

1 **Effect of spacing, parental genotype and harvesting cycle on biomass production in two half-sib progenies**
2 **of *Robinia pseudoacacia* L.**

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9 **Abstract**

10 This study investigated the influence of spacing, parental genotype and harvesting cycle on woody biomass
11 production in open-pollinated families of the locally selected black locust (*Robinia pseudoacacia* L.) clones
12 ‘Tsarevets’ and ‘Srebarna’ at specific site conditions.

13 A Nelder experiment with 16, varying along the spokes, nearly-square spacings and two black locust families
14 arranged in alternating sectors, was established in North Central Bulgaria. Alternative harvesting options
15 (‘harvesting cycles’), consisting of one, two or three successive coppicings, were applied and the amounts of dry
16 shoot dendromass collected per plant were accumulated to calculate and analyse the total yield of woody biomass,
17 achievable within 2-, 3- and 4-year periods (‘production period’).

18 The one-coppicing cycle proved superior for both families, regardless of the length of the production period.
19 Biomass yield in the family of ‘Srebarna’ exceeded that of ‘Tsarevets’ at the wider spacings, when 1 and 2 year-
20 old shoots were harvested, while dendromass production of ‘Tsarevets’ was substantially higher in the 4-year-old
21 shoots. The woody biomass yield per plant increased with spacing, and growth tended to saturation at the lowest
22 densities, enabling derivation of optimal planting densities. The total dendromass yield per hectare was maximized
23 at growing space of around 0.5 m² per plant for the 2-year production period, while spacing of 1.2–2 m² was
24 required for production periods of 3 and 4 years. If the results from this experiment can be reliably scaled up in
25 practice then short rotation crops of ‘Srebarna’ and ‘Tsarevets’ families at comparable site conditions should be
26 able to produce annual biomass yields of 9–11 Mg.ha⁻¹.year⁻¹ within a 4-year period, given the optimal planting
27 densities and harvesting regimes prescribed.

28 **Introduction**

29 The widespread popularity of the black locust tree (*Robinia pseudoacacia* L.), which is not native to Europe, has
30 led to the species being the third most planted hardwood tree worldwide (De Gomez and Wagner, 2001). Among
31 its numerous uses, this species shows strong promise for use in energy crops, because it is easily propagated even
32 on marginal lands (Gruenewald et al., 2007; Böhm et al., 2011; Carl et al., 2017), requires little agricultural input,
33 produces high biomass yields and is efficiently converted into a variety of forms of energy (Straker et al., 2015).
34 The frequently expressed notion of rapid growth rate in association with low wood density has clearly been
35 disproved by the black locust (Rédei and Veperdi, 2009). Other properties of black locust such as the vigorous
36 growing potential of the juvenile phase, along with the favourable combustibility, relatively fast drying and
37 processing of the wood have been emphasized and explain the excellent potential of the species for use in short-
38 rotation plantations for biomass production (Rédei and Veperdi, 2009).

39 The black locust tree was introduced to Bulgaria in the middle of the 19th century and was widely cultivated in
40 parks, gardens and at roadsides (Petkova et al., 2017). By the end of the century, the mass propagation of black
41 locust seedlings began in state and municipal nurseries (Naydenov and Dimitrova, 2018). This was the start of a
42 process of expanding afforestation, especially in the lower forest belt, after native *Quercus cerris* L. stands were
43 felled and uprooted (Panayotov et al., 2006). Black locust currently occupies an area of more than 150 000 hectares,

44 which represents around 4 per cent of the total forest area in the country (Petkova et al., 2017), over an altitudinal
45 range of 0 to 1500 m a.s.l. (Kalmukov, 2006; Panayotov et al., 2006). Since the black locust breeding program
46 was initiated in Bulgaria in the 1970 s, more than 80 trees have been phenotypically selected. Together with elite
47 clones imported from Hungary, Serbia, Slovakia and Romania, the selected trees have been used to establish more
48 than 30 clonal trials and more than 25 progeny tests at different sites throughout the country (Kalmukov, 2014).
49 This was followed by establishment of more than 25 seed orchards, occupying over 60 ha in different parts of
50 Bulgaria (Kalmukov, 2014).

51 The results of the black locust progeny trials in Bulgaria revealed a high degree of heritability of important
52 quantitative and qualitative tree traits (Kalmukov, 2002, 2014). The outcomes of the clonal experiments identified
53 more than 20 locally selected black locust genotypes, including ‘Tsarevets’ and ‘Srebarna’, as being of proven
54 superiority (Kalmukov, 2014). Rédei et al. (2011) did not find differences in biomass yield between the selected
55 black locust clones and non-selected (common) black locust in Hungary and concluded that using expensive black
56 locust clonal material to establish short rotation plantations is of no added value. However, since the research
57 results in Bulgaria showed that black locust is able to inherit valuable characteristics, such as productivity
58 (Kalmukov, 2002, 2014), we believe that half-sib progenies of selected parental genotypes could potentially be
59 used to establish energy crops of improved yield and at low cost, using propagules, already available from the
60 seed orchards.

61 According to Namkoong (1965), it is reasonable to expect that genotypes within species will vary in their
62 responses to density, and therefore testing under single spacing regimes is satisfactory only if there is little or no
63 genetic variance in response to a range of densities. The author inferred that to study the relations between density
64 and parameters of growth, branching, and other traits, it is necessary to sample an adequately wide range of
65 densities in any single field experiment. An appropriate design for such experiment was proposed by Nelder (1962)
66 and is a circular plot containing concentric circumferences, with spokes connecting the centre with the furthest
67 circumference, where the trees are planted at the intersections of spokes and circumferences. Nelder (1962) sug-
68 gested four circular (‘wheel’) designs and according to Namkoong (1965) two of them could be adapted for small
69 family or genotypic plots of interest to tree breeders. Of these two, in Nelder’s design ‘Ia’ the size of the growing
70 space varies along the spokes and the genetic factor changes along the concentric circumferences of the wheel.
71 Nelder’s design overcomes some of the disadvantages of the full factorial design, such as differential precision of
72 estimate and a great waste in trees (if the plot areas per density level are equal) and error heterogeneity (if the
73 numbers of trees per density level are equal) (Namkoong, 1965). The design is efficient also because of the small
74 size of the plot area, allowing homogeneity of the site conditions to be ensured. A trial plantation was established
75 in the autumn of 2013 in North Central Bulgaria using Nelder wheel design with 2 half-sib families of the locally
76 selected black locust clones ‘Srebarna’ and ‘Tsarevets’ and 16 nearly-square spacings. The experiment was
77 designed to study the effect of parental genotype, spacing and harvesting regime on the short-term dendromass
78 production.

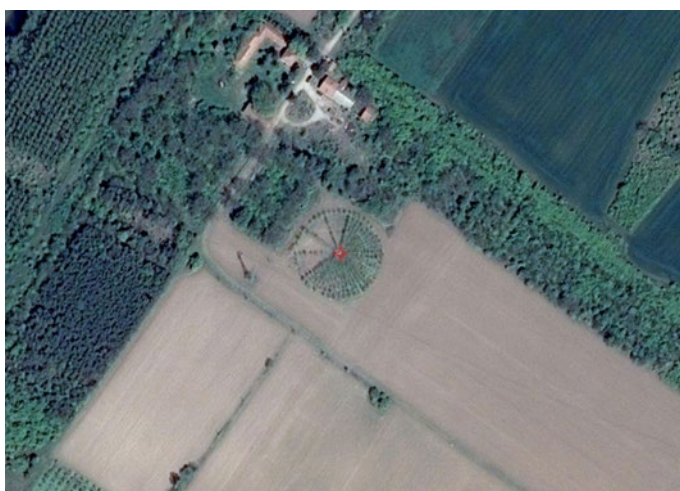
79 It has been suggested that research on short rotation forestry should focus on genetic improvement, plantation
80 design, rotation length and tending operations (Cañellas et al., 2012). In order to address the first of these priorities,
81 species and genotypes have been compared in relation to their juvenile growth potential under different site
82 conditions (Bernardo et al., 1998; Armstrong et al., 1999; Geyer, 2006; Gruenewald et al., 2007; Stolarski et al.,
83 2013). Furthermore, the effect of spacing on biomass production has been explored in selected poplar clones
84 (Armstrong et al., 1999; Cañellas et al., 2012), black locust (Rédei and Veperdi, 2009, Rédei et al. 2010a,
85 Kalmukov, 2013), willow (Willebrand and Verwijst, 1993) and eucalypts (Bernardo et al., 1998). Different aspects
86 of how age and length of rotation influence the amount of harvested biomass have also been investigated
87 (Willebrand and Verwijst, 1993; Armstrong et al., 1999; Geyer, 2006; Gruenewald et al., 2007).

88 In relation to the importance of *Robinia pseudoacacia* as a short-rotation crop and considering the state-of-art of
89 the research on energy plantations as well as the advances in the black locust breeding program in Bulgaria, the
90 present study aimed to explore the influence of spacing, parental genotype and harvesting cycle on woody biomass
91 production in open-pollinated families of the locally selected black locust clones ‘Tsarevets’ and ‘Srebarna’ at the
92 specific soil and climate conditions presented in the experimental plantation in North Central Bulgaria.

