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IMPACT OF SPACING, CLONE AND COPPICING ON BIOMASS PRODUCTION OF TWO BLACK POPLAR HYBRIDS

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Abstract

The main objective of this study was to investigate the influence of genotype, spacing, age and harvesting cycle on the woody biomass production from coppiced plants of the black poplar hybrids (*Populus* × *euramericana* (Dode) Guinier) 'Agathe F' and 'Guardi'. The experiment was carried out in a Nelder wheel trial plantation with 11 nearly-square spacings, ranging from 1.0 to 11.5 m^2 and data collection took place at the end of the 2nd, 4th and 5th year from plantation establishment. Spacing significantly affected dendromass production in both clones and all harvesting cycles considered. The individual plant biomass generally increased with spacing, but growth approached saturation at the lowest densities for the plants of clone 'Agathe F'. Comparable dendromass amounts were recorded for both clones after 1 and after 3 years of shoot growth, and marginally significant genotype effect was found for the 4-year-period of dendromass production, when the plants of 'Guardi' attained higher biomass yields. No significant effect of the harvesting cycle was distinguished within 3-year production period, the accumulated amounts of 1- and 2-year-old shoots yielding similar quantities to those of 3-year-old sprouts for both clones. The black poplar hybrids did not differ significantly in the number of sprouts produced at any of the investigated ages. The shoot number increased with growing space and with stump age, the influence of age being obscured only at the highest planting densities. The plants of clone 'Agathe F' showed substantial growth decline in the 5th year from plantation establishment due to worsen environmental conditions and biotic pests, while clone 'Guardi' proved higher suitability for cultivation as a short rotation crop under Mediterranean climate, because of its steady growth even at worsen site conditions. Under unfavourable alterations in the water availability and without additional irrigation, coppiced plantations of 'Guardi' would produce above 4 Mg/ha/year dry dendromass, if cultivated on light arable Fluvisols.

Key words: dendromass production, Nelder wheel design, plantation density, *Populus* × *euramericana* 'Agathe F', *P.* × *euramericana* 'Guardi', short-rotation crops.

Introduction

Poplars (*Populus* spp.) are recognized as the fastest-growing forest tree species at temperate continental climate. They possess many other valuable characteristics, such as ease of propagation, aptitude to hybridize, pleasing appearance and many uses (Isebrands and Richardson 2014) and, together with eucalypts (*Eucalyptus* spp.) and black locust (*Robinia pseudoacacia* L.), are among the most widely planted hardwood trees in the world (DeGomez and Wagner 2001). The ‘eucalypts of the temperate geographic belt’, have attracted the attention of Bulgarian foresters, primarily in pursue of intensified timber production and numerous studies have been conducted to fulfill the needs for modern poplar sylviculture in Bulgaria (Kolarov 1974; Marinov et al. 1982; Krastanov et al. 1984, 1986, 1987a, 1987b).

Poplars are among the most appropriate species for use in short-rotation (energy) crops for biomass production due to their fast growth and ability to sprout. Traditional poplar clones selected for productivity and stress resistance have been used in preliminary experiments in Italy to evaluate their potential in short-rotation forestry; however, according to Paris et al. (2011) the results were not encouraging, mainly because these genotypes were not specifically selected for short-rotation plantations. On the other hand, Sixto et al. (2013) tested different interspecific poplar hybrids for their performance in short-rotation crops and concluded that clones showing intermediate reactivity to the test environments may be of interest for use in a large target population of environments, or in areas where environmental conditions are very heterogeneous, provided the mean performance is above average. The study findings showed that such intermediate clones were the black poplar hybrids ‘I-214’ and ‘Guardi’: the first is widely used in industrial plantations and the second is becoming more popular. Although traditional poplar clones have not been specifically selected for use in short-rotation crops, the advantages of their application in such plantings are that they have already been tested in various environments and their mass propagation is inexpensive. The black poplar hybrids *Populus* × *euramericana* (Dode) Guinier were first introduced to Bulgaria in the 1950s (Naydenov and Stoyanov 2017) and at the end of the last century a dozen clones of this group have already been successfully approbated for broad use in the afforestation practice (Tsanov and Mikov 1997). ‘Agathe F’ is a female clone imported from the Netherlands in 1974, characterized by a straight stem and better growth than ‘I-214’ and in 1997 accounted for around 6 % of the poplar plantations (Tsanov and Mikov 1997; Naydenov and Stoyanov 2017). The female clone ‘Guardi’ was selected in Italy, but imported from Romania in 1979. It was characterized by ecological plasticity in terms of drought, salted or excessively moist soils, and by multipurpose timber (Naydenov and Stoyanov 2017).

It has been suggested that research on Short Rotation Forestry should focus on genetic improvement, plantation design, rotation length and tending operations (Cañellas et al. 2012). Ceulemans and Deraedt (1999) reported that optimum rotation time and plantation density for poplar energy plantations are generally 4 years and 2500 to 10,000 plants per hectare, respectively. An experimental plantation was established in the spring of 2013 in South-Western Bulgaria using the Nelder wheel design with the *Populus* × *euramericana* clones ‘Agathe F’ and ‘Guardi’ and 11 nearly-square spacings (initial densities from 870 to 10,000 plants per hectare). The experiment was designed to study the effect of genotype, spacing and rotation length on the biomass production. The main objectives of this study were: 1) to investigate the influence of genotype and spacing on the woody biomass yielded per plant (at the end of the 2nd, 4th and 5th year from plantation establishment, respectively); 2) to examine the effect of root age on the amount of dendromass yield from 1-year-old shoots at 2- and 5-year-stump age; 3) to compare the woody biomass yields, obtained by 1- and 2-cut harvesting cycles; 4) to study the effect of spacing, genotype and age on the number of shoots per stump.

