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SUSCEPTIBILITY OF PEAR CULTIVARS AFTER INOCULATION WITH BULGARIAN STRAINS OF *ERWINIA AMYLOVORA*

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Abstract

Pear production in Bulgaria was limited because of the occurrence of fire blight. There is no certain chemical control of this disease. In terms of integrated management of bacterial diseases, it is very important to grow resistant cultivars. The aim of this study was to examine the reaction of 15 pear cultivars grown in Bulgaria. Terminal shoots of these cultivars grown in a greenhouse and grafted on quince BA29 rootstock were inoculated with two Erwinia amylovora isolates. The bacteria were isolated from apple and pear orchards in various regions of Bulgaria. After artificial inoculation degree of severity of diseases on the leaves and terminal shoots were recorded. For all tested cultivars the degree of infection on the leaves varied from 11.0% for 'Alexander Lucas' to 82.3% for 'Packham's Triumph'. Twenty-five days after the inoculation the tested cultivars were distributed in five susceptibility classes by their degree of severity of diseases on terminal shoots. 'Beurré Bosc' was considered as susceptible with 61.1% necrosis on terminal shoots, 'Dr. Jules Guyot' was considered as very low susceptible with 18.4% terminal shoots showing symptoms. Six of the tested cultivars were low susceptible with recorded degree of infection 20-40%. The very low susceptible and low susceptible cultivars could be recommended for commercial pear orchards establishment.

Key words*: bacteria, Erwinia amylovora, quince rootstock, single strain.*

INTRODUCTION

Fire blight, caused by *Erwinia amylovora* is one of the most destructive diseases affecting pome fruit trees (Sobiczewski et al., 2015; van der Zwet 2006; Thomson 2000; Bonn and van der Zwet, 2000; Vanneste, 2000). In 1989, the pathogen causing fire blight was recorded for the first time in Bulgaria (Bobev et al., 1999). Now the disease spread 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 sprays (Bobev, 2010).

One of the most promising approaches for sustainable fire blight management is the planting of tolerant or highly resistant cultivars and rootstocks (Van der Zwet et al., 2012; Korba et al., 2008). The pear breeding for resistance to fire blight is a priority in obtaining new cultivars (Gunen and Misirli, 2003). For this reason, it is necessary to identify resistant genotypes which can be used as genitors in artificial hybridisations (Zwet et al., 1974; Zwet and Bell, 1990; Sestras et al., 2008).

The quince rootstock BA29 is commonly used rootstock in European pear. It is a vegetative (*Cydonia oblonga* L.), suitable for intensive pear orchards, with a possibility of intensive fruiting of pear cultivars. This rootstock has some serious disadvantages - it is highly susceptible to fire blight, often attacked by *Psylla pyri* and shows graft incompatibility with some pear cultivars (Dondini, L. and Sansavini, S., 2012).

The aim of this study was to determine for a first time in Bulgaria the reaction of fifteen pear cultivars grafted on BA29 after inoculation with *E amylovora* strains.

MATERIALS AND METHODS

The studies were conducted in 2014-2017 at the Fruit Growing Institute Plovdiv, Bulgaria. Pear cultivars were grafted on a quince rootstock (BA29) and grown in an experimental greenhouse as potted one-year-old pear trees. The cultivars 'Dr. Jules Guyot', 'Beurré Hardy', 'Beurré Giffard', 'Santa Maria', 'Curé', 'Ranna Bolyarka', 'Passe Crassane', 'Beurré Hardenpont' 'Conference', 'Beurré Bosc', 'Alexander Lucas', 'Packham's

'Triumph', 'Red Williams', 'Williams', 'Abate Fetel'. Each of pear cultivar was planted in seven potted in repetition.