93 **Methods**

94 **Experimental plantation and data collection**

95 The study was carried out in an experimental black locust plantation established in the autumn of 2013 in the
96 Nikopol Forestry Estate nursery in Gulyantsi, North Central Bulgaria (Fig. 1). The nursery is situated at 29 m a.s.l.
97 in a flat area in the Vit valley. The climate is temperate continental, with cold winters, primarily due to western
98 winds, and hot summers. Spring begins early but is characterized by late frosts, until 18–20 April. The autumn is
99 warmer than the spring, and the temperature drops below 10°C at around 24–30 October. The growth period lasts
100 for about 7–8 months. The mean annual temperature is 11.3°C and the total annual precipitation is about 515 mm,
101 with maximum levels in June and minimum levels in February–March. July is the hottest month of the year (mean
102 temperature 22–24°C), with long dry periods, while January is the coldest month (mean temperature –2.2°C).
103 Snow cover is unstable and lasts around 48–53 days a year (Silva 2003 Ltd., 2016). The soil is Calcic Chernozem,
104 slightly to moderately alkaline, of sandy clay texture and high bulk density, but with good porosity and water
105 permeability. It is characterized by low nitrogen, very low phosphorus and high potassium contents, an optimal
106 C:N ratio, and low amounts of soil organic matter. The groundwater level is below 2 m (Silva 2003 Ltd., 2016).



107

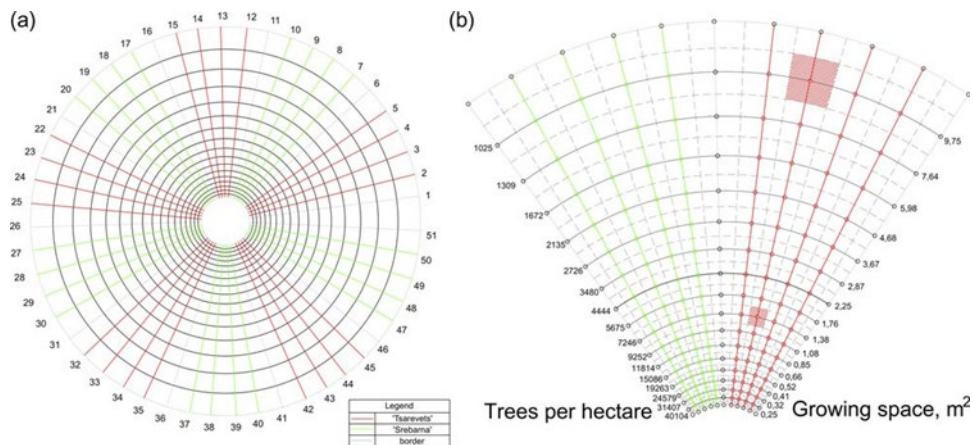
108 Figure 1 The experimental black locust plantation in North Central Bulgaria (43°37'36.4" N, 24°41'39.1" E).

109 The trial plantation was established following a Nelder wheel experimental design (Nelder, 1962; Namkoong,
110 1965) with 16 nearly-square spacings, ranging from 0.25 to 9.75 m² and corresponding to initial densities of 40
111 000 to 1000 plants per hectare (Fig. 2b). The half-sib families of the locally selected black locust clones 'Srebarna'
112 and 'Tsarevets' were arranged in 10 four-spoke alternating sectors, separated by border spokes (51 spokes in total)
113 (Fig. 2a). The planting density was varied along the spokes, the intersections of the spokes and circumferences
114 being the planting point locations, to form 18 concentric circumferences, with the innermost and the outermost
115 circumferences acting as borders (Fig. 2). This specific layout of the experiment prevented between-family-
116 competition and secured isolation of the different harvesting scenarios (i.e. sectors coppiced at different times).
117 To provide site conditions of maximum soil homogeneity, an area comprising 3500 m² of flat nursery land of
118 approximately square form was tilled to a depth of 35 cm and further prepared by triple disking before the
119 experiment was established. Standard two-year-old seedlings were planted in holes (30×30×30 cm), marked
120 according to the design. Combined NPK fertilizer was applied around the trees, and the plots were watered twice
121 immediately after trial establishment, to ensure maximum survival rate of the planted stock. This is particularly
122 important for Nelder plots, because of the sensitivity of the analysis to tree mortality (Parrott et al., 2011) and it
123 has been recommended that seedling survival should be a primary concern when establishing Nelder plantings.
124 Managers should consider double plantings, intensive weed control, and other site preparation methods to increase
125 survival (Parrott et al., 2011). The seedlings were coppiced at the beginning of 2014 to restart their growth at
126 different growing spaces.

127 Data were collected four times throughout the study: at the beginning of spring in 2015 and 2018, and in autumn
128 in 2015 and 2016, i.e. four growth periods were comprised in total. To reduce, as much as possible, the effect of
129 random variation due to probable site heterogeneity resulting from, e.g. presence of vegetation belts in north-
130 eastern and northwestern directions (Fig. 1), adjacent sectors from both families were harvested simultaneously
131 (Fig. 3). In addition, the plantation was inspected visually each time before harvesting and the half-wheels in
132 north-southern and east-western directions were compared to avoid systematic growth heterogeneity due to
133 microsite differences. The sprouts of each sample tree were cut as close as possible to the stump. All shoots on
134 each stump were weighed in situ, to the nearest 0.005 kg. Separate samples of lignified biomass (100–300 g),
135 combining parts of stems and branches proportionally to their share in the whole (i.e. all harvested shoots of the
136 plant), were obtained from three of the sampled at that time trees in each sector, across the spacing range (i.e.
137 from plants at low, medium and high density). The fresh weights of the samples were measured, they were oven-
138 dried at 105°C to constant mass and weighed again to the nearest 0.001 kg. Proportions of dry mass relative to the
139 fresh weight of the samples were averaged per sector and were subsequently used to estimate the total amount of
140 dry dendromass of the harvested shoots of each tree in the corresponding sector.

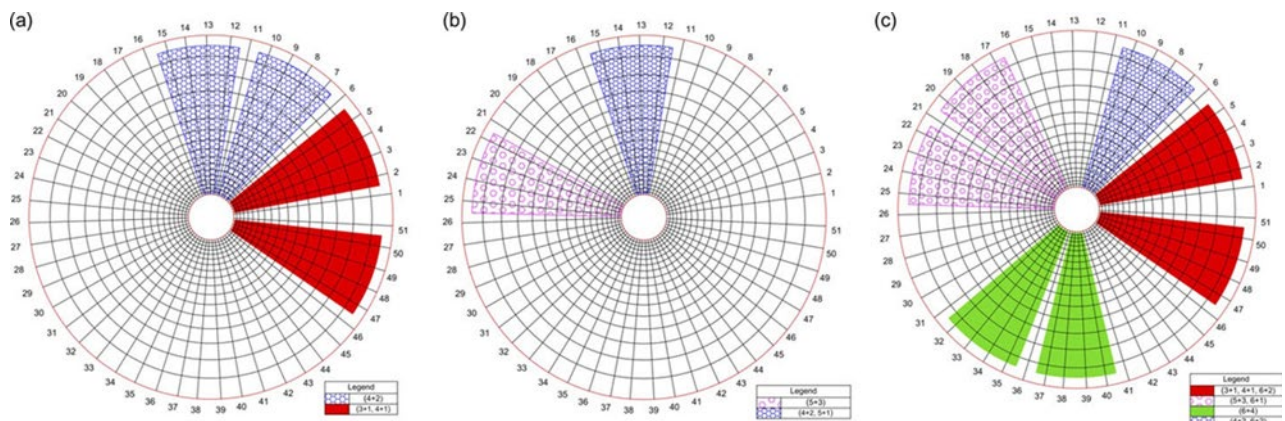
141 The amounts of dry dendromass, harvested from each plant until the end of the second (2015), the third (2016)
142 and the fourth (2017) year, were accumulated to estimate the total yield of woody biomass per plant achievable
143 within 2-, 3- and 4-year periods of biomass production, respectively, referred hereafter as ‘production periods’
144 (Table 1). According to the sampling design, these total biomass yields resulted from various number of cuts: one
145 or two coppicings within 2- and within 3-year periods of dendromass production, one, two or three successive
146 coppicings within the 4-year production period. Each particular mode of biomass harvesting (i.e. one, two or three
147 cuts) within the respective production period is referred hereafter as ‘harvesting cycle’ (Table 1). The total yield
148 of dry dendromass from the shoots, harvested per plant, achievable within particular production period (i.e. 2, 3
149 or 4 years) was the principal investigated variable in this study.

