AGRICULTURAL ACADEMY - SOFIA
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"Reaction of pear cultivars, caused by *Erwinia amylovora* (Burril). Methods and managements of control. "

EXTENDED ABSTRACT

Of PhD Thesis for awarding educational and scientific degree "Doctor"

Professional field 6.2. Plant protection

Scientific specialty: "Phytopathology"

Supervisor: associate professor Petar Chavdarov, PhD

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The research was conducted during the period 2015-2019 in the laboratory of phytopathology and in the experimental orchard of the Fruit Growing Institute – Plovdiv, Bulgaria. The subject of the study is 25 cultivars of pears, and 2 strains of the bacteria *Erwinia amylovora*.

The dissertation is written on 129 pages, contains 20 tables, 13 figures and 21 photos. The cited literature includes 166 sources, of which 3 are in Cyrillic and 163 are in Latin.

The defense of the dissertation will be consist on ..... ,at.....o'clock... in the conference hall in Fruit Growing Institute. The Specialized Scientific Jury was appointed by order № РД 05-277/17.09.2020.

1. INTRODUCTION

Fire blight is among the most dangerous diseases registered in Bulgaria and worldwide. It is caused by the bacterium *Erwinia amylovora*. The pathogen infects leaves, flowers, fruits and branches, the stronger multiplication of bacteria leads to complete drying of the trees, reduced yields and reduces the quality of production.

The pathogen has been detect in Bulgaria for more than 30 years. The bacterium has been found in almost all areas of the country where seed fruit crops are grown. The hosts of fire blight is wide and includes over 130 species, mainly from the Rosaceae family, among the most susceptible species is the pear. To conduct a successful management it is necessary to combinatione of measures, one of the most effective methods to reduce the disease is through the selection of tolerant or resistant cultivars.

2. OBJECTIVE AND TASKS OF THE RESEARCH

The aim of the dissertation is to study the reaction of pear cultivars grafted on two rootstocks to the bacterial disease fire blight.

For achieving the objective, the following tasks were formulated:

1.To determine of susceptibility of pear cultivars grafted on quince rootstock BA29 or pear rootstock OHF333 under field conditions.
2.To determine of susceptibility of pear cultivars grafted on quince rootstock BA29 or pear rootstock OHF333 after artificial inoculation.

- To determine the degree of leaf infestation, in cultivars grafted on quince rootstock BA29 and pear rootstock OHF333
- To determine the degree of attack on shoots in cultivars grafted on quince rootstock BA29 and pear rootstock OHF333.
- To determine the location of the damage from the bacterium *Erwinia amylovora*.
- Study of the reaction of pear cultivars in two different strains of the bacterium and a combination of them.
- Monitoring the influence of the rootstock on the degree of susceptibility in cultivars combinations.

3.To determine of the effectiveness of systemic and contact fungicides in *in vitro* for the prevention and control of *Erwinia amylovora.*
4. The study the effectiveness of the biostimulator Regoplant.

3. MATERIALS AND METHODS

The research was conducted in the period 2015-2019 in the laboratory of phytopathology and in the experimental bases of the Institute of Fruit Growing - Plovdiv. The subject of the study are 25 pear cultivars grafted on two rootstocks, as well as 5 of the varieties grown on their own roots.

3.1. Susceptibility of pear cultivars in a field conditions

To establish the susceptibility of the cultivars to naturally occurring infection, in the spring of 2015 a pear orchard was established. The trees were planted on experimental orchards of the Asenovgrad at the Fruit Growing Institute – Plovdiv. The experience includes 25 pear cultivars grafted on a two rootstock, as well as 5 of the varieties grown on their own root. Five trees have been planted in a repetition of each variety.

On the BA 29 (*Cydonia oblonga*) rootstock were grafted 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'

On the OHF333 (*Pyrus communis;* 'Old Home x Fermingdale') rootstock were grafted cultivars: 'Beurré Bosc', 'Williams', 'Red Williams', 'Abate Fetel', 'Packham's Triumph', 'Starkrimson', 'Highland', 'Carmen', 'Tosca', 'Etrusca'

Own roots plants: 'Beurré Bosc', 'Williams', 'Red Williams', 'Abate Fetel', 'Packham's Triumph'.

During the experimental years, were performed the usual care for the culture and age of the trees, they were not used a bactericidal and fungicidal sprays due to immunity research.

The emergence and development of fire blightin a field under in the period 2015-2019 were evaluated under Van der Zwet (1970) USDA (United States Department of Agriculture), a scale on which three indicators are reported:

- Number of infected shoots and branch;
- Age of wood reached by necrosis;
- Percentage of infection of each tree.

3.2. Susceptibility of pear cultivars after artificial inoculation.

3.2.1 Degree of attack on the leaves (%).

The degree of attack on the leaves was presented in section 4.1. Cultivars was established under greenhouse conditions. To do this, the trees were planted in 3-liter plastic pots, 5 pots in repetition, in the spring of 2015. After the growth of one-year-old shoots about 20 cm with

actively growing tips, the pots were moved under controlled conditions. They were provided with a constant temperature of 25°C and 80-90% relative humidity.

To create an artificial infection, strains were used:

- Strain Ea3325 – isolated from apple on 16.05.2013 in Petrich
- Strain Ea3345 – isolated from pear on 27.06.2013 in Botevgrad.

The artificial inoculation about immunity study was performed with each of the two strains and a combination of them, for each cultivar combination. The strains were cultivated for 48 hours at 26 °C, on a nutrient medium King's B. The bacterial was washed to agar surface with distilled and autoclaved water, after which the bacterial suspension is brought to a turbidity of $3x10^8$ CFU/ml.

Artificial infection was performed with scissors, which is immersed in the bacterial suspension, cuts 1/3 of the surface of the leaf. The subjects of artificial inoculation were the last three well-developed leaves. Infected plants were placed at a temperature of 25°C and 80-90% relative humidity, conditions suitable for the occurrence of infection.

To establish the infection on the leaves, were monitored two reports in dynamics for the infection occurring, on the tenth and fifteenth day respectively after the artificial inoculation of the plants.

Susceptibility is recorded on the 5-stage Zeller et al.1990 scale to determine the degree of susceptibility at which:

Class 0 – infected plants no visible symptoms of infection;
Class 1 – visible blackening only on the site of the cut;
Class 2 – symptoms at the site of the cut and nerve;
Class 3 – blackening of leaves and leaf handle;
Class 4 – symptoms of necrosis on the peak parts of the plant;
Class 5 – covering the vegetation peak.

The results of the fifteenth day of infection were reported on the susceptibility scale cited above. For the calculation of the index of the disease, the data on the rates of the attack recorded on the tenth and fifteenth day after artificial inoculation in all varieties were used. The result has been transformed by MC Kiney's formula.

The results were presented for the three consecutive years, calculating the average for the attack by leaf for all years of study.

3.2.2 Degree of attack on shoots (%)

To monitor the dynamics of the development of the disease after the passage of bacteria into the shoots, a reading of the twenty-fifth day after artificial infection was performed. The necrosis of the one-year growth for each variety combination and the accounting for direct, visible losses after inoculation were calculated using the formula:

$X = a \cdot 100 / A$:

x – percentage of infection
a – infected part of the shoots (cm)
A – length of the shoots (cm)

The rate of infected shoots is determined according to the percentage of infected part of the shoots and on the basis of this indicator the cultivars were divided into the following groups according to the five-stage scale Le Lezec et al. (1997):

Degree of Susceptibility (%)

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).

3.2.3 Localization of diseases on shoots, stem and rootstock.

The important stage of disease tracking is the penetration of the bacteria from one-year-old shoots to older wood and reaching the infection to the rootstock. This monitored was performed on the forty-fifth day of inoculation for each cultivar of the study combinations.

3.3 Influence of the rootstock on the degree of susceptibility of the cultivar after inoculation.

Infection and reporting of results were performed as in the described sections 3.2.1 Degree of attack on leaves (%); 3.2.2 Degree of attack on shoots (%) and 3.2.3 Localization of diseases on shoots, stem and rootstock.

In order to confirm the bacterium nature of the observed symptoms of all cultivar, a sample was taken at the border of infect and healthy tissue. The bacterium was isolated on the nutrient medium King's B. After 24h incubation at 23ºC, the bacteria were washed on agar surface and applied for biological tests, by manifestation of a hypersensitive reaction on tobacco leaves and the development of symptoms on immature pear fruit.

3.4 Testing of chemical and biological metods to control of *Erwinia amylovora*

3.4.1 Chemicals control of the *Erwinia amylovora in vitro*

The study included five fungicides, which, according to literary data, have a bactericidal effect as follows:

- Bordeaux mix 20 TH (200 g/kg coppercalcium sulphate) at a concentration of 0,6 % solution.
- Funguran 50 NG (87,7% copper hydrooxide) at a concentration of 0.15% solution.
- Aliette Flash (800 g/kg fozetil – aluminium), at a concentration of 0.30% solution.
- Alfil Duplo (350g/kg mancoceb, 350g/kg fozetil – aluminium), at a concentration of 0.35% of the preparation.
- Ditan M 45 (800 g/kg mancoceb) at a concentration of 0.3% solution.

The test was performed at the laboratory conditions and for this purpose 5 mm of wells (4 in Petri dishes) were made in a 90 mm petri dish containing the King's B medium. The bacterial suspension of the two strains was spread, a combination of them (24h culture), and then 100µl of the working solution was poured into each well. Each variant was performed in 5 repetitions, and Petri dishes with the addition of water were used for the control. The incubation of bacteria occurred at 20°C for 48 hours. The available inhibition zones were measured twice the diameter of a cross.

3.4.2 Biological agents to control of *Erwinia amylovora.*

The study of biological control possibilities was conducted by applying the biostimulator Regoplant. The experiment included the cultivars: 'Packham's Triumph', 'Red Williams', 'Williams', 'Abate Fetel'. The cultivar were grafted to the two study rootstock BA29 and OHF 333, subject to artificial inoculation was the one-year growth that reached about 20cm. Biostimulator Regoplant was used in 2 concentrations of 100µl/l and 200µl/l, with plants not treated for control. Five days after treatment, the plants were inoculated with a combination of Ea3325 and Ea3345 strains at a concentration of $3x10^8$ CFU/ml. For this purpose, the actively growing peak of the one-year growth was cut with scissors, after which the incision was treated with bacterium suspension. Regular reports of assault rates were performed, with results reported every seven days. For all variants studied, a calculation was made according to the degree of attack of the shoots and the effectiveness of the concentration used.

