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American black currant as donor of leaf disease resistance in black currant breeding

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Fungal diseases such as powdery mildew (*Sphaerotheca mors-uvae*), Septoria leaf spot (*Mycosphaerella ribis*) and anthracnose (*Pseudopeziza ribis*) are among the major problems for black currant growers. Interspecific hybridisation is one of the most effective methods to solve this problem by creating resistant cultivars. Hybrids of *Ribes nigrum* × *R. americanum* and *R. americanum* × *R. nigrum* were obtained after interspecific hybridisation and culture of isolated embryos *in vitro*. The fertility of plants was very low in F_1 and increased in F_2 and F_3. There were resistant plants in all families of hybrids obtained by reciprocal crosses, but the resistance level of hybrids with *R. americanum* cytoplasm was considerably higher. In F_3 generation plants, vitality and agronomical traits were increased. The resistance to fungal diseases of the same hybrids in F_3 generation was higher than of *R. nigrum* and equal to that of *R. americanum*.

Key words: fungal diseases, interspecific hybrids, *Ribes*

INTRODUCTION

Sensitivity to fungal diseases is a major problem for commercial black currant growing [1]. Distant hybridisation between different species provides qualitatively new material for currant breeding [2]. It has been shown that *Ribes americanum* is very resistant to antracnose [3] and its resistance to Septoria leaf spot is controlled by the oligogene [4]. The aim of this work was to create interspecific hybrids between *R. nigrum* and *R. americanum* in reciprocal crosses, to evaluate the fertility of hybrids and their resistance to fungal diseases, and to investigate the possibilities to use *R. americanum* as a resistance donor to powdery mildew and antrachnose.

MATERIALS AND METHODS

The work was performed at Plant Genetic and Biotechnology Department of Lithuanian Institute of Horticulture in 1989–2004. The following cultivars and species of *Ribes* were used for crossings: *R. nigrum* 'Vakariai' (Lithuania), 'Belorusskaya sladkaya' (Belarus), *R. americanum* wild form 'As-03'.

For crossing combinations, 100–250 flowers were emasculated. Because collected seeds were unable to germinate, for embryo rescue F_1 isolated embryos were placed on White [5] nutrition media, which was modified for currant isolated embryos [6]. The embryos were maintained in the culture room at a temperature of 21–25 °C and 50 μmol $m^{-2}s^{-1}$ PPF with a 16-h photoperiod using cool white fluorescent light. F_2 hybrids were obtained from selected most fertile plants in reciprocal hybrid families after open pollination. Families of F_3 hybrids were obtained from plants selected according to complex valuable agronomical traits after open pollination. Two-year-old hybrids from the greenhouse were planted outside in a breeding plot at a distance of 3 × 1 m been grown without fungicides.

Hybrid resistance to the fungal diseases powdery mildew (*Sphaerotheca mors-uvae*), Septoria leaf spot (*Mycosphaerella ribis*) and antrachnose (*Pseudopeziza ribis*) have been observed in the first and second fruiting seasons. The extent of leaf damage caused by fungal diseases was evaluated on a 5-point scale, with 0 denoting undamaged leaves and 5 denoting 100% of leaf surface unable to assimilate in a natural infected background in the field.

The data were analysed by analysis of variance (ANOVA) and grouped by the Duncan test, standard errors were calculated.

RESULTS

On average, 54.4% of flowers in cross combination (*R. nigrum* × *R. nigrum*) yielded berries and there were about 28.8 well-developed seeds in each berry (Table 1). Seeds were obtained in each interspecific crossing combination. The number of berries in each

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Table 1. **Results of *Ribes nigrum* interspecific reciprocal crossings with *Ribes americanum* (pooled data of crossings *R. nigrum* cv. 'Vakariai' and *R. nigrum* cv. 'Beloruskaya sladkaya' with *R. americanum*, 1989–1991)**

Crossing combination	Number of flowers	Number of berries	Fertility* (%)	Number of seeds	Number of seeds in a berry
Ribes nigrum cv. 'Vakariai' × *R. nigrum* cv. 'Belorusskaya sladkaya'	101	55	54.4a	1584	28.8
R. nigrum × *R. americanum*	108	6	5.5b	36	6.0
R. americanum × *R. nigrum*	255	19	8.4b	132	6.9

* Means marked with the same letter do not differ significantly (1% LSD).

