Resistance of Apple Varieties and Selections to *Erwinia amylovora* in the Czech Republic

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Abstract

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Sixty-four apple cultivars and selections of potential interest to apple producers and plant breeders in the Czech Republic were tested for their relative resistance to the fire blight pathogen over six years. Level of fire blight resistance was evaluated according to the extent of lesion development on the shoots tips after artificial inoculation in experimental plots under insectproof nets. Cultivars Quinte (resistant) and Yellow transparent (high susceptible) were included in the tests. Of 64 apple cultivars and selections tested, none were high resistant, 3.1% were evaluated as resistant, 10.9% moderately resistant, 57.8% moderately susceptible, 21.9% susceptible and 6.3% high susceptible. Resistant apple genotypes, showing blight necrosis of shoots of 11–12%, were only cultivars Selena and Quinte. Moderately resistant genotypes (blight necrosis 13.1–25.0%) were Kordona, Golden Smoothee, Julia, HL 323, Melodie, HL 421 and S 634/3. High susceptible genotypes (blight necrosis more then 80.1%) were comprised cultivars Vesna, Topas, Yellow transparent and Vanda. The remaining genotypes were moderately susceptible (blight necrosis 26.1–60.0%) and susceptible (blight necrosis 60.1–80.0%). During six experimental years, quantitative variability was recorded in the blight score. Differences between cultivars in susceptibility to fire blight were often statistically significant.

Keywords: apple cultivars; *Erwinia amylovora*; fire blight resistance

Fire blight, caused by the bacterium *Erwinia amylovora*, is regarded as one of the most economically important diseases of several plant species that belong to the Rosaceae family. In 1986, the fire blight pathogen was recorded for the first time in the Czech Republic (Kůdela 1988). The disease is now found on approximately three-quarters of the apple and pear production area in the country.

Up to now, the control measures used in fire blight contaminated areas consisted the removal of diseased host plants or their parts (orchard sanitation), cultural practices, and application of chemical sprays (Kůdela *et al.* 2002). However, these measures are not always satisfactory. In an integrated control programme, the growing of relatively resistant tree scion varieties on resistant rootstocks is the most efficient control method for fire blight. The need for fire blight-resistant cultivars of fruit and ornamental trees is more pressing than ever. Chemical control is unsatisfactory, and modern orchard management practices, such as high density of trees, results in

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increased vulnerability of orchards to fire blight (Vanneste 2002).

In the last three decades, a few reviews on fire blight resistance and breeding programmes were published (e.g. Aldwinckle & Beer 1978; van der Zwet & Keil 1979; Sobiczewski *et al.* 1997; Lespinasse & Aldwinckle 2000).

Following the appearance of fire blight in Central and Eastern Europe, research on fire blight, including testing of domestic apple and pear cultivars, have been started in Poland (Sobiczewski & Suski 1988), the Czech Republic (Blažek 1999; Korba & Kůdela 2004; Paprštein *et al.* 2004; Fischer *et al.* 2004), Austria (Keck *et al.* 1996) and Hungary (Toth *et al.* 2006).

The objective of this study was to determine the level of resistance (or susceptibility) of Czech apple cultivars and selections in comparison to selected standards. For this aim, trees were inoculated with the strains of fire blight pathogens from the Czech Republic and the values for evaluation of resistance of apple genotypes were obtained by measurement of shoot blighted lesions during next six years. Recently, several programmes are using genetic engineering to enhance fire blight resistance Norelli and Aldwincle (2000).

MATERIAL AND METHODS

Plant material. Three-years-old trees composed of M.9 rootstocks and specific scion cultivars or selections were planted at spacing of 1 × 1 m in an experimental plot under insect-proof net at Slaný Research Station of Crop Research Institute Prague-Ruzyně. The plant material was obtained from the Plant Breeding Station Litoměřice; Research and Breeding Institute of Pomology Holovousy, Ltd., and Experiment Station Střížovice, Institute of Experimental Botany, AS CR and from breeders. All cultivars and selections tested are given in Table 1.

