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Nursery screening of poplar and willow clones for biofuel application in Ukraine

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Poplars and willows are fast-growing trees that can be effectively grown as a renewable energy source. This study was devoted to the preliminary screening of poplar and willow clones for biofuel application in a fast-growing tree nursery established in the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine. The nursery includes 19 *Populus* and 10 *Salix* clones, with many hybrids of Ukrainian origin. The clones were assessed in the first two years in the nursery using growth parameters, biomass fuel criteria, and susceptibility to pathogens. Using total rank for evaluation, the highest rank was found in the poplar clone "Kanadska × balsamichna" followed by the clones "Ivantiivska", "Volosystoplidna", "Perspektyvna", and "Nocturn". Among the willows, the highest rank was recorded for the clone "Zhytomyrska-1", followed by clone "Zhytomyrska-2". High ranks were also found in the poplars "Strilopodibna", "Mobilna", "Novoberlinska-7" and "Keliberdynska", and the willows "Lisova pisnya" and "Vinnytska". Thus, the above-mentioned clones may be recommended as promising trees, though they should be further evaluated under field conditions for growth performance within the short-rotation cycles. The clones with the lowest total rank were poplars "Bolle", "Gradizka" and "Kytaiska × piramidalna" and willows "Lukash", "Olimpiisky vohon" and "Pryberezhna" are not recommended for bioenergy short-rotation plantations. Evaluation of plants in the nursery allowed us to carry out rapid and cost-effective preliminary screening. Such multiclonal screening of bioenergy trees for planting in short rotations was described for the first time in Ukraine.

Keywords: Tree Biomass, *Populus*, *Salix*, Short-Rotation Plantations, Growth Parameters, Wood Biofuel Properties, Pathogen Tolerance

Introduction

Poplars have been planted as timber, fuel, and windbreak trees for centuries in Ukraine. Willows have been mostly used for basket and furniture production, and both genera are exploited in traditional medicine and as ornamental plants. During the years 50-70s of the 20th century, many prospective poplar and willow clones were obtained by Ukrainian breeders to meet the needs of traditional long-rotation forestry (Starova 1975, Patlai & Rudenko 1990, Los 2013). At that time, plantations were mostly grown under Soviet directives, without knowledge about the fundamental principles of cultivation, and frequently with no commercial success. Today, the total area of conventional poplar forests covers less than 30,000 ha, mostly having functions as recreation, defence or natural conservation forests (98%), while only 2% are used for wood production (Vysotska 2017) covering a quite small area – 600 ha.

There is a great interest in the study of short-rotation forestry based on fast-growing bioenergy trees – mainly poplars and willows – in many countries (Bonosi et al. 2013, Lazdina et al. 2014, Sabatti et al. 2014, Kutsokon et al. 2015, Stochlová et al. 2015, Mir et al. 2017, Pilipović et al. 2019, Stolarski et al. 2020). Up to now, implementation of short-rotation forestry was poorly established in Ukraine, despite a large extension of unused, non-agricultural land. By estimates, there are 3-4 Mha of such lands in Ukraine, and 0.7 Mha could be recommended to be employed for the cultivation of bioenergy trees (Geletukha et al. 2014). Therefore, marginal areas require searching for tree clones with high biomass productivity. However, field experiments for the clonal assessments of short-rotation plantations (SRP) have been performed only recently in Ukraine, being *Salix* species more common then poplars (Los 2013, Kharytonov et al. 2017, Fuchylo et al. 2018, Kutsokon et al. 2018, Hrytsulyak 2019). Currently, the Ukrainian state registry for plant varieties (2022) contains 17 clones of willows, including 11 clones of *Salix viminalis*, 3 of *S. fragilis*, 2 *S. triandra*, and 1 *S. alba*, but no poplar clones are now included (SRPV 2022). Thus, despite the high potential of poplar clones as alternative energy source, it is clear that the bioenergy trees are much understudied in Ukraine.

Recently, few Ukrainian companies have started to cultivate willows for producing solid fuel – pellets, chips, and briquettes – and currently very few pilot SRP of poplars have been established (Geletukha et al. 2014).

Tree plantations of Salicaceae can be advantageously established in areas not suitable for agriculture such as areas with eroded, degraded, infertile or polluted soils, thus avoiding the competition with food production. However, to successfully establish tree plantations resistant to envi-

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Tab. 1 - General characterization of the plantation site.

Characteristic	Value
Location	M. M. Gryshko National Botanical Garden of NAS of Ukraine, Kyiv
Latitude, longitude	50° 25′ 01.2″ N, 30° 33′ 25.2″ E
Altitude (m a.s.l.)	100-200
Soil type	Dark grey podzol
pH	6.5-7.0
Soil humus content (%)	2.4-2.6
Annual mean temperature (°C)	8.4
Growing season mean temperature (°C)	16.3
Average number of days below 0°C	100-120
Annual rainfall (mm)	580-590
Growing season rainfall (April-September, mm)	350-370
Previous land use	Research area
Earlier crops	Grasses
Weed control in establishment year	Mechanical

Tab. 2 - Identification of the poplar and willow clones.

Genus	Clone name	Species/Hybrids
Populus	Bolle	*Populus alba* var. "Bolleana" Lauche
	Deltopodibna	*P. deltoides* Marsh. ssp. *monilifera* Henry (Torosova et al. 2015)
	Gulliver	*P. deltoides* Marsh. (free pollination) (Patlai & Rudenko 1990)
	Volosystoplidna	*P. trichocarpa* Torr. Et Gray (Torosova et al. 2015)
	Tronko	*P.* × *canadensis* (Patlai & Rudenko 1990)
	Gradizka	*P. nigra* L. × *P. deltoides* Marsh. (Patlai & Rudenko 1990)
	Robusta-16	*P. nigra* × *P. deltoides* (Los 2013)
	Keliberdynska	*P. nigra* × *P. deltoides* (Patlai & Rudenko 1990)
	Kanadska × balsamichna	*P. deltoides* Marsch. × *P. balsamifera* L. (Torosova et al. 2015)
	Kytaiska × piramidalna	*P. simonii* × *P. pyramidalis* (Starova 1975)
	Strilopodibna	*P. deltoides* × *P. pyramidalis* (Starova 1975)
	Perspektyvna	*P.* × *canadensis* (Dode) Guinier cv. "Regenerata" × *P. lasiocarpa* Oliv. (Los 2013)
	Lubenska	*P. piramidalis* × *P. trichocarpa* (Starova 1975)
	Nocturn	*P. trichocarpa* × *P. lasiocarpa* Oliv. (Starova 1975)
	Novoberlinska-3	*P. pyramidalis* × *P. laurifolia* (Torosova et al. 2015)
	Novoberlinska-7	*P. pyramidalis* × *P. laurifolia* (Torosova et al. 2015)
	Ivantiivska	*P. suaveolens* × *P. berolinensis* (Starova 1975)
	Mobilna	*P. trichocarpa* Torr. Et Gray × *P.* ×*canadensis* (Los 2013)
	Slava Ukrainy	*P. nigra* cv. "Pyramidalis"(Los 2013)
Salix	Lisova pisnya	*Salix alba* × *Salix fragilis* (Patlai & Rudenko 1990)
	Lukash	*S. alba* × *S. fragilis* (Patlai & Rudenko 1990)
	Mavka	*S. alba* × *S. fragilis* (Patlai & Rudenko 1990)
	Olimpiisky vohon	*S. alba* × *S. fragilis* (Patlai & Rudenko 1990)
	Pryberezhna	*S. alba* × *S. fragilis* (Patlai & Rudenko 1990)
	Pechalna	*S. alba* × *S. fragilis* (Patlai & Rudenko 1990)
	Verba na biomasu	*Salix* sp.
	Vinnytska	*Salix* sp.
	Zhytomyrska-1	*Salix* sp.
	Zhytomyrska-2	*Salix* sp.