150 The trees in two sectors (one four-spoke sector per family) were harvested 3 times: after the first (2014), the
151 second (2015) and the fourth (2017) growth period. They provided data on dendromass yield from a two-coppicing
152 cycle (denoted as S11 and T11 in Table 1) within 2-year period of production (2014–2015) (Fig. 3a) and from
153 three-coppicing cycle (denoted as S112 and T112 in Table 1) within 4-year period of production (2014–2017)
154 (Fig. 3c). Another two sectors (one sector per family, denoted as S2 and T2 in Table 1) were sampled in the
155 autumn of 2015, yielding woody biomass data from coppiced plants of 4-year-old roots and 2-year-old shoots,
156 which were compared as one-cut alternative to the two-coppicing cycle described earlier, when a 2-year period of
157 dendromass production (2014–2015) was considered (Fig. 3a). The sector of ‘Srebarna’, from the latest two
158 described, was resampled in 2018 and data were utilized to estimate the total harvest per plant by means of two
159 successive cuts (denoted as S22 in Table 1) within the 4-year period of production (Fig. 3c). The sector of
160 ‘Tsarevets’, on the other hand, was resampled in the autumn of 2016 to yield data, used to estimate the total
161 harvest per plant by means of two successive cuts (denoted as T21 in Table 1) for comparison at 3-year period of
162 dendromass production (2014–2016) (Fig. 3b). The trees in 2 sectors (denoted as S31 and T31 in Table 1) were
163 sampled for the first time in the autumn of 2016, resampled 1 year later and the total dendromass harvested per
164 plant by means of the two consecutive cuts formed the data set for the respective two-coppicing cycle (5 + 3, 6 +
165 1 in Table 1) within 4-year period of production (Fig. 3c). In addition, the data of ‘Tsarevets’ family from plants
166 of 5-year-old roots, which present one-cut and two-cut harvest- ing cycles (i.e. T3 and T21 in Table 1) were
167 compared at 3-year period of dendromass production (2014–2016) (Fig. 3b). Finally, sectors planted with both
168 families, which had not been cut after the initial coppicing, were harvested in 2018 and dendromass data from
169 one-coppicing cycle (denoted as S4 and T4 in Table 1) within the 4-year period of production (2014–2017) became
170 available for comparison (Fig. 3c).



171

172 Figure 2 Design of the Nelder wheel experimental plantation (a) Arrangement of the families in alternating four-
 173 spoke sectors; (b) Distribution of the planting densities along the spokes. The dotted lines indicate border spokes
 174 and circumferences. The planting spots are indicated with circles and the dashes lines delineate the corresponding
 175 growing spaces. The hatched areas exemplify the growing spaces corresponding to plants at spacings 1.38 m² and
 176 9.75 m².



177

178 Figure 3 Sampling design (a) 2-year production period; (b) 3-year production period ('Tsarevets' clone); (c) 4-
 179 year production period. The different sector patterns indicate the compared harvesting cycles within the respective
 180 production periods, while the different families treated with the same harvesting cycle have the same pattern.

Table 1 Description of the data on coppiced black locust plants.

PC	Production period	HC'	PCxHC''	Spokes	Number of plants'''	Dry weight of woody biomass per plant, kg Mean (Minimum – Maximum)''''
Srebarna	2 years	3 + 1, 4 + 1	S11	47–50	59	1.1419 (0.0184–3.1192{
		4 + 2	S2	7–10	64	2.0406 (0.2410–5.6783{
	4 years	3 + 1, 4 + 1, 6 + 2	S112	47–50	40	3.6901 (0.0643–13.5932{
		4 + 2, 6 + 2	S22	7–10	64	5.5173 (0.2694–19.8648{
		5 + 3, 6 + 1	S31	17–20	47	5.3348 (0.0919–17.2836{
Tsarevets	2 years	3 + 1, 4 + 1	T11	2–5	57	0.7771 (0.1317–2.2850{
		4 + 2	T2	12–15	52	1.8711 (0.0384–6.3674{
	3 years	4 + 2, 5 + 1	T21	12–15	43	3.3067 (0.2862–9.2659{
		5 + 3	T3	22–25	64	4.6859 (0.0419–17.1705{
	4 years	3 + 1, 4 + 1, 6 + 2	T112	2–5	56	3.0716 (0.1550–8.1914{
5 + 3, 6 + 1		T31	22–25	59	6.6045 (0.0735–19.3880{	
		6 + 4	T4	32–35	56	9.7930 (0.0218–33.3736{

181

182 Abbreviations: PC – parental clone, HC – harvesting cycle, PC x HC - parental clone– harvesting cycle
 183 combination.

184 * The harvesting cycle is presented as a sequence of Root age + Shoot age combinations.

185 ** PC x HC is coded as: Clone name (T for ‘Tsarevets’, S for ‘Srebarna’), harvested shoot age (1, 2, 3, 4 years)
186 at each successive coppicing.

187 *** The number of plants in the sector at the end of the production period.

188 **** Presents the total biomass yields resulted from various (one, two or three) number of successive coppicings.

189 Prior to the analysis, we excluded measurements from those trees which had not resprouted or which were adjacent
190 to missing plants or to plants that had not re-sprouted, and for which the space available therefore did not
191 correspond to the design. Due to the relatively high survival rate after establishment and regrowth after coppicing,
192 data on a pooled across the spacings average of 3.4 plants (minimum 2.5 plants) per clone-harvesting cycle-
193 spacing combination were available for ‘Srebarna’ and of 3.6 plants (minimum 2.7 plants) for ‘Tsarevets’ (Table
194 1).

195 **Statistical analysis**

196 Firstly, we investigated how the amount of dendromass, harvested per plant within certain interval of time
197 (production period), varied between families or harvesting cycles and according to the different spacing. Growing
198 space, which ranged from 0.25 to 9.78 m², was considered a continuous variable and was treated as covariate to
199 the main factors ‘parental genotype’ and ‘harvesting cycle’. One-factor analysis of covariance (ANCOVA) was
200 used to examine the influence of the factor parental genotype and the covariate spacing on the dendromass yield
201 by harvesting cycles (Table 2) and, afterwards, we explored in similar manner the effect of the factor harvesting
202 cycle and the covariate spacing in the different families (Tables 3 and 4). Following the standard ANCOVA pro-
203 cedure, we first examined the full model, including the factor, the covariate and their interaction. Significant factor
204 x covariate interaction indicates that the factor effect is dependent upon the value of the covariate and it is
205 impossible to claim significance or non-significance of the factor throughout the range of the covariate under
206 consideration. Consequently, the interpretation of the factor effects per se in the presence of significant factor x
207 covariate interaction is misleading (Quinn and Keough, 2002) and therefore is not performed. Considering the
208 above, when there was no statistically significant factor x covariate interaction, the significance of the factor effect
209 throughout the range of the covariate was tested. When there was statistically significant factor x covariate
210 interaction, the significance of the factor effect at each particular level of the covariate (i.e. growing space) was
211 examined and interpreted. Tukey multiple comparison test was used to identify significantly different groups of
212 observations according to factor levels.

213 We performed graphical examination of the data, following the procedure, described by Picard et al. (2012), to
214 check for possible violations of the assumptions for the residuals of the ANCOVA model. When we plotted the
215 dependent variable plant dendromass yield (denoted as ‘woody biomass’ on the charts) against the covariate
216 growing space by factor levels (Figs 4–7), we noticed that the data points were not evenly distributed on both
217 sides of the visualized main relationships, but their variance increased with the increase in spacing. Since this
218 observation diagnoses heteroscedasticity of errors (Picard et al., 2012), we used in our study the non-parametric
219 rank transform ANCOVA method, proposed by McSweeney and Porter (1971). This method involves separate
220 transformation of the covariate and the dependent variable, substituting ranks across treatment groups for the
221 original observations. The ranked data can then be analysed using the same procedures as for parametric
222 ANCOVA. The resulting test statistic has an F-distribution with the same degrees of freedom as those associated
223 with the parametric ANCOVA test statistic (Olejnik and Algina, 1984).

224 On the next step, we examined how harvested biomass increased with increasing growing space for each particular
225 family-harvesting cycle combination (Figs 6–7) by fitting a sigmoidal regression model:

$$y = b_0 e^{(-b_1/x)} \quad (1)$$

226

227 where y is the total biomass yielded per plant (kg) for the respective family-harvesting cycle combination, x is the
 228 growing space per plant (m^2), b_0 is the asymptote and b_1 is the scale parameter ($b_1 > 0$). The model was used to
 229 estimate the total dendromass per hectare Y (Mg. ha^{-1}) for each particular density:

$$Y = y \frac{10000}{x} \frac{1}{1000} = 10b_0 e^{(-b_1/x)} x^{-1} = b_0' x^{-1} e^{(-b_1/x)} \quad (2)$$

230

231 where $b_0' = 10b_0$. The multiplier $\frac{1}{1000}$ converts kg into Mg and the multiplier $\frac{10000}{x}$ estimates the number of plants
 232 per hectare (10 000 m^2), assuming square growing space of x m^2 per plant. The function for Y (Equation (2))
 233 attains the maximum value $y_{\max} = \frac{b_0'}{b_1} e^{-1}$ at a growing space $x_{\max} = b_1$, which yields the optimal planting density
 234 $\text{Nopt} = 10000/b_1$ for each family-harvesting cycle combination.

