also recorded and can be attributed to rainfall amount periodicity. First and third year of experiment received more rainfall during the vegetative growth, compared to second experimental year (Table 1).

Table 6. Repeated-measures ANOVA for the effect of agricultural management system on soybean yield and yield components.

Variable	df	Soybean yield		Number of pods per plant		1000 kernel weight	
		F	Sig.	F	Sig.	F	Sig.
Between-subject source							
Row spacing (RS)	2	14.925	0.000	6.196	0.002	8.926	0.000
Duration of weed interference (DWI)	10	7.919	0.000	49.326	0.000	2.223	0.022
RS * DWI	20	1.182	0.286	4.421	0.000	1.668	0.052
Error	99						
Within-subject source							
Year (Y)	2	722.981	0.000	871.558	0.000	366.170	0.000
Y * RS	4	9.334	0.000	16.919	0.039	3.085	0.017
Y * DWI	20	26.470	0.000	114.835	0.000	13.502	0.000
Y * RS * DWI	40	0.604	0.970	4.569	0.072	1.316	0.114
Error	198						

Notes: df = the degrees of freedom; F-value and significance levels of effects are shown for each variable. Within-subject analysis used Geisser-Greenhouse adjusted probabilities. *P<0.05: ** P<0.01; *** P<0.001. Boxplots for duration of weed interference are shown in Fig. 2, 3 and 4 supplementary materials).

The significantly highest soybean yield was observed in weed free crop growing in 70 cm rows, while in 50 cm rows yield was reduced 10%, and in 25 cm rows yield reduction was 20% (Table 7). However, when weeds were present throughout the growing season, yield reduction were 52, 53 and 60% in 50 cm 25 cm and 70 cm soybean rows, respectively.

Number of pods per plant in weed free soybean does not significantly differ, but allowing weeds to interfere the whole season, dramatic reduction (70%) in number of pods per plants

were observed in 70 cm rows. A 1000 kernel weight was also not significantly different among the crop rows in weed free plots. Presence of weeds reduced 1000 kernel weight about 10%.

Table 7. Soybean yield, number of pods per plant and 1000 kernel weight as influenced by soybean row spacings (25, 50 70 cm), and duration of weed interference (averaged over 2014-2016).

Soybean growth stage	Soybean yield (kg/m²)			Number of pods per plant - row spacing -			1000 kernel weight (g)		
	25 cm	50 cm	70 cm	25 cm	50 cm	70 cm	25 cm	50 cm	70 cm
Weed-free	586.6 a	650.7 a	724.8 a	68 a	63 a	67 a	154.6 a	163.7 a	160.8 a
V2	480.4 b	542.9 b	625.8 b	64 b	55 b	64 a	154.1 a	159.4 ab	160.5 a
V4	473.2 b	540.5 b	622.6 b	53 c	54 b	54 b	154.9 a	158.8 ab	159.8 a
R1	460.1 c	526.5 c	618.4 bc	51 c	53 b	52 b	154.5 a	157.1 b	159.5 a
R2	431.4 d	502.6 c	611.7 c	51 c	53 b	43 c	152.5 b	154.6 bc	156.1 b
R3	429.1 de	490.0 d	604.0 c	51 c	53 b	40 c	151.3 b	151.8 c	155.7 b
R4	421.4 e	447.2 e	543.6 d	48 cd	51 bc	27 d	151.1 b	151.3 c	155.6 b
R5	413.6 f	435.7 ef	529.1 e	44 d	48 c	22 e	147.6 c	149.9 d	154.3 bc
R6	351.8 g	424.1 f	525.5 e	37 e	45 c	21 e	144.5 cd	148.3 d	152.6 c
R7	350.8 g	339.8 g	385.7 f	36 e	43 d	20 e	141.9 d	147.1 d	150.2 c

*within a column, the same letter indicates that the yield or yield components are not significantly different

Weed infestation until second trifoliate (V2) stage had no detrimental effect on soybean yield regardless the row spacing. Number of pods per plants started to significantly decrease at the same stage in 25 and 50 cm rows, but in 70 cm wide soybean rows this process started later, at four unfolded trifoliate leaves (V4 stage). A 1000 kernel weight was less sensitive to weed infestation and significantly decreased at full flowering (R 2 stage) in 25 and 70 cm rows, while in soybean growing in 50 cm rows decrease at four unfolded trifoliate leaves (V4 stage).

3.4. Effect of weed management in soybean on economic return

Following the experimental results (study 2) a Monte-Carlo simulation was constructed to forecast the distribution of the difference in obtaining the profit between six weed control strategies in soybean (see Materials and Methods, study 2) growing in 25, 50 and 70 cm rows. Figure 3. shows a flow diagram representing the sequence of calculations performed. The difference in profits between weed control strategies were presented for each study year separately. However, in the second year (very arid growing season) all examined weed management strategies failed and variable costs (dotted line in Figure 3) were higher than Gross Margin.