Bacteria of two *E. amylovora* strains were cultivated on a King's B medium for 24 h at 25°C, then washed off the medium with sterile distilled water. The concentration of the bacteria suspension was regulated to 3×10^8 cfu/ml (Mc Farland). The artificial inoculation of the pear cultivars was done by the application of two different bacterial isolates and a combination of them:

- Ea 3325 - isolated from apple trees on 16.05.2013 in Petrich (Bulgaria);
- Ea 3345 - isolated from pear trees on 27.06.2013 in Botevgrad (Bulgaria).

The artificial inoculation was done by cutting 1/3 of the leaf blade of three leaves on each terminal shoot with scissors dipped in the bacterial suspension. On the 10th and 15th day after the artificial inoculations, the reaction of the leaves was classified using the grading scale of disease severity of Zeller and Wolf (1996):

Class 0 - no visible symptoms of an infection;
Class 1 - the place of the cut is black;
Class 2 - visible symptoms on the place of the cut and leaf veins;
Class 3 - necrosis observed on the leaf blade;
Class 4 - necrosis observed on petiole;
Class 5 - necrosis is spread on the whole shoot tip.

To determine the severity of diseases of leaves, the results were transformed by the McKinney formula.

$$I=\frac{\sum(n\times k)\times100}{N\times K}$$

I - percentage Index of attack (%);
E (n.k) - the sum of the number of infected plants or organs (n) in the corresponding attack class (k);
N - the total number of plants examined (organs);
K - the highest grade in the corresponding scale.

Twenty-five days after the inoculation susceptibility level of the shoots to fire blight, was calculated by the formula below (Thomson et al., 1975):

Length of infected part (cm) = Susceptibility of shoots/Total shoots length (cm) × 100

The tested cultivars were classified in 5 susceptibility classes according to the calculated susceptibility level of the terminal shoots (Le Lezec et al. 1997):

1 Very Low Susceptible: 0-20;
2 Low Susceptible: > 20-40;
3 Moderately Susceptible: > 40-60;
4 Susceptible: > 60-80;
5 Very Susceptible: > 80-100.

On the 45th day after the artificial inoculation, on all pear cultivars, *E. amylovora* development to stem and rootstock was observed.

To prove the damage is causal by *E. amylovora* was taken the samples of infected tissue for resolution of bacteria in each cultivar.

The results were statistically analyzed by variance analysis of the SPSS 19 / column shows a significant difference ($p < 0.05$). The mean values were compared by using Dunkan test.

RESULTS AND DISCUSSIONS

The infection of the leaves of the tested pear cultivars was evaluated in dynamics through the 10th and 15th day after the inoculation was done.

The inoculated leaves with strain Ea3325 on the 10th days with the lowest percentage of infection was registered the cultivar 'Alexander Lucas' (11.0%) on the 15th day, the value of the infection with the same strain increased to 30.0% (Table 1) in 2016 year. The cultivars 'Santa Maria' (11.5%) and 'Beurré Giffard' (12.0%) had a similar severity of diseases. The most affected after inoculation with this strain were the cultivars 'Beurré Bosc' (76.5%) in 2015 year and 'Packham's Triumph' (60.4%) in 2017 year. The increased of value on the 15th day reached a 100% infected leaves.

The results after artificial inoculation with strains Ea3345 showed that with the lowest values respectively on 10th and 15th day after the infection were the cultivars 'Beurré Hardy' (from 13.6% to 30.9%) in 2017 year, 'Santa Maria' (from 13.0% to 30.8%) in 2016, and 'Alexander Lucas'(from 10.6% to 30.6) in 2016 year. The most percentage of necrosis of leaves was calculated in cultivars 'Beurré Bosc' (from 52.1% to 100.0%) in 2017 year, 'Williams' (from 43.0% to 100.0%),'Red Williams' (from 67.4% to 100.0%) in 2016

year and ‘Packhams Triumph’ (from 54.6% to 100.0%) in 2016 year.