235 The function for the harvested woody biomass per plant (Equation (1)) is monotonically ascending and saturating
 236 at an upper asymptote b_0 . At a growing space equal to 5 times the optimal growing space x_{\max} , the respective plant
 237 biomass $y(5x_{\max})$ makes 82 per cent of the asymptotic biomass value b_0 , which is a feasible to achieve maximum
 238 dendromass yield per plant for practical purposes. However, the total dendromass yield per hectare for this spacing
 239 $Y(5x_{\max})$ amounts to 45 per cent from the maximum yield Y_{\max} attainable at the optimal growing space (x_{\max}).
 240 Considering the above, we used the 5 times optimal growing space ($5x_{\max}$) to define a minimum planting density
 241 that can be recommended for each family-harvesting cycle combination, such that the individual plants take
 242 advantage of the available growing space, but also the yield loss due to the lower stocking rate is bearable.

243 Equation (1) was fitted using ordinary least squares regression. The normality of errors was evaluated according
 244 to the Shapiro–Wilk analytical test, the skewness and kurtosis values and by inspection of the Quantile–Quantile
 245 plot. Homoscedasticity of errors was estimated by plotting the residuals against predicted values and by using
 246 White and Breusch–Pagan analytical tests. If homoscedasticity was not unequivocally demonstrated by all tests,
 247 the Heteroscedasticity Consistent Covariance Matrix estimator was applied to ensure the efficiency of the
 248 regression estimates (Long and Ervin, 2000). Regression models were examined for bias by t-test for a mean error
 249 equal zero, and the goodness-of-fit was assessed using the adjusted coefficient of determination (R^2_{adj}) and the
 250 root mean squared error (RMSE).

251 Results

252 The non-parametric ANCOVA used to investigate the effect of clone and spacing for each mode of harvesting
 253 revealed a significant ($P < 0.05$) or marginally significant ($P < 0.1$) interaction between genotype and growing
 254 space in most of the cases (Table 2). This was because both half-sib families produced similar amounts of biomass
 255 at high densities, but substantially different biomass quantities when the spacing became sufficiently large (Figs
 256 4a and 4b, 5b and 5d). We observed that the open-pollinated ‘Srebarna’ trees exploited the growing space better
 257 at younger shoot ages (1- and 2-year-old shoots), when its increase in biomass exceeded that of the other family
 258 at the wider spacing (Figs 4a and 4b, 5d). However, biomass production in the 4-year-old shoots of the ‘Tsarevets’
 259 progeny was substantially higher than that of ‘Srebarna’ for a growing space above 2 m^2 (Fig. 5b).

260 We also compared the biomass yields in the different har-vesting cycles within each family. This revealed a
 261 significant interaction between growing space and harvesting cycle in only one case (Tables 3 and 4). For the two-
 262 year production period, the biomass amounts for both clones were significantly greater when 2-year-old shoots
 263 were harvested (Figs 4c and 4d). For the ‘Tsarevets’ progeny, harvesting of 3-year-old shoots yielded significantly
 264 more biomass than the consecutive harvesting of one- and two-year old shoots in the 3-year production period
 265 (Table 3, Fig. 5a). The same trend was observed in four-year production period, and the cumulative yield from
 266 one- and two-year old shoots was lower than the dendromass of the 4-year- old shoots (Table 4). ‘Tsarevets’ takes
 267 advantage of the wider spacing to produce significantly higher amount of aboveground biomass at more advanced
 268 age, while the total dendromass production from the different harvesting cycles varied within narrow margins for
 269 ‘Srebarna’ (Table 4).

270 All analyses revealed that the covariate ‘growing space’ had a significant effect on biomass production (Tables
 271 2–4). The increase in biomass with growing space increase was well distinguished (Figs 6 and 7) and the linear
 272 relation assumed by ANCOVA showed a reasonably good approximation (data not shown). However, a tendency
 273 to growth saturation at the lowest densities also could have been observed (Figs 6 and 7), which suggested the
 274 examination of a non-linear regression model. The sigmoidal model (Equation (1)) fitted reasonably well to the
 275 data subsets for the 3- and 4-year production periods, yielding coefficients of determination greater than 0.6 in 5
 276 and greater than 0.5 in 2 of nine cases (Table 5). The poorer fit of the regression models (Equation (1)) for the 2-
 277 year production period was resolved by fitting Equation (2) instead of Equation (1) (Table 5). Symmetric residual
 278 distributions, with average values not differing significantly from zero, were revealed by both graphical and
 279 analytical tests, although a tendency for leptokurtic deviation from normality was noted for five of the 13 data
 280 subsets. Nevertheless, the effect of non-normality of errors on the regression inferences is not considered serious,
 281 as the means will follow the normal distribution more closely than the distribution of the variates themselves
 282 (Sokal and Rohlf, 1995; Draper and Smith, 1998; Wheeler, 2013) and provided that the residual distribution is not
 283 severely asymmetric (Sokal and Rohlf, 1995). The optimal growing space was around 0.5 m² per plant for the 2-
 284 year production period, while spacing of 1.2–2 m² per plant was required for production periods of 3 and 4 years
 285 in most cases (Table 5). A total woody biomass yield of 11–22 Mg.ha⁻¹ is expected within a 2-year production
 286 period, while the predicted amount of harvest at 4-year production period ranged from 27 to 46 Mg.ha⁻¹ (Table 6).
 287 The maximum annual increment of lignified biomass, estimated for both families, exceeded 11 Mg.ha⁻¹.year⁻¹
 288 (Table 6). The recommended minimum planting densities ranged from 1000 to 4500 ha⁻¹ depending on the parental
 289 clone and har-vesting regime (Tablet 6).

290 Table 2 Results on the effect of parental clone and growing space on the total harvested dendromass by harvesting
 291 cycles, for 2- and 4-year production periods.

Production period		DF	MS	F-value	P-value	
2 years	<i>One coppicing (4 + 2)</i>					
	Clone	1	647.077	0.849	0.359	
	Clone x Growing space	1	2244.82	2.947	0.089	
	Growing space	1	33 429.6	43.886	0.001	
	Residuals	112	761.743			
	<i>Two coppicings (3 + 1, 4 + 1)</i>					
	Clone	1	1257.15	1.377	0.243	
	Clone x Growing space	1	5411.60	5.927	0.016	
	Growing space	1	17 964.5	19.675	0.001	
	Residuals	112	913.053			
	4 years	<i>One coppicing (6 + 4)</i>				
		Clone	1	1061.27	2.561	0.113
		Clone x Growing space	1	2908.66	7.020	0.009
		Growing space	1	44 951.3	108.486	0.001
Residuals		99	414.352			
<i>Two coppicings (5 + 3, 6 + 1)*</i>						
Clone		1	8.55266	0.025	0.876	
Clone x Growing space		1	68.9249	0.198	0.658	
Growing space		1	61 269.3	175.652	0.001	
Residuals		102	348.811			
Clone		1	522.029	1.508	0.222	
Growing space		1	62 148.8	179.572	0.001	
Residuals		103	346.094			
<i>Three coppicings (3 + 1, 4 + 1, 6 + 2)</i>						
Clone	1	743.566	2.353	0.128		
Clone x Growing space	1	1256.00	3.975	0.049		
Growing space	1	44 489.6	140.797	0.001		
Residuals	92	315.983				

293 Abbreviations: DF – degrees of freedom, MS – mean square.

294 *When there was no statistically significant factor x covariate interaction, the significance of the factor effect
 295 throughout the range of the covariate was tested.

296 Table 3 Results on the effect of harvesting cycle (HC) and growing space on the total harvested dendromass by
 297 clones, for 2- and 3-year production periods*.

Production period		DF	MS	F-value	P-value
2 years	<i>Clone 'Srebarna'</i>				
	HC	<u>1</u>	<u>890.991</u>	<u>1.269</u>	<u>0.262</u>
	HC x Growing space	<u>1</u>	<u>1390.28</u>	<u>1.980</u>	<u>0.162</u>
	Growing space	<u>1</u>	<u>52 487.6</u>	<u>74.748</u>	<u>0.001</u>
	Residuals	<u>119</u>	<u>702.195</u>		
	HC	<u>1</u>	<u>19 249.9</u>	<u>27.192</u>	<u>0.001</u>
	Growing space	<u>1</u>	<u>54 556.9</u>	<u>77.066</u>	<u>0.001</u>
	Residuals	<u>120</u>	<u>707.929</u>		
	<i>Clone 'Tsarevets'</i>				
	HC	<u>1</u>	<u>778.354</u>	<u>1.104</u>	<u>0.296</u>
	HC x Growing space	<u>1</u>	<u>1219.86</u>	<u>1.730</u>	<u>0.191</u>
	Growing space	<u>1</u>	<u>7133.81</u>	<u>10.115</u>	<u>0.002</u>
	Residuals	<u>105</u>	<u>705.289</u>		
	HC	<u>1</u>	<u>20 705.3</u>	<u>29.156</u>	<u>0.001</u>
Growing space	<u>1</u>	<u>6065.11</u>	<u>8.541</u>	<u>0.004</u>	
Residuals	<u>106</u>	<u>710.144</u>			
3 years	<i>Clone 'Tsarevets'</i>				
	HC	<u>1</u>	<u>21.5496</u>	<u>0.051</u>	<u>0.822</u>
	HC x Growing space	<u>1</u>	<u>850.993</u>	<u>2.013</u>	<u>0.159</u>
	Growing space	<u>1</u>	<u>47 593.6</u>	<u>112.581</u>	<u>0.001</u>
	Residuals	<u>103</u>	<u>422.750</u>		
	HC	<u>1</u>	<u>5553.92</u>	<u>13.011</u>	<u>0.001</u>
	Growing space	<u>1</u>	<u>56 465.7</u>	<u>132.279</u>	<u>0.001</u>
Residuals	<u>104</u>	<u>426.867</u>			

298

299 Abbreviations: HC – harvesting cycle, DF – degrees of freedom, MS – mean square.

300 *When there was no statistically significant factor x covariate interaction, the significance of the factor effect
 301 throughout the range of the covariate was tested.

