

Reactions of different plant organs of pear cultivars to *Erwinia amylovora* infection

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Summary: Research project has been initiated in 1999 with the aim of evaluating the degree of susceptibility/resistance of pear cultivars grown in Hungary to fire blight disease caused by *Erwinia amylovora*. The recently selected promising cultivars were also examined. Inoculation experiments were conducted in controlled greenhouse conditions because of quarantine regulations in Hungary. Following the disease process, development of symptoms of plant organs (shoots, flower parts, fruits) was observed. Suspension of two *E. amylovora* strains (Ea 21, Ea 23) isolated from pear was used in a mixture (5×10^8 cells \times ml⁻¹) for the inoculation. Twenty-six pear cultivars were examined and grouped into four categories: low susceptibility, moderately susceptible, susceptible and very susceptible. Most of the cultivars were susceptible or very susceptible while some promising 'Eldorado', 'Harrow Delight' and 'Hosui' showed low susceptibility.

Key words: pear cultivars (Japanese and European), fire blight susceptibility, *in vitro* testing, plant organs

Introduction

The causal agent of the fire blight disease, *Erwinia amylovora* bacterium appeared in Hungary in 1996. The bacterium is one of the most invading pathogens in apple, pear and quince orchards. No chemical can completely control the bacterium so disease resistant cultivars are important for the integrated pest management. The degree of susceptibility/resistance of the host plants is needed to know. Completely resistant cultivars are unknown in relevant literature. Vanneste (2000) found only 11% resistant type cultivars in *Pyrus communis*. After the invasion of the pathogen – following a shorter or longer incubation – the disease symptom will expand, as a sign of susceptibility. Lack of disease symptom is a consequence of resistance of the host plant. Between these extreme categories a wide range of susceptibility/resistance exists as well as low susceptibility, moderate resistance etc.

When evaluating disease resistance of pear cultivars to fire blight data from abroad must taken into account (Arsenijević & Panič, 1992, Le Lezec & Belouin, 1991, van der Zwet & Bell, 1990, 1995, Spotts & Mielke, 1999, Sobiczewski et al., 1997, Thiboult et al., 1989). These data sometimes show inconsistencies depending on country and different ecological circumstances. The aim of our experiments was to evaluate the degree of susceptibility/resistance of pear cultivars grown in Hungary and recently selected promising cultivars to *Erwinia amylovora*. The present research project was started in 1999 at the Department of Pomology, in a special laboratory for *Erwinia amylovora*. Inoculation experiments were conducted in controlled greenhouse conditions because of the strict quarantine regulations in Hungary.

Susceptibility was reported to vary (van der Zwet & Keil, 1979) widely depending on the organs affected that are usually flowers or shoots. Following these observations we described the host-parasite relationships between different pear cultivars and strains of *Erwinia amylovora*, with the evaluation of plant organs (shoots, flower parts and fruits). Infection of blossom is usually the first appearance of the disease symptoms.

The blossoms first appear water-soaked and turn brownish, finally fall down. Leaves and shoot are infected through the peduncle. While in some literature (Le Lezec & Belouin, 1991) the resistance of the whole cluster is described, we were interested in the potential differences in the susceptibility of different flower parts. After the blossom, the succulent twigs or shoots are the most susceptible parts of the plant (van der Zwet & Keil, 1979). There are only few research data on the susceptibility of fruits. Infection spreads directly through lenticels in the skin or by injury (after storm or hail). The natural infection of unripe fruits is possible in early summer in Hungary.

Material and method

For the artificial inoculation we have compared ten *Erwinia amylovora* strains of pear isolated from different locations determining its virulence in unripe pear fruits ('Kaiser Alexander' and 'Conference'). Two strains (Ea 21, Ea 23) were chosen and their mixture was used for inoculation in a suspension of 5×10^8 cells \times ml⁻¹. Bacteria were grown in King-B media. Bacterial strains were frozen at -18 °C in glycerol-nutrient broth (15% final concentration) or strains were lyophilised for long-term storage.

In the experiments, pear cultivars approved by the state (14 varieties) and other new varieties (12) were examined. To define the degree of susceptibility, shoots of forced grafted plants, blossoms (parts) and unripe fruits collected from orchards were used.

The methods of inoculation and evaluation were adapted from the literature (*van der Zwet & Keil, 1979*) or developed by us (*Hevesi et al., 2000*).