ronmental stresses, the application of best management practices is an important issue (Euring et al. 2016). Those marginal lands are to be reclaimed due to the ability of trees to phytoremediation (Hrytsulyak 2019, Salam et al. 2019). To achieve an acceptable level of SRPs' yields in such conditions, knowledge of the growth dynamics of various clones, optimizing the interaction of plant genotype with site, and cultural management, including prolonging rotations, are important tasks (Niemczyk et al. 2016, Sabatti et al. 2014).

On the other hand, monoclonal/monospecific plantations involve also several drawbacks, particularly regarding the reduced biodiversity of plantations and their adverse environmental impacts (Freer-Smith et al. 2019). Therefore, increased attention should be paid to mixed-species planting and agroforestry systems with poplars and willows as the key tree species, which positively affect tree growth and ecosystem functions and is beneficial for both trees and ecosystems by restoring and sustaining soil health, enhancing soil organic carbon and nutrient availability, and improving soil biota (Bardhan & Jose 2012, Dollinger & Jose 2018, Gold & Garrett 2009, Liu et al. 2018).

The production of biomass in SRP may be considered a "relatively new issue" (Stolarski et al. 2020), and the requirements for planting material for bioenergy SRP differ from those for traditional forestry (Bartko 2011), which suppose the selection of trees with long, straight stems with poor branching and strong apical control (Sabatti et al. 2014). Indeed, for biomass production in SRP different genotypes should be selected and improved for fast juvenile growth, rapid biomass production, high survival rate, easy primary rooting and coppicing ability (Bartko 2011, Sabatti et al. 2014). Some key energy-related characteristics such as cellulose, lignin, and hemicellulose content, bark content, moisture content, heating value, ash content etc., are also relevant for bioenergy production (Sannigrahi et al. 2010).

Additional characteristics of bioenergy trees may be drought tolerance, resistance to pests and insects, the ability to produce high biomass yields on different types of land (Sannigrahi et al. 2010), tolerance to abiotic stresses and ability to phytoremediation. Some of the above-mentioned characteristics can be enhanced by genetic modifications (Kutsokon 2011).

Studies on clones of fast-growing trees suitable to effective biomass production are even more relevant in Ukraine, where SRPs are currently being introduced in the country. The present study was aimed to carry out a preliminary screening on a number of clones available in Ukraine, as well as to evaluate growth parameters, fuel biomass characteristics and susceptibility to pathogens of 19 *Populus* and 10 *Salix* clones growing in the nursery for their suitability to cultivation in SRPs.

Materials and methods

Site description and plant material

A fast-growing tree nursery was established at the National Botanical Garden of National Academy of Sciences of Ukraine in Kyiv. It is located on the border of Forest-Steppe and Polissya geographical zones, on the right bank of the Dnipro River, close to the city centre. The climate in Kyiv is temperate continental with relatively mild winters and warm summers. Detailed information about the site, such as location, climate, soil type etc. is shown in Tab. 1.

Nineteen *Populus* and ten *Salix* clones were manually planted in the nursery. Planting material was mostly provided by the Institute of Forestry and Forest Melioration (Kharkiv, Ukraine). Many of the poplars and willows provided were hybrids of Ukrainian origin. Identification of the clones is presented in the Tab. 2. All the agricultural practices on the site (pre-planting fertilisation, planting, irrigation, weed control, etc.) were carried out according to common practice.

Cuttings of 20-25 cm in length and up to 1.5 cm in diameter were planted in rows in May with a very dense scheme, an average of about 50 × 50 cm, depending on the availability of planting material which varied between six and twenty seven cuttings (Tab. 3). During the first growing season, the plot was watered when required through the spring and summer periods, usually every week because of the extreme early summer drought.

Tab. 3 - Leave measurements and susceptibility of poplar and willow clones to the pathogens: clones susceptible (++), low susceptible (+), and with no signs of disease (-) to poplar rust (PR), or witches' broom disease (WB). (LL): leaf length (cm); (LW): leaf width (cm); (a): analyses were performed in the first growing season; (b): analyses were performed in the second growing season.

Genus	Clone/hybrid	No. Plants	LL[a]	LW[a]	Susceptibility to pathogens[b]
Populus	Bolle	7	8.1	9.1	PR +
	Deltopodibna	6	15.1	14.9	PR ++
	Gradizka	6	9.5	14.1	PR +
	Gulliver	16	17.1	18.1	PR -
	Ivantiivska	13	19.6	9.6	PR +
	Kanadska × balsamichna	7	17.5	18.2	PR ++
	Keliberdynska	9	10.2	11.2	PR ++
	Kytaiska × piramidalna	14	10.4	7.1	PR -
	Lubenska	21	13.7	10.2	PR ++
	Mobilna	14	16.4	14.6	PR ++
	Nocturn	11	23.1	16.5	PR +
	Novoberlinska-3	12	13.1	10.1	PR ++
	Novoberlinska-7	22	15.1	11.5	PR ++
	Perspektyvna	16	17.1	16.1	PR ++
	Robusta-16	16	11.6	13.6	PR -
	Slava Ukrainy	17	9.1	12.1	PR +
	Strilopodibna	16	15.5	14.1	PR ++
	Tronko	14	18.4	17.1	PR +
	Volosystoplidna	8	20.1	8.1	PR ++
	Mean ± SE	-	*14.8 ± 1.0*	*12.9 ± 0.8*	-
	Coefficient of variation (%)	-	*28*	*26*	-
Salix	Lisova pisnya	23	11.4	3.1	WB +
	Lukash	25	9.4	2.9	WB ++
	Mavka	27	10.5	3.5	WB -
	Olimpiisky vohon	17	10.3	2.6	WB ++
	Pryberezhna	17	11.3	2.7	WB +
	Verba na biomasu	18	11.1	1.4	WB -
	Vinnytska	12	16.2	1.6	WB -
	Zhytomyrska-1	13	17.2	2.6	WB -
	Zhytomyrska-2	9	16.1	1.6	WB -
	Mean ± SE	-	*12.6 ± 1.0*	*2.4 ± 0.3*	-
	Coefficient of variation (%)	-	*24*	*31*	-