302 Table 4 Results on the effect of harvesting cycle (HC) and growing space on the total harvested dendromass by
 303 clones, for a 4-year production period.

	DF	MS	F	P-value	Harvesting cycle	Growing space (m ²)	Homogenous groups (P < 0.05 ^{***})
<i>Clone 'Srebarna'</i> ^{**}							
HC	3	4351.13	3.134	0.027			
HC x Growing space	3	2173.56	1.566	0.199	3 + 1, 4 + 1, 6 + 2		a
Growing space	1	335 447	241.623	0.001	5 + 3, 6 + 1		ab
Residuals	190	1388.30			4 + 2, 6 + 2	0.25–9.75	ab
HC	3	4598.04	3.283	0.022	6 + 4		b
Growing space	1	351 974	251.319	0.000			
Residuals	193	1400.51					
<i>Clone 'Tsarevets'</i> ^{***}							
HC	2	292.838	0.334	0.717	3 + 1, 4 + 1, 6 + 2	0.25–0.66	a
HC x Growing space	2	7381.11	8.418	0.001	5 + 3, 6 + 1		a
Growing space	1	206 592	235.605	0.001	6 + 4		a
Residuals	165	876.860			3 + 1, 4 + 1, 6 + 2	0.66–2.25	a
					5 + 3, 6 + 1		ab
					6 + 4		b
					3 + 1, 4 + 1, 6 + 2	>2.25	a
					5 + 3, 6 + 1		b
					6 + 4		c

304

305 Abbreviations: HC – harvesting cycle, DF – degrees of freedom, MS – mean square.

306 *When there was no statistically significant factor x covariate interaction, the significance of the factor effect
307 throughout the range of the covariate was tested.

308 ** When there was statistically significant factor x covariate interaction, the significance of the factor effect at
309 each particular level of the covariate (i.e. growing space) was examined.

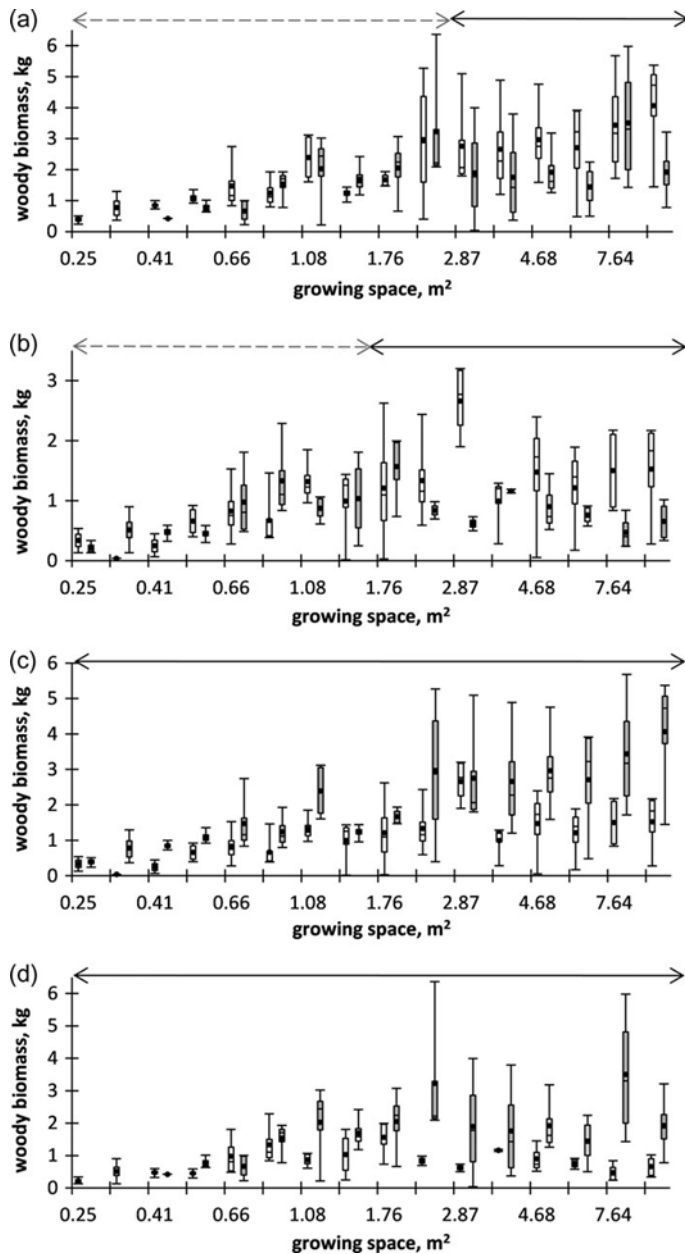
310 *** Groups with the same letter are not significantly different at P < 0.05.

311 Discussion

312 Individual coppiced plant from the families under study produced on average 2 kg of dry shoot biomass at 2 years
313 of age and a dendromass yield of up to 7–9 kg from 4-year-old shoots (Table 1). The annual increment at optimal
314 stocking rates was estimated to vary between 5.78 and 11.56 Mg.ha⁻¹.year⁻¹ (Table 6). We need to point out that,
315 according to the observations by Kalmukov (2002, 2014), sites favourable for growing black locust in Bulgaria
316 require low carbonate content in the upper soil horizon, and therefore the soil conditions in the experimental
317 plantation appear to be sub-optimal for cultivating the species. In a study on short-term biomass production in
318 black locust grown at different densities, the annual dendromass increment under poor site conditions amounted
319 to 2–3 Mg.ha⁻¹.year⁻¹, while on alluvial soil it was around 5–7 Mg.ha⁻¹.year⁻¹ at 6 years of age (Kalmukov, 2013).
320 Rédei et al. (2010a, b) reported that selected black locust clones propagated from cuttings produced around 7–9
321 Mg.ha⁻¹.year⁻¹ dry shoot biomass in a 5–7 year growth period, and the annual increment in black locust of coppiced
322 origin had similar amounts at 4 years. In a recent study combining data on black locust of different ages and grown
323 in mining and agricultural areas, the annual dry biomass yield varied from 1.0 to 13.8 Mg.ha⁻¹.year⁻¹ (Carl et al.,
324 2017). Quinkenstein et al. (2012) measured annual dendromass growth of 0.3–4.8 Mg.ha⁻¹.year⁻¹ on reclamation
325 land in Lusatia (Germany) and concluded that when used in harsh growth conditions *R. pseudoacacia* is very
326 productive compared to willow and poplar, and therefore a promising tree for biomass production. Dickmann et
327 al. (1985), on the other hand, reported about comparable four-year mean annual increment of 4.6 Mg.ha⁻¹.year⁻¹
328 for *Platanus occidentalis* L. and *Robinia pseudoacacia* L. on eroded upland site in the Georgia Piedmont (US).