The impact of the biostimulant on the development of strains was found on the King's B nutrient medium with the addition of Regoplant in the same concentrations. The turbidity of the suspension was determined with spectrophotometers the second and fifth days after the incubation of the bacteria, the turbidity is determined at 600nm wavelength (λ). To transform the results of a percentage concentration into cells per milliliter, an Mc Farland standard was used to calculate approximate cell density.

3.4.3 Statistical methods

The data from the observations and analyzes were processed using the statistical software IBM SPSS Statistics 19 and Analysis ToolPak in MS Excel. One-way analysis of variance was performed using the Duncan's Multiple Range Test (MRT).

4. RESULT AND DISCUSSION

4.1. Susceptibility of pear cultivars in a field conditions

Picture 1 Fourth vegetation of the studied pear varieties

For susceptibility testing infield, the studied cultivars were planted in orchards. The orchard managements no included chemical preparation for plant protection. The plants are given the usual age and culture care such as watering, pruning and agronomic measures. The orchard was established on 01.04.2015 and so far there has been no attack of fire blight.

The susceptibility of pear varieties is a constant value, but in order to develop an infectious process, the pathogen needs to combine of few conditions. Depending on the climatic data of the particular season, the age of the trees, the aggressiveness of the strains and the concentration of the inoculum in the orchard are decisive for the presence or absence of an infectious process.

4.2. Susceptibility of pear cultivars after artificial inoculation.

Our studies on the response of cultivars to pears under controlled conditions are included to three stages in the development of the disease. The infections ability of the pathogen on the leaves, development of bacteria on shoots and the penetration of the pathogen to the stem and rootstock.

4.2.1. Degree of attack on the leaf (%)

The ability of the bacterium infection on the leaves, the accumulation of inoculum in the infected leaves, which would ensure the development of the bacterium on shoots, stem and rootstock, were monitored.

Leaf infection in cultivars grafted on a quince rootstock (BA29)

From the obtained results (Fig. 1) it is evident that when infected with a combination of the two bacterial strains, the percentage of infestation were reported in almost all studied cultivars. An exception is the cultivar ‘Beurré Bosc’ (83.84%), which has the highest percentage of infected leaves after inoculation with strain Ea3345. The results show that the cultivar ‘Alexander Lucas’ after inoculation with Ea3325 on average for the three tested years was reported with an infection of 24,16%, and 23,11% after inoculation with Ea3345, the percentage of mixed infection it is 24, 63%.

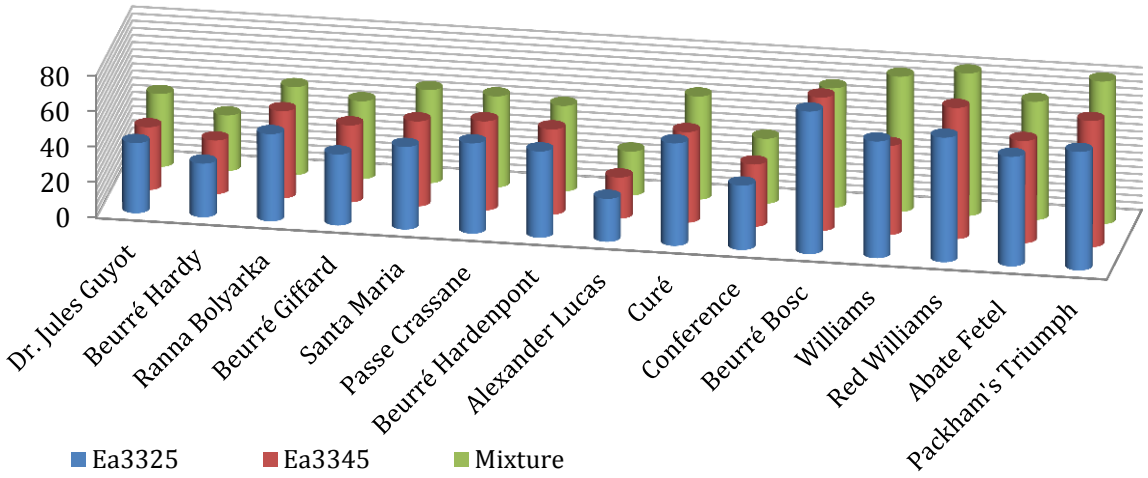


Figure 1. Percentage of leaf infestation after artificial inoculation with single strain or combination of them.

The cultivar ‘Beurré Hardy’ was reported with a slightly higher percentage of infection than ‘Alexander Lucas’, after inoculation on average for the years of tested 30.12% after inoculation with Ea3325 after artificial infection with the other strain 30.13%, the attack on the leaves has reached up to 31.21% after infection with mixed strains. The cultivar ‘Pakhams Triumph’ was reported as an among the most affected by the disease. The infection after inoculation with strain Ea3325 is 66.51%, another strain Ea3345 shown results as a more virulent with percentage of infection 70.89%, the combination of the two strains was reported with the highest value 82.33%. Another cultivar with high percentage of infection was ‘Red William's’, after inoculation with Ea3325 strain shown 69.93% infected leaves, inoculation with the other studied strain the cultivar reached with 73.63% infection, and combination of them strain is a high percentage of infection 82.82%.

On the tenth days after artificial infection in some of the cultivars there are differences between the used single strains of the bacterium and a combination of them (Table 1). The cultivars ‘Dr. Jules Guyot’, ‘Beurré Hardy’ and ‘Ranna Bolyarka’, the artificially infected leaves have statistically significant differences in the degree of attack. In the cultivars ‘Conference’ and

'William's', a statistical difference was statistically significant differences between a combination of the two strains and strain Ea3325. Non statistical differences for the other studied cultivars.

Table. 1. Reaction of pear cultivar ten days after inoculation with a single or a combination of two strains of *Erwinia amylovora*. Cultivar grafted on rootstock BA29.

Cultivar	Dr. Jules Guyot	Beurré Hardy	Ranna Bolyarka	Beurré Giffard	Santa Maria
Ea3325	19,10 b	19,10 b	30,77 b	26,05 a	27,92 a
Ea3345	25,70 b	19,33 b	32,83 b	29,56 a	30,00 a
Mixture	47,54 a	28,67 a	48,25 a	42,81 a	56,86 a
Cultivar	Passe Crassane	Beurré Hardenpont	Alexander Lucas	Curé	Conference
Ea3325	29,57 b	30,77 a	23,40 a	34,53 a	22,83 b
Ea3345	31,41 b	33,99 a	27,51 a	39,46 a	26,66 ab
Mixture	53,27 a	49,84 a	37,69 a	73,67 a	37,84 a
Cultivar	Beurré Bosc	Williams	Red Williams	Abate Fetel	Packham's Triumph
Ea3325	47,68 a	3,53 b	49,80 a	38,26 a	43,46 a
Ea3345	50,54 a	39,82 ab	60,21 a	48,60 a	53,43 a
Mixture	67,67 a	51,69 a	61,92 a	53,88 a	64,66 a

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

Table. 2. Reaction of pear cultivars fifteen days after inocilation with a single or a combination of two strains of *Erwinia amylovora*. Cultivars grafted on rootstock BA29.

Cultivar	Dr. Jules Guyot	Beurré Hardy	Ranna Bolyarka	Beurré Giffard	Santa Maria
Ea 3325	44,86 a	40,90 b	64,95 b	53,66 b	64,83 a
Ea3345	59,70 a	41,16 b	67,21 b	56,80 b	65,46 a
Mixture	75,40 a	64,94 a	100,0 a	88,43 a	100,0 a
Cultivar	Passe Crassane	Beurré Hardenpont	Alexander Lucas	Curé	Conference
Ea 3325	69,90 b	61,64 a	36,90 b	75,63 a	43,30 a
Ea3345	70,53 b	66,10 a	38,23 b	82,91 a	49,50 a
Mixture	100,0 a	94,20 a	60,83 a	100,0 a	71,46 a
Cultivar	Beurré Bosc	Williams	Red Williams	Abate Fetel	Packham's Triumph
Ea 3325	86,62 a	68,46 a	85,33 a	74,26 a	88,33 a
Ea3345	99,17 a	91,13 a	90,05 a	76,46 a	89,55 a
Mixture	100,0 a	100,0 a	100,0 a	78,75 a	100,0 a

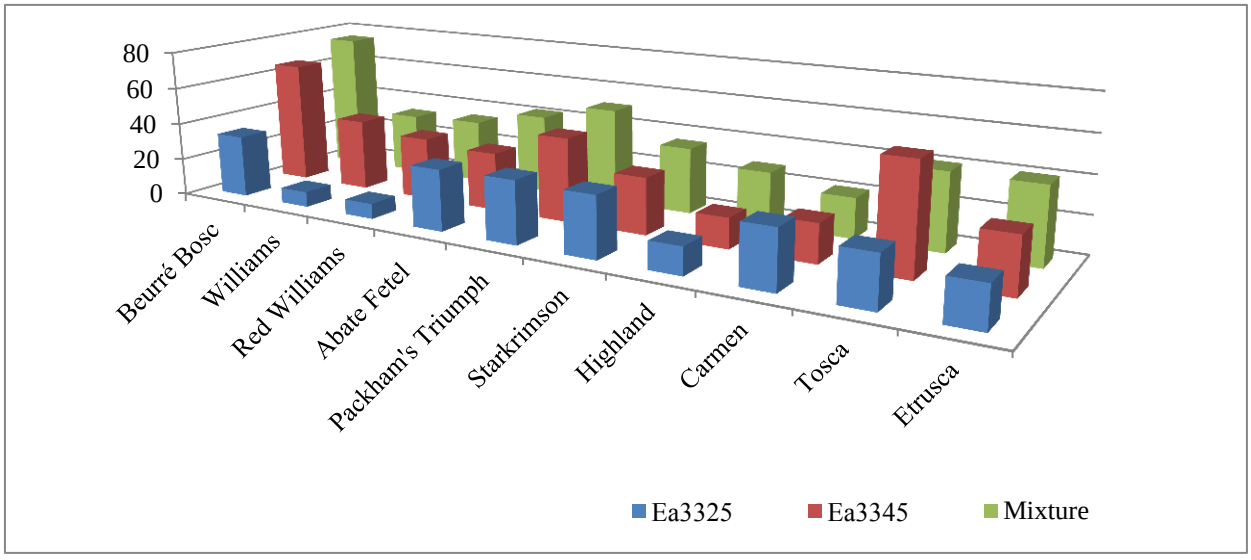
*Different letters in the same column indicated significant difference ($p<0.05$) were compared by using Dunkan test.

