

## IMPACT OF DIFFERENT FERTILIZATION REGIMES ON THE BIOMASS PRODUCTION OF PERENNIAL GRASS *MISCANTHUS* × *GIGANTEUS* IN SLOVAKIA

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**Abstract.** *Miscanthus* energy grass, being one of the renewable energy sources, has suitable soil-climatic conditions for growth in the regions of Slovakia. The lifespan of a *Miscanthus* plantation is 15-20 years. In the specific conditions of southwestern Slovakia, the plantation productivity was studied under the application of NPK fertilizer after one third of the plantation's lifespan. The measurement interval was 14 days throughout the growing period of the monitored years (2016-2018). Morphometric and selected ecophysiological and production parameters were analysed under three fertilization variants (C – control, V1 – variant with the application of 50 g NPK per individual and V2 – variant with the application of 100 g NPK per individual). Variant V2 had the highest values of the studied parameters in all monitored years and out of all fertilization variants. The statistical significance of differences in growth indicators between the years of cultivation and total biomass production between variants and years was confirmed. Dynamics of stomatal density per mm<sup>2</sup> were determined by microrelief method in three randomly selected clumps of *Miscanthus* × *giganteus* in control and in each variant of fertilization in 2016. There are significant differences in the density stomatal density and width between the control and variants of fertilization in 2016.

**Keywords:** NPK fertilizer, energy grass, growth parameters, production, stomata

### Introduction

The use of biomass as a renewable energy source is rapidly increasing worldwide. Further increases in the use of these resources can be achieved through technological development, long-term planning, implementation of integration strategies and appropriate investment. Biomass as an energy source is an easily accessible that is both technologically and ecologically acceptable. The focus on biofuels is increasing due to the reduction of dependence on fossil fuels and the stimulation of rural development and agriculture.

*Miscanthus* × *giganteus* is a perennial, warm-season Asian grass with the C<sub>4</sub> photosynthetic pathway. In the search for ideal bioenergy crops following the oil crisis of the 1970s, evaluations to determine the biomass yield potential of giant miscanthus began across Europe. *Miscanthus* × *giganteus* grows to a height of 4 m, produces shoots with a length of up to 4 m and a diameter of approx. 10 mm, and can be grown in one location for up to 15–20 years (Dubis et al., 2017).

*Miscanthus* plantations have several environmental benefits compared to annual crops. These plantations can use water and nutrients more efficiently, they are resistant to pests and diseases and do not require annual soil cultivation (Cadoux et al., 2012). *Miscanthus* × *giganteus* grows very rapidly and produces a high yield of aboveground biomass at relatively low input costs (Clifton-Brown et al., 2015; Roncucci et al., 2015).

The cultivation and high yield of *Miscanthus* × *giganteus* are characterized in various climatic and environmental conditions. Higher productivity has also been identified on less productive and marginal soils (Stavridou et al., 2017). *Miscanthus* × *giganteus* has low soil fertility requirements, tolerates cultivation on light soils and has no high nitrogen requirements (Kołodziej et al., 2016).

Several authors have observed the impact of *Miscanthus* plantation fertilization on its production (Maughan et al., 2012; Dierking et al., 2016). Larsen et al. (2014) reported that fertilization of the *Miscanthus* × *giganteus* did not increase the yield. Other authors suggest that yields can be increased by applying annual fertilizers to older stands (Arundale et al., 2014).

To predict the production potential of energy plant species in a particular region, it is necessary to identify the basic morphometric and ecophysiological parameters and their changes due to environmental factors. The shape of organs, especially leaves, often shows the ability to adapt to environmental conditions. These include irradiation density, drought, and extreme temperatures, change anatomy and leaf morphology, skin thickness, stomata density, size, and degree of stomata openness. The stomata density is a relatively plastic feature that is potentially adaptable to environmental changes (Killi et al., 2018; Mwendia et al., 2019).

This article presents the results of the influence of various fertilization variants applied after the sixth growing period of the stand on the morphometric, selected ecophysiological and production parameters of the *Miscanthus* × *giganteus* plantation in the conditions of southwestern Slovakia.

