Evaluation of susceptibility level of pear cultivars to fire blight (*Erwinia amylovora*) in the Czech Republic

J. Korba[1], J. Šillerová[1], F. Paprštein[2], J. Sedlák[2], E. Prokinová[3], P. Hošková[4]

[1]*Department of Bacteriology, Disision of Plant Health, Crop Research Institute, Prague, Czech Republic*
[2]*Research and Breeding Institute of Pomology Holovousy Ltd., Holovousy, Czech Republic*
[3]*Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
[4]*Department of Statistics, Faculty of Economics and Management, University of Life Sciences Prague, Prague, Czech Republic*

Abstract

Korba J., Šillerová J., Paprštein F., Sedlák J., Prokinová E., Hošková P., 2013. **Evaluation of susceptibility level of pear cultivars to fire blight (*Erwinia amylovora*) in the Czech Republic**. Hort. Sci. (Prague), *40*: 58–64.

Thirty-three pear cultivars and selections of potential interest to pear producers and plant breeders in the Czech Republic were tested for relative field susceptibility to the fire blight (*Erwinia amylovora*) over five years. Level of fire blight susceptibility was evaluated according to the extent of lesion development on the shoot tips after artificial inoculation in experimental plots under insect proof nets. Old cvs Alexander Lucas (as resistant standard), Conference (as moderately resistant standard) and Beurré Bosc (as susceptible standard) were included in the tests. The 33 pear cultivars and selections were tested, only one of them was highly resistant (3.0%), 3.0% were evaluated as resistant, 12.1% moderately resistant, 36.4% moderately susceptible, 18.2% susceptible and 27.3% highly susceptible. Breeding selection US 625-63-10 was the only highly resistant pear genotype (necrosis of shoots of 0–7.0%). Resistant genotype group (necrosis 7.1–13.0%) comprised US 625-63-4. Moderately resistant genotypes (necrosis 13.1–25.0%) included cvs Alexander Lucas, Alfa, Bohemica and HL 31-50-31. Highly susceptible genotypes (necrosis more than 80.1%) included cvs Vonka, Karina, Bona, Decora, Elektra, Milka, Regina, Alice and TE 4763. The remaining genotypes were moderately susceptible (necrosis 26.1–60.0%) and susceptible (necrosis 60.1–80.0%).

Keywords: bacterial disease; pome fruit trees; fire blight susceptibility

Fire blight caused by the bacterium *Erwinia amylovora* (Burrill) Winslow et al., is regarded as one of the most economically important and destructive 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). Now the disease is found in all parts of the country. Up to now, control measures used in the fire blight contaminated areas consisted of removal of diseased host plants or their parts (orchard sanitation), adherence to cultural practices, and application of chemical

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QH91148.

sprays (Kůdela et al. 2002). However, these measures are not always satisfactory. In an integrated control programme, growing of relatively resistant tree scion cultivars on corresponding 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, result in increased vulnerability of orchards to fire blight (Vanneste 2002).

In the last three decades, a large numbers of reviews on fire blight resistance and breeding programmes were published (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, started in Poland (Sobiczewski, Suski 1988), Czech Republic (Blažek 1999; Fischer et al. 2004; Korba, Kůdela 2004; Paprštein 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 susceptibility of Czech pear cultivars and selections in comparison to selected standards. To accomplish this aim, trees were inoculated with strains of *E. amylovora* from the Czech Republic and values for evaluation of pear genotype susceptibility were obtained by measurement of blighted lesions on annual shoot basis for five years.

MATERIAL AND METHODS

Plant material. Three-year-old trees composed of suitable rootstocks and specific scion genotypes were planted at a spacing of 1 × 1 m in an experimental plot under insect proof net at the Slaný Research Station of the Crop Research Institute in Prague. Plant material was obtained from the Plant Breeding Station Litoměřice and Research and Breeding Institute of Pomology Holovousy Ltd., Czech Republic.

One year after planting, 33 pear cultivars and selections were tested for susceptibility to *E. amylovora* during five consecutive years. Inoculations were carried out on 10 to 30 randomly chosen shoots on three trees for each cultivar or selection. Old cvs Alexander Lucas (resistant), Conference (moderately resistant) and Beurré Bosc (susceptible) were included in the tests (Kutina 1992; van der Zwet, Beer 1995) as resistant, moderately resistant and susceptible standards.