Measurements of growth and biofuel characteristics

Growth measurements were performed for 6 to 10 plants per clone in October-November at the end of the first and second growing seasons. The tree basal stem diameter (D, cm) was measured with a digital calliper for the thickest shoot, and the maximal shoot height (H, m) was measured with a measuring pole in both growing seasons. Leaf length (LL, cm) and leaf width (LW, cm) were measured with a ruler on the leaves of the main shoot at the middle tree level in September of the first growing season. For the ranking estimations, leaf indexes (LI, cm^2) were calculated as LI = LL × LW, where LL is the leaf length and LW is its width. The number of shoots per plant (NoSP) and the fresh weight of a shoot (FW, g) were determined at the end of the first growing season.

Shoot sampling at the end of the first vegetation year was performed. One single shoot of average size was collected randomly from each of three different distant plants for each clone. The shoots were weighted immediately after cutting using a 1g precision scale and then air dried.

Wood samples were collected from the base, middle and top parts of each stem. The samples containing the bark were dried, ground with a laboratory mill and homogenized by thorough mixing. The calorific value (CalV, MJ kg^{-1}) was measured using a calorimeter (C200®, IKA-Werke, Staufen, Germany). The ash content (AC, %) was determined by burning in the muffle furnace (SNOL 7.2/1100, ThermoLab, Ukraine) at 300-700 °C for two hours (Hrytsayenko et al. 2003). AC was estimated as a weight percentage of ashes remaining after burning the wood samples. The dry matter content (DMC, %) was estimated after drying the samples at 105 °C until reaching a permanent weight as a percentage of the dry mass of the sample to its fresh biomass.

All the vital parameters (D, H, NoSP, LL, and LW) were analysed on ten plants. Fewer growing plants (6-9) were available for 7 of the total of 29 clones. Thus, in this case analyses were performed on all available plants (Tab. 3). All the other parameters (FW, CalV, AC, and DMC) were estimated with the use of three shoot samples from the different plants.

To estimate the total potential of clones for application as biofuel, both growth (D, H, NoSP, LI, FW) and fuel (CalV, AC and DMC) characteristics during the two years of nursery evaluation were ranked separately for poplars (ranks 1-19) and willows (ranks 1-9). All the parameters, excluding ash content, were ranked in ascending order based on the values obtained (Mir et al. 2017).

In August of the second growing season, all plants in the nursery were screened for the presence of phytopathogens. Clones were recorded as susceptible ("++", ranking score = 0), low susceptible ("+", score = 1) or with no signs ("–", score = 2) of poplar rust (PR), or witches' broom disease (WB).

Statistical analyses

The results were processed using the software Microsoft Excel® (Redmond, WA, USA) and OriginPro® 9 (Northampton, MA, USA) tools using common statistical methods. Variable values were expressed as the mean ± standard error of the mean (SE). Average values for all samples were calculated separately for poplars and willows and then compared to each individual clone variable. The Mann-Whitney U-test for statistical analysis was used to determine which clones had higher or lower parameter values compared to average levels. Divergences were evaluated as statistically different if $p < 0.05$.

Results and discussions

Growth parameters

Stem diameter

Variations in basal stem diameter were exhibited between the clones as well as between the first and second vegetation years (Fig. 1). After the first year of cultivation, the poplar clones "Kanadska × balsamichna" had a maximal D followed by the clone "Nocturn" while the lowest values were produced by the poplar clones "Bolle", "Gradizka" and "Gulliver' (Fig. 1a). Among the willows, maximal D was recorded for the clone "Zhytomyrska-1" followed by the clone "Vinnytska" (Fig. 1b). The willow clones "Lukash", "Mavka" and "Olimpiisky vohon" had even lower basal stem diameters than the poplars. The rest of the clones did not differ from the average levels (Fig. 1a, Fig. 1b). It is necessary to note that henceforth, if the differences between clones and corresponding average levels are noted, then they are statistically significant ($p < 0.05$), otherwise not mentioned.

After the second year, higher diameters were attained by the poplar clones "Kanadska × balsamichna", "Volosystoplidna", "Perspektyvna" and "Ivantiivska", with D more than 2.50 cm. The highest value among the willows was produced by the clone "Zhytomyrska-1", with D=2.13 cm. The lowest rates of base stem diameter, less than 1.3 cm, were seen in poplars "Bolle", "Kytaiska × piramidalna" and "Gradizka" and willows "Verba na biomasu" and "Olimpiisky vohon". The ranks for the diameters of different clones of fast-growing trees are presented in Tab. 4 and Tab. 5.

A comparison of the average total poplar and willow diameters showed that poplars had larger diameters than willows both in the first and second years ($p < 0.01$ – Fig. 1a, Fig. 1b).

The differences in diameter growth may be most likely related to clonal variability, as was shown by several poplar cultivars (Niemczyk et al. 2016). However, the clonal variability may not always be an obvious factor, and sometimes variations are more dependent on the environmental conditions including growing season (Lazdina et al. 2014). The soil properties is also a factor greatly affecting tree productivity (Fontana et al. 2016), but in our study the soil composition has been assumed to be invariant within the single plot considered.