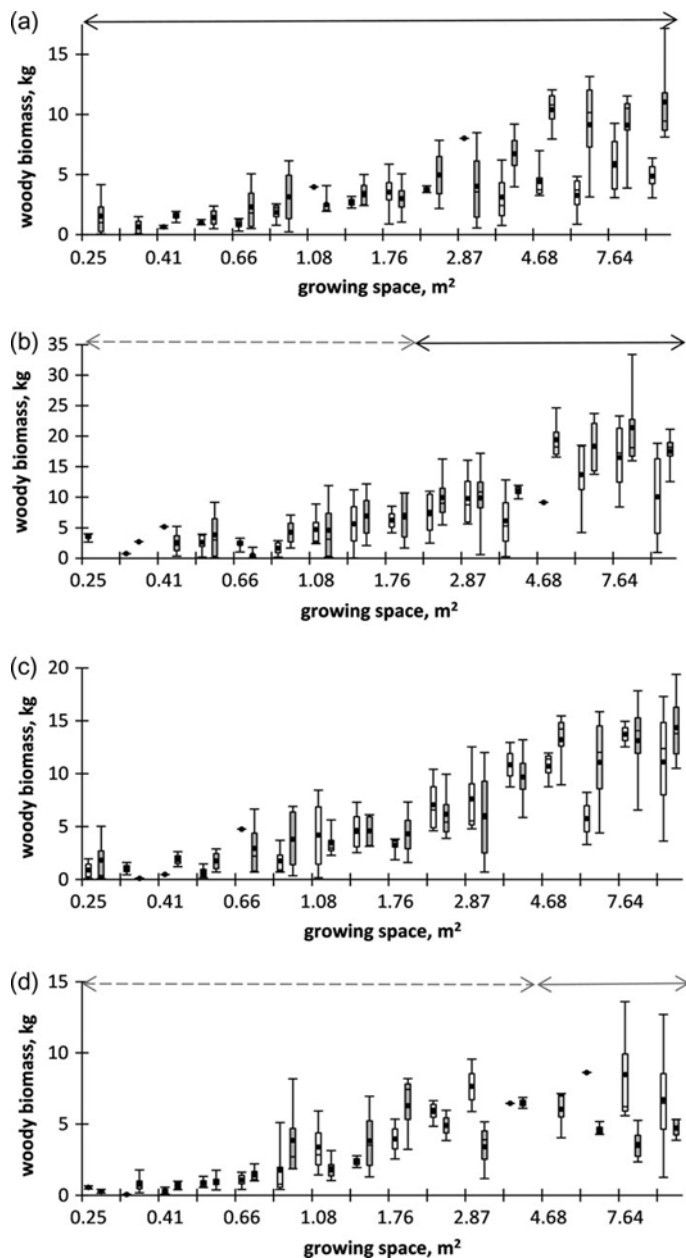
329 As genotype (species) is a major determinant of plant growth and productivity, the parental clone of the half-sib
330 black locust progenies was one of the factors investigated in this study. Trials have been carried out with various
331 species and under different site conditions to compare the growth and production potential of black locust and
332 other fast-growing broadleaf species. For example, *Salix viminalis* clone and a *Populus nigra* x *P. Maximowiczii*
333 hybrid produced notably higher yields than black locust for 2-year rotation period under different soil enrichment
334 regimes on poor site in Poland (Stolarski et al, 2013). On the other hand, in a study of the yield potential of
335 different poplar (*Populus spp.*), willow (*Salix viminalis* L.) and black locust (*Robinia pseudoacacia* L.) clones in
336 Central Germany, the performance of the black locust was superior, matching or even exceeding previously

337 reported yields (Gruenewald et al., 2007). According to these authors, *R. pseudoacacia* is well adapted to nutrient-
 338 poor sandy substrates and low precipitation. Another comparative study in an experimental plantation with silver
 339 maple (*Acer saccharinum* L.), cottonwood (*Populus deltoides* Bartr.) and black locust on alluvial, old-field site
 340 near Manhattan, also showed that black locust was the most productive species in a 5-year rotation period, with
 341 an annual yield of 11 Mg.ha-1.year-1 (Geyer, 2006). In the present investigation, we found that the biomass of
 342 the individuals from the two half-sib families was similar over a particular high-density range, but differed
 343 significantly when the growing space became sufficiently large (significant spacing x family interaction).
 344 Similarly, a significant clone x density interaction was reported in relation to the breast-height diameter at ages 3
 345 and 6 years of 7 poplar clones grown in a Nelder wheel experiment with 5 planting densities (Panetsos, 1980).



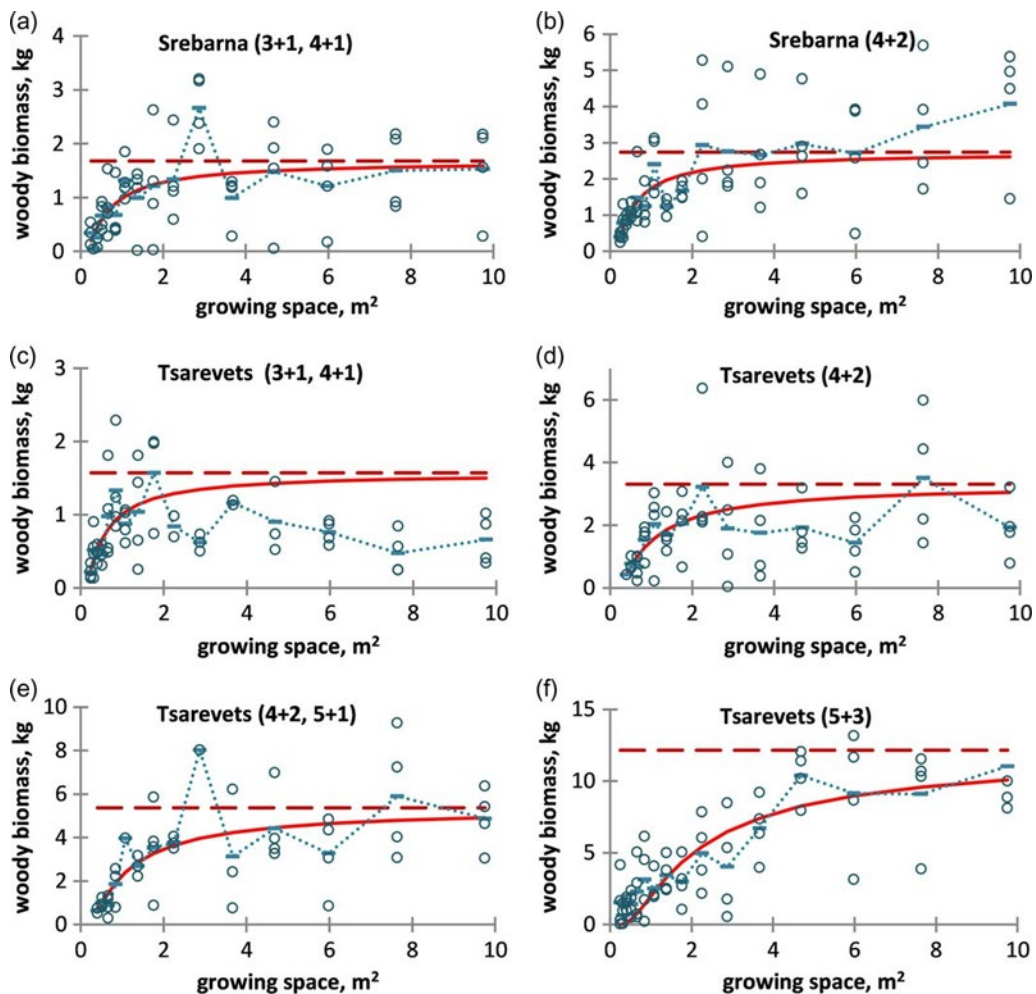
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347 Figure 4 Box-plot charts for 2-year production periods. A) 'Srebarna' (white boxes) vs. 'Tsarevets' (grey boxes)
 348 at Harvesting cycle (HC): 4 + 2; b) 'Srebarna' (white boxes) vs. 'Tsarevets' (grey boxes) at HC: 3 + 1, 4 + 1; c)
 349 HC: 3 + 1, 4 + 1 (white boxes) vs. HC: 4 + 2 (grey boxes) for clone 'Srebarna'; d) HC: 3 + 1, 4 + 1 (white boxes)
 350 vs. HC: 4 + 2 (grey boxes) for clone 'Tsarevets'. Mean values are shown with black dots, medians – with lines,
 351 25 per cent and 75 per cent quartiles – with boxes, minimum and maximum-with whiskers. Dashed arrows indicate
 352 insignificant differences, solid arrows indicate differences significant at $P < 0.05$.



353

354 Figure 5 Box-plot charts for 3- and 4-year production periods. (a) Harvesting cycle (HC): 4 + 2, 5 + 1 (white
 355 boxes) vs. HC: 5 + 3 (grey boxes) for 'Tsarevets'; (b) 'Srebarna' (white boxes) vs. 'Tsarevets' (grey boxes) at
 356 HC: 6 + 4; c) 'Srebarna' (white boxes) vs. 'Tsarevets' (grey boxes) at HC: 5 + 3, 6 + 1; d) 'Srebarna' (white boxes)
 357 vs. 'Tsarevets' (grey boxes) at HC: 3 + 1, 4 + 1, 6 + 2. Mean values are shown with black dots, medians-with
 358 lines, 25 per cent and 75 per cent quartiles-with boxes, minimum and maximum-with whiskers. Dashed arrows
 359 indicate insignificant differences, solid black arrow indicates differences significant at $P < 0.05$, solid grey arrow
 360 indicates differences significant at $P < 0.1$.