Bacterial strains and inoculum. Strains of *E. amylovora* used in this work are listed in Table 1. Inoculum was prepared as a mixture from five strains of *E. amylovora* of 24-h-old cultures, cultivated on nutrient sucrose agar (SNA) in sterile distilled water. Concentration 10^6 CFU/ml was chosen as optimal concentration of inoculum for plant inoculation. Inoculum was shaken and used within 2 h of dilution.

Inoculation techniques. Each year, 10 to 30 actively growing shoots per cultivar/selection were inoculated. Inoculation was performed with a bacterial suspension composed of five selected strains of *E. amylovora* from the Czech Republic (Table 1). Before inoculation, 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 had 20 to 40 cm in length. The upper leaves of shoot tips were cut off using scissors immersed in *E. amylovora* suspen-

Table 1. Strains of *Erwinia amylovora* (Ea) used in this work

Strains of *Ea*	Place of origin	Host plant	Characteristic
H 8/95	CZ Tuchoměřice	*Crataegus* × *monogyna*	high virulent isolate from seedling of hawthorn (1995)
H 10/96	CZ Slaný	*Pyrus communis*	high virulent isolate from pear cv. Dita (1996)
H R 3A	CZ Tuchoměřice	*Crataegus* × *monogyna*	high virulent isolate from seedling of hawthorn (1995)
R 1617	HU Budapest	unknown	standard strain of bacteriology collection of Crop Research Institute, Prague (1996)
R 30/97	DE Ascherleben	unknown	standard strain of bacteriology collection of Crop Research Institute, Prague (1997)

Table 2. Blight scores and classes of susceptibility to fire blight

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

sion at a concentration of approximately 10^6 CFU/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 susceptibility evaluation. Forty days after inoculation, total length of shoot and the number of visually blighted parts of shoot were recorded. A fire blight score for each cultivar was determined by dividing the average length of necrotic tissue by the average total length. Higher percentage of blighted shoot length reflects higher level of susceptibility. Six classes of blight susceptibility were defined from the blight score in percentage (Table 2).

Statistical analyses of our experiments were performed with the statistic program STATISTICA v. 10 (Statsoft Inc., Tulsa, USA).

RESULTS AND DISCUSSION

Variability in the level of susceptibility to fire blight among pear cultivars and selections in our test was high. Of the 33 tested pear cultivars and selections, 3.0% were evaluated as highly resistant, 3.0% as resistant, 12.1% as moderately resistant, 36.4% as moderately susceptible, 18.2% as susceptible and 27.3% as highly susceptible. Cultivars and selections, arranged according to the level of susceptibility in descending order, are shown in Table 3.

The only highly resistant pear genotype, showing a mean necrosis of 3–4%, was breeding selection US 625-63-10 (USA). Resistant genotype (necrosis 8–12%) was breeding selection US 625-63-4 (USA). Moderately resistant genotypes (necrosis 13–26%) were cvs Alexander Lucas (FR), Alfa (CZ), Bohemica (CZ) and HL 31-50-31. Moderately susceptible genotypes (necrosis 26–60%) were cvs Morava (CZ), Jizera (CZ), Eldorado (USA), Nitra (SK), Beurré Hardy (FR), Nela (CZ), Delisa (CZ), David (CZ), Amfora (CZ), Delta (CZ), Conference (GB) and Isolda (CZ). Susceptible genotypes (necrosis 61–79%) were cvs Dicolor (CZ), Manon (CZ), Red Bartlett (GB), Beurré Bosc (FR), Boro (CZ) and Highland (USA). Highly susceptible genotypes (necrosis more than 80%) were cvs Vonka (CZ), Karina (CZ), Bona (CZ), Decora (CZ), Elektra (CZ), Milka (CZ), Regina (CZ), Alice (CZ) and TE 4763 (CZ).

The variability of blight scores in years (mean blight scores ranged from 54.28 to 65.94) indicates that there are other factors than *E. amylovora* suspension at a concentration 10^6 CFU/ml to influence the results. The ability of pear genotypes to exclude penetration of the fire blight pathogen, or to suppress activity after penetration can be strongly affected by age, vigour, and nutrition of the host; environmental factors, particularly temperature and humidity; orchard location; soil types; orchard moisture levels, cultural practices; and combinations of one or all of these factors (van der Zwet, Keil 1979).

In accordance with our tests, relative susceptibility of pear cultivars to fire blight is usually assessed by visual observation of fire blight lesions. Based on fire blight lesion formation, more than 80% of tested genotypes were evaluated as moderately susceptible, susceptible or highly susceptible.