One year after planting, 64 apple cultivars and selections were tested for resistance to *Erwinia amylovora* during six consecutive years. Inoculations were carried out on shoots, randomly chosen on three trees. Like resistant or susceptible standards, cultivar Quinte (resistant) and Yellow Transparent (high susceptible) were included in the tests (Kutina 1992).

Inoculation techniques. Each year, 10 to 30 actively growing shoots on three trees per cultivar/selection were inoculated. Inoculation was performed with a bacterial suspension composed of five selected strains of *E. amylovora* from the Czech Republic. Before inoculation, the virulence of pathogen strains was verified by testing on shoots of *Pyrus ussuriensis* or *Crataegus monogyna*.

Artificial inoculations were carried out during a period of strong shoot growth when shoots were 20 to 40 cm in length. The upper leaves of shoot tips were cut off using scissors immersed in an *E. amylovora* suspension at a concentration of approximately 10^6 cells/ml, and a drop of inoculum was subsequently put on wounded tissues. Following inoculation, trees were misted to create a higher relative humidity.

Scoring and blight resistance evaluation. Forty days after inoculation, the total length of the shoots and visually blighted parts of the shoots were measured. A fire blight score for each cultivar was determined by dividing average length of necrosis tissue by the average total length. The higher percentage of blighted shoot length is the higher level of susceptibility. From the blight score in percentage, 6 classes of blight resistance were defined (Table 1).

Differences in fire blight resistance of the cultivars and selections (percentage of visually blighted parts of shoots) were analysed using an analysis of variance of double classification and Tukey's method.

Table 1. Blight scores and classes of fire blight resistance

Percentage of blighted shoot length (blight scores or severity)	Resistance class and its abbreviation	
0–7.0	high resistant	hR
7.1–13.0	resistant	R
13.1–26.0	moderately resistant	mR
26.1–60.0	moderately susceptible	mS
60.1–80.0	susceptible	S
80.1–100	high susceptible	hS

Table 2. Fire blight scores of apple cultivars and selections after artificial inoculations

No.	Cultivars/selections		Year of inoculation						Mean blight score (%)	Resistance class
			1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}		
1	Selena	CZ	2	36	2	9	10	9	**11**	R
2	Quinte		5	53	7	3	6	0	**12**	
3	Kordona	CZ	2	40	12	20	10	14	16	mR
4	Golden Smoothee		13	26	23	17	13	18	**18**	
5	Julia	CZ	2	65	17	10	13	13	**20**	
6	HL 323	CZ	26	61	13	17	7	0	**21**	
7	Melodie	CZ	3	35	17	45	12	22	**22**	
8	HL 421	CZ	2	64	5	38	13	14	**23**	
9	S 634/3	CZ	3	18	67	20	8	28	**24**	
10	HL 888	CZ	3	61	66	7	12	5	**26**	mS
11	S 781/6	CZ	8	33	17	44	8	47	**26**	
12	Sir Prize		23	31	22	42	22	24	**27**	
13	Red Boskoop		32	35	27	16	45	14	**28**	
14	HL 902	CZ	2	77	11	14	53	20	**30**	
15	HL 1577	CZ	16	25	93	9	27	12	**30**	
16	HL 938	CZ	2	67	72	15	15	22	**32**	
17	HL 390	CZ	2	67	19	23	74	12	**33**	
18	HL 1677	CZ	14	42	24	29	67	26	**34**	
19	Prima		50	29	25	60	27	17	**35**	
20	Macresa	CZ	2	58	12	30	88	23	**36**	
21	HL 1860	CZ	10	49	33	56	27	39	**36**	
22	HL 795	CZ	8	44	14	19	76	53	**36**	
23	Idared		19	26	48	38	22	73	38	
24	Golden Delicious	CZ	13	46	34	55	57	26	**38**	
25	Primadela	CZ	33	46	9	56	79	14	**40**	
26	Karmína	CZ	12	39	26	76	51	39	**40**	
27	Dezert	CZ	25	35	27	53	13	97	**42**	
28	HL 138 A	CZ	2	33	49	72	57	43	**43**	
29	S 584/1		5	8	46	71	82	45	**43**	
30	HL 1971	CZ	7	27	14	42	74	99	**44**	
31	Resista	CZ	45	22	32	57	66	42	**44**	
32	Blaník	CZ	18	67	39	86	48	14	**45**	
33	Aneta	CZ	5	48	53	65	32	70	**45**	
34	Goldstar	CZ	75	50	20	79	45	4	**46**	
35	HL 186/A	CZ	37	44	51	47	98	4	**47**	
36	Hana	CZ	13	27	47	95	74	30	**48**	
37	Jantar	CZ	27	98	50	44	16	62	**49**	
38	HL 2219	CZ	41	42	74	96	33	14	**50**	
39	HL 102	CZ	12	67	32	89	47	87	**56**	
40	Nela	CZ	25	63	41	78	75	58	**57**	
41	Priam		39	39	67	80	53	70	**58**	
42	Rosana	CZ	5	86	41	100	19	100	**59**	
43	Rubinola	CZ	29	59	33	56	88	89	59	
44	HL 369	CZ	43	76	60	60	68	51	**60**	
45	HL 384	CZ	42	53	59	41	100	65	**60**	
46	Corint	CZ	37	69	46	95	55	59	**60**	

