JOURNAL OF FOREST SCIENCE, *51*, 2005 (4): 161–167

Selection of clones of *Populus nigra* L. ssp. *nigra* for resistance to *Melampsora larici-populina* Kleb. Rust

V. Benetka, I. Šálková, F. Vrátný

Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Průhonice, Czech Republic

ABSTRACT: A possibility of selection of clones of the species *Populus nigra* L. with higher resistance to the rust *Melampsora larici-populina* Kleb. was tested in a set of 29 clones of *P. nigra* originating from individuals selected in various localities of the CR. Clones were tested in three localities for four years. Rust infection was evaluated according to a five-point scale. The two most resistant (R_1; R_2) and two most sensitive (S_1; S_2) clones were tested in laboratory conditions. The results of this test confirmed to be consistent with those obtained under field conditions. Significant differences between the progenies of resistant ($R_1 \times R_2$) and sensitive ($S_1 \times S_2$) parents proved the genetic control of resistance. Intermediate inheritance of resistance was found in the progenies $S_1 \times R_2$ and $R_1 \times S_2$. Genetic effect of the paternal component was also expressed. A possibility of selection for resistance to *M. larici-populina* rust in *P. nigra* was proved.

Keywords: genetic control; resistance; selection; *Populus nigra* L.; *Melampsora larici-populina* Kleb.

Resistance to diseases is one of the most important traits that are currently in the focus of plant breeders' interest. Diseases reduce yields or they cause plant death, diminish product quality and sometimes they may have adverse effects on human health.

Black poplar, which belongs to the group of fast-growing tree species, becomes an important tree species in programmes of renewable energy resources. The species *Populus nigra* is one parental component in about 60% of interspecific hybrids (Directory of Poplar 2000). In addition, black poplar as a domestic tree species has an irreplaceable position in landscape planning programmes.

The rust caused by *Melampsora larici-populina* Kleb. is the most widespread and most frequent disease of poplars (Pinon 1992; Pei et al. 2003). The occurrence of this rust in black poplars in the Czech Republic does not appear important in solitary trees. But in black poplar plantings with dense spacing the rust occurrence is high already in the middle of the growing season. Besides the oldest race E1 other races of this rust were identified (Steenackers 1982; Pinon et al. 1987; Pinon, Peulon 1989; Pinon, Frey 1997). These new races can infect poplar clones that are resistant to race E1 only (Pinon, Frey 1997; Legionnet et al. 1999).

Most clones of the genus *Populus* grown in the last two centuries in Europe seem not to have ever been immune to rust; it indicates non-specific resistance of poplar to rust (Pinon 1992), and as reported by Legionnet et al. (1999), specific resistance was not detected in *Populus nigra*. On the contrary, some interspecific hybrids show immunity that may however be paralysed by the adaptation of physiological races, particularly of the rusts *Melampsora larici-populina* and *M. medusae* (Pinon 1992). The species *Populus nigra* does not transfer increased resistance to *Melampsora larici-populina* in interspecific hybridisation (Pinon 1992); Lefèvre et al. (1994) reported that in resistant hybrids the species *Populus deltoids* Marsh was probably the resistance donor.

The aim of the study was to test a possibility of selection of genotypes with detected higher resist-

Supported by the Ministry of Agriculture of the Czech Republic, Grant No. QD 0172.

ance to rust in the black poplar and the transfer of this resistance to the progeny.

MATERIAL AND METHOD

Selection of clones with different resistance

Twenty-nine trees of black poplar were chosen in several areas of the CR. Clones derived from these trees were tested in three localities (Tables 1 and 2) to determine the yield potential of the species *Populus nigra* L. ssp. *nigra* (Benetka et al. 2002). Besides growth characteristics the trait resistance to *Melampsora larici-populina* Kleb. rust was examined. This trait was evaluated in field conditions by a five-point scale:

0 – leaves absolutely free of rust infection symptoms;
1 – small patches covered with rust sori on a half of the leaves;
2 – small patches covered with rust sori on a majority of the leaves;
3 – large patches or complete coverage with rust on all leaves;
4 – entire leaves covered with rust, incipient leaf necrosis;
5 – all leaves necrotised or shed.