When the leaves were inoculated with mixture of strains the severity of diseases on the 15th day after inoculation is more than 50% of the tested pear cultivars were calculated 100.0% infected leaves. The lowest percentage of necrosis on 10th day were determine in cultivars Beurré Hardy (23.6%), Conference (30.0%), and Beurré Giffard (30.0%). From the result after the artificial infection was determined the difference in the percentage of infection witch depend on tested cultivars, this result was confirmed by other authors.

The leaves infection can be used for reliable rapid screening for the sensitivity of the varieties (Fazio et al., 2006, 2008), which can be used for early diagnostics.

Other researchers support this view by explaining different pathogenic susceptibility due to different structural and biochemical features in the leaves (Viljevac et al., 2009).

Table 1. Fire blight development (%) on pear leaves, inoculation with two different strains of *Erwinia amylovora* and a mixture of them

Cultivar	Year	Strain Ea3325		Strain Ea3345		Mixture of Ea3325 and Ea3345 strains	
		Percentage of leaves infection after (days)					
		10	15	10	15	10	15
Dr. Jules Guyot	2015	20.2	87.9	26.0	51.5	55.0	80.0
	2016	20.4	50.7	30.0	43.1	33.3	50.0
	2017	16.7	40.5	21.1	40.0	54.9	96.1
Beurré Hardy	2015	17.4	34.8	23.2	42.5	31.7	67.4
	2016	18.4	38.1	20.5	50.1	23.6	63.6
	2017	22.2	49.8	13.6	30.9	30.7	63.8
Ranna Bolyarka	2015	30.0	70.8	20.0	37.8	50.0	100.0
	2016	24.7	60.4	36.2	73.1	46.7	100.0
	2017	37.6	70.4	42.3	83.9	48.1	100.0
Beurré Giffard	2015	33.3	69.8	21.2	47.1	57.1	93.3
	2016	12.0	30.8	27.5	63.2	30.0	85.0
	2017	32.8	60.4	40.0	60.1	41.3	87.0
Santa Maria	2015	40.3	80.0	34.0	78.4	64.7	100.0
	2016	31.9	79.6	13.0	30.8	43.3	100.0
	2017	11.5	34.9	43.0	87.2	62.6	100.0
Passe Crassane	2015	33.3	78.1	23.5	68.2	53.8	100.0
	2016	37.6	76.3	34.9	73.4	53.3	100.0
	2017	23.3	55.3	30.3	70.0	52.7	100.0
Beurré Hardenpont	2015	43.3	89.1	46.7	70.0	51.7	82.6
	2016	27.1	66.3	30.2	68,1	48.6	100.0
	2017	21.9	42.9	25.1	46.8	49,3	100.0
Alexander Lucas	2015	33.3	40.2	38.0	54.0	40.0	60.0
	2016	11.0	30.0	10.7	30.6	30.3	56.8
	2017	38.2	40.5	21.5	30.1	42.8	65.7
Curé	2015	20.0	48.9	24.0	69.4	43.3	100.0
	2016	50.3	90.5	36.4	89.0	86.7	100.0
	2017	48.1	87.5	43.2	90.3	91.0	100.0
Conference	2015	30.0	55.6	30.0	51.0	40.0	85.9
	2016	25.7	53.0	23.0	41.1	30.0	40.0
	2017	12.8	39.9	27.0	37.8	43.5	88.5
Beurré Bosc	2015	76.5	100.0	48.3	97.5	64.0	100.0
	2016	70.2	100.0	51.2	100.0	24.4	73.3
	2017	56.3	100.0	52.1	100.0	54.6	86.5
Williams	2015	44.0	76.4	20.0	40.0	53.3	100.0
	2016	35.0	100.0	43.0	100.0	50.0	100.0
	2017	40.5	97.0	31.6	65.4	51.8	100.0
Red Williams	2015	56.9	83.0	53.3	76.0	86.7	100.0
	2016	42.5	100.0	67.4	100.0	50.0	100.0
	2017	50.0	87.1	65.0	80.0	43.9	100.0
Abate Fetel	2015	46.0	54.3	37.8	60.0	75.0	100.0
	2016	51.1	100.0	32.0	89.9	24.4	46.7
	2017	48.7	68.5	45.0	79.5	62.2	89.6
Packham's Triumph	2015	30.0	68.7	41.1	65.0	80.0	100.0
	2016	40.0	100.0	54.7	100.0	37.5	100.0
	2017	60.4	100.0	64.6	100.0	76.5	100.0

