Scientific Papers. Series B, Horticulture. Vol. LXIV, No. 2, 2020
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

FIRE BLIGHT SUSCEPTIBILITY OF PEAR CULTIVARS GRAFTED ON OHF 333 ROOTSTOCK

Diyana ALEKSANDROVA[1], Petar CHAVDAROV[2], Marieta NESHEVA[1]

[1]Fruit Growing Institute, Ostromila 12 Str., Plovdiv, Bulgaria
[2]Institute for Plant Genetic Resources "K. Malkov" - Sadovo, Bulgaria

Corresponding autor email: aleksandrova_diyana@abv.bg

Abstract

The rootstock OHF 333 ('Old Home' × 'Farmingdale') is characterized by high yield efficiency and moderate resistance to fire blight (Erwinia amylovora) in Bulgarian environmental conditions. The aim of this study was to examine the reaction of 10 pear cultivars grafted on OHF 333 rootstock to Erwinia amylovora. Their susceptibility was determined after artificial inoculation of leaves in a greenhouse. Several vigorously growing shoots per tree were inoculated with two pathogenic local isolates of Erwinia amylovora by cutting the leaves. The two bacterial suspensions of Bulgarian strains were used single and mixed inoculum (3 x 10^8 cfu/ml). Based on the artificial inoculation the degree of attack of leaves and shoots was calculated. All cultivars were grouped into 5 categories - very low susceptible, low susceptible, moderately susceptible, susceptible and very susceptible. In twenty-five days after inoculation with isolate Ea3325, 6 of the studied cultivars reacted as very low susceptible showing less than 20% shoot necrosis. Two of the cultivars, artificially inoculated, with mixed inoculum showed terminal shoots necrosis over 60%.

Key words: *bacteria, Erwinia amylovora, pear rootstock, single strain, mixture of two strains.*

INTRODUCTION

During the recent 15 years, the Bulgarian pear production has collapsed. In 1980 Bulgaria has produced 96 thousand tons pears and was on 18th place in the world. In 2005 our pear fruit production reached its lowest level - 750 tons, and today Bulgarian pear production is only 1,974 tons. The areas occupied by pear orchards are much reduced and the fruit production is limited. The main reasons for that are the use of old and inefficient technologies for growing them and low efficiency of the applied plant protection products against the major pest *Cacopsilla pyri* L. and bad health condition of the orchards (Gandev et al., 2014). The most economically important disease for the pears is fire blight. This disease is caused by the bacterium *Erwinia amylovora*. Fire blight is apparently indigenous to North America and it was first noticed in the late 18th century in New York state. Later during 1921 and 1970 fire blight was included in the list of the plant diseases in Central and South America, Europe, Oceania and Africa. In Bulgaria, it was discovered for the first time 17 years ago (Bobev et al., 1999).

Erwinia amylovora is a pathogen of more than 130 plant species belonging to 40 genera, mainly from the family Rosaceae (Van der Zwet and Keil, 1979). The disease occurs on pear (*Pyrus* spp.), apple *(Malus* spp.) and quince (*Cydonia* spp.) trees as well as on hawthorn (*Crataegus* spp.), sorb (*Sorbus* spp.), cotoneaster (*Cotoneaster* spp.). It is generally believed that *E. amylovora* is a homogeneous species and does not show pathogenic specialization. This means that each isolate of the pathogen is potentially able to infect any of the known host plants (Momol and Aldwinckle 2000). However, in an artificial inoculation experiment, De Ley et al. (1983) have shown that different isolates may exhibit some variations in host range. Isolates from plants of the genus *Rubus* are particularly noteworthy because they are incapable of infecting apple and pear trees (Asselin et al., 2008; De Ley et al., 1983; Evans, 1995; Ries and Otterbacher, 1977; Starr et al., 1951).

Biodiversity of bacterial plant pathogens is a result of their variability. This variability is caused by both environmental conditions leading to not fully hereditary changes in phenotype, and variation in the genetic material

(Agrios,1988; Puławska and Sobiczewski, 2012).