An experiment was established in a randomised block design, with five plants per replication. Well-known clones – interspecific hybrids were used as controls. The experiment was evaluated in four successive years (1999–2002). The data were processed by one-way analysis of variance (ANOVA) and multiple range test (Multifactor ANOVA) of homogeneous groups using the programme Statgraphics Plus for Windows 1.4 (1994–1995 by Statistical Graphics Corporation). Differences are significant on a significance level $\alpha < 0.05$.

Based on the results, two clones with the highest resistance and the two most sensitive clones were selected while the representation of males and females in each group was observed.

Laboratory test of parent clones

The resistance level of the four clones selected for genetic analysis of the trait resistance to rust was tested in laboratory conditions.

To prevent uncontrolled infection of leaves before the test started one-year plants from woody cuttings were grown in a greenhouse. Leaves taken from these plants were rinsed with running water. Disks 25 mm in diameter were cut out from the leaves and placed on wet filter paper in Petri dishes. The four

Table 1. Origin of clones and their distribution in the experiment

Origin of clones	Clone	Study site		
		Smilkov SM	Svojšice SV	Bystřice BY
Elbe river basins	101	×	–	–
Elbe river basins	102	×	–	×
South Moravia	104	×	–	–
South Moravia	105	×	–	–
South Moravia	107	×	×	×
South Moravia	108	×	–	–
South Moravia	110	×	–	×
cv. NE – 42 *P. maximowiczii* × *P. trichocarpa*	201	×	×	×
Brno	202	×	×	×
Praha	203	×	×	–
Ivachnová	204	×	–	×
Ostravice – Baška	205	×	×	×
South Moravia	206	×	×	×
Moravia river basins	209	×	–	×
South Moravia	210	×	×	×
cv. Blanc du Poitou P. × *canadensis*	211	×	×	–
South Moravia	301	×	×	×
South Moravia	302	×	×	×
South Moravia	303	×	–	–

Table 2. Description of the study sites

Study site	Latitude N	Longitude E	Altitude (m a.s.l.)	Temperature		Precipitation (mm)		
				Mean annual 1901–1950	Mean IV–IX 1901–1950	Total IV–IX	Total IV–IX	
						1901–1950	1999	2000
Smilkov (SM)	49°36´	14°36´	515	6.8	12.6	393	352	393
Svojšice (SV)	49°14´	13°26´	606	6.9	12.9	411	418	405
Bystřice (BY)	49°21´	12°48´	551	5.7	12.0	448	–	–

tested clones and control clone – interspecific hybrid *Populus* × *canadensis* cv. Pannonia with high resistance were put into each dish. Besides the variant with inoculation the experiment also comprised a control variant without inoculation. The experiment had 6 or 5 replications. Dishes were placed into an air-conditioned chamber, temperature 20°C and photoperiod 12 hours light/12 hours dark.

Inoculation. To prepare the inoculum leaves were taken from black poplar plants in Průhonice-Michovky locality also. Progenies of the tested clones were planted in this locality. The rust species was determined in a phytopathological laboratory of Silva Tarouca Research Institute for Landscape and Ornamental Gardening in Průhonice. Uredospores were transferred with a brush from the leaves onto agars (0.01%). The inoculum was applied to leaf disks with a brush. The number of uredospores in the inoculum was not determined.

The experiment was evaluated several times. The degree of infection was expressed as the number of uredia that grew on leaf disks.

Genetic analysis

We carried out reciprocal crossing of selected clones R_1 (206 f); R_2 (301 m); S_1 (205 f); S_2 (303 m) and we obtained four progenies:

$R_1 \times R_2$ – progeny of parents with partial resistance,

$S_1 \times R_2$ – progeny of susceptible female and resistant male parent component,

$R_1 \times S_2$ – progeny of resistant female and susceptible male parent component,

$S_1 \times S_2$ – progeny of susceptible parents.