In our study in each studied year, the experiment was conducted with the same cultivars, the artificial inoculations were done with the same strains and inoculation plant were maintained in similar conditionals.
The result showed that in different years were reported a variance in the percentage of infection of pear leaves.
The differences were explained by other authors. Following infection, the activity of phytoalexins becomes increasingly important in host plant resistance (Kuć et al., 1999).
Phytoalexins are low-molecular-weight antimicrobial compounds that are synthesized and accumulate in host tissue following exposure to microorganisms (Ebel, 1986).
The same author noted that some phytoalexins accumulate in both resistant and susceptible cultivars (Kuć et.al., 1999).
However, the accumulation of higher concentrations and the timing of their synthesis is critical for them to be effective (Bell, 2004).
The reacted of each plant is different after inoculation and the accumulation of phytoalexins in the plants may be different.

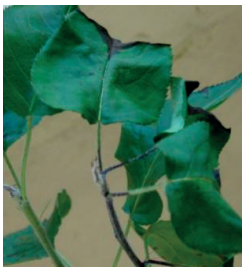

Figure 1. Infected leaves of Beurré Hardy cultivar

On the 10th after the inoculation six of the tested cultivars had different reaction which was statistically significant between inoculation with single or a mixture of two *E. amylovora* strains - 'Dr. Jules Guyot', 'Beurré Hardy', 'Ranna Bolyarka', 'Passe Crassane', 'Conference' and 'Williams' (Table 2). For the rest pear cultivars were observed non-significant differences. As a more virulent from both single strain in 10th days after inoculation is Ea3345. The averages rate for three studied years in all tested cultivar were reported the less damage after inoculation with Ea3325 but the differences non-significant.

Table 2.The average value of fire blight development (%) on the pear leaves after 10th days from inoculation with two different strains of *Erwinia amylovora* and a mixture of them

Type of inoculum	Cultivar				
	Dr. Jules Guyot	**Beurré Hardy**	**Ranna Bolyarka**	**Beurré Giffard**	**Santa Maria**
Ea3325	19.1* b	19.1* b	30.8*b	26.1*a	27.9*a
Ea3345	25.7 b	19.3 b	32.8 b	29.6 a	30.0 a
Mixture	47.5 a	28.7 a	48.3 a	42.8 a	56.9 a
Type of inoculum	**Passe Crassane**	**Beurré Hardenpont**	**Alexander Lucas**	**Curé**	**Conference**
Ea3325	29.6*b	30.8* a	23.4* a	34.5* a	22.8 *b
Ea3345	31.4 b	34.0 a	27.5 a	39.5 a	26.7 ab
Mixture	53.3 a	49.8 a	37.7 a	73.7 a	37.8 a
Type of inoculum	**Beurré Bosc**	**Williams**	**Red Williams**	**Abate Fetel**	**Packham's Triumph**
Ea3325	47.7* a	31.5* a	49.8* a	38.3* a	43.5* a
Ea3345	50.5 a	39.8 ab	60.2 a	48.6 a	53.4 a
Mixture	67.7 a	51.7 b	61.9 a	53.9 a	64.7 a

*Different letters in the same column indicated significant difference ($p<0.05$) were compared by using Dunkan test.