An important question is, whether inoculation should be performed with a single strain or a mix of strains (Norelli et al. 1987; Crepel et al. 1997; Lespinasse, Aldwinckle 2000; Richter, Fisher 2000). All the above described methods of artificial inoculation were verified in our conditions and the most suitable was the method of decapitation of upper leaves of shoot tips by scissors immersed in *E. amylovora* suspension at a concentration of approximately 10^6 CFU/ml. The drop of inoculum was subsequently put on wounded tissues. The mix of strain was applied for eliminating false results of interaction susceptibility between genotype and the strain of *Erwinia amylovora* (Norelli et al. 2000).

Table 3. Pear shoot susceptibility to *Erwinia amylovora* after artificial inoculation

Order	Pear cultivar/selection	Blight scores or severity (%)					Mean of blight score (%)	Resistance class
		2004	2005	2007	2008	2009		
1	US 625-63-10	0.00	2.86	7.20	0.00	0.00	2.01	hR
2	US 625-63-4	20.00	0.00	27.72	0.00	0.00	9.54	R
3	HL 31-50-31	21.30	7.45	36.37	32.95	11.44	21.90	mR
4	Alfa	9.35	19.40	37.91	24.19	6.25	19.42	mR
5	Alexander Lucas	18.47	6.62	37.13	14.38	10.92	17.50	mR
6	Bohemica	17.33	8.67	27.95	26.16	13.15	18.65	mR
7	Morava	39.50	39.52	33.42	42.97	17.76	34.63	mS
8	Beurré Hardy	54.20	49.40	28.39	50.24	23.60	41.17	mS
9	Nitra	58.50	58.47	37.99	44.63	15.77	43.07	mS
10	Eldorado	36.50	41.03	28.40	52.10	44.90	40.59	mS
11	Jizera	51.40	41.80	48.87	21.30	49.30	42.53	mS
12	David	61.80	23.71	45.70	63.95	82.50	55.53	mS
13	Conference	41.80	39.83	54.03	55.23	53.16	48.81	mS
14	Nela	49.30	63.20	48.61	62.94	30.50	50.91	mS
15	Amfora	61.90	61.90	46.30	42.10	62.30	54.90	mS
16	Isolda	56.89	66.17	59.71	74.10	35.67	58.51	mS
17	Manon	69.80	65.10	86.15	67.83	41.29	66.03	S
18	Beuré Bosc	87.30	73.59	46.50	76.88	43.70	65.59	S
19	Delta	78.80	76.00	74.28	58.33	45.71	66.62	S
20	Red Bartlett	80.40	81.80	64.25	73.04	83.10	76.52	S
21	Vonka	88.35	71.13	92.31	87.69	77.52	83.40	hS
22	Bona	92.00	89.30	94.40	73.57	79.10	85.67	hS
23	Highland	91.80	54.18	71.53	100.00	62.26	75.95	hS
24	Karina	95.00	100.00	49.66	100.00	80.00	84.93	hS
25	Dicolor	96.60	81.60	74.28	92.80	100.00	89.06	hS
26	Delisa	100.00	100.00	50.13	33.71	100.00	76.77	hS
27	Boro	100.00	53.70	46.54	100.00	67.51	73.55	hS
28	Decora	100.00	78.00	98.00	100.00	72.00	89.60	hS
29	TE 4763	100.00	100.00	100.00	90.59	100.00	98.12	hS
30	Milka	97.60	100.00	100.00	100.00	86.06	96.73	hS
31	Elektra	100.00	100.00	100.00	87.03	95.91	96.59	hS
32	Regina	100.00	100.00	100.00	96.59	100.00	99.32	hS
33	Alice	100.00	100.00	100.00	100.00	100.00	100.00	hS
Mean of blight scores in years		65.94	59.23	59.20	61.98	54.28		

for resistance class abbreviations, see Table 1

Most of the results, obtained after natural infection in orchards in East Bohemia, are comparable with our results of artificial inoculation (Blažek 1999; Korba, Kůdela 2004). Highly susceptible and susceptible cultivars showed high susceptibility or susceptibility (cvs Delta, Highland and Eldorado) in both cases.