For shoot inoculation, six hand-grafted plants grown under greenhouse conditions were prepared until the shoots had become 30–40 cm long of each cultivar. Inoculation was carried out at the third leaf axil introducing the suspension by a hypodermic needle. Then shoots were covered by polyethylene bags to maintain high relative humidity. After seven days of incubation development of symptoms was evaluated four times in every three days. The fire blight symptom (browning) was evaluated separately on the leaves, petioles and shoots by a 0–5 grade scale (disease category) (*Horsfall-Barratt concept, 1945*).

Disease categories:

0. lack of browning
1. browning of shoots below and above pricking
2. extension of size of browning on shoots, browning of central leaf vein
3. complete browning and curving of shoots above pricking
4. browning of shoots below pricking with some browned and healthy leaves
5. complete browning of shoots and leaves.

The calculation was followed the method of *Bertrand & Gottwald (1978)* with a slight modification (*Hevesi et al., 2000*).

Disease rating on shoots:

$$DR_s = \frac{\Sigma\{(N1 \times 1) + (N2 \times 2) + (N3 \times 3) + (N4 \times 4) + (N5 \times 5)\}}{\Sigma N}$$

N1–5: Number of shoots in each disease category.

By the calculation of DR_s we established four susceptibility groups. $DR_s = 0-1.25$ (0–25%) low susceptible, 1.26–2.5 (26–50%) moderately susceptible, 2.51–3.75 (51–75%) susceptible, 3.76–5 (>76%) very susceptible ($DR_s = 5$ equals to 100%).

Data were processed with the aid of Statgraph 5.1. software; letters (A, B, C) mean homogeneity groups.

For flower inoculation, short and middle length bearing twigs were forced in 20 °C water. Open flowers and young leaves were sprayed with the bacterial suspension while control plants with sterile distilled water. After one-week incubation disease severity was determined by browning of receptacle, calyx, petal, stamen and pistil.

The disease severity was evaluated on a 0–3 grade scale (disease category) on flower parts.

0. lack of browning
1. 1/3 browning
2. 2/3 browning
3. complete browning

Disease rating on flowers (DR_f) was calculated by the formula:

$$DR_f = \frac{\Sigma\{(N1 \times 1) + (N2 \times 2) + (N3 \times 3)\}}{\Sigma N}$$

N1–3: Number of flowers in each disease category.

On the bases on browning of flower parts (receptacle: f_{re} , calyx: f_c , petal: f_{pe} , stamen: f_s and pistil: f_{pi}) we established four susceptibility groups. $DR_{f_{re,c,pe,s,pi}} = 0-0.75$ (0–25%) low susceptible, 0.76–1.5 (26–50%) moderately susceptible, 1.51–2.25 (51–75%) susceptible, 2.26–3 (>76%) very susceptible ($DR_s = 3$ equals to 100%).

Susceptibility of unripe fruits was recorded in years 2001 and 2002. In 2002 we could collect less cultivars than in 2001 because of the spring frost damage. Six fruits per cultivars (2–2.5 cm in diameter) were used for inoculation by six pricking per fruits with a needle immersed into the suspension of *E. amylovora*. We evaluated the susceptibility or resistance of cultivars from the different characteristics of symptoms and the diameter of spots. Water-soaked, continuously growing spots with diffused margin meant susceptibility while smaller, sinking, dark brown spots with strict margin signified resistant characters.

The fruits were evaluated on a 0–5 grade scale (disease category) by the diameter of the brown spots.

Disease categories:

0. lack of browning
1. 1–5 mm
2. 6–10 mm
3. 11–20 mm
4. 21–30 mm
5. <31 mm

For determination of disease rating of fruits (DR_{fr}) and grouping the cultivars, the same formula was used as before.

Results and discussion

On the basis of shoot infestation, during ten days disease process (evaluation 4 times) the cultivars showed different behaviour. The cultivars were separated from each others to homogeneity groups. We could conclude that 'Kieffer' is in the low susceptibility category, rises from the others. Among main market varieties, 'Kaiser Alexander' and 'Clapp's Favorite' show moderately susceptible. 'Harrow Sweet' is in the most resistant category by literature (*Bellini, 1993, Hunter & Gordon Bonn, 1992*). In contrast, it takes place after 'Kaiser Alexander' and 'Clapp's Favorite' as to our results. We are in agreement with other authors (*van der Zwet & Bell, 1995*) that 'Conference', 'Dr. Jules Guyot',

Table 1 Susceptibility of shoots on grafted pear trees to *Erwinia amylovora* (2002)

