

PAPER • OPEN ACCESS

## Field trials of micropropagated clones of triploid white and grey poplars

To cite this article: O M Korchagin *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **226** 012007

View the [article online](#) for updates and enhancements.

# Field trials of micropropagated clones of triploid white and grey poplars

O M Korchagin<sup>1</sup>, O S Mashkina<sup>2\*</sup>, O V Tregubov<sup>3</sup>

<sup>1</sup>All-Russian Research Institute of Forest Genetics, Breeding and Biotechnology, 105 Lomonosov street, Voronezh 394087 Russia

<sup>2</sup>Voronezh State University, 1 Universitetskaya square, Voronezh 394018 Russia

<sup>3</sup>Voronezh State University of Forestry and Technologies named after G.F. Morozov, 8 Timirjazeva street, Voronezh 394087 Russia

\*E-mail: mashkinaos@mail.ru

**Abstract.** In this article we present, for the first time, the results of 20 years field trials of triploid white poplar (*Populus alba* L.) and grey poplar (*Populus x canescens* Sm.) clones propagated by *in vitro* culture in different edaphic conditions (6 clones in total). The soils of the test plots differ in fertility, chemical and physical-chemical characteristics, which had a significant impact on the plants' capacity for survival, health and growth. Low fertile, dry, acidic sandy loam and sandy soils are the least suitable for cultivating of *in vitro* micropropagated clones. In this case we had sandy loam soils on glaciofluvial monomineral quartz sands. The response to adverse growing conditions largely depended on the genotypic characteristics of the plants. The grey poplar clone named Hopersky 1 cultivar has shown the highest ecological plasticity as it has broad genetic basis (it is a hybridogenic species and allotriploid).

## 1. Introduction

Poplar (*Populus* L.) is one of the fast growing and economically valuable tree species. White poplar (*Populus alba* L.) and grey poplar (*Populus x canescens* Sm.) are the most productive and resistant in central Russia. There is a number of valuable hybrids, polyploids and varieties obtained from them [1, 2]. White and grey poplars are difficult to reproduce by stem cutting which makes the large-scale implementation of valuable forms and varieties into forestry practice difficult. That is the reason why forestry specialists in Europe usually obtain planting material of valuable genotypes by micropropagation (*in vitro* cloning) [3-6]. There are field tests conducted in order to study the micropropagated plants, their growth, physiological characteristics and wood ontogeny in terms of their suitability for pulp and paper industry [7-9].

For purposes of silvicultural and economic effect, we ought to create plantations not only using certified planting material with improved hereditary characteristics but also establishes them in optimal for each species soil conditions. It is well known that soil conditions have a great impact on the growth of all tree species, including poplar [10-14]. For example, in Bryansk region (Russia) it has been reported that the growth of the northern red oak is significantly influenced by such soil characteristics as soil phosphorus and potassium storage, silt and clay content and pH value [13]. In



Northern Europe it has been demonstrated that the rapid growth of hybrid aspen takes place only on fertile ground with good hydrophysical characteristics [12]. Chemical and physical characteristics of soils also have a strong influence on the growth of hybrid poplar in the Terme-Golardi region of Turkey [11].

However, our knowledge of the impact of soil conditions on the growth and productivity of micropropagated plants is still fragmentary. Besides, the cultivation of the same clone (which consist of genetically identical plants) in contrasting edaphic conditions allows us to assess its norm of reaction (ecological plasticity) and to identify optimal conditions for the maximum realization of potential of different poplar's species and genotypes.

This article presents the data on survival and growth of micropropagated clones of triploid white and grey poplars cultivated for 20 years on different soil types: black soil (chernozem) and sandy soil. Triploid poplars are of considerable practical importance due to their fast growth, high productivity and sterility (in the majority of cases they do not produce seeds).

