Verification of new *Populus nigra* L. clone improvement based on their performance over three rotations

Kateřina Novotná,
Petra Štochlová,
Vojtěch Benetka

Populus nigra is an important autochthonous woody plant that can be grown as a renewable energy source. The possibility of its improvement through intraspecific hybridization was tested. Differences in biomass production, growth parameters, *Melampsora larici-populina* rust resistance and drought tolerance were evaluated among 19 intraspecific hybrids from controlled crosses, 2 clones selected from natural populations and the "MAX 4" clone (*P. nigra* × *P. maximowiczii*). These *P. nigra* clones from controlled crosses were chosen from more than 2000 hybrid individuals whose parents were selected from natural populations in the Czech Republic. A field trial was set up in Průhonice, Czech Republic (320 m a.s.l., 591 mm rainfall annually, mean annual temperature of 9.5 °C). The planting density was 6061 plants ha^{-1}, and the plants were coppiced three times at 3-year intervals. The trial was irrigated only during its establishment. Among the clones, significant differences were found in all the evaluated traits over three rotations. An average dry matter yield of the best clone "MAX 4" was 12.8 t ha^{-1} yr^{-1} over three harvests. The best black poplar clone reached up to 9.4 t ha^{-1} yr^{-1} in three harvests. Rust resistance was constant over 9 years and high for the three *P. nigra* clones (two from controlled crosses and one from natural populations). Moreover, the best *P. nigra* clones from controlled crosses showed higher drought tolerance than the "MAX 4" clone. Breeding progress was confirmed, and most of the *P. nigra* clones from controlled crosses performed better than the clones selected from natural populations. The trial validated the suitability of natural populations for use as gene sources for intraspecific hybridization and as sources of clones with traits comparable with those of interspecific clones. These new *P. nigra* clones can replace allochthonous clones in areas where autochthonous *P. nigra* populations are threatened by introgression.

Keywords: Plant Breeding, Black Poplar, Intraspecific Hybridization, Short Rotation Coppice Culture, *Melampsora larici-populina*, Biomass Production, Tree Regeneration

Introduction

In recent years, the importance of fast-growing trees cultivation intensively managed as coppices with 2- to 5-year rotation cycles and potential lifetimes of 30 years (DEFRA 2004), the so-called short-rotation coppice (SRC) cultures, has increased with the increasing demand for woody biomass as a renewable energy source (Bentsen & Felby 2012). This trend is apparent in the Czech Republic, where the total SRC area has increased by more than eight times

within the last 8 years and is now at 2862 ha (Ministry of Agriculture of the Czech Republic 2018).

Similar to those in other European countries (Herve & Ceulemans 1996, Verwijst 2001, Langeveld et al. 2012), Czech SRC cultures are usually based on poplar or willow species, and the most commonly grown poplars in Europe are primarily the result of interspecific crosses. In countries with the highest SRC area (France, Italy and Spain), the most popular parental combinations of

these clones are *Populus deltoides* Marsh. × *P. nigra* L. (= *P.* ×*canadensis* Moench), *P. trichocarpa* Torr. & A. Gray × *P. deltoides*, *P. nigra* × *P. maximowiczii* Henry and *P. maximowiczii* × *P. trichocarpa* (FAO 2016); however, in central Europe, the trend in recent years has been to grow the "MAX 4" hybrid clone of *P. nigra* × *P. maximowiczii* (Štícha et al. 2016). The popularity of F1 interspecific hybridization is due to the predominance of heterosis and the ease with which it can be economically exploited through vegetative propagation (Stanton et al. 2010).

P. nigra is an autochthonous woody plant that covers a large area of Europe (Vanden Broeck 2003), and its populations can be threatened by introgression from the aforementioned interspecific hybrid clones and other clones of allochthonous poplar species (Smulders et al. 2008). The relevant causes are cultivated poplar trees grown to the adult stage, which is the case not only for clones coming from *P. deltoides* and *P. trichocarpa* but also for interspecific hybrids of *P.* ×*canadensis*-type that can spontaneously interbreed with *P. nigra* (Benetka

☐ Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Publ. Res. Inst., Květnové náměstí 391, CZ-252 43 Průhonice (Czech Republic)

@ Kateřina Novotná (novotna@vukoz.cz)

et al. 2002b). The contamination of *P. nigra* gene pools has led to restrictions on inter-specific hybrid cultivation in certain locations (Ministry of the Environment of the Czech Republic 1992).

The original autochthonous species *P. nigra* is not commonly grown in its pure form, and few cultivars are available. Furthermore, these clones are usually selected from natural populations (IPC 2000). According to previously published studies (Nielsen et al. 2014, Verlinden et al. 2015), *P. nigra* clones are the least productive, although certain clones derived from genetically pure *P. nigra* produce yields comparable to or exceeding those of interspecific hybrid clones (Benetka et al. 2002a, Al Afas et al. 2008, Dillen et al. 2013). Additionally, pure *P. nigra* clones are not commonly grown because of their generally reported susceptibility to leaf rust caused by *Melampsora larici-populina* Kleb., which is the case in Western Europe (Steenackers 1972).

M. larici-populina leaf rust is one of the main diseases of cultivated poplars and can cause severe damage and economic losses (Frey et al. 2005). This disease negatively affects biomass production (Gastine et al. 2003, Benetka et al. 2012, Štochlová et al. 2015), resulting in yield losses of up to 66% in *P. nigra* clones (Štochlová et al. 2015). The resistance of *P. nigra* to *M. larici-populina* shows a quantitative inheritance (Dowkiw et al. 2012), as demonstrated by a genetic analysis of this trait (Benetka et al. 2005). This type of resistance does not ensure complete resistance but produces more durability than qualitative resistance. In contrast, the qualitative resistance that occurs in some *P. deltoides* clones ensures complete or near-complete resistance to some *M. larici-populina* strains (Pinon 1992, Dowkiw & Bastien 2004), although this resistance can be suddenly overcome (Dowkiw et al. 2012). Due to the transitory nature of resistance based on qualitative

resistance genes, plant breeders now focus their efforts on quantitative resistance genes, for which selection is slower but the risk of a sudden loss of resistance is reduced (McDonald 2010).

Another goal in poplar breeding has arisen with the projected dryer summer climate in Central-Western Europe (Schär et al. 2004, Seneviratne et al. 2006, Kreuzwieser & Gessler 2010). Poplars are especially sensitive to water deficiency and could be threatened by these climatic extremes. The growth of their leaves and shoots is affected by water shortage (Monclus et al. 2009), affecting the total biomass, leaf area, shoot height and diameter (Monclus et al. 2006). Therefore, these traits are used as indicators of drought, and their evaluation can be used to select genotypes with higher drought tolerance.