Cultivars	IV. 22.		IV. 25.		IV. 29.		V. 2.	
	Disease rating*	Homogeneity groups	Disease rating*	Homogeneity groups	Disease rating*	Homogeneity groups	Disease rating*	Homogeneity groups
Kieffer	0.14	A	0.14	A	0.57	A	0.57	A
Kaiser Alexander	0.71	AB	1.14	BC	1.14	AB	1.29	AB
Clapp's Favorite	0.33	A	0.75	AB	1.67	B	1.67	B
Harrow Sweet	1.00	BC	1.50	BCD	2.00	B	2.00	B
Packham's Triumph	2.20	EF	2.70	EFG	3.00	C	3.20	C
Harvest Queen	1.80	DE	2.80	FG	3.20	CD	3.30	C
Conference	1.38	CD	1.87	CD	3.38	CDE	3.88	CD
Baki Bosc	2.52	F	2.96	FG	3.96	DEF	3.96	CD
Wicar of Winkfield	1.71	DE	2.28	DEF	3.57	CDEF	4.00	CD
Orsolya	3.63	G	3.88	H	4.25	EF	4.38	D
Beurre Durondeau	2.41	F	3.25	GH	4.00	DEF	4.42	D
Dr. Jules Guyot	2.14	EF	3.14	GH	4.29	EF	4.43	D
Nijisseiki	1.25	BCD	2.75	EFG	4.50	F	4.50	D
Flemish Beauty	1.25	BCD	2.00	DE	4.50	F	4.75	D
		SZD _{5%} =0.58		SZD _{5%} =0.79		SZD _{5%} =0.93		SZD _{5%} =0.95

*counted DR_s-value

'Nijisseiki' and 'Beurre Durondeau' infected seriously so we put them into the very susceptible category (Table 1).

At evaluation of flowers, different signs of disease symptoms were observed among flower parts so that their reactions were determined separately (Table 2). The symptoms were the strongest in pistil while petals were the least infected. We came to the conclusion that browning of calyx showed mainly the degree of susceptibility characteristics of cultivars, which came near to the data of literature as well. Cultivars 'Dr. Jules Guyot', 'Harrow Delight', 'Magness', 'Fertilia Delbard' and the Japanese cultivars, 'Hosui' and 'Nijisseiki' were in the low susceptible category. It was evident that vast differences existed between cultivars by the infestation of flower parts. For example 'Moonglow' is less susceptible to fire blight according to other authors. However, it proved to be very susceptible due to the infestation of receptacle and calyx in our experiments.

Summarizing the data of flower parts, 'Packham's Triumph', 'Baki Bosc', 'Star' and 'US 6506313' are very susceptible-, 'Harrow Delight', 'Magness', 'Fertilia Delbard' and 'Hosui' are low susceptible as to our categories. Nearly the same susceptibility categories existed by the infestation of the calyx (Figure 1).

Characterization of susceptibility/resistance of unripe pear fruits is not successful only by the growth of infected area and appearance of bacterial exudates. The character of the infected fruit tissue (resistant or susceptible type) is very important. In very susceptible cultivars, secondary drops of bacterial exudates appear far from the place of inoculation. This phenomenon was observed in the 'Fertilia Delbard', 'Kaiser Alexander' and 'Dr. Jules Guyot' cultivars. Data of

Table 2 Susceptibility of pear flower organs to *Erwinia amylovora* (2002)

Cultivars	Disease rating*				
	Pistil	Petal	Calix	Receptacle	Stamen
Harrow Delight	0	0	0.26	0	0.29
Fertilia Delbard	0	0	0.18	0.42	1.45
Magness	0.26	0	0.24	0.24	0.78
Eldorado	0.27	0.50	1.50	1.50	1.60
Conference	0.67	0.64	2.06	2.06	0.67
Flemish Beauty	0.95	1.00	1.25	1.93	0.95
Clapp' Favourite	1.06	1.61	1.65	1.72	1.85
Beurre Hardy	1.22	0.17	1.13	1.78	1.55
Hosui	1.36	0.33	0.18	0.18	1.36
Beurre Hardenpont	1.71	0.57	1.71	1.71	1.71
Kaiser Alexander	2.00	0.52	1.06	2.26	2.28
Dr. Jules Guyot	2.02	0.17	0.70	0.90	1.90
Moonglow	2.05	0.20	2.80	2.77	2.05
Bonne Louise d'Avranches	2.13	1.27	2.38	2.33	2.38
Williams Pear	2.25	0.46	1.60	1.11	2.15
Beurre Durondeau	2.27	0.10	1.83	1.83	2.27
Star	2.37	1.17	2.83	2.83	2.35
Beurre Giffard	2.51	0.80	2.27	2.20	2.60
Nijisseiki	2.61	0.42	0.64	0.39	1.93
Max Red Bartlett	2.63	0	1.50	1.55	1.17
Árpával érő	3.00	0.38	3.00	3.00	3.00
US 6506313	3.00	1.78	3.00	3.00	3.00
Baki Bosc	3.00	3.00	2.84	2.84	3.00
Packham's Triumph	3.00	2.80	3.00	3.00	2.92