## Materials and Methods

### *Plant materials*

Perennial grass *Miscanthus* × *giganteus* is a vital triploid hybrid (57 chromosomes) often used in the field trials. The planting material consisted of rhizomes from Hannes Stelzhammer Austria. The fresh weight of the rhizomes was different (on average 2.61 g), the length of the rhizomes was on average 67.5 mm.

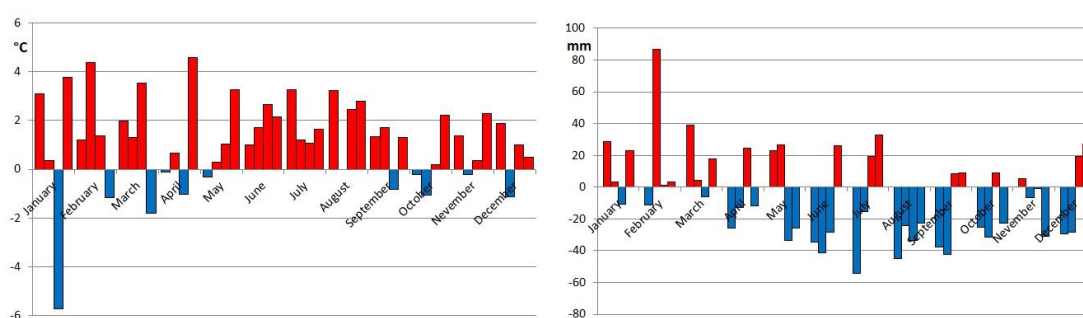
### *Model locality of the research area*

The model area of experimental research of growing fast-growing plants on agricultural soils is located in Slovakia in Nitra region in the cadastral area of Koliňany (48°21'21" N 18°12'25" E). The area has an altitude of 80 m above sea level. The soil is moderate (loam), pH values range from 7.18 to 7.35, humus content is 1.16 to 2.50% (Demo et al., 2013). In terms of exposure, it is a plane without manifestation of areal erosion. According to climatic conditions the research area belongs to the warm, very dry, lowland climate region. The basic climatic parameters (*Table 1*) were evaluated based on the measurements of the Meteorological station located near the research area. The values were compared with the long-term average values of the 50-year period of 1951-2000 in the Nitra region, according to Slovak Hydrometeorological Institute (*Fig. 1*). The average annual air temperature for the period 2015 to 2018 was 10.99°C (the deviation from the long-term average was higher by 1.13°C) and the average annual precipitation was 483.67 mm (the deviation from the long-term annual total was lower by 63.93 mm). The average length of the main growing period during the monitored years in the research area is 186 days.

The research plot was established in 2010. The plant material was planted in a 1 m × 1 m planting distance on an area of 100 m<sup>2</sup>. The optimum *Miscanthus* stand density is 10,000 plants per hectare, depending on the abiotic conditions of the habitat – temperature and water. In the research area, *Miscanthus* × *giganteus* rhizomes were planted in wet soil at the beginning of the growing period at a depth of up to 10 cm.

**Table 1.** Average monthly precipitation and course of average monthly temperatures in 2015-2018 in the research area in Koliňany, southwestern Slovakia

4 year average / month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
mm	40.20	50.10	45.34	35.20	53.50	46.65	54.75	22.90	27.48	23.35	43.95	40.25
°C	-1.02	1.95	6.05	11.42	16.27	20.17	21.77	21.81	16.39	10.48	5.55	1.05



**Figure 1.** Average monthly deviations of temperature and average monthly deviations of total precipitation in 2015-2018 from the long-term average in research area in Koliňany

### Application of different fertilization regimes

The mineral granular fertilizer N P K (15-10-10), type designation B.1, was applied before to the beginning of the growing period (beginning of April in 2016 – after the sixth growing year since the plantation was established). The fertilizer contained 15% of total nitrogen [N], 10% of total phosphorus [P<sub>2</sub>O<sub>5</sub>] and 10% of water-soluble potassium [K<sub>2</sub>O]. The fertilizer was applied at the centre of the tillering circle of each individual before the growth of new shoots (Fig. 2).