## 2. Methods and materials

The study is based on field observations and measurements of *in vitro* micropropagated clones of 5 triploid ( $2n=3x=57$ ) hybrids of white poplar (# 101/83, 155/83, 143/82, 11/83, 22/83) [15] and one clone of grey poplar (Hopersky 1 cultivar) [1]. The grey poplar is a natural hybrid of the white poplar (*P. alba*) and the aspen (*P. tremula*). Its allotriploid Hopersky 1 cultivar is characterised by great growth rates with a pronounced heterosis effect, as well as in productivity and wood quality [1]. We obtained white poplar autotriploids through hybridization with unreduced diploid ( $2n$ ) pollen obtained artificially by increasing temperature. Then we selected fast growing and productive hybrids among them [15].

*In vitro* poplar propagation was carried out via organogenesis using the method elaborated by us [16]. We used stem segments from herbaceous summer shoots with one axillary bud as explants. They were taken from 15-year-old trees. To induce shoots, we used Murashige and Skoog mineral salts medium (MS) [17] supplemented by 0.2 mg/L 6-benzylaminopurin (BAP) and 0.2 mg/L gibberellic acid. To reduce the likelihood of somaclonal variation at stages of multiplication and root formation, we used hormone-free half-strength MS medium ( $\frac{1}{2}$  MS) with activated charcoal (2%). Microplants were then transferred to the greenhouse soil (peat-sand mixture, 1-1 ratio), where they continued to grow for one more year.

1-year-old seedlings of the same clones were planted in contrasting edaphic conditions in 1997. The first site is located in the Semiluki forest breeding nursery in Voronezh region (Russia). It lies within the high part of watershed boundary of Central Russian upland in forest-steppe zone. The second site is located in Stupino (Ramon district, Voronezh region) situated above flood lands in its terrace part.

Only homogeneous plants in terms of growth and morphology (without signs of damage and variability), with well-developed roots were selected for planting. Up to twenty regenerants per clone were planted on each test site. Then at the age 2-20 following planting (in 1999, 2002, 2008, 2011, 2014 and 2017) we conducted analysis of plants' survival and growth (height, diameter at breast height - DBH). Stems volume was defined according to poplar volumes table [18].

The average annual temperature in Voronezh region is +6.9 °C, the average annual precipitation is 531 mm, and the average air humidity is 74%. The average annual air temperature in dry years reached: +8.6 °C (2007 and 2010), +9.1 °C (2012). In 2010 there was an abnormally hot summer drought (both atmospheric and soil) in Voronezh region. Three temperature records were broken that year: June 28: +38.9 °C (which exceeds the climatic norm of the last decades by + 3.9 °C), July 29: +40.1 °C (exceeds it by +6.7 °C), August 2: +40.5 °C (exceeds it by +7.1 °C) [19]. In addition to this it didn't rain for about 2 month. There were only 49% of normal rainfall in June, 47% – in July and 56% – in August [19]. The upper layer of sandy soils (10-15 cm) considerably dried up and its temperature reached 60 °C.

Our test sites are located within a typical forest-steppe zone in the same climatic conditions. Therefore, the main limiting factors affecting plants' growth and development are soil conditions, such as ground water level, its nutrient content, pH, and grain composition.