*counted DR_f-value

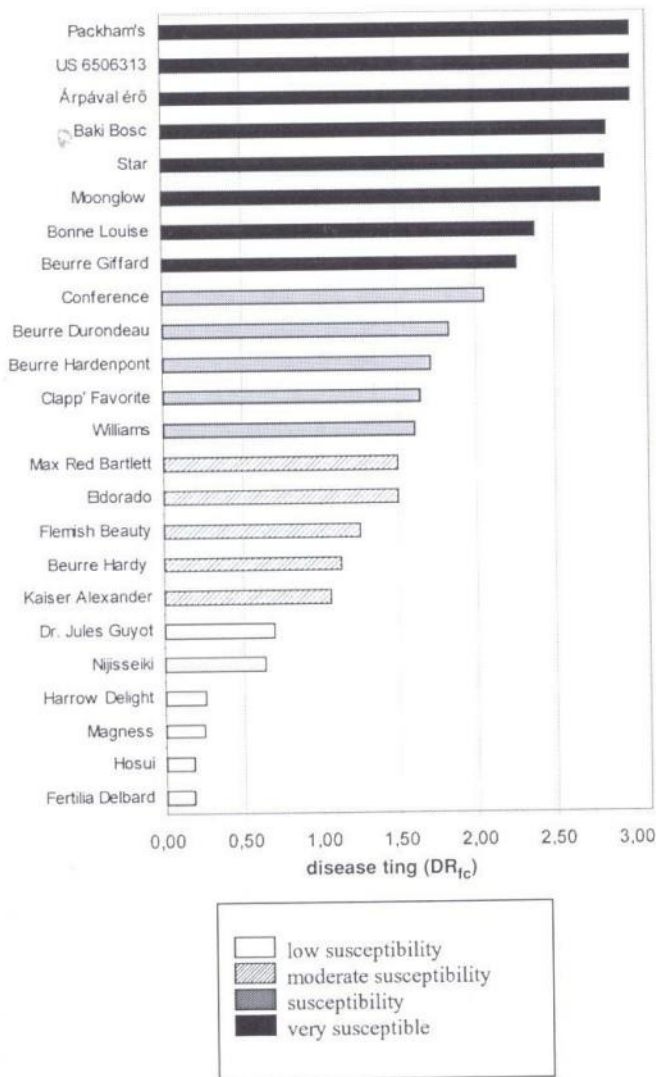


Figure 1 Susceptibility of pear calyx to *Erwinia amylovora* (2002)

Table 3 show that fire blight symptoms of unripe fruits in 2001 were stronger than in 2002, when the fruits were collected two weeks later. It is in agreement with Crosse et al. (1958) who observed that fire blight symptoms develop quicker on younger fruits than on older ones. In 2002, the necrotic area on unripe fruits was 70%, the ooze size 44% in the average of cultivars compared to data of 2001 (100%). The cultivars were grouped based on the disease rating of unripe fruits (DR_{fr}) in the means of 2 years (Figure 2). 'Fertilia Delbard' was in the very susceptible category, most of the cultivars were susceptible (9 cultivars). 'Moonglow' and 'Magness' also proved to be susceptible contrary other authors, who found them low susceptible previously. The members of 'Harrow'-series, 'Wicar of Winkfield', 'Star' and 'Beurre Durondeu' were moderately susceptible. Resistant type spot without development was on fruits of 'Beurre Hardenpont', 'Eldorado', 'Packham's Triumph' and the Japanese cultivars, 'Hosui' and 'Nijisseiki' showed low susceptibility as well.

Pear cultivars examined at least on the basis two organs were grouped into four susceptibility categories on the basis