On the 15th day, five of tested cultivars were statistically significant differences – ‘Beurré Hardy’, ‘Ranna Bolyarka’, ‘Beurré Giffard’, ‘Passe Crassan’ and ‘Alexander Lucas’ (Table 2). 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.

Leaf infection in cultivars grafted on a pear rootstock (Old Home x Farmingale 333)

When comparing the three variants of infection (Fig. 2) and the response of infected cultivars, on average for leaf attack for pear varieties grafted on OHF333, again with the highest percentage of infection is the variant in which the inoculation was made with a combination of the two strains of the bacterium. The cultivar ‘Tosca’ only is exception, with the highest percentage of infection reported after inoculation with strain Ea3345. In cultivar ‘Abate Fetel’, the response of infected leaves in all three variants was very similar 32.50% after inoculation with Ea3325, a lower percentage of 30.96% after inoculation with Ea3345, and in mixed inoculation 42.24%. As more virulent for the cultivar ‘Williams’ is strain Ea3345, the attack on the leaves is 38.32% for the three years of testing, while after inoculation with the other strain Ea3325 the reported result is 8.19%, and after inoculation with a combination of both strains, the attack of leaves was 32.88%.

Figure 2. Percentage of leaf infestation after inoculation with single and a combination of two strains of *Erwinia amylovora*.



Cultivars grafted on OHF333 rootstock (Table 3) on the tenth day after artificial inoculation with single or a combination of the two strains showed statistically significant differences in the degree of infestation, only in the cultivar ‘Pakhams Triumph’, with the highest percentage of infection. has been reported with a combination of the two strains. The cultivar

‘Beurré Bosc’ after inoculation with strain Ea3325 compared to the other single strain used and a combination of the two strains of the bacteria was statistically significant. For the other cultivars non-statistically differences in the degree of infestation.

The cultivars ‘Beurré Bosc’, ‘Abate Fetel’, ‘Pakhams Triumph’, ‘Highland’ and ‘Etrusca’ were reported with hight percentage of infection after inoculation with combination of the two strains. In the cultivars ‘Red Williams’, ‘Starkrimson’, ‘Carmen’ and ‘Tosca’, the most virulent strain was Ea3345, but the differences were non- statistically significant in all cultivars.

Table 3 Reaction of pear cultivars ten days after inoculation with a single or a combination of two strains of *Erwinia amylovora*. Cultivars grafted on OHF333 rootstock.

Cultivar	Beurré Bosc	Williams	Red Williams	Abate Fetel	Pakhams Triumph
Ea 3325	15,76 b	7,50 a	8,67 a	34,88 a	29,18 b
Ea3345	48,25 a	25,00 a	34,33 a	27,84 a	34,16 b
Mixture	49,93 a	31,17 a	32,67 a	45,66 a	43,16 a
Cultivar	**Starkrimson**	**Highland**	**Carmen**	**Tosca**	**Etrusca**
Ea 3325	21,02 a	14,93 a	20,50 a	29,36 a	17,86 a
Ea3345	23,55 a	13,82 a	37,43 a	61,73 a	22,65 a
Mixture	20,51 a	20,66 a	20,00 a	35,55 a	25,56 a

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

Table 4 Reaction of pear cultivars fifteen days after inoculation with a single or a combination of two strains of *Erwinia amylovora*. Cultivars grafted on OHF333 rootstock.

Cultivar	Beurré Bosc	Williams	Red Williams	Abate Fetel	Pakhams Triumph
Ea 3325	50,94 b	14,33 b	12,92 a	51,78 a	59,37 b
Ea3345	83,56 a	62,66 ab	55,00 a	54,70 a	85,00 a
Mixture	100,0 a	71,00 a	58,66 a	66,96 a	92,36 a
Cultivar	**Starkrimson**	**Highland**	**Carmen**	**Tosca**	**Etrusca**
Ea 3325	65,00 a	23,50 a	44,13 a	41,32 a	38,93 a
Ea3345	56,40 a	28,95 a	33,70 a	89,80 a	55,23 b
Mixture	73,33 a	54,60 a	36,76 a	73,33 a	82,50 c

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

On the fifteenth day after artificial infection in ‘Beurré Bosc’ was a statistically significant difference after inoculation with strain Ea3325. Statistically significant differences was found the percentage of infection in the cultivar ‘Pakhams Triumph’, as compared to the tenth day in this cultivar of rootstocks there are differences in the development of the disease. The ‘Etrusca’ was with significant differences in all studied variants of infection, the variety shown progressive

development of the disease during the reporting period and on average for all years by 82.5% is statistically significant with infected leaves of single strains. The varieties ‘Red William’, ‘Abate Fetel’, ‘Starcrimsson’, ‘Highland’, ‘Carmen’ and ‘Tosca’ have non-statistically significant differences between the degree of infestation and the strains used for infection.

4.2.2Degree of infection on shoots (%)

In all of the studied twenty-five cultivars grafted on a different rootstock, the successful transition of the bacteria from the leaves to the one-year growth was reported. Based on the length on shoots, was determines the degrees of attack in each individual cultivar.

Degree of infection on shoots in cultivars grafted on quince rootstock (BA29)

The lowest percentage of infection after inoculation with strain Ea3325 was reported ‘Beurré Giffard’ 12.4%, the variety has statistically significant differences with ‘Beurré Hardy’, ‘Ranna Bolyarka’, ‘Santa Maria’, ‘Passe Crassane’, Beurré Hardenpont’, ‘Conference’, ‘Beurré Bosc’, ‘Red Williams’, ‘Abate Fetel’ and ‘Packham's Triumph’

Table 5 Degree of infection on shoots (%) after inoculation with single of strain or a combination of them. The cultivars grafted on a BA29 rootstock.

Cultivars	Ea3325	Ea3345	Микс
Dr. Jules Guyot	13,33 g	6,6 g	18,41 f
Beurré Hardy	30,34 bcde	20,82 cdefg	32,99 def
Ranna Bolyarka	32,76 bcd	8,43 fg	30,07 def
Beurré Giffard	12,40 g	6,92 g	26,08 ef
Santa Maria	28,44 bcde	28,36 bcde	44,99 abcd
Passe Crassane	37,54 abc	25,06 cdef	45,49 abcd
Beurré Hardenpont	39,74 abc	31,59 bcd	45,3 abcd
Alexander Lucas	18,94 efg	11,73 efg	36,40 cde
Curé	15,31 fg	18,38 defg	34,84 cde
Conference	27,89 cde	28,3 bcde	45,77 abcd
Beurré Bosc	45,12 a	93,36 a	61,17 a
Williams	20,20 efg	36,93 bc	43,18 bcd
Red Williams	36,90 abcd	33,32 bcd	50,52 abc
Abate Fetel	25,37 def	21,17 cdefg	39,84 cde
Packham's Triumph	33,39 bcd	43,01 b	57,40 ab

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

The highest percentage of infection was reported in the variety ‘Beurré Bosc’ 45.12%, the result was statistically significant compared to ‘Dr. Jules Guyot’, ‘Beurré Hardy’, ‘Ranna

Bolyarka', 'Beurré Giffard', 'Santa Maria', 'Alexander Lucas', 'Curé' , 'Conference', 'Williams', 'Abate Fetel' and 'Packham's Triumph'.

Inoculated with a strain Ea3345 the low percentage of necrosis of shoots in close range showed a 'Dr. Jules Guyot' 6.6% and 'Beurré Giffard' 6.92%, in two cultivar have statistically significant differences with 'Santa Maria', 'Passe Crassane', 'Conference', etc. (Table 5). With the most highs percentage of length on shoots was reported the cultivar 'Beurré Bosc' with 93.36%, the variety showed a statistical significant differences of all varieties. The 'Pachams Triumph' is the next variety after 'Beurré Bosc' on affected shoots 43.01%.

The analysis after artificial infection with a combination of two strains of bacteria showed that 'Dr. Jules Guyot' and 'Beurré Giffard' was reported with lowest percentage of infection. The most affected were again 'Beurré Bosc' and'Pachams Triumph'. The results showed that the two varieties have statistical significant differences in the percentage of necrosis with 'Abate Fetel', 'Conference', 'Curé', 'Alexander Lucas', 'Beurré Giffard', 'Ranna Bolyarka', 'Beurré Hardy' and 'Dr. Jules Guyot'.

Non-statistically significant differences after used of single and a combination of two strains of the bacteria in the cultivars 'Beurré Hardy', 'Beurré Hardenpont', 'Williams' and 'Red Williams' (Table 6). The analysis of the obtained results in the varieties 'Beurré Gifards', 'Santa Maria', 'Curé', 'Conference' and 'Abate Fetel' shows that the combination of the two strains is more virulent compared to the use of single strains of the bacteria. In some of the studied cultivars, such as 'Ranna Bolyarka', there is a statistically significant difference in strain Ea3345 and the other two variants.

Table 6. Difference in the degree of attack by shoots compared to the used single or combination of the two strains. The cultivars grafted on a BA29 rootstock.

Cultivar	**Dr. Jules Guyot**	**Beurré Hardy**	**Ranna Bolyarka**	**Beurré Giffard**	**Santa Maria**
Ea3325	13,33 ab	30,34 a	32,76 a	12,40 b	28,44 b
Ea3345	6,60 b	20,82 a	8,43 b	6,92 b	28,36 b
Mixture	18,41 a	32,99 a	30,07 a	26,08 a	44,99 a
Cultivar	**Passe Crassane**	**Beurré Hardenpont**	**Alexander Lucas**	**Curé**	**Conference**
Ea3325	37,54 a	39,74 a	18,94 ab	15,31 b	27,89 b
Ea3345	25,06 b	31,59 a	11,73 b	18,38 b	28,30 b
Mixture	45,49 a	45,30 a	36,40 a	34,84 a	45,77 a
Cultivar	**Beurré Bosc**	**Williams**	**Red Williams**	**Abate Fetel**	**Packham's Triumph**
Ea3325	45,12 b	20,20 a	36,90 a	25,37 b	33,39 b
Ea3345	93,36 a	36,93 a	33,32 a	21,17 b	43,01 ab
Mixture	61,17 b	43,18 a	50,52 a	39,84 a	57,40 a

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

The results of the ‘Alexander Lucas’ variety show that when infected with a combination of the two strains, the highest values of infection were reported, but only the difference with strain Ea3345 was statistically significant. The virulence of strain Ea3345 to ‘Beurré Bosc’ was statically significant compared to the other two variants in the study.