In the V1 variant, 50 g of fertilizer per individual (500 kg per hectare of which 75 kg of nitrogen, 50 kg of phosphorus and 50 kg of potassium per ha) was applied. In the V2 variant, 100 g per individual (1000 kg per hectare of which 150 kg of nitrogen, 100 kg of phosphorus and 100 kg of potassium per ha) was applied and variant C (control) remained without fertilization. A schematic representation of the research area with variants of fertilization is shown in Fig. 3.

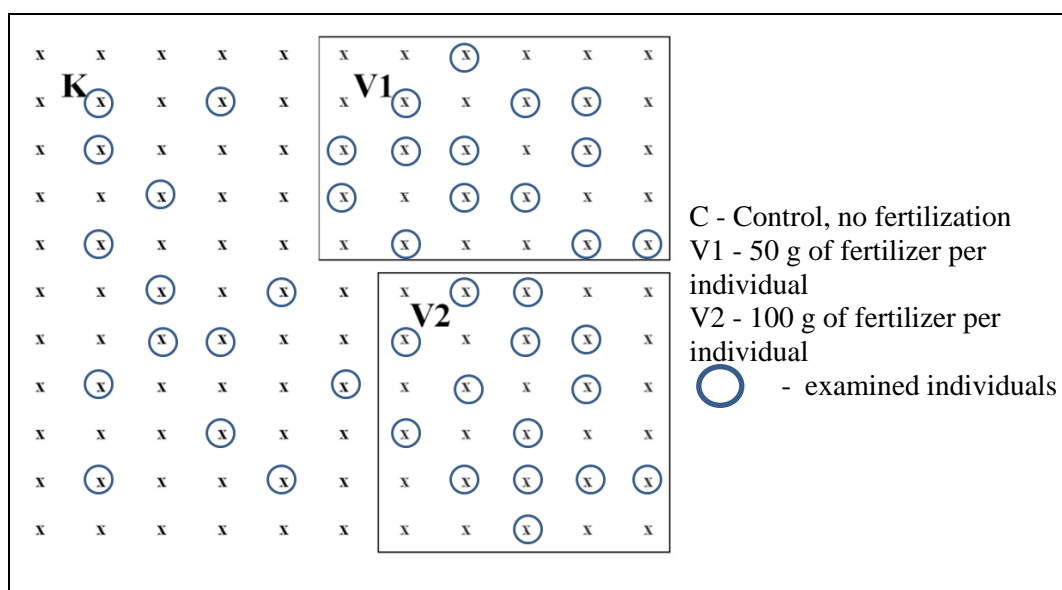
### Monitored the growth parameters and production yield

Basic morphometric characteristics of *Miscanthus* × *giganteus* were studied for all three variants. The interval of measurement of morphometric parameters was every 14 days during the whole growing period of the monitored years (2016–2018) at 14 selected individuals. The height of the individuals was determined as the distance from the soil surface to the top of the longest shoot. The number of shoots on individual plants

was determined by counting the shoots in the clump. The shoot thickness was measured on the selected shoot of the studied individuals. The number of leaves on the shoot and the number of leaves in the clump were monitored. Accordingly, the senescence of the leaves on the shoot was determined. In the case of energy plants, a destructive determination of the biomass weight is carried out outside the growing period. After cutting, the total weight of the selected individuals (seven clumps from each variant) was determined. The biomass sample was oven-dried at 105°C and the dry weight was determined. The moisture content of *Miscanthus x giganteus* ranges from 19 to 22%. The total weight of the above-ground biomass was calculated (t/ha).



**Figure 2.** The tillering circle of *Miscanthus x giganteus* before the application of the fertilizer in the beginning of the seventh growing period



**Figure 3.** The schema of application of different fertilization regimes in the research area of *Miscanthus x giganteus*

The stomatal density per mm<sup>2</sup> in the leaves was determined non-destructively in three selected clumps in each fertilization variant in 2016. The indicated number of the stomata refers to one treatment. The analysis was performed on the designated stem on the senescence leaf located in the middle of the stand (approximately fifth leaf from the

bottom) exposed to western direction by a micro-relief method. Samples were collected in the middle part of the leaf outside the main vein on the adaxial (top) and abaxial (bottom) skin surface in August 2016. The evaluation of preparations was carried out by optical microscope Axiostar plus, Carl Zeiss lens, CP-Achromat 40 × / 0.65, 10 × eyepiece PI / 18, Canon Utilities software Zoom Browser EX 4.6 and hardware Acer Travel Mate 4600, Canon Power Shot A 95. In total, 144 microscopic view-fields were considered in the counting. Stomata length and width were measured on 162 randomly selected stomata.