Table 2. **Performance of interspecific hybrids (F_1–F_3) to fungal diseases (pooled data of crossings *R. nigrum* cv. 'Vakariai' and *R. nigrum* cv. 'Belorusskaya sladkaya' with *R. americanum*, 2003–2004, rating based on 0–5 scale)**

Pedigry	No. of plants	Powdery mildew		Septoria leaf spot		Anthracnose	
		Average damage (points)	Uninfected plants* (%)	Average damage (points)	Uninfected plants* (%)	Average damage (points)	Uninfected plants* (%)
*R. nigrum***	20	1.72 ± 0.16	0f	2.29 ± 0.43	0e	2.88 ± 0.23	0f
R. americanum	20	0	100a	0.36 ± 0.10	66.7a	0.26 ± 0.07	45a
R. nigrum × *R. americanum* F_1	11	0.65 ± 0.36	17.3e	1.23 ± 0.38	45.5b	1.28 ± 0.33	18.2bc
R. nigrum × *R. americanum* F_2	33	0.65 ± 0.30	54.5c	0.83 ± 0.21	15.1d	1.73 ± 0.32	12.1cde
R. nigrum × *R. americanum* F_3 family 83	40	1.50 ± 0.46	63.6bc	1.13 ± 0.31	27.3c	1.50 ± 0.20	9.0de
R. nigrum × *R. americanum* F_3 family 88	53	1.35 ± 0.59	66.7b	0.83 ± 0.40	0e	1.00 ± 0.25	25.0b
R. nigrum × *R. americanum* F_3 family 95	33	0.50 ± 0.20	65.0b	1.63 ± 024	0e	0.50 ± 0.01	5.0ef
R. nigrum × *R. americanum* F_3 family 96	32	0.28 ± 0.08	69.2b	1.48 ± 0.10	23.1c	1.30 ± 0.12	15.4cd
R. nigrum × *R. americanum* F_3 family 97	28	1.13 ± 0.24	40.0d	1.0 ± 0.20	10.0d	1.15 ± 0.41	40.0a

* Means marked with the same letter do not differ significantly (1% LSD)
**Seedlings of crossing *Ribes nigrum* cv. 'Vakariai' × *R. nigrum* cv. 'Belorusskaya sladkaya'.

interspecific combination was significantly lower than in intraspecific combination and depending on crossing direction varied from 5.5% (*R. nigrum* × *R. americanum*) to 8.4% (*R. americanum* × *R. nigrum*). On average, there were 6.0–6.9 seeds in a berry. Embryo rescue methods were used, because seeds from interspecific crossings did not grow using common methods.

Fungal diseases differently injured parental species. All plants of *Ribes nigrum* were damaged by powdery mildew, Septoria leaf spot and antrachnose. All *R. americanum* plants were undamaged by powdery mildew ant slightly damaged by Septoria leaf spot and antracnose (Table 2). Seedlings of F_1 and F_2 generations were in-between parental forms by disease resistance and slightly close to the maternal component of the crossings. Five *R. nigrum* × *R. americanum* F_3 hybrid families did not differ by the percentage of uninjured plants, except No 97 in which the number of uninjured plants was considerably lower. The hybrid families varied in powdery mildew injury (average extent from 0.28 to 1.50 points). There were 5 to 40% of plants among all hybrid families absolutely resistant to antracnose, though septoria leaf spot not uninjured plants were observed in 3 of 5 hybrid F_3 families only. Disease resistance in

Table 3. **Performance of interspecific hybrids (F_1–F_3) to fungal diseases (pooled data of crossings *R. americanum* with *R. nigrum* cv. 'Vakariai' and cv. 'Belorusskaya sladkaya', 2003–2004, rating based on 0–5 scale)**

Pedigry	No. of plants	Powdery mildew		Septoria leaf spot		Anthracnose	
		Average damage (points)	Uninfected plants* (%)	Average damage (points)	Uninfected plants* (%)	Average damage (points)	Uninfected plants* (%)
R. americanum	20	0	100a	0.36 ± 0.10	66.7b	0.26 ± 0.07	45d
*R. nigrum***	20	1.72 ± 0.16	0f	2.29 ± 0.43	0g	2.88 ± 0.23	0g
R. americanum × *R. nigrum* F_1	12	0	100a	0.31 ± 0.08	41.7d	0.1 ± 0.00	58.3c
R. americanum × *R. nigrum* F_2	21	0	100a	1.06 ± 0.17	28.6e	0.23 ± 0.07	80.9b
R. americanum × *R. nigrum* F_3 family 45	33	0.40 ± 0.12	36.7d	1.88 ± 0.13	3.0g	1.25 ± 0.43	21.2e
R. americanum × *R. nigrum* F_3 family 46	31	0.28 ± 0.11	77.4b	1.75 ± 0.14	29.0e	1.380.35	9.7f
R. americanum × *R. nigrum* F_3 family 47	27	0.63 ± 0.19	55.6c	1.25 ± 0.13	11.1f	2.08 ± 0.17	0g
R. americanum × *R. nigrum* F_3 family 48	28	0	100a	0.67 ± 0.20	53.6c	0.38 ± 0.10	57.1c
R. americanum × *R. nigrum* F_3 family 50a	38	1.38 ± 0.55	39.5d	1.03 ± 0.39	36.8ed	2.38 ± 0.21	0g
R. americanum × *R. nigrum* F_3 family 50b	40	0	100a	0.26 ± 0.06	80.0a	0.1 ± 00	92.5a
R. americanum × *R. nigrum* F_3 family 52	38	2.05 ± 0.18	8.3e	1.20 ± 0.12	12.5f	2.30 ± 0.13	0g

