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Follow-up effect of white clover (*Trifolium repens* L.) intercropping system on biomass and morphology of willow (*Salix viminalis* L.)

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Abstract

In 2010, a split-plot experiment was set up in Poland in order to determine the follow-up effect of the white clover intercropping system on biomass and morphology of willow. In 2010 willow was planted in a pure stand and intercropped with white clover. In 2013, after willow achieved a proper growth stage (2010–2012), plant measurements were made in the years 2013–2014. The experiment was carried out with two factors in a split-plot design: the first factor was the cultivation system (main plot) (a) without white clover and (b) with white clover, and the second factor was three basket willow clones: 1047, 1052 and 1057 (sub-plot). The investigated characters were biomass yield, height of plants, shoot diameter and plant loss. No N fertilization or pesticides were used. The intercropping system (willow with white clover) reduced the number of willow plants, but the willow height was lower in the pure stand (368 cm) than in the intercropping system (409 cm). The highest dry matter yield (30.8 t ha^{-1}), crude ash yield (434 kg ha^{-1}) and macronutrient (N, P, K and Mg) content were obtained with clone 1047.

Introduction

Biomass is the third largest natural source of energy in the world and in Poland it is considered as an important renewable energy resource (Nowak and Jasiewicz, 2012). Simultaneously, expectations related to the energy use of straw and wood 'waste' from forests need a new rethink.

The area of cereal crops production does not increase, and the systematic use of straw for heating purposes results in lowering the content of humus and soil depletion.

Forests should provide technological wood for processing and construction, small assortments obtained during felling of technological wood should be crushed and left on felling areas.

For this reason, since the beginning of the 1990s, we have seen an increase in the area of cultivation of plants used for energy purposes (Nowak *et al*., 2011), among which, basket willow is one of the more important species in countries with moderate climate (Wojciechowski *et al*., 2011; Nissim *et al*., 2013). Cultivation of this crop can help to protect the natural environment (Toome *et al*., 2010; Jama and Nowak, 2012; Holm and Heinsoo, 2013; Kubátová *et al*., 2018).

Like some other perennial plants, willow prevents soil erosion and is sometimes used in the rehabilitation of degraded land (Zydroń and Skórski, 2018). By taking up heavy metal ions, it contributes to their removal from soil (Dos Santos Utmazian *et al*., 2008; Kubátová *et al*., 2018). The interest in willow (*Salix viminalis* L.) as a renewable source of energy results from its low soil and thermal requirements as well as high yields (Korniak, 2007). In addition, its wide prevalence is due to the ease of vegetative reproduction and a wide range of biological variability (Christersson *et al*., 1993; Mitchell, 1995). It can be grown even in submontane conditions (Kozak and Sowiński, 2004). What can limit willow cultivation is mainly water and nutrients deficiency, including nitrogen, which is the strongest yielding factor (Nowak *et al*., 2011).

The optimal dose of nitrogen has a beneficial effect on yield and technological value of the willow. Nitrogen deficiency inhibits plant growth by reducing the chlorophyll content in the leaves, which, in turn, lowers the photosynthesis efficiency, and consequently, affects yields. On the other hand, too high N content deteriorates the quality of the raw material, through excessive branching and brittleness of the shoots (Nowak *et al*., 2011). Excessive N doses lead to N gas losses to the atmosphere, and nitrates leak to ground and surface water, which lead to financial losses, and create ecological threat (Cavanagh *et al*., 2011; Staniszewski, 2012).

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In the first years of willow vegetation, this problem may be solved by willow intercropping with white clover. White clover can be treated as a living litter that limits weed infestation (Adiele, 2012; Helios, 2013), improves soil properties by enriching it with organic matter and reduces soil erosion (Arevalo *et al.*, 2005; Adiele, 2012; Rogowska and Rybczyński, 2012). Also, nitrogen fixed by nodule bacteria is released into the soil gradually, and therefore, its leaching from the soil is small, unlike in the case of ammonium and nitrate ions in mineral fertilizers (Stevenson *et al.*, 1998). Interactions between clover and willow in intercropping system cultivation are still poorly understood. Therefore, the aim of the present study was to determine the subsequent influence of white clover on willow plant density, number of shoots, height and yield of willow plants. The study was of practical nature and served to understand the possibility of limiting nitrogen fertilization at willow plantations by intercropping it with white clover.