Materials and Methods

Populus × euramericana 'Agathe F' is characterized with high ecological plasticity and long growth period, while fast initial growth and tolerance to adverse environments is specific to *P. × euramericana* 'Guardi' (Tsanov and Mikov 1997). Therefore, we chose to examine the potential of these clones for short-term biomass production under Mediterranean climate in South-western Bulgaria. Experimental plantation with the two *Populus × euramericana* (Dode) Guinier clones 'Agathe F' and 'Guardi' was established in March 2013 on the territory of Mikrevo nursery of Strumyani Forestry Estate in South-western Bulgaria (Fig. 1). The nursery is situated at altitude 138 m a.s.l in the valley of river Struma. The climate is transitional Mediterranean, with hot summer of maximum average monthly temperatures exceeding 25°C and mild, warm winter of minimum average monthly temperatures usually above 0 °C (Fig. 2). The annual amount of precipitations, registered in the last 5 years, ranged from 460 mm (in 2016) to above 700 mm (in 2014). The soil is arable Fluvisol, of low bulk density and slight alkalinity, characterized by good water permeability. The Nelder wheel experimental design (Nelder 1962, Namkoong 1965) was adopted for the trial plantation, with 11 nearly-square spacings, ranging from 1.0 to 11.5 m^2, and corresponding to initial densities of 10,000 to 870 plants/ha. The two clones were arranged in alternating spokes and the planting densities varied along the spokes (Fig. 1, Table 1). Plantation was established on prepared nursery land using standard lignified cuttings (18–20 cm). Irrigations (10 L per plant) were carried out weekly 8 times during the first growth period and nitrogen fertilizer (20 g NH_4NO_3 per plant) was applied once. More than 95 % rooting was achieved and the empty spots were replanted in the autumn of 2013 with standard 1-year-old ramets from the same clones. Infestation of the wood-boring insect *Paranthrene tabaniformis* Rot. was detected during the first (2013) and the second (2014) growth periods, causing harms to around 1/3 of the plants each time. This was one of the reasons to coppice the entire plantation in the winter of 2013–2014 and again in the winter of 2014–2015. Severe root damage of the poplar plants, caused by the beetle *Capnodis tenebrionis* L., was found in the summer of 2017 and the test plantation was cut and uprooted at the beginning of 2018.

Data collection took place in the winters of 2014–2015 and 2016–2017, and in the autumn of 2017. The trees of six spokes (3 spokes per clone) were harvested after the second growth period (2014) and provided dendromass data from coppiced plants of 2-year-old roots and 1-year-old shoots (Fig. 1). The same spokes were sampled again after the 4th (2016) and after the 5th (2017) growth periods, which assured leafless biomass data from coppiced plants of 4-year-old roots, 2-year-old shoots and from plants of 5-year-old roots, 1-year-old shoots (Fig. 1).

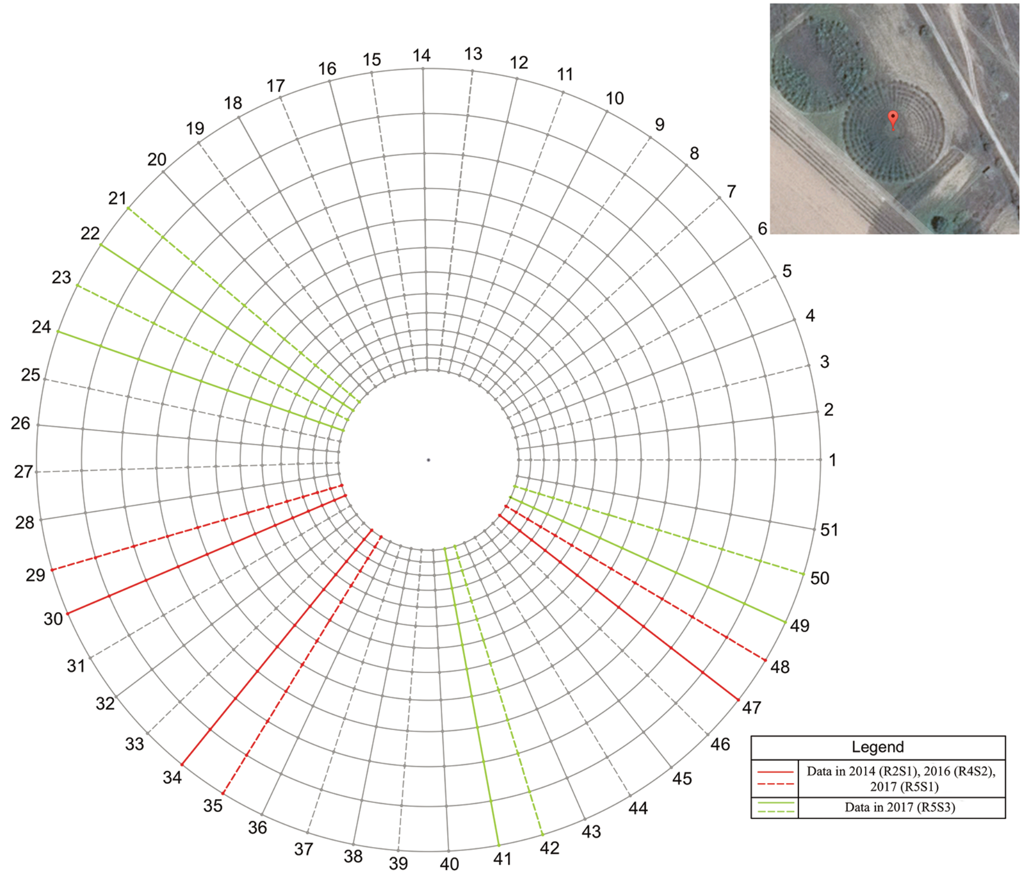
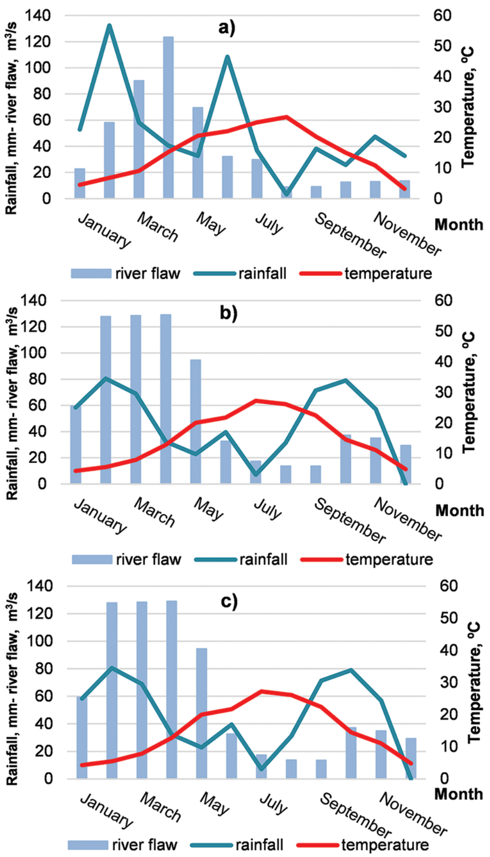