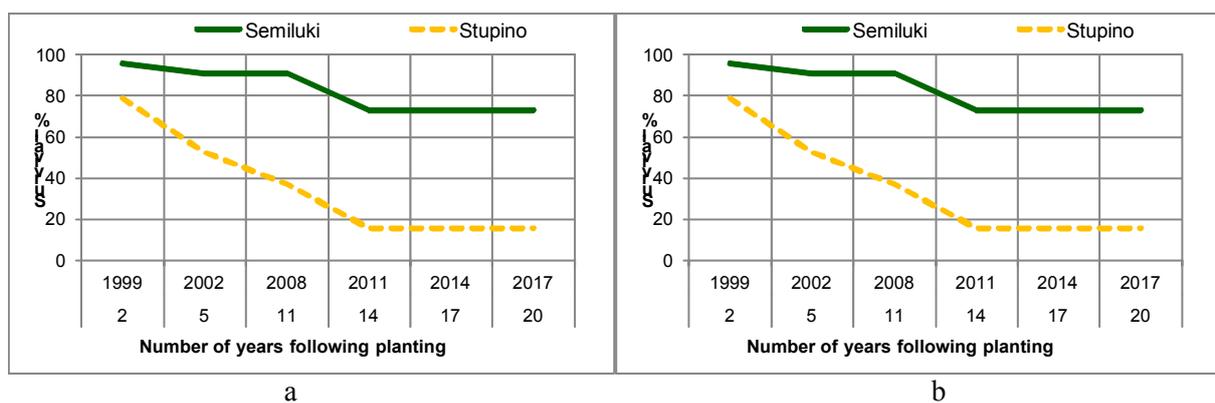
We used 2 soil cross sections to characterize soil conditions of the test sites and analyzed 12 soil samples in accordance to the generally accepted methods in soil science [20]. The analysis determined the composition of soil, its morphological characteristics, grain-size distribution of soil, chemical and physico-chemical composition. We also analyzed the following parameters: the humus content, the total absorbed bases, the amount of mobile potassium and phosphorus, the amount of absorbed calcium and magnesium and soil acidity. On 2 sites the water regime was registered.

Statistical data processing was done according to Lacin [21] using statistical software package "Stadia".

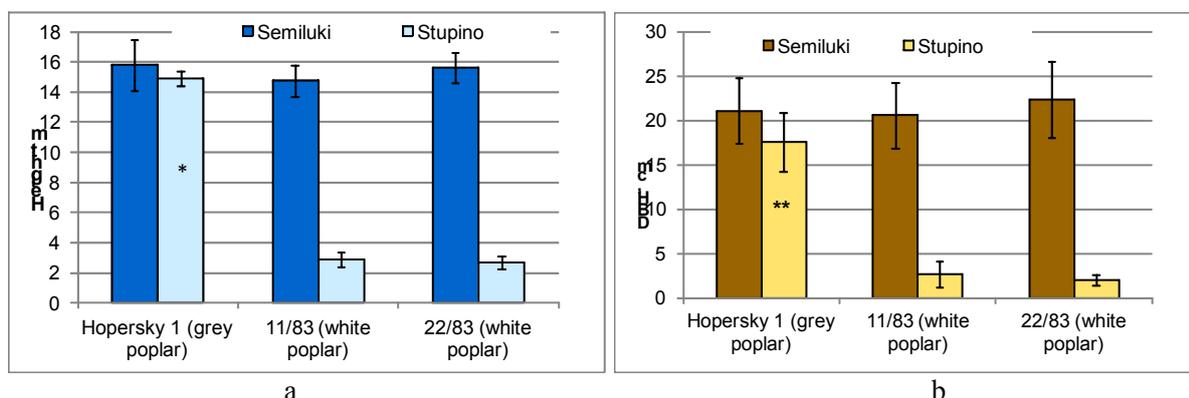
### 3. Results and discussion

#### 3.1. Survival and growth of *in vitro* micropropagated clones

The results of field trials showed that the micropropagated poplar clones had higher survival index, growth and intraclonal homogeneity in the Semiluksky nursery (figures 1 and 2).



**Figure 1.** Survival dynamics of *in vitro* propagated clones of triploid grey poplar (a) and white poplar (b) (average figures for 5 clones) grown under different growth conditions: Semiluki (chernozem) and Stupino (sandy loam).



**Figure 2.** Growth data of micropropagated clones (a) and diameter at breast height DBH (b) of triploid grey and white poplar at the age of 20 years grown under different growth conditions: Semiluki (chernozem) and Stupino (sandy loam). The differences between the clones from the Semiluksky nursery and Stupino are statistically significant (\*  $P < 0.05$ , \*\*  $P < 0.01$ ).