Table 2 to be continued

Nº	Cultivars/selections		Year of inoculation						Mean blight score (%)	Resistance class**
			1st	2nd	3rd	4th	5th	6th		
47	Bláhova reneta	CZ	64	32	18	93	93	64	**61**	S
48	Rajka	CZ	13	49	57	97	69	88	**62**	
49	Victorie	CZ	22	91	65	91	94	18	**64**	
50	HL 820	CZ	69	22	63	81	63	100	**66**	
51	Bohemia	CZ	54	67	50	93	56	85	**68**	
52	Angold	CZ	47	86	50	88	45	97	**69**	
53	Delor	CZ	77	92	53	85	100	18	**71**	
54	Golida		68	22	72	96	86	89	**72**	
55	HL 598	CZ	34	77	51	99	87	93	**73**	
56	Lena	CZ	34	77	47	84	100	100	**74**	
57	Otava	CZ	44	84	66	89	63	100	**74**	
58	HL 495	CZ	37	90	64	63	100	98	**75**	
59	Dalila	CZ	52	90	60	82	84	100	**78**	
60	Lotos	CZ	2	78	92	99	100	100	**79**	
61	Vesna	CZ	25	85	85	100	100	100	**83**	hS
62	Topaz	CZ	55	69	73	100	100	100	**83**	
63	Yellow Transparent		58	82	92	89	98	100	87	
64	Vanda	CZ	88	84	55	100	100	100	**88**	
Mean blight score (%)			26	54	42	59	55	51	48	

CZ = cultivar or selections of the Czech origin

Resistance class: R = resistant; mR = moderately resistant; mS = moderately susceptible; S = susceptible; hS = high susceptible

Red Booskop = Boskoopské červené; Yellow Transparent = Průsvitné letné

RESULTS

Of 64 apple cultivars and selections tested, none were highly resistant, 3.1% were evaluated as resistant, 10.9% moderately resistant, 57.8% moderately susceptible, 21.8% susceptible and 6.3% high susceptible. Cultivars and selections arranged according to the level of resistance in descending order from resistant to high susceptible are shown in Table 2.

Resistant apple genotypes, showing a mean blight necrosis of 11–12%, were only Selena and Quinte. Moderately resistant genotypes (blight necrosis 13–25%) were Kordona, Golden Smoothee, Julia, HL 323, Melodie, HL 421 and S 634/3. High susceptible genotypes (blight necrosis more then 80%) were cultivars Vesna, Topas, Yellow transparent and Vanda. The remaining genotypes were moderately susceptible (blight necrosis 26–60%) and susceptible (blight necrosis 61–79%).

Results of multiple comparisons using Tukey's method gave proof a statistical significance of 65 differences in the means of blight scores among cultivars and selections (Table 3).

The quantitatively variability was recorded in the blight score during six experimental years (Table 2). In the second till sixth experimental years, the mean blight scores ranged from 42 to 59%, and it surprisingly reached only 26% in the first year.