Fig. 1. Nelder wheel experimental plantation (41º37´59.6˝ N, 23º11´38.2˝ E)

Note: Spokes of clone ‘Agathe F’ are indicated with dashed lines, while spokes of clone ‘Guardi’ with solid lines. The planting locations are at the crossing points of the spokes and the circumferences. Sampled spokes are described in the Legend. Plant age is abbreviated as a sequence of Root age (Rx) Shoot age (Sy) – (RxSy).

Poplar trees in eight more spokes (4 spokes per clone) were also harvested and measured in the autumn of 2017, providing information for woody biomass from coppiced plants of 5-year-old roots and 3-year-old shoots (Fig. 1).

The sprouts of each sample tree were cut as close as possible to the stump and were counted. Stems and branches of all shoots on each stump were weighted *in situ*, to the nearest 0.005 kg. One sample of lignified biomass (100–300 g) per spoke was obtained and its fresh weight was 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 spoke.



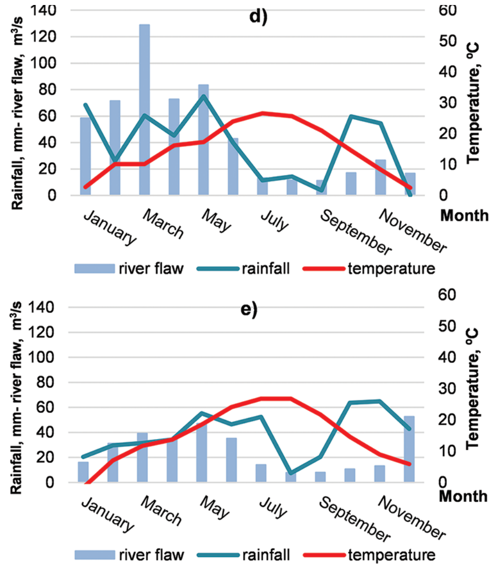


Fig. 2. Hydrological and meteorological data for 2013–2017: a) 2013; b) 2014; c) 2015; d) 2016; e) 2017.

Note: Temperature (°C) and precipitations (mm) were recorded at the station of Sandanski and are available on: https://www.stringmeteo.com/synop/index.php. Data on the flow of river Struma (m^3/s) were recorded at the station 'Kresnensko hanche' (41°47'02" N, 23°09'19.8" E) and are provided by the National Institute of Meteorology and Hydrology of Bulgaria.

The dendromass, harvested from each plant in the initially selected in 2014 spokes (Fig. 1), was additionally summed to estimate the total yield of woody biomass per plant as a result of one-, two- or three-coppicing cycles, referred hereafter as 'harvesting cycles'. These harvesting cycles were carried out within periods of variable duration (1-, 3- and 4-years), referred hereafter as 'production periods' (Table 1). To achieve the first research objective, we investigated the effect of clone and spacing on the accumulated per plant woody biomass production at the end of the 2nd, 4th and 5th year (harvesting cycles R2S1; R2S1, R4S2 and R2S1, R4S2, R5S1, respectively, as denoted in Table 1). The influence of root age along with spacing (second research objective) was studied by clone for the dendromass yield from 1-year-old shoots (harvesting cycles R2S1 and R5S1 in Table 1). The effect of harvesting cycle, expressed by the number of cuts within certain production period, together with the impact of spacing on the woody biomass production of the examined poplar clones, was explored for 1- and 2-cut cycles (harvesting cycles R5S3 and R4S2, R5S1, respectively, as denoted in Table 1) (third research objective).

The number of shoots, as affected by spacing and clone, was examined at each root age – shoot age combination (R2S1, R5S1, R5S3 and R4S2 in Table 1) and the shoot counts of the different root age – shoot age combinations (R2S1, R5S1 and R4S2 in Table 1) were compared by clone (fourth research objective). To assist the interpretations and the comparisons with other studies, the total dendromass yield per hectare (Mg/ha) was calculated for each level of spacing by multiplying the dendromass, harvested per plant, with the product $\frac{10000}{Spacing}\frac{1}{1000}$, where 1/1000 converts kg into Mg and 10,000/Spacing estimates the number of plants per hectare (10,000 m^2), assuming square growing space of 'Spacing' m^2 per plant (Table 1). 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.

Table 1. Description of the analyzed data.

Clone	Production period, years	Harvesting cycle*	Number of trees	***Spacing, m^2	Dry woody biomass per plant, kg mean (min–max)	Shoot number mean (min–max)
'Agathe F'	1 year	R2S1	33	1.00, 1.28, 1.63, 2.08, 2.66, 3.40, 4.34, 5.54, 7.07, 9.03, 11.53	0.705 (0.119–1.561)	5 (2–6)
		R5S1	33		0.330 (0.008–0.877)	21 (2–43)
	3 years	R2S1, R4S2**	33		4.110 (0.579–10.045)	14 (1–30)
		R4S2, R5S1	33		4.736 (0.159–9.386)	
		R5S3	44		4.623 (0.808–11.267)	17 (5–46)
	4 years	R2S1, R4S2, R5S1	33		4.441 (0.593–10.756)	
'Guardi'	1 year	R2S1	33		0.727 (0.021–1.951)	5 (3–8)
		R5S1	33		0.824 (0.061–7.968)	26 (8–57)
	3 years	R2S1, R4S2**	33		5.124 (1.272–14.439)	14 (4–31)
		R4S2, R5S1	33		5.221 (1.357–22.179)	
		R5S3	44		5.674 (0.205–25.993)	17 (2–30)
	4 years	R2S1, R4S2, R5S1	33		5.947 (1.607–22.407)	

Note: * – the harvesting cycle is coded as a sequence of Root age (Rx) Shoot age (Sy) – (RxSy) combinations, with the total shoot age equal to the duration of the production period; ** – the stem number corresponds to R4S2 Root age – Shoot age combination; *** – the spacing variants are presented in all Clone-Harvesting cycle combinations (column 1 × column 3).