Methods

The field experiment with willow planted in a pure stand and intercropped with white clover was set up in Poland (51° 10′35″N, 17° 07′13″E) in 2010 on light soil defined as a very light alluvial soil, on loose sand and sandy gravel. On the 6th of March 2013 willow plants were cut and first measurements started at the beginning of May.

A year prior to willow planting, weeds were controlled with a double spray of Roundup 360 SL at a dose of 5 l ha^{-1}, next autumn ploughing was performed. An experiment was carried out in a split plot design with two variable factors with four replications. The first factor was the cultivation system (main plot) (a) without white clover and (b) with white clover (hand-sown at the rate of 10 kg ha^{-1} during plantation set-up). The second factor was three basket willow clones: 1047, 1052 and 1057 (subplot). Willow was planted in rows 65 cm apart with plant spacing every 40 cm (38.5 thousand of cuttings per ha). The area of the harvesting plot was 6.76 m^2.

Every year the analysis of two soil samples (with and without clover) was carried out. Samples were taken at 0–30 cm depth at the beginning of vegetation period. The pH of the soil and the content of macronutrients are shown in Table 1. The data show that the soil was acidic, the content of phosphorus and potassium was from high to very high (Egner, 1940; Riehm, 1940, 1943), and the amount of Mg was from very low to low (Schachtschabel, 1954).

From the moment of willow planting to the end of the experiment, no weed, disease and pest controls were performed. Every year, fertilizers were applied in doses of 17.4 kg P and 49.8 kg K per hectare. Nitrogen fertilization was not used. In the years of study, after starting and before the end of the growing season, all plants and shoots were counted on the plots, and the shoot diameters were measured. Every 4 weeks throughout the growing season, the height of the willow was measured on the sample of ten plants from each plot. Each year in June, weed infestation was determined on each plot in four randomly selected places of 0.25 m^2. Water content of weeds and their dry matter was determined after 5 h drying process at 105 ± 2°C. The fresh willow matter was collected on 23/01/2015.

Analyses of the chemical composition of willow sprouts were made using the following methods:

Table 1. Macronutrients in the soil (mg kg^{-1}) and the pH of the soil

Cultivation system	Years	pH in 1 M KCl	P	K	Mg
Without white clover	2013	5.4	97.8	234.5	16.2
	2014	5.5	87.8	320.1	15.7
With white clover	2013	4.9	94.6	297.9	16.6
	2014	4.8	86.8	311.3	15.2

- total nitrogen—modified Kjeldahl's method
- crude ash—by burning plant material in an electric furnace at 600°C
- magnesium, potassium, calcium and sodium—by flame photometry using a flame spectrophotometer (Spectra 220 FS, Candela: http://www.candela.com)
- phosphorus—colorimetric method using an appropriate equipment (Cecil CE 2011-P)

The water content in the willow shoots was determined by the drying method at 105 ± 2°C for 5 h. Statistical analyses were carried out with the 'AWA' programs (Bartkowiak 1978) and Statistica 10.

Results and discussion

The weather conditions affect the growth of willow. Moderate temperatures and frequent rainfall are suitable conditions for growing willows. Willow has very high evapotranspiration requirements. In Sweden, for optimal growth at mid-summer 5–6 mm of available water per day are needed (Perttu, 1999). The climate in Poland with an average annual temperature of 9.2°C and mean annual precipitation of 523.8 mm is classified by Köppen as cold (D), without a dry season (f) and with warm summers (b) (Peel *et al.*, 2007). Detailed climatic data on the Wrocław region in Poland are described in Gąsiorek *et al.* (2012). As observed on 22/04/2013 (the stage where new leaves started to appear), lower air temperatures, maintained from January to mid-April, in comparison with multiyear averages, delayed the onset of willow growing season. Also, in the first year of the study, the number of shoots was higher at the beginning of the growing season than in the corresponding period next year (Table 5). The reason for this might be harvesting willow prior to the experiment and higher water availability in soil (Styszko *et al.*, 2011) due to higher precipitations in February and March 2013 than in 2014. The sum of precipitations in 2013, compared to the long-term averages, was higher by 207.3 mm. Due to poor transpiration, the precipitation deficit observed in October and December did not have a negative impact on willow growth. Extremely little rainfall in February 2014 with a temperature above 0°C was not conducive to water storage in the soil before the vegetation began. The shortage of rainfall in June and July, however, had an adverse effect on the growth of willow (Table 2).