Statistical analysis of morphometric and production data was performed using STATISTICA 12 by StatSoft. Inc. ANOVA analysis was used to analyse the statistical significance of morphometric and production indicators between years and variants. The result is statistically significant if  $p < 0.05$  or statistically highly significant if  $p < 0.001$ . Statistical significance of stomatal differences was evaluated by LSD-test.

## Results and Discussion

The basic prerequisite for the efficiency of growing plants for energy purposes is the ability of the species to adapt to the region's climate. Without fertilization, *Miscanthus x giganteus* has produced an economically efficient above-ground biomass every year since planting in 2010 (Kotrla et al., 2017; Kotrla and Prčík, 2018). In 2016, fertilization was applied to identify the need for fertilization in relation to the growth and production of biomass of the energy grass.

### *Morphometric analysis of Miscanthus x giganteus*

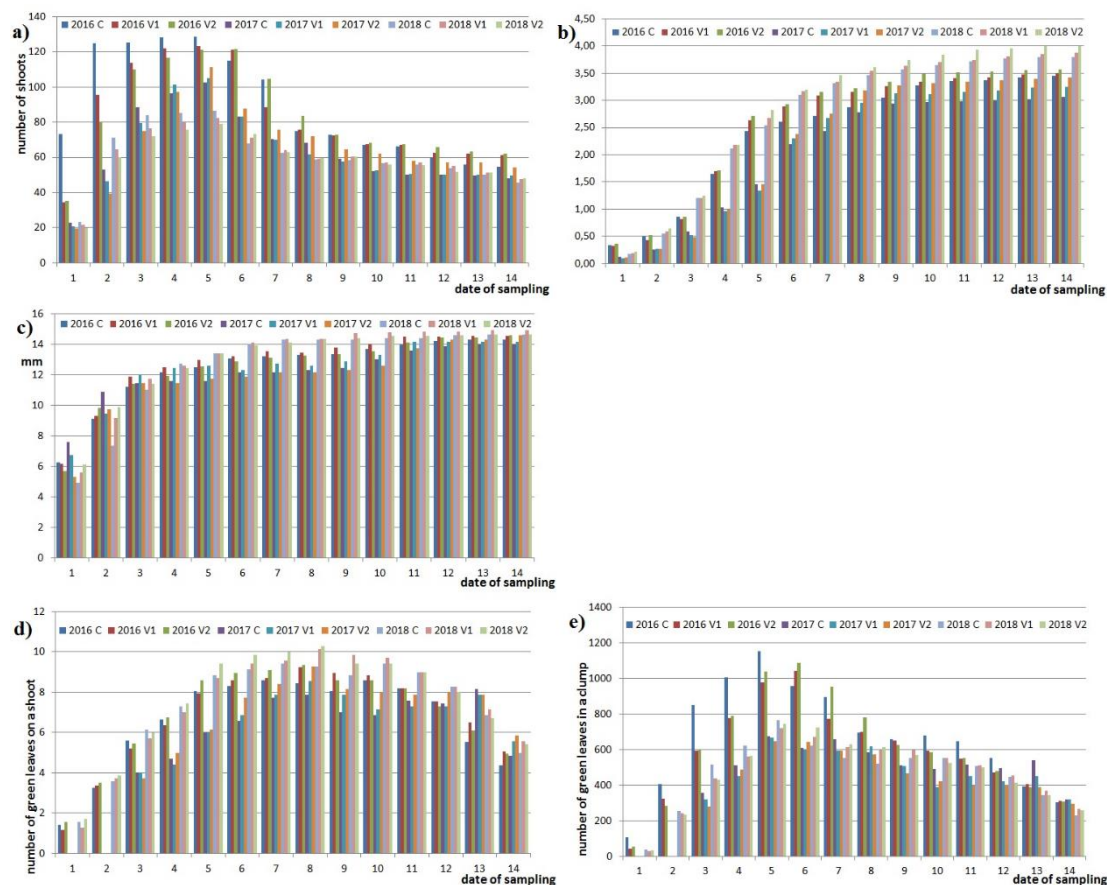
Production of usable biomass for energy use is realized through the formation of above-ground organs. The growth of above-ground organs is limited by the length of the growing period. In 2016 and 2017, the growing period was 176 days and in 2018 it was 219 days. In 2018, average daily temperatures above 10°C began at the beginning of April and ended at the beginning of November compared to previous years.