Stem height

The average total poplar and willow height values were almost the same at the end of the first growing season, while they were statistically different after the second year (Fig. 1c, Fig. 1d). At the end of the first year, the greatest stem height was shown by the poplars "Volosystoplidna", "Ivantiivska", "Novoberlinska-7" and "Kanadska × balsamichna" (H = 2.19-2.06 m) and willows "Zhytomyrska-1" and "Lisova pisnya" (H = 2.38 and 1.99 m, respectively). In contrast, significantly lower heights were found in poplars "Kytaiska × piramidalna", "Deltopodibna", "Gradizka" and "Bolle" (H = 1.39-0.74 m) and willows "Lukash", "Olimpiisky vohon", with H = 1.40 and 1.31 m. During the second plantation year, poplar clones demonstrated similar patterns when the same four clones ("Volosystoplidna", "Kanadska × balsamichna", "Novoberlinska-7" and "Ivantiivska") showed the highest rate (H = 4.43-3.69 m). Similarly, the same three out of four clones ("Gradizka", "Kytaiska × piramidalna", "Bolle") demonstrated the lowest rate (H = 2.04, 1.91, 1.11 m, respectively) and they ranked the lowest for stem height (Tab. 4). Among the willows, the tallest clone was "Zhytomyrska-1" (H = 4.12

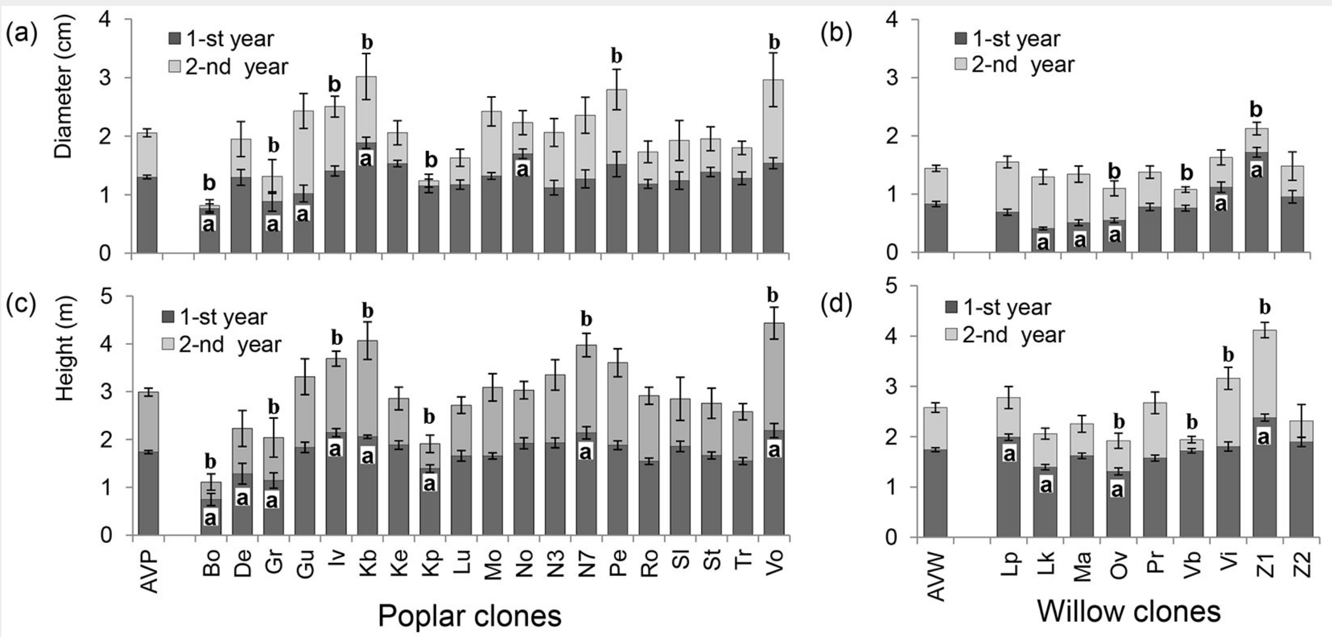

Fig. 1 - Stem diameter and height of poplar (a, c) and willow (b, d) clones in the first and second growing seasons. Clones demonstrated significant (p<0.05) differences after Mann-Whitney test comparing the average levels for poplars (AVP) and willows (AVW) in the first (a) and second (b) vegetation years. Poplar clones: Bo - "Bolle"; De - "Deltopodibna"; Gr -"Gradizka"; Gu- "Gulliver"; Iv - "Ivantiivska"; Kb - "Kanadska x balsamichna"; Ke - "Keliberdynska"; Kp - "Kytaiska x piramidalna"; Lu - "Lubenska"; Mo - "Mobilna"; No - "Nocturn"; N3 - "Novoberlinska-3"; N7 - "Novoberlinska-7"; Pe - "Perspektyvna"; Ro - "Robusta-16"; Sl - "Slava Ukrainy"; St - "Strilopodibna"; Tr - "Tronko"; Vo - "Volosystoplidna". Willow clones: Lp - "Lisova pisnya"; Lk - "Lukash"; Ma - "Mavka"; Ov - "Olimpiisky vohon"; Pr - "Pryberezhna"; Vb - "Verba na biomasu"; Vi - "Vinnytska"; Z1 - "Zhytomyrska-1"; Z2 - "Zhytomyrska-2".

Tab. 4 - Ranking of the growth and biofuel parameters of poplar clones. (D): tree basal stem diameter; (H): shoot height; (NoSP): the number of shoots per plant; (FW): fresh weight of a shoot; (CalV): calorific value; (AC): ash content; (DMC): dry matter content; (LI): leaf index calculated as LI = LL × LW; (PR): susceptibility to the poplar rust recorded as: clones susceptible (score 0), low susceptible (score 1) or without signs of disease (score 2); (a): analyses were performed at the first growing season; (b): analyses were performed at the second growing season.

No	Clone/hybrid	D[a]	D[b]	H[a]	H[b]	NoSP[a]	FW[a]	CalV[a]	AC[a]	DMC[a]	LI[a]	PR[b]	Total score	Total rank
1	Bolle	1	1	1	1	4	1	3	15	12	1	1	41	1
2	Deltopodibna	11	8	3	4	13	7	2	7	10	13	0	78	7
3	Gradizka	2	3	2	3	1	2	6	8	10	6	1	44	2
4	Gulliver	3	15	8	13	2	5	5	10	2	16	2	81	8
5	Ivantiivska	14	16	14	16	11	7	9	8	14	11	1	121	18
6	Kanadska × balsamichna	19	19	13	18	7	13	2	9	11	18	0	129	19
7	Keliberdynska	16	10	10	9	14	15	3	4	4	4	0	89	11
8	Kytaiska × piramidalna	5	2	4	2	5	7	7	11	7	2	2	54	3
9	Lubenska	6	4	6	6	10	7	8	3	13	7	0	70	5
10	Mobilna	12	14	6	12	2	9	6	13	7	14	0	95	13
11	Nocturn	18	12	11	11	6	6	11	6	5	19	1	106	15
12	Novoberlinska-3	4	11	12	14	1	12	7	14	3	5	0	83	10
13	Novoberlinska-7	9	13	14	17	6	10	1	10	1	10	0	91	12
14	Perspektyvna	15	17	10	15	2	3	9	12	9	15	0	107	16
15	Robusta-16	7	5	5	10	10	8	4	5	9	8	2	73	6
16	Slava Ukrainy	8	7	9	8	9	10	2	2	8	3	1	67	4
17	Strilopodibna	13	9	7	7	8	11	8	11	10	12	0	96	14
18	Tronko	10	6	5	5	12	14	5	1	6	17	1	82	9
19	Volosystoplidna	17	18	15	19	3	4	10	10	9	9	0	114	17

m) followed by "Vinnytska" with 3.16 m. The smallest clones were "Verba na biomasu" and "Olimpiisky vohon" with H=1.94 and 1.92 m, respectively. Thus, the height growth dynamics in willows was more diverse during both years.

