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First results of five pear cultivars after artificial inoculation with *Erwinia amylovora* (Burrill)

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ABSTRACT

Fire blight, caused by the bacterium *Erwinia amylovora* (Burrill) is a very serious and most perplexing disease of pome fruit. It is most destructive to dessert pear and less to quince and apples. Fire blight is apparently indigenous to North America and it was first noticed in the late of 18th century in New York state. In our country it was discovered for first time in 1989 on quince and pear trees in the region of Plovdiv. Several years later, this bacterial disease has spread in different regions of the country and causes significant damage with blighted terminal shoots mostly on pome fruit and some ornamental species. The investigation were carried out at the Fruit Growing Institute in Plovdiv during 2015-2016. Pear cultivars were grafted on BA29 quince rootstock and on OHF 333 (Old Home x Farmingdale) pear rootstock.

The best results after artificial inoculation with the bacteria were received for cultivars Williams and Red Williams on OHF 333 rootstock, where 75% of the shoots were reported with grade 0 of infection on the 10th day after inoculation. The most sensitive to the bacteria were proved the plants of Red Williams on BA29 rootstock-84.62% of the shoots were with class 3 level of inoculation. On the OHF 333 rootstock the cultivars Beurre Bosc and Abate Fetel reacted with third degree of infection, i. e. respectively with 14.29 and 11.11 %. These first results indicate that the plants grafted on OHF 333 rootstock have a higher resistance to the bacteria *Erwinia amylovora*. Selection of resistant or tolerant cultivars and rootstocks is the main aim of our breeding programs.

Key words: pear, cultivars, inoculation, *Erwinia amylovora*

Introduction

The pear is one of the major fruit tree crop and occupies the second place in the world fruit production after apples. Production of pears in the world is characterized by sustainable rates of increasing. For 30 years it has increased almost three times and now according to FAOSTAT (2014) almost 24 million tons are produced in the world. During the recent 15 years the Bulgarian pear production collapsed. In 1980 Bulgaria produced 96 thousand tons and was on 18 places in the world. In 2005 our pear fruit production reached its lowest level - 750 tons, and today Bulgarian pear production is 1974 tons only. The areas occupied by pear orchards are much reduced and the fruit production is limited. The main reasons for that are the using of old and inefficient technologies for growing them and low efficiency of the applied plant protection products against major pest *Cacopsilla pyri* L. and bad health condition of the orchards (Gandev et al., 2014). The most economically important disease for 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 of 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 first time 17 years ago (Bobev & Deckers, 1999). During the period 2003-2007 the disease is enlarged area all over the country and causes significant damages to many pear, quince and apple orchards.

This bacterial disease can destroy apple and pear orchards for one season. Over the last few years, fire blight has caused serious loses around the world. The economic importance of this disease is likely to increase because it is still spreading in new apple and pear areas (Vanneste, 2000). Fire blight is devastating for apple and pear orchards and the only agent for control is antibiotic treatments with streptomycin. After streptomycin registration in the United States, in 1950, it was used extensively for controlling the disease on pears and apples. In 1971 the control of fire blight with the antibiotic sprays failed in Sacramento Valley, California because the prevalent strains of the bacteria were resistant (Schroth & Thompson, 1981). In 1991 streptomycin - resistant mutants were isolated from apple orchard in Michigan state (Chiou & Jones, 1991). Due to the occurrence of resistant strains, in Europe antibiotic treatments are not allowed. The bacterial diseases cannot be controlled by plant protection with other

chemicals. Another effective way of limiting the spread of this dangerous disease is breeding of tolerant or resistant cultivars and rootstocks. The aim of our study is to select tolerant or resistant cultivars and rootstocks suitable for our country. In the world are conducted different experiments. In one of them Van der Zwet and Keil (1979) classified about 40% of the 287 known varieties of pears as sensitive and another 40% as moderately susceptible or resistant and 11% were classified as resistant to highly resistant. In this group were included the cultivars Fouqueray, Old Home, Ore, Professeur Molon, Sudduth. Nine percent had variable resistance - Douglas, Garber, Le Conte, Lincoln, Winter Nelis. The rootstocks Old Home (OH), OH x Farmingdale (OHF) *P. calleryana*, *P. betulaefolia* were classified as resistant (Van der Zwet & Keil, 1979; Van der Zwet & Beer, 1991). Simard and Michelesi (2002) in 1966 in France started a breeding program for control the growth of grafted cultivar using the rootstock. From open pollination of Fieudiere 3 were obtained seedlings as rootstocks compatible with Williams and Passe Crassane. The productive capacity and the susceptibility to fire blight of Kirchensaller-Mostrebime, Beurre Hardy and Old Home were studied. One of this genotypes (OH 11), obtained out of open pollination of OH, had very good results. In 1997 it is now known as Pyriam rootstock (*Pyrus* resistant to *Erwinia amylovora*). When tested in different regions of France the cultivar Williams grafted on OH 11 had good and constant yield. The grafted cultivars on BA29 rootstock they died after a few years.