A prerequisite for successful breeding for resistance to drought is variability in the traits observed in the genus *Populus* L.; namely, in *P. nigra*, variability was observed between populations originating from contrasting climatic conditions within Europe (Viger et al. 2016). There are consistent genotypic differences in the water-use efficiency (WUE) of poplar species and hybrids, along with a lack of correlation between the WUE and productivity of poplar clones (Marron et al. 2005, Monclus et al. 2005, 2006, Navarro et al. 2018). This indicates that there is the potential for the selection of poplar clones that combine high WUE and high productivity, which would be an advantage for the growth of poplar trees in moderately drought-prone areas (Braatne et al. 1992, Jones et al. 2016).

The black poplar clones used in studies by Benetka et al. (2002a, 2014) and Dillen et al. (2013) were derived from natural populations and did not represent the products of any type of selective breeding. Newly released clones arising from cross-pollination might be promising and have a potential

production value comparable to that of commercial hybrid poplar clones when parental clones originate from contrasting conditions (Benetka et al. 2012).

The aim of the present study was: (i) to verify whether black poplar clones originating from intraspecific hybridization (controlled crossings) of parental trees from local populations were improved in yield and growth traits and leave rust resistance in comparison with selected clones from natural populations; (ii) to verify whether their performance was comparable with that of commercial interspecific hybrid clones; and (iii) to choose black poplar clones that are convenient for growing in SRC systems under suboptimal conditions.

Materials and methods

Materials

Twenty-one clones of *P. nigra* L. ssp. *nigra* from the black poplar breeding programme of Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Publ. Res. Inst. (Czech Republic), including 19 clones originating from 9 intraspecific hybrid combinations (Benetka et al. 2012), were used in a field trial. These clones were chosen among 2227 individuals for their good yield potential and high level of resistance to *M. larici-populina* leaf rust (Benetka et al. 2012). An additional, leaf rust-susceptible intraspecific clone (98/37) was only used to confirm rust infection and represented an additional rust source. The parental trees used in our crossings were plus trees selected from natural populations, and crosses were performed between parents from different climatic and geographic regions, with their genetic diversity previously confirmed by microsatellite DNA analysis (Benetka et al. 2012).

Two other *P. nigra* clones (202 and 301) selected from a set of approximately 200 plus trees originating from natural populations (Benetka et al. 2007) were chosen as reference clones based on long-term yield trials (Benetka et al. 2014) to evaluate the improvement in *P. nigra* clones from controlled crosses. Furthermore, clone 202 is one of the parental trees of several tested *P. nigra* clones (00/234, 00/237, 00/239, and 00/274). In addition to these two clones, the "MAX 4" clone, an interspecific hybrid of *P. nigra* × *P. maximowiczii*, was used as a reference clone.

Locality and experimental design

The trial was established at the Silva Tarouca Research Institute for Landscape and Ornamental Gardening in Průhonice, Czech Republic (Central Bohemia – 49° 59′ 26″ N, 14° 34′ 40″ E) in spring 2009 (Benetka et al. 2012). The plantation site is in a flat location at an altitude of 320 m a.s.l. facing slightly towards the south, and it consists of arable land that was grassed over before the establishment of the plantation. The soil type is modal brown earth found on loess, and the ground water level
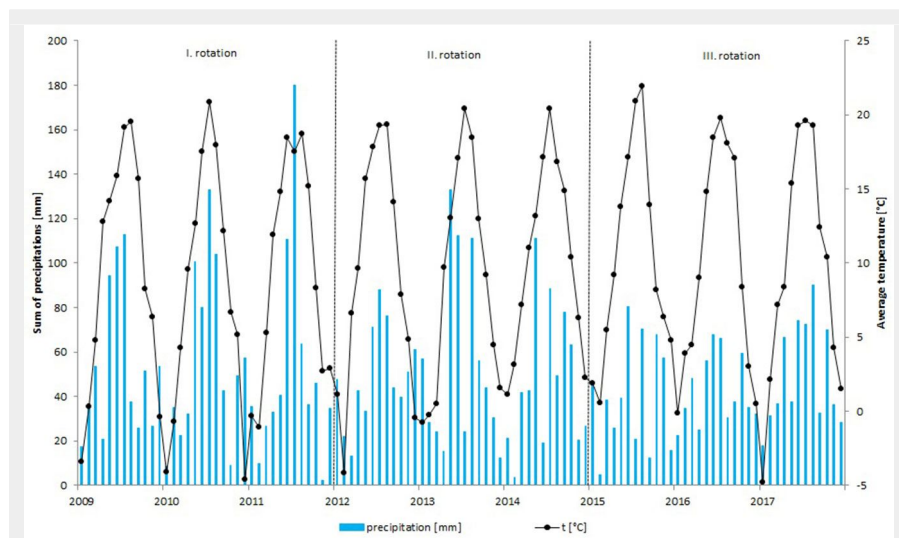

Fig. 1 - Monthly cumulative precipitation (blue bars) and average temperatures (black points) during the experimental period (2009-2017).

is approximately 2.1 m deep. The location is a suitable but rather dry area with frequent drying winds. The trial was not fertilized and was irrigated only before the establishment of the plantation and at sapling planting, respectively. The monthly average temperatures and cumulative precipitation during the 2009-2017 experimental periods are shown in Fig. 1, and Lang's rain factor is shown in Fig. 2. The mean annual temperatures and mean annual cumulative precipitation were 9.0, 9.5 and 9.9 °C and 643.6, 601.4 and 528.8 mm in first (2009-2011), second (2012-2014) and third (2015-2017) rotations, respectively.

One-year-old saplings, obtained by cultivating 0.2 m-long hardwood cuttings, were cut at a height of 0.5 m and planted with a planting machine at a spacing of 3.0 × 0.55 m (*i.e.*, a plant density of 6061 plants per ha) in spring 2009. A randomized block design with two to five (predominantly four) blocks for each *P. nigra* clone and eight blocks for the "MAX 4" reference clone was used, and four or five plants of each clone were included in each block. Border rows were planted around the experimental plot to prevent any border effect, and the plot was also fenced to prevent browsing by wild animals.