Such differences in height growth rates were certainly related to clonal variability, as was demonstrated for the willow cultivars (Fuchylo et al. 2018). However, in another experiment in Latvia, the height of the poplars depended more on the year of planting and fertilisation treatment than on the clonal origin (Lazdina et al. 2014). These variations may also be linked to different growth sensitivities to weather conditions, as shown for a young hybrid aspen (Senhofa et al. 2018).

Number of shoots per plant

Most willow clones produced several shoots, as demonstrated by a 34% higher total level of shoots per plant compared to the poplars ($p < 0.01$). A recent study also showed that willow clones differed from poplars for the production of a greater number of shoots (Stolarski et al. 2015), probably because of weaker apical dominance in willows (Liu et al. 2017).

We determined that clonal values in willows varied between 1.2-2.3, and the average equalled 1.79 ± 0.14, while the poplars' ranges (1.0-2.2) and the mean (1.34 ± 0.07) were lower (Fig. 2, Fig. 3). The coefficient of NoSP variation between the clones was the same (24%) for both the averages of poplars and willows.

It can be seen from the ranks in Tab. 4 and Tab. 5 that the poplars "Keliberdynska", "Deltopodibna" and "Tronko" and the willows "Olimpiisky vohon", "Zhytomyrska-2", "Zhytomyrska-1" and "Lukash"

Tab. 5 - Ranking of growth and biofuel parameters of willow clones. (D): tree basal stem diameter; (H): shoot height; (NoSP): the number of shoots per plant; (FW): fresh weight of a shoot; (CalV): calorific value; (AC): ash content; (DMC): dry matter content; (LI): leaf index, calculated as LI = LL × LW; (WB): susceptibility to witches' broom disease recorded as: clones susceptible (score 0), low susceptible (score 1) or with no signs of disease (score 2); (a): analyses were performed at the 1-st growing season; (b): analyses were performed at the 2-nd growing season.

No	Clone/hybrid	D[a]	D[b]	H[a]	H[b]	NoSP[a]	FW[a]	CalV[a]	AC[a]	DMC[a]	LI[a]	WB[b]	Total score	Total rank
1	Lisova pisnya	4	7	8	7	3	3	3	6	5	7	1	54	7
2	Lukash	1	3	2	3	6	1	1	4	7	5	0	33	1
3	Mavka	2	4	4	4	2	4	5	5	4	8	2	44	5
4	Olimpiisky vohon	3	2	1	1	9	5	4	2	6	4	0	37	2
5	Pryberezhna	6	5	3	6	4	3	2	1	1	6	1	38	3
6	Verba na biomasu	5	1	5	2	5	2	6	5	8	1	2	42	4
7	Vinnytska	8	8	6	8	1	6	3	3	2	3	2	50	6
8	Zhytomyrska-1	9	9	9	9	7	7	3	7	3	9	2	74	9
9	Zhytomyrska-2	7	6	7	5	8	5	5	8	9	2	2	64	8

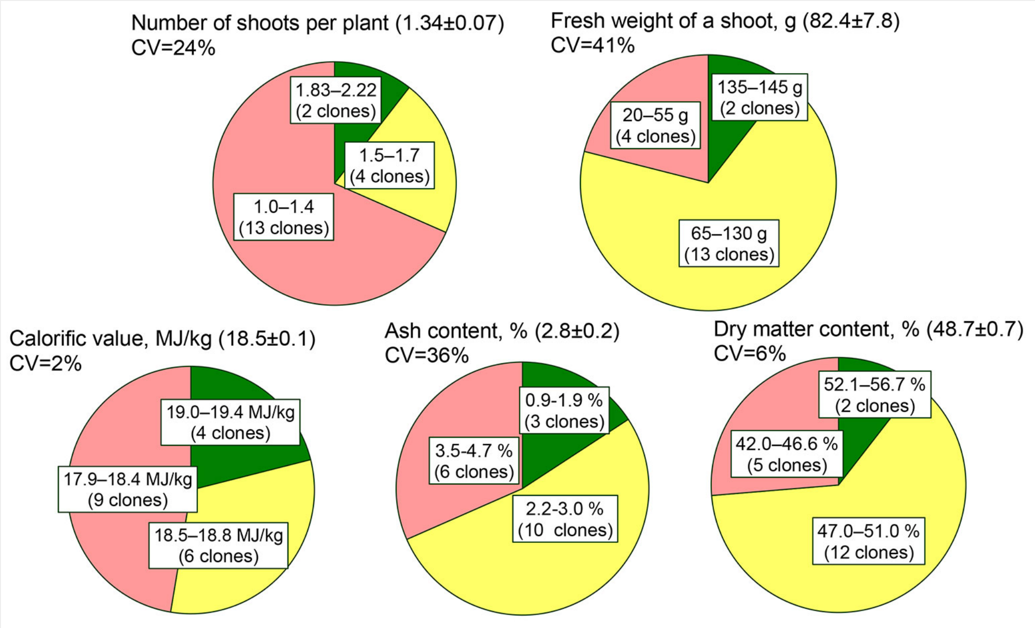

Fig. 2 - Growth and biofuel characteristics of poplars at the end of first growing season. Clones are grouped in three equal intervals for each variable analysed: high (green), average (yellow) and low (red) values; mean and SE are presented in parentheses; (CV): coefficient of variation.

were the clones with the greatest number of shoots. The lowest NoSP was produced by poplars "Novoberlinska-3" and "Gradizka", with only one shoot per plant, and willow clones "Mavka" and "Vinnytska", with 1.3 and 1.2 shoots per plant, respectively (Tab. 4, Tab. 5).

The number of shoots per plant is an important parameter for biomass productivity in bioenergy SRP, often being as important as height or radial growth, as it allows an increase in the total biomass (Fuchylo et al. 2018). Additionally, high regeneration ability is necessary for producing powerful shoots after pruning. Actually, the approach used for ranking in this study is the opposite to that applied in conventional forestry where the trees with fewer stems and side branches are preferable for planting (Mir et al. 2017, Sabatti et al. 2014). Clones are frequently variable in stem number (Mosseler et al. 2014, Sabatti et al. 2014), and the number of shoots per plant seems to be mostly dependent on clonal peculiarities and planting density (Fuchylo et al. 2018).