Growing space that varied from 1.0 to 11.5 m^2 was regarded as a continuous variable and was treated as a covariate to each of the factors 'clone', 'root age', 'harvesting cycle' and 'root age – shoot age combination', analysed separately by study objective. The graphical examination of the data (see figs 3–6) 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 and 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. Significant factor × covariate interaction indicates that the factor effect is dependent upon the value of the covariate and it is impossible to claim significance or non-significance of the factor throughout the range of the covariate under consideration. When there was no statistically significant factor × covariate interaction, the significance of the factor effect throughout the range of the covariate was tested.

Results

Tracking the monthly amounts of rainfall in the area during the growth period from April to October showed irregular distribution of precipitations, with well-expressed summer minimum, which correlates with the annual minimum of Struma river flow, while coinciding with the maximum of the average monthly temperatures (Fig. 2). This observation suggests limited water availability to plants during the period of active development, which acts as a growth-impeding factor. Therefore, our results present the growth and productivity of the studied poplar hybrids under moderate drought (Fig. 2).

The results on the influence of clone and growing space on the woody biomass yield from the selected poplar genotypes at 1-, 3- and 4-year-production periods did not reveal clone × spacing interaction. Growing space influenced significantly the amount of harvested biomass per plant, which increased with the decrease of density (Table 2, Fig. 3). Comparable dendromass amounts were recorded for the two clones at 1 and 3 years duration of growth, and marginally significant genotype effect was found at the 4-year-production period, when the plants of ‘Guardi’ attained higher biomass yields (Table 2, Fig. 3).

Table 2. Results of ANCOVA, testing the effect of clone and growing space on the total harvested dendromass by harvesting cycle.

Harvesting Cycle*	Factors and covariates	*DF*	*SS*	*MS*	*F*-value	*P*-value
‘R2S1’	Clone	1	40.1455	40.1455	0.210	0.649
	Clone × Growing space	1	48.5470	48.5470	0.254	0.616
	Growing space	1	12,040.5	12,040.5	62.93	5.180×10^{-11}
	Residuals	62	11,863.3	191.343		
	Clone	1	0.13636	0.13636	0.001	0.979
	Growing space	1	12,040.5	12,040.5	63.68	3.867×10^{-11}
	Residuals	63	11,911.8	189.076		
‘R2S1, R4S2’	Clone	1	40.4369	40.4369	0.214	0.645
	Clone × Growing space	1	20.7409	20.7409	0.110	0.741
	Growing space	1	11,735.0	11,735.0	62.18	6.250×10^{-11}
	Residuals	62	11,700.4	188.716		
	Clone	1	496.379	496.379	2.668	0.107
	Growing space	1	11,735.0	11,735.0	63.07	4.508×10^{-11}
	Residuals	63	11,721.1	186.050		

Harvesting Cycle*	Factors and co-variates	*DF*	*SS*	*MS*	*F*-value	*P*-value
'R2S1, R4S2, R5S1'	Clone	1	66.0427	66.0427	0.381	0.539
	Clone × Growing space	1	21.4561	21.4561	0.124	0.726
	Growing space	1	12,488.9	12,488.9	72.00	5.721×10^{-12}
	Residuals	62	10,754.8	173.464		
	Clone	1	687.409	687.409	4.019	0.049
	Growing space	1	12,488.9	12,488.9	73.01	3.995×10^{-12}
	Residuals	63	10,776.2	171.051		

Note: *DF* – degrees of freedom, *SS* – sum of squares, *MS* – mean square, * – the harvesting cycle is coded as a sequence of Root age (Rx) Shoot age (Sy) – (RxSy) combinations, with the total shoot age equal to the duration of the production period

Growing space affected substantially the dendromass, yielded from 1-year-old shoots of both clones, but root age (2 vs. 5 years) exerted significant influence only on the growth of 'Agathe F' plants (Table 3, figs 4a and 4b). While the mean