The area covered with white clover and weed infestation

No significant losses of white clover were observed (Helios, 2013) in the first years after the plantation was set up. Clover dying was observed because of little access to light in the first year (2013) of the experiment after the harvest of 3 year old willow plants

Table 2. Weather conditions in 2013–2014

	Temperature (°C)			Precipitation (mm)		
Month	2013	2014	Average 1981–2010	2013	2014	Average 1981–2010
I	−1.6	0.0	−0.8	51.3E	35.8D	31.9
II	0.05	3.7	0.3	29.5D	1.2A	26.7
III	−0.9	7.0	3.8	43.0E	40.1E	31.7
IV	9.2	10.6	8.9	42.7E	55.2E	30.5
V	14.6	13.3	14.4	135.9G	101.4F	51.3
VI	17.7	16.6	17.1	171.7G	40.2C	59.5
VII	20.5	21.2	19.3	36.3C	52.9C	78.9
VIII	19.0	17.3	18.3	68.2D	75.0E	61.7
IX	12.9	15.5	13.6	105.8E	72.2E	45.3
X	10.8	10.7	9.1	7.8B	59.4E	32.3
XI	5.6	6.6	3.9	25.8D	15.5C	36.6
XII	3.0	2.2	0.2	13.0C	17.5C	37.4
Average temperature or total precipitation	9.2	10.4	9.0	731.0	566.4	523.8

Data collected by Wrocław-Swojec weather station.
Classification of monthly precipitation averages for Wrocław (Gąsiorek *et al*., 2012), RPI, relative precipitation index.
A–extremely dry (RPI: <19), B–very dry (RPI: 19–31.5), C–dry (RPI: 31.5–68), D–normal (RPI: 68–120), E–wet (RPI: 120–192), F–very wet (RPI: 192–235), G–extremely wet (RPI: >235).

Table 3. White clover cover as (%) total area (average for the interaction of the cultivation system and willow clones)

		Observation date					
		10.08.2012		15.05.2013		08.08.2013	
Cultivation system	Clone	%	Bliss	%	Bliss	%	Bliss
Without white clover	1047	1.5	4.1	2.3	6.1	0.0	0.0
	1052	2.0	4.1	2.5	4.6	0.0	0.0
	1057	0.0	0.0	0.0	0.0	0.0	0.0
With white clover	1047	93.8	77.6	37.5	34.7	0.0	0.0
	1052	95.0	80.8	49.3	44.0	0.0	0.0
	1057	96.3	84.3	20.0	25.8	0.0	0.0
LSD ($P = 0.05$)		–	NSD	–	NSD	–	–
MS		–	61.50	–	103.01	–	0.0
SE (standard error)		–	4.3	–	6.2	–	–
P-Value		–	0.463	–	0.496	–	–
Calculated value of *F* statistic (Fisher)		–	0.8	–	0.7	–	–

NSD, no significant difference.

(Table 3). It was caused by a large number of new willow shoots at the beginning of the growing season (Table 5).

In the years 2013–2014, the number of weeds per 1 m^2 was lower than in the first years after the experiment was set up. Similar results were obtained by other authors (Borkowska and Molas, 2010). Despite comparable water content and the number of weeds in both cultivation systems, the dry weed matter per 1 m^2 was higher in willow pure stand than in the intercropped stand, which indicates that less light reaches the soil surface in the latter cultivation system (Borkowska and Molas, 2010). The lowest weed dry matter was observed in clone 1047. Similar trends were recorded in 2010–2012 (Helios, 2013). Higher weed infestation occurred in the second year of the study, which was caused by the partial loss of leaves due to lower precipitation and the occurrence of giant willow aphid (*Tuberolachnus salignus* Gmel.) (Table 4).

The yield of willow biomass from 1 ha depends on the length of shoots and their number per root (Nowak *et al*., 2011). In our

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Table 4. Effect of the cultivation system and willow clones on weed infestation in the following clones of willow