Crossing was carried out on separated flower-bearing branches under controlled conditions in a greenhouse in 2003. Harvested seeds were sown into seed boxes, and after germination seedlings were transplanted onto a bed at a spacing of 0.12 × 0.30 m. The progenies were planted in a randomised block design with six replications. Rust infection was evaluated by the 5-point scale (see above) on one-year (2003) and two-year (2004) seedlings.

Table 3. Resistance of *Populus nigra* clones to the rust *Melampsora larici-populina* observed in 1999–2002 in Smilkov locality (degree evaluation – extreme values)

Clone	Number	LS mean	Homogeneous groups
211	12	0.78	x
301	16	1.44	x
206	16	1.62	xx
201	14	1.71	xxx
108	15	1.76	xxx
202	12	1.78	xxxx
210	16	1.84	xxxx
102	16	2.75	xxx
204	19	2.78	xxx
209	16	2.91	xx
205	16	2.94	xx
101	12	2.96	xx
303	13	3.06	x

Table 4. Occurrence of the rust *Melampsora larici-populina* in four selected clones of *Populus nigra* (average degree evaluation)

Clone	Smilkov					Svojšice				Bystřice		
	99	00	01	02	Ø	99	00	01	Ø	99	00	Ø
Date of evaluation	22.9.	25.9.	18.9.	19.9.		21.9.	19.9.	29.9		15.9.	20.9.	
301	1.2	1.2	0.9	2.5	1.44	0.50	2.1	1.00	1.20	1.60	1.60	1.6
206	1.1	1.8	0.6	3.0	1.63	1.30	2.7	0.75	1.58	1.50	1.10	1.3
205	2.5	2.5	2.4	4.0	2.94	1.75	2.8	2.40	2.32	2.80	2.80	2.8
303	2.1	2.3	3.8	4.0	3.06							
201	1.5	1.5	1.25	2.6	1.71	1.90	1.1	2.00	1.67	1.10	1.25	1.7

Table 5. Laboratory test of selected clones of *Populus nigra* for resistance to the rust *Melampsora larici-populina* in four selected clones *Populus nigra* (average number of uredia)

Date of inoculation /Date of evaluation	22.7./6.8.03			22.7./6.8.03			11.8./23.8.04			16.8./30.8.04		
Clone	*n*			*n*			*n*			*n*		
PAN	6	0.0	a	6	0.0	a	5	0.0	a	6	4.8	a
301	6	2.2	a	6	5.8	a	5	11.2	ab	6	68.2	c
206	6	3.3	a	6	4.2	a	5	4.4	ab	6	39.0	b
303	6	7.0	ab	6	23.0	b	5	32.6	b	6	75.0	c
205	6	11.5	b	5	26.4	b	5	92.6	c	6	112.8	d

RESULTS

Selection of clones with different resistance

Table 3 summarises the evaluation of rust occurrence in Smilkov locality over four years in 19 clones of black poplar and two control clones (201 and 211). Significant differences were determined between the separate clones of black poplar, and between the clones of black poplar and control clones. The degree of rust infection of the hybrid clone Blanc du Poitou (211) was significantly lower than in all other clones. Five black poplar clones with the lowest infection did not differ significantly from the other control clone NE – 42 (201). A female clone (206) and a male clone (301) were selected out of these five clones. Out of the six black poplar clones with the highest rust infection a female clone (205) and a male clone (303) were also selected.

Table 4 shows the results of evaluation of four selected clones in three localities over four, three or two years. Clone 303 was not planted in the two remaining localities. In Svojšice and Bystřice localities the degree of rust infection of clone 205 was obviously higher than on clones 206 and 301. Rust infection of clones 206 and 301 was similar to the infection of the control clone 201.