In thirteen of the fifteen cultivars studied, after artificial infestation with a combination of strains, the highest percentage of necrosis was reported, with the exception of the ‘Ranna Bolyarka’and ‘Beurré Bosc’cultivars. with strain Ea3345, in which up to 93% length of shoots was reported, and the difference was statistically significant compared to the other two variants of infection.

The largest number of cultivars were classified as moderately susceptible to bacterial disease with up to 60% shoot blight. The cultivar‘Beurré Bosc’ reacted as susceptible to the disease.

Table 7 Degree of susceptibility of pear cultivars, according to artificial infestation with single or a combination of *E. amylovora strains*. The cultivars grafted on a BA29 rootstock

Degree of susceptibility				
Type of inocolum	**Very Low susceptibility**	**Low susceptibility**	**Medium susceptibility**	**Susceptibility**
	0-20%	**20-40%**	**40-60%**	**60-80%**
Mixture	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	**80-100%** Beurré Bosc

After artificial inoculation with the Ea3325 strain, some of the cultivar changes their position. Four cultivar, Dr. 'Beurré Giffard', Alexander Lucas and 'Curé', reacted as very low susceptibility. The largest number of cultivars were categorized of low susceptibility with up to 40% blackened of shoots. The variety 'Beurré Bosc' was again the most affected in the study and reacted as moderately susceptibility with up to 60% infected shoots.

The classification according to the degree of susceptibility after infection with Ea3345 retains almost the same structure as in the case of artificial infection with the other strain of the bacteria. Five of the studied cultivar 'Dr. Jules Guyot', 'Ranna Bolyarka', 'Beurré Giffard', 'Alexander Lucas' and 'Curé' were reported with up to 20% infected. An exception is the 'Pakhams Triumph' variety, which is classified as moderately susceptibility. The highest percentage of necrosis is the variety 'Beurré Bosc', classified in the highest degree by the scale used or up to 100% necrosis of shoots.

Degree of infection on shoots in cultivars grafted on pear rootstock (OHF333).

The results after inoculation with strain Ea3325 of the studied combinations of cultivars showed that the cultivars Red Williams is statistically significant difference in terms of shoot attack compared to'Beurré Bosc', 'Abate Fetel' , 'Pakhams Triumph', 'Starkrimso', 'Tosca' and 'Etrusca'. The variety 'Beurré Bosc' was statistically significant differences with all other cultivars, followed by 'Pakhams Triumph', which was a statistically significant difference with the 'Williams', 'Starkimson', 'Highland' and 'Etrusca'.

Table 8 Degree of infection on shoots (%) after inoculation with single of strain or a combination of them. The cultivars grafted on a OHF333 rootstock

Cultivars	Ea3325	Ea3345	Mixture
Beurré Bosc	63,08 a	37,27 abc	86,4 a
Williams	6,33 ef	23,01 cd	23,66 c
Red Williams	0,00 f	12,10 de	48,24 b
Abate Fetel	22,19 bcd	5,00 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	4,40 e	28,61 c
Tosca	28,05 bc	43,91 ab	77,65 a
Etrusca	16,45 cde	28,84 bcd	53,66 b

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

Inoculation cultivars with strain Ea3345 showed that the cultivars 'Abate Fetel' and Carmen were the lowest value of infection and were statistically different from 'Beurré Bosc',

‘Williams’ , Toska and Etrusca. The variety Pakhams Triumph was reported as a most affected by the disease.

After inoculation with a combination of the two strains of bacteria, the cultivars were grouped according to the percentage of infection in three groups. The least affected by the disease were ‘Williams’, ‘Abate Fetel’, ‘Highland’ and ‘Carmen’ with statistically significant differences with all other cultivars (Table 8). The next outlined group consists of the cultivars ‘Red Williams’, ‘Starkrimson’ and ‘Etrusca’. The most affected by the disease were the varieties ‘Beurré Bosc’ 86.4%, ‘Pakhams Triumph’ 72.86% and Tosca with 77.65% affected shoots of the disease.

In seven of the ten cultivars studied - ‘Beurré Bosc’, ‘Red Williams’, ‘Pakhams Triumph’, ‘Starkimson’, ‘Carmen’, ‘Tosca’ and ‘Etrusca’, statistically significant differences in shoot attack were found in terms of artificial infection with one or a combination of two strains of the studied pathogen. An exception is Williams , this variety there are statistically significant differences between strain Ea3325 and the other two variants between which there is no statistical difference. The variety Beurré Bosc’, there is a statistically significant difference between all variants of infection. Ea3345 proved to be more virulent of the two strains depending on the degree of attack on the shoots, as a higher percentage of necrosis was reported in six of the varieties. When infected with the other strain Ea3325, four of the varieties, ‘Beurré Bosc’, ‘Abate Fetel’, ‘Starkrimson’ and ‘Carmen’, reacted with a higher degree of attack. The difference in virulence was non- statistically significant in all varieties.

Table 9 Difference in the degree of attack by shoots compared to the used single or a combination of both strains. The cultivars grafted on a OHF333 rootstock.

Cultivar	Beurré Bosc	Williams	Red Williams	Abate Fetel	Pakhams Triumph
Ea 3325	63,08 b	6,33 b	0 b	22,19 a	34,52 b
Ea3345	37,27 c	23,01 a	12,1 b	5 b	48,55 b
Mixture	86,4 a	23,66 a	48,24 a	13,12 ab	72,86 a
Cultivar	**Starkrimson**	**Highland**	**Carmen**	**Tosca**	**Etrusca**
Ea 3325	19,74 b	8,48 b	6,95 b	28,05 b	16,45 b
Ea3345	14,53 b	15,68 ab	4,4 b	43,91 b	28,84 b
Mixture	47,74 a	29,57 a	28,61 a	77,65 a	53,66 a

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

The cultivar ‘Red Williams’ after inoculations with Ea3325 and in the three experimental years the percentage of attack was 0%, the cultivar reacted by localizing the infestation on the leaves, and in the cultivar ‘Abate Fetel’ 5% necrosis of shoots was reported when infected with the same strain. . In all varieties, the combination with both strains was reported with the highest value of necrosis shoots, with the results showing a large variation in the percentage of attack, for example the Tosca variety.

Degrees of susceptibility were determined after artificial infection with both strains, showed that only the cultivar Abate Fetel as very low susceptibility with shoot blight up to 20%. Three of the studied varieties grafted on this rootstock are defined as low susceptibility: Williams , Highland and Carmen. Three of the varieties were determined of 40 to 60% of the one-year growth (Table 10). With the highest class, three varieties are again determined.

The distribution in classes (Table 10) after infection with strain Ea3325 of the bacterium, the very low susceptibility varieties include 'Williams', 'Red Williams', 'Starcrimson', 'Highland', 'Carmen' and 'Etrusca'. Two of the cultivars 'Pakhams Triumph' and 'Tosca' were low susceptibility to the disease and only cultivar 'Beurré Bosc' corresponds up to 40 to 60% necrosis on shoots.

The distribution of a cultivars after inoculation with strain Ea3345 was a similar - the cultivars, 'Red Williams, Abate Fetel, Carmen and Highland retain their position as very low susceptibility with 0 to 20% infected on annual growth. Infected with this strain, only two varieties, 'Pakhams Triumph' and 'Tosca', were classified as moderately susceptible.

Table 10 Degree of susceptibility of pear cultivars, according to artificial infestation with single or a combination of *E. amylovora* strains. The cultivars grafted on a OHF333 rootstock

	Degree of susceptibility			
Type of inocolum	**Very Low susceptibility**	**Low susceptibility**	**Medium susceptibility**	**Susceptibility**
	0-20%	**20-40%**	**40-60%**	**60-80%**
Mixture	Abate Fetel	Williams Highland Carmen	Red Williams Starcrimson Etrusca	Beurré Bosc Packham's Triumph Tosca
Ea3325	Williams Red Williams Starcrimson Highland Carmen Etrusca	Abate Fetel Packham's Triumph Tosca	Beurré Bosc	
Ea3345	Red Williams Abate Fetel Starcrimson Carmen Highland	Beurré Bosc Williams Etrusca	Packham's Triumph Tosca	

4.2.3 Localization of diseases on shoots, stem and rootstock.

The reported results on the forty-fifth days after inoculation showed that in all cultivars grafted on the quince rootstock BA29, a one-year growth was infect (Table 11). In all studied cultivars there is an attack on the stem of the plant. The infection passed into the rootstock in

most of the tested rootstock combinations, with the exception of the cultivars ‘Red Williams’, ‘Dr. Jules Guyot’, ‘Alexander Lucas’, ‘Cure’ and ‘Abate Fetel’.

The pathogenicity of the bacterium was also manifested in the varieties grafted on the pear rootstock OHF333. In all varieties, *E. amylovora* was successfully penetrated from the leaves to the annual growth.

Table. 11 Development of *E. amylovora* on shoots, stem and rootstock

Rootstock	Cultivars	Shoots	Stem	Rootstock
BA29	Beurré Bosc	+	+	+
	Williams	+	+	+
	Red Williams	+	+	-
	Dr. Jules Guyot	+	+	-
	Beurré Hardy	+	+	+
	Ranna Bolyarka	+	+	+
	Beurré Giffard	+	+	+
	Santa Maria	+	+	+
	Passe Crassane	+	+	+
	Beurré Hardenpont	+	+	+
	Alexander Lucas	+	+	-
	Curé	+	+	-
	Packham's Triumph	+	+	+
	Conference	+	+	+
	Abate Fetel	+	+	-
OHF333	Beurré Bosc	+	+	-
	Williams	+	-	-
	Red Williams	+	-	-
	Abate Fetel	+	-	-
	Packham's Triumph	+	-	-
	Starkrimson	+	-	-
	Highland	+	-	-
	Carmen	+	-	-
	Tosca	+	+	-
	Etrusca	+	-	-

In only two of the studied varieties, ‘Tosca’ and ‘Beurré Bosc’, the infestation reached the stem of the artificially infected plants. In the other varieties – ‘Williams’, ‘Red Williams’, ‘Abate Fetel’, ‘Pakhams Triumph’, ‘Starkrimson’, ‘Highland’, ‘Carmen’ and ‘Etrusca’, after the artificial infection, were reported a slowly movement of the bacteria is observed in the infected shoots. Wasn’t observed infection of rootstock in any of the tested cultivars.