Table 3. The average value of fire blight development (%) on the pear leaves after 15th days from inoculation with two different strains of *Erwinia amylovora* and a mixture of them

Type of inoculum	**Dr. Jules Guyot**	**Beurré Hardy**	**Ranna Bolyarka**	**Beurré Giffard**	**Santa Maria**
Ea3325	44.9* a	40.9* b	65.0* b	53.7* b	64.8* a
Ea3345	59.7 a	41.2 b	67.2 b	56.8 b	65.4 a
Mixture	75.4 a	64.9 a	100.0 a	88.4 a	100.0 a
Type of inoculum	**Passe Crassane**	**Beurré Hardenpont**	**Alexander Lucas**	**Curé**	**Conference**
Ea3325	69.9* b	61.64* a	36.9* b	75.6* a	43.3* a
Ea3345	70.5 b	66.1 a	38.2 b	82.9 a	49.5 a
Mixture	100.0 a	94.2 a	60.8 a	100.0 a	71.5 a
Type of inoculum	**Beurré Bosc**	**Williams**	**R. Williams**	**Abate Fetel**	**Packham's Triumph**
Ea3325	86.6* a	68.5* a	85.3* a	74.3* a	88.3* a
Ea3345	99.2 a	91.1 a	90.1 a	76.5 a	89.6 a
Mixture	100.0 a	100.0 a	100.0 a	78.8 a	100.0 a

*Different letters in the same row/column indicated significant difference ($p<0.05$)) were compared by using Dunkan test.

On the 15th day, five of tested cultivars had statistically significant differences – 'Beurré Hardy', 'Ranna Bolyarka', 'Beurré Giffard', 'Passe Crassan' and 'Alexander Lucas' (Table 3). The difference in the reaction of the ten cultivars: 'Dr. Jules Guyot', 'Santa Maria', 'Beurré Hardenpont', 'Curé', 'Conference', 'Beurré Bosc', 'Williams', 'Red Williams', 'Abate' 'Fetel' and 'Packham's Triumph' was statistically non-significant. As a more virulent from both single strain in 15th days after inoculation is again strain Ea3345. The averages rate for three studied years in all tested cultivar were reported the less damage after inoculation with Ea3325 but the differences non-significant. These results confirmed earlier findings (Sobiczewski et al., 2004; 2006) from experiments with application of single strain inoculum or inoculated either with a mixture of 4 strains or with each of them separately showed some
differences in strain virulence, which was reflected in the severity of fire blight caused by mixed inoculum. Other studies (Norelli et al., 1984) clearly indicate that differential interactions occur between apple cultivars and strains of *E. amylovora*. On the other hand,
(Paulin and Lespinasse, 1990) pointed that using the mixture of strains for inoculation of apple shoots did not always give a higher overall disease incidence and severity than
the most virulent strain alone.
On the 25th day after the inoculation, the length of the lesions on shoots was measured for each cultivar. The inoculated pear cultivars with the mixture of the two strains or with each of them separately showed some differences in strain virulence, which was reflected in the severity of fire blight (Table 4). Both single strains showed a lower virulence level. The percentage of infected shoots after inoculation with single strain Ea3325 was lower for 'Dr. Jules Guyot' and 'Beurré Giffard'. Similar results on these cultivars were observed and after the artificial inoculation with single strain Ea3345. After artificial inoculations with the mixture of both *E. amylovora* strains only 'Dr. Jules Guyot' reacted with low percentage of necrosis. In each artificial inoculation variants, the cultivar 'Beurré Bosc' reacted with the highest percentage of necrosis. After inoculation with single Ea3325 strain the cultivar 'Dr. Jules Guyot' showed severity of disease with statistically significant difference with 'Beurré Hardy', 'Ranna Bolyarka', 'Santa Maria', 'Passe Crassane', 'Beurré Hardenpont', 'Conference', 'Beurré Bosc', 'Red Williams', 'Abate Fetel' and 'Packham's Triumph'.