Measurements

Biomass was harvested three times, and these harvests were separated by a three-year interval. Plants were coppiced in February 2012 for the first time, in January 2015 for the second time, and in February 2018 for the third time. Individual plants were cut by hand with a chainsaw at a height of approximately 0.1 m above the ground at the first harvest and at ~0.05 m above the previous cut at the following harvests. During the harvests, the fresh woody aboveground biomass produced by all plants of a given clone in each block was pooled and weighed. To simultaneously estimate the dry weight yield, a representative sample of the harvested material (equally from 1-, 2- and 3-year-old part), including the main and lateral shoots, was collected from each block and weighed separately. The size of these samples ranged from 300 to 1000 g depending on the diameter of the shoots. The samples were dried at 105 °C until their weights were constant, and the average dry matter weight of the individual plants (DMIP), expressed as kg yr^{-1}, was calculated from the weight of the harvested fresh biomass from a given block, multiplied by the appropriate value of the percent dry matter, and divided by the number of plants in a given block and by the length of the rotation. The total dry matter yield per unit area (ha) and the percentage of living plants were then calculated.

At the end of first rotation cycle before harvesting, the main shoot diameter was measured with a digital calliper at a height of 0.5 m above the ground. In the following rotation cycles before harvesting, all shoots thicker than 0.01 m at this height
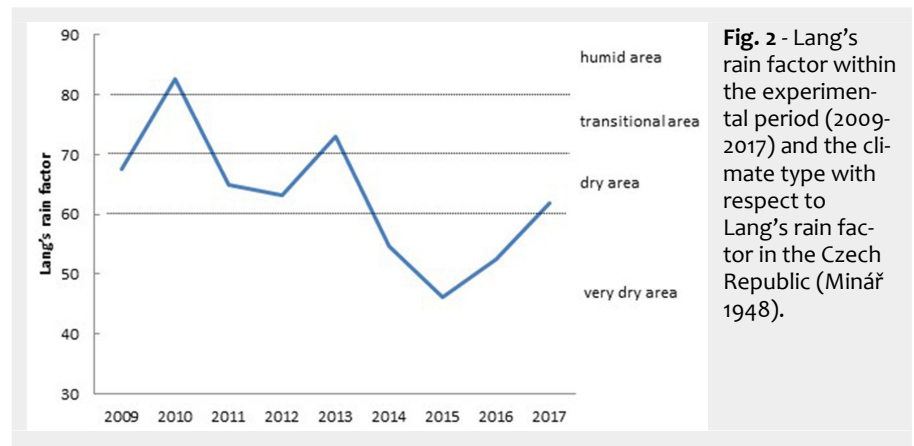

Fig. 2 - Lang's rain factor within the experimental period (2009-2017) and the climate type with respect to Lang's rain factor in the Czech Republic (Minář 1948).

were counted and their diameters were measured. The shoot with the highest diameter in each plant was considered the main shoot. Before the second and third harvest, the combined total cross-sectional area (TCA) of all measured shoots was calculated and expressed as the sum of the per plant area in mm². In the autumn before harvesting, plant mortality was determined as the percentage of dead plants relative to the total number of plants planted.

The incidence of *M. larici-populina* leaf rust was evaluated under field conditions, in a previous study (Štochlová et al. 2015) virulences 2, 3, 4, 5 and 7 were detected on test clones. Evaluations were made using a 6-point scale ranging from 0 = no symptoms of rust infection on the leaves to 5 = all leaves necrotized or shed (Štochlová et al. 2015) at least once during a vegetation period (April-September). The point values presented are the means of the point evaluations performed for each replication in mid-August, and the mean point value of

rust incidence was calculated based on an annual rust evaluation over a nine-year period.

Statistical analysis

Statistical evaluations were carried out using software R (R Core Team 2019) or Statistica® v. 11.0 (StatSoft Inc., Tulsa, OK, USA). Clones (fixed factor) and blocks (random factor) were used as the independent variables, and the parameters of the number of shoots per plant, diameter of the main shoots, TCA, DMIP and dry matter yield per unit area were used as the dependent variables in linear mixed-effects model of ANOVA (Bates et al. 2015). The parameter mortality was not statistically evaluated due to its minimal variability. The square root transformation (Anděl 1998) was applied to the number of shoots per plant to obtain an approximately normally distributed variable. When a significant difference was found, a *post hoc* comparison was carried out using Tukey's test. The
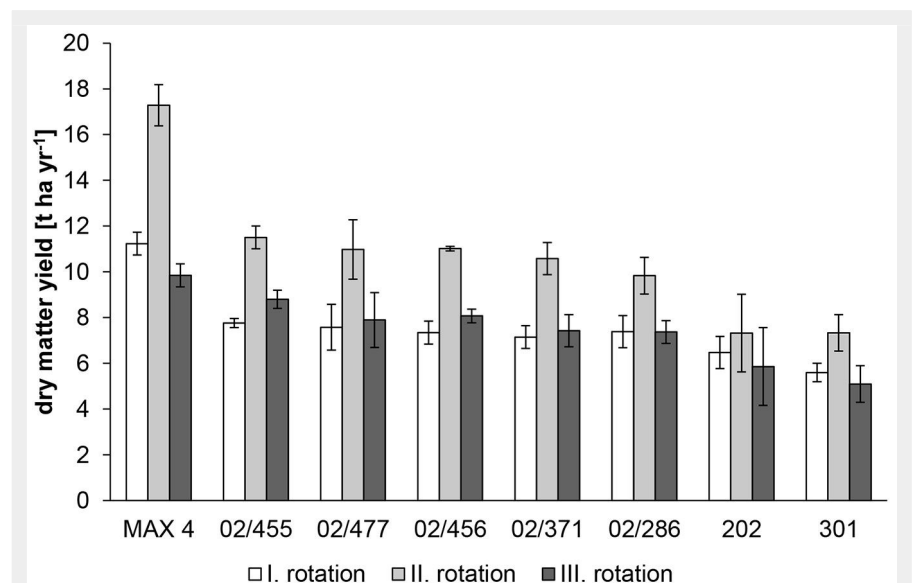

Fig. 3 - Mean value of the dry matter yield for the five best *P. nigra* clones from controlled crosses (02/455, 02/477, 02/456, 02/371, 02/286), *P. nigra* reference clones from natural populations (202, 301) and the *P. nigra* × *P. maximowiczii* reference clone "MAX 4" within the first (I.), second (II.) and third (III.) rotations. The bars represent the standard errors of the mean values.

Tab. 1 - P-values of the linear mixed-effects model of ANOVA (effects of the clone or block on dependent variables) in the first (I.), second (II.) and third (III.) rotations. (TCA): total cross-sectional area; (DMIP): dry matter weights of individual plants.