Picture 2 Development of *Erwinia amylovora* on stem and rootstock, cultivars grafted on BA29. From left to right, Ranna Bolyarka, Dr. Jules Guyot and Beurré Hardy.

4.3 Influence of the rootstock on the degree of susceptibility of the cultivar after inoculation.

The presented results (Table 12) showed that after inoculation with strain Ea3325 the statistically significant differences in leaf infection between the cultivars Beurré Bosc, Williams and Red Williams, grafted on a pear rootstock and the same varieties grafted on BA29 and own root plants.

Таблица 12 Degree of infection of leaves in pear cultivar grafted on a quince (BA29), pear (OHF333) rootstocks and own root plants.

Cultivars	Beurré Bosc	Williams	Red Williams	Packham's Triumph	Abate Fetel
Ea3325					
Own Roots	78,02 a	49,05 b	65,92 a	57,83 ab	55,30 ab
Ba29	83,84 a	65,48 a	69,93 a	61,43 a	66,51 a
OHF 333	33,35 b	10,91 c	10,79 b	43,33 b	44,28 b
Ea3345					
Own Roots	70,87 a	78,24 a	61,62 ab	52,32 a	70,44 a
BA29	74,86 a	50,00 b	73,63 a	57,36 a	70,89 a
OHF 333	65,91 b	51,09 b	44,66 b	41,27 a	59,58 a
Mixture of Ea3325/Ea3345					
Own Roots	70,18 a	77,61 a	40,99 b	66,59 a	72,33 a
BA29	67,08 a	75,84 a	82,81 a	66,32 a	82,33 a
OHF 333	74,96 a	43,83 b	45,66 ab	56,32 a	67,76 a

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

In the varieties ‘Pakhams Triumph’ and ‘Abate Fetel’, the varieties grafted on OHF333 and those grafted on BA29 were statistical differences in the degree of attack by the bacteria. The ‘Pakhams Triumph’ variety, grafted on BA29, on the tenth day after inoculation showed a third degree of infestation, expressed in necrosis that reached the leaf petioles. The same cultivar grafted on the pear rootstock OHF333 reacted with necrosis only at the cut site, symptoms of the first degree of attack.

After inoculation with strain Ea3345 in the varieties ‘Pakhams Triumph’ and ‘Abate Fetel’, the lowest percentage of infection were reported in the cultivars grafted on the pear rootstock, but the differences were non-statistically significant. ‘Red Williams’ there were statistical differences in leaf attack between the rootstocks BA29 and OHF 333, while in the variety grown on its own root there were non-statistically significant differences with any of the other studied rootstocks. Statistically significant differences in the degree of infestation were found in ‘Williams’, this cultivar has the highest percentage of infection with 78.24%, grown on its own root compared to plants grafted on quince or pear rootstocks. In four of the five cultivars studied, the highest degree of infestation was observed in rootstock BA29, with the exception of the cultivar ‘Williams’, in which the cultivar grown on its own root was most affected.

In four of the five cultivars studied, after inoculation with a combination of the two strains of the bacteria, the lowest leaf infestation was reported in the cultivars grafted on the pear rootstock OHF 333, with the exception of the ‘Beurré Bosc’ cultivar. In this variant of the study, statistically significant differences were reported in the two cultivar ‘Williams ‘and ‘Red Williams’. In the other varieties there were non-statistically found differences in the attack on the leaves.

Picture 3 Packhams Triumph cultivar, left to right grafted on BA29 and OHF333

In the reported damages on shoots on the twenty-fifth day after the artificial inoculation (Table 13) there are differences and changes in the data compared to the leaves. The cultivars with their own roots have the highest percentage of attack, with the exception of several rootstock

combinations: ‘Beurré Bosc’ grafted on BA29 and ‘Pakhams Triumph’ grafted on OHF333 after inoculation with strain Ea3345. When infected with both strains at the same time, the same two cultivars showed a similar reaction.

The summarized three-year results on the assault on the one-year growth of five cultivars of pears grafted on two rootstock and own root plants was reported that after artificial inoculation with Ea3325 strain showed the highest percentage of infection were cultivars witch was grown on their own root. The cultivars ‘Williams’ and ‘Red Williams’, there were statistically significant differences between rootstock BA29 and OHF333 in the study. The cultivar ‘Abate Fetel’ showed a statistically significant differences in the attack on shoots between own root plants and a grafted on other two rootstock. The inoculated pear cultivars with the strain Ea 3345 showed that statistical differences in the attack were found in pear rootstock and cultivars ‘Williams’ and ‘Red Williams’ of their own root plants and BA29 grafts.

‘Beurré Bosc’ cultivar was reported with statistically significant difference between all studied variants. The statistically significant difference between studied variants was found in cultivars ‘Abate Fetel’ graftedon pear rootstock OHF 333, quince rootstock BA29 and own root plants. The ‘Pachams Triumph’cultivars, was non-statistically significant differences in percentage of necrosis on shoots.

Table 13 Shoots infection, cultivars grafted on of pears(OHF333), quince (BA29)rootstocks and own root plants.

Cultivars	Beurré Bosc	Williams	Red Williams	Packham's Triumph	Abate Fetel
Ea3325					
Own Root	69,25 a	51,76 a	43,76 a	43,72 a	56,02 a
BA29	45,12 b	20,20 b	36,90 b	33,39 a	25,37b
OHF 333	63,08 a	6,33 c	0,00 c	22,19 b	34,52 b
Ea3345					
Own Root	67,33 b	46,29 a	51,47 a	33,65 a	59,77 a
BA29	93,36 a	36,93 a	33,32 a	43,01 a	21,17 b
OHF 333	37,27 c	23,01 b	12,10 b	48,55 a	5,00 c
Mixture Ea3325/Ea3345					
Own Root	83,30 a	78,67 a	36,03 b	32,91 b	75,70 a
BA29	61,17 b	43,18 b	50,52 a	57,40 ab	39,84 b
OHF 333	86,40 a	23,66 c	48,24 ab	72,86 a	13,12c

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

Forty-five days after the artificial inoculation the bacteria development was reported from shoot to rootstock (Table 14). Necrosis on shoots was observed for each of the tested cultivars in all variants. Bacteria development was determined on the stem for all tested cultivars witch grafted on a Ba29 rootstock. The bacteria were spread on rootstock in three cultivars ‘Beurré

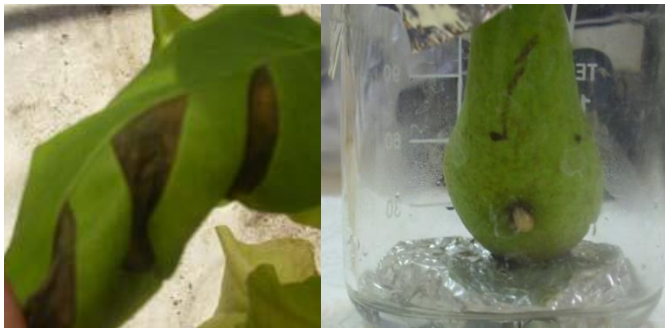
Bosc', Williams' and 'Abate Fetel', the infection wasn't covered in two cultivars Red Williams' and 'Packham's Triumph'.

Таблица. 14 Развитие на *E. amylovora* по летораст, стъбло и подложка

Rootstock	Cultivar	Shoots	Stem	Rootstock
BA29	'Beurré Bosc'	+	+	+
	'Williams'	+	+	+
	'Red Williams'	+	+	-
	'Packham's Triumph'	+	+	-
	'Abate Fetel'	+	+	+
OHF333	'Beurré Bosc'	+	+	-
	'Williams'	+	-	-
	'Red Williams'	+	-	-
	'Packham's Triumph'	+	-	-
	'Abate Fetel'	+	-	-
Own roots	'Beurré Bosc'	+	+	+
	'Williams'	+	+	+
	'Red Williams'	+	+	-
	'Packham's Triumph'	+	+	+
	'Abate Fetel'	+	+	+

In cultivar grafted on an OHF333 the symptoms of infection was reported only in one cultivar 'Beurré Bosc', localized infection on shoots were monitored in another tested cultivars. The rootstock wasn't infected in all five studied cultivars. The infection of bacterial diseases was localized on shoots and stem. To prove the influence of rootstock in cultivar, they were grown on own-root. In all tested cultivars the infection of bacteria was monitored on shoots and stems. The fire blight caused by Erwinia amylovora was identified of all studied cultivars, the exception was 'Red Williams'.

After completion of the experimental, samples have been taken for reisolation of all tested cultivars. The bacteria *E. amylovora* was successfully isolated from inoculation pear cultivars in the study.



Picture 4 From left to right hypersynthetic reaction in tobacco and test for pathogenicity of immature pear fruit.

The necrosis of a tree are the result caused by of the bacterial disease, to prove the pathogenicity of the strains and a test for a hypersynsive reaction in tobacco was carried out. The method allows the differentiation of phytopathogenic bacteria that cause a hypersensitive reaction (HR) in the mesophilia of the leaves in tobacco, unlike saprophytes bacteria that do not induce this reaction.

The use of unripe pear fruit complements the characteristic in terms of virulence of the bacteria *Erwinia amylovora*. In all varieties studied, a positive reaction was reported after three days of incubation of the bacterium.

4.4 Testing of chemical and biological methods to control of *Erwinia amylovora*

4.4.1 Chemicals control of the *Erwinia amylovora in vitro*

Concentrations of plant protection products were selected in accordance with the recommendations of the manufacturers. From the results obtained and the dispersion analysis carried out, it was found that the preparation Bordo Mix 20VP showed the largest inhibition zones (table 15). There were significant differences in all other preparations, the exception is only the experiment with Funguran and strain E3325, the inhibition zones were within close range and have no statistically significant differences. In strain E3325 with inhibition zones are the preparations Ditan M45, Alfil NG and Alfil Duplo.

Table 15 Influence of chemical products *on in vitro* development of *Erwinia amylovora*

Preparats	Concentartion (%)	Strain/ Inhibition zone (%)		
		Ea3325	Ea3345	Ea3325/Ea3345
Bordo Mix 20	0.6	13,58 a	14,00 a	13,37 a
Funguran OH 50	0.5	11,08 ab	10,66 b	9,83 b
Ditan M 45	0.3	8,29 b	10,45 b	5,47 c
Alfil	0.3	9,58 b	8,50 bc	1,37 cd
Alfil Duplo	0.35	8,50 b	5,95 c	3,25 c
Control	water	0,00 c	0,00 d	0,00 d

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

The control option was with statistically significant differences from all other variants.

