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## Adaptive potential and productivity of two *Salix viminalis* L. clonal varieties grown in North Central Bulgaria

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### Abstract

*Salix viminalis* L. is a broadly cultivated willow species that has been intensively used for breeding purposes, particularly in selection of clones and hybrids for biomass production. The present study aimed to explore and compare growth and adaptability of two basket willow (*Salix viminalis* L.) clonal varieties - *rubra* and *purpurea* - at the specific edaphic and climate conditions and cultivation practice presented in an experimental plantation in North Central Bulgaria. The influence of spacing, genotype, root age and rotation on their productivity were also investigated and allometric relationships for non-destructive estimation of woody plant biomass were suggested.

The trial plantation was established in the spring of 2018 as 4 sectors of a Nelder wheel plot with 15 nearly-square spacings, ranging from 0.25 to 9.80 m<sup>2</sup> (40000–1020 plants per hectare). The two varieties were arranged in 4 four-spoke sectors (2 sectors per clone). Data collection took place from 2018 to 2020 and comprised 1- and 2-year rotations.

The study showed that *Salix viminalis* var. *rubra* was more susceptible to desiccation-causing adverse climatic changes, poorly adapted to the specific conditions of the study site and gradually died out during the second and the third year. *Salix viminalis* var. *purpurea* exhibited steady survival rate (97 - 77%) and annual biomass productivity varying from 3 to 17 Mg/ha/year across the densities, sectors and years. During the first year of growth *purpurea* variety had a significantly higher plant weight and sprout number than *rubra* variety. Growing space affected positively the number of shoots and tree dendromass, and sprouting was reinforced by coppicing that doubled the shoot number during the subsequent rotation. This was reflected in the development of the stump-level allometric models that were differentiated according to rotation. The stump-level allometric model of higher predictive power was based on the main shoot length and shoot number. An exponential relationship to the breast-height shoot diameter was also suggested for biomass estimation of each sprout separately.

**Key words:** basket willow, spacing, rotation, allometry, site conditions

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## Introduction

Poplars and willows, the members of the *Salicaceae* family, have been used and cultivated by people for many centuries. It has been suggested that willow baskets and containers were probably among the first articles manufactured by humans (Isebrands & Richardson, 2014). These species not only provide wood, fibre, fuelwood and other forest products, but also benefit the society in the rehabilitation of degraded land, restoration of forest landscapes and mitigation of climate change (Isebrands & Richardson, 2014).

The genus *Salix* comprises 330–500 species, appearing in several life forms: upright trees, shrubs, prostrate plants or groundcovers, with the majority of taxa occurring as shrubs (Dickmann & Kuzovkina, 2014). Willows have shown to possess numerous advantages for cultivation such as fast growth, remarkable capacity of vegetative regeneration, very high sprouting capacity, high productivity, short rotation cycle, broad genetic base, low economic investments after establishment (Hernea et al., 2015). The species of willow (*Salix* spp.) are known to exhibit significant genotypic variability in regard to growth and adaptability traits, such as survival (Kopp et al., 2001), number of shoots, shoot length and diameter (Tsanov, 1974a; Labrecque & Teodorescu, 2005; Demo et al., 2013), pest resistance (Labrecque & Teodorescu, 2005; Demo et al., 2013; Rogers et al., 2019), aboveground biomass production (Tsanov, 1986; Bergante & Facciotto, 2011; Sevel et al., 2012; Miller, 2018), root-shoot ratio (Rogers et al., 2019), tree allometry (Heinsoo et al., 2002), elemental composition of bark and wood (Fabio & Smart, 2020), response to weed competition (Johnson et al., 2018), duration of growth period (Tsanov, 1974b). Breeding and improvement programs for species of genus *Salix* have been developed by research organizations in many countries across Europe such as, UK, Sweden, Russia, Italy, Poland, with priority selection objectives being biomass production, stem form, rod quality for wicker use, tannin content and quality, ornamental value, resistance to frost, leaf rust and insect pests (Stanton et al., 2014). In Bulgaria, extensive studies have been dedicated to species of genus *Salix* by Tsanov (1965a, b; 1974a, b; 1984, 1986) who investigated growth, productivity, phenology and adaptability of various willow species, varieties and their hybrids and the influence of plantation density and site conditions on their cultivation. He reported about the establishment of the first *Salix* spp. nursery stock in Vardim nursery near Danube, North-Eastern Bulgaria, in 1969 (Tsanov, 1984). This nursery stock has been periodically rejuvenated by replanting of the best locally adapted varieties that nowadays compose the Vardim willow collection. Dimitrova (2019) investigated growth and biomass production of *Salix*

*viminalis* var. *rubra*, one of the clonal varieties cultivated there.

Breeding of willows for energy purposes started after 1970-s and the species most actively used for selection were *Salix viminalis* L., *Salix dasyclados* Wimmer, and *Salix schwerini* E. Wolf (Rodzkin et al., 2015). The common osier or basket willow *S. viminalis* L. is a species with very wide natural distribution throughout Eurasia, occurring from sea level to 600 m in the Urals, 900 m in the Carpathians, and even to 1800 m in the Altai, that has been introduced and cultivated also in North America, South America (Brazil and Chile), Pakistan, India and Australasia (Richardson et al., 2014). *S. viminalis* clones have been used as parental genotypes or progenitors of commercial willow varieties such as *Inger*, *Jorr*, *Olof*, *Sven*, *Tordis*, *Tora* etc. bred for use in short-rotation crops by the UK/Swedish European Willow Breeding Partnership and the Swedish company Svalöf-Weibull AB (Caslin et al., 2012). The present study aimed to explore and compare growth and adaptability of two *S. viminalis* clonal varieties from Vardim clonal collection as affected by the specific soil and climate conditions and cultivation practice presented in an experimental plantation in North Central Bulgaria, to study the influence of spacing, genotype, root age and rotation on their productivity and to suggest allometric relationships for non-destructive estimation of woody plant biomass.

## Materials and methods

### Experimental plantation and data collection

The study was carried out in an experimental willow plantation (43°37'36.0"N, 24°41'41.7"E) established in the spring of 2018 in Nikopol Forestry Estate nursery in Gulyantsi, North Central Bulgaria. The nursery is situated at 29 m a. s. l. in a flat area in the valley of river Vit. The climate is temperate continental, with cold winters, primarily due to western winds, and hot summers. Spring begins early but is characterised by late frosts, until 18–20 April. The growth period lasts for about 7–8 months. The mean annual temperature is 11.3°C and the total annual precipitation is about 515 mm, with maximum levels in June and minimum levels in February–March (“Silva 2003” Ltd., 2016). The soil is Calcic Chernozem, slightly to moderately alkaline, of sandy clay texture and high bulk density, but with good porosity and water permeability. It is characterized by low nitrogen, very low phosphorus and high potassium contents, an optimal C : N ratio, and low amounts of soil organic matter. The groundwater level is below 2 m (“Silva 2003” Ltd., 2016).

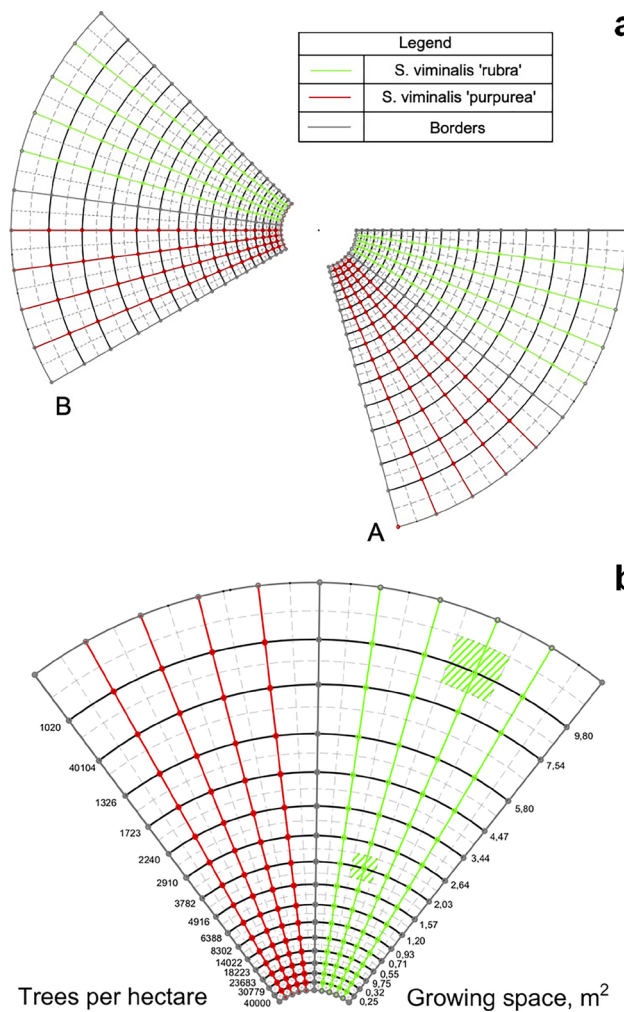


Fig. 1. Design of the experimental plantation. a. Arrangement of the varieties in four-spoke sectors; b. Distribution of the planting densities along the spokes. The planting spots are indicated with circles and the dashed lines delineate the corresponding growing spaces. The hatched areas exemplify the growing spaces corresponding to plants at spacings 2.03m<sup>2</sup> and 9.8m<sup>2</sup>. Location of sectors A and B are indicated with capital letters

The trial plantation was established as 4 sectors of a Nelder wheel plot (Nelder, 1962; Namkoong, 1965) with 15 nearly-square spacings, ranging from 0.25 to 9.80 m<sup>2</sup> and corresponding to initial densities of 40000 to 1020 plants per hectare (Fig. 1). Two basket willow (*Salix viminalis* L.) clonal varieties, *rubra* and *purpurea*, were arranged in 4 four-spoke sectors (2 sectors per clone), surrounded by border spokes. The planting density was varied along the spokes and the intersections of the spokes and circumferences were the planting point locations (Fig. 1). One-year-old saplings grown from cuttings *in situ* were planted in holes, marked according to the design. The cuttings were obtained at the beginning of 2018 from one-year-old shoots of the mother clones grown in the *Salix* spp. nursery stock in Vardim nursery (43°36'28"N, 25°29'30.5"E). The trees were coppiced immediately after planting to restart their growth at different growing spaces. The trial plantation was subjected to regular weeding and was irrigated several times during the growth periods of 2018 and 2019, considering the meteorological conditions and the soil water availability. Fertilization with ammonium nitrate was done once, during the summer of 2019 and treatments against pests were carried out according to the established silvicultural practices.

Data collection took place in the autumns of 2018, 2019 and 2020. The trees of sectors A (Figs 1a, 2a) were harvested after the first growth period (2018), providing dendromass data from coppiced plants of 2-year-old roots and 1-year-old shoots. The willow saplings of sectors B (Figs 1a, 2b) were sampled in 2019, supplying data from plants of 3-year-old roots and 2-year-old shoots. However, the second inventory showed that the sector planted with the *rubra* clonal variety was compromised, with numerous missing plants throughout the entire density range and therefore, its data (except for survival) were disregarded. The third plantation inventory

Table 1. Description of the data from coppiced willow (*Salix viminalis* L.) plants grown in an experimental plantation in North-Central Bulgaria

Clone	Root age + shoot age <sup>a</sup>	<sup>b</sup> Plant number	<sup>c</sup> d <sub>0</sub> , cm	<sup>c</sup> dbh, cm	<sup>c</sup> h, m	<sup>c</sup> w <sub>1</sub> , kg	<sup>c</sup> w, kg	<sup>c</sup> Shoots per stump	<sup>c</sup> W, t/ha
<i>S. viminalis</i> var. <i>purpurea</i>	2+1 (A)	58 (97%)	1.3 (0.3–2.1)	0.7 (0.2–1.1)	2.5 (0.4–3.3)	0.060 (0.003–0.131)	0.745 (0.003–3.202)	13 (1–34)	4.28 (0.06–24.04)
	3+2 (B)	55 (92%)	1.9 (0.8–3.3)	1.1 (0.3–1.5)	2.8 (0.5–3.4)	–	2.452 (0.009–6.770)	16 (1–36)	13.96 (0.04–48.56)
	4+1 (B)	48 (80%)	1.1 (0.8–1.7)	0.7 (0.3–1.1)	2.3 (1.6–2.9)	0.047 (0.015–0.134)	0.949 (0.150–2.264)	48 (14–97)	4.46 (0.91–16.90)
	4+2 (A)	46 (77%)	1.7 (1.0–2.4)	1.0 (0.5–1.4)	2.7 (1.8–3.3)	0.137 (0.030–0.306)	1.442 (0.041–4.322)	23 (4–90)	5.18 (0.18–9.91)
<i>S. viminalis</i> var. <i>rubra</i>	2+1 (A)	55 (92%)	0.8 (0.4–1.8)	0.5 (0.1–1.0)	1.5 (0.5–2.8)	–	0.234 (0.003–2.199)	7 (1–38)	1.47 (0.01–10.41)

Denotations: *dbh* – breast-height diameter of the main shoot; *d*<sub>0</sub> – basal diameter of the main shoot; *h* – total height of the main shoot; *w*<sub>1</sub> – dry weight of the main shoot; *w* – aboveground dendromass of the plant (dry weight); *W* – total aboveground woody biomass per hectare.

<sup>a</sup> Plantation sectors are abbreviated with capital letters in parentheses. <sup>b</sup> Survival rate, expressed as a percentage from the initial number of plants, is presented in parentheses. <sup>c</sup> Mean and minimum – maximum variable values in parentheses are presented.



showed that, practically, only the plants of *purpurea* variety survived in A and B sectors. The latter were harvested (second rotation) providing data on coppiced trees of 4-year-old roots and 2-year-old shoots (sector A) and 4-year-old roots and 1-year-old shoots (sector B).

The sprouts of each sample tree were cut as close as possible to the stump and were counted. Total length (to the nearest 1.0 cm), basal and breast-height diameters (to the nearest 0.1 cm) of the main shoot were measured. The stems and branches of all shoots on each stump and the main shoots alone were weighted *in situ*, to the nearest 0.005 kg. Three samples of lignified biomass (100–300 g) per sector were obtained and their fresh weights were measured. The samples were oven-dried at 105°C to constant weight and measured to the nearest 0.001 kg. Proportion of dry mass relative to the fresh weight of a sample was used to estimate the total amount of dry dendromass of each tree in the sector. All collected data are summarised in Table 1.

## Data analyses

To assist the interpretations and the comparisons with other studies, the potential total dendromass yield per hectare (Mg/ha) was calculated for each level of spacing, assuming square growing space (Fig. 1b). Thereafter, the annual biomass increment (Mg/ha/year) was determined by division of the total dendromass yield per hectare to the years of shoot growth. Survival was calculated as the ratio in percentage between the live plants at the time of inventory to the initial number of planted saplings in the sector (60 trees/clone/sector).

Growing space (0.25–9.80 m<sup>2</sup>) was regarded as a continuous variable and was treated as a covariate to each of the factors ‘clone’, ‘rotation’ and ‘root age’ and their influence on the individual plant variables ‘number of shoots’ and ‘aboveground dendromass’ were analyzed by one-way Analysis of covariance (ANCOVA). The graphical examination of the data (see Figs 4–5) showed that the data points were not evenly distributed on both sides of the visualized main relationships, but their variance increased with the increase in spacing. Since this observation diagnoses heteroscedasticity of errors (Picard et al., 2012), rank transformed non-parametric Analysis of covariance method (Olejnik & Algina, 1983) was applied to fulfil the study objectives, while assuring unbiased and efficient statistical inferences. Following the standard ANCOVA procedure, we first examined the full model, including the factor, the covariate and their interaction. When there was no statistically significant factor  $\times$  covariate interaction, the significance of the factor effect throughout the range of the covariate was tested.



Fig. 2. Views to the experimental plantation, April 2019. a. Sectors A; b. Sectors B

The method of conditioning (Picard et al., 2012; Stankova et al., 2017, 2018) and empirical piecewise regression modelling were employed to derive stump-level allometric models for estimation of the aboveground woody biomass. In line with the recommendations for biomass modelling of shrub-like plants (Burkhart & Tomé, 2012) and the results of earlier investigations (Menéndez-Miguélez et al., 2013), we chose as principal predictor variables the measurements of the main shoot on the stump: basal and breast-height diameters ( $d_0$  and  $dbh$ , respectively) and total height ( $h$ ) and we explored graphically their relationships to the aboveground woody biomass. We formulated one- and two-predictor “base models” involving power or exponential functions of the principal predictors as well as their products. Root age, shoot age and number of shoots per stump were considered as secondary predictors that served to expand the slopes or intercepts of the log-transformed forms of the base models deriving two- and three-predictor biomass relationships. Both base and expanded models were fitted in log-transformed form and their adequacy was assessed according to a set of criteria derived from Gadow and Hui (1999), Parresol (1999), Picard et al. (2012) and Sileshi (2014). All regressions that reached convergence involved number of shoots as a secondary predictor, but none of the resultant models met the requirements of all goodness-of-fit tests. Close examination of the residual plots of the fitted relationships as well as the results of the ANCOVA for the effect of coppicing on shoot number (Table 2) suggested

Table 2. Results of ANCOVA, testing the effect of Clone, Rotation and Growing space on the number of shoots and the aboveground dendromass (where relevant) of *Salix viminalis* L. clonal varieties

a		Variable		
Factors and covariates	Number of shoots per stump		Aboveground dendromass of the plant	
	F-test	Pr (>F)	F-test	Pr (>F)
Clone	20.25	< 0.001	34.60	< 0.001
Clone × Growing space	0.238	0.627	0.098	0.755
Growing space	9.376	0.003	10.46	0.002
Clone	20.43	< 0.001	34.97	< 0.001
Growing space	9.461	0.003	10.58	0.002
b		Variable		
Factors and covariates	Number of shoots per stump, Ages 2+1, 4+2*		Number of shoots per stump, Ages 3+2, 4+1	
	F-test	Pr (>F)	F-test	Pr (>F)
Rotation	17.25	< 0.001	116.9	< 0.001
Rotation × Growing space	0.058	0.811	0.064	0.801
Growing space	9.702	0.003	48.86	< 0.001
Rotation	17.49	< 0.001	49.53	< 0.001
Growing space	9.835	0.003	118.5	< 0.001

Denotations: *F*– Fisher’s coefficient, *P* – probability

<sup>a</sup> Factor ‘Clone’ with levels *Salix viminalis* var. *purpurea* and *S. viminalis* var. *rubra* is studied at age 2+1.

<sup>b</sup> Factor ‘Rotation’ with levels 2+1 vs. 4+2 and 3+2 vs. 4+1 is examined for *Salix viminalis* var. *purpurea*.

\* Age is abbreviated as a sequence of Root age + Shoot age.

the development of empirical piece-wise regression models. Therefore, we refitted the one-, two- and three-predictor regression models, which included as independent variables main shoot length, basal or breast height diameter and number of shoots, by subsets defined by the root age-shoot age combinations (Table 1) and those that showed appropriate for at least 3 of the 4 data subsets, were selected for further examination. Consecutive combination of any two data subsets for concomitant fitting, with different sets of parameters for each subset, revealed that while the regression coefficients of the main shoot variables appeared invariant, the coefficients related to the shoot number differed. In agreement with the results from ANCOVA, the empirical break-point, in regard with the number of sprouts, which was suggested during the process of regression examination, was between the consecutive rotations, i.e. (2+1, 3+2) data subset vs. (4+1, 4+2) subset. Therefore, we examined further the selected models with the entire data set estimating different parameters of the predictor “shoot number” for the first and the second rotation data. This was achieved by expanding the parameter through inclusion of an associated parameter and a dummy variable to differentiate the two rotations:  $a_1 + a_2 \cdot \text{Dummy}$ , where  $a_1$  is a parameter common for both rotations,  $a_2$  is the associated parameter of the expanded model, and *Dummy* is a dummy variable which value is equal to 1 for the second rotation and 0 for the first.

Simple one-predictor relationship for the shoot biomass was also developed. Data of the main shoots were used for this purpose and exponential and/or power relationships of  $d_0$ ,  $dbh$  and  $h$  were tested in log-transformed form, applying the same

goodness-of-fit criteria for model selection as for the stump-level models.

Normality of errors was examined by Shapiro-Wilk analytical test, through the skewness and kurtosis estimates and by inspection of the Quantile-Quantile plot. Homoscedasticity of errors was evaluated according to Breusch-Pagan analytical test and by exploring the plot of residuals against predicted values. Stability of parameter estimates was assessed by the Percent Relative Standard Error (PRSE%), which is the ratio (in percent) between the standard error and the absolute value of the regression parameter and must attain values below 25%. The Percent Relative Standard Error served indirectly also to control the effect of possible outliers and influential observations. Collinearity was handled through the condition number that must obtain estimates below 30. Ratio correction for bias (Snowdon, 1991) was applied to convert the predicted values to arithmetic, untransformed units. Unbiasedness of the back-transformed predicted data was judged according to the t-test for mean error equals zero and simultaneous *F*-test for slope equal to 1 and zero intercept of the linear regression relating observed and predicted values. Cross-validation was performed through leave-one-out regression, assessing the coefficient of determination (*P*-square) based on the predicted residual error sum of squares (PRESS) and a test for the mean paired difference residuals - predicted residuals equals zero.

Statistical analyses were carried out using packages MASS, lmtest, moments, stats, car, agricolae, olsrr, sandwich, MPV and were illustrated with ggplot2 package of R software environment (Venables & Ripley, 2002; Zeileis & Hothorn, 2002; Komsta &

Novomestky, 2015; Wickham, 2016; R Core Team, 2019; Fox & Weisberg, 2019; de Mendiburu, 2020; Hebbali, 2020; Zeileis et al., 2020; Braun & MacQueen, 2021).

## Results

### Survival and potential total yield per unit area

The inventory of sectors A (Fig. 1a) in the autumn of 2018 showed high percentage of survival of both willow clones after the first growth period: 97% for *purpurea* variety and 92% for *rubra* variety (Table 1). The situation changed drastically in the next two years, when the number of live plants from *rubra* clonal variety decreased to 58% after the second period of growth (data not shown) and only a few specimens could have been found in both A and B sectors in the autumn of 2020. Therefore, our analyses of productivity of *S. viminalis* var. *rubra* were limited to the first-year data. A steady decrease in the survival rate was recorded for *purpurea* clonal variety: 77% of the plants in sector A had remained after the third year of growth, while 92% and 80% of the willows in sector B survived after the second and the third growth periods, respectively (Table 1).

*Purpurea* variety showed higher productivity per unit area than *rubra* variety at the end of the first year of aboveground biomass growth (2018), yielding more than 5 Mg/ha/year woody biomass at the best performing densities (1.20–3.44 m<sup>2</sup> growing space) (Fig. 3a). The maximum dendromass yield from the trees of *rubra* variety amounted to around 3 Mg/ha/year and was limited to a narrow range of higher densities (0.42–1.20 m<sup>2</sup> growing space) as compared to *purpurea* variety (Fig. 3a). The growth of *purpurea* plants in sector A slowed down in the next two years. Its annual rate estimated in 2020 was of maximum values 3–5 Mg/ha/year achieved at spacings 0.93–4.47 m<sup>2</sup>, while the trees at the densest planting schemes did not survive (Fig. 3b). At the same time, the willows at the highest densities (0.32–0.42) of sector B survived and produced the maximum annual dendromass yield, which was up to 14.5 Mg/ha/year at the end of 2019 and up to nearly 17 Mg/ha/year after the 3-rd year of growth (Fig. 3c).

### Effect of spacing, clone, rotation and root age on productivity of plants

The steady plant growth of both clonal varieties during the first year allowed comparison of the aboveground plant biomass and the number of sprouts

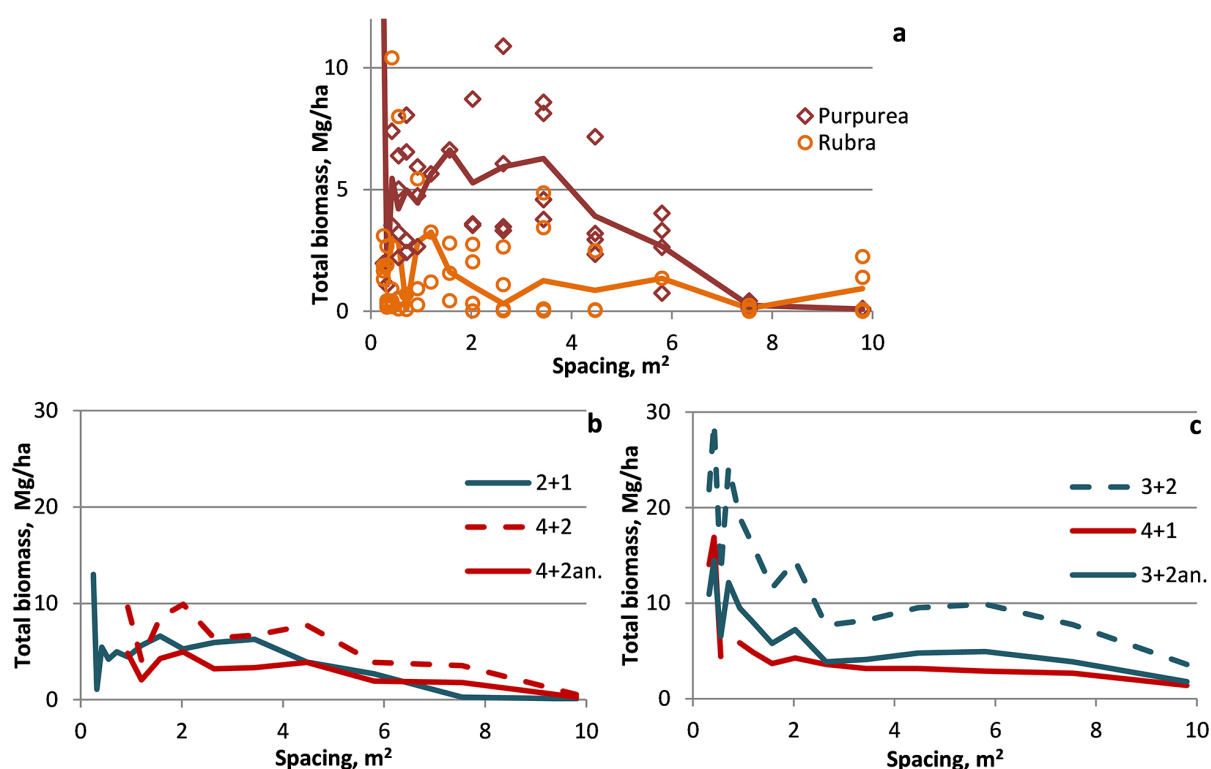


Fig. 3. Total aboveground woody biomass of: a. the two willow varieties after the first year of growth (2+1); b. *Salix viminalis* var. *purpurea* plants in sector A at successive rotations; c. *Salix viminalis* var. *purpurea* plants in sector B at successive rotations

The age is abbreviated as a sequence of Root age + Shoot age, an. denoting annual growth of 2-year-old shoots.



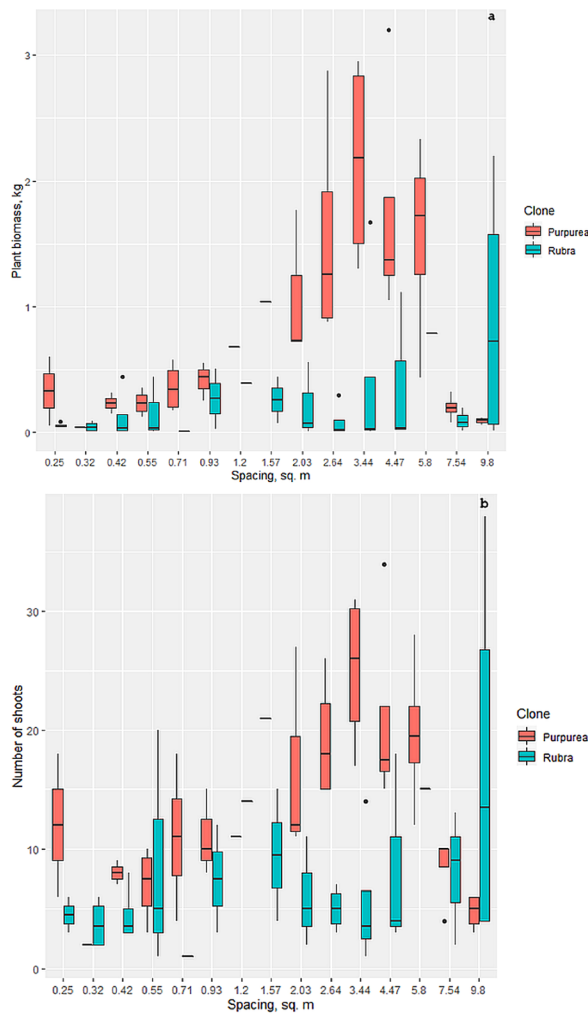


Fig. 4. Comparison of bioproductivity of the two willow varieties after the first year of growth (2+1) a. Aboveground woody biomass per plant; b. Shoot number per stump

at age (2+1) according to genotype and spacing. No significant clone  $\times$  growing space interaction was found for both investigated variables (Table 2). Growing space had a significant and positive influence on plant biomass and shoot number of both clones (Fig. 4), but the statistically significant effect of the genotype was more convincingly proven (Table 2). Indeed, the saplings of variety *purpurea* not only showed higher total yield at age (2+1) (Fig. 3a), but also had a significantly higher amounts of woody biomass and number of sprouts of individual plants (Fig. 4; Tables 1, 2).

As both planted with *Salix viminalis* var. *purpurea* sectors were harvested twice, it was interesting to investigate how the number of shoots changed at the second rotation. The aboveground dendromass weights of the successive rotations were not compared because of the different shoot age. The number of sprouts was strongly affected by the rotation (Table 2), yielding more than double number of sprouts at the time of the second harvesting (Figs 5c, 5d, Table 1). Growing space had a significant influence on the examined parameter, with a stronger positive effect for the second rotation when the roots grew older and bigger and required more space and resources (Figs 5c, 5d).

The influence of root age and spacing on shoot number and biomass could have been investigated for 1- and 2-years of aboveground growth of *purpurea* clonal variety. Significant root age effect on the number of sprouts was found, which led to a proportional increase of the shoot counts in time (Figs 5c, 5d, Table 3). No significant root age  $\times$  growing space interaction with regard to aboveground plant dendromass was distinguished, while large variation of the plant weights around their means was observed and the influence of root age was less pronounced than that

Table 3. Results of ANCOVA, testing the effect of Root age and Growing space on the number of shoots and the aboveground dendromass of *Salix viminalis* var. *purpurea*

a	Variable			
Factors and covariates	Number of shoots per stump		Aboveground dendromass of the plant	
	F-test	Pr (>F)	F-test	Pr (>F)
Root age	125.1	< 0.001	3.518	< 0.065
Root age × Growing space	2.465	0.121	2.838	0.096
Growing space	11.95	0.001	15.39	<0.001
Root age	122.6	< 0.001	3.430	0.068
Growing space	11.71	0.001	15.00	< 0.001
b	Variable			
Factors and covariates	Number of shoots per stump		Aboveground dendromass of the plant	
	F-test	Pr (>F)	F-test	Pr (>F)
Root age	4.896	0.030	10.23	0.002
Root age × Growing space	1.326	0.254	3.316	0.073
Growing space	21.54	< 0.001	16.09	< 0.001
Root age	4.872	0.031	9.879	0.003
Growing space	21.43	< 0.001	15.55	< 0.001

Denotations: F – Fisher's coefficient, P – probability

<sup>a</sup> Factor 'Root age' with levels 2 and 4 years, and 1-year-old shoots.

<sup>b</sup> Factor 'Root age' with levels 3 and 4 years, and 2-year-old shoots.

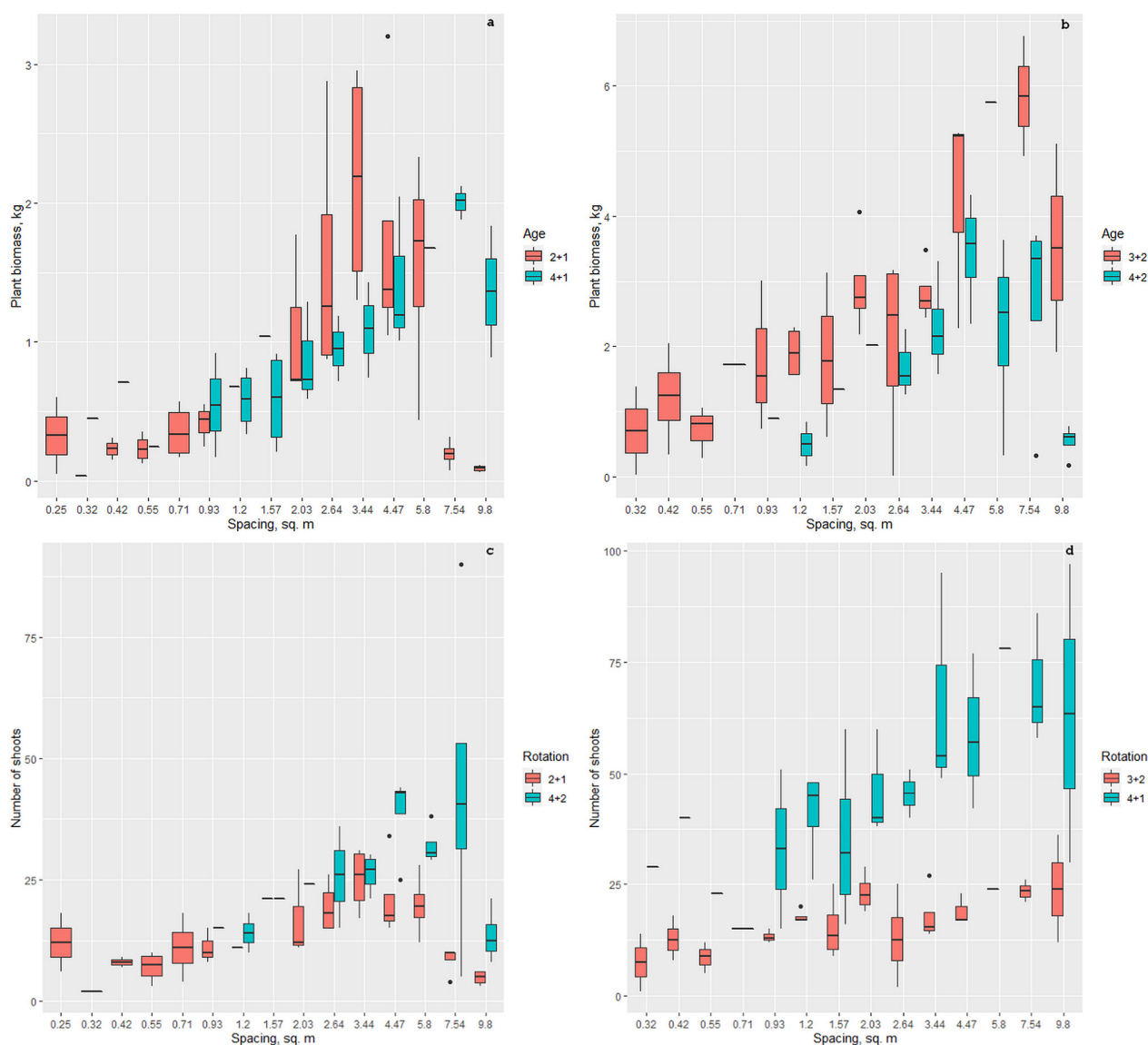


Fig. 5. Plant biomass and shoot number of *Salix viminalis* var. *purpurea* according to age and rotation. a. Plant biomass of 1-year-old shoots; b. Plant biomass of 2-year-old shoots; c, d. Change in the shoot number at two successive rotations. The age is abbreviated as a sequence of Root age + Shoot age and rotation is denoted by Root age + Shoot age in the year of harvesting

of spacing (Table 3). While the 1-year-old shoots of 2-year-old root system had, although insignificantly, lower weights than those of 4-year-old root system, the opposite was true for the 2-year-old shoots of the younger root system (Figs 5a, 5b). The last observation can be probably ascribed to the prevailing effect of the microsite heterogeneity and the variation in the meteorological conditions across the years.

### Allometric models for estimation of aboveground woody biomass

As already mentioned, only *Salix viminalis* var. *purpurea* provided sufficient and representative growth and productivity data and therefore, specific

allometric models for aboveground dendromass estimation were derived only for this clonal variety.

Following the protocol for model fitting, verification and validation, described in the methods, we derived 2 biomass models at stump-level, based on shoot number and breast-height diameter of the main shoot (M1) and shoot number and length of the main shoot (M2). Both models revealed good predictive potential and were validated through the leave-one-out regression and tests (Table 4). In addition, the percent relative standard error of the parameter estimates proved their stability, attaining values below 20% (Table 5). Because of diagnosed heteroscedasticity of the regression residuals, Heteroscedasticity-Consistent Covariance Matrix Estimator (HCCME, Table 5) was employed to ensure the



Table 4. Aboveground dendromass models for *Salix viminalis* var. *purpurea* – goodness-of-fit and validation statistics\*

	Model	RMSE	R <sup>2</sup> adj	P-square	Res – PRes**
Models at stump level					
M1	$\ln w = a_0 + SN(a_1 + a_2 \text{Dummy}) + a_3 \ln(dbh)$ $w = CF \times e^{(a_0 + SN(a_1 + a_2 \text{Dummy}))} \times dbh^{a_3}$	0.504	0.799	0.789	$1.01 \times 10^{-6}$
M2	$\ln w = a_0 + \ln SN(a_1 + a_2 \text{Dummy}) + a_3 h$ $w = CF \times SN^{(a_1 + a_2 \text{Dummy})} \times e^{(a_0 + a_3 h)}$	0.562	0.821	0.814	–0.0015
Model at shoot level					
M3	$\ln w_1 = a_0 + a_3 dbh$ $w_1 = CF \times e^{(a_0 + a_3 dbh)}$	0.203	0.908	0.904	0.0003

Denotations: *dbh* – breast-height diameter of the main shoot, cm; *h* – total height of the main shoot, m; *SN* – number of shoots on the stump; *w* – aboveground dendromass of the plant, kg; *w*<sub>1</sub> – dry weight of a shoot, kg; *CF* – ratio correction coefficient; *a*<sub>0</sub>, *a*<sub>1</sub>, *a*<sub>2</sub>, *a*<sub>3</sub> – regression parameters, where *a*<sub>1</sub> + *a*<sub>2</sub>·*Dummy* is an expanded parameter with *a*<sub>1</sub> – common component, *a*<sub>2</sub> – associated component and *Dummy* – dummy variable of value 1 for the second rotation (of root age 4 years and 1- and 2-year-old shoots) and value 0 for the first rotation (of root age 2 or 3 years and 1- and 2-year-old shoots); RMSE – root mean squared error, kg; R<sup>2</sup>adj – adjusted coefficient of determination; P-square – coefficient of determination based on predicted residual error sum of squares (PRESS); Res – PRes – mean paired difference between residuals and predicted residuals.

\* The goodness-of-fit statistics concern the fitted log-transformed model, while the validation statistics are estimated for the corrected back-transformed predicted values.

\*\* The null hypothesis that the mean paired difference (residuals-predicted residuals) equals zero is accepted in all cases.

Table 5. Parameter estimates, their goodness-of-fit statistics and the ratio correction coefficients of the aboveground dendromass models for *Salix viminalis* var. *purpurea*

Model	CF	Parameter*	a <sub>0</sub>	a <sub>1</sub>	a <sub>2</sub>	a <sub>3</sub>
Models at stump level						
$w = CF \times e^{a_0 SN(a_1 + a_2 Dummy)} \times dbh^{a_3}$	1.093	Estimate	−0.818	0.072	−0.043	1.869
		HCCME	0.088	0.005	0.004	0.144
		PRSE %	10.82	7.334	9.833	7.684
$w = CF \times SN^{(a_1 + a_2 Dummy)} \times e^{(a_0 + a_3 h)}$	1.177	Estimate	−6.423	1.121	−0.212	1.264
		HCCME	0.267	0.073	0.038	0.113
		PRSE %	4.149	6.473	17.956	8.960
Models at shoot level						
$w_1 = CF \times e^{(a_0 + a_3 dbh)}$	1.006	Estimate	−4.764			2.613
		SE	0.056			0.069
		PRSE %	1.170			2.630

Denotations: *dbh* – breast-height diameter of the main shoot, cm; *h* – total height of the main shoot, m; *SN* – number of shoots on the stump; *w* – aboveground dendromass of the plant, kg; *w*<sub>1</sub> – dry weight of the main shoot; *CF* – ratio correction coefficient; *a*<sub>0</sub>, *a*<sub>1</sub>, *a*<sub>2</sub>, *a*<sub>3</sub> – regression parameters, where *a*<sub>1</sub> + *a*<sub>2</sub>·*Dummy* is an expanded parameter with *a*<sub>1</sub> – common component, *a*<sub>2</sub> – associated component and *Dummy* – dummy variable of value 1 for the second rotation (of root age 4 years and 1- and 2-year-old shoots) and value 0 for the first rotation (of root age 2 or 3 years and 1- and 2-year-old shoots); SE – standard error, HCCME – Heteroscedasticity-Consistent Covariance Matrix Estimator; PRSE % – percent relative standard error.

\* PRSE % was defined as a criterion for stability of parameter estimate and must obtain values below 25%.

efficiency of the regression estimates (Long & Ervin, 2000).

Additionally, a simple exponential relationship of shoot biomass to the breast-height shoot diameter was also derived, verified and validated according to the standard procedure and can be employed for estimation of the biomass of each sprout separately.

All allometric biomass models can be applied with confidence within the size range of the data used for their approximation.

## Discussion

Along with the significant genotypic variability, willow varietal performance is also known to vary from place to place, i. e. strong genotype by

environment interaction is pertinent to this genus (Miller, 2018). Our study revealed that *Salix viminalis* var. *rubra* not only showed significantly poorer growth and sprouting performance than *Salix viminalis* var. *purpurea* during the first growing season, but also a long-term establishment on the experimental site in the valley of river Vit was not possible and this clonal variety gradually declined and dropped off during the second and the third growing seasons. Similar result was reported in the study by Kopp et al. (2001), where two of the six tested clones of *Salicaceae* species were eliminated from the experiment. A statistically significant interclonal variation regarding plant biomass was found in a study by Mleczek et al. (2010) comparing 8 *S. viminalis* and 1 *S. alba* clones grown in the lowland of Poland. The authors reported annual shoot growth of the basket

willow clones within the range 0.06–2.33 kg/year dry weight for 3-year production period. The average annual growth of woody biomass recorded for *Salix viminalis* var. *rubra* in our study was 0.234 kg/plant, which is comparable to the third least productive basket willow clone from Poland and ranged from 0.721 to 1.226 kg/plant for *S. viminalis* var. *purpurea* approaching the weight of the second-best Polish clone of *S. viminalis*.

The edaphic characteristic of our experimental site is very similar to that of the Vardim nursery where the cuttings were collected and where both clonal varieties grew and developed well. Tahvanainen and Rytkönen (1999) observed that *S. viminalis* clones they studied grew rather well on a wide variety of soil types, and Tsanov (1974b) pointed out that the clones and hybrids of *Salix viminalis* demonstrated ecological plasticity regarding soil conditions, growing on Chernozems even better than on Fluvisols. Tahvanainen and Rytkönen (1999) attributed the difference in productivity of the investigated *S. viminalis* genotypes to susceptibility of most of them to early autumn frosts before growth cessation. In addition, Kopp et al. (2001) distinguished strong positive correlations between the number of growing degree days and the annual biomass production of the tested willow and poplar clones. On the other hand, in a study on 15 willow hybrids, grown on five sites in Michigan, Miller (2018) observed that yield was positively related to precipitation and to a lesser extent to the number of growing degree days. Similarly, Nerlich et al. (2016), after examination of a number of potential biomass-producing species and clones on four sites in south-western Germany, concluded that willow growth is mainly promoted by adequate precipitation and cool temperatures. Harayama et al. (2020) found that weather conditions, such as hours of sunshine during growing season, air temperature in May (when leaf flushing occurs), and precipitation in July (when vigorous height growth occurs) have significant positive effects on willow yield. The study of Tsanov (1974b) showed that *S. viminalis* has a long growth period commencing at the end of March and lasting until mid-October. Early autumn or late spring frosts were not observed during the 3-year-period of our experiment. However, the long-term climatic records showed that the yearly number of days with strong winds (above 14 m/s) at the study site is twice as high as that specific for the region of the nursery near Vardim village and the average speed of the prevailing winds range from 6 to 11 m/s (Kyuchukova, 1982). According to De Martoone's global classification index  $I_{DM}$  (adapted after Baltas, 2007) the climate during the study period can be classified in 2018 as humid ( $I_{DM}$  2018 = 31), in 2019 as moderate arid ( $I_{DM}$  2019 = 20) and in 2020 as semi-humid ( $I_{DM}$  2020 = 26). Data on average temperatures

and precipitation amounts (Fig. 6) revealed a pronounced period of summer drought in 2019, with annual rainfall sum (482 mm) below the average. The total precipitation amounts during the growth periods of 2019 and 2020 remained around 370–420 mm that is far below the necessary water supply of 550 mm for fast growing woody crops (*sensu* Demo et al., 2013). Labrecque and Teodorescu (2003) postulated that once the willow crop is established, temperature and precipitation become the most limiting factors and Rodzkin et al. (2015) paid attention to the fact that willows belong to the group of phreatophytes, therefore having an increased demand for water during the period of growth. In agreement with the latest and considering the climatic information, we can conclude that *Salix viminalis* var. *rubra* is more susceptible than *S. viminalis* var. *purpurea* to adverse, desiccation-causing environmental conditions related to summer droughts and, likely, to spring winds. Tsanov & Broshtilova (1987) reported that decrease in soil moisture to 55% of the saturation capacity would decrease height growth of willow species such as *S. viminalis* by 7–18%. We need to mention also that the increased weed growth, promoted partially by the fertilization applied in 2019, probably has also played a role for *rubra* variety suppression and decline. Strong weed pressure as a reason for differential growth and survival of willow clones has been suggested also in the studies by Tahvanainen and Rytkönen (1999) and Johnson et al. (2018).

Clonal variety *S. viminalis* var. *purpurea* showed a steady survival rate during the 3-year-period of growth. Tsanov (1965a) reported that *S. viminalis* plants, grown on Gleyic Fluvisols, exhibited lower mortality than *S. purpurea* and Bergante and Faccioto (2011) found that willow survival after repeated coppicing was generally better than poplars. Tsanov (1986) examined five species of willows at growing space corresponding to the highest density of our experiment and found that when harvested annually *S. viminalis* was the second most productive species after *S. triandra*, yielding on average 18 Mg/ha/year fresh weight and achieving a maximum of 23–25 Mg/ha/year between the third and the seventh year of growth. Converted to oven-dry weight (at 40% average water content), these numbers are comparable to our data obtained for *S. viminalis* var. *purpurea* at the densest survived planting schemes (Fig. 3). The estimated biomass amounts of our study are comparable also with those from a trial with 10 willow clones in southern Quebec grown for 4 years at density 18000 plants per hectare (spacing 0.56 m<sup>2</sup>), which attained annual yield between 6.21 and 16.90 Mg/ha/year (Labrecque & Teodorescu, 2005). Similar to our data was reported also for four commercial willow clones cultivated in Denmark for 2 years at density 12000 plants per hectare (spacing 0.83 m<sup>2</sup>) that produced

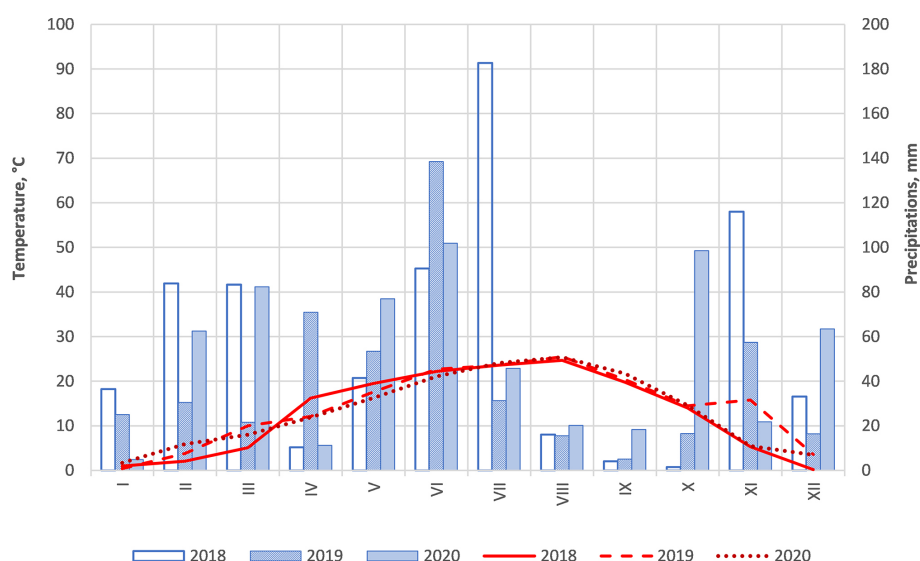


Fig. 6. Meteorological data for 2018–2020. Average monthly temperatures are connected with lines and monthly precipitation amounts are indicated with columns.

Retrieved from <https://www.sinoptik.bg/gulyantsi-bulgaria-100730982?search>.

aboveground biomass of 5.2 to 8.8 Mg/ha/year (Sevel et al., 2012). Demo et al. (2013), on the other hand, examined eight commercial willow clones at density 8889 plants per hectare (spacing 1.12 m<sup>2</sup>) on arable land in South-Western Slovakia for 3 years. Under first dry and second humid growing seasons, at plant age 3+2, they observed yield of 8.3–18.5 Mg/ha, with *purpurea* variety of our study exceeding 6 of the tested clones at the same age and comparable spacing (15.9 Mg/ha at 8333 plants per hectare, spacing 1.2 m<sup>2</sup>, Fig. 3c).

We must admit, however, that total biomass growth varied largely between years and sectors that probably can be ascribed to both differing meteorological and microsite conditions. Our observation is in agreement with the remark by Tsanov (1986) on the influence of the climatic variation across the years and with the finding of Miller (2018) who reported a significant effect of block within site explaining 7% of the total observed variation in yield. The annual yields, estimated from the second rotations in both sectors showed a decline in comparison with the first rotations for a large part of the density range (Figs 3b, 3c). These second rotations, however, included the dry growth period of 2019 (Fig. 6) and the growth period without irrigation (2020). Bergante and Facciotto (2011) also observed a decrease in the annual biomass growth of six willow clones in experimental plantation (10000 plants per hectare) in Italy after the first biannual cycle when the average yield was as high as 12.9 Mg/ha/year (compared to at most 9.5 Mg/ha/year of our study at spacing 0.93 m<sup>2</sup>, Fig. 3c). The authors emphasized on the importance of the drip irrigation in the first two years and the rainy period during the first year to obtain high production. The second rotation of *purpurea* variety

in sector A started during the second, dry growth period (2019), under severe weed competition and continued in the year without irrigation (2020). The plants of the first rotation of sector B were well supplied with water at the beginning (2018) and their growth was reinforced by the fertilization in the second growing season (2019). The differences described could be the possible explanation of the significantly higher biomass of the 2-year-old shoots of younger root system (Table 3, Fig. 5b). In a short rotation coppice with 18 willow clones in Northern Japan, observed over 10 years, Harayama et al. (2020) estimated that the yield loss per stool per rotation was equivalent to approximately 5% of the initial average yield and consequently in 10 consecutive years of harvesting the yield could be halved. On the contrary, Miller (2018) found that the willow yield increase from the first to the second triennial rotation varied from 0 to 183% across the five study sites. In connection to the above, Harayama et al. (2020) considered also the factor “rotation length” and derived the conclusion that biannual harvesting had a significant positive effect on willow yield in comparison to annual harvesting.

Our study showed that shoot number was significantly affected by all investigated factors and covariates: clone, rotation, root age and spacing. *Salix viminalis* var. *rubra* exhibited a lower number of shoots than *S. viminalis* var. *purpurea* across the entire density range. Tsanov (1965a) reported a higher number of sprouts per stump for *Salix purpurea* than *S. viminalis* and Labrecque and Teodorescu (2003) found that the native to Canada *Salix discolor* had a higher shoot number than the introduced *S. viminalis*. Demo et al. (2013), on the other hand, reported a significant variation in the number of shoots among the eight



commercial willow varieties investigated in an experiment in south-western Slovakia. The increase in shoot number at the subsequent rotations found in our study is in line with the observations on willow re-sprouting by Tsanov (1965a, 1974a), Demo et al. (2013), Hernea et al. (2015), Miller (2018). In agreement with our results, Miller (2018) registered a shoot number increase by 50 to 100% after coppicing, while Tsanov (1965a) recorded tripling of *Salix viminalis* sprout number and a four-fold increase for *Salix rubra*. Tsanov (1974a) emphasized on the ability of willows to abundantly develop new shoots after coppicing ascribing this specificity of the genus to the large number of dormant and auxiliary buds they produce, in addition to the buds remained on the stump after cutting. Our study confirmed also the increase of shoot number with spacing, reported by Tsanov (1974a, b) for willow clones and hybrids examined at 4 planting densities.

In our study, we derived clone- and site-specific allometric models for woody biomass estimation of *S. viminalis* var. *purpurea* at stump and shoot level. The study of Heinsoo et al. (2002), developing allometric relationships for shoot biomass of six *Salix viminalis* and one *S. dasyclados* clones grown on 2 sites in Estonia, suggested the presence of clone-related, but not site-related differences in the models. On the other hand, Berner et al. (2015) who investigated biomass allometry of several shrub genera in four ecoregions of North Central Alaska and Yakutia concluded that alder and willow populations exhibited differences in allometric relationships across ecoregions. Arevalo et al. (2007) derived diameter-based shoot weight models for four willow clones at two sites in central New York and reported that biomass amount was affected by clone, age and site variables. However, they admitted that stem diameter measurements using intermediate data (clone and age alone) was sufficient to predict aboveground biomass efficiently without compromising accuracy, while the improvements on the predictive ability were relatively small when clone, age, and site-specific equations were derived.

Both, Heinsoo et al. (2002) and Arevalo et al. (2007), whose observations comprised 4 years of growth, found that shoot biomass allometry was age-related. Applying the general allometric equation (Huxley, 1972) to shoot diameter, Heinsoo et al. (2002) observed that the exponent increased with age, while Arevalo et al. (2007) reported decrease of the exponent and increase of the model intercept as the shoots grew older. While modelling basket willow allometry, Żyromski et al. (2016) also described variation related to shoot age, which was manifested through biomass distribution change. The allocation of shoot weights evolved from a simple gamma distribution (2-year-old shoots) to a mixture of two gamma distributions (2–4-year-old shoots), the first

including shorter and more massive shoots and the other - longer shoots with evidently smaller diameter. In our study, we also found a change in the basket willow allometry in time, but it was manifested differently. While the above-mentioned authors explored an interval of time when some of the shoots grew bigger and heavier at the expense of the loss of the thinnest and the weakest scions, we studied 1- and 2-year-old sprouts that experienced little or no mortality. We did not find rotation- or age-related variation in the diameter-biomass relationships, but the number of shoots was strongly affected by coppicing. The specific ability of basket willows to develop numerous new shoots after cutting affected the derivation of the stump-level biomass models of our study that were differentiated according to rotation (first vs. second).

Unlike the conclusion in the study by Annighöfer et al. (2016) that root collar diameter was a better single predictor of biomass growth than the height, the stump-level allometric relationship of our study based on main shoot length showed a higher predictive ability than that based on the breast-height diameter. Both diameter-including models (M1 and M3) incorporated breast-height rather than basal diameter of the main shoot, which is not common particularly for shrubs and coppiced plants (e.g. Blujdea et al., 2012; Ciuvăt et al., 2012, 2013; Paul et al., 2013; Matula et al., 2015). Although willows are fast-growing species, using breast-height diameter can be particularly inconvenient when all, not just the leading, shoots must be measured (e.g. model M3). We must point out however, that the root-collar diameter of the coppiced plant and the basal diameter of its main shoot do not coincide. Not always the size of the main shoot is representative for the size of the whole plant and its basal diameter can also be weakly correlated with the root-collar diameter of the whole bush. Therefore, the high predictive power of the root-collar diameter attributed to the significant share of the crown of the shrubby plants, with sprouts branching from the surface, cannot be directly transferred to the main shoot basal diameter. Measuring the root-collar diameter, on the other hand, is sometimes impossible without clipping the plant close to the surface that limits the application of allometric relationships based on the root-collar diameter. Investigators choose to measure shoot diameter of coppiced or shrub-like plants at various heights from the base: 10 cm (Paul et al., 2013), 30cm (Arevalo et al., 2007), 55cm (Heinsoo et al., 2002). Nordh and Verwijst (2004) investigated allometry of 12 willow clones deriving relationships between shoot dry weight and stem diameter at 55, 85 and 105 cm from the shoot base. In line with our results, the authors found that for most clones, the best fit of the model relating shoot dry weight and

stem diameter was found at 105 cm above shoot base. To achieve harmonization of biomass relationships Annighöfer et al. (2016) proposed conversion of all diameter measurements to root collar-diameter by deriving of correction factors from data sets including diameter measurements at various heights.

## Conclusion

*Salix viminalis* var. *rubra* is poorly adapted to the specific soil and climate conditions of the region of study and is more susceptible to desiccation-causing adverse climatic changes. At the opposite, *Salix viminalis* var. *purpurea* exhibited steady survival rate and biomass productivity that was affected by the density, climatic and micro-environmental variations. The latter clonal variety showed a very high potential to re-sprout and produced more than double number of shoots after coppicing. This was reflected in the developed stump-level allometric models that were differentiated according to rotation. The stump-level allometric model of higher predictive power was based on the main shoot length and shoot number. An exponential relationship to the breast-height shoot diameter was also suggested for biomass estimation of each sprout separately.

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