Laboratory test of parent clones

Table 5 shows the results of laboratory tests for resistance to the rust *Melampsora larici-populina* in four clones of black poplar designed for genetic analysis of the given trait. All four observations revealed significant differences in the number of uredia between clone 205 and less infected clones (206 and 301). The number of uredia in clone 303 was twice significantly higher and twice insignificantly higher than in clone 206. The number of uredia in clone 301 was always lower than in clone 303 but the difference was significant in one observation only. No uredia were observed in the variant without inoculation.

The results of laboratory tests confirmed the differences between less infected and more infected clones that were found out in field conditions.

Genetic analysis

The aim of the genetic analysis of four clones was to confirm an assumption that the examined clones differed in their resistance to rust and that they conferred this trait to their progeny. As indicated by two-year observations of four progenies (Table 6), the progeny of parents with the highest assumed resistance, $R_1 \times R_2$ (clone 206 and 301), had the lowest average degree of infection (2.59 and 1.95). The

Table 6. Genetic analysis of the progenies of four clones of *Populus nigra* and their sensitivity to the rust *Melampsora larici-populina*

		Sensitivity to rust Average point evaluation		Percentage of degree scale classes			
Parents	*n*	one-year seedlings	two-year seedlings	1	2	3	4
$R_1 \times R_2$	41	2.59a	1.95a	29.3	46.3	24.4	0.0
$S_1 \times R_2$	88	2.72a	2.23a	25.0	36.0	30.0	9.0
$R_1 \times S_2$	116	3.57b	2.89b	4.3	25.9	46.5	23.3
$S_1 \times S_2$	107	4.00c	3.34c	1.8	10.3	40.2	47.7

R_1 clone 206, S_1 clone 205
R_2 clone 301, S_2 clone 303

highest average degree of infection (4.00 and 3.34) was found in the progeny of parents with the highest susceptibility to rust, $S_1 \times S_2$ (205 and 303). Differences between these two progenies were significant in both years. The average degree of infection in progenies $S_1 \times R_2$ and $R_1 \times S_2$ was between the values of progenies $R_1 \times R_2$ and $S_1 \times S_2$. It indicates intermediate inheritance of the examined trait. There were significant differences in the degree of infection between the progenies $S_1 \times R_2$ and $R_1 \times S_2$. It is to note that $S_1 \times R_2$ combination was composed of parent clones with higher resistance (206 and 303) compared to the parents of $R_1 \times S_2$ combination (205 and 301). Such a result would suggest the genetic effect of paternal component.

The frequency distribution of the degree classes was similar. The relative frequency of individuals with lowest infection (degree class 1) was highest in $R_1 \times R_2$ combination (29.3%) and lowest in $S_1 \times S_2$ combination (1.8%). On the contrary, the frequency of $R_1 \times R_2$ combination in class 4 was zero while $S_1 \times S_2$ combination showed the highest frequency (47.7%).

DISCUSSION

Variability in rust resistance was examined in black poplar clones at three sites for two to four years. The examined clones were distributed in a randomised block design at four replications in each locality. This experimental design sufficiently eliminated the environmental influence on the host/pathogen relationship. Resistance of selected clones with the extreme values of resistance (sensitivity) that were determined by field evaluation was also checked in laboratory tests. The differences were identical in both environments. In the course of laboratory testing the rust species, not the rust race, was determined. The frequency of new race occurrence is low in *Populus nigra* from natural sites (Pinon, Frey 1997). In experiments with black poplar clones and with progenies of selected clones the infection was natural under field conditions where the origin of the species and race was not examined. Field sensitivity investigated on the basis of natural infection was also described by Legionnet et al. (1999). Nevertheless, identical sensitivity to rust in field and laboratory conditions is important.

In the genetic analysis of the trait resistance in four genotypes (clones) and in four progenies of these genotypes (clones) the observed sensitivity to rust was divided into six classes while the two extreme classes (0 and 5) did not occur in the evaluated progenies. Such discontinuous distribution does not correspond to the type of genetic constitution of resistance because resistance in *Populus nigra* is non-specific with quantitative variability (Legionnet 1999).