The pear breeding for resistance to fire blight is a priority in obtaining new cultivars (Gunen et al., 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 cultivar performance on different rootstocks particularly on OH x F clones, including effects on the size, vigour, compatibility and precocity, have been studied in the USA and Canada (Westwood et al., 1976; Lombard and Westwood, 1976; Larsen and Fritts, 1984; Kappel and Quamme, 1988)

The pear rootstock OH × F (*P. communis* L.), bred in the USA, shows 10% more vigorous growth compared to BA29. The rootstock is characterized by high yield efficiency and moderate resistance to fire blight (*Erwinia amylovora*) (Dzhuvinov et al., 2008).

The aim of the following study was to examine the reaction of pear cultivars when grafted on OHF 333 to *Erwinia amylovora* and to compare the reaction of the cultivars when inoculated with different strains.

MATERIALS AND METHODS

This study was conducted in 2014-2017 at the Fruit Growing Institute - Plovdiv. The cultivars 'Beurré Bosc', 'Williams', 'Red Williams', 'Abate Fetel', 'Packham's Triumph', 'Starkrimson', 'Highland', 'Carmen', 'Tosca', 'Etrusca' were grafted on the pear rootstock Old Home x Fermingdale (OHF333) and grown in an experimental greenhouse as potted one-year-old trees.

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

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

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

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

To determine the disease attack index of leaves, the results obtained by the scale were transformed by the Mc Kiney formula.

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 the infected part (cm)= Susceptibility of shoots/Total shoots length (cm) x 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, fire blight symptoms development to stem and rootstock was observed. To prove the damage was caused by *Erwinia amylovora* a reisolation of infected tissue was done.

RESULTS AND DISCUSSIONS

OHF 333 is a pear rootstock tolerant to fire blight, drought, chloroses and nematode (Erdal & NazlI, 2019). In Bulgarian environmental conditions it is also characterized by high yield efficiency and moderate resistance to fire blight. The experiments with artificial inoculation of grafted on OHF 333 cultivars give us information about their susceptibility to

fire blight when a tolerant rootstock is used for producing the planting material.

Ten days after artificial inoculation with Ea 3325 strain the lowest disease severity index was recorded for the cultivars 'Williams' and 'Red Williams' (Table 1). The differences in the disease severity index with 'Carmen', 'Tosca', 'Abate Fetel' and 'Packham's Triumph' were statistically significant. The highes pecentage of infected with the single Ea3325 strain leaves was recorded for 'Carmen' cultivar.

Fifteen days after the artificial inoculation the calculated indexes for the cultivars 'Williams' and 'Red Williams' were still the lowest.

Table 1. Pear leaves infection to fire blight after inoculation with a single *Erwinia amylovora* strain or a mixture of 2 strains

Cultivar/Day	Strain Ea3325		Strain Ea3345		Mixture of both strains	
	10	15	10	15	10	15
Beurré Bosc	15.77 bc	50.94 abc	48.26 ab	83.57 a	49.93 a	100.00 a
Williams	7.50 c	14.33 c	31.17 bc	71.00 a	25.00 a	62.67 ab
Red Williams	8.67 c	12.92 c	34.33 bc	55.00 ab	32.67 a	58.67 ab
Abate Fetel	34.89 ab	51.78 ab	27.84 bc	54.71 ab	45.67 a	66.97 ab
Packham's Triumph	29.18 ab	59.37 a	34.17 bc	85.00 a	43.16 a	92.37 ab
Starkrimson	21.02 abc	65.00 a	23.56 c	56.40 ab	20.51 a	73.33 ab
Highland	14.93 bc	23.50 bc	13.82 c	28.96 b	20.66 a	54.60 ab
Carmen	37.43 a	44.13 abc	20.50 c	33.71 b	20.00 a	36.77 b
Tosca	29.37 ab	41.32 abc	61.73 a	89.80 a	35.56 a	73.33 ab
Etrusca	17.87 abc	38.93 abc	22.65 c	55.23 ab	25.57 a	82.50 ab

After artificial inoculation with Ea3345 strain the lowest percentage infected leaves was recorded for the cultivars 'Highland' and 'Carmen'. This was observed and 15 days after the inoculation. When infected with Ea3345 the cultivars 'Williams' and 'Red Williams' reacted with a higher percentage of necrosis and 15 das after the inoculation 'Williams' is even one of the most affected cultivars.