With the reported results of strain E3345, the preparation Bordeaux Mix 20VP is again of the highest value and with proven statistical differences compared to other preparations. With close inhibition values on the agar environment after Bordeaux Mix by efficiency fall the preparations Funuran, Ditan and Alfil NG, the latter having no statistically proven differences with Alfil Duplo. Statistical differences are found in Alfil Duplo and Funuran, Ditan and Bordeaux Mix In all variants statistically proven differences in the inhibition zone there is with the control variant.

The variant in which a combination of the two strains of bacteria Ea3325 and Ea3345 on the agar medium is placed repeats the trend outlined above that the preparation Bordoa Mix suppressed the development of bacteria in the area of application of the preparation and the difference has been significant statistically.

The preparation Funguran in this variant was exhibited high bactericidal activity and was significant differences among all other strains.

In the control variant with the addition of water, weren't showed a inhibition of zone the growth of bacteria on the agar medium is observed in all tested plant protection products.

In our research, in the first two variants, Bordo Mix and Funguran, there were non-significant differences in terms of suppressing the development of the bacterium relative to different strains or a combination of them. Both options reported results within close range that non-significant statistical differences between of them (Fig. 3).

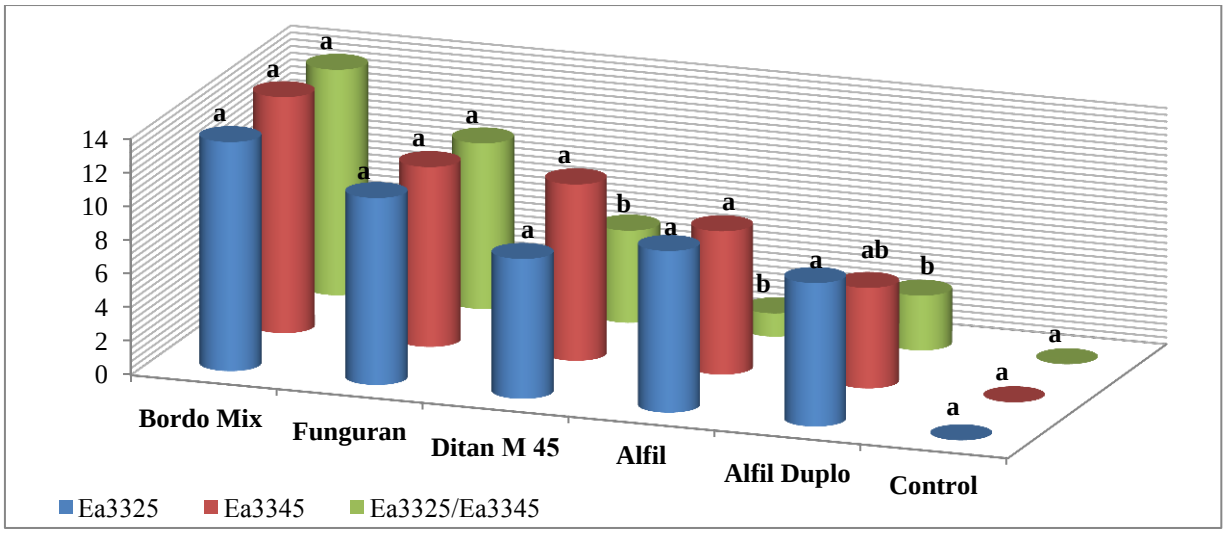


Figure 3 Zones of inhibition in different strains of *Erwinia amylovora* and combination of them

Statistically significant differences in the action of preparations after the use of Ditan M45 and Alfil, in variants with the two phytopathogen strains were grouped as statistically the same, in combination with the two strains the lowest inhibition zone values were recorded.

The different strains in the preparation Alfil Duplo indicate that the inhibition zone of the strain E3325 was a statistically significant difference from the combination of the two strains. While for strain Ea3345 there were non- significant differences in the other variants of the studied.

When comparing the inhibition zone of the after used of individual strains and the combination between the two, very little variation was observed, with each tested preparations the inhibition zone being smaller in a combination of the two strains. Compared to the single strains of the bacterium, the variation is within very small limits, for example Bordo Mix and strain E3325 zone is 13.58%, and for strain E3345 the area is 14%.

4.4.2 Biological agents to control of *Erwinia amylovora.*

In the control variant in varieties grafted on quince rootstock BA29 at ‘Abat Fetel’, one day after artificial infection, the first symptoms of the onset of the disease, necrosis at the site of the cut were reported.’Williams’ cultivar is reported during the second day of infection (Table 16). With the latest manifestation of the disease is ‘Packhams Triumph’ on the fourth day after inoculation. The incubation period of the pathogen was of different durations in the individual varieties grafted on the pear rootstock, for example, the first symptoms of the disease in the varieties ‘Abat Fetel’ and ‘Red Williams’ were recorded on the second day after the inoculations, and in ‘Williams’ and ‘Packhams Triumph’ on the third day.

In the variant with application of Regoplant in 200µl/l, the varieties grafted to BA29 were reported with a duration of the incubation period ‘Abat Fetel’, ‘Red Williams’ and ‘Packhams Triumph’ on the second day after the artificial inoculation. The variety ‘Williams’ on the third day after artificial inoculation. In the same variant PR 2, but in the cultivar grafted on OHF333, ‘Williams’ and ‘Packhams’ Triumph reacted on the third day, while ‘Abat Fetel’ and ‘Red Williams’ on two day incubation period.

In variant PR1 and cultivars of quince rootstock, the variety ‘Abat Fetel’ is recorded after the first day of the incubation period, as in the control. The other three varieties of ‘ Williams’, Red Williams and Packhams Triumph were reported with the first symptoms of the disease on the second day after inoculation with a combination of the two strains of the bacterium.

The cultivar ‘Abat Fetel’ grafted on a quince rootstock, was monitored earliest manifestation of the disease on the first day after artificial infection in the control variant and PR1. ‘Red Williams grafted on BA29 in the two variants studied and the control manifestation of the first symptoms, were observed in the same period.

The significant difference in reported incubation periods was found in varieties grafted on to the pear pad in PR1 variant, where the ‘Packhams Triumph’ variety was reported on day four with the first symptoms of necrosis around the site of the cut.

Picture 5 Packhams Triumph cultivar grafted on BA29 left to right RP1 and RP2 variant.

Table 16 Incubation period after infection with *Erwinia amylovora*.

Incubation period					
Variants	**Подложка**	**Days of first symptoms**			
		1	**2**	**3**	**4**
PR1	Ba 29	Abat Fetel	Williams		
			Red Williams		
			Packhams Triumph		
	OHF333	Abat Fetel		Red Williams	Williams
				Packhams Triumph	
PR 2	Ba 29		Abat Fetel	Williams	
			Red Williams		
			Packhams Triumph		
	OHF333		Abat Fetel	Williams	
			Red Williams	Packhams Triumph	
Control	Ba 29	Abat Fetel	Williams		Packhams Triumph
			Red Williams		
	OHF333		Abat Fetel	Williams	
			Red Williams	Packhams Triumph	

To establish the damage of attack in all variants, were monitored development in dynamics of the bacterium in each cultivars and rootstock combination were tracked after treatment with biostimulant Regoplant and artificial inoculation with a combination of two strains of the bacterium Ea3325 and Ea3345.

Figure 4 presents data on the 'Abat Fetel' variety grafted on a BA29 quince rootstock and the three variants of infection in the study. In the control variant, the rate of attack on the infected on shoots was 9.64%, compared to the treated variants with Regoplant, where the infection reached 16.94% necrosis. The same variety grafted on a pear rootstock OHF333 (Fig. 5) showed that the most affected by the bacterial disease is the control and by the last reported stage the infection reaches 18.35% necrosis. In both variant of application with the biostimulator, lower percentage of infection was reported.

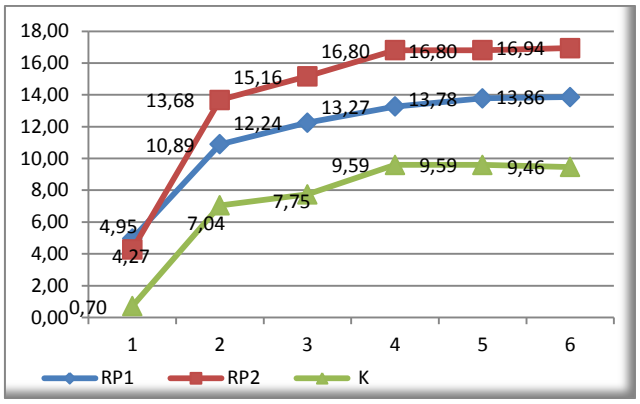


Figure 4 Development of *Erwinia amylovora* in Abat Fetel cultivar grafted on BA29

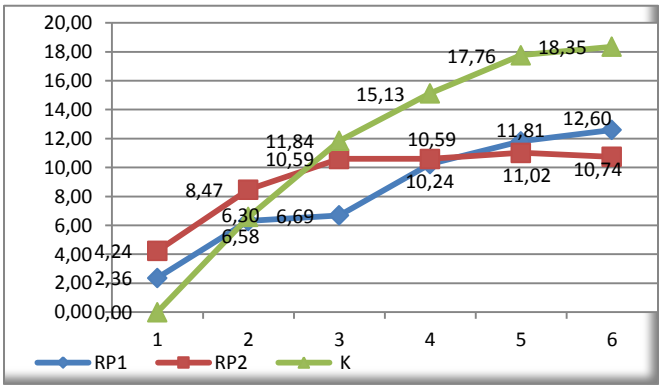


Figure 5 Development of *Erwinia amylovora* in Abat Fetel cultivar grafted on OHF333