Cultivation system	Clone	Dry weight of weeds per 1 m^2 (g)	Number of weeds per 1 m^2	The water content in the fresh weight of weeds (%)
Without white clover	1047	39.4	188	77.0
	1052	71.3	210	73.4
	1057	42.8	238	79.9
With white clover	1047	28.5	213	75.3
	1052	20.7	143	71.2
	1057	54.7	206	71.6
LSD ($P = 0.05$)		11.6	39	NSD
MS		3997.21	8625.33	53.19
SE (standard error)		3.89	11	2.4
P-Value		<0.001	<0.001	0.326
Calculated value of *F* statistic (Fisher)		29.8	10	1.2
Means for the factors				
Without white clover		51.2	212	76.8
With white clover		34.6	187	72.7
LSD ($P = 0.05$)		6.3	NSD	NSD
MS		3286.83	7600.33	197.60
SE (standard error)		1.98	9	1.3
P-Value		<0.001	0.086	0.062
Calculated value of *F* statistic (Fisher)		35.1	4	4.5
	1047	34.0	200	76.2
	1052	46.0	176	72.3
	1057	48.7	222	75.8
LSD ($P = 0.05$)		8.5	22	NSD
MS		984.68	8289.33	73.62
SE (standard error)		2.90	7	1.7
P-Value		0.003	<0.001	0.217
Calculated value of *F* statistic (Fisher)		7.3	9	1.6
2013		35.3	163	75.3
2014		50.5	236	74.2
LSD ($P = 0.05$)		6.3	30	NSD
MS		2745.19	63,656.33	15.58
SE (standard error)		1.98	9	1.3
P-Value		<0.001	<0.001	<0.564
Calculated value of *F* statistic (Fisher)		29.3	31	0.4

NSD, no significant difference.

study, the density of willow plants at the beginning of the growing season did not depend significantly on the cultivation system. At the end of the growing season, a significantly higher number of plants per 1 m^2 was observed on control plots. Probably it was caused by both competitions from white clover in the first years of cultivation, and drought during the period of the study. There were no significant differences in the number of willow plants in particular years of the experiment. The number of plants per 1 m^2 in clones 1047 and 1057 at the end of the growing season was significantly higher than in the clone 1052. According to other authors, the experiments with a density of 40 thousand plants per ha resulted in 3.7–3.8 plants per 1 m^2 in 4th and 5th year after the experiment was established (Szczukowski *et al.*, 2004). The density of willow shoots in the

Table 5. Density of plants and shoots (average by factors and years)

			Number at the beginning of the growing season (pcs·m^2)		Number at the end of growing season (pcs·m^2)	
Cultivation system	Clone	Years	Plants	Main shoots	Plants	Main shoots
Without white clover			3.38	42.4	3.37	34.0
With white clover			3.36	38.5	3.31	32.3
LSD ($P = 0.05$)			NSD	NSD	0.05	NSD
MS			0.01	182.52	0.04	36.93
SE			0.02	2.4	0.01	0.6
P-Value			0.434	0.284	0.019	0.069
Calculated value of *F* statistic (Fisher)			0.66	1.30	8.07	4.3
	1047		3.41	40.2	3.38	35.1
	1052		3.32	41.1	3.27	34.0
	1057		3.38	40.1	3.37	30.3
LSD ($P = 0.05$)			NSD	NSD	0.08	3.6
MS			0.04	5.18	0.06	99.68
SE			0.03	1.8	0.03	1.2
P-Value			0.080	0.908	0.010	0.029
Calculated value of *F* statistic (Fisher)			10.4	0.1	5.63	4.11
		2013	3.40	48.5	3.34	32.4
		2014	3.34	32.4	3.33	33.9
LSD ($P = 0.05$)			NSD	7.8	NSD	NSD
MS			0.03	3094.44	<0.01	27.45
SE			0.02	2.4	0.01	0.6
P-Value			0.137	0.001	0.763	0.109
Calculated value of *F* statistic (Fisher)			2.67	22.0	0.10	3.16

NSD, no significant difference.

present experiment, at the beginning of the growing season, did not depend on the cultivation system or the genotype, but it varied in years. In the first months of the study, after cutting the plants in the previous year, willow developed 48.5 shoots per 1 m^2 (Table 5).

In the following months (2013), weaker willow shoots withered due to the lack of enough light. In the second year of the experiment, it was observed that willow plants did not die as much as in the first year, and the number of willow shoots increased during the growing season (Table 6). In our study plant losses were smaller on the experimental plots than in natural growth stand (Jakubowski, 2005) as there was no damage caused by humans or animals. The willow losses were also lower in our study compared to experiments with a density of 40 thousand plants per 1 hectare, in which willow was harvested annually (Szczukowski *et al*., 2004).