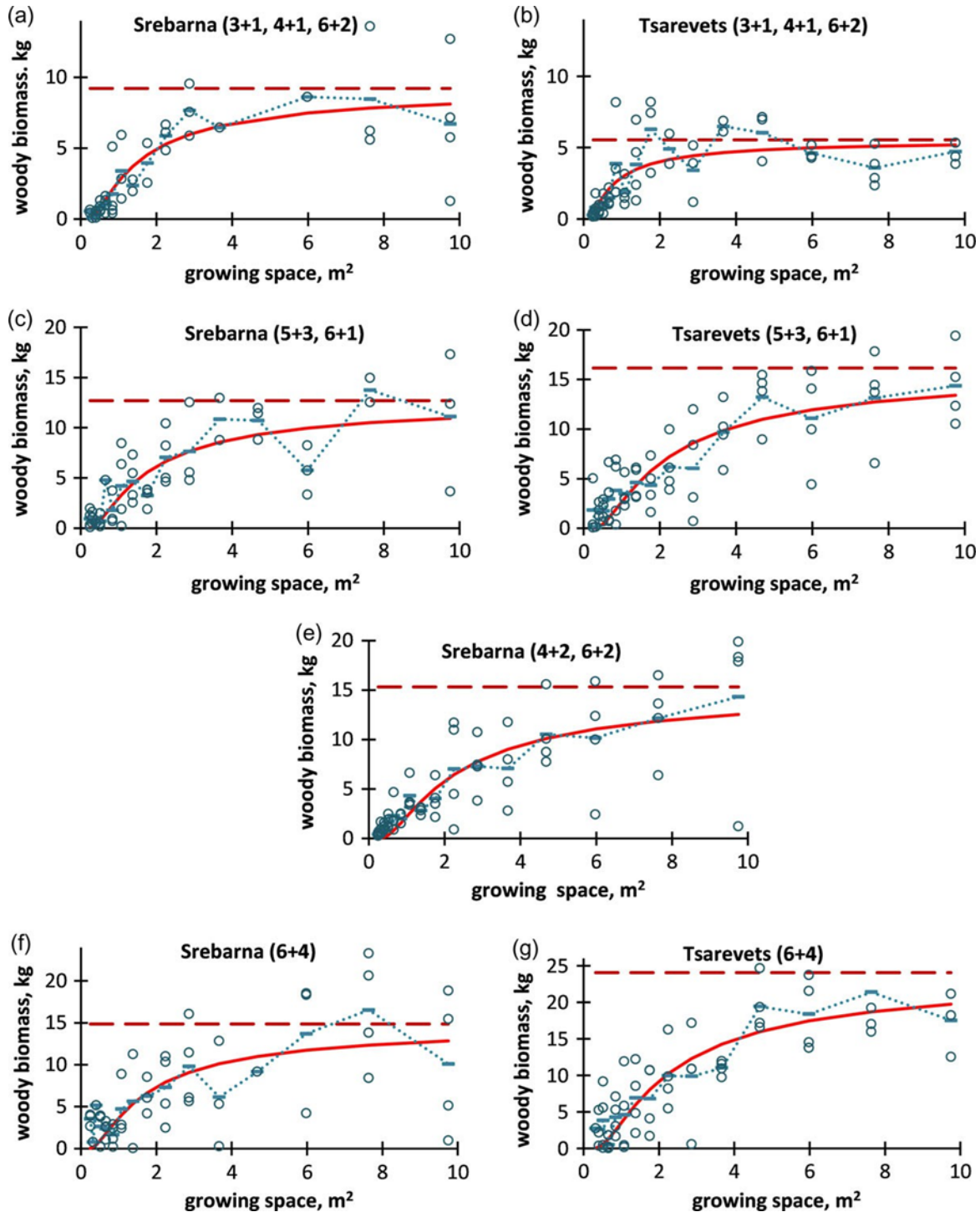
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362 Figure 6 Trends of woody biomass with growing space by parental clone and harvesting cycle for 2- and 3-year
 363 production periods. The dotted line connects the biomass values averaged by density. The solid line represents
 364 the function of Equation (1), while the dashed line is the upper asymptote b_0 .

365 However, the same study demonstrated similar height growth patterns in the poplar genotypes investigated,
 366 although depending on the growing space, with the tallest trees measured at intermediate spacing, and with no
 367 significant clone \times density interaction for this growth parameter. A survey evaluating the performance at age 17
 368 years of five genotypes of *Pinus radiata* D. Don grown at 10 stocking rates in a Nelder plot concluded that there
 369 was no statistically significant interaction between breed and stocking in relation to height, diameter, slenderness
 370 or crown length (Waghorn et al., 2007). For the range of densities at which we observed substantial differences
 371 in productivity of the half-sibs, the productivity of the ‘Srebarna’ offsprings was greater than that of ‘Tsarevets’
 372 over a 2-year-long period, but the opposite was true for a dendromass harvesting period of 4 years. In a
 373 comparative study of 4 *Eucalyptus* clones cultivated in two sites in Kenya under short rotation in a Nelder wheel
 374 experiment with 10 planting densities, genotype had a significant or marginally significant effect on the growth
 375 parameters basal and breast-height diameter and height at 3 years of age; the *Eucalyptus grandis-camaldulensis*
 376 (GC 10) clone showed overall superiority, and the local landrace performed least well (Kironjo et al., 2012).

377 Most studies investigating the effects of the length of the production period on the amount of harvested biomass
 378 from short rotation crops compare yields from single cuts for different growth periods. The results usually indicate
 379 higher yields when the number of years before harvesting is increased (e.g. Willebrand and Verwijst, 1993;
 380 Armstrong et al., 1999). In the present study, we preferred to compare the cumulative yields, obtained through
 381 different number of coppicings within a certain period, because we considered it to be a fairer test, and also
 382 because time intervals with the same climatic conditions are comprised. We found that a one-coppicing cycle was
 383 significantly more productive than the combination of two coppicings of one-year-old shoots within a 2 year-

384 period for both black locust families (Table 3, Figs 4c and 4d). The general tendency of lower yield from multiple
 385 harvesting of 1- and 2-year-old shoots was also observed for the 4-year period, but the advantage of the increas-
 386 ing shoot age was more pronounced only for the ‘Tsarevets’ progeny at spacing above 2.25 m² (Table 4).
 387 Nevertheless, the one-cut harvesting cycle proved superior for both families and production periods (Figs 4c and
 388 4d, Table 4).



389

390 Figure 7 Trends of woody biomass with growing space by parental clone and harvesting cycle for 4-year
 391 production period. The dotted line connects the biomass values averaged by density. The solid line represents the
 392 function of Equation (1), while the dashed line is the upper asymptote b_0 .

393 In a study of biomass production in 3 poplar clones grown at 2 densities on 3 sites in the UK, a single harvest in
 394 a four year cutting cycle yielded more biomass than two harvests on a two year cycle for all clones at all sites and
 395 both spacings considered (Armstrong et al., 1999). In *Populus trichocarpa*, a single harvest after 8 years was one
 396 third more productive than two rotations of 4 years (Blake, 1983). By contrast, in a study on the yield potential
 397 and sustainability of yields in different clones of poplar, willow and black locust in Central Germany, the rotation
 398 period did not significantly affect tree biomass accumulation, except in the poplar clone Hybride 275 (P.
 399 maximowicii Henry × P. trichocarpa Torr. et Gray), and two 3-year cycles produced the same amounts of
 400 dendromass as one 6-year harvesting cycle (Gruenewald et al., 2007). In addition to the length and the number of
 401 the harvesting cycles, the number of the successive coppicings that can be carried out without notable stump loss
 402 and a reduced ability to sprout is also important. However, we were not able to evaluate this effect in the present
 403 study.

404 Table 5 Regression models for plant dendromass yield as a function of growing space.

Parental clone	Production period	Harvesting cycle	R ² adj	RMSE	^a b ₀ (b ₀)	^a b ₁
$Y = b_0' x^{b_1} e^{-b_2/x}$						
Srebarna'	2 years	3 + 1, 4 + 1	0.313	4.677	16.779 (3.383)	0.534 (0.147)
		4 + 2	0.516	6.188	27.442 (3.334)	0.458 (0.059)
Tsarevets'	2 years	3 + 1, 4 + 1	0.493	4.826	15.725 (2.706)	0.445 (0.082)
		4 + 2	0.431	5.663	33.160 (5.069)	0.799 (0.120)
$v = b_0 e^{-b_1/x}$						
Tsarevets'	3 years	4 + 2, 5 + 1	0.420	1.689	5.374 (0.632)	0.873 (0.163)
Srebarna'	4 years	5 + 3	0.605	2.527	12.157 (1.501)	1.811 (0.322)
		3 + 1, 4 + 1, 6 + 2	0.639	2.129	9.229 (1.730)	1.248 (0.291)
		5 + 3, 6 + 1	0.584	2.939	12.693 (2.181)	1.449 (0.363)
		4 + 2, 6 + 2	0.610	3.299	15.317 (2.639)	1.939 (0.424)
		6 + 4	0.384	4.819	14.852 (2.941)	1.410 (0.384)
Tsarevets'	4 years	3 + 1, 4 + 1, 6 + 2	0.506	1.668	5.554 (0.438)	0.644 (0.144)
		5 + 3, 6 + 1	0.634	3.164	16.154 (1.689)	1.805 (0.283)
		6 + 4	0.655	4.551	24.036 (2.493)	1.913 (0.317)

405

406 Abbreviations: R²adj – coefficient of determination, RMSE – Root Mean Squared Error, y - total yielded biomass
 407 per plant (kg), Y - total yielded biomass per hectare (Mg.ha⁻¹), x - growing space (m²), b₀, b₁, b₀' – regression
 408 parameters, b₀' = 10b₀.