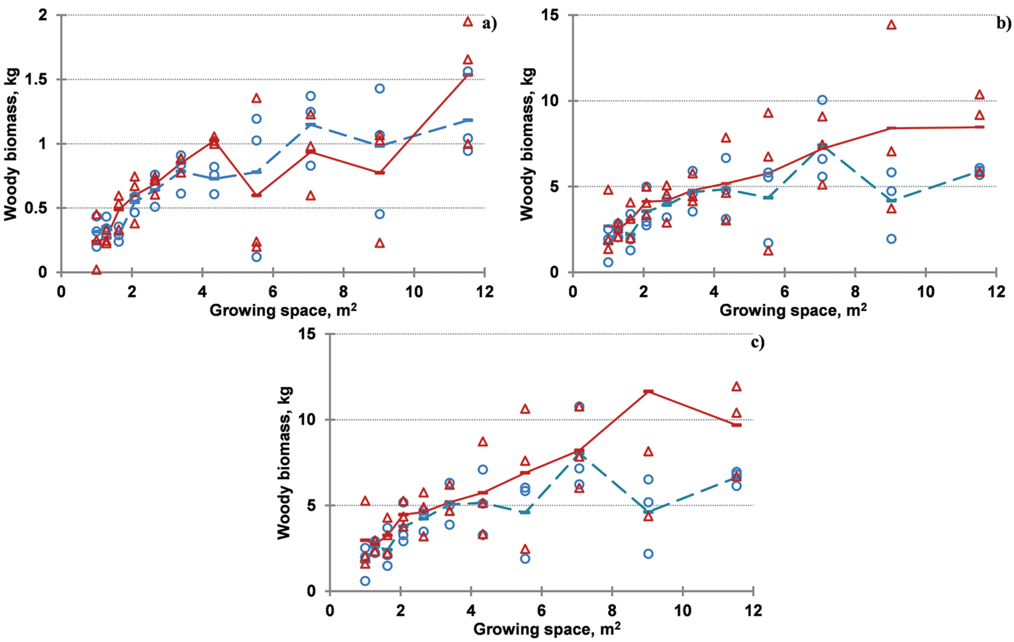


Fig. 3. Trends of cumulated woody biomass with growing space for different production periods. a) 1-year production period, harvesting cycle R2S1; b) 3- year production period, harvesting cycle R2S1, R4S2; c) 4-year production period, harvesting cycle R2S1, R4S2, R5S1.

Note: Circles and dashed line indicate the biomass values and their average, respectively, for clone 'Agathe F', while triangles and solid line – for clone 'Guardi'. The harvesting cycle is coded as a sequence of Root age (Rx) Shoot age (Sy) – (RxSy) combinations, with the total shoot age equal to the duration of the production period.

shoot weight amounted to around 2 kg dry mass for 'Guardi' at both root ages and 'Agathe F' at root age 2 years, the mean plant mass across the densities did not exceed 1 kg for 'Agathe F' at root age 5 years.

Table 3. Results of ANCOVA, testing the effect of harvesting cycle and growing space on the total harvested dendromass by clones.

Clone	Factors and covariates	*DF*	*SS*	*MS*	*F*-value	*P*-value
1-year production period ('R2S1' vs. 'R5S1')						
'Agathe F'	HC	1	1686.70	1686.70	14.21	3.675×10^{-4}
	HC×Growing space	1	7.63788	7.63788	0.064	0.801
	Growing space	1	9721.35	9721.35	81.88	6.129×10^{-13}
	Residuals	62	7360.95	118.725		
	HC	1	6862.56	6862.56	58.67	1.405×10^{-10}
	Growing space	1	9721.35	9721.35	83.12	4.068×10^{-13}
	Residuals	63	7368.59	116.962		
'Guardi'	HC	1	690.922	690.922	3.796	0.056
	HC×Growing space	1	371.250	371.250	2.040	0.158
	Growing space	1	11,904.3	11,904.3	65.41	2.79×10^{-11}
	Residuals	62	11,284.3	182.004		
	HC	1	392.742	392.742	2.123	0.150
	Growing space	1	11,904.3	11,904.3	64.34	3.272×10^{-11}
	Residuals	63	11,655.5	185.008		
3-year production period ('R2S1, R4S2' vs. 'R5S3')						
'Agathe F'	HC	1	5.85847	5.85847	0.020	0.888
	HC×Growing space	1	228.486	228.486	0.779	0.380
	Growing space	1	14,980.0	14,980.0	51.05	5.608×10^{-10}
	Residuals	73	21,421.34	293.443		
	HC	1	551.727	551.727	1.886	0.174
	Growing space	1	15,836.4	15,836.4	54.13	2.109×10^{-10}
	Residuals	74	21,649.8	292.565		
'Guardi'	HC	1	297.554	297.554	0.810	0.371
	HC×Growing space	1	329.926	329.926	0.899	0.346
	Growing space	1	11,220.0	11,220.0	30.56	4.762×10^{-7}
	Residuals	73	26,802.17	367.153		
	HC	1	6.41667	6.41667	0.018	0.895
	Growing space	1	10,899.5	10,899.5	29.73	6.274×10^{-7}
	Residuals	74	27,132.1	366.650		

Note: *DF* – degrees of freedom, *SS* – sum of squares, *MS* – mean square, HC – harvesting cycle; * – the harvesting cycle is coded as a sequence of Root age (Rx) Shoot age (Sy) – (RxSy) combinations, with the total shoot age equal to the duration of the production period.

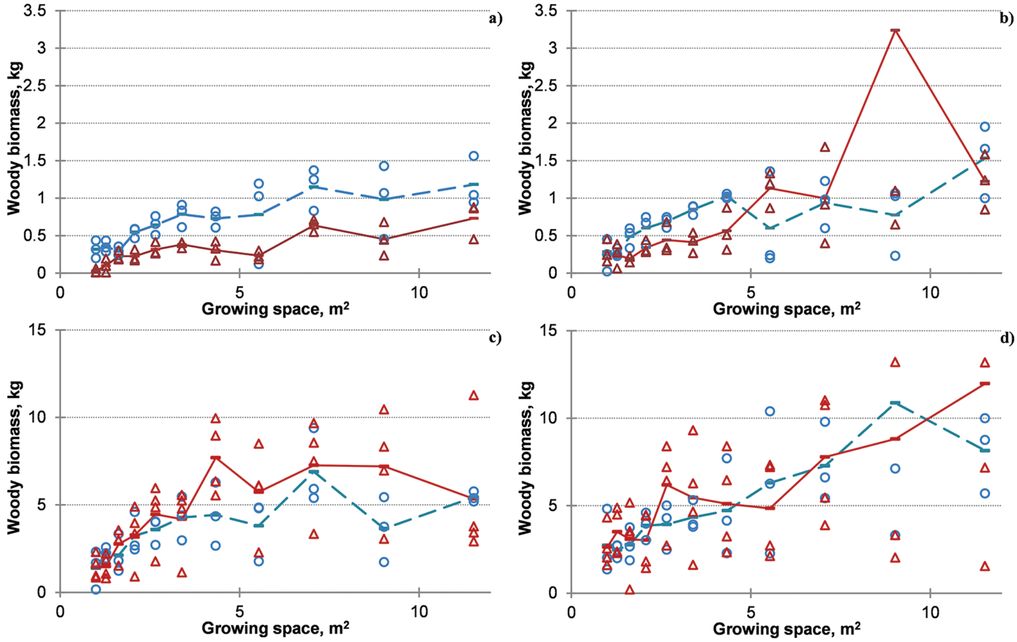


Fig. 4. Trends of cumulated woody biomass with growing space compared for different root ages or harvesting cycles.