With preemergence herbicide application, as an individual weed control strategy, soybean growing in 25 cm rows had not obtained positive financial results in all three study years. In 50 cm rows, probability of achieving a profit were 20% in 2014 and 40% in 2016, while in 70 cm rows, positive financial results with preemergence herbicide application had 60% of probability in 2014 and 30% in 2016.

Combination of preemergence herbicide and one inter-row cultivation at 1st trifoliate stage in 50 cm rows received only in 2014 a positive financial results (20% probability). Soybean growing in 70 cm rows with one inter-row cultivation, and with two inter-row cultivation were the best option for achieving profit. There was a 50% and 70% possibility for positive financial results with preemergence herbicide and one inter-row cultivation in 2014, and 2016 respectively. The best option in this study was the strategy with preemergence herbicide application followed by two inter-row cultivation. In this treatment, probability for not receiving a profit was only 10% in 2014.

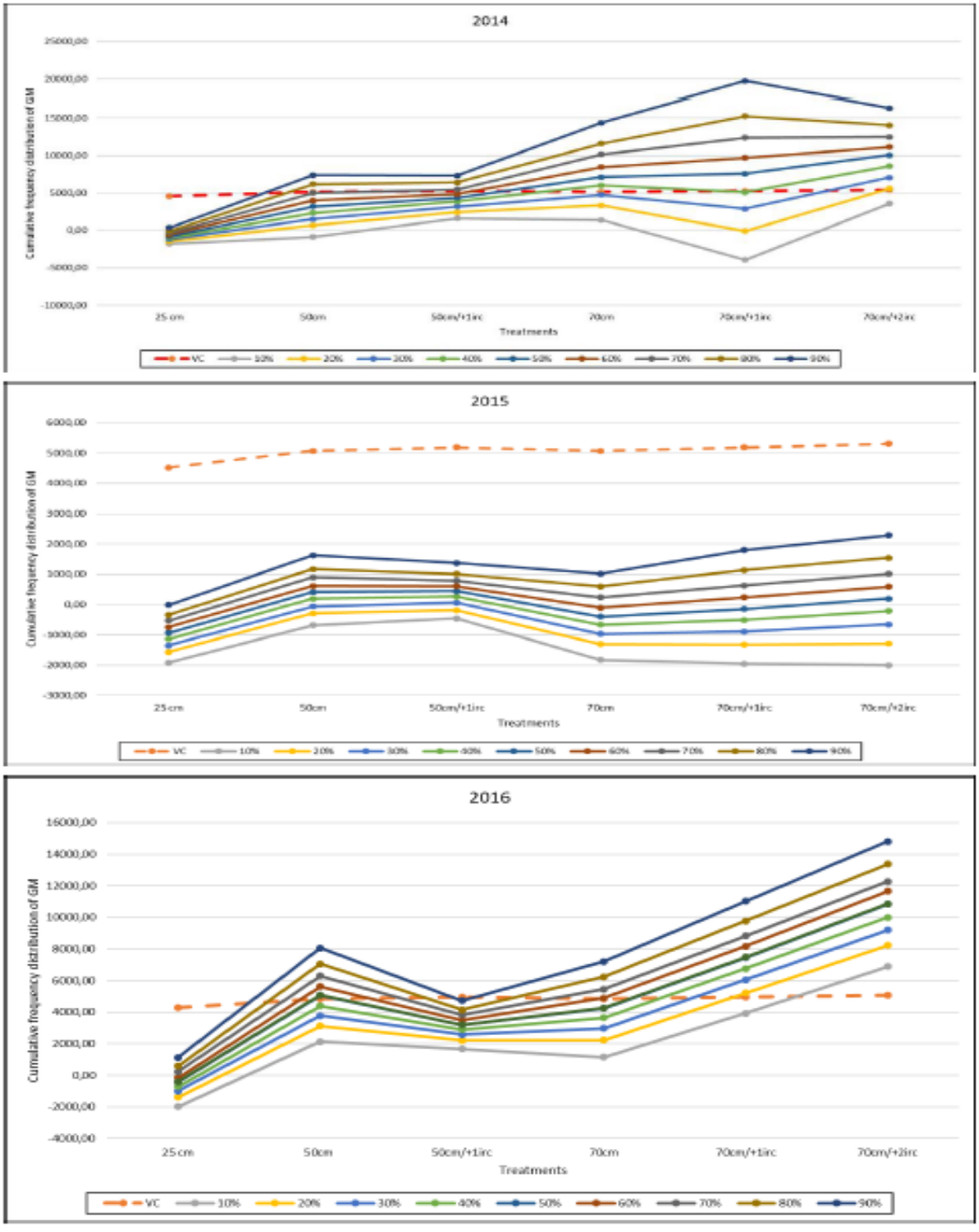


Figure 3. Probability (%) of increase in profit by controlling weeds in soybean growing in 25, 50 and 70 cm rows. Weed control options are: i) pre-emergence application of metribuzin +flufenacet (2 kg/ha^{-1}) in 25 cm soybean; ii) pre-emergence application in 50 cm soybean; iii) pre-emergence application in 50 cm soybean and cultivation at first trifoliate; iv) pre-emergence application in 70 cm soybean; v) pre-emergence application in 70 cm soybean followed by cultivation at first trifoliate; vi) pre-emergence application in 70 cm soybean followed by cultivation at first trifoliate and at R1 stage. GM=Gross Margin; VC=variable costs of production