409 * Standard errors in brackets.

410 ** Equation (2) was fitted instead of Equation (1) to resolve the problem with poorer fit of the regression models
 411 for the 2-year production period.

412 Table 6 Estimated optimal and minimal planting densities in relation to parental clone, harvesting cycle and
 413 production period.

Parental clone	Production period	Harvesting cycle	Optimal planting density (ha ⁻¹)	Maximum expected yield (Mg.ha ⁻¹)	Maximum expected annual yield (Mg.ha ⁻¹ . year ⁻¹)	Recommended minimum planting density (ha ⁻¹) ^a
Srebarna'	2 years	3 + 1, 4 + 1	18 742	11.57	5.78	3748 (2.57)
		4 + 2	21 847	22.05	11.03	4369 (4.91)
Tsarevets'	2 years	3 + 1, 4 + 1	22 488	13.01	6.50	4498 (2.90)
		4 + 2	12 508	15.26	7.63	2502 (3.40)
Tsarevets'	3 years	4 + 2, 5 + 1	11 449	22.63	7.54	2290 (3.36)
		5 + 3	5523	24.70	8.23	1105 (3.66)
Srebarna'	4 years	3 + 1, 4 + 1, 6 + 2	8014	27.21	6.80	1603 (3.03)
		5 + 3, 6 + 1	6901	32.22	8.06	1380 (3.59)
		4 + 2, 6 + 2	5156	29.05	7.26	1031 (3.23)
		6 + 4	7095	38.76	9.69	1419 (4.31)
Tsarevets'	4 years	3 + 1, 4 + 1, 6 + 2	15 524	31.72	7.93	3105 (3.53)
		5 + 3, 6 + 1	5542	32.93	8.23	1108 (3.66)
		6 + 4	5228	46.23	11.56	1046 (5.14)

414

415 ^aExpected annual yield (Mg.ha⁻¹.year⁻¹) for the recommended minimum planting density in brackets.

416 The age-related decrease in the ability of trees to sprout after cutting, which has been observed in many species,
417 may be attributable to a decline in tree vigour with age and/or the loss of viable dormant buds (Blake, 1983). In a
418 study on biomass production under various coppice regimes in conventional spacing trials (average spacing
419 1.8×1.8 m), boxelder (*Acer negundo* L.), black locust (*Robinia pseudoacacia* L.), cottonwood (*Populus deltoides*
420 Bartr.), honey locust (*Gleditsia triacanthos* L.), silver maple (*Acer saccharinum* L.) and Siberian elm (*Ulmus*
421 *pumila* L.) were able to be cut up to four times without loss of yield, as three subsequent harvests at five-year
422 intervals showed remarkably consistent growth over time and the oven-dry weight yields remained high after four
423 cuts (Geyer, 2006). However, in high- density trials (0.3×0.3 m), survival decreased significantly to about 22 per
424 cent after six annual cuts for black locust, cotton- wood, and silver maple but remained high (about 82 per cent)
425 for honey locust and Siberian elm. Although black locust initially produced the greatest yield, after many annual
426 harvests, the coppice yields for Siberian elm were 37–57 per cent greater than for the other species. These
427 outcomes raise the question as to the sustainable number of harvests for short rotation black locust crops, an aspect
428 that requires further investigation.

429 The effects of spacing can be viewed at population, individual or organ level and despite the general principles
430 driving the processes of growth and intraspecific competition, findings on the influence of density vary depending
431 on the species, site and range of spacing considered (Kerr, 2003). Basal and breast height diameters, height,
432 volume and biomass are the individual level variables that have attracted most interest in spacing trials. Studies
433 exploring plant biomass or volume in relation to growing space have generally reported positive correlations
434 between biomass/volume of individual plants and spacing (Bernardo et al., 1998; Jinks and Mason, 1998; Erkan
435 and Aydin, 2016), while the total volume or biomass per unit area increases with stocking rate (Bernardo et al.,
436 1998; Jinks and Mason, 1998; Rédei et al., 2008; Rédei and Veperdi, 2009; Erkan and Aydin, 2016). We found
437 that spacing significantly affected tree dendromass in both families and all harvesting cycles considered. The
438 woody biomass yield per plant generally increased with spacing, and growth tended to saturation at the lowest
439 investigated densities. In addition, the total biomass yield per unit area was reasonably well described by a function
440 in which maximum values peaked within the range of the planting densities examined. These findings are
441 consistent with previously reported tendencies to constancy in growth traits at wider spacings, for the individual
442 tree variables diameter and height, and which was coupled with basal area per hectare of maximum values in the
443 middle of the range of the investigated densities for eucalypt (Ferrere et al., 2005). Our findings are also in
444 agreement with previously reported age-related trends of individual biomass growth saturation in juvenile red
445 alder at different stocking levels (Giordano and Hibbs, 1993).

446 In a Nelder wheel plot attempting to determine the optimal planting densities for *Quercus mongolica* var.
447 *grosseserrata*, an exponential decrease function provided a good description of the relation between survival, stem
448 and crown diameters of the 17-year-old oaks and density, while the clear stem length seemed to increase
449 exponentially with density (Imada et al., 1997). In a study of juvenile coppiced willows, the total yield per unit
450 area increased across the range of densities considered and the relationship was expressed by the Mitscherlich
451 function, which allows asymptotic biomass values to be reached when density tends to infinity (Willebrand and
452 Verwijst, 1993). In agreement with their concept, Cañellas et al. (2012) distinguished different patterns of density-
453 total biomass relation according to age for the coppiced black poplar hybrid ‘I 214’. The authors concluded that
454 higher density would be advantageous during the first year, but after three years, the effect of density on growth
455 and production would become insignificant as a result of increased competition among plants for resources. We
456 must point out, that in our study, we did not aim to model the size-density relationship, but rather to describe the
457 observed tendency in the accumulated, within a given period of time, amount of harvested dendromass from the
458 individual plants with the increase in spacing. Beyond a certain density, no further increase in individual plant
459 yield was achieved by increasing the spacing, although reduction in the total harvest may be expected due to the
460 smaller number of plants harvested. We therefore proposed initial planting densities that would yield maximum
461 amounts of dendromass per unit area according to the harvesting regime, bearing in mind that the density ranges
462 derived are indicative and that the strength of the estimated relationships should also be considered. We assumed
463 that at an early age, competition due to the high stocking levels will primarily lead to growth suppression and that
464 the mortality rate will be negligible, at least for the investigated species. In addition, as the cost of establishing
465 short rotation crops increases with the number of propagules planted, and lower densities are therefore preferable,

466 we advised that some minimum number of seedlings per unit area should be considered for achievement of
467 admissible yields (Table 6).

468 In a study on competition and growth partitioning among *Robinia pseudoacacia* L. trees, grown on 10 sites of
469 similar climate, but varying soil conditions in Germany Carl et al. (2018) found that phosphorus is the most
470 important nutrient for black locust growth in areas with sandy soils, and increasing phosphorus and water
471 availability leads to increased competition for above-ground resources, primarily sunlight. The authors con-
472 cluded that under the increased competition for sunlight on water and phosphorus-rich sites, the optimal tree
473 density for a high biomass production decreases. Quantitative traits of selected trees, such as growth and
474 productivity, on the other hand, are usually examined in test plantations at various site conditions and the results
475 derived on their evaluation and comparison are also site-specific. The site conditions of our experimental plot are
476 described by temperate continental climate and Calcic Chernozem soil type, which are characteristic for an area
477 of around 590 000 ha in the northernmost part of Bulgaria. Therefore, we must point out that the outcomes of our
478 study, if reliably scaled up in practice, should be considered for application only at comparable climate and soil
479 conditions.

480 **Conclusions**

481 The study findings showed that under the particular soil and climate conditions the progenies of the selected black
482 locust clones ‘Srebarna’ and ‘Tsarevets’ grow and produce biomass in very different ways and these differences
483 should be considered in their use in short rotation crops. Trees of the open-pollinated ‘Srebarna’ family can be
484 harvested in a more regular manner, e.g. biannually, without great loss of dendromass yield. Although the
485 ‘Tsarevets’ progeny must be harvested at a more advanced age, the delay will be offset by a significantly higher
486 biomass yield. Nonetheless, one-cut harvesting cycle of 4-year- old shoots is the most productive option for both
487 families, and a growing space of 1.5–2 m² per plant would provide the maximum amount of harvested dendromass
488 within a 4-year period. Short rotation crops established with seedlings from the half-sib families of ‘Srebarna’ and
489 ‘Tsarevets’ and managed according to the recommended optimal growing space and harvesting cycle should
490 produce, if the results reported here can be scaled up in practice, under continental temperate climate on the
491 particular soil type an annual biomass yield of 9–11 Mg.ha⁻¹.year⁻¹.

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497 **Conflict of interest statement**

498 None declared.

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