When infected with a mixture of both *Erwinia amylovora* strains non-significant differences between the ractions of all pear cultivars were observed on the 10th day. Fifteen days after the artificial inoculation the differences were statistically significant between the least and the most affected cultivars - resp. 'Carmen' and 'Beurré Bosc'.

Twenty-five days after the artificial inoculation was recorded the spread of the disease from the cultivars leaves to the shoots. The result shown in Table 2 express the average length of the shoots lessions calculated for the 3 years of experiments. When 'Red Williams' was inoculated with Ea3325 the infection was localized in the leaves.

Figure 1. Infected leaves of 'Packham's Triumph' cultivar after inoculation with mixture of strains

Table 2. Length of the observed lesions on shoots after inoculation with single or mixture of *Erwinia amylovora* strains (%)

Cultivar	Ea3325	Ea3345	Mixture of both strains
Beurré Bosc	63.08 a	37.27 abc	86.4 a
Williams	6.33 ef	23.01 cd	23.66 c
Red Williams	0 f	12.1 de	48.24 b
Abate Fetel	22.19 bcd	5 e	13.12 c
Packham's Triumph	34.52 b	48.55 a	72.86 a
Starkrimson	19.74 cde	14.53 de	47.74 b
Highland	8.48 def	15.68 de	29.57 c
Carmen	6.95 ef	4.4 e	28.61 c
Tosca	28.05 bc	43.91 ab	77.65 a
Etrusca	16.45 cde	28.84 bcd	53.66 b

The result for this cultivar's reaction were statistically significant with 'Beurré Bosc', 'Abate Fetel', 'Packham's Triumph', 'Starkrimson', 'Tosca' and 'Etrusca'. The longest lesions were measured on the 'Beurré Bosc' cultivar - 63.08% of the total length of the shoot.
For two of the tested cultivars ('Carmen' and 'Abate Fetel') inoculated with Ea3345 strain the infection was in the lowest percentage. The highest percentage of shoots with lesions was observed for 'Packham's Triumph' fllowed by 'Tosca' and 'Beurré Bosc'.

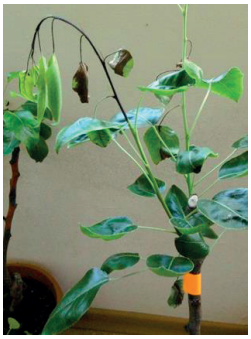

Figure 2. Infected shoot of 'Red Williams' cultivar inoculation with sigle strain Ea3325

When for the inoculation was used mixure of both *Erwinia amylovora* strains were observed distinct differences between the cultivars. The most damaged cultivars were 'Beurré Bosc', 'Tosca' and 'Packham's Triumph'. The cultivars with the lowest reaction to the mixture were - 'Abate Fetel', 'Williams', 'Carmen' and 'Highland'. 'Starkrimson', 'Red Williams' and 'Etrusca' had a medium value of the length of infected part of the shoots.
The results described, show that a variation in the reaction of each pear cultivar was observed when the inoculation was done using the separate strains. Some of them are susceptible to Ea3325. For example 'Williams' and 'Red Williams'. When both strains cause mixed infection they are more virulent. For screening genotype's susceptibility to fire blight some authors consider using inoculum containing a mixture of several highly virulent strains originating from various hosts as more reliable and more reflective of the situation occurring in nature where cross infections are common (Lespinasse and Aldwinckle, 2000; Norelli et al., 1987, 2003).
The five grade scale of Le Lezec et al. (1997) gave us the opportunity to classify the tested cultivars according to their terminal shoots susceptibility level. According to their susceptibility to Ea3325 strain, 6 of the cultivars were classified as very low susceptible, 3 - as low susceptible and 'Beurré Bosc' was the only cultivar showing moderate susceptibility. The five cultivars 'Red Williams', 'Abate Fetel', 'Starkrimson', 'Highland' and 'Carmen' were classified as very low susceptible to Ea3345 strain. Moderately susceptible to this strain were 'Tosca' and 'Packham's Triumph'. In a combination as mixture both strains are more aggressive and the susceptibility of the cultivars increased. The moderately and low susceptible to the single strains cultivars were classified as susceptible to the mixture - 'Beurré Bosc', 'Packham's Triumph' and 'Tosca'. The cultivars with best results were 'Williams', 'Abate Fetel', 'Highland' and 'Carmen'. Their susceptibility was very low to low when inoculated with the single strains or the mixture (Table 3).