*Fig. 4* represents the course of growth of selected parameters between 2016 and 2018 in the different fertilization variants. The V2 variant had the highest values of the growth parameters in all studied years and fertilization variants. The comparison of the monitored growth parameter values at the end of the growing period 2016 to 2018 is shown in *Table 2*.

The number of shoots in the clump increases at the beginning of the growing season culminates in the second half of June and subsequently decreases (*Fig. 4a*). The shoots continue with intense growth and higher production of green leaves in June, resulting in the drying of shoots in the middle of the clump. The reason for reducing the number of shoots is the competitive struggle of shoots for space, especially light.

In each year, V2 variant has reached the highest height of the clump. When comparing the variants, V1 variant was on average lower by 3.5% and the C variant was on average lower by 6.5% than V2 variant at the end of the growing period. Based on the analysis of the shoot thickness, it can be stated that the shoots in the V1 and C variants were on average thinner by 1.8% and 2.7%, respectively compared to the variant V2. The number of leaves is closely related to the height of the shoots. The dynamics of the number of green leaves on the shoots is shown in *Fig. 4d and e*. Senescence of the leaves (yellowing and drying) begins in the second half of June and continues until the end of the growing period. The ratio of green and dry leaves on the shoot intensely changes in early October

in favour of dry leaves. This trend continues until the end of the growing period when the photosynthetic activity of the leaves ends. The average number of leaves in the variant V2 was 3.3% higher than in the variant V1 and 15.2% higher compared to the variant C. Dražić et al. (2017) examined the impact of fertilization on *Miscanthus* stand in Serbia. They confirmed the increase in morphometric characteristics of fertilized variants. Pogrzeba et al. (2017) also monitored plant growth parameters for fertilized and non-fertilized varieties in Poland. They found no significant differences between these variants.



**Figure 4.** Morphometric analysis of *Miscanthus* × *giganteus* stand in monitored years and fertilization variants [a) number of shoots in a clump, b) height of shoots in a clump, c) shoot diameter, d) number of green leaves on a shoot, e) number of green leaves in a clump]

The analysis confirmed the statistical significance of the growth parameters between the variants and years. The statistical significance of the growth rate values over the growing period is statistically highly significant only between the monitored years. The reason is the different climatic conditions during the growing period of these years. In each year, the values of the growth parameter between variants C, V1 and V2 are statistically non-significant (Table 3).

### **Production potential of *Miscanthus* × *giganteus***

Annual production of the shoots of *Miscanthus* × *giganteus* represents an amount of biomass usable as an alternative energy source. Fig. 5 shows the variability of the biomass

samples collected at the end of February in the following year. Differences in the above-ground biomass weight between years and variants are statistically highly significant (Fig. 6). The statistical significance between years ( $p = 0.00029$ ) and also between variants ( $p = 0.00019$ ) was confirmed at the significance level of 0.001.