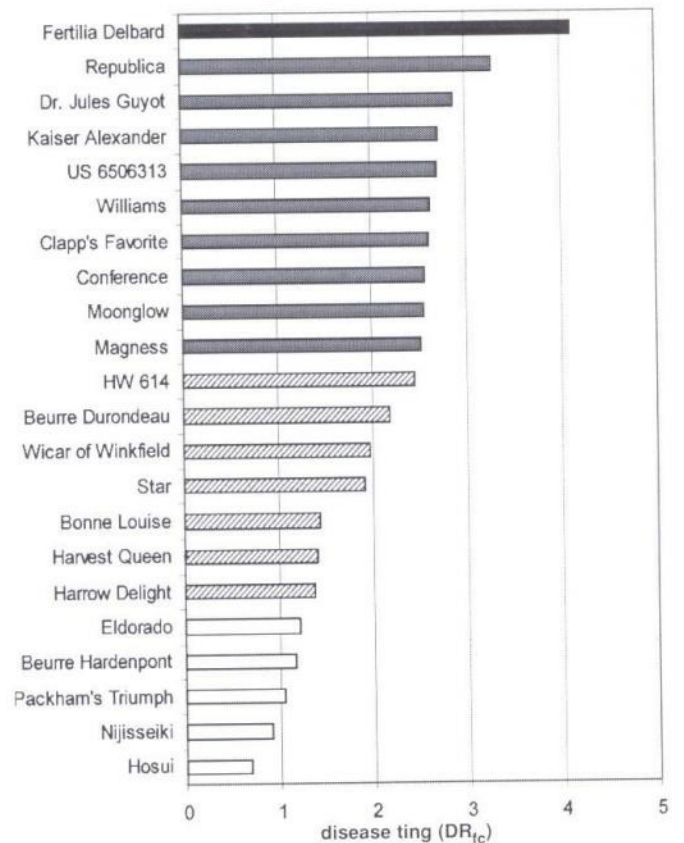


Figure 2 Susceptibility of unripe pear fruits to *Erwinia amylovora* (average of 2001 and 2002)

of disease severity (Table 4). Evaluation was difficult in a few cases where different organs displayed inhomogeneous reactions. For example, the shoots of 'Packham's Triumph' were susceptible-, the flower organs were very susceptible- and unripe fruits showed low susceptibility. The flower parts and unripe fruits of 'Nijisseiki' were low susceptible, while shoots very susceptible as well.

One cultivar was very susceptible, eleven cultivars susceptible, six cultivars moderately susceptible and only three cultivars low susceptible out of twenty-one cultivars. We could propose by our preliminary results cultivars of these two latter groups for the growers as candidates useful for the integrated pest management.

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Table 3 Susceptibility of unripe pear fruits to *Erwinia amylovora* (2001, 2002)

Cultivars	Necrotic area (mm)		Ooze ϕ (mm)	
	2001	2002	2001	2002
Packham's Triumph	1.96 A	6.90 AB	0.25 A	0.68 AB
Nijisseiki	1.38 A	4.58 A	0.66 A	0.01 ABC
Hosui	0.83 A	3.33 A	1.00 A	0.90 BC
Eldorado	3.70 AB	4.88 A	2.12 B	0.55 AB
Harrow Delight	5.45 AB	—*	4.25 DE	—
Harvest Queen	5.81 AB	—	5.87 GH	—
Bonne Louise d'Avranches	5.87 AB	—	4.62 DEF	—
Beurre Hardenpont	7.85 BC	2.63 A	3.50 CD	0.01 A
Star	8.33 BC	—	5.00 EF	—
Wicar of Winkfield	11.68 CD	7.46 AB	2.04 B	0.00 A
HW 614	13.83 DE	—	5.25 FGH	—
Clapp' Favorite	14.31 DE	14.83 CD	3.48 CD	0.30 AB
Williams	12.66 CD	13.52 C	3.25 C	4.55 E
Kaiser Alexander	11.93 CD	15.52 D	3.62 CD	5.02 E
Moonglow	14.10 DE	10.16 BC	5.12 FG	0.01 A
US 650613	19.22 EFG	13.61 CD	5.00 EF	0.01 A
Dr. Jules Guyot	16.68 DEF	14.16 CD	4.60 EF	2.66 D
Republica	20.70 FG	—	5.00 EF	—
Magness	20.77 FG	6.36 AB	5.00 EF	1.47 C
Beurre Durondeau	21.50 FG	2.75 A	5.12 FG	0.01 A
Conference	22.29 G	7.62 AB	5.00 EF	2.79 D
Fertilia Delbard	31.25 H	—	6.00 H	—
	SZD _{5%} = 5.39	SZD _{5%} = 5.16	SZD _{5%} = 0.84	SZD _{5%} = 0.77

*no fruits existed

Table 4 Relative fire blight susceptibility of pear varieties

Susceptibility			
Low	Moderately	Susceptible	Very
Eldorado	Beurre Hardenpont	Beurre Durondeau	Baki Bosc
Harrow Delight	Clapp' Favorite	Beurre Giffard	
Hosui	Harvest Queen	Conference	
	Kaiser Alexander	Flemish Beauty	
	Magness	Dr. Jules Guyot	
	Nijisseiki	Moonglow	
		Packham's Triumph	
		Star	
		US 6506313	
		Wicar of Winkfield	
		Williams	

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