4. Discussion

Natural weed community that developed in soybean crop during the study were typical of row crops flora in this region [28,29]. Dominant weeds that appear in the study are, likewise, a major limiting factor of optimum soybean production in the region [30, 31], as well as worldwide [32,33,34].

It is well documented that management practice and environmental conditions affect soybean and weed competition. Weeds impact on soybean by competing for limited light, water, nutrient resources, and space. The manipulation of row spacing, as one of the management tactics, can have a sizable impact on weeds in soybean [35]. However, our results suggest that significant effects on weed community composition and structure in soybean had environmental factors followed by management practice. The variation in weed community composition were significantly explained by seasonal aspect. Amount of precipitation during the growing season was the most important factor determining weed species composition. Similar conclusions can be found in other studies [36,37,38,39] where authors suggested that changes in weed communities were influenced more by year than by management practices, indicating fluctuational rather than directional or consistent changes in community composition. However, some other studies suggest that management factors play more important role than environmental ones [40,41] revealed that different crop types and their associated management have more influence on weed composition than the relative importance of climatic variables.

Weed biomass growth and accumulation rate increased with increasing duration of weed infestation. The absence of herbicide application, or other weed control measure in these weeded plots, gives the weeds competitive advantage over crop growing in all three row spacings. Since dominant weeds *Amaranthus retroflexus*, *Ambrosia artemisiifolia*, *Chenopodium album*, *Datura stramonium*, *Setaria viridis*, and *Sorghum halepense* were spread over all row spacings, formed the main biomass because of their size. Moreover, they emerge with the crop resulting with vigorous competition, since soybean is not a strong competitor in the early part of the season [42]. It is well documented that weeds that germinate and emerge with soybeans usually are better competitors for light, water and nutrients [43, 44]. Particularly, broadleaf weeds, such as *Amaranthus retroflexus*, *Ambrosia artemisiifolia*, *Chenopodium album*, *Datura stramonium* are more competitive with soybean than grass weeds, and many late emerging weeds [45]. Moreover, soybeans are very sensitive to moisture deficiencies in late summer, and even a few large weeds left in the field can severely reduce yield potential [46].

Weed infestation is one of main causes of low soybean yield [47]. Cultural practices such as narrow row spacing has been reported as a management tactic that reduce the amount of light that reaches the soil surface, and that reduce the amount of time that needs soybean to reach full canopy closure [48,49]. A number of previous published studied showed yield increases when soybean was planted in narrow rows [35,50]. Our results are in contrast to reports where narrow row spacing improve weed control efficacy, productivity and profitability of soybean. The reason for this lay in fact that we did not manipulate with seeding density (soybean was sown to achieve recommended population of 500 000 plants per hectare).

In a given crop population, soybean planted in 70 cm rows gave the best economic results, according to Monte Carlo simulation. Probability distribution of achieving a profit showed the best results for soybean growing in 70 cm rows, with preemergence herbicide application and 2 inter-row cultivation (in V1 and R1 soybean development stage). Individual weed control methods generally do not provide complete control of weeds [51] and therefore, integrated weed management is often considered to be the most effective approach, as was confirmed by this research, as well. Preemergence herbicide application followed by mechanical cultivation can increase soybean yield, receive the positive financial result. Although Harder et al. [6] reported that gross margins were usually greater in 19 and 38 cm soybean rows, but in 76 cm rows at low soybean population the gross margin was greatest. Combination of preemergence herbicide application and mechanical weed

control is not a fixed process and needs to be adjusted depending on crop type, farming operation, and seasonal conditions [52].

5. Conclusions

This study confirmed that weed interference in soybean is a major limiting factor in successful soybean production. Differences in weed community were more influenced by year (humid – arid environment) followed by compositional differences between 25 cm vs. 50 and 70 cm row spacings. There were no differences in weed biomass accumulation with reduction in row spacing from 70 to 50 and 25 cm since dominant weeds *Amaranthus retroflexus*, *Ambrosia artemisiifolia*, *Chenopodium album*, *Datura stramonium*, *Setaria viridis*, and *Sorghum halepense* formed the main biomass and were spread over all row spacings. Row spacing, duration of weed interference and year had significant influence on soybean yield and yield components. The best financial results were evident in soybean growing in 70 cm rows with preemergence herbicide application following two inter-row cultivation during V1 and R1 stage.

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