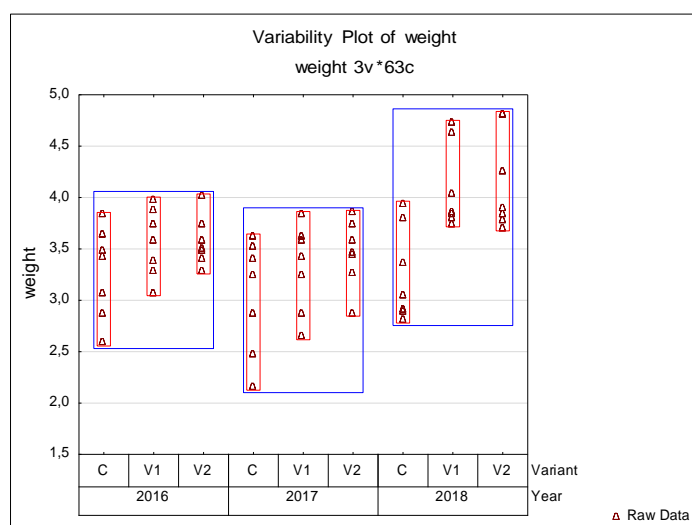
**Table 2.** Comparison of growth parameter values at the end of the growing period 2016 to 2018 in different fertilization variants

Year	2016			2017			2018		
Variant / growth parameter	C	V1	V2	C	V1	V2	C	V1	V2
number of shoots in a clump	54.64	61.29	62.29	48.05	49.71	54.29	45.71	47.57	48.14
height of shoots [m]	3.44	3.50	3.57	3.06	3.25	3.42	3.80	3.87	4.00
shoot diameter in a clump [mm]	14.31	14.54	14.60	14.00	14.14	14.57	14.62	14.63	14.93
number of green leaves on a shoot	4.37	4.94	5.07	4.86	5.57	5.86	5.00	5.43	5.57
number of green leaves in a clump	238.67	302.75	315.79	233.39	276.98	317.96	228.57	258.24	268.22

**Table 3.** Statistical significance of selected growth and production parameters of *Miscanthus x giganteus* organs in the studied years and fertilization variants

Parameter	Years		Variants	
	p-value	S	p-value	S
number of shoots	0.00001	***	0.85937	n
height of shoots	0.04148	*	0.79439	n
shoot diameter	0.03071	*	0.09866	n
number of green leaves on a shoot	0.01412	*	0.80012	n
number of green leaves in a clump	0.00032	***	0.73284	n
weight of an individual	0.00029	***	0.00019	***

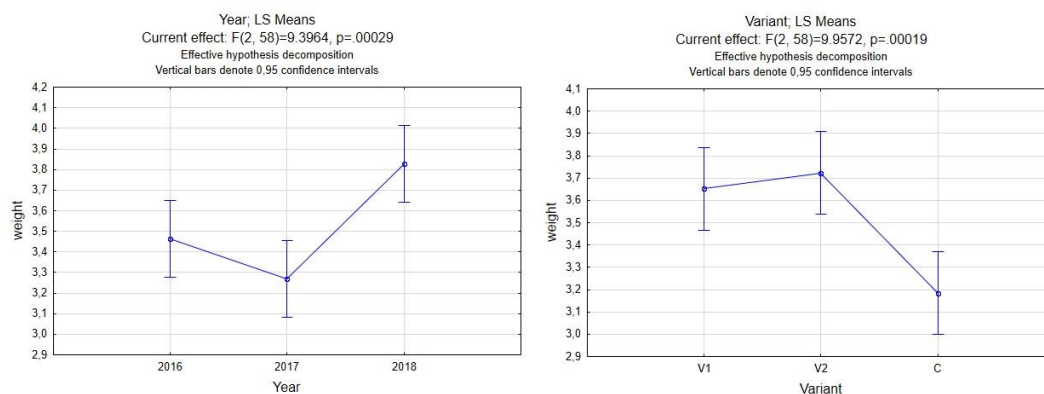
S - Level of significance is defined as: n: non-significant impact, \*: significant impact in  $P \leq 0.05$ , \*\*:  $P \leq 0.01$  and \*\*\*:  $P \leq 0.001$



**Figure 5.** Variability of biomass samples of the above-ground organs of *Miscanthus x giganteus* in the studied years and variants of fertilization



Biomass production increased every year in the research area. Since 2015, slight fluctuations in total biomass production have been monitored (Table 4). In 2015, the summer was characterized by low precipitation compared to the long-term average. After the application of fertilizer at the beginning of the 2016 growing period, an increase in biomass was observed in fertilized variants compared to the control variant. The variant without fertilization also produced 8.5% more biomass than in the previous year (2015). The difference between the yield in 2015 and biomass yield without fertilization in 2016 is statistically significant ( $p = 0.01560$ ). When comparing the produced biomass between the non-fertilized and fertilized variants, it is possible to state the average increase in fertilized variants by 7.6% in 2016, 10.1% in 2017 and 13.5% in 2018.