Rust infection of the progeny of more resistant parents was significantly lower than the infection in the progeny of susceptible parents. Similar conclusions were reported by Rajora et al. (1994) and Legionnet et al. (1999).

Our results do not permit an exact analysis of the inheritance of the trait resistance, nevertheless we may draw these conclusions:

– significant effect of the parents (genotype) on the transfer of the trait resistance to the progeny and so genetic control of the trait resistance to rust were confirmed;
– inheritance between the susceptible and resistant parent was intermediate. Similar results were reported by Pinon (1992);
– genetic effect of the paternal component was expressed. Similar results were published by Rajora et al. (1994).

The results confirm that the black poplar can be selected for its resistance to the rust *Melampsora larici-populina* even though full resistance – immunity is hard to achieve, it will be only partial and non-

specific resistance. Significant genetic variability of the trait resistance and medium or high heritability of this trait (Rajora et al. 1994) are prerequisites of successful breeding for this trait.

References

BENETKA V., BARTÁKOVÁ I., MOTTL J., 2002. Productivity of *Populus nigra* ssp. *nigra* under short-rotation culture in marginal areas. Biomass and Bioenergy, *23*: 327–336.

DIRECTORY OF POPLAR AND WILLOW EXPERTS, 2000. Register of *Populus* L. cultivars. Instituto di Sperimentazione per la Pioppicultura, Casale Monferrato, Italy.

LEFÈVRE F., PICHOT C., PINON J., 1994. Intra- and interspecific inheritance of some components of resistance to leaf rust (*Melampsora larici-populina* Kleb.) in poplars. Theoretical and Applied Genetics, *88*: 501–507.

LEGIONNET A., MURANTY H., LEFÈVRE F., 1999. Genetic variation of the riparian pioneer tree species *Populus nigra*. II. Variation in susceptibility to folia rust *Melampsora larici-populina.* Heredity, *82*: 318–327.

PEI M.H., RUIZ C., HARRIS J., HUNTER T., 2003. Quantitative inoculations of poplars with *Melampsora larici-populina*. European Journal of Plant Pathology, *109*: 269–276.

PINON J., 1992. Variability in the Genus Populus in Sensitivity to Melampsora Rust. Silvae Genetica, *41*: 25–34.

PINON J., FREY P., 1997. Structure of *Melampsora larici-populina* populations on wild and cultivated poplar. European Journal of Plant Pathology, *103*: 159–173.

PINON J., PEULON V., 1989. Mise en évidence d´une troisième race physiologique de *Melampsora larici-populina* Kleb. en Europe. Cryptogamie Mycologie, *10*: 95–106.

PINON J., VAN DAM B.C., GENETET I., DE KAM M., 1987. Two pathogenic races of *Melampsora larici-populina* in north-western Europe. European Journal of Forest Pathology, *17*: 47–53.

RAJORA O.P., ZSUFFA L., YEH F. C., 1994. Variation, Inheritance and Correlations of Growth Characters and Melampsora Leaf Rust Resistance in Full-Sib Families of Populus. Silvae Genetica, *43*: 219–225.

STEENACKERS V., 1982. Nouvelle race physiologique de *M. larici-populina* en Belgique. FAO/CIP, 22e réunion du Groupe de Travail des maladies, Casale Monferrato: 6.

Received for publication September 30, 2004
Accepted after corrections December 3, 2004

Selekce klonů druhu *Populus nigra* L. ssp. *nigra* na rezistenci vůči rzi *Melampsora larici-populina* Kleb.