Variable	Rotation	Block	Clone
Diameter of strongest shoot	I.	> 0.5	<0.001
	II.	0.127	<0.001
	III.	0.055	<0.001
Average shoot diameter	II.	> 0.5	<0.001
	III.	0.104	<0.001
Number of shoots per plant	II.	0.4	<0.001
	III.	0.241	<0.001
TCA	II.	0.004	<0.001
	III.	0.001	<0.001
DMIP	I.	> 0.5	<0.001
	II.	> 0.5	<0.001
	III.	0.058	<0.001
Dry matter yield per unit area	I.	> 0.5	<0.001
	II.	0.5	<0.001
	III.	0.086	<0.001

Kruskal-Wallis test was used for testing differences in the percentage variations between first and second and the second and third harvests. When a significant difference was found, a *post hoc* comparison was made using multiple comparisons of mean ranks. Significant differences in wood production between the rotations were established based on the results of *t*-tests, and Spearman's coefficient of rank correlation was calculated between the particular dates of rust incidence evaluations and between the TCA and DMIP in the second and third harvest. The relationship between the clones and rust incidence was tested using Pearson's coefficient of contingency. All results are presented using a significance level of $\alpha < 0.05$.

Results

Biomass production

The amount of biomass production was evaluated based on the results from all three harvests. The biomass production increased between the first and second harvests in all clones, and the increases were significant in nine of these clones (Fig. 3, Tab. S1 in Supplementary material). In contrast, biomass production decreased between the second and third harvest, and the decreases were significant for thirteen of these clones. The higher part of observed variability in yield was explained by the variable of clone. Significant clonal differences were found in every harvest (Tab. 1, Tab. S1 in Supplementary material).

The "MAX 4" clone displayed the highest DMIP in all rotations (Tab. S1 in Supplementary material), presenting mean values of 1.9, 2.9 and 1.6 kg yr^{-1} in the first, second and third harvests, respectively. The DMIP of the *P. nigra* clones ranged from 0.9 to 1.3 kg yr^{-1} in the first harvest, from 1.1 to 1.9 kg yr^{-1} in the second and from 0.8 to 1.5 kg yr^{-1} in the third. Significant differences among the "MAX 4" clone and *P. nigra* clones were found in every harvest, but the DMIP of 5 intraspecific clones (02/455, 02/456, 02/477, 02/371, 02/476) did not differ significantly

Tab. 2 - Health status of the tested clones (mortality within the first (I.), second (II.) and third (III.) rotations and rust incidence within the trial observation period, 2009-2017). *P. nigra* × *P. maximowiczii* reference clone is "MAX 4", *P. nigra* reference clones from natural populations are 202 and 301, the rest clones are *P. nigra* clones from controlled crosses from which only clone 98/37 is leaf rust susceptible. Values are means ± standard error.

Clone	Mortality (%)			Rust incidence (points)									
	I.	II.	III.	2009	2010	2011	2012	2013	2014	2015	2016	2017	2009-2017
MAX 4	0	0	0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.6 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.9 ± 0.1	0.4 ± 0.1	0.8 ± 0.1	0.4 ± 0.0
00/234	0	0	0	0.1 ± 0.0	1.1 ± 0.2	1.6 ± 0.1	0.7 ± 0.2	0.5 ± 0.0	0.4 ± 0.1	0.3 ± 0.2	1.0 ± 0.5	1.0 ± 0.0	0.7 ± 0.1
02/477	0	0	0	0.1 ± 0.0	0.8 ± 0.1	2.3 ± 0.3	0.9 ± 0.1	1.0 ± 0.2	0.5 ± 0.0	0.9 ± 0.1	1.3 ± 0.1	1.0 ± 0.0	1.0 ± 0.1
202	0	0	0	0.2 ± 0.1	1.8 ± 0.3	1.5 ± 0.1	0.8 ± 0.3	1.0 ± 0.0	0.5 ± 0.0	0.8 ± 0.3	1.3 ± 0.3	1.0 ± 0.0	1.0 ± 0.1
00/441	0	0	0	0.1 ± 0.0	1.3 ± 0.1	2.0 ± 0.1	0.5 ± 0.0	1.0 ± 0.3	0.5 ± 0.0	0.5 ± 0.0	1.6 ± 0.1	2.0 ± 0.0	1.1 ± 0.1
00/237	0	0	0	0.2 ± 0.1	1.8 ± 0.2	1.7 ± 0.2	1.3 ± 0.3	1.0 ± 0.0	0.5 ± 0.0	1.0 ± 0.3	0.8 ± 0.2	1.3 ± 0.3	1.1 ± 0.1
02/21	0	0	0	0.2 ± 0.1	1.4 ± 0.1	1.6 ± 0.4	0.6 ± 0.1	1.3 ± 0.1	0.5 ± 0.0	1.0 ± 0.0	1.5 ± 0.0	1.8 ± 0.3	1.1 ± 0.1
00/239	0	0	0	0.3 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	0.9 ± 0.1	1.3 ± 0.3	0.8 ± 0.1	0.9 ± 0.2	1.4 ± 0.2	1.0 ± 0.0	1.1 ± 0.1
02/264	0	0	0	0.4 ± 0.1	0.9 ± 0.1	2.1 ± 0.6	0.6 ± 0.1	1.3 ± 0.3	0.6 ± 0.1	2.1 ± 0.4	1.6 ± 0.1	1.3 ± 0.3	1.2 ± 0.1
02/286	0	0	0	0.3 ± 0.1	1.7 ± 0.1	2.6 ± 0.2	0.9 ± 0.1	1.1 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	1.8 ± 0.1	1.0 ± 0.0	1.2 ± 0.1
301	0	0	0	0.1 ± 0.0	1.8 ± 0.1	2.2 ± 0.4	1.7 ± 0.3	1.5 ± 0.0	0.5 ± 0.0	0.8 ± 0.2	1.7 ± 0.2	1.0 ± 0.0	1.3 ± 0.1
02/476	0	0	0	0.4 ± 0.1	1.1 ± 0.2	2.0 ± 0.5	1.3 ± 0.3	1.0 ± 0.2	0.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.1	1.0 ± 0.0	1.3 ± 0.1
02/278	0	0	0	0.3 ± 0.1	1.5 ± 0.3	2.5 ± 0.5	1.0 ± 0.0	1.3 ± 0.1	0.9 ± 0.1	1.5 ± 0.3	1.6 ± 0.1	1.3 ± 0.3	1.3 ± 0.1
00/274	0	0	0	0.4 ± 0.1	1.8 ± 0.1	2.1 ± 0.4	0.8 ± 0.1	1.8 ± 0.3	0.9 ± 0.1	0.6 ± 0.1	2.0 ± 0.0	1.5 ± 0.3	1.3 ± 0.1
02/378	0	0	0	0.6 ± 0.1	1.5 ± 0.2	1.8 ± 0.5	1.4 ± 0.2	1.7 ± 01	0.8 ± 0.1	1.1 ± 0.2	1.6 ± 0.1	1.6 ± 0.2	1.3 ± 0.1
02/103	5	5	10	0.3 ± 0.1	1.9 ± 0.1	2.3 ± 0.6	0.9 ± 0.1	1.6 ± 0.1	0.5 ± 0.0	1.1 ± 0.1	1.5 ± 0.2	2.3 ± 0.3	1.4 ± 0.1
02/456	0	0	0	0.4 ± 0.1	1.1 ± 0.1	2.4 ± 0.5	1.5 ± 0.3	1.6 ± 0.2	0.8 ± 0.1	1.8 ± 0.3	2.0 ± 0.3	1.8 ± 0.3	1.5 ± 0.1
02/455	0	0	0	0.4 ± 0.1	1.6 ± 0.1	2.6 ± 0.6	1.8 ± 0.3	1.5 ± 0.2	1.3 ± 0.3	2.0 ± 0.2	1.8 ± 0.3	1.0 ± 0.0	1.5 ± 0.1
02/102	0	0	5	0.6 ± 0.1	1.9 ± 0.1	2.6 ± 0.6	1.3 ± 0.3	1.8 ± 0.1	0.8 ± 0.1	1.9 ± 0.4	1.9 ± 0.1	2.5 ± 0.3	1.7 ± 0.1
02/371	0	0	0	0.9 ± 0.1	2.1 ± 0.1	3.3 ± 0.1	2.3 ± 0.3	2.0 ± 0.0	1.3 ± 0.1	1.9 ± 0.3	2.4 ± 0.4	2.5 ± 0.3	2.1 ± 0.1
02/40	0	0	5	1.3 ± 0.2	1.9 ± 0.1	2.9 ± 0.3	2.3 ± 0.3	1.8 ± 0.1	1.6 ± 0.2	2.1 ± 0.2	2.3 ± 0.1	2.8 ± 0.3	2.1 ± 0.1
02/383	0	0	0	0.9 ± 0.2	1.8 ± 0.1	3.4 ± 0.3	2.0 ± 0.0	2.5 ± 0.2	1.3 ± 0.1	2.5 ± 0.2	2.1 ± 0.1	2.8 ± 0.3	2.1 ± 0.1
98/37	9	19	19	1.4 ± 0.2	2.4 ± 0.1	3.8 ± 0.1	3.0 ± 0.0	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.2	3.0 ± 0.0	3.0 ± 0.0	2.8 ± 0.1