*Means marked with the same letter do not differ significantly (1% LSD)
**Seedlings of crossing *Ribes nigrum* cv. 'Vakariai' × *R. nigrum* cv. 'Belorusskaya sladkaya'.

all generations of the *R. americanum* × *R. nigrum* combination was higher than in other combinations (Table 3). There were plants not injured by Septoria leaf spot in F_1, F_2 and F_3 hybrid families, whereas in the family 50b, which had been received from a branch of a chimeric plant morphologically close to *R. americanum*, even 80% of plants were not injured by this disease. It considerably exceeds the resistance of both parental components. In seedlings of the family 50a, received from a branch of the same chimeric plant morphologically closer to *R. nigrum*, only 36.8% of plants were not injured by Septoria leaf spot, while plants not infected by antracnosis were not found at all. All seedlings of seven F_3 generation hybrid families were more or less injured by antracnose. Antracnose-resistant plants were more numerous among the two families' plants than in maternal component (*R. americanum*) plants.

DISCUSSION

Our data have proved the results reported in [4, 7, 8] that species of the section *Eucoreosma* may be easily involved to interspecific hybridization. However, it is difficult to obtain hybrids between *R. nigrum* and *R. americanum*. The hybridisation barrier between these two species emerges from hybrid endosperm developmental disorders in the initial stages of embryo development [8]. After pollination of 1403 *R. nigrum* flowers by *R. americanum* pollen, only two hybrids were obtained by Melekhina [8]. One of them developed berries, but seeds did not germinate. We succeeded in regenerating plants from 70–100% seeds and selecting partially fertile F_1 plants solely when culture of isolated embryos was employed [6]. The F_2 and F_3 hybrid families were obtained from open pollination of F_1. A large number of plants in these families were

disease-resistant. The number of disease-resistant plants was significantly higher in hybrid families having a cytoplasm from *R. americanum*. Moreover, the injury level of plants in these hybrid families was considerably lower. Some plants from this crossing combination exhibited resistance to all investigated diseases. Our results show that in interspecific, like in intervarietal [9], crosses a resistant progeny could be obtained when the components of the crosses are selected according to the phenotype.

The interspecific currant hybrids are recommended in further breeding for inducing resistance to powdery mildew, Septoria leaf spot and antracnose.

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AMERIKINIAI SERBENTAI – ATSPARUMO LAPØ LIGOMS DONORAI JUODØJØ SERBENTØ SELEKCIJOJE

Santrauka

Grybinës ligos – miltligë (*Sphaerotheca mors-uvae*), šviesmargë (*Mycosphaerella ribis*) ir antraknozë (*Pseudopeziza ribis*) – daro þalà juodøjø serbentø uogynams. Tarprûðinë hibridizacija yra vienas efektyviausiø metodø sprendþiant ðià problemà. *Ribes nigrum* × *R. americanum* ir *R. americanum* × *R. nigrum* hibridai gauti tarprûðinës hibridizacijos ir izoliuotøjø gemalø kutûros *in vitro* metodais. F_1 kartos augalø vaisingumas buvo þemas, o F_2 ir F_3 kartø padidëjo. Atspariø augalø buvo visose hibridø ðeimose, gautose abipusio (F_1) ir grá þtamojo (F_2; F_3) kryþminimo bûdø. Hibridø su *R. americanum* citoplazma atsparumo lygis buvo þenkliai aukðtesnis. Augalø gyvybingumas ir ûkiniø poþymiø vertë buvo didþiausi F_3 kartos. Kai kuriø F_3 hibridø atsparumas grybinëms ligoms buvo didesnis uþ *R. nigrum* ir prilygo *R. americanum*.