V. Benetka, I. Šálková, F. Vrátný

Výzkumný ústav Silva Taroucy pro krajinu a okrasné zahradnictví, Průhonice, Česká republika

ABSTRAKT: Možnost selekce klonů druhu *Populus nigra* L. s vyšší rezistencí ke rzi *Melampsora larici-populina* Kleb. byla testována na souboru 29 klonů topolu černého, pocházejících od jedinců vybraných v různých lokalitách ČR. Klony byly testovány na třech lokalitách po čtyři roky. Napadení rzí bylo hodnoceno pětibodovou stupnicí. Dva nejodolnější (R_1; R_2) a dva nejcitlivější (S_1; S_2) klony byly zkoušeny i v laboratorních podmínkách. Tato zkouška potvrdila shodu s výsledky v polních podmínkách. Průkazné rozdíly mezi potomky rezistentních ($R_1 \times R_2$) a citlivých ($S_1 \times S_2$) rodičů potvrdily genetickou kontrolu rezistence. U potomstev $S_1 \times R_2$ a $R_1 \times S_2$ byla dědičnost rezistence intermediální. Zároveň se projevil genetický efekt otce. Potvrdila se možnost výběru na rezistenci proti rzi *M. larici-populina* u druhu *P. nigra*.

Klíčová slova: genetická kontrola; rezistence; výběr; *Populus nigra* L.; *Melampsora larici-populina* Kleb.

Odolnost vůči chorobám je jeden z nejdůležitějších znaků, na který se soustřeďuje současné šlechtění rostlin. Topol černý, který patří do skupiny rychle rostoucích dřevin, se stává významnou dřevinou v programech obnovitelných zdrojů energie. Vedle toho má topol černý jako domácí dřevina

nezastupitelné místo v krajinotvorných programech. Rez, způsobená druhem *Melampsora larici-populina* Kleb., je nejvíce rozšířenou a nejčastěji se vyskytující chorobou topolů. Výskyt rzi u topolu černého v podmínkách ČR se nezdá být významný u solitérních stromů. Avšak při pěstování topolu černého v kultuře s hustým sponem lze pozorovat vysoký výskyt rzi již uprostřed vegetačního období. U jmenovaného druhu rzi vedle původní rasy E1 je dnes známo asi pět nových ras. U topolu černého byla pozorována nespecifická rezistence s projevem částečné rezistence na rozdíl od některých mezidruhových hybridů. Možnost výběru klonů topolu černého s vyšší odolností vůči rzi *M. larici-populina* byla testována na souboru 29 klonů *P. nigra,* pocházejících od jedinců vybraných v různých lokalitách ČR. Klony byly testovány na třech lokalitách po čtyři roky ve znáhodněných blocích ve čtyřech opakováních. Napadení rzí bylo hodnoceno pětibodovou stupnicí. Dva nejodolnější (R_1 a R_2) a dva nejcitlivější (S_1 a S_2) klony, vždy opačného pohlaví, byly vybrány v uvedeném pokusu a byly zkoušeny i v laboratorních podmínkách. Infekce byla provedena uredosporami, které byly odebrány na listech topolu černého na lokalitě, kde rostla i potomstva testovaných čtyř klonů. Laboratorní zkouška potvrdila shodu laboratorních testů s hodnocením v polních podmínkách. Čtyři klony byly zkříženy za účelem provedení genetické analýzy znaku rezistence. Průkazné rozdíly mezi potomky rezistentních ($R_1 \times R_2$) a citlivých ($S_1 \times S_2$) rodičů potvrdily genetickou kontrolu rezistence. U potomstev $S_1 \times R_2$ a $R_1 \times S_2$ byla dědičnost resistence intermediální. Zároveň se projevil genetický efekt otce. Výsledky potvrzují možnost šlechtit na rezistenci vůči rzi druhu *Melampsora larici-populina* i u topolu černého, i když nepůjde o úplnou odolnost – imunitu, ale pouze o částečnou nespecifickou rezistenci. Významná genetická proměnlivost znaku rezistence a střední až vysoká heritabilita tohoto znaku (Rajora et al. 1994) jsou předpokladem pro úspěšné šlechtění na rezistenci vůči rzi.

Corresponding author:

Ing. Vojtěch Benetka, CSc., Výzkumný ústav Silva Taroucy pro krajinu a okrasné zahradnictví,
252 43 Průhonice, Česká republika
tel.: + 420 296 528 253, fax: + 420 267 750 440, e-mail: benetka@vukoz.cz