Our results were performed with the statistic program STATISTICA. The percentages of pear trees affected by *E. amylovora* were compared using one-way ANOVAs followed by the Fisher's LSD test. After rejection the null hypothesis, data obtained in our experiment were sorted out for two

Table 4. Evaluation of variability of level susceptibility to *Erwinia amylovora*

Order	Pear cultivar/ selection	Mean	Group													Resistance class
			A	B	C	D	E	F	G	H	I	J	K	L	M	
1	US 625-63-10	2.0120	****													hR
2	US 625-63-4	9.5440	****	****												R
3	Alexander Lucas	17.5040	****	****	****											mR
4	Bohemica	18.6520	****	****	****											mR
5	Alfa	19.4200	****	****	****											mR
6	HL 31-50-31	21.9020		****	****											mR
7	Morava	34.6340			****	****										mS
8	Eldorado	40.5860				****	****									mS
9	Beurré Hardy	41.1660				****	****	****								mS
10	Jizera	42.5340				****	****	****								mS
11	Nitra	43.0720				****	****	****								mS
12	Conference	48.8100				****	****	****	****							mS
13	Nela	50.9100				****	****	****	****	****						mS
14	Amfora	54.9000					****	****	****	****						mS
15	David	55.5320					****	****	****	****						mS
16	Isolda	58.5080						****	****	****	****					mS
17	Beuré Bosc	65.5940							****	****	****	****				S
18	Manon	66.0340							****	****	****	****	****			S
19	Delta	66.6240								****	****	****	****			S
20	Boro	73.5500									****	****	****	****		S
21	Highland	75.9540									****	****	****	****		S
22	Red Bartlett	76.5180										****	****	****		S
23	Delisa	76.7680										****	****	****		S
24	Vonka	83.4000											****	****	****	hS
25	Karina	84.9320												****	****	hS
26	Bona	85.6740												****	****	hS
27	Dicolor	89.0560												****	****	hS
28	Decora	89.6000												****	****	hS
29	Elektra	96.5880													****	hS
30	Milka	96.7320													****	hS
31	TE 4763	98.1180													****	hS
32	Regina	99.3180													****	hS
33	Alice	100.0000													****	hS

for resistance class abbreviations, see Table 1, **** – statistically significant differences among averages of relative resistance level of pear cultivar

criteria, for cultivar and for year of observation. The basic characteristics are presented in Table 4. Comparing variability dates according to the coefficient of variation (CV), stability results were assessed. The great differences between results were found in the case of breeding selections US 625-63-10 (156,736%) and US 625-63-4 (139,885%), which consists in observation of fire blight symptoms on

two shoots in two years per selection. Susceptibility level of moderately susceptible, susceptible and highly susceptible cultivars was increasing and stabilized over the years.

The detailed evaluation of cultivars was made according to the analysis of variance by Fisher's LSD test. The differences among tested cultivars were shown in Table 4. Cultivars were classified into 13 groups (A–M). Statistically significant differences in classes were found among the tested cultivars ($LSD_{5\%}$ = 21.0101). Differences among classes are approximately corresponding with our classification of level susceptibility to fire blight.

Having compared the results according to the second criterion – year, the average values are relatively balanced ($LSD_{5\%}$ = 6.6406). The fluctuation of the level of susceptibility of particular year is affected by weather conditions.

The level of resistance may be affected by both factors at the same time. In this case, analysis of variance – double classification for cultivar/year was made ($LSD_{5\%}$ = 20.3010). The null hypothesis was rejected. Means were different for cultivars and also for years.

CONCLUSIONS

These results should be useful to pear breeders and growers for a future selection of new cultivars more resistant to fire blight.

The most resistant cultivars from Czech breeding programmes are cvs Alfa and Bohemica.

Description of cv. Alfa – the summer cultivar

- Origin: open pollination of cv. Bonne Louise d'Avranches
- Time of ripening: harvest maturity in first decade of August, prime quality in end of August, and keeps well until September
- Fruit: medium to large, conical-pyriform
- Tree growth: medium vigorous
- Fruit quality: sweet, crisp and aromatic
- Resistance: resistant to fungal diseases and frost
- Notices: suitable for all pear growing areas

Description of cv. Bohemica – the late winter cultivar

- Origin: cv. Comtesse de Paris × Fondante de Charneu
- Time of ripening: harvest maturity in mid-October, prime quality in January, and keeps well until April
- Fruit: medium to large, conical-pyriform
- Tree growth: vigorous, later moderate
- Fruit quality: sweet, crisp and aromatic
- Resistance: resistant to fungal diseases, medium frost hardy
- Notices: suitable for mild growing areas

References

Aldwinckle H.S., Beer S.V., 1978. Fire blight and its control. Horticultural Reviews, *1*: 423–474.

Blažek J., 1999. Hodnocení citlivosti odrůd a genotypů hrušní po přirozené infekci spálou růžovitých (*Erwinia amylovora*) (Assessment of susceptibility of pear cultivars and genotypes to a natural Infection by fire blight). Vědecké práce ovocnářské, *16*: 91–101.