**Figure 6.** Statistical analysis of the above-ground biomass weights in the studied years and fertilization variants

**Table 4.** The production of biomass of *Miscanthus × giganteus* in the fertilization variants since 2016 and compared with 2015 in the research plot in Koliňany

Year /Variant	2015	2016	2017	2018
Variant C (without fertilization)	30.1	32.69	30.39	32.97
Variant V1		35.03	33.13	37.17
Variant V2		35.74	34.51	39.1

Lower levels of the biomass produced in 2017 are the result of the climatic situation, especially low temperatures at the beginning of the growing period and spring frosts in late April and early May. The night frost, as a negative phenomenon in 2017, was recorded in the first decade of May (the minimum temperature of  $-0.196^{\circ}\text{C}$  was on the 10th of May). These spring frosts negatively affected the growth of the shoots in 2017.

Iqbal et al. (2015) analyzed the stands of energy grasses in a period of ten years. They confirmed that the application of fertilizers did not significantly increase the yield of *Miscanthus*. On the contrary, Dubis et al. (2017) monitored the effect of different fertilization methods on *Miscanthus* productivity. The application of NPK fertilizer increased the biomass yield by 7.7% compared to the non-fertilized variant. Arundale et al. (2014) applied fertilization to older energy grass stand in Illinois, USA. They confirmed an increase in the yield of the fertilized variant compared to the non-fertilized variant by 25%. In the Midwest of the USA, Lee et al. (2017) confirmed a statistically highly significant increase in *Miscanthus* yield compared to the non-fertilized variant, but they did not confirm the differences in the total production between higher and lower

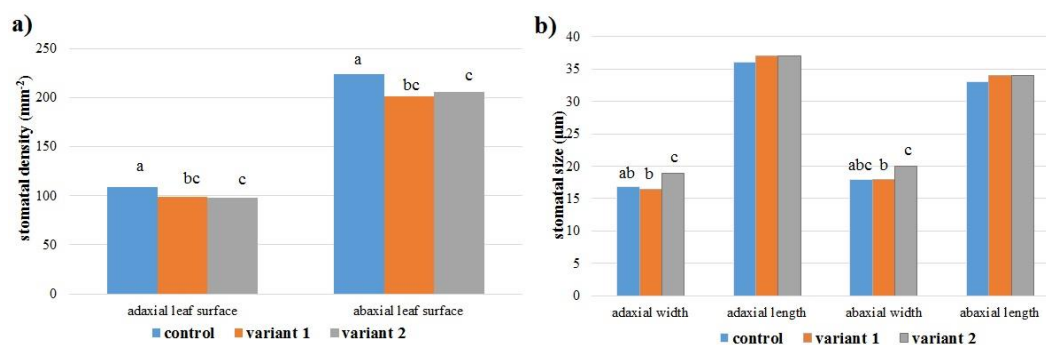


fertilizer doses. Several authors have done fertilization experiments in the first years after planting. However, in the conditions of southwestern Slovakia, it was not necessary to apply fertilizer in the first years, as the energy grass had been reaching an economically profitable yield. The *Miscanthus* stand had shown the culmination of the yield after the sixth year since the establishment, so the fertilization was applied at the beginning of the seventh growing period (which is about a third of the plant's lifespan). Maksimović et al. (2018) observed the effect of fertilization in the first years of the establishment of the *Miscanthus* plantation in Serbia, but they did not find significant differences in biomass production. They confirmed that the profitability of the plantation is affected also by climatic conditions in individual years.

### *Analysis of the stomata of Miscanthus × giganteus leaves*

Photosynthetic performance of *Miscanthus* leaves and their production ability is also influenced by the anatomical structure of assimilation organs. The density and the size of stomata are critical. The stomata play an irreplaceable role in venting the gases between the plant and the atmosphere and they are the place of regulated water discharge in the transpiration process. Their development and movements are indicated by environmental factors.