from that of the "MAX 4" clone in the third harvest. Both black poplar reference clones exhibited lower mean values than most of the *P. nigra* clones from controlled crosses, although significant differences were not found in any of the harvests. Descendants of clone 202 exhibited up to 22%, 34% and 11% higher DMIP values than did their parent during the first, second and third harvests, respectively (Tab. S1 in Supplementary material). The DMIPs were very strongly correlated with the TCA in both the second and third harvest (R = 0.877, p < 0.001 and R = 0.918, p < 0.001, respectively).

Similar to the DMIP results, the "MAX 4" clone showed the highest dry matter yield per unit area in all harvests (Fig. 3, Tab. S1 in Supplementary material) because negligible mortality was observed during the trial (Tab. 2). The biomass yields of most black poplar clones were significantly low, only the 5 above mentioned *P. nigra* clones did not differ from the interspecific reference clone in the third harvest. The highest increment of dry matter yields per unit area between the first and second harvest was observed in the "MAX 4" clone (*i.e.*, 6.1 t ha^{-1} yr^{-1}), for which the yield of the second harvest increased by approximately 54% relative to that of the first harvest (Fig. 4). In the *P. nigra* clones, the yield increment ranged between 0.3 and 3.8 t ha^{-1} yr^{-1}, and the yield in the second harvest increased by approximately 32% on average. In one *P. nigra* clone (02/378), the yield increased by approximately 62%. However, the yield increment of the black poplar reference clones was below average. The differences in the dry matter yields per unit area between the "MAX 4" clone and the best *P. nigra* clones increased in the second harvest, with the highest-yielding *P. nigra* clones exhibiting dry matter yields per unit area that were approximately 71% (clone 00/274) and 66% (clone 02/455 – Tab. S1 in Supplementary material) of those of the interspecific reference clone in the first and second harvests, respectively. The dry matter yields per unit area decreased in the third harvest (Fig. 3, Tab. S1). The yields in the third harvest relative to that in the first harvest were equivalent in one clone and even lower in 13 clones. These clones included both reference *P. nigra* clones and the "MAX 4" reference clone, although its yield remained 10% higher than the best black poplar clone (02/455). The yield decrease between the second and third harvest was significant in 13 clones, with the highest decrease in the most productive "MAX 4" clone (Fig. 4), while the decrease in low-yielding black poplar clones was insignificant (Tab. S1 in Supplementary material). The percentage of yield decrease between the second and third harvest of the "MAX 4" reference clone was significantly higher than that of some high-yielding *P. nigra* clones (Fig. 4).