The weather conditions in 1997 (the year of planting) were quite favorable or, that is to say, in conformity with the regional norm. Nevertheless, the plants' survival rate was 1.2 times higher in Semiluki, than in Stupino: 95.5% as opposed to 78.9% for grey poplar and 83.2% (from 71.4% to 92.3% depending on the clone) as opposed to 66.8% (57.1-84.6%) for white poplar (figure 1). By the age of 17 plants' survival rate in Semiluki was 3-4.5 times higher than in Stupino: 59.4% (16.7-76.9%) as opposed to 18.6% (from 0 to 40%) for white poplar and 72.7% as opposed to 15.8% for grey poplar (figure 1). The highest number of dead trees was registered after abnormally hot and dry summer of 2010, when the micropropagated clones were 14 years old. By this age on the second site (in Stupino) there were no plants of two white poplar clones (#155/83 and 143/82), and only one plant of the clone 101/83 left.

A similar trend was observed in the plants' growth in height and diameter (figure 2). The differences are particularly striking in the case of white poplar clones. Thus, at the age of 20, the plants of two clones (11/83 and 22/83) in Semiluki were about 5 times higher than in Stupino, their average height was 14.8 and 15.6 m as opposed to 2.9 and 2.7 m. In Stupino we registered higher intracolon heterogeneity (variability of characteristics), as evidenced by higher coefficient of variation (45.3-51.8% as opposed to 2.7-6.0% in Semiluki). What is more, all plants in Stupino had some apparent defects usually manifested in recurrent dying of the main shoot (especially after the drought of 2010) and the formation of new shoots from the lower parts of the stem.

In general, the grey poplar clones were healthier and grew better than the white poplar clones. The difference in growth of grey poplar on the two sites was not so significant: at the age of 20 years the average height of plants was 15.8 m in Semiluki and 14.9 m in Stupino. The findings are statistically significant (figure 2).

Thus, the growth conditions are proved to have a significant effect on the survival, growth and intracolon homogeneity of *in vitro* propagated poplar clones.

### 3.2. Characteristics of soil conditions

The soil of the first site (Semiluki forest breeding nursery) is typical clay loam black soil (chernozem) underlain by a light clay (i.e. clay with plasticity index from 0.17 to 0.27). The silt and clay content in a 2-meter layer of soil was 9.103 tons/ha. The soil's nutrient status lets us place the plot into a D2 site type according to Pogrebnyak's classification which means mesophytic (medium-humid) deciduous forest typically with sedge and ground elder in herbage cover. There are clay loam layers present in the soil profile (table 1) Soil-forming material is light clay (i.e. clay with plasticity index from 0.17 to 0.27).

Humus content of the soil is 4.1%, which is an average number (table 2).

As for exchange acidity, the upper layers of soils have a slightly acidic pH of about 5.5 at the topsoil lever (0-30 cm), a neutral pH of 6.2 at a depth of 30-40 cm and a slightly alkaline pH of 7.5-7.8 at a depth of 60-170 cm (table 2).

The content of labile phosphorus in the upper layers of soil (at a depth of 0-25 cm) can be considered high as it amounts to 148 mg/kg. At a depth of 20-40 cm, however, it is average (59-62 mg/kg) while at a depth of 60-170 cm it is very low (7-8 mg/kg). The soil labile potassium supply in the upper layers is very high and at a depth of 0-10 cm it amounts to 226 mg/kg, at a depth of 20-130 cm it is average while in deeper layers – quite low (table 2).

The soil of the second site (Stupino) is forest sandy loam formed on glaciofluvial monomineral quartz sands. The silt and clay content in a 2-meter layer of soil is 2.692 tons/ha. The plot's site type is B2 according to Pogrebnyak's classification which means mesophytic (medium-humid) mixed forest with oak stands and grassy areas.

Soils here are mostly gray especially in the upper layers. Going deeper the color of the soil becomes lighter and a brown shade appears, which indicates a certain amount of iron and manganese oxides.