Note: a) Harvesting cycle R2S1 (circles and dashed line) vs. R5S1 (triangles and solid line) for clone 'Agathe F'; b) Harvesting cycle R2S1 (circles and dashed line) *vs.* R5S1 (triangles and solid line) for clone 'Guardi'; c) Harvesting cycle R4S2, R5S1 (circles and dashed line) vs. R5S3 (triangles and solid line) for clone 'Agathe F'; d) Harvesting cycle R4S2, R5S1 (circles and dashed line) vs. R5S3 (triangles and solid line) for clone 'Guardi'. The markers indicate the individual plant values, while the line – their average The harvesting cycle is coded as a sequence of Root age (Rx) Shoot age (Sy) – (RxSy) combinations, with the total shoot age equal to the duration of the production period.

Significant spacing effect was also proven when 1- and 2-cut harvesting cycles were compared at 3-year production period (Table 3), showing dendromass increase with growing space (figs 4c and 4d). However, no significant effect of the harvesting cycle was distinguished, the accumulated amounts of 1- and 2-year-old shoots yielding similar quantities to those from 3-year-old poplar sprouts for both clones.

Increasing with growing space number of shoots was found at all investigated root age – shoot age combinations, but the compared poplar hybrids did not differ significantly in the number of sprouts produced (Table 4, Fig. 5). Shoot number increased with stump age, leading to manifestation of substantial differences regarding this parameter for both clones (Table 1, Fig. 6).

The statistically significant age x growing space interaction (Table 5) was related to the highest planting densities where no significantly different shoot number according to stump age was distinguished (at 1 m^2 for 'Agathe F') or only the youngest (R2S1) and the oldest (R5S1) plants had significantly different number of sprouts (at 1.28 m^2 for 'Agathe F' and at 1 m^2 for 'Guardi'). The number of shoots differed significantly according to stump age at the wider spacings for both clones (Fig. 6). No decrease in the sprouting ability was distinguished for both clones after 3 successive coppicings.

Table 4. Results of ANCOVA, testing the effect of clone and growing space on the number of shoots by root age – shoot age combinations.

Root age + shoot age	Factors and co-variates	*DF*	*SS*	*MS*	*F*-value	*P*-value
'R2S1'	Clone	1	3.64269	3.64269	0.144	0.709
	Clone×Growing space	1	20.4045	20.4045	0.808	0.381
	Growing space	1	390.223	390.223	15.44	0.001
	Residuals	18	454.827	25.2682		
	Clone	1	20.0455	20.0455	0.801	0.382
	Growing space	1	390.223	390.223	15.60	0.001
	Residuals	19	475.232	25.0122		
'R4S2'	Clone	1	598.470	598.470	1.599	0.210
	Clone×Growing space	1	520.828	520.828	1.391	0.242
	Growing space	1	24,729.8	24,729.8	66.06	3.369×10^{-12}
	Residuals	84	31,447.3	374.373		
	Clone	1	84.0455	84.0455	0.223	0.638
	Growing space	1	24,729.8	24,729.8	65.75	3.418×10^{-12}
	Residuals	85	31,968.2	376.096		
'R5S1'	Clone	1	12.4175	12.4175	0.074	0.786
	Clone×Growing space	1	64.2970	64.2970	0.385	0.537
	Growing space	1	13,007.4	13,007.4	77.83	1.502×10^{-12}
	Residuals	62	10,362.2	167.133		
	Clone	1	518.561	518.561	3.133	0.082
	Growing space	1	13,007.4	13,007.4	78.59	1.108×10^{-12}
	Residuals	63	10,426.5	165.500		
'R5S3'	Clone	1	79.1527	79.1527	0.133	0.716
	Clone×Growing space	1	8.21023	8.21023	0.014	0.907
	Growing space	1	6616.56	6616.56	11.12	0.001
	Residuals	84	49971.1	594.893		
	Clone	1	186.182	186.182	0.317	0.575
	Growing space	1	6616.56	6616.56	11.25	0.001
	Residuals	85	49,979.3	587.991		

Note: *DF* – degrees of freedom, *SS* – sum of squares, *MS* – mean square; * – plant age is abbreviated as the sequence of Root age (Rx) Shoot age (Sy) – (RxSy).

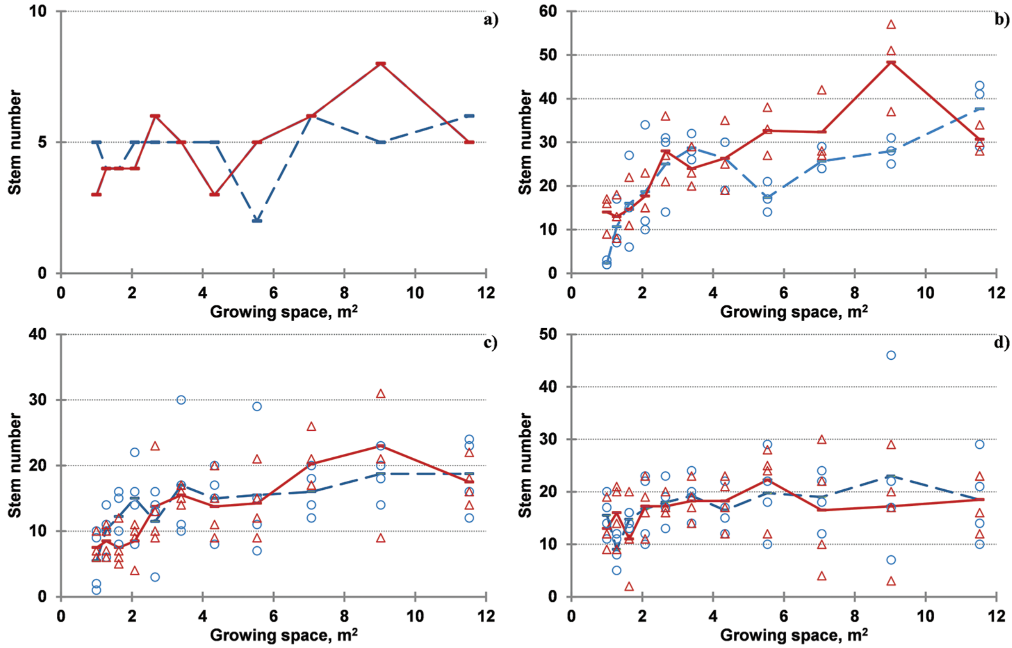


Fig. 5. Trends in number of stems with growing space by root age-shoot age combinations.