In the reported experiments (Arevalo *et al*., 2005), it was observed that during the first 2 months after ploughing of a 34-day old clover, willow grew better in a pure stand. After 3 months of the experiment, a higher biomass was obtained in clover intercropped plots, and at the end of the vegetation period, the control plots showed the highest yields. In the present experiment, we observed that the influence of 3-year intercropping (2010–2012) on the height of plants and an increase in plant height was maintained for the next 2 years (2013–2014) (Figs 1 and 2, Table 7), and the most significant increases in plant height were recorded between 30 and 120 days of vegetation in the first year after harvesting the crop (Fig. 2).

The diameter and number of willow main shoots did not depend on the cultivation system. A similar number of shoots (9.6 per plant) was obtained by other authors (Szczukowski *et al*., 2004). The clone 1057 was characterized by the highest height and diameter of shoots, and at the same time, by the lowest number of shoots on the plant. In the second year (2014) of the experiment, there was a significant increase in the plant height and shoot diameter with a similar number of shoots per plant (Table 7).

The dry matter of main shoots, side shoots and of the whole plant did not depend on the cultivation system. The highest dry matter of side shoots and of the entire plant was observed in clone 1047 after 2 years of the experiment. Due to the large differences in the weight of branching of individual plant shoots, the effect of the cultivation method on shaping this morphological characteristic was not shown (Table 8).

Table 6. The loss of plants and shoots during the vegetation period (average for factors and years)

			The loss of plants and shoots on the plant during the vegetation period (%)	
Cultivation system	Clone	Years	Plants	Shoots
Without white clover			0.37	13.6
With white clover			1.25	10.5
LSD ($P = 0.05$)			NSD	NSD
MS			9.32	117.81
SE			0.32	4.1
P-Value			0.083	0.603
Calculated value of *F* statistic (Fisher)			3.81	0.3
	1047		0.81	6.7
	1052		1.35	11.4
	1057		0.28	17.9
LSD ($P = 0.05$)			NSD	NSD
MS			4.60	507.36
SE			0.46	3.5
P-Value			0.278	0.095
Calculated value of *F* statistic (Fisher)			1.4	2.6
		2013	1.44	29.4
		2014	0.18	−5.4
LSD ($P = 0.05$)			1.02	13.2
MS			18.99	14,511.61
SE			0.32	4.1
P-Value			0.021	<0.001
Calculated value of *F* statistic (Fisher)			7.77	35.7

NSD, no significant difference.

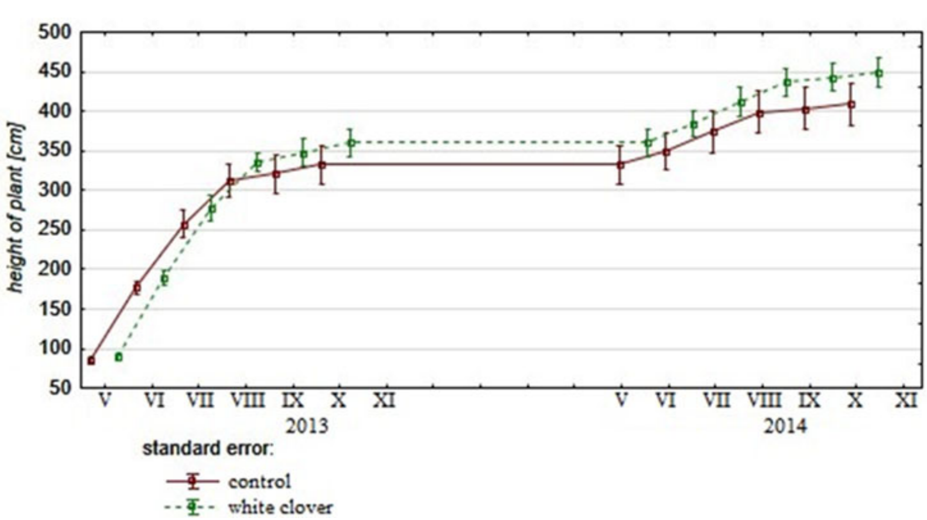

Fig. 1. The successive effect of white clover on the height of the willow plants.

The dry matter of side shoots of 1047 clone was higher when grown with white clover (Table 8).

Yield components and the number of first-order branches were not affected by the cultivation system. In clone 1047, side shoots made the largest part of the dry matter yield, but the number of main branches in the investigated genotypes was similar (Table 9).

Willow dry matter yield from 1 ha determines the energy value (Nowak *et al.*, 2011). The yields of willow in the present study did

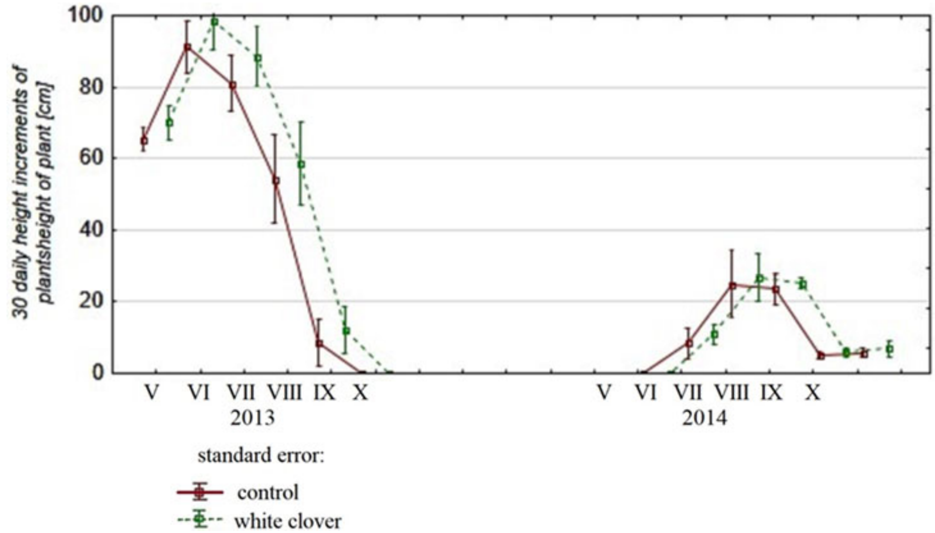

Fig. 2. The successive effect of white clover on the growth dynamics of the willow plants.

Table 7. Effect of cultivation system on the morphological characteristics of three willow clones at the end of the growing season in 2013–2014 (average for factors and years)

Cultivation system	Clone	Years	Height of plant (cm)	Main shoots diameter (mm)	Number of main shoots on the plant
Without white clover			368	13.3	10.1
With white clover			409	13.6	9.7
LSD ($P = 0.05$)			22	NSD	NSD
MS			19,886.02	0.96	1.92
SE (standard error)			7	0.3	0.2
P-Value			0.002	0.577	0.172
Calculated value of *F* statistic (Fisher)			18	0.3	2.2
	1047		382	13.1	10.4
	1052		382	13.1	10.4
	1057		402	14.3	9.0
LSD ($P = 0.05$)			16	NSD	1.0
MS			2166.94	7.63	10.32
SE (standard error)			5	0.53	0.3
P-Value			0.022	0.206	0.009
Calculated value of *F* statistic (Fisher)			4	1.7	5.7
		2013	350	11.9	9.7
		2014	428	15.1	10.2
LSD ($P = 0.05$)			22	1.1	NSD
MS			71,842.69	124.17	2.52
SE (standard error)			7	0.3	0.2
P-Value			<0.001	<0.001	0.124
Calculated value of *F* statistic (Fisher)			66	43.1	2.9

NSD, no significant difference.

not depend significantly on the cultivation system and per one year was lower than in other studies with a 2-year harvesting cycle (Szczukowski *et al*., 2011*a*, 2011*b*). The 1047 clone yielded the best. The yield of side shoots was lower on the control plots than at stand where white clover was previously grown. The largest dry matter yield of side shoots was obtained in clone 1047.

Table 8. Effect of cultivation system on dry matter of one shoot and one plant (g) of three willow clones after 2 years of cultivation

Cultivation system	Clone	Mean weight of 2-year-old shoot			
		Main shoot	Side shoots	Together	Weight of 1 plant
Without white clover	1047	80.6	3.1	83.7	926
	1052	59.3	1.8	61.1	659
	1057	64.7	3.7	68.4	600
With white clover	1047	67.5	18.7	86.2	888
	1052	63.7	10.9	74.6	770
	1057	75.2	6.3	81.6	737
LSD ($P = 0.05$)		NSD	11.2	NSD	NSD
MS		303.50	85.09	77.63	17,504.54
SE (standard error)		5.9	2.8	6.8	71
P-Value		0.215	0.033	0.693	0.298
Calculated value of *F* statistic (Fisher)		1.8	4.6	0.4	1.3
Means for the factors					
Without white clover		68.2	2.8	71.0	728
With white clover		68.8	12.0	80.8	798
LSD ($P = 0.05$)		NSD	NSD	NSD	NSD
MS		2.47	500.32	569.40	29,540.17
SE (standard error)		2.4	2.2	3.4	54
P-Value		0.864	0.063	0.141	0.427
Calculated value of *F* statistic (Fisher)		<0.1	1.1	4.0	<1
	1047	74.1	10.9	85.0	908
	1052	61.5	6.3	67.8	714
	1057	70.0	5.0	75.0	668
LSD ($P = 0.05$)		NSD	4.7	NSD	124
MS		330.13	75.02	591.76	100,000
SE (standard error)		4.7	1.5	5.1	40.4
P-Value		0.191	0.045	0.095	0.003
Calculated value of *F* statistic (Fisher)		1.9	4.1	2.9	9.9

NSD, no significant difference.

Genetic properties of clones and the cultivation method did not affect the water content in willow shoots (Table 10). No interaction was observed in the yield between the cultivation method and the tested genotypes. Water content ranged between 530–550 g kg^{-1} (Table 11), which are similar values to those reported in other studies (Stolarski *et al.*, 2008).

The average crude ash content was 14.8, the contents of: nitrogen was 4.04, phosphorus was 0.85, potassium was 2.17 and calcium was 3.28 g kg^{-1}. Similar values of ash and macronutrients in willow wood were recorded by Jama and Nowak (2012). Almost twice higher content of phosphorus and potassium was observed by Nowak and Jasiewicz (2012). Differences in chemical composition may depend on the variety and the harvesting cycle (Jakubiak, 2010). Detailed chemical composition of 2-year-old shoots is given in Table 11.

The accumulation of nutrients is a function of yield and chemical composition that allows determining optimal doses of fertilizers in the future. In the studies of Sevel *et al.* (2014), these values were as follows: nitrogen 35–61, phosphorus 9–12, potassium 30–40, calcium 26–35 and magnesium 4–5 kg ha^{-1} yr^{-1}. In the present study similar results were recorded. The accumulation of nutrients in the biomass of plants did not depend on the cultivation system. The largest accumulation of macronutrients was noted in clone 1047 (Table 12).

Conclusion

On the basis of the obtained data, it is possible to conclude:

(1) There was no long-term follow-up effect of intercropping cultivation on the yield and nutrient accumulation of willow.
(2) There was a beneficial effect of white clover on the height of willow plants.

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Table 9. Effect of cultivation system on yield components (%) and number of first order shoots in three willow clones after 2 years of cultivation (average by factors)

		2-year-old shoot		
Cultivation system	Clone	Main shoot	Side shoots	Number of first order shoots
Without white clover		96.0	4.0	2.26
With white clover		85.8	14.2	3.60
LSD ($P = 0.05$)		NSD	NSD	NSD
MS		616.11	616.11	10.73
SE (standard error)		2.3	2.3	0.55
P-Value		0.052	0.052	0.184
Calculated value of *F* statistic (Fisher)		9.7	9.7	0.39
	1047	87.3	12.7	2.08
	1052	91.9	8.1	3.50
	1057	93.4	6.6	3.22
NIR (0.05)—LSD ($P = 0.05$)		4.9	4.9	NSD
MS		80.68	80.682	4.54
SE (standard error)		1.6	1.6	0.41
P-Value		0.048	0.048	0.070
Calculated value of *F* statistic (Fisher)		4.0	4.0	3.35

NSD, no significant difference.

Table 10. Effect of cultivation system on yield and water content of three willow clones after 2 years of cultivation (average for factors)

		Yield of dry matter ($t\ ha^{-1}$)			
Cultivation system	Clone	Main shoot	Side shoots	Σ	The water content ($g\ kg^{-1}$)
Without white clover		21.3	3.2	24.5	532
With white clover		20.8	5.8	26.6	541
LSD ($P = 0.05$)		NSD	1.6	NSD	NSD
MS (Mean squares)		1.98	42.80	26.25	486.00
SE (standard error)		1.4	0.3	1.7	11
P-Value		0.784	0.012	0.450	0.614
Calculated value of *F* statistic (Fisher)		0.1	29.4	0.8	0.3
	1047	24.7	6.1	30.8	540
	1052	19.1	4.2	23.3	531
	1057	19.4	3.2	22.6	537
LSD ($P = 0.05$)		4.1	1.5	4.4	NSD
MS		78.58	17.77	165.15	144.88
SE (standard error)		1.32	0.5	1.4	13
P-Value		0.019	0.003	0.003	0.898
Calculated value of *F* statistic (Fisher)		5.7	9.6	10.1	0.1

NSD, no significant difference.

(3) The intercropping system of willow and clover reduced the number of willow plants at the end of the growing season when precipitation was low.

(4) Clone 1047 gave the highest biomass yields and it can be recommended for broad agricultural practice as a source of renewable energy.

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Table 11. Chemical composition (g kg^{-1})of 2-year-old willow shoots after vegetation period

Cultivation system	Clone	Crude ash (g kg^{-1})	N	P	K	Ca	Mg	S	Na (mg kg^{-1})
Without white clover	1047	13.0	4.43	0.84	2.16	2.76	0.56	0.33	0.02
	1052	15.7	4.05	0.84	2.20	3.94	0.46	0.33	0.02
	1057	16.2	3.57	0.84	2.25	3.55	0.58	0.39	0.04
With white clover	1047	15.2	4.91	0.89	2.26	3.17	0.58	0.36	0.03
	1052	15.7	3.91	0.90	2.10	3.73	0.47	0.36	0.04
	1057	12.7	3.38	0.76	2.03	2.55	0.42	0.36	0.03
Means for the factors									
Without white clover		15.0	4.02	0.84	2.20	3.42	0.53	0.35	0.03
With white clover		14.5	4.07	0.85	2.13	3.15	0.49	0.36	0.04
	1047	14.1	4.67	0.87	2.21	2.97	0.57	0.35	0.03
	1052	15.7	3.98	0.87	2.15	3.84	0.47	0.34	0.03
	1057	14.5	3.48	0.80	2.14	3.05	0.50	0.38	0.04

Table 12. Nutrients' accumulation (kg ha^{-1}) in 2-year-old willow shoots after vegetation period

Cultivation system	Clone	Crude ash (kg ha^{-1})	N	P	K	Ca	Mg	S	Na
Without white clover	1047	407	139	26.3	67.7	86.4	17.5	10.3	0.75
	1052	333	86	17.8	46.7	83.6	9.8	6.9	0.48
	1057	338	75	17.5	47.0	74.1	12.1	8.2	0.87
With white clover	1047	460	149	26.9	68.4	96.0	17.6	10.9	1.00
	1052	398	99	22.8	53.2	94.5	11.9	9.0	1.00
	1057	307	82	18.3	49.0	61.6	10.1	8.6	0.84
LSD ($P = 0.05$)		NSD	NSD	NSD	NSD	NSD	NSD	NSD	0.31
MS		5502.04	18.16	11.99	18.06	346.37	8.41	1.72	0.15
SE		36	10	2.1	5.2	8.2	1.2	0.8	0.08
P-Value		0.28	0.947	0.423	0.801	0.217	0.209	0.463	0.008
Calculated value of *F* statistic (Fisher)		1	<1	0.9	0.2	1.7	1.8	0.8	7.51
Means for factors									
Without white clover		360	100	20.6	53.8	81.4	13.1	8.5	0.70
With white clover		388	110	22.7	56.9	84.0	13.2	9.5	0.94
LSD ($P = 0.05$)		NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
MS		4930.67	602.00	27.09	57.66	41.34	0.015	6.36	0.36
SE (standard error)		26	7	1.5	3.7	5.9	0.8	0.6	0.06
P-Value		0.491	0.379	0.610	0.596	0.773	0.969	0.318	0.058
Calculated value of *F* statistic (Fisher)		<1	1	1.1	0.4	0.1	<0.01	1.4	8.92
	1047	434	144	26.6	68.0	91.2	17.5	10.6	0.87
	1052	366	93	20.3	50.0	89.1	10.8	7.9	0.74
	1057	322	78	17.9	48.0	67.8	11.1	8.4	0.85
LSD ($P = 0.05$)		68	20	3.9	9.7	15.4	2.4	1.6	NSD

(*Continued*)

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Table 12. (*Continued.*)

Cultivation system	Clone	Crude ash (kg ha^{-1})	N	P	K	Ca	Mg	S	Na
MS		25,126.79	9520.44	161.28	975.34	1335.77	115.02	16.21	0.04
SE		22	6.5	1.3	3.2	5.0	0.8	0.5	0.05
P-Value		0.013	<0.001	0.001	0.001	0.011	<0.001	0.007	0.189
Calculated value of *F* statistic (Fisher)		6	29	12.5	12.2	6.7	24.5	7.7	1.92

NSD, no significant difference.

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