The resistant or tolerant genotypes may not have good qualities of the fruits but they can be used as donors of resistance in the breeding programs.

Materials and Methods

The Five pear cultivars have been used as plant material: Bosc's, Williams, Red Williams, Packham's Triumph and Abbat Fettel. The cultivars were grafted on BA29 quince and on OHF 333 pear rootstocks.

The grafted trees on two rootstock were planted in containers with 5 trees per cultivar/rootstock combination.

The trees have grown under conditions to achieve the growing of shoots about 20 cm length. The artificial inoculation were performed in laboratory under controlled conditions by maintaining an optimal conditions for bacteria growth. The bacterium was cultured in an incubator for 48 hours at 26°C on a nutrient medium King's B (King et al., 1954). After the bacteria were washed from the agar surface and the suspension is brought to turbidity 3x10⁸ Cl/ml. The inoculation was performed with scissors dunk in the bacterial suspension, which was cut 1/3 of the leaf length. The object of inoculation was the last three well developed leaves on the top. The inoculated plants were placed at 25 °C

and 80-90% relative humidity. First reporting on the degree of sensitivity was made 10 days after inoculation.

Sensitivity is reported in 5 point scale (Zeller & Wolf, 1996) to determine the degree of sensitivity in which:

Class 0 - infected plants are no visible symptoms of infection

Class 1 - blackening only in place of the cut

Class 2 - visible symptoms of the place of cut and stem

Class 3 - browning of leaf and petiole

Class 4 - from symptoms necrosis on apices of the plant

Class 5 - blackening the whole plant

The plants are infected with isolate Ea 3325 - isolated from apple tree on 16.05.2013 in Petrich town, Sout-West Bulgaria.

Results

The The strain Ea 3325 of the bacteria was used for artificial inoculation. The plants were put under optimal conditions for multiplication of the bacteria. Ten days after that degree of attack was monitored.

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The best results were obtained from cultivars Bosc's (66.67%), Williams (40%) and Pakhams Triumph (47.06%). Reactions with level 1 attack or blackening place the cut, 10 days after inoculation.

With the high level 4 of sensitivity were cultivars Bosc's (33.33%), blackening the entire peak of the infected plant.

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).

In these cultivar/rootstock combination with the lowest 0 class of sensitivity were cultivars: Williams -75% and Red Williams- 75%. Ten days after the artificial inoculation of plants, there were no visible symptoms of infection.

At the high class 3 of sensitivity reacted varieties Bosc's- 14.29% and Abate Fettel with 11.11% necrosis handale of infected leaf.

Conclusions

The tested cultivars and rootstocks are widely cultivated in the world. Two of the varieties on the OHF 333 rootstock 10 days after artificial inoculation showed no visible damages from the disease. These cultivar/rootstock combinations were observed localization of the infection at the incision site.

RESEARCH ARTICLE

Table 1. Sensitivity of 5 cultivars on BA29 rootstock, 10 days after artificial inoculation.

Cultivar	Degree of attack (%)					
	0	1	2	3	4	5
Beurre Bosc	0	66.67	0	0	33.33	0
Williams	0	40	0	60	0	0
Red Williams	0	0	15.38	84.62	0	0
Packhams's Triumph	0	47.06	29.41	23.53	0	0
Abate Fetel	0	0	70	30	0	0

Table 2. Sensitivity of 5 cultivars on OHF333 rootstock, 10 days after artificial inoculation.

Cultivar	Degree of attack (%)					
	0	1	2	3	4	5
Beurre Bosc	0	0	85.71	14.29	0	0
Williams	75	25	0	0	0	0
Red Williams	75	25	0	0	0	0
Packhams's Triumph	0	75	25	0	0	0
Abate Fetel	0	16.67	72.22	11.11	0	0

So far the study shows that the grafted on OHF 333 cultivars are less sensitive than those grafted on BA29 rootstock. The cultivar/rootstock combinations with OHF 333 are infected, but the disease is localized to shoots only and not infected the fruiting wood. The cultivars grafted on OHF 333 rootstock are with low sensitivity to *Erwinia amylovora* (Burrill) and will be good as rootstock to be included in future breeding programs for improving its resistance or tolerance to fire blight disease.

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