Table 3. Susceptibility classes according to calculated susceptibility level of the terminal shoots

Cultivar	Susceptibility class Ea3325	Susceptibility class Ea3345	Susceptibility class Mix
Beurré Bosc	Moderately Susceptible	Low Susceptible	Susceptible
Williams	Very Low Susceptible	Low Susceptible	Low Susceptible
Red Williams	Very Low Susceptible	Very Low Susceptible	Moderately Susceptible
Abate Fetel	Low Susceptible	Very Low Susceptible	Very Low Susceptible
Packham's Triumph	Low Susceptible	Moderately Susceptible	Susceptible
Starkrimson	Very Low Susceptible	Very Low Susceptible	Moderately Susceptible
Highland	Very Low Susceptible	Very Low Susceptible	Low Susceptible
Carmen	Very Low Susceptible	Very Low Susceptible	Low Susceptible
Tosca	Low Susceptible	Moderately Susceptible	Susceptible
Etrusca	Very Low Susceptible	Low Susceptible	Moderately Susceptible

Fourty five days after the artificial inoculation the bacteria development was monitored from shoot to rootstock (Table 4).

Necrosis on shoots was observed for each of the tested cultivars. For 'Beurré Bosc' and 'Tosca' necrosis was detected on the stem. For all of the other pear cultivars, the bacteria has localized in the shoots. No symptoms were observed on the rootstock.

Table 4. Bacteria development in shoots, stem and rootstock

Cultivar	Shoots	Stem	Rootstock
Beurré Bosc	+	+	-
Williams	+	-	-
Red Williams	+	-	-
Abate Fetel	+	-	-
Packham's Triumph	+	-	-
Starkrimson	+	-	-
Highland	+	-	-
Carmen	+	-	-
Tosca	+	+	-
Etrusca	+	-	-

CONCLUSIONS

The cultivars with best results were 'Carmen', 'Williams', 'Abate Fetel' and 'Highland'. Their susceptibility to the bacterial disease was low when inoculated with the single strains and the mixture of both. These cultivars could be recommended for establishing production orachards.

After this experiment, 'Beurré Bosc' cultivar grafted on OHF 333 could be classified as the most susceptible among the 10 tested.

The mixture of *Erwinia amylovora* strains is more virulent than the single strains and caused the highest percentage of damages of all studied cultivars.

REFERENCES

Agrios, G. N. (1988). Introduction to plant pathology. *Plant Pathology, 3rd ed. Academic Press, San Diego, CA*, 3-40.

Asselin, J. E., Yip, K. N., & Beer, S. V. (2007, August). eop1 differs in strains of *Erwinia amylovora* that differ in host specificity. In *XI International Workshop on Fire Blight, 793* (pp. 213-214).

Bobev, S., Garbeva, P., Crepel, C., Maes, M. and Hauben, L. (1999). Fire Blight In Bulgaria – Characteristics Of *E. amylovora* Isolates. *Acta Hortic., 489, 121-126*

De Ley, J., Vantomme, R., Swings, J., Kersters, K., Rijckaert, C., Goor, M., ... & Geenen, J. (1983, September). Research report on *Erwinia amylovora*. In *III International Workshop on Fire Blight, 151* (pp. 241-248).

Dzhuvinov VT, Nacheva LR, Gercheva PS. (2008). Efficient shoot regeneration system of pear rootstock OHF 333 (*Pyrus communis* L.) leaves. I International Symposium on Biotechnology of Fruit Species: *Biotechfruit*, 839

Erdal, İ., & Nazli, İ. (2019). Influence of Rootstock and Variety on Leaf Nutrient Concentration of Pear Grown on a Nutrient-Sufficient Soil. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 22, 141-148.