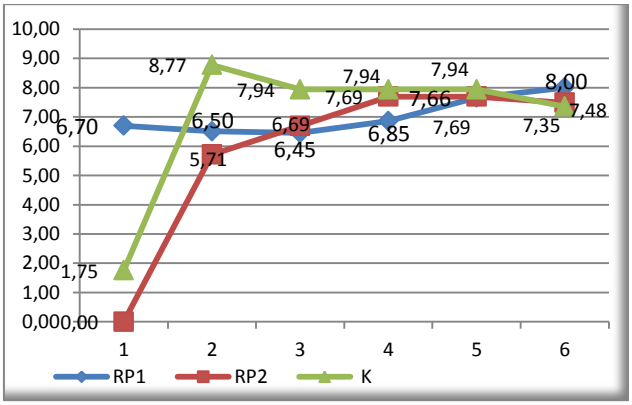


Figure 6 Development of *Erwinia amylovora* in Williams cultivar grafted on BA29

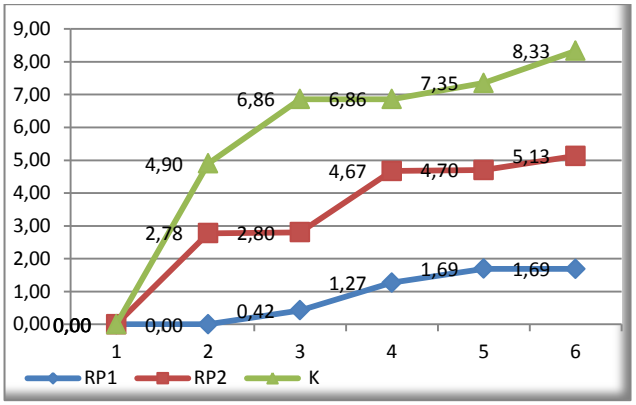


Figure 7 Development of *Erwinia amylovora* in Williams cultivar grafted on OHF333

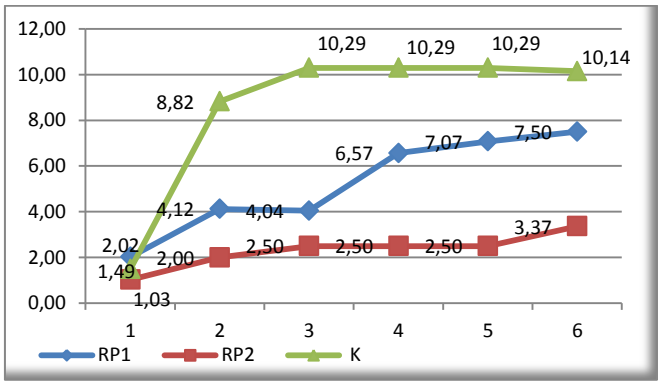


Figure 8 Development *of Erwinia amylovora* in Red Williams cultivar grafted on BA29

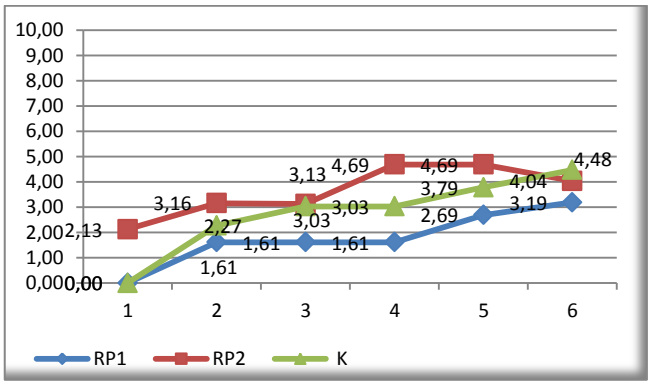


Figure 9 Development of Erwinia amylovora in Red Williams cultivar grafted on OHF333

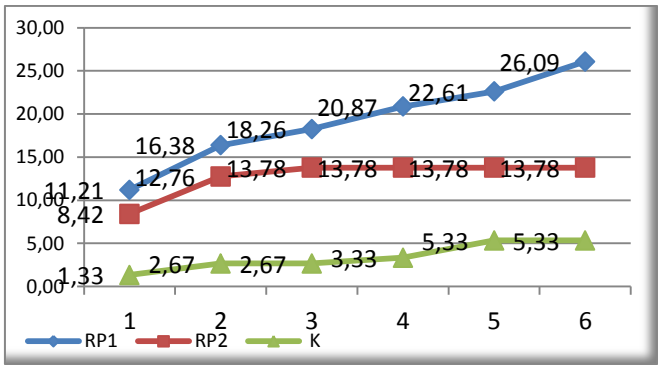


Figure 10 Development of *Erwinia amylovora* at Packhams Triumph cultivar grafted on BA29

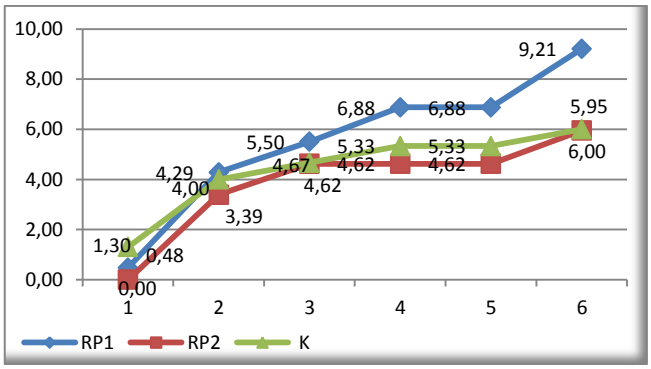


Figura 11 Development of Erwinia amylovora at Packhams Triumph cultivar grafted on OHF333

The variety 'Williams' grafted on BA29 (Fig. 6) showed differences from the first reading in a variant treated with 200µl/l Regoplant, the shoots showed no inoculation, while in the variant with 100µl/l biostimulator, the recorded value was 6,70 % attack. The last reading made 5 weeks after infection in all variants studied reported close values in infection. The same variety grafted on to the pear rootstock, in all the variants studied on the first reading on the fifth day after infection were without visible symptoms of infection. In variant RP1 and the second reported value in the dynamics is 0 and the maximum value for the variant is 1.69%. Variant RP2 was reported with a maximum infection value of 5.13%. The most affected is the control variant, in which already in the second reading of the results was reported with rapid penetration of the bacterium into the shoots 4.90% (Fig. 7).

Снимак 21 Control variant of Red Williams left to right grafted on BA29 and OHF333

The 'Red Williams' cultivar grafted on a quince rootstock was reported with the attack 10.14%, with lower infection reported with the biostimulant in variant RP1, rapid development of the disease was reported after the third reporting in dynamics with a maximum development of up to 7.50%. In the other variant studied and the lowest values for the variety combination at RP2 with a maximum development of up to 3.37%. The variant with rootstock OHF333 the results in another direction, here all the resulting values are within close range, the one with the lowest

percentage of necrosis being treated shoots with 100µl/l bio regulator before artificial inoculation. In the control variant without the use of Regoplant, duplicate values were reported with respect to RP2 variant infection, but in the last reading the necrosis rate was higher than the treated shoots with a biostimulant.

The dynamics in the development of fire blight in the variety 'Packhams Triumph', established as an average disease susceptibility cultivar in the control variant, were found to be the lowest values, which until the last reported moved at a slow rate of development. The penetration of the bacterium into variant RP1 is the fastest and reaches the highest infection rates, with necrosis reaching 20.87% on the third reading of the dynamics. With lower values, the other option was taken into account in the experience, with necrosis reaching 13.78% assault on the one-year growth. Similar is the dynamics in the development of the bacterium with the same cultivar grafted on a pear rootstock. The most affected by the disease was the variant with a 100µl/l biotimulator, in the first monitoring on the fifth day after inoculation and 0.48% attack, after only two days necrosis reached 4.29% (Fig. 11). Control is the other variants in the experience were accounted for with close percentage of necrosis, with the maximum development of the disease reaching up to 6% necrosis on the shoots.

Based on average study results after spraying biostimulant Regoplant in two concentrations and artificial inoculation with a combination of two strains of the bacterium, a dispersion analysis was carried out for comparison. In the case of varieties grafted to BA29, only the 'Williams' cultivar non-significant statistically differences in the variants used and the control (Table 17). There were statistically significant differences in the reaction of the 'Abat Fetel' variety between RP2 and the control, with the control variant having the lowest recorded value of necrosis.

Table 17. Percentage of shoot attack (%), after treatment with biostimulator Regoplant.

Variants	Abat Fetel	Williams	Red William	Packhams Triumph
Ba 29				
Control	7,35 b	6,94 a	8,55 a	3,44 c
RP1	11,49 ab	7,02 a	5,22 b	19,23 a
RP2	13,94 a	5,87 a	2,31 b	12,71 b
OHF333				
Контрола	11,61 a	8,55 a	1,78 b	4,43 a
RP1	8,33 a	5,22 b	2,76 ab	5,53 a
RP2	9,27 a	2,31 b	3,63 a	3,86 a

*RP1-Regoplant at a concentration of 100µl/l *RP2-regoplant at a concentration of 200µl/l

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

Statistical differences are found in the variety 'Red Williams' between the control and the treated shoots with Regoplant. In the case of the 'Packhams Triumph' variety, proven differences were reported in the two variants studied and the control, which also recorded the lowest value of 3,44 % necrosis in the one-year growth. In two varieties on rootstock OHF333 'Abat Fetel' and 'Packhams Triumph', there were non-statistically significant differences between the biostimulant used in two concentrations and the control, but for the two varieties with the lowest infection rate was variant RP2 with 200µl/l. In the case of 'Red Williams' cultivar, there is a statistically significant difference between the control and variant RP2.

To track the direct impact of the biostimulator on the development of the bacterium, two measurements of the concentration of bacteria in 1 ml have been made. Bacterial cultures were incubated at 23°C and measured on a spectrophotometer at 600nm wavelength.

The reported value for the turbidity of the bacterial suspension through 48 hours of incubation in a thermostat showed the most active growth of the bacterium was recorded in variant RP2 (1,86%), with a subsequent value being the control variant by 1,804%, and with the lowest value was RP1 with 1,779.

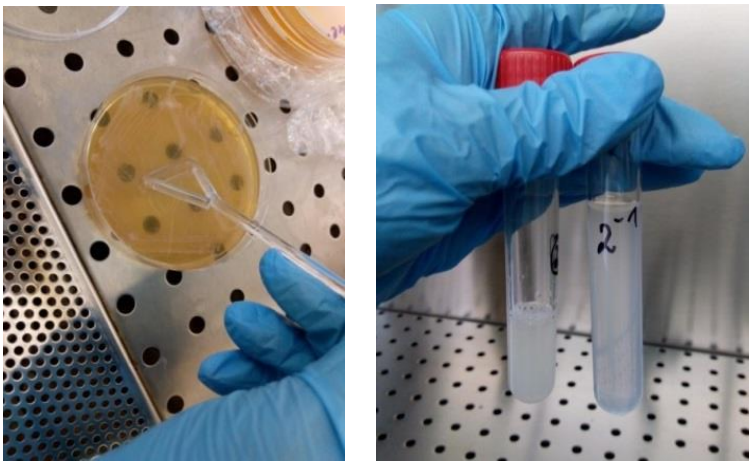


Photo 6 Left to right washing the bacteria from the agar surface and the turbidity of the suspension

The results of the fifth day after incubation of bacteria on a nutrient medium show that bacteria per milliliter decrease in both variants, as well as in the control. Within close range, but with the highest suspension density, was reported in variant RP1 (1.75%) compared to the control, the difference is minimal. The variation between the second and fifth days of bacteria incubation is greatest in the variant with the addition of 200 µl/l Regoplant.