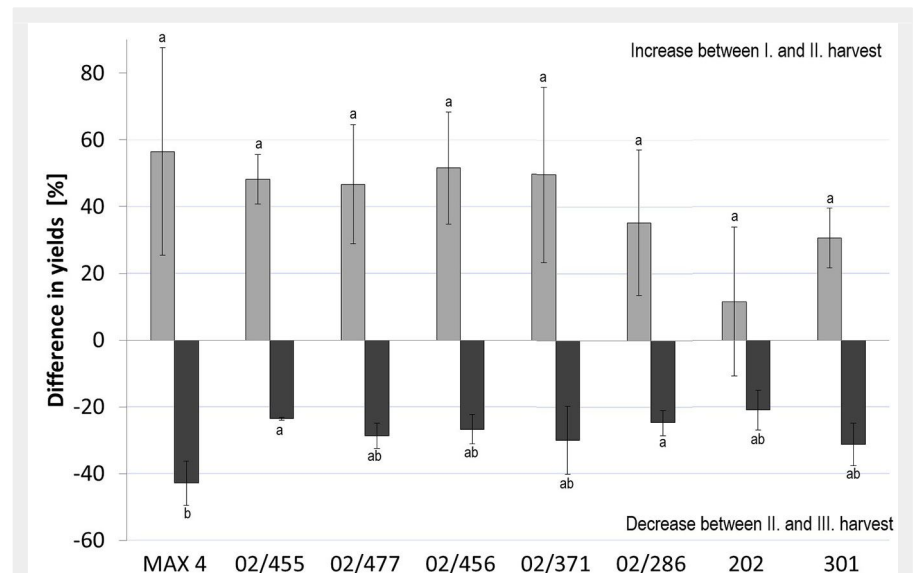The average annual production of the "MAX 4" reference clone over three rota-

Fig. 4 - Percentage differences in the dry matter yield for the five best *P. nigra* clones from controlled crosses (02/455, 02/477, 02/456, 02/371, 02/286), *P. nigra* reference clones from natural populations (202, 301) and the *P. nigra* × *P. maximowiczii* reference clone "MAX 4" between subsequent harvests. Different letters are related to the results of multiple comparisons of the mean ranks through a *post hoc* test. The bars represent the standard errors of the mean values.

tions was 12.8 t ha^{-1} yr^{-1}, while this value in the black poplar clones ranged between 5.8 and 9.4 t ha^{-1} yr^{-1}. The black poplar reference clones (202, 301) were among the clones with the lowest annual production (Tab. S1 in Supplementary material).

Growth traits

The growth traits were measured at the end of the vegetation period just before each harvest, and significant differences were observed in all the evaluated traits (Tab. 1, Tab. S2 in Supplementary material),
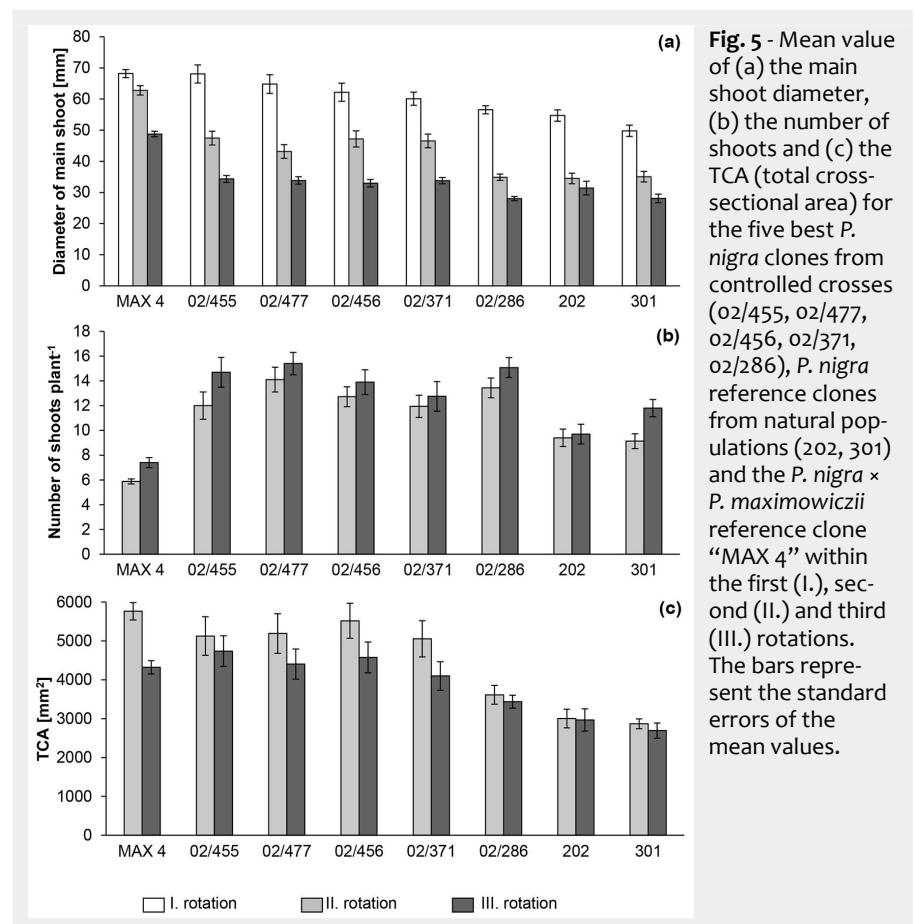

Fig. 5 - Mean value of (a) the main shoot diameter, (b) the number of shoots and (c) the TCA (total cross-sectional area) for the five best *P. nigra* clones from controlled crosses (02/455, 02/477, 02/456, 02/371, 02/286), *P. nigra* reference clones from natural populations (202, 301) and the *P. nigra* × *P. maximowiczii* reference clone "MAX 4" within the first (I.), second (II.) and third (III.) rotations. The bars represent the standard errors of the mean values.

with the independent variable of clone having the greatest effect. A significant block effect was observed only in TCA.

The diameter of the main shoot was the highest in all clones before the first harvest, followed by that before the second and third harvest (Fig. 5a, Tab. S2 in Supplementary material). The "MAX 4" clone had the greatest mean diameter of the main shoot before all three harvests at 68.2, 62.8 and 48.8 mm, respectively, and it differed significantly from all *P. nigra* clones in the second and third rotation. The best *P. nigra* clones had main shoot diameters of 68.1, 47.5 and 34.7 mm in the three rotations, respectively, with clone 02/455 showing the best results in the first two rotations and 02/264 showing the best results in the third rotation. Both *P. nigra* clones selected from nature were among the clones that showed the lowest main shoot diameter in all three rotations. Compared with the parental clone, the descendants of clone 202 exhibited greater shoot diameters during the first, second and third harvests of up to 16%, 29% and 4%, respectively (Tab. S2 in Supplementary material). Similar to the main shoot diameter, higher average shoot diameters were found before the second harvest than the third harvest, and the best diameters in these harvests were found in the "MAX 4" clone, at 30.5 and 24.6 mm, respectively. The average shoot diameter of the *P. nigra* clones ranged between 17.0 and 22.5 in the second rotation and 16.2 and 19.0 mm in the third harvest (Tab. S2 in Supplementary material). The average shoot diameters of the *P. nigra* clones were 26% to 44% and 23% to 34% smaller than that of the "MAX 4" clone before the second and third harvest, respectively.