Evans, I. R. (1995, August). Fire blight of raspberries in Alberta. In *VII International Workshop on Fire Blight*, *411* (pp. 69-72).

Gandev S., Arnaoudov B., Milusheva S., Manolova V., Domozetov D., Gercheva P., Natcheva L., Popov S., Kornov C. (2014). Status, problems and prospects of production of pears in Bulgaria.

Günen, Y., Yağmur, B., Misirli, A., & Gülcan, R. (2003). Ateş Yanıklığına Duyarlı Ve Dayanıklı Bazı Armut Çeşitlerinin Bitki Besin Maddesi İçeriklerinin Belirlenmesi. *Ege Üniversitesi Ziraat Fakültesi Dergisi*, *40(3)*.

Kappel, F., & Quamme, H. A. (1988). Growth and yield of pear cultivars on several rootstocks. *Canadian Journal of Plant Science*, *68*(4), 1177-1183.

Larsen, F. E., & Fritts Jr, R. (1984). Rootstock influence (1965–1980) on yield, yield efficiency and tree size of 'Bartlett' and 'd'Anjou'pear. *Scientia horticulturae*, *24*(3-4), 271-278.

Lombard, P. B., & Westwood, M. N. (1976). Performance of six pear cultivars on clonal Old Home, double rooted, and seedling rootstocks [Tolerance to pear decline, a mycoplasma-induced disease]. *Journal American Society for Horticultural Science*.

Le Lezec M., Lecomte P., Laurens F. Michelesi J.C. (1997). Sensibilite varietale au feu bacterien. L' Arbor. *Fruitiere*, 503: 57-62.

Momol, M. T., & Aldwinckle, H. S. (2000). Genetic diversity and host range *of Erwinia amylovora*. Fire Blight. The Disease and its Causative Agent, *Erwinia amylovora* ed. Vanneste, JL, 55-72.

Puławska, J., and Sobiczewski P. (2012) "Phenotypic and genetic diversity of *Erwinia amylovora*: the causal agent of fire blight." Trees 26.1: 3-12.

Ries, S. M., SM, R., & AG, O. (1977). Occurence of fire blight on thornless blackberry in Illinois.

Starr, M. P., Cardona, C., & Folsom, D. (1951). Bacterial fire blight of raspberry. Phytopathology, 41(10).

Sestras, R., Sestras, A., Barbos, A., & Harsan, E. (2008). New cultivars obtained at Cluj-Napoca, Romania: 'Saruman'and 'Sauron' (apple); 'Arvena'(pear). Modern Variety Breeding for Present and Future Needs. Prohens, J. & ML Badenes (eds.). Ed. Univ. Politécnica Valencia, Spain pp. 722-723. *Proc. 18th Eucarpia Gen. Congr*, 9-12.

Thompson SS, Janick J, Williams EB (1975). Evaluation of resistance to fire blight of pear, p. 610. Janick J, Moore JN (Eds.). Purdue University Press, West Lafayette, Indiana

Westwood, M. N., Lombard, P. B., & Bjorstand, H. O. (1976). Performance of 'Bartlett' pear on standard and Old Home X Farmingdale clonal rootstocks [Breeding for resistance to bacterial fire blight (*Erwinia amylovora*) and mycoplasma related decline]. *Journal-American Society for Horticultural Science (USA)*.

Van der Zwet, T., & Bell, R. L. (1990). Fire blight susceptibility in *Pyrus* germplasm from Eastern Europe. HortScience, 25(5), 566-568.

Van der Zwet T, Keil H. 1979. Fire Blight: a bacterial disease of rosaceous plants. Agriculture Handbook 510. United States Department of Agriculture *Journal of mountain agriculture on the Balkans,* 16(6): 1534 -1552

Zeller, W. and B. Wolf, 1996. Studies on Biological Control of Fire Blight. *Acta Hortic.*, *411: 341-345*.