Shoot fresh weight

The fresh weight of a shoot was estimated at the end of the first growing season. No significant differences were found between poplars and willows; although the average rates differed (82.4 ± 7.8 g and 70.0 ± 12.2 g, respectively), this parameter greatly varied among different clones with a high percentage of variation for both poplar (41%) and willow clones (52% – Fig. 3, Fig. 4). Mosseler et al. (2014) also demonstrated high variability in single stem and total above-ground fresh biomass in willow clones.

The greatest annual increase in single stem fresh biomass was produced by the poplars "Keliberdynska" and "Tronko" and the willow "Zhytomyrska-1", whereas poplars "Bolle" and "Gradizka" and willows "Lucash" and "Verba na biomasu" produced the least biomass during the first growing year (from 20 to 40 g). Subsequent ranks are represented in Tab. 4 and Tab. 5.

Leaf measurement

The length of poplar leaves from the different clones varied in the range 8.1-23.1 cm, with a mean rate of 14.8 ± 1.0 cm, while the length of willow leaves varied between

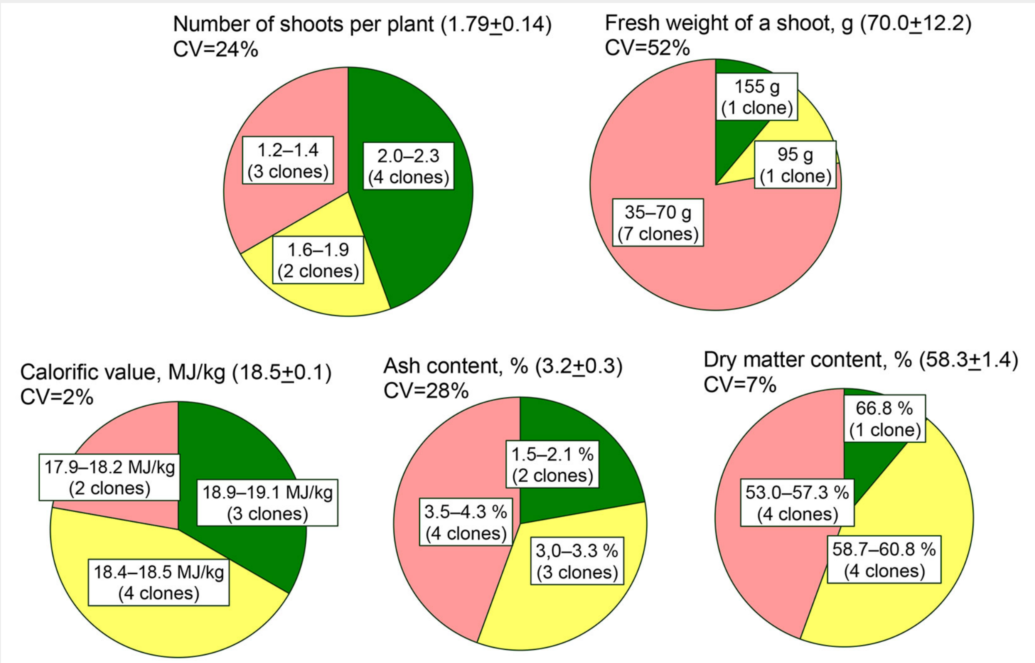

Fig. 3 - Growth and biofuel characteristics of willows at the end of first growing season. Clones are grouped in three equal intervals for each variable analysed: high (green), average (yellow) and low (red) values; mean and SE are presented in parentheses; (CV): coefficient of variation.

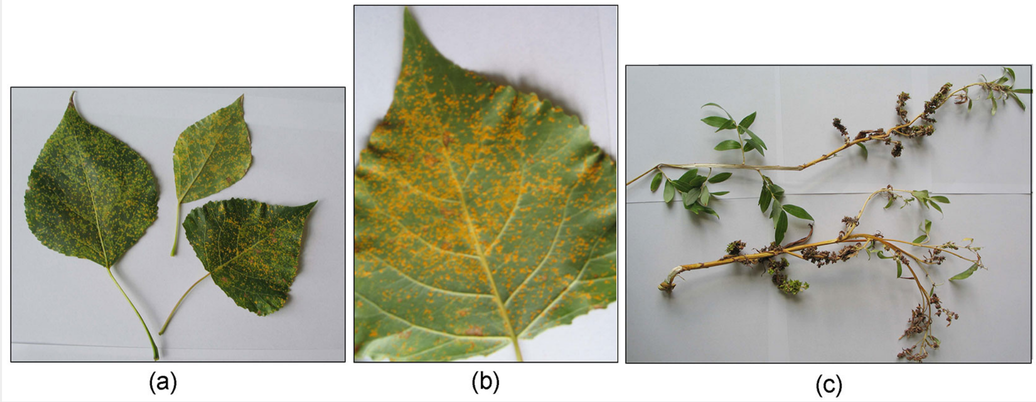

Fig. 4 - Poplar rust *Melampsora laricis-populina* determined in *Populus* (a, b) and witches' broom disease founded in *Salix* (c) clones.

9.4-17.2 cm, with an average mean of 12.6 ± 1.0 cm (Tab. 3). These differences were not statistically significant after Mann-Whitney test. In contrast, the mean width of leaves in poplars was significantly greater than in willows by five times ($p < 0.001$). Such a great difference in width is due to well-known morphological traits of *Populus* and *Salix* leaves.

For the ranking estimations, the leaf index (LI) was used (multiplication of leaf length by width). Due to LI values, the smallest total square sizes were recorded in poplars "Bolle" and "Kytaiska × piramidalna" and willow "Verba na biomasu", while the greatest values were obtained for the poplars "Nocturn", "Kanadska × balsamichna", "Tronko" and "Gulliver" and willow "Zhytomyrska-1" (Tab. 4, Tab. 5). Leaf size parameters (including leaf length, width, and area) are important variables determining stand productivity in poplar (Ceulemans et al. 1993) and shoot fresh biomass in mulberry genotypes (Cao et al. 2019). They also correlated with the primary productivity in woody ecosystems (Li et al. 2020).

However, it should be mentioned that the leaf size may be considered as a climate-driven trait reflecting how natural selection modifies it across varying climates (Li et al. 2020). For instance, the cultivars with larger leaves may be better biomass producers in optimal water conditions, whereas the cultivars with smaller leaves might be satisfactory producers in drylands and cold regions (Cao et al. 2019), although not superior in biomass production.