DISCUSSION

The degree of fire blight resistance for any species and combination of scion and rootstock can be tested in different environments (using either natural infection in orchard/nursery or artificial inoculation in greenhouses) with various techniques, (using either needle inoculation of the succulent shoot tip or spray inoculation of blossoms) with various rating systems (Aldwinckle & Beer 1978; van der Zwet & Keil 1979; Sobiczewski *et al.* 1997; Lespinasse & Aldwinckle 2000).

In breeding programmes, artificial inoculation of shoots in greenhouses has been used for selection

Table 3. Results of multiple comparisons in the means of blight scores among cultivars and selections using Tukey´s method

Cultivar	Selena	Quinte	Kordona	Golden Smoothee	Julia	S 781/6	Melodie	S 634/3	HL 421	HL 323	Sir Prize
Lena	0.030699										
Golida	0.027320										
Otava	0.024278										
HL 598	0.021545										
HL 495	0.014911	0.036467									
Victorie	0.008404	0.021545	0.038592								
Dalila	0.006050	0.015866	0.028966	0.045634							
Lotos	0.004928	0.013156	0.024278	0.038592							
Vesna	0.000913	0.002650	0.005273	0.008968	0.022875	0.027320	0.027320	0.030699			
Topaz	0.000792	0.002303	0.004605	0.007874	0.020269	0.024278	0.024278	0.027320	0.048218		
Delor	0.000396	0.001131	0.002303	0.004017	0.010877	0.013156	0.013156	0.014911	0.027320	0.030699	
Yellow Transparent	0.000153	0.000485	0.000981	0.001735	0.004928	0.006050	0.006050	0.006905	0.013156	0.014911	0.038592
Vanda	0.000524	0.001427	0.002779	0.004675	0.011865	0.014179	0.014179	0.015944	0.028144	0.031405	

of relatively resistant genotypes and for exclusion of very susceptible or susceptible ones. However, several observations a year of shoot, blossom, limb and trunk disease symptoms of cultivars growing under nursery or orchard conditions is still worthwhile. Additionally, according to Thibault and Le Lézec (1990), the correlation between susceptibility of shoots and flowers is weak.

To minimise variability in the resistance levels among genotypes in our tests, artificial inoculation of shoots with a standard inoculum concentration of trees grown in experimental plots was carried out. In spite of this, quantitative variability was recorded in the blight score during six experimental years (Table 1). In the second till sixth experimental years, the mean blight scores ranged from 42 to 59%, but reached only 26% in the first year. Therefore, it appears that there are other factors than inoculum concentration, which influence results. The ability of apple genotypes to exclude penetration of the fire blight pathogen, or suppresses activity after penetration can be strongly affected by the age, vigour, and nutrition of the host; environmental factors, particularly temperature and humidity; location of the orchard; soil types; orchid moisture levels, cultural practices; and combinations of one or all of these factors (van der Zwet & Keil 1979). For example, in tests carried out by Richter and Fisher (2000), the length of blighted apple shoots markedly decreased because of lower temperature in the greenhouse following inoculation.

As in our tests, the relative resistance of fruit cultivars to fire blight is usually assessed by the visual observation of fire blight lesions. Based on fire blight lesion formation, nearly 80% of genotypes tested were evaluated as low susceptible or susceptible. The differences in susceptibility between the genotypes in these groups are rather small (Table 2). Therefore, it is desirable to search for another parameter(s) suitable for differentiation of this large group of host genotypes. Among examples of such parameters include movement of the fire blight pathogen inside the host plant (Paulin & Lespinasse 1987; Crepel *et al.* 1997; Richter & Fisher 2000; Bogs *et al.* 2004); frequency and the severity of infection; the use of medium infective dose (ID_{50}); quantitative trait loci analysis (Khan *et al.* 2006). An important question is whether the inoculation should be performed with a single strain or a mix of trains (Norelli *et al.* 1987; Crepel *et al.* 1997; Lespinasse & Aldwinckle 2000; Richter & Fisher 2000).

Our results should be useful to apple breeders and growers, because genotypes that are resistant and susceptible to fire blight were identified.

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