In order to calculate the turbidity of the bacterial suspension in CFU/ml, a dilution of one in ten was made in order to be able to use the Mc Farland standard scale. After dilution, the results shifted in a different direction, namely that the greatest growth of bacteria was recorded in a variant with the addition of 100 µl/l of bioregulator on both reporting days. Another difference was found that in the control variant the turbidyti of the suspension was the lowest percentage.

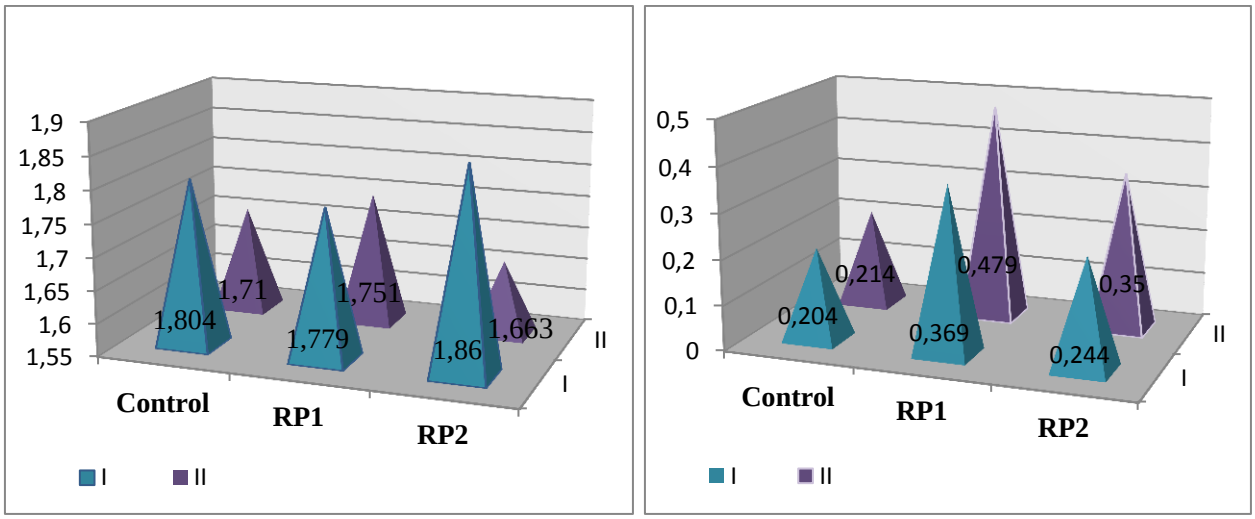


Figure 12 Turbidity of bacterial suspension (%) two and five days after incubation of bacteria.

The second and fifth days after incubation of bacteria on a nutrient medium with the addition of Regoplant in the cultural media, there were non-significant statistically differences in the concentration of bacteria in 1ml suspension. The data obtained in both the reporting periods and the dispersion analysis data indicate that there are no statistically proven differences in the control, which is without Regoplant in the media and the two variants studied with different concentrations of the biostimulator. There were also non-significant differences between RP1 with the addition of 100µl/l and RP2 with the addition of a 200 µl/l stimulator.

Table 18 Turbidity of bacterial suspension

Variants	I (%)	I^{-1}(%)	CFU/ml	II (%)	II^{-1} (%)	CFU/ml
Control	1,804 a	0,204 b	$3x10^8$	1,710 a	0,214 b	$3x10^8$
RP1	1,779 a	0,369 a	$\underline{6x10^8}$	1,751 a	0,479 a	$\underline{6x10^8}$
RP2	1,860 a	0,244 b	$3x10^8$	1,663 a	0,35 a	$3x10^8$

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

Statistically proven differences were found after the dilution of bacterial cultures. On the second day proven differences there are between option RP1 and the other option, as well as the control. After three-fold measurement after dilution, one to ten on day five statistically proven difference was determined between the control and the two regoplant concentrations studied.

After determining the turbidity of the bacterial suspension in CFU/ml, the results in both reported periods were the same. Administration of 100 µl/l Regoplant in variant RP1 resulted in faster growth of bacteria on the agar medium up to $6x10^8$CFU/ml compared to the control and variant RP2, which valued at $3x10^8$CFU/ml.

5. CONCLUSIONS

1. The absence of damage caused by fire blight in natural conditions is due to a lack of primary inoculum and a source of infection in the orchard.
2. In the cultivar grafted on BA29 with the high percentage of infection were reported the cultivar 'Beurré Bosc', and with the lowest percentage of infection 'Alexander Lucas'.
3. In the cultivars grafted to OHF333 with the highest affected on the leaf was 'Beurré Bosc', and the weakest affected cultivars in relation to the strain used.
4. The highest percentage of infection on a shoots of cultivars grafted on the quince rootstock was 'Beurré Bosc' with the lowest values were the cultivars 'Dr. Jules Guyo' and 'Beurré Giffard'.
5. In eleven of the fifteen cultivars studied, the attack with the combination of the two strains of the bacteria was manifested as more virulent.
6. In the cultivars grafted on the pear rootstock with the highest values of infection by shoots was the cultivar 'Beurré Bosc', and with the lowest values were the cultivar 'Red Williams', 'Williams' and 'Carmen'.
7. The infected of shoots in all cultivars grafted on OHF 333 was with statistically significant differences in terms of the single strains used and a combination of both strains of the bacteria.
8. The distribution of cultivars to the degrees of susceptibility depending on the single or combination of the two strains of the bacteria used.
9.The infection was passed into the rootstock in the majority of studied cultivars grafted on a BA29, with the exception were the cultivars 'Red Williams', 'Dr Jules Guyo', 'Alexander Lucas', 'Cure' and 'Abate Fetel'.
10. In only two of the cultivars studied – 'Tosca' and 'Beurré Bosc' grafted on the pear rootstock, the infections were reached the stem of the artificially infected plants.
11. The rootstock affects the degree of susceptibility the differences can be seen still at inoculation leaves, with the lowest susceptibility were the cultivars grafted on the pear rootstock OHF333.
12. Synergism is manifested between a combination of the two strains of the bacteria and the cultivars inoculations with them show the highest percentage of infection.
13. The largest inhibition zones were reported in the copper preparations Bordeaux Mix and Funguran.
14. The bio stimulator Regoplant has no effect on the incubation period of the disease, the exception is only the cultivar 'Abate Fetel' in PR2 variant.
15. Regoplant doesn't provide good protection for plants after inoculation with a combination of both strains of bacteria.
16. The media Kings' B in addition to 100µl/l Regoplant stimulates the growth of the bacterium.

6.CONTRIBUTIONS

6.1 Original contributions

- For the first time in the country, twenty-five pear varieties have been evaluated after artificially infecting local strains of the erwinia amylovora bacterium.
- For each cultivar, was determined the degree of attack on the leaves, the degree of attack on the shoots and the development of the bacterium on the rootstock were established.
- A selection of cultivars with tolerance to bacterial disease was performed.
- There was a difference in the virulence of different isolates of *Erwinia amylovora* after artificial infection, as well as the virulence of a mixed inoculum from the bacterium.
- The effect of the biostimulator Regoplant in two concentrations as an inducer of immunity was monitored.

6.2 Confirmatory contributions

- It is confirmed that the cultivars 'Dr. Jules Guyot' and 'Beurré Giffard' grafted on BA29 were tolerant to the disease, and the cultivar 'Beurré Bosc' is very susceptibility.
- It is confirmed that *Erwinia amylovora* strains were found to vary in virulence.
- It is confirmed that mixing of the two strains of the bacterium increases the virulence of the inoculum.
- The influence of the rootstock on the susceptibility to bacterial disease has been confirmed
- The bactericidal action of copper preparations in *in vitro* conditions was confirmed.

6.3 Applied contributions.

- The tolerant cultivars were determined for growing suitable in the country.
- The cultivars grafted on quince rootstock were determined as a more susceptible to bacterial disease as those grafted on pear rootstock OHF333.
- The effect of copper preparations, which can be used as a preventive measure against bacterial disease in orchards, was evaluated.

Publications connected with he dissertation:

1. Alexandrova, D., & Dzhuvinov, V. (2017). First results of five pear cultivars after artificial inoculation with Erwinia amylovora (Burrill). Journal of BioScience & Biotechnology.

2. Alexandrova D., Chavdarov P., Chemical control agents of *Erwinia amylovora* (Burill) in in vitro conditions. Field Crop Studies XII(3): 121-128.

3. Aleksandrova, D., Chavdarov P. & Nesheva M. (2020). Fire blight susceptibility of pear cultivars grafted on OHF 333 rootstock. Scientific Papers. Series B (in print).

SUMMARY

The aim of this study was to determine the reaction of pear cultivars grafted on a two rootstock to the bacterial disease fire blight. It was traced the percentage of pear leaves infection, length of lesions on shoots, susceptibility classes, and bacteria development in shoots, stem, and rootstock. It was studied fungicides for the prevention and control to *Erwinia amylovora*. To determine the efficiency of a biostimulator Regoplant.

The researches were conducted in a pear orchard and laboratory of phytopathology at the Fruit Growing Institute – Plovdiv, during the period 2015 -2019. The objectives of the study were 15 pear cultivars grafted on a quince rootstock BA29 and 10 pear cultivars grafted on a pear rootstock OHF333. It was to compare the reaction of the cultivars when inoculated with different strains of *Erwinia amylovora* and a combination of them.

From the results was conclude that after infection with a single or mixture of bacterial suspension, the susceptibility of leaves was different in depends on the used strain and cultivar. According to the reactions to inoculation with a single or mixture of strains the cultivars were classified in a different class of susceptibility. In 45th days after inoculation was researched to spread of necrosis distribution to shoots, stem and rootstock were observed in all cultivars. It was confirmed that when the combination of two strains of the bacteria the virulence of the bacteria suspension increased.

It was proved the antibacterial action of copper preparations.and the limited action of biostimulator Regoplant.