Note: a) R2S1; b) R5S1; c) R4S2; d) R5S3. Circles and dashed line indicate number of shoots and their average, respectively, for clone 'Agathe F', while triangles and solid line – for clone 'Guardi'. Plant age is abbreviated as the sequence of Root age (Rx) Shoot age (Sy) – (RxSy).

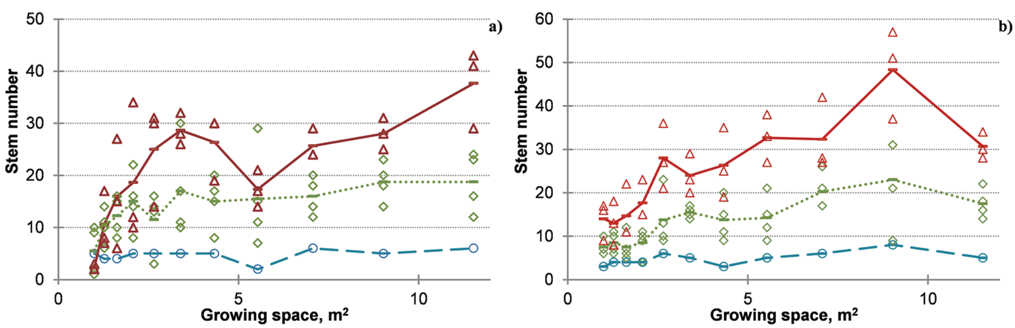


Fig. 6. Trends in number of stems with growing space by clones.

Note: a) clone 'Agathe F'; b) clone 'Guardi'. Circles and dashed line indicate stem number and their average, respectively, for plant age (R2S1). Diamonds and dotted line indicate number of shoots and their average, respectively, for plant age (R4S2), while triangles and solid line – for plant age (R5S1). Plant age is abbreviated as the sequence of Root age (Rx) Shoot age (Sy) – (RxSy).

Table 5. Results of ANCOVA, testing the effect of root age - shoot age and growing space on the number of shoots by clones.

Clone	Factors and co-variates	*DF*	*SS*	*MS*	*F*-value	*P*-value
‘Agathe F’	Age	2	446.148	223.074	0.866	0.424
	Age × Growing space	2	2673.93	1336.96	5.192	0.008
	Growing space	1	6874.93	6874.93	26.70	1.642×10^{-6}
	Residuals	82	21,116.8	257.522		
‘Guardi’	Age	2	3543.97	1771.98	12.77	1.489×10^{-5}
	Age × Growing space	2	1042.02	521.010	3.754	0.028
	Growing space	1	7082.76	7082.76	51.03	3.374×10^{-10}
	Residuals	82	11,381.1	138.794		

Note: *DF* – degrees of freedom, *SS* – sum of squares, *MS* – mean square, Age – Root age – Shoot age combination, abbreviated as the sequence of Root age (Rx) Shoot age (Sy) – (RxSy) with the levels R2S1, R5S1 and R4S2.

Discussion

Populus × *euramericana* ‘Agathe F’ is a black poplar hybrid, which has been described as a very perspective cultivar for wood production, having high ecological plasticity and long growth period (Tsanov and Mikov 1997). The potential of *Populus* × *euramericana* ‘Guardi’ for cultivation in short-rotation plantations has been recognized due to its fast initial growth. It has been described as a cultivar of the future, because of its ability to succeed in relatively adverse growth conditions (Tsanov and Mikov 1997). Although the ecological and biological characteristics of both clones suggest that they would fit well the transitional Mediterranean climate of the South-western Bulgaria plains, the plants of clone ‘Guardi’ appeared more suitable for the particular growth environment. While the trees of ‘Agathe F’ showed well distinguished growth decline in 2017, steady growth and increased dendromass yield was recorded for the plants of ‘Guardi’ (figs 3c, 4a, and 4b).

Brief examination of the meteorological and hydrological data for the period 2013–2017 (Fig. 2) showed worsening of the water availability to plants in 2017 that could have caused growth diminishing in ‘Agathe F’ plants resulting in significantly different biomass production between the clones at the end of the 4-year production period (Table 2, Fig. 3c). This outcome confirmed also that clone ‘Guardi’ resisted better the worsen growth conditions and its coppiced plants produced average annual yield of 4.0 Mg/ha/year dry mass in the last 4 years. Paris et al. (2018), who studied the biomass production of poplar short rotation crops with clone ‘Monviso’, established under Mediterranean climate and irrigated during the summer season, admitted that it was lower than the production from the same clone, but grown under non-irrigated, fertile alluvial soils of northern, continental Italy. The authors reported that the biomass yield varied according to the applied water amount from 4.1 to 5.3 Mg/ha/year for the poplar plants of 5-year-old roots and 2-year-old shoots, while it ranged from 3.8 to 6.8 Mg/ha/year for poplars of 6-year-old roots and 3-year-old shoots. They also derived the conclusion that solutions to improve the yield such as, choosing alternative species or clone and scheduling irrigation more efficiently, must be searched since biomass yield of 10 Mg/ha/year represents the break-even point to get financial profitability of poplar Short Rotation Crops in Italy. This advice should be considered also by the prospective biomass producers at the transitional Mediterranean conditions of South-western Bulgaria, since our estimated yields are comparable with the data of Paris et al. (2018), obtained for the control treatment of clone 'Monviso', irrigated with average amount of 115 mm during the summer. In addition, care should be taken to minimize the infestation by wood-boring insects, such as *Paranthrene tabaniformis* Rot. and *Saperda populnea* L., which destroy the invaded shoots, diminishing the usable amount of the biomass harvest and weakening the plants, making them susceptible to other pests. Furthermore, protection from *Capnodis tenebrionis* L., which is known to attack even healthy plants, must be ensured to avoid yield decrease and losing the entire plantation.