Table 4. Pears shoots susceptibility to fire blight after 25th days from inoculation with two different strains of *Erwinia amylovora* and a mixture of them

Pear shoots susceptibility to fire blight (%)			
Cultivar	Strain Ea3325	Strain Ea3345	Mixture of Ea3325 and Ea3345 strains
Dr. Jules Guyot	13.3*g	6.6* g	18.4*f
Beurré Hardy	30.3 b-e	20.8 c-g	32.9 d-f
Ranna Bolyarka	32,76 b-d	8.4 fg	30.1 d-f
Beurré Giffard	12.4* g	6.9* g	26.1 ef
Santa Maria	28.4 b-e	28.4 b-e	45.0 a-d
Passe Crassane	37.5 a-c	25.1 c-f	45.5 a-d
Beurré Hardenpont	39.7 a-c	31.6 b-d	45.3 a-d
Alexander Lucas	18.9 e-g	11.7 e-g	36.4 c-e
Curé	15.3 fg	18.4 d-g	34.8 c-e
Conference	27.9 c-e	28.3 b-e	45.8 a-d
Beurré Bosc	45.1 a	93.4 a	61.2 a
Williams	20.2 e-g	36.9 bc	43.2 b-d
Red Williams	36.9 a-d	33.3 b-d	50.5 a-c
Abate Fetel	25.4 d-f	21.1 c-g	39.8 c-e
Packham's Triumph	33.4 b-d	43.0 b	57.4 ab

*Different letters in the same row/column indicated significant difference ($p<0.05$)) were compared by using Dunkan test.

The most affected cultivar after inoculation with the single strain Ea3345 was 'Beurré Bosc'. Its difference in the severity of diseases was statistically significant compared to all other cultivars. When inoculated with the mixture of both strains the lowest level of infection showed again 'Dr. Jules Guyot'. The differences were statistically significant with 'Santa Maria', 'Passe Crassane', 'Beurré Hardenpont', 'Alexander Lucas', 'Curé', 'Conference', 'Beurré Bosc', 'Williams', 'Red Williams', 'Abate Fetel' and 'Packham's Triumph' (Table 4).
Compared to the outlined trend of leaves infection the results obtained of respect to the virulence of the spread strain of *E. amylovora* on the twenty-fifth day after inoculation, the results showed a different away.

The differences were reported in ten cultivars 'Beurré Hardy', 'Dr. Jules Guyot', 'Beurré Giffard', 'Ranna Bolyarka',' Passe Crassane', 'Beurré Hardenpont', 'Alexander Lucas', 'Red Williams'and 'Abate Fete'l to compared in both single strains, the result showed strain Ea3325 as a more virulence. From the results was conclude that after infection with a single or mixture of bacterial suspension, the susceptibility of individual plant organs is different in depends on the used strain, this comment was confirmed by other authors (Zwet and Beer, 1992).

According the reactions to inoculation with a single or mixture of strains the cultivars were classified in a different class of susceptibility. The distribution of each cultivar among susceptibility classes reflected their general susceptibility of fire blight (Table 5). In all variants of artificial inoculation, 'Dr. Jules Guyot' was classified as a very low susceptible. When inoculated with single strain Ea3325 ten of the cultivars had similar results. The necrosis observed on their shoots was 20-40% and they were also classified as low susceptible. The similar results were observed and when these cultivars were infected by Ea3345. For seven of the tested cultivars the observed necrosis was 40-60% and they were classified as moderately susceptible. As the most susceptible was evaluated 'Beurré Bosc' cultivar.

Similar results were reported by other authors (Toth et al., 2004) after artificial infection with the *E. amylovora*. The cultivar 'Dr. Jules Guillot', was classified as very sensitive and in our study as a very low susceptible. Greek researchers (Tsiantos and Psallidas, 2004) studied the cultivars 'Williams' and 'Santa Maria' were determine as highly susceptible after artificial infection with a mixture of six local strains of *E. amylovora*, in our study the cultivars were identified with the same degree of susceptibility after infection with a mixture of both the strains. The cultivars 'Williams', 'Santa Maria', 'Abate Fetel', 'Passe Crassane', Beurré Hardy were classified as susceptible (Demir and Gündogdu, 1992) in our result were determine as a moderately susceptible..