The number of stomata in leaves was statistically highly significantly affected by adaxial and abaxial leaf surface of the epidermis of *Miscanthus × giganteus* (LSD<sub>0,05</sub> test ± 12.87). The ratio of stomata was 32:68 on the adaxial and abaxial leaf surface, respectively. The number of stomata per mm<sup>2</sup> of area varied from 97.8 ± 15.0 to 223.6 ± 28.2. Between control and variants of fertilization are significant differences in the density of stomata (LSD<sub>0,05</sub> test ± 7.19 on the adaxial skin, 11.24 on the abaxial skin) (Fig. 7a). On the contrary, Weng and Hsu (2001) found that the N treatment could increase the stomatal frequency on the adaxial surface in most tested *Miscanthus* clones in Taiwan, however, no significant effect was observed on that of the abaxial surface.



**Figure 7.** a) The number of stomata per mm<sup>2</sup> of the adaxial and abaxial surface depending on the variants of fertilization. b) Statistical evaluation of significant differences in the size of stomata (µm) depending on fertilization. Values with different letters (a, b, c) in columns indicate statistically significant difference according to LSD test (P < 0.05)

The stomatal length ranged from 33.5 ± 2.1 µm to 37.4 ± 4.0 µm, and stomatal width from 16.6 ± 1.8 µm to 19.8 ± 2.6 µm (Fig. 7b). Rayburn et al. (2009) also compared the size of stomata of two *Miscanthus* genotypes in the USA and confirmed genotypic conditionality of the monitored indicator expressed by the average length of stomata of

*Miscanthus × giganteus* 24.4 µm. Moon et al. (2018) recorded the average length of stomata of *Miscanthus × giganteus* ranging from 21 to 28.5 µm. In our observations, the average length of stomata of *Miscanthus × giganteus* was higher 35.6 µm.

Differences in stomatal width of leaves between control and variants of fertilization on the adaxial surface and between variants V of fertilization on the abaxial surface and V2 were highly significant (LSD 0,05 test ± 1.07 on the adaxial skin, 1.49 on the abaxial skin), while no significant differences were observed in stomatal lengths (Fig. 7b).

## Conclusion

The plantation of *Miscanthus × giganteus* grown in soil-climatic conditions in southwestern Slovakia produced an effective yield (more than 30 t/ha) of the above-ground biomass every year. At the beginning of the seventh growing period, NPK mineral granular fertilizer was applied in order to determine the effect of fertilization on crop yield after the first third of the plantation lifespan. The *Miscanthus* stand was divided into three variants, two with different fertilizer doses and one without fertilization. In the growing periods of 2016-2018, morphometric, selected ecophysiological and production parameters were measured. The morphometric analysis confirmed higher growth performance of the fertilized variants. The statistical analysis of the growth parameters is highly statistically significant only between the monitored years. In each year, the values of the growth parameters between the variants C, V1 and V2 were statistically non-significant. Differences between variants and years were statistically highly significant in total biomass production. Statistical significance between the years ( $p = 0.00029$ ) and also between the variants ( $p = 0.00019$ ) in the biomass production was confirmed at the significance level of 0.001.

As a factor affecting the production ability of the *Miscanthus* plantation, the density and size stomata of the *Miscanthus* leaves were analysed. Significant differences in the density of stomata (LSD 0,05 test ± 7.19, 11.24) and stomatal width (LSD 0,05 test ± 1.07, 1.49) were found between the control and variants of fertilization.

*Miscanthus* cultivated in southwestern Slovakia produced economically efficient yield despite fertilization. The difference in fertilized and non-fertilized variations ranges from 7.6 to 13.5%. The positive effect of fertilization on above-ground biomass production has been demonstrated. A possible negative phenomenon for the *Miscanthus* plantations production is the climatic conditions in each year of cultivation. However, fertilization at certain stages of the energy grass lifespan can maintain plantation production performance even in the later years of cultivation. The aim of further studies will be to find out the period of culmination and decrease in biomass production of *Miscanthus × giganteus* in the conditions of southwestern Slovakia and subsequently to apply another dose of fertilization.

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