After the first harvest, the number of shoots per plant increased due to coppicing and varied greatly. The number of shoots was greater in the black poplar clones than in clone "MAX 4" (Fig. 5b, Tab. S2 in Supplementary material) and ranged between 5.9 and 14.2 shoots per plant. The mean number of coppiced shoots in the *P. nigra* clone with the greatest shoot number (02/476) was 2.4 times higher than that of the interspecific reference clone "MAX 4" just before the second harvest. In the third rotation, the number of shoots in all clones was higher than that in the second rotation, and the lowest number of shoots was observed in the "MAX 4" clone at 7.4, while the highest number was found in clone 02/476 at 16.1.

Total cross-sectional area (TCA) accounts for the number of shoots and their diameters. In this trial, the TCA values ranged between 2867 and 5764 mm² before the second harvest and showed high variability, particularly among the tested clones (Fig. 5c, Tab. S2 in Supplementary material). The "MAX 4" clone exhibited the highest TCA value in the second harvest. Before the third harvest, the TCA values were lower in most of the clones than before the second

harvest and ranged between 2692 and 4738 mm² with highest TCA observed in *P. nigra* clone 02/455. Compared with the parental clone, the descendants of clone 202 showed up to 50% and 31% higher TCA values during the second and third harvest, respectively, and the lowest values were observed in both black poplar reference clones.

Health status

Plant mortality was negligible after the establishment of the trial (Tab. 2). Before the first harvest, only one clone (02/103) exhibited 5% mortality. Before the third harvest, it exhibited 10% mortality, and two additional clones (02/40, 02/102) exhibited 5% mortality.

The occurrence of *M. larici-populina* leaf rust was evaluated under field conditions (Tab. 2). The particular dates of rust incidence evaluations were positively correlated with themselves (R = 0.382-0.741, p < 0.001). The results show that the rust incidence is strongly dependent on the clone; the Pearson's coefficient of contingency was C = 0.692, p < 0.001.

Although the lowest rust incidence was observed in the "MAX 4" reference clone, some of the individual black poplar clones showed an equally low incidence. The mean point value of leaf rust incidence in the "MAX 4" clone was 0.4 ± 0.1 over 9 years, and the mean point values in the black poplar clones showing minimal rust incidence (00/234, 02/477 and 202) were between 0.7 ± 0.1 and 1.0 ± 0.1. In contrast, the clones showing the poorest results for this parameter were 02/383, 02/371, and 02/40, with mean point values 2.1 ± 0.1. The susceptible clone 98/37 showed the highest values of rust incidence over all 9 years with a mean point value of 2.8 ± 0.1.

In the year with the highest rust incidence (2011), the mean point values for most of the clones with the highest resistance did not exceed 2 points, while the clones that showed the highest rust incidence had 3 or more points. The susceptible clone 98/37 received 3.8 points.

Discussion

The performance of 19 *P. nigra* clones originating from intraspecific crossings were compared to that of 2 clones from natural populations and the interspecific clone "MAX 4" in SRC systems over three three-year rotations. The *P. nigra* clones used in the current study are the result of more than 20 years of breeding, the goal of which was to obtain genotypes suitable for biomass production that could replace interspecific *Populus* hybrids threatening autochthonous *P. nigra* species through gene introgression (Heinze 1997, Benetka et al. 2002b, Vanden Broeck et al. 2005, Smulders et al. 2008). The other goal was to obtain clones resistant to existing virulences of *M. larici-populina* in the Czech Republic, in which the resistance would be stable, as expected in *P. nigra* (Legionnet et al. 1999,

Dowkiw et al. 2012). The first step was the selection of natural *P. nigra* populations to obtain plant material with high genetic diversity, and extensive crossing of geographically different genotypes generated intraspecific hybrid populations, from which the selection was made (Benetka et al. 2012). The current study focused on the selection of clones with favourable biomass production, higher *M. larici-populina* resistance and drought tolerance. Compared with the previous research of Bastien et al. (2014), the selection was performed based on field evaluations, without the use of molecular markers and laboratory evaluations of rust resistance.

Biomass production

In this study, the *P. nigra* clones from controlled crosses showed higher growth and yield parameter values than did the clones selected from natural populations that had previously been confirmed to be suitable for SRC plantations (Benetka et al. 2014, Štochlová et al. 2019). The best intraspecific descendants of clone 202 displayed up to 34% greater dry matter yield per unit area and up to 29% greater shoot diameter for the main shoots compared with their parent during the second harvest. In *P. ×canadensis*, Dillen et al. (2009) obtained an increase of 26.5% in the 2-year-old shoot diameter, and Marron & Ceulemans (2006) reported an increase of 23.3%. These results cannot be compared due to the different study conditions involved, although the observed increases are satisfactory from a breeding perspective.

Higher growth parameter values of the tested *P. nigra* clones in comparison with clones used in Štochlová et al. (2015) were achieved under the same rust disease attack, thereby excluding different effects of the pathogen and confirming that breeding progress has been achieved through intraspecific hybridization. The lower values obtained for clones from natural populations could be affected by the water regime at the locality, which is characterized by lower ground water levels and precipitation, potentially revealing drought tolerance in the best *P. nigra* clones from controlled crosses.

In this study, many black poplar clones were evaluated, although the presented data were obtained in small plots at only one locality. However, a similar trial with the same clones has been established by the Central Institute for Supervising and Testing in Agriculture at Stachy, Czech Republic (South Bohemia – 49° 06′ 06″ N, 13° 39′ 60″ E) in 2010. The plantation site is located at an altitude of 742 m a.s.l. and slopes slightly towards the northeast, and it was used as pasture land before the establishment of the plantation. The soil is dystric Cambisol on paragneiss with a pH from 6.2-6.5. The average annual temperature is 6.4 °C (in growing period 14.2 °C), and the average cumulative precipitation is 742 mm (growing period 378 mm). The

plantation was harvested for the first time in autumn 2015. A significant strong correlation (R = 0.666, p = 0.001) was found between rank of clones according to the yields at Stachy and the average annual dry matter production in Průhonice. Among the best-performing clones under marginal growing conditions were clones 02/456, 02/455 and 02/383 (Reininger et al. 2015); therefore, two of the three best clones from both trials are the same, thus confirming their high adaptability. However, clones with a distinct rank were observed in this study, including clones 02/383 and 301, which may be less sensitive to marginal growing conditions and lower temperatures (at Stachy) and present much better growth than that observed in the current trial (at Průhonice). In contrast, clone 02/477 belonged to the clones with a low yield under marginal growing conditions; therefore, this clone likely requires better growing conditions. Nevertheless, further evaluations of the best clones must be made *via* field tests using larger plots in more localities to determine the genotype × site interactions of the clones.

