STUDY REGARDING THE RESPONSE OF SOME APPLE VARIETIES TO THE ATTACK THE BACTERIUM *Erwinia amylovora* (Burrill) Winslow

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Abstract.*Erwinia amylovora is a very dangerous plant pathogen bacterium that produces the disease named fire blight. Due to its gravity was mentioned in the category of the phytosanitary quarantine organism in the Annex II from H.G. 563/2007. The pathogen has entered on the territory of Romania in the '90 and slightly was expanded in all the fruit tree cultivation areas. In the last years there was noticed an increase of the frequency and intensity in apple, pear and quince. Erwinia amylovora represents a risk for the fruit tree sector, mainly in pear and apple but also in nurseries. The topic approached in this study is present-day and is looking to bring support for the fruit tree farmers from Banat's area because they are confronting often with problems that are regarding the pathogens control from the apple orchards and mainly the control of the fire blight that is almost impossible to control, mostly in the years with hot and humid weather. The main objective of the research was the monitoring of six apple varieties from a fruit tree farm from Banat, where the bacterium Erwinia amylovora is present for several years. There was monitored the response of these varieties to the attack of the bacterium during two years. The studied apple varieties monitored were Florina, Granny Smith, Generos, Mutsu, Idared and Golden Spur. The mentioned varieties have different resistance to the attack of fire blight. The obtained results following this research show that there is a relