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Long-term study on utilization of Virginia fanpetals – a valuable biomass

Wieloletnie użytkowanie ślazowca pensylwańskiego – wartościowego źródła biomasy

Summary. Next-generation biomass feedstocks are needed to optimize sustainability in a wide range of soils and climates. Species that has been recently noticed in Europe is Virginia fanpetals (*Sida hermaphrodita* L. Rusby). A critical question with research of this species is its field propagation. A long-term (2003–2012), field experiment was conducted to determine the impact of propagation method on yields and productivity this species. The hypothesis was higher yields of *S. hermaphrodita* biomass from vegetative propagations than from generative propagations, also over the long term. On average, from first 10 years of lifespan of Virginia fanpetals (2003–2012), biomass dry matter (DM) yields were significantly higher by vegetative propagation (16.8 Mg ha^{-1}) as for generative (10.9 Mg ha^{-1}). The average gross energy yield obtained by the vegetative propagation reached 304 GJ ha^{-1} while by the generative propagation was 196 GJ ha^{-1}. The determined heat of combustion reached 18.1 GJ Mg^{-1} DM, the ash content was 28 g kg^{-1}, and the nitrogen (N), sulphur (S), and chlorine (Cl) contents were 1.9 g kg^{-1}, 0.52 g kg^{-1} and 0.23 g kg^{-1}, respectively, regardless of propagation methods.

Key words: propagation method, Virginia fanpetals, *Sida hermaphrodita*, biofuels

INTRODUCTION

Wood and lignocellulosic crops have been used for centuries as an energy source [Somerville et al. 2010]. Results of energy-efficiency analyses have shown [Bonin and Lal 2014] that perennials are much more efficient than northern hemisphere annual crops (generative), like, e.g., corn (*Zea mays*). Also, some tuber-crops (vegetative), are still the potential energy source, e.g., Jerusalem artichoke, sweet potato [Sawicka and Kalembasa

2013, Sawicka et al. 2018]. What has changed diametrically, is that the high-yielding perennials' biomass is not only a fed to animals but also converted to a liquid biofuel that is then used to power the internal-combustion engines [Anderson et al. 2008, Kryzeviciene et al. 2011, Borkowska and Molas 2013]. The biomass of perennial herbaceous species, however, does have some drawbacks. It is characterized by lower bulk density and calorific value than wood [Mani et al. 2006]. Despite these drawbacks, it does have beneficial qualities, one of which is the low moisture content at the time of winter (I–III) harvest: 10 g kg^{-1} [Chołuj et al. 2008], effect on significant improved logistics and reduction biomass processing cost. Indeed, in many cases, biomass can be granulated direct from the field, without the need for long-term storage or energy-intensive drying stages [Sharma et al. 2009, Borkowska and Molas 2013].

Virginia fanpetals (*Sida hermaphrodita*. L. Rusby); further in the manuscript as "VF"; is native to the USA and Canada (now is becoming as a rare and endangered species), formerly was cultivated as a forage in Russia. Professor B. Styk (University of Life Sciences in Lublin) first in 1955 received seeds of this species from one of the Soviet Crop Research Institutes, being at an agricultural exhibition in Moscow. Since 1995 VF is utilized as fodder and bio-energy crop, studied in Poland and other countries [Borkowska and Styk 2006, Jablonowski et al. 2017]. This species is *a* high-yielding perennial dicot plants, that once planted can grow for many years; additionally, as an excellent fodder [Purwin et al. 2019]. Yields of VF are varying to a great extent, reduced when is cultivated on rain-fed sandy soils with low water capacity [Borkowska et al. 2009]. VF yield can be higher than of Switchgrass (*Panicum virgatum* L.), Miscanthus (*Miscanthus* × *giganteus*), which require growing in warm climates with yields obtained by Christian et al. [2008], Kryzeviciene et al. [2011] and, higher than of coppice willow [Arundale et al. 2014]. VF is well suitable for the production of cellulosic ethanol [Jablonowski et al. 2017]. The VF' cellulose is composed of pentosans (average 170 g kg^{-1}) and average 260 g kg^{-1} of lignin [Wróblewska et al. 2009]. In accordance with research, after saccharification procedure of VF, bio-ethanol content in DM reached 261 mg g^{-1} [Mialon 2012]. According to Gubisowa et al. [2013], the heat of combustion of VF biomass can attain 18.2 MJ kg^{-1} DM, and it has very low contents of the particularly harmful element S (average 0.03 g kg^{-1}) and 0.25 g kg^{-1} Cl [Stolarski et al. 2005, Gubisowa et al. 2013]. Wróblewska et al. [2009], Michalska et al. [2015] were reported that, VF biomass contained 60 g kg^{-1} of hydrogen and 410 g kg^{-1} cellulose.

Studies on improving the quality and efficiency of VF biomass production, through the appropriate selection of technology components, were conducted in a Felin Experimental Farm from many years. Of particular interest, is the search for agronomic factors affecting the performance and reproduction of VF. Besides generative reproduction (seed sowing), this species is capable of vegetative propagation. The most expensive method is *in vitro* production. Another method, albeit inefficient and costly, is the cutting of the rhizome (at least biennial) of the parental plants into four large parts. A more efficient way of producing seedlings is a cutting of the root system into small parts (50–100 mm) and selecting the roots with visible growth buds. This vegetative material allows the growth of strong plants within the first year of cultivation. Earlier study [Borkowska and Styk 2006] reported that vegetative propagation allowed to be produce higher yields than of generative, however, they only lasted spanned three years. Other studies [Kurucz et al. 2014, Gansberger et al. 2016, Jablonowski et al. 2017, Matyka and Kuś 2018] are giving a wide range of methods, yields and periods, however, there was still no assessment of the yield stability of this species in the longer as three years' time. Therefore, it was necessary to test the hypothesis of higher yields from vegetative parts than by generative, also over the longer term.

Therefore, in current manuscript it is presented some results (2003–2012) first ever comprising the period of consecutive 10 years of VF cultivation. In other studies, depending of research objectives and hypothesis, from 10 to 150 thousand of root cuttings, and seeding rate from 0.25 kg to 10 kg of VF' seeds per hectare was practiced. Such a large span in seed rates is mostly caused by soil condition, low domestication of VF (the open-pollinated species), and different quality of seeds and roots. It worth be noted that cultivation management of VF is the same as for row crops. Proper VF number of successfully established crops in the shortest possible time, should cause the 100% canopy closure to reduce weed-infestation. Efficient purpose field-numbers (crop-density) is in a wide range; for heat or 3G- biofuel is 40–80 thousand, but for fodder or biogas production, is 80–120 thousand per hectare.

The objective of current study was to check long-term yield stability and examine the pros and cons of two ways of reproduction of VF on yield productivity. The test spanned ten years.

MATERIALS AND METHODS

The single-factor (reproduction) field studies were conducted using a randomized block design. Research was carried out in the Felin Experimental Farm, at the University of Life Sciences in Lublin, Poland. Propagating material was collected from the parental plantation located also in Felin Experimental Farm (lat.: 51°14' N; long. 22°38' E; 215 m a.s.l.).

For propagation of VF, two propagation methods (vegetative and generative) were compared. Four replicates were obtained for each method, for eight plots, each one consisting of a harvest area of 12.6 m^2. In early April 2003, 40 thousand pieces per one hectare of root cuttings were planted to a depth of 80–100 mm. Root cuttings, which are a much richer source of nutrients compared to small seeds, caused the development of strong plants in the first year of cultivation. In late April, 1 kg ha^{-1} untreated seeds (240,000 pcs) were sown (thousand seeds weighed 4.17 g). It was used a seeding depth of 10–15 mm. Seeds and root cuttings were obtained from the parental plantation. In both cases (sowing seeds and planting of roots), it was applied a 0.70 m row spacing. Based on previous experiences, it was used the medium quantity of the propagation material. Depending on the quality of VF seeds, the correct dose per hectare is from 0.5 kg to 2.0 kg of untreated seeds, and from 30 to 60 thousand of root cuttings needed to get the right cultivated crop quantity per hectare for bioenergy. The mass of a thousand seeds (MTS) of cultivated VF, prepared by the proper ISTA-1985 method, ranges from 3.2 to 4.2 g. After emergence and at the end of the growing season, the plant numbers were counted. The field emergence and survival rates of plants in the first year of cultivation were calculated. In 2003, fertilizer was not applied. From 2004 onward, every spring at the start of the growing season, 100 kg ha^{-1} N, 39 kg ha^{-1} P, and 75 kg ha^{-1} K was applied to the soil and mixed in the inter-rows by hoes. Every year in November, all eight plots were harvested, and the yield of biomass determined. The number of shoots per one square meter was counted, and length measured. Plant samples (2 kg) from each plot were collected to determine the humidity and the biomass yield of dried VF (dried for five hours at 105°C). In 2007 at the Institute of Wood Technology in Poznań, biomass of VF was analysed. Sample was prepared by collecting and mixing them 3.0 kg of shoot cutting from each plot. The following tests were performed: heat of combustion with Polish/EU standard of calorimetry [PN-EN ISO 18125:2017-7], ash content (calcination at 550°C), and the content of N, S, and Cl was measured in accordance with the procedures of Thermo Fisher Scientific, Waltham, Massachusetts, USA. The determined heats of combustion were used to calculate the energy values of the annual VF biomass yields. Hydrogen (fig. 1), here calculated by weight in biomass yield of VF according to Wróblewska et al. [2009], and Michalska et al. [2015]. The experiment was conducted on sandy loam soil (Haplic Luvisol) susceptible to crust after rain, with the following composition particle size: 1.0–0.1 mm, 220–230 g kg^{-1}; 0.1–0.02 mm, 400 g kg^{-1}; and <0.02 mm, 370–380 g kg^{-1}. The soil contained the following forms: P_2O_5 – 1.50–1.52, K_2O – 1.83–1.90 and Mg – 0.41–0.43 mg kg^{-1} (Mehlich 3 extraction Protocol).

CLIMATE CONDITIONS

On a latitude of the current research site, usually it is very cold in winter time. In December 2011 and January 2012, the temperatures were four degrees higher than the 50 year average. The plants began to grow in winter time. The effect of a sudden climate change was visually stated. The meteorological data, including the average daily air temperature and precipitation, were obtained from the Agro-Meteorological Observatory at Felin (the location of the recent research). The Sielianinov hydrothermal coefficient (K) was calculated by dividing 10-time of the total rainfall by the total mean circadian temperature (quotient of the total rainfall and 0.1 of the sum of average temperatures) for the given period (tab. 1). K values less than 1.0, less than 0.5, and less than 0.25 represent a shortage of precipitation, drought, and disastrous drought, respectively. The optimum growing season (April to October) was determined based on the mean value of hydrothermal coefficients over the last 50 years (K = 1.56).

Table 1. The Sielianinov's (K) coefficient for growing seasons and the several-year (SY) average (1951–2010)

Month	Year of research										
	1	2	3	4	5	6	7	8	9	10	SY
April	2.15	1.61	0.68	1.16	0.67	1.99	0.08	0.87	0.98	1.19	1.76
May	1.42	1.03	2.39	1.41	1.73	2.58	1.69	3.49	0.95	1.21	1.51
June	0.75	1.05	1.16	0.75	1.62	0.49	2.55	1.21	1.22	1.21	1.35
July	1.61	1.62	1.79	0.10	1.46	1.36	0.93	1.51	3.31	0.79	1.47
August	0.48	0.85	2.07	3.67	0.66	0.75	0.93	2.12	1.12	0.63	1.33
Septtember	0.73	0.37	0.40	0.23	3.33	2.70	0.46	3.17	0.12	0.76	1.42
October	3.23	0.63	0.35	0.45	0.75	1.77	4.84	0.65	1.15	3.58	1.70
Average	1.21	1.03	1.39	1.12	1.50	1.51	1.39	1.96	1.35	1.13	1.56

K < 0.25 – disastrous drought, K < 0.5 – drought, K < 1.0 – shortage of precipitation, K = 1.3–1.6 – the optimum, K > 2 – humid, K > 3 – extremely humid

The vegetation periods in the years 2003, 2004, 2006, and 2012 were quite dry (K ranged from 1.03 to 1.21) yet 2010 was rather damp (K = 1.96). In the years 2005, 2007–2009, and 2011, the ratio of precipitation to temperature was optimal (K ranged from 1.35 to 1.51). In every year of the study, however, the growing season experienced unfavourable ratios of precipitation to temperature for the optimal growth and development of plants (tab. 2).

Table 2. Air temperature and precipitation (P) of winter 2011/2012 and average of several (1951–2010) year (SY) according to the Agro-meteorological Observatory in Felin (Lublin)

Month	Days	Temperature (°C)			Days with		Snow cover (cm)	Total P (mm)
		min.	max.	average	rain	snow		
December	1–10	0.8	5.6	2.7	6	1	0	21.2
	11–20	0.2	4.5	2.3	6	0	0	8.6
	21–31	–1.7	3.2	0.9	2	5	0–10	4.7
Year average	2012	–0.3	4.4	1.9	14	6	–	34.5
	SY	–3.9	0.9	–1.6	–	–	–	31.4
January	1–10	0.4	4.1	2.1	7	3	0	13.9
	11–20	–2.9	1.5	–1.1	2	7	0–8	14.7
	21–31	–8.9	–3.3	–6.5	0	5	5–10	5.0
Year average	2012	–3.9	0.6	–1.9	9	15	–	33.6
	SY	–6.3	–0.9	–3.7	–	–	–	23.4
February	1–10	–18.4	–11.9	–15.6	0	2	5–10	0.8
	11–20	–10.0	–2.7	–6.8	0	4	4–10	6.7
	21–29	–1.5	4.1	1.0	5	4	0–10	14.6
Year average	2012	–10.3	–3.7	–7.4	5	10	–	22.1
	SY	–5.6	0.4	–2.8	–	–	–	25.8

The max. temp. in December 2012 were between 3.2°C and 5.6°C, which are quite unusual in our latitude. Since the snowfalls occurred in positive temperature, the snow melted during the day and could not provide the plantations with a protective snow covering. After this extended period of relatively warm weather for this time of year (plants were started to grow), February was marked by sudden frosts with average temperatures between minus 15.6°C and minus 18.4°C. Those conditions killed most of the plants (tab. 2).

STATISTICAL ANALYSIS

Analyses were performed with the mixed procedure of Statistical Analysis System (SAS) software. Analysis of significance, variance and the least square means of crop yield, plant quantity, were performed by two-factor ANOVA procedures with HSD (Tukey's Honest Significant Difference) procedure, used the $\alpha = 0.05$ to determine if there were significant differences between method of reproduction on yield. Calculations also include relative standard deviation (RSD) with Fisher-Snedecor test (F) and *p*-value.

In addition, the analysis of the research results was enriched with descriptive statistics, simple correlation and polynomial, linear and partially non-linear regression. The descriptive statistics showed the following characteristics: mean, standard deviation, median, range, minimum and maximum, coefficient of variation V, where the coefficient of variation was calculated from the formula:

$$V = S / c \cdot 100\%,$$

where: S – standard deviation, x – arithmetic mean. It is a measure of the dispersion of the obtained results. The lower its value, the more stable a given feature is [Koronacki 2016].

RESULTS

Variability of plant density in the first year of cultivation

In the first year of the study, the results concerning the number of planted seedlings/ sown seeds, plant emergence, the number of harvested shoots, their survival and other features that significantly affect the VF yield were higher in the plots with vegetative than generative reproduction (tab. 3).

Table 3. Changes of the number of Virginia fanpetals plants in the first year of cultivation (2003), depending on the propagation methods

Propagation method	Planted (pcs m^{-2})	Emerged* (pcs m^{-2})	Appeared (%)	Collected (pcs m^{-2})	Survival (%)	Success** (%)
Generative	24.0	5.7	23.75	3.9	68.4	16.3
Vegetative	4.0	4.0	100.0	4.0	100.0	100.0

pcs – pieces; *from emergence to harvest, **from sowing to harvest

A comparison of the two propagation method’s impact on VF yields, has not been previously conducted. Effectively, 100% of the vegetative parts survived first season (tab. 3).

A very low seed field-emergence was observed, despite sowing 24 of seeds m^{-1}, and resulted in less than as 40% plants germination. After emergence, was observed a decrease in the number of plants occurred in the vegetation season. In November 2003, similar plant number for both reproductive methods were found: 4.0 and 3.9 plants m^{-1} for vegetative and generative propagation way, respectively (tab. 3). The difference in biomass yield, despite similar starting numbers of living plants per unit area, resulted by the plants grown from root cuttings, compared to those grown from seeds.

Biomass yield

In the current experiment, biomass yields of the two methods of VF reproduction were significantly different (tab. 4). With all variables (traits), significant higher mean values are associated with vegetative propagation methods. A significant increase in biomass yields, in comparison to the first year, occurred in the second and third year of cultivation (2004, 2005) (tab. 4). The following year (the fourth year, 2006), a substantial increase in yield was expected, meanwhile, their decrease was observed as compared to the previous year of the study. The highest increase in the above-ground biomass yield was observed in 2007, which was characterized by optimal meteorological conditions during the period of the most intensive plant growth in May–September. In the 7th year, the obtained biomass was lower than the fifth and sixth year. Low biomass crop yields in the 7th year (2009) are most likely as the result of negative weather conditions, where extreme drought occurred in April and September (tab. 1). The greatest reduction of *S. hermaphrodita* productivity took place in the last 10 year of the research. It was a decrease in the yield of aboveground biomass by 49,2% compared to the previous year. This is mainly attributed to the unfavourable distribution of rainfall, because in the months of July–September, which determine the increase in yield biomass, there was a significant shortage of precipitation (drought), but the general health condition of plants also contributed to this condition.

When assessing the interaction of summer × methods of reproduction, it was found that in objects with generative reproduction homogeneous biomass yield was recorded in 2004 and 2012. In the combinations of experiment with vegetative reproduction, a homogeneous yield was observed in 2007, 2008 and 2010.

Table 4. Yield and humidity of Virginia fanpetals biomass at harvest time depending on the propagation method and cultivation year

No	Year of cultivation	Yield (Mg ha^{-1} DM)			Humidity (%)	
		propagation method		average	propagation method	
		generative	vegetative		generative	vegetative
1	2003	1.68^{a}	2.80^{a}	2.24^{a}	50.69	50.69
2	2004	5.99^{b}	14.35^{efh}	10.17^{c}	22.16	22.81
3	2005	12.43^{cef}	19.45^{km}	15.94^{e}	30.43	38.93
4	2006	11.82^{cd}	16.05^{hi}	13.93^{d}	30.44	35.20
5	2007	16.67^{hj}	22.73^{n}	19.70^{h}	23.70	32.45
6	2008	12.92^{dfg}	22.15^{n}	17.53^{fg}	27.80	27.80
7	2009	12.68^{df}	17.98^{ijk}	15.33^{de}	28.54	28.54
8	2010	15.29^{gh}	22.26^{n}	18.77^{gh}	37.56	37.63
9	2011	12.72^{df}	20.65^{mn}	16.68^{ef}	36.53	25.77
10	2012	6.42^{b}	10.00^{c}	8.21^{b}	29.70	28.00
Average		10.86^{y}	16.84^{z}	13.85	–	–

The same letter index at numbers value, mean no significant difference between them by $p = 0.05$

Vegetative propagation provided more than 20 Mg ha^{-1} of dry biomass in four out of the ten years of research (2007, 2008, 2010, and 2011). In 2006, the most unfavourable year, yields in combination with vegetative multiplication amounted to 16 Mg ha^{-1}, whereas yields in seeded plots was approximately about 12 Mg ha^{-1}. The tenth year of cultivation, 2012, was extremely disadvantageous for yields. For both generative and vegetative multiplications, biomass yield amount were half of those from the previous year (2011). These results were attributed to weather anomalies associated with sudden climate change. The highest yield of dry matter, in combination with a generative, was obtained in the fifth (2007) and eighth (2010) year of cultivation with 16.7 Mg ha^{-1} and 15.3 Mg ha^{-1}, respectively. These years contained favourable rainfall distributions during the growing season. Plants from the vegetative propagation had significantly higher yields.

On the basis of the coefficient of variation V, it can be concluded that the greatest variability regarding the yielding of VF in Poland in the years 2003–2012 was characteristic of the biomass obtained in generative reproduction (V = 42.91%) – table 5.

The most stable yield in those years was shown by VF grown on a vegetative reproductive plantation, as its value was most concentrated around the average. Its coefficient of variation was equal to V = 37.84%. The minimum and maximum were the smallest and largest elements, respectively, and determined the yield range for each size. The skewness coefficient, in both methods of reproduction, with a value below 0 indicates the left-hand asymmetry of the distribution (otherwise called a negative skew distribution). Kurtosis is a measure of the concentration of results. Kurtosis tells us to what extent our observations, results are concentrated around the mean, and also how large the ‘scatter’ of the obtained results is, whether most of them are concentrated around the mean – the values are close to the mean value. In our case, there is a significant concentration of results around the mean (kurtosis takes a value above 0) and we can say that a large part of the results are similar to each other and the results significantly different from the mean are few (tab. 5).

Tabela 5. Descriptive statistics of the Virginia fanpetals biomass yield

Specification	Propagation method	
	generative	vegetative
Average	10.86	16.84
Median	12.56	18.72
Standard deviation	4.66	6.37
Kurtosis	0.12	1.51
Skewness	–0.92	–1.35
Range	14.99	19.93
Minimum	1.68	2.80
Maximum	16.67	22.73
Coefficient of variation V (%)	42.91	37.84

Variability of shoots density in the cultivation years

Years of study significantly differentiated the means of the number of shoots (tab. 6). High statistically significant differences in the analysed variables were found depending on the factors studied (years, reproduction) and their interactions. Plant density was significantly higher in objects from vegetatively propagated by 24.8%. This gave a great advantage to this vegetative method over the generative one and allowed for a significantly higher yield of above-ground biomass. The highest plant density per area unit was obtained in the fourth year of the study (2006). In the following years, a decrease in the plant density per unit area was observed. In 2005 and 2010, a homogeneous plant population was obtained. The significantly lowest plant density, compared to the first year of research, was achieved in the last year of 2012 research. The interaction of years and methods of propagation of the propagating material was also found (tab. 6). In objects with generative reproduction, a homogeneous number of shoots was obtained in the years: 2009, 2010, 2011, and 2007 and 2008. In turn, in combinations with vegetative reproduction, the number of shoots turned out to be homogeneous in 2004 and 2009 (tab. 6).

Plants in generative reproduction (V = 37.12%) were characterized by the highest variability of plant density in the VF canopy in the years 2003–2012 (tab. 7). The most stable density was that of VF plants grown from vegetative reproduction (V = 35.02%), the value of which was most concentrated around the average. The minimum and maximum were the smallest and largest elements, respectively, and defined the range for each method of reproduction. A skewness coefficient in both reproduction methods below 0 indicates a left-hand asymmetry of the distribution (called a negative skew distribution). Kurtosis, as a measure of the concentration of results, tells about how the results are centered around the mean as well as how large their 'spread' is. In this case, there is a significant concentration of results around the mean (kurtosis takes a value above 0) and we can say that a large part of the results are similar, and the results significantly different from the mean are few (tab. 7).

Table 6. Average for shoot density of Virginia fanpetals depending on reproduction way and years

No	Year of cultivation	Propagation method		Average
		generative	vegetative	
1	2003	3.9^{a}	4.0^{a}	4.0^{a}
2	2004	17.5^{bc}	29.8^{egh}	23.7^{c}
3	2005	22.3^{bd}	32.0^{fghi}	27.1ce
4	2006	35.0^{hj}	41.0^{j}	38.0^{h}
5	2007	26.0^{def}	35.0^{hj}	30.5^{eg}
6	2008	27.0^{def}	37.3^{ijk}	32.1^{fg}
7	2009	24.0^{de}	30.0^{egh}	27.0^{ce}
8	2010	25.3^{de}	34.0^{hk}	29.6^{def}
9	2011	24.0^{de}	27.8^{dg}	25.9^{cd}
10	2012	16.4^{b}	22.6^{cd}	19.5^{b}
Average		22.1^{y}	29.4^{z}	25.7

The same letter index at numbers value, mean no significant difference between them by $p = 0.05$

Table 7. Descriptive statistics of the shoot density of Virginia fanpetals

Specification	Propagation method	
	generative	vegetative
Average	22.14	29.35
Median	24.00	31.00
Standard deviation	8.22	10.28
Kurtosis	2.38	4.25
Skewness	–1.00	–1.82
Range	31.10	37.00
Minimum	3.90	4.00
Maximum	35.00	41.00
Coefficient of variation V (%)	37.12	35.02

Variability hight of shoots in the cultivation years

The average height of shoots from vegetative reproduction was over 292 cm, while from the generative generation they were significantly lower by 25 cm (tab. 8). Indeed, in three years the shoots were significantly shortest: in the first (2003), second (2004) and tenth (2012) cultivation years. Therefore, even with a greater number of short shoots, it was not possible to obtain high yields of biomass. In the years: 2005, 2007, 2008 and 2010 the shoots exceeded 300 cm in height (average up to 327 cm). In the years 2004 and 2006 the shoot height was homogeneous. The interaction of years with the methods of reproduction was found. However, only in 2009 and 2012, there was no significant difference in the number of shoots between the methods of propagation (tab. 8).

Table 8. Average for shoot length in cm of Virginia fanpetals depending on reproduction way and years

No	Year of cultivation	Propagation metod		Average
		generative	vegetative	
1	2003	157^{a}	183^{b}	170^{a}
2	2004	263^{de}	285^{fg}	274^{c}
3	2005	315^{hj}	339^{k}	327^{f}
4	2006	260^{d}	283^{ef}	272^{c}
5	2007	305^{gh}	335^{jk}	320^{ef}
6	2008	284^{e}	332^{ijk}	308^{e}
7	2009	286^{fg}	298^{fg}	292^{d}
8	2010	307^{gh}	333^{jk}	320^{ef}
9	2011	287^{f}	311^{hi}	299^{de}
10	2012	208^{c}	224^{c}	216^{b}
Average		267^{y}	292^{z}	280

The same letter index at numbers value, mean no significant difference between them by $p = 0.05$

Tabela 9. Descriptive statistics of the shoot length of Virginia fanpetals

Specification	Propagation method	
	generative	vegetative
Average	267.20	292.30
Median	285.00	304.50
Standard deviation	49.49	52.04
Kurtosis	1.84	0.96
Skewness	–1.49	–1.29
Range	158.00	156.00
Minimum	157.00	183.00
Maximum	315.00	339.00
Coefficient of variation V (%)	18.52	17.80

Plants in generative reproduction (V = 18.52%) were characterized by greater variability of *S. hermaphrodita* plant height in the years 2003–2012 (tab. 9), while VF plants grown from vegetative reproduction had a more stable value (V = 17.80%), the height of which was more concentrated around the average. The minimum and maximum were the smallest or largest elements, respectively, and defined the range for plant height characteristic of each method of reproduction. The skewness coefficient in both methods of reproduction was below 0, which indicates a left-hand asymmetry of the distribution (negative skew distribution). Kurtosis, on the other hand, indicates how the results are centered around the mean, and also how large their ‘spread’ is. In this case, there is a significant concentration of the results around the mean (the value of kurtosis is >0) and a large part of the results are similar, and the results significantly different from the mean are few (tab. 9).

DEPENDENCE OF BIOMASS YIELD ON INDEPENDENT VARIABLES

In the statistical study of regression analysis, the dependent variables (*y*) were: *yn* value – biomass yield and independent variables *x*1 – plant density, *x*2 – plant height. Based on the simple correlation coefficients, the variables for the polynomial regression were selected. The regressions were calculated according to the formula: $y = a + bx$, where *y* – the dependent variable, *a* – the intercept with the axis, *b* – the value of the regression coefficient, *x* – the independent variable. Partial regression coefficients (*bj*) indicate how much the biomass yield changes when *a* given factor changes by one unit. Regression analysis provides information on whether individual variables introduced into the model are statistically significant, i.e. whether any of them are 'redundant' when estimating the dependent variable. This analysis gives the magnitude of the error in estimating the value of the results (with a predetermined probability) and provides information about the extent to which the linear regression model explains the observed variance of the dependent variable results (coefficient of determination). If the model is statistically significant, then it is 'useful' for estimating the value of the dependent variable from the value of the predictor (variable) or the predictors.

In the regression model carried out in the form In the regression model carried out in the form:

$$y = 0.014710x1 + 0.3910x1 + 0.00001$$

With the significance level of $p = 7.23E\text{-}13$ and the coefficient $R^2 = 0.9606$, the polynomial dependence of the 2nd degree of biomass yield on the plant density and plant height was proved. Figures 1 and 2 show the partial dependence of the yield on individual dependent variables, which took the form of a parabolic regression equation, 2-degree, on the analyzed features.

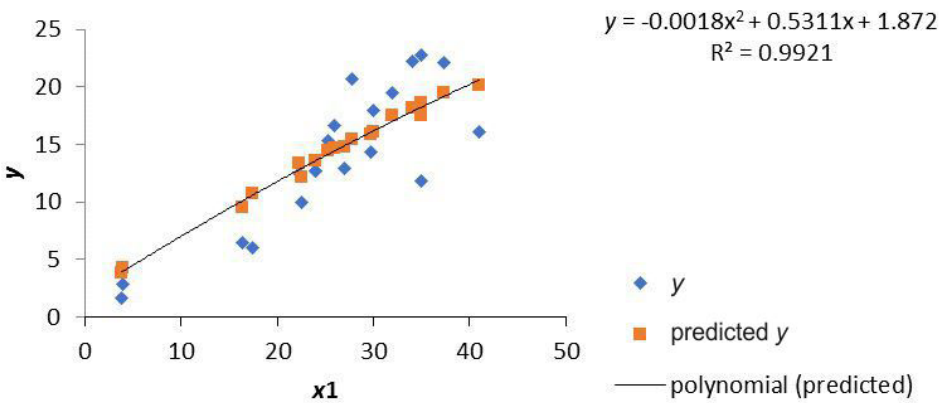

Fig. 1. Values of partial regression of Virginia fanpetals biomass yield from plant density

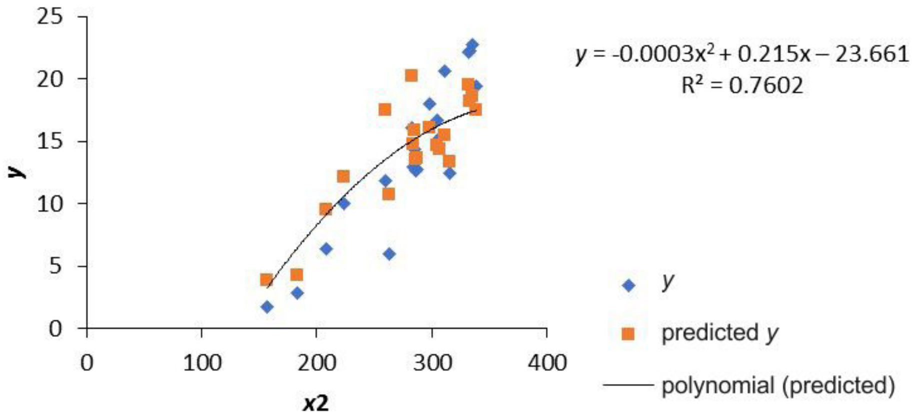

Fig. 2. Values of partial regression of Virginia fanpetals biomass yield from plant height

Energy value of biomass

Using the heat of combustion determined in the Wood Technology Institute in Poznan, it was calculated the energy content of dry biomass yields from each year. Differences in the amount of energy obtained per hectare depended directly on the differences in biomass yields. Cultivating VF from root cuttings resulted in over 55% more energy than cultivating from generative (tab. 10). Every year, the differences in obtained energy yields were significantly higher (>35%) from vegetative propagation, even in 2006, the driest year (tab. 1). Omitting the first year, in the case of vegetative propagation, the smallest amount of energy (180 GJ ha^{-1}) was obtained in the tenth year (2012) due to the destruction of the crops by frost. The highest (more than 400 GJ ha^{-1}) was obtained in the fifth (2007), sixth (2008), and eighth year (2010). With generative propagation, the lowest an accumulated biomass energy yield (108 GJ ha^{-1}) was obtained in the second year (2004) and the highest (302 GJ ha^{-1}) in the fifth (2007).

Table 10. Calorific value (GJ) of biomass yield of Virginia fanpetals per hectare, depending on the propagation method and cultivation year

No	Year of cultivation	Propagation method		Average	D:C (%)
		generative (C)	vegetative (D)		
1	2003	30.39^{a}	50.65^{a}	40.52^{a}	166.7
2	2004	108.35^{b}	259.56^{efh}	183.95^{c}	239.5
3	2005	224.83^{cef}	351.81^{km}	288.32^{e}	156.5
4	2006	213.80^{cd}	290.31^{hi}	251.97^{d}	135.8
5	2007	301.53^{hj}	411.14^{n}	356.33^{h}	136.4
6	2008	233.70^{dfg}	400.65^{n}	317.08^{fg}	171.4
7	2009	229.36^{df}	325.22^{ijk}	277.28^{de}	141.8
8	2010	276.57^{gh}	402.64^{n}	339.51^{gh}	145.6
9	2011	230.08^{df}	373.52^{mn}	301.71^{ef}	162.3
10	2012	116.12^{b}	180.88^{c}	148.50^{b}	155.8
Average		196.47	304.64	250.56	196.5

The same letter index at numbers value mean no significant difference between them by $p = 0.05$

Depending on the propagation method, the energy yield during the ten-year period was between 196 and 305 GJ ha^{-1}. In the current study, the determined heat of combustion was above 18 GJ Mg^{-1} DM, a significantly high value. This allows for the harvest of more than 200 GJ ha^{-1} per annum (tab. 10), even with low biomass yields exceeding 12 Mg ha^{-1}. The content of ingredients that released by the conversion of biomass should be as low as possible.

Results of chemical analysis, documented VF as the high-quality feedstock. The contents of ash were not high (28 g kg^{-1}), with low N (1.9 g kg^{-1}), S (0.52 g kg^{-1}) and particularly low of the harmful Cl (0.23 g kg^{-1}). These advantages have very positive environment's aspects as the quality [DIN EN 14961-2:2011-09] requirements (e.g., burning of pellets). The additional result of experiment is a certainty, that productive lifespan of VF exceeds 10 years.

DISCUSSION

Sida hermaphrodita is a particularly interesting energy plant, especially in the last decade a growing number of researchers, mainly from Poland and Germany, realized the potential of VF as an ecologically valuable raw material for various paths of use and studied it from different research perspectives [Borkowska and Styk 2006, Borkowska and Molas 2012, 2013, Veste et al. 2016, Nahm and Morhart 2018, Molas et al. 2018, Siwek et al. 2019, Stolarski et al. 2019, Cumplido-Marin et al. 2020, Tilvikieneet al. 2020].

The effectiveness of the long-term cultivation of VF decreased significantly in the 10^{th} year of cultivation, which can be explained by unfavourable weather conditions, as well as lower vigour of plants, and their worse overwintering in winter. Such long-term research results are found only sporadically in the literature. Borkowska et al. [2015] in long-term use, the lowest yield of VF biomass was obtained in the second year, and twice as much in the fifth and eighth cultivation years. Banach [2019] explains the fact of reducing the production efficiency of perennial plants by the existence of the FVE gene. It is one of the better described proteins of the autonomic flowering induction pathway and is part of the HDAC complex that recruits histone deacetylase. It has no direct ability to interact with chromatin. It binds to it via the Transposable Element Silencing Via At-Hook (TEK) protein [Jeon and Kim 2011]. Every few months they are presented instead reports on the interaction of FVE with proteins with an increasingly established role. Such proteins include Swinger/Curly Leaf Interactor1 (SCI1) assigned to the family of polycomb proteins involved in the regulation of embryo development and cell cycle control [Kenzior and Folk 2015]. FVE expression is regulated by De-Etiolated1 (DET1) and under short day conditions leads to inhibition of FVE transcription. This results in an increase in trimethylation and a decrease in FLC chromatin methylation. These changes in turn induce an increase in FLC expression which induces a further decrease in FT and SOC1 expression. Demonstrated in addition, the DET1 protein also influences the photoperiodic pathway by inhibiting functional protein (GI) transcription. Reduction in the amount of functional GI protein induces decrease in FT transcriptional activity. DET1 is part of the complex Constitutive Photomorphogenic 10 (COP10) and Damaged DNA Binding Protein 1 (DDB1) inducing ubiquitination of target proteins and lowering their activity. Consequently, the regulator of FVE expression is a repressor of short-day flowering photomorphogenesis and is involved in regulating the circadian cycle. One way of acting may be to simultaneously control these changes by modulating the activity of the autonomic and photoperiod pathways [Kang et al. 2015].

As presented in current paper, the results from the ten-year study VF yielded an average of about six Mg ha^{-1} more biomass using the vegetative propagation method. This indicates that vegetative propagation has positive effects lasting longer than three years of cultivation. The full yield potential of perennial plant species considered efficient sources of bioenergy become apparent in the third to fourth year of cultivation [Christian et al. 2008, Borkowska and Molas 2012, Arundale et al. 2014]. The generative (sowing the seeds) and vegetative (root cuttings) propagation of VF had an impact on biomass yields in the first years of its cultivation. Research by von Gehren and Gansberger [2017] describes possible reasons for the low germination capacity of VF generative and shows a method to increase their quality.

Other long-term studies on bio-energy crop candidates are inconsistent with methods in current research of VF yields. Arundale et al. [2014] found the average yield of *Miscanthus* to be 24 MG ha^{-1} D.M., higher than that observed in vegetatively propagated VF. It is worth noting the reason of the larger *Miscanthus* yield. In this study (*ibidem*), however, *Miscanthus* was reproduced in a greenhouse in 100 mm tops, and plots were irrigated in the first year. Such treatments strengthen plant growth and increase crop yields. In certain circumstances, irrigation is a necessity. Arundale et al. [2014] noticed that breeding *Miscanthus* in large pots and greenhouses did not make economically sense at an industrial scale. Switchgrass yields are similar to generative-propagated VF. It should be noted, however, that even this crop was artificially irrigated in the most difficult year of establishment. Irrigation also increased the yield, or even it is a necessary condition to establish the plantation of Switchgrass (*ibidem*). Due to these conditions, a direct comparison of the yields between VF, *Miscanthus* and Switchgrass is useless. In another long-term study (12 years) reported by Angelini et al. [2009], determined high annual yields of *Miscanthus* (28.7 Mg ha^{-1}) and *Arundo donax* L. (Giant reed) 37.7 Mg ha^{-1} without the use of irrigation [Angelini et al. 2009]. Due to climate differences between eastern Poland and central Italy, and a lack of VF research in warmer European regions, direct comparisons of yields are impossible. In a 14 year study on the yields of *Miscanthus* × *giganteus* cultivated in a similar altitude to our site [Christian et al. 2008] showed an average yield of 12.8 Mg ha^{-1}, a value much lower than the average VF yield (16.8 Mg ha^{-1}). The highest yield of *Miscanthus* was 17.7 Mg ha^{-1}, compared to 22.7 Mg ha^{-1} of VF [Christian et al. 2008]. Research of Arundale et al. [2014] showed that the yield of unfertilized *Miscanthus* and Switchgrass declined with the stand age, after the fifth year of planting. Christian et al. [2008] showed that *Miscanthus* yields declined after the sixth year of planting. In recent study it was not observed a linear decrease in VF yields. The only decreases observed were due to droughts, and the damage caused by climate change. Previous studies on VF have shown winter hardiness (up to minus 35°C) of the species in the Eastern Polish conditions, as a good trait [Borkowska and Styk 2006]. The unexpectedly harsh winter of 2011–2012, however, proved detrimental to the survival of the plants. This led to a 50% reduction in yields compared to the previous year [2011]. Besides the first two and the last year of cultivation (after the destruction of the crops by frost), the energy yields obtained from the use of vegetative material ranged from 290 GJ ha^{-1} to 411 GJ ha^{-1}. Such yields of biomass energy can substitute 11–16 t of medium quality coal. Research by Stolarski et al. [2005] showed that willow harvested each year cumulated 240 GJ ha^{-1} of biomass yield, and generatively propagated VF, 220 GJ [Stolarski et al. 2005]. Angelini et al. [2009] showed results in the range of

470 GJ ha^{-1} and 640 GJ ha^{-1} for *Miscanthus* and *Arundo*, respectively. Collected biomass of VF presented in the current study, characterized by high heat of combustion (18.09 kJ kg^{-1} DM); however, lower within the range of values in other studies, from 18.11 kJ kg^{-1} to 19.22 kJ kg^{-1} DM [Stolarski et al. 2005, Borkowska and Molas 2012, Gubisova et al. 2013].

The issue of generative reproduction of *S. hermaphrodita* discussed in the conducted research is very important both in terms of costs and logistics solutions. Jankowski et al. 2019 stressed the urgent need to investigate seed production technology in order to maximize the energy efficiency of *S. hermaphrodita*. As it results from our own research, the yield obtained from generatively reproduced plantations was significantly lower and with greater variability (V = 43.2%) than the vegetatively reproduced plantation (V = 37.2%).

Planting of VF from root cuttings proved to be an effective method of reproduction under the conditions of the Felin experiment. This is confirmed by the results of Borkowska and Molas [2013] and Šiaudinis et al. [2015], because the germination of seeds of this species turns out to be very low (10%) [Borkowska and Wardzińska 2003]. Gehren and Gansberger [2017] claim that could be significantly increased with proper pre-treatment. Jablonowski et al. [2017] undertook cost estimates for VF root cuttings, which are generally not available as it is not an established energy crop. On the other hand, miscanthus rhizomes are sold for about 0.16 EUR per piece, and it should be noted that the roots of *S. hermaphrodita* and their collection and preparation of seedlings for planting are more complicated, and the demand for them is lower, so he estimated their price at 0.20 EUR per head, which is respectively 2000, 4000 or 8000 EUR per ha, depending on the planting density (1, 2 or 4 plants per 1 m^2). It is therefore possible to roughly estimate the costs of preliminary field preparation and planting VF seedlings of 375, 450 and 600 EUR respectively, as the correct planting density of a perennial energetic species is essential when establishing a plantation. Borkowska and Molas [2012] report the success of establishing such vegetatively propagated plantations and claim that it is possible to obtain over 20 annual harvests. By dividing the high cost of establishing a VF plantation by 20 harvests, the financial burden is reduced. The costs of preparing and running energetic VF Šiaudinis et al. [2015] using vegetative reproduction (including planting and annual harvest) reached 8 630–29 264 MJ/ha, while Jablonowski et al. [2017] calculated 10 200 MJ ha/1 year/1, taking into account only the main cultivation processes, i.e. fertilization and harvest.

Over the ten-year study period, yields in vegetative plots were six Mg ha^{-1} higher than yields in seeded plots. Higher crop yields mean the possibility to be produced more bioproducts like e.g. fibre, crude, chemicals, liquid (cellulosic ethanol) and gaseous fuels, e.g., hydrogen, here calculated by weight in biomass yield of VF according to Wróblewska et al. [2009], and Michalska et al. [2015]. The specific species used for bioenergy purposes influences the obtained biomass yield and its quality. Some of the indicators of quality are heat of combustion, ash content, and the elements polluting the atmosphere [Tvikiene et al. 2010, Franzaring et al. 2014, Jablonowski et al. 2017]. Following obligatory standards for pellets from debarked hardwood e.g. German DIN _standards should be used to evaluate the quality of the biomass from field cultivation. The standards for agricultural biomass, however, have yet to be not widely known. An unfavourable (if compared with wood of most tree species) trait of VF biomass is the ash content, here 28.4 g kg^{-1}, which is higher than in one-year willow shoots (16.3 g kg^{-1})

showed by Stolarski et al. [2005]. Wróblewska et al. [2009] reported a lower ash content of VF 18.0 g kg^{-1}, low N 2.0 g kg^{-1}, Cl (0.23 g kg^{-1}), and S (0.45 g kg^{-1}); elements contributing to acid rain.

VF mainly is cultivated in Poland, however, in many other countries, progress has been made by research VF, and very interesting results are reported [Franzaring et al. 2014]. Cultivation of VF and *Miscanthus* produces greater annual yields of dry biomass than the yearly, and long-term growth of forest wood [Borkowska et al. 2009, Borkowska and Molas 2012, 2013]. Many studies confirmed the excellent ecological traits and chemical characteristics of VF biomass and high yields, which may lead to admit VF as one of the most important biomass species for bioenergy [Šiaudinis et al. 2012, 2017, Kurucz et al. 2014, Jablonowski et al. 2017, Molas et al. 2018] and eco-friendly feedstock [Emmerling 2014].

Perennial crops grown for bioenergy purposes should provide high and stable yields, continue supply, simultaneously with steady quality required by the biorefineries. High biomass yield is still one of the main criteria in selecting the type of crop species used for renewable-energy purposes. Only few studies have shown long-term of second-generation energy-crop yields, and there are almost none on VF. Current study is the first ever on the long-term (10 years) VF yields.

Our research shows that the most sustainable option, compared to annual crops, for renewable energy is perennial energy crops, including *Sida hermaphrodita* Rusby, also known as Virginia mallow, mallow or Virginia fanpetals [Cumplido-Marin et al. 2020], which can meet all three criteria related to the use of energy crops. These are: (1) this species does not require annual planting and can be harvested successively for many years, as our many years of research have proved, (2) it can be harvested using existing agricultural equipment, (3) it can increase biodiversity at the field and farm level and prevents soil erosion.

CONCLUSIONS

1. Higher yields of Virginia fanpetals biomass ensured the use of vegetative propagation compared to the generative method of cultivation.

2. The research confirmed that the yield of VF biomass remains high for at least 10 years and does not decrease with the age of the plants.

3. VF biomass contains small amounts of nitrogen, sulfur, chlorine and ash.

4. The biomass yields of VF do not decrease with the age of the plantation. This species can be cultivated successfully for at least 10 years on one sit

5. The polynomial dependence of the 2^{nd} degree of *S. hermaphrodita* biomass yield on the plant density and plant height was proved. This will make it possible to predict yield based on these characteristics. The coefficients of variation indicate that more stable yield characteristics as well as the number of shoots and their height were obtained by vegetative propagation of *S. hermaphrodita* plants. Plant height turned out to be the most stable traits.

6. The energy value of dry biomass yield per hectare depended on the differences in biomass yields. Growing VF from root cuttings gave over 55% more energy than growing this species in a generative way, from seed.

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Streszczenie: Źródła biomasy trzeciej generacji są konieczne do optymalizacji zrównoważonego rozwoju upraw w szerokiej gamie jakości gleb i warunków klimatycznych. Gatunkiem, na który ostatnio zwrócono uwagę w Europie, jest ślazowiec pensylwański (*Sida hermaphrodita* L. Rusby). Wieloletni (2003–2012) eksperyment polowy został założony w Gospodarstwie Doświadczalnym UP w Felinie, w układzie bloków losowanych w 4 powtórzeniach. Celem doświadczenia było zbadanie wpływu rodzaju materiału rozmnożeniowego na wydajność i plon biomasy i wydajność energetyczną ślazowca. Hipoteza badawcza zakładała uzyskanie wyższych plonów z sadzonek korzeniowych niż z siewu nasion, także w dłuższym okresie użytkowania. Średnio w pierwszych 10 latach użytkowania plantacji (2003–2012) uzyskano istotnie wyższe plony z rozmnożeń wegetatywnych (16,8 Mg h^{-1}) niż z generatywnych (10,9 Mg h^{-1}). Wydajność energii brutto średnio z hektara wynosiła 304 GJ z rozmnożeń wegetatywnych, zaś 196 GJ z siewu nasion. Obliczone ciepło spalania wyniosło 18,1 GJ, zawartość popiołu 28 g kg^{-1}, azotu, siarki i chloru natomiast odpowiednio: 1,9 g, 0,52 g i 0,23 g kg^{-1}, niezależnie od sposobu rozmnażania.

Słowa kluczowe: metoda rozmnażania, ślazowiec pensylwański, *Sida hermaphrodita*, biopaliwa