The interspecific clone "MAX 4" performed better for most of the evaluated traits. The species combination giving rise to the "MAX 4" clone appears to be very efficient compared with other interspecific combinations (Zamora et al. 2013). Observed differences in evaluated traits can be important for selection of right harvesting method and rotation length (optimal diameter of main shoots for mechanical harvesting), wood assortment and its utilization (chips, logs, pulp, etc. – Marron et al. 2012). Also differences in growth and yield traits can affect wood properties as higher number of shoots in *P. nigra* clones could lead to a higher proportion of bark and therefore differences in combustion heat (Geyer et al. 2000, Benetka et al. 2002a).

The biomass production of new *P. nigra* clones coming from controlled crosses was higher than production of clones from natural population in the study by Benetka et al. (2014) with exception of the third harvest of certain clones grown in optimal or marginal conditions. This difference could be explained by different growing conditions, the lower amount of precipitation in the third rotation, and different spacing.

The intraspecific hybridization success is evident compared to the results of other studies in which black poplar clones were also used (Dillen et al. 2013, Benetka et al. 2014, Nielsen et al. 2014, Verlinden et al. 2015). However, different growing conditions, spacing and management were implemented, and the best black poplar clones showed mostly comparable or even higher biomass yields.

Rust resistance

In a previous work by Štochlová et al. (2015), a negative correlation was demonstrated between the shoot thickness, which depends on the amount of nutrient storage, and the incidence of *M. larici-populina*. The decrease in the growth rate of plants with rust incidence from 1 to 2.25 points (using the same point evaluation) was not important, and the higher rust incidence in plants from the end of August did not negatively affect the shoot thickness (amount of nutrient storage – Štochlová et al. 2015). These findings indicate that the incomplete rust resistance observed in certain *P. nigra* clones is sufficient to defend against the decrease in biomass yield caused by rust. In the current trial, a lower rust incidence in comparison with that in the study of Štochlová et al. (2015) was observed in 2009 and 2010, although the trials were situated very close to each other. In 2011, the rust incidence in both trials was comparable (resistant clones 97/152 and 97/157 were evaluated as 2.25 points and susceptible clone as 4.33 points). This difference could be explained mainly due to the use of different clones, with a low number of susceptible clones in the current study and a looser spacing. According to several years of field observations, the stability of the higher rust resistance was confirmed in the best clones. These clones were even better than clones with higher levels of rust resistance identified in a previous work (Štochlová et al. 2015). However, a high variability in leaf rust susceptibility was observed among the tested clones, which supports the notion of resistance based on several quantitative trait loci (Legionnet et al. 1999, Benetka et al. 2005, Štochlová et al. 2015). Intraspecific clones with a high level of resistance were identified, which are important for follow-up resistance breeding programmes because rust-resistant clones could achieve higher biomass production (Dillen et al. 2013, Štochlová et al. 2015). Moreover, *P. nigra* is one of the parents of most of the most commonly grown poplar clones; therefore, the best *P. nigra* clones could be used for other interspecific crosses.

Drought tolerance

Both low amounts of precipitation and high temperatures characterized by a low Lang's rain factor during the third rotation (Fig. 2) enabled the evaluation of the drought resistance in the trialled clones, although the trial was not established with this goal. The biomass yield of the black poplar clones and "MAX 4" in the present study decreased in the third harvest in comparison with that in the second harvest. This is in contrast to the results obtained at the Rosice site (Benetka et al. 2014), in which a similar spacing was used and the biomass yield was the same or higher in the third or fourth harvest than in the second harvest. The observed decrease in Průhonice in the present study was caused by drought, whereas at the Rosice site, the soil was always sufficiently supplied with groundwater coming from a nearby river, and therefore, plant growth was not dependent on the amount of precipitation (Benetka et al. 2014). An increasing yield in the third or fourth rotation was also confirmed in other experiments in some clones (Al Afas et al. 2008, Dillen et al. 2013).

As a result of the drought, the yield decrease between second and third harvest was the highest and was significant in the most productive "MAX 4" clone, while the decrease in low-yielding black poplar clones was non-significant. The same response was achieved even in poplar genotypes originating from *P. deltoides* and *P. trichocarpa* (Monclus et al. 2009). In contrast, the yield decrease of some high-yielding black poplar clones was significantly lower than that of the "MAX 4" clone, indicating their higher drought tolerance compared with "MAX 4" clone. It will be very interesting to observe the biomass yield tendency and differences among intraspecific black poplar clones and reference clones in a series of years with continuous low Lang's rain factors.

Prospects and utilization

The current study indicates a high potential for further intraspecific *P. nigra* breeding. Obtaining clones that are relatively highly resistant to *M. larici-populina* is highly valuable and can disprove the notion of the unsuitability of *P. nigra* in SRC cultures due to its susceptibility to this pathogen.

In particular, pure *P. nigra* and *P. trichocarpa* clones appeared to be most suitable for growth under suboptimal conditions (*i.e.*, planted on degraded land – Dillen et al. 2013), which meets the requirements for growing SRC cultures in areas that are not suitable for intensive agricultural production.

Conclusions

In the present study, the significance of natural populations (subpopulations) as a source of genetic diversity for further *P. nigra* breeding was confirmed. It was shown that the first generation of controlled, intraspecific cross-pollination among selected clones of *P. nigra* was effective and convenient for breeding with the goals of high biomass yields and resistance to *M. larici-populina*. It was shown that *P. nigra* can be a genetic source of durable resistance to this pathogen.

Most *P. nigra* clones from controlled crosses exhibited higher values of all evaluated traits than those of clones selected from natural populations. Three high-yielding clones (02/455, 02/477 and 02/456) that were highly resistant to leaf rust and had higher drought tolerance were selected and can be recommended for growth in SRC cultures mainly in specific areas. Further intraspecific breeding of *P. nigra* has considerable potential, although the interspecific clone "MAX 4" performed better than did most of the *P. nigra* clones. The *P. nigra* clones presented herein will be used in further intraspecific breeding cycles with

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the goal of further yield increases. The possibility of transferring relatively durable resistance towards *M. larici-populina* to interspecific hybrids must be verified.

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Supplementary Material

Tab. S1 - Yield parameters of the tested clones in the first (I.), second (II.) and third (III.) harvests.

Tab. S2 - Comparison of the growth parameters.

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