Crepel C., Moermans R., Maes M.,1997. Evaluation of fire blight susceptibility of host plants under natural conditions. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent, *62*: 15–21.

Fischer C., Richter K., Blažek J., 2004. Testing of Czech cultivars and advanced selections of apples for fire blight (*Erwinia amylovora*) resistance. Horticultural Science (Prague), *31*: 8–11.

Keck M., Chartier R., Lecomte R., Reich H., Paulin J.P., 1996. First characterization of *Erwinia amylovora* isolates from Austria and fire blight susceptibility of some apple genotypes from Central Europe. Journal of Plant Diseases and Protection, *104*: 17–22.

Korba J., Kůdela V., 2004. Evaluation of the fire blight susceptibility of pear genotypes following inoculation. In: Proceedings of the XVI. Slovak and Czech Plant Protection Conference, September 16–17, 2003, Nitra. Acta Fytotechnica et Zootechnica, *7* (Special Issue): 144–146.

Kůdela V., 1988. *Erwinia amylovora*, původce spály růžovitých rostlin v Československu (*Erwinia amylovora*, causative agent of fire blight in Czechoslovakia). Ochrana rostlin, *24*: 173–182.

Kůdela V., Novacky A., Fucikovsky L., 2002. Rostlinolékařská bakteriologie (Bacteria and Plant Health). Academia, Prague: 348.

Kutina J. et al., 1992. Pomologický atlas 2 (Pomology 2). Zemědělské nakladatelství Brázda, Prague.

Lespinasse Y., Aldwinckle H.S., 2000. Breeding for resistance to fire blight. In: Vanneste J.L. (ed.), Fire Blight: The Disease and its Causative Agent, *Erwinia amylovora*. CABI Publishing, Wallingford: 253–273.

Norelli J.L., Aldwincle H.S., Beer S.V., Lamb R.C., 1987. The effects of virulence of *Erwinia amylovora* on the evaluation of fire blight resistance in *Malus*. Phytopathology, *77*: 1551–1555.

Norelli J.L., Aldwincle H.S., 2000. Transgenic varieties and rootstocks resistant to fire blight. In: Vanneste J.L.

(ed.): Fire Blight: The Disease and its Causative Agent, *Erwinia amylovora*. CABI Publishing, Wallingford: 275–292.

Paprštein F., Korba J., Kosina J., Šillerová J., 2004. Evaluation of resistance to fire blight in Czech pear cultivars. Acta Horticulturae (ISHS), 704: X. International Workshop on Fireblight. Available at http://www.actahort.org/books/704/704-577.htm, 577–582.

Richter K., Fisher C., 2000. Stability of fire blight resistance in apple. Acta Horticulturae (ISHS), 538: 267–270.

Sobiczewski P., Deckers T., Pulawska J., 1997. Fire Blight (*Erwinia amylovora*). Some Aspects of Epidemiology and Control. Research Institute of Pomology of Pomology and Floriculture, Skierniewice, Poland: 71.

Sobiczewski P., Suski Z.W. 1988. Fire blight in Poland. EPPO Bulletin, *18*: 375–379.

van der Zwet, Keil H.L., 1979. Fire Blight: A Bacterial Disease of Rosaceous Plants. USDA Agriculture Handbook No. 510, Washington: 83.

Toth M., Kasa K., Gondor M., Honty K., Hevesi M., 2006. First results of fire blight resistance screening in a Hungarian apple breeding programme. Acta Horticulturae (ISHS), 704: 545–549.

van der Zwet T., Beer S.V., 1995. Fire blight – its nature, prevention, and control: a practical guide to integrated disease management. U.S. Department of Agriculture. Agriculture Information Bulletin, *631*: 83.

van der Zwet T., Keil H.L., 1979. Fire blight a bacterial disease of rosaceous plants. Agricultural Handbook No. 510, U.S. Department of Agriculture, Washington.

Vanneste J.L., 2002. Fire Blight: The Disease and its Causative Agent, *Erwinia amylovora*. CABI Publishing, Wallingford: 370.

Received for publication December 28, 2012
Accepted after corrections February 2, 2013

Corresponding author:

PharmDr. Jana Šillerová, Crop Research Institute, Division of Plant Health, Department of Bacteriology, 161 06 Praha 6-Ruzyně, Czech Republic
phone: + 420 312 522 675, fax: + 420 233 311 592, e-mail: vsslany@seznam.cz