Biomass fuel criteria

Calorific value

The calorific value of wood samples from different clones was estimated at the end of the first growing season. Interestingly, both poplars and willows demonstrated the same mean rates (18.5 MJ kg^{-1}) and the same low coefficients of variation of CalV (2%).

Among the poplars, the highest calorific value (> 19 MJ kg^{-1}) was found for the samples of clones "Nocturn", "Volosystoplidna", "Perspektyvna" and "Ivantiivska"; among willows, the highest rank was reached by the clone "Verba na biomasu" (Tab. 4, Tab. 5). In contrast, the lowest calorific value (17.9 MJ kg^{-1}) was detected in poplar "Novoberlinska-7" and willow "Lucash". Variation of heating values in different poplar genotypes was also found by Sabatti et al. (2014). Calorific values of the wood samples of all clones ranged within 17.9-19.4 MJ kg^{-1} (Fig. 2, Fig. 3), close to the typical calorific value of wood (Francescato et al. 2009). Interestingly, these levels exceeded the allowable minimum of calorific value (16.5 MJ kg^{-1}), for pellets in the EU according to the standards of ENplus (EPC 2015).

The calorific value of different wood species varies mainly due to the lignin content, which has a higher CalV compared to cellulose and hemicellulose (Francescato et al. 2009). Lipids and terpenes are other components greatly affecting the wood energy content (Jamnická et al. 2014). The calorific value of wood also differs between the various parts of the tree (Alakangas 2005, Jamnická et al. 2014) and depends on the condition of wood used for chip production (Pecenka et al. 2020). Heat content is an important factor affecting utilisation of any material as a fuel, while high ash content makes it less desirable.

Ash content

Ash content was measured in the air-dried wood samples after the first growing season. In the poplar samples, AC ranged within 0.9-4.7%, with a mean level of 2.8 ± 0.2%. In the willows, the ash content was in the range 1.5-4.3%, with a mean rate of 3.2 ± 0.3%. Such differences were not statistically significant.

The lowest amounts of ash (up to 2%) were determined in the poplar samples "Bolle", "Novoberlinska-3", and "Mobilna" and the willow sample "Zhytomyrska-2". These clones had the highest ranks (Tab. 4, Tab. 5), while the biomass samples from poplars "Lubenska", "Slava Ukrainy" and "Tronko" and willows "Olimpiisky vohon" and "Pryberezhna" showed the highest ash contents (4.0 to 4.7%).

The typical ash value of the wood from short-rotation coppices of willow and poplar is usually up to or around 2% (Francescato et al. 2009, Sannigrahi et al. 2010) and may vary depending on the clone, rotation cycle and other factors (Sabatti et al. 2014). According to the standards of ENplus for pellets, ash content should be in the range of 0.7-2%, depending on the category of the pellets (EPC 2015). Our data demonstrated that only some part of the analysed samples contained ash amounts of 2% or less. We suggest that the higher ash amounts in our experiments may be explained not only by clonal variability. Indeed, it is likely that true ash production is overestimated by analysing the biomass samples from young one-year plants. Such shoots are thinner compared to three-year plants, typically used in SRP for producing wood chips and pellets in temperate climates. A large shoot diameter generally improves fuel quality for combustion, but thinner shoots have a higher bark proportion (Liu et al. 2017) and, consequently, higher ash content compared to shoots with larger diameter. Additionally, the fuel quality of biomass from SRP may be improved by planting clones having low element concentrations (Liu et al. 2017).

Dry matter content

Dry matter content (DMC) measured at the end of the first growing season was on average 20% higher in willows compared to poplars, and was significantly different between them ($p < 0.001$). The values varied within 42.0-56.7% in the poplar samples, and 53.0-66.8% in the willows, with similar variation coefficients between clones for both (6-7% – Fig. 2, Fig. 3).

The highest percentage of DMC among the poplars was produced in the sample of clone "Ivantiivska" (56.7%). The willow samples with the highest ranks, "Zhytomyrska-2", "Verba na biomasu" and "Lukash" had more than 60% of DMC (Tab. 5). The lowest rates of DMC among the poplars were detected in the samples of clones "Novoberlinska-7" and "Gulliver", and among the willows in the samples of clones "Pryberezhna" and "Vinnytska". Significantly different clonal variation in poplar biomass moisture content was also found in other studies (Stolarski et al. 2020, Sabatti et al. 2014).

It is well known that a higher percentage of dry matter and lower moisture content increases the energy yield of wooden biomass (Alakangas 2005), which is an important parameter for biofuel efficiency. Several authors reported the moisture content in poplar wood to vary 41-59% (Di Matteo et al. 2012, Monedero et al. 2017, Rosso et al. 2013, Stolarski et al. 2020), similar to the rates obtained in our experiments. Likewise, the same authors determined significantly higher moisture content from the poplar compared to the willow samples (Monedero et al. 2017).

Assessment of phytopathogens presence

During the first growing season, no visible signs of phytopathogens were determined on the plants from the nursery. However, during the second summer season, natural phytopathogens were found on the plants, with a high variation in susceptibility among clones. Many poplar clones were infected with the leaf rust *Melampsora laricis-populina* Kleb. (Fig. 4a, Fig. 4b), while a few willow clones had witches' broom disease (Fig. 4c) (EPPO 2008). The susceptibility of poplar clones from the nursery to the rust disease mostly ranged from low (6 clones) to high levels (10 clones). Only three poplar clones, "Robusta-16", "Gulliver" and hybrid "Kytaiska × piramidalna", among the 19 studied, had no visible signs of rust infection (Tab. 3).

The willow clones "Lukash" and "Olimpiisky vohon" demonstrated the highest susceptibility to witches' broom disease followed by the clones "Lisova pisnya" and "Pryberezhna", with lower symptoms of infection. The remaining five willow clones did not show any sign of witches' broom disease.

Most likely, close spacing in the nursery planting contributed greatly to the spreading of phytopathogens. This hypothesis may be confirmed by the results of our other experiment (not presented here), where we did not find any sign of infection during the three years in which several of the same clones were planted on a widely-spaced field plantation (Kutsokon et al. 2018). The causal agent of witches' broom disease is still unclear; it might be caused by intracellular parasite phytoplasma (EPPO 2008, Alfaro-Fernández et al. 2011) but also by other factors such as fungi infection, mite infestation, genetic mutations or even adverse environmental conditions killing the terminal bud of the tree shoot (MBG 2012). For instance, witches' broom disease was frequently encountered close to the epicentre of the Chornobyl NPP disaster due to damage of the apical meristems of plants by high doses of irradiation (Rashydov et al. 2012).

Ranking of growth and biofuel parameters of poplar and willow clones

Our experiments were intended to identify the clones with the fastest growth at a young age; additionally, it is also important that those plants have valuable biofuel parameters. Thus, an overall ranking approach for selecting the best clones was used. Growth parameters such as D, H, NoSP, LI and FW, biomass fuel criteria such as CalV, AC and DMC and susceptibility to pathogens varied differently among the clones of fast-growing trees. Similarly, both variations in the growth traits and wood properties of white poplar and willow clones to identify the superior clones were studied (Ma et al. 2015, Rosso et al. 2013).

Some clones demonstrated high growth performances, but their biofuel parameters were scored at low values and *vice versa*. For instance, the biomass sample from the poplar clone "Bolle" had the highest rank for ash content, but its growth parameters (D, H, FW, and LI) demonstrated the lowest ranks. In contrast, the poplar "Kanadska × balsamichna" demonstrated high ranks for growth parameters, but it was susceptible to poplar rust, and its calorific value was low ranked (Tab. 4).

Nevertheless, the maximal total score was reached by the poplar clone "Kanadska × balsamichna", ranked 19th, followed by the clones "Ivantiivska", "Volosystoplidna", "Perspektyvna" and "Nocturn", ranked 18th-15th, respectively. Among the willows, the highest rank (9th) was recorded for clone "Zhytomyrska-1", followed by clone "Zhytomyrska-2" (ranked 8th). The willow "Zhytomyrska-1" was recently selected from the natural flora at the National Botanical Garden; it could be a very promising clone for short rotations, particularly in a very dense planting. Additionally to the highest diameter and height growth values, the clone demonstrated tolerance to witches' broom disease. Moreover, the plants had many powerful shoots, which greatly improves productivity. Interestingly, four of the best willow clones were tolerant to witches' broom diseases besides only the clone "Lisova pisnya", which demonstrated average susceptibility to WB (WB+ in Tab. 5).

High total ranks were also demonstrated by the poplars "Strilopodibna", "Mobilna", "Novoberlinska-7" and "Keliberdynska", ranked 14th-11th respectively, and willows "Lisova pisnya", "Vinnytska" (ranked 7th and 6th, respectively). Thus, the abovementioned clones are recommended as promising for SRP. However, it is important to note that susceptibility to poplar rust should be taken into consideration in poplar plantations with very close spacing. In our experiment, some highly productive clones were susceptible to poplar leaf rust (Tab. 3). Although we did not find any significant growth suppression *via* infection at the current stage, adverse effects may increase in the future. The rust fungus *Melampsora larici-populina* can decrease poplar growth parameters up to 66% (Stochlová et al. 2015).

The lowest total ranks (1st-3rd) were attained by poplars "Bolle", "Gradizka" and "Kytaiska × piramidalna" and willows "Lukash", "Olimpiisky vohon" and "Pryberezhna". Therefore, these clones are not recommended for bioenergy SRP, but they may be appropriate for conventional forestry, defensive field belts, ornamental purposes, etc.

Ranking the male clones by their growth parameters after two years of evaluation allowed to recommend the best clones for mass multiplication and gradual replacement of female cultivars in the Kashmir Valley (Mir et al. 2017). We used more different parameters to screen and rank the clones available in Ukraine.

Our results showed that despite the very dense planting of cuttings, after being watered during the rooting time, the plants showed a high level of survival and intensive growth during the first two seasons in the nursery. Similarly, Bergante & Facciotto (2011) reported that irrigation in the first two years was crucially important for plant survival and high biomass production.

To some extent, our approach for planting spacing is differed from the methodology used in the SRP for clonal trials where randomised complete block design with three block replicates applied (Niemczyk et al. 2016). Usually, poplar nurseries may be established at different spacing, for instance, 0.6 × 0.6 m (Mir et al. 2017), but less or more dense schemes are also applicable. However, we believe that in our study the slightly differed spacing has affected less the growth parameters as compared to the origin of clone.

The existing range of varietal diversity of poplars and willows allows for the selection of clones that will be more productive in certain conditions. The water regime can be crucial for the productivity of plantations (Bonosi et al. 2013). While poplars and willows are not generally considered drought-tolerant, the existing clonal variations in their drought resistance allow them to survive under severe conditions. Interestingly, our other experiments (data unpublished) showed that the best willow clone, "Zhytomyrska-1", performed poorly under stressful conditions, such as water deficiency and salinity; being highly productive in terms of biomass, this clone is quite demanding of water and soil quality. Thus, for biomass purposes it should be planted only in suitable plots. This example, as well as others, demonstrates that it is quite important to estimate the effects of local climate and soil conditions before deploying clones on a commercial scale (Niemczyk et al. 2016). Therefore, we suggest that promising clones in the nursery screening should be further evaluated under field conditions for growth performance within the rotation cycles.

Conclusions

The current study evaluated 19 poplar and 10 willow clones mostly of Ukrainian origin. The clones were screened for growth parameters, biomass fuel criteria and susceptibility to pathogens in the first two years in the nursery. Using the total rank for evaluation, the maximal score was reached by the poplar clone "Kanadska × balsamichna", followed by the clones "Ivantiivska", "Volosystoplidna", "Perspektyvna", and "Nocturn". Among the willows, the highest rank was recorded for the clone "Zhytomyrska-1", followed by the clone "Zhytomyrska-2". A high rank was also demonstrated by the poplars "Strilopodibna", "Mobilna", "Novoberlinska-7" and "Keliberdynska", and the willows "Lisova pisnya" and "Vinnytska". Thus, the above-mentioned clones may all be recommended as promising, but they should be evaluated under field conditions for growth performance in short-rotation cycle experimental plantations. Clones with the lowest total rank, such as poplars "Bolle", "Gradizka" and "Kytaiska × piramidalna" and willows "Lukash", "Olimpiisky vohon" and "Pryberezhna", are not recommended for bioenergy SRP, as they demonstrated slow growth during the two years.

In this study, ranking all the parameters was considered as equally weighted in the final choice of the clones. In the case of other interests or different purposes of cultivation, processing, or using biomass as biofuels, such an approach may be corrected by including weighed coefficients in rank calculation.

Such multiclonal screening of bioenergy trees for planting in short rotations was performed for the first time in Ukraine. The evaluation of plants in the nursery allowed us to carry out rapid and cost-effective preliminary screening. We believe that the results obtained as well as establishment of this nursery collection will provide new perspectives for developing short-rotation bioenergy plantations in the country.

Author's contribution

NK carried out the field measurements, performed the statistical analysis and wrote the manuscript; DR conceived the study, performed field measurements and sampling, and helped to draft the manuscript; SR conceived the study, performed field measurements and sampling; LK carried out the field measurements and helped to draft the manuscript; NR helped to draft and revised the manuscript.

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