Table 5. Distribution of the pear cultivars in five susceptibility classes, in 25th days from inoculation.

Type of inoculum	Susceptibility classes				
	Very Low Susceptible*	**Low Susceptible**	**Moderately Susceptible**	**Susceptible**	**Very Susceptible**
	0-20%	**20-40%**	**40-60%**	**60-80%**	**80-100%**
Mixture of 2 strains	Dr. Jules Guyot	Beurré Hardy Beurré Giffard Ranna Bolyarka Alexander Lucas Curé Abate Fetel	Santa Maria Passe Crassane Beurré Hardenpont Conference Williams Red Williams Packham's Triumph	Beurré Bosc	-
Ea3325	Dr. Jules Guyot Beurré Giffard Alexander Lucas Curé	Beurré Hardy Ranna Bolyarka Santa Maria Passe Crassane Beurré Hardenpont Conference Williams Red Williams Abate Fetel Packham's Triumph	Beurré Bosc	-	-
Ea3345	Dr. Jules Guyot Ranna Bolyarka Beurré Giffard Alexander Lucas Curé	Beurré Hardy Santa Maria Passe Crassane Beurré Hardenpont Conference Williams Red Williams Abate Fetel	Packham's Triumph	-	Beurré Bosc

*Susceptibility classes (Le Lezec, 1997)

Figure 2. Infected shoot of Beurré Bosc cultivar

In 45th days after inoculation was researched to spread of necrosis distribution to shoots and stem was observed in all cultivars. Symptoms of the infection of rootstock were observed in each pear, the result showed five of the tested cultivars -'Red Williams', 'Abate Fetel', 'Curé' 'Alexander Lucas' and 'Dr. Jules Guyot' less to symptoms of infection. For the rest of pears cultivar the disease was spread to the rootstock.

Table 6. Fire blight infection of shoots, stem and rootstock after inoculation with mixture of two *Erwinia amylovora* strains

Cultivar	Shoots	Stem	Rootstock
Dr. Jules Guyot	+	+	-
Beurré Hardy	+	+	+
Ranna Bolyarka	+	+	+
Beurré Giffard	+	+	+
Santa Maria	+	+	+
Passe Crassane	+	+	+
Beurré Hardenpont	+	+	+
Alexander Lucas	+	+	-
Curé	+	+	-
Conference	+	+	+
Beurré Bosc	+	+	+
Williams	+	+	+
Red Williams	+	+	-
Abate Fetel	+	+	-
Packham's Triumph	+	+	+

Figure 3. Infection on a stem on 'Dr. Jules Guyot'

The fire blight has recognized as the most dangerous disease of pear orchards in the world. Timely diagnosis is very important for the detection of this disease. Visual assessment and reaction of the pear cultivar after the infection is important for limiting the diseases.
Under conditions of heavy infection during bloom, the yield can be considerably reduced or even destroyed. The need to remove fire blight infected parts from the plant forces the grower to prune away large portions of the tree, which has a long-term severe impact on the plant itself (Al-Dahmashi and Khlaif, 2004; Kuflik et al., 2008).

CONCLUSIONS

Using the mixture of two strains of *Erwinia amylovora* for inoculation of pear leaves always gives a higher percentage of disease incidence and severity than the single strain of bacteria.
The cultivar 'Dr. Jules Guyot' in all variants of artificial inoculation give the lowest percentage of necrosis on leaves and shoots. 'Beurré Bosc' reacted with the highest – 93.36% infected shoots.
The distribution of susceptibility classes depends on inoculation with single or mixed *Erwinia amylovora* strains.
On a 45th after inoculation development of bacteria was reported on shoots, stem and rootstock. Only five cultivars reacted with localization of the infection to the stem.

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