**Table 1.** Granulometric composition of soils on test sites.

Depth, cm	Grain-size distribution of soil, %							Category
	1-0.25 mm	0.25-0.05 mm	0.05-0.01 mm	0.01-0.005 mm	0.005-0.001 mm	< 0.001	< 0.01	
Site 1. Typical clay loam black soil (chernozem) with average humus content underlain by a light clay (Semiluki forest breeding nursery, Voronezh region)								
0-10	16.20	19.03	19.72	8.40	13.37	23.28	45.05	clay loam
20-30	20.06	24.69	14.54	7.70	10.88	22.19	40.71	clay loam
30-40	22.56	18.30	16.50	5.30	11.73	25.61	42.64	clay loam
60-70	23.12	17.03	20.43	4.70	10.71	24.01	39.42	medium-textured loam
120-130	29.81	22.23	17.24	2.77	8.34	19.61	30.72	medium-textured loam
160-170	40.48	21.92	12.75	2.79	5.28	16.78	24.85	light loam
Site 2. Sandy-loam soil on glaciofluvial monomineral quartz sands (Stupino, Ramon district, Voronezh region)								
0-25	51.12	28.45	4.90	3.86	4.06	7.61	15.53	sandy loam
25-35	55.21	29.72	3.62	3.88	2.30	5.27	11.45	sandy loam
40-50	56.40	28.59	3.94	3.66	2.88	4.53	11.07	sandy loam
70-80	43.35	46.98	3.38	1.00	0.80	4.49	6.29	sand cohesive
150-160	59.26	26.63	5.00	2.74	1.62	4.75	9.11	sand cohesive

**Table 2.** Physicochemical and chemical properties of soil.

Depth, cm	Humus, %	Labile, mg/kg		pH (KCl)	Exchange bases, mg-eq /100 g of soil		Total absorbed bases	Hydrolytic acidity	Base saturation, %
		P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O		Ca <sup>2+</sup>	Mg <sup>2+</sup>			
Site 1. Typical clay loam black soil (chernozem) with average humus content underlain by a light clay (Semiluki forest breeding nursery, Voronezh region)									
0-10	4.10	148	226	5.5	22.7	6.5	29.2	3.40	90
20-30	2.30	62	50	5.5	14.8	11.5	26.3	3.56	87
30-40	1.10	59	48	6.2	9.4	4.7	14.1	2.74	85
60-70	0.60	7	50	7.5	-	-	-	-	-
120-130	0.25	8	43	7.6	-	-	-	-	-
160-170	0.01	7	32	7.8	-	-	-	-	-
Site 2. Sandy-loam soil on glaciofluvial monomineral quartz sands (Stupino, Ramon district, Voronezh region)									
0-25	3.50	50	39	3.6	4.4	2.0	6.4	3.05	68
25-35	1.54	51	16	3.9	4.0	0.8	4.8	1.53	31
40-50	1.11	24	13	4.1	8.0	4.4	12.4	2.86	81
70-80	0.10	18	15	4.3	9.2	6.0	15.2	2.99	83
150-160	-	20	16	4.7	-	-	-	-	-

Note: “-“ means that the analysis was not carried out.

Light granulometric composition (table 1) of the soil has led to a low level of base saturation of the upper part of the soil profile. Humus content of the soil is 3.5%, which can be considered quite low

and drops even lower with the depth which is typical for sandy and sandy loam soils (table 2). As for exchange acidity, the upper layers of plot's soils have a highly acidic pH of 3.6-3.9 at the topsoil lever (0-35 cm), a rather acidic pH of 4.1-4.3 at the middle part at a depth of 40-80 cm and medium acidic pH at a depth of 150-160 cm (table 2). The content of labile phosphorus in the upper layers of soil (at a depth of 0-25 cm) can be considered low (50 mg/kg). The soil labile potassium supply is also low (39 mg/kg) in the humus accumulation horizon and extremely low (13-16 mg/kg) in deeper layers (table 2).

It is known that the optimal conditions for poplar growth are alluvial soil with a close groundwater occurrence with high ground water levels [10,11]. Holing through the soils of test sites to a depth of 175 and 250 cm showed no high ground water table. This fact indicates that the previous dry years (2007, 2010 (when there was especially severe drought), 2012) had a really strong impact on the water regime and atmospheric precipitation that usually goes to soil water, evaporation and deeper layers of groundwater.

Furthermore, the high content of quartz sand and, correspondingly, the low amount of aluminosilicates in Stupino leads to high mineralization of soil organic matter. As a result, the plant nutrient content drops sharply and the role of soil moistening increases equally sharply. Thus, during periods of drought, plants do not receive the required amount of nutrients. The high acidity of the soil also has a negative impact on the plants' growth and development.

Poplar clones in the Semiluki forest breeding nursery are growing within the calciferous type of geochemical landscape (pH (KCl) – 5.5; 6.2; 7.5; 7.6; 7.8). Poplar clones in the Stupino are growing within a type of geochemical landscape where a significant role is played by silicon and iron in highly acidic and acidic conditions (pH(KCl) – 3.6; 3.9; 4.1; 4.3; 4.7) (table 2).

According to Kaňuchová and Ďurkovič [7] grey poplar is quite tolerant to groundwater level fluctuations. It is also tolerant to acidic soils and clay-loam soils with high soil moistening. This, however, is not the case with the white poplar. Our research has shown that soil conditions play a decisive role in the growth of micropropagated white poplar clones.

It's known that mineral and nutrient concentrations need to be in certain ranges in soil to support plant growth. The excess amounts of nutrients in soil cause toxicity for plants while lower amounts create deficiency and impede plant growth [10,11].

The soil conditions of the first test site are quite favorable, but they worsen as the soil moistening reduces. The micropropagated triploid poplar clones (especially white poplar clones) most fully realize their genetic potential on the fertile black soils of the Semiluki forest breeding nursery. At the age of 20 years the average height of plants was 14.8-15.8 m, stem diameter at breast height – 20.6-22.4 cm, stem volume – 0.231-0.266 m<sup>3</sup>. On low fertile, dry, acidic sandy loam soils (Stupino) the corresponding figures for grey poplar, for example, were 14.9 m, 17.6 cm and 0.170 m<sup>3</sup> respectively as opposed to 15.8 m, 21.1 cm and 0.242 m<sup>3</sup> in Semiluki (chernozem).

#### 4. Conclusion

The edaphic conditions have a significant impact on the capacity for survival and growth of *in vitro* micropropagated clones of white and grey poplar. The soil of the Semiluki forest breeding nursery site (a typical clay-loam black soil (chernozem) underlain by a light clay) is more favourable for white and grey poplar than that of the Stupino site (sandy-loam soil on glaciofluvial monomineral quartz sands).

The response to adverse growing conditions (on Stupino site) depended largely on the genotypic characteristics of a clone. Capacity for survival, growth and intraclonal homogeneity of grey poplar clones were better than those of white poplar clones.

The productive grey poplar clone has a broad genetic basis as it is allotriploid and a interspecific hybrid of the white poplar (*P. alba*) and the aspen (*P. tremula*). So it shows the higher ecological plasticity than an autotriploid white poplar clones, which has three haploid genomes of the same species. This expands the possibilities of its practical use in different edaphic conditions. To better realize the potential of triploid white poplar clones we should grow them in optimal soil conditions (alluvial and black soils).

## References

- [1] Sivolapov A I, Politov D V, Mashkina O S, Belokon M M, Sivolapov V A, Belokon Y S and Tabatskaya T M 2014 Cytological, molecular-genetic and silvicultural-selection research of polyploid poplars *Siberian J. Forest Sci.* [in Russian] **4** 50
- [2] Tsarev A P, Wühlisch G and Tsareva R P 2017 Hybridization of poplars in the Central Chernozem region of Russia *Silvae Genetica* **66** (6) 1
- [3] Peternel Š, Gabrovšek K, Gogala N and Regvar M 2009 *In vitro* propagation of European aspen (*Populus tremula* L.) from axillary buds via organogenesis *Scientia Horticulturae* **121** (1) 109
- [4] Žiauka J and Kuusienė S 2014 Multiplication and growth of hybrid poplar (*Populus alba* × *P. tremula*) shoots on a hormone-free medium *Acta biologica Hungarica* **65** (3) 346
- [5] Zontikov D, Zontikova S, Sergeev R, Shurgin A and Sirotina M 2014 Micropropagation of highly productive forms of diploid and triploid aspen *Adv.Mater. Res.* 962-963 pp 681-690
- [6] Keseru Z, Balla I, Antal B and Redei K 2015 Micropropagation of Leuce-poplars and evaluation of their development under sandy site conditions in Hungary *Acta Silv. Lign. Hung.* **11** (2) 139
- [7] Kaňuchová A and Ďurkovič J 2013 Wood ontogeny during *ex vitro* acclimatization in micropropagated hybrid poplar clones *Biologia Plantarum* **57** 144
- [8] Kulagin D V, Konstantinov A V, Boginskaya L A, Ostrikova M Ja and Padutov V E 2016 The comparisons of growth parameters on clonally propagated seedlings of different clones of woody species in forest plantations *Proc. BGTU* [in Russian] **1** 110
- [9] Ďurkovič J, Husárová H, Javoříková L, Čaňová I, Šuleková M, Kardošová M, Lukáčik I, Mamoňová M and Lagaňad R 2017 Physiological, vascular and nanomechanical assessment of hybrid poplar leaf traits in micropropagated plants and plants propagated from root cuttings: A contribution to breeding programs *Plant Physiol. Biochem.* **118** 449
- [10] Kelly J M and Ericsson T 2003 Assessing the nutrition of juvenile hybrid poplar using steady state technique and a mechanistic model *Forest Ecol. Manag.* **180** 249
- [11] Tufekcioglu A, Kalay H Z and Yilmaz M 2005 Effects of some soil properties on the growth of hybrid poplar in the Terme-Golardi region of Turkey *Turkish Journal of Agriculture and Forestry* **29** 221
- [12] Tullus A, Rytter L, Tullus T, Weih M and Tullus H 2012 Short-rotation forestry with hybrid aspen (*Populus tremula* L.×*P. tremuloides* Michx.) in Northern Europe *Scand. J. Forest Res.* **27** (1) 10
- [13] Shoshin V I and Graborov A V 2014 Influence of soil conditions on growth of the quercus borealis in forest cultures of the Bryansk region *Forestry Bull.* **4** 97
- [14] Stefanou S and Papazafeiriou A Z 2014 The effect of soil physical properties of an Entisol on the growth of young poplar trees (*Populus* sp.) *Bulg. J. Agric. Sci.* **20** 807
- [15] Mashkina O S 2016 Testing of triploid poplar hybrids in Voronezh region *Siberian Journal of Forest Science* [Sibirskii lesnoi zhurnal – in Russian] **5** 72
- [16] Mashkina O S, Tabatskaya T M, Morkovina S S and Panyavina E A 2016 Growing seedlings white poplar (*Populus alba* L.) based on the collection *in vitro* and evaluation of its cost For. Tech. J. [Lesotekhnicheskii zhurnal – in Russian] **1** (21) 28
- [17] Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassay with tobacco tissue cultures *Physiol. Plant.* **15** 473
- [18] Hadži-Georgiev K and Goguševski M 1972 Dvolazne tabele mass za topola klona *Populus euramerican* cv. I-214 u geveliskom području *Topola* **16** (90) 25
- [19] Weather and Climate, web-page <http://www.pogodaiklimat.ru>
- [20] Vorobyova L A 1998 *Chemical Analysis of Soils* (Moscow: University Press) p 272 ([http://www.pochva.com/?content=3&book\\_id=0078](http://www.pochva.com/?content=3&book_id=0078))
- [21] Lakin G F 1990 *Biometrics* (Moskow: Higher School) p 352