To explore the influence of root age on the biomass productivity of the selected poplar clones, we examined the dendromass yield from 1-year-old shoots at 2 and 5 years of stump age. Decreased with stump age dendromass production was found for the plants of 'Agathe F' in our study, but the growth of 'Guardi' remained stable after three successive coppicings (Table 3, figs 4a and 4b). From analysis of the environmental data, we found that in the 5th year from the establishment of the plantation (2017), the flow of river Struma, which is tightly connected with the underground water level, dropped nearly twice below the values measured in the preceding years (average annual flow 25 m^3/s vs 40–60 m^3/s). At the same time, the annual rainfall amount was below 500 mm and the average monthly temperature in January dropped below zero, which is unusual for the transitional Mediterranean climate (Fig. 2). These observations indicate that in 2017 in our trial plantation the highly water-demanding poplar plants were exposed to abiotic stress, leading to declined vigor and weakened resistance to pests. Our conclusion agrees with the inference by Bergante and Facciotto (2011) that water, obtained from irrigation and from rainfall seems to be of crucial importance for biomass production from short-rotation crops, established with Salicaceae species. The outcome of the analysis suggested also higher vulnerability of clone 'Agathe F' in comparison with 'Guardi' at the particular site conditions as well as its susceptibility to the root destroying insect *C. tenebrionis*.

As it has been found in studies by other investigators (Laureysens et al. 2003, Paris et al. 2015), the number of shoots per plant increased significantly with stump age for both clones. In agreement with Laureysens et al. (2003), the increased number of shoots observed can be attributed to the bigger stump diameter at more advanced age. Unlike the study by Štochlová et al. (2019), our results did not reveal statistically significant difference in the number of shoots, produced by the two poplar clones. Parallel comparison of the findings on the shoot number and the biomass amounts for the 1-year-old shoots (figs 4a, 4b, 6a, and 6b, tables 3 and 5) suggested that the significantly increased number of the one-year-old sprouts at 5 years stump age, although positively related to plant biomass production (Stankova et al. 2017), does not directly derive its increase. This result is in concordance with the observation by Oliveira et al. (2017) that there is greater uncertainty in the biomass estimation in the year following coppicing, because this growth stage is related to the shoot, rather than to the root age. In addition, the study by Paris et al. (2015) showed that coppicing significantly decreased shoot dimensions. Finally, the aforementioned plant weakening due to the worsened environmental conditions suggested and the experimental data provided in Stankova et al. (2017) confirmed that the increased number of 1-year-old shoots, recorded in 2017, was related to a decreased shoot size of the poplar plants, as compared to 2014.

Our results showed that the one cut of 3-year-old shoots and the two successive cuts of 2- and 1-year-old shoots yielded similar amounts of woody biomass for both clones (Table 3). This finding is particularly important, if we consider that for the same final yield, a greater number of cuts implies higher cost and more energy consumption. The average amount of dendromass cumulated per plant of 5-year-old roots within the last 3 years of growth was around 4.2 kg for the ramets of 'Agathe F' and 5.4 kg for the ramets of 'Guardi' (figs 4c and 4d). The outcome of the comparison between the harvesting cycles in our study agrees with the finding about different clones of poplar, willow and black locust in Central Germany, where the rotation period did not significantly affect tree biomass accumulation and two 3-year cycles produced the same amounts of dendromass as one 6-year harvesting cycle (Gruenewald et al. 2007). By contrast, Blake (1983) found that in *Populus trichocarpa*, a single harvest after 8 years was one third more productive than two rotations of 4 years. Similarly, Armstrong et al. (1999) reported that a single harvest in a 4-year cutting cycle yielded more biomass than two harvests on a 2-year cycle in a study of biomass production in 3 poplar clones grown at 2 densities on 3 sites in the UK. Furthermore, the study by Nassi o di Nasso et al. (2010) with the clone 'Lux' of *Populus deltoides* Bartr. at temperate climate conditions derived the conclusion that the biomass production at 3-year cutting cycle is significantly higher than the dendromass harvested at 1- and 2-year periods of coppicing. The results from the present study disagree also with the finding by Stankova et al. (2019) about the open-pollinated families of two black locust clones, which showed that one-coppicing cycle resulted in a significantly higher yield than the combination of two or three coppicings within 2- or within 4-year production periods for both black locust families.

Studies investigating plant biomass or volume in relation to growing space have generally reported positive correlations between biomass/volume of individual plants and spacing (Jink and Mason 1998, Bernardo et al. 1998, Erkan and Aydin 2016). We found that spacing significantly affected tree dendromass in both clones and all harvesting cycles considered (tables 2 and 3). The individual plant biomass generally increased with spacing, but growth approached saturation at the lowest densities for the plants of clone 'Agathe F', showing that no advantage of wider growing area has been taken by trees at spacing above 7 m^2 (figs 3 and 4).

Conclusions

The plants of clone 'Agathe F' revealed substantial growth decline in the 5th year of plantation establishment due to worsened environmental conditions and biotic pests. Clone 'Guardi' proved its higher suitability for cultivation as a short rotation crop under Mediterranean climate, because of its steady growth even at worsen site conditions. Under unfavourable alterations in the water availability and without additional irrigation, coppiced plantations of 'Guardi' would produce above 4 Mg/ha/year dry dendromass, if cultivated on light arable Fluvisols. Growing space proved to be a major determinant of dendromass yield from juvenile coppiced plants of the investigated black poplar hybrids. The biomass yield of 'Guardi' increased steadily across the spacings from 1.0 to 11.5 m^2, while that of 'Agathe F' showed tendency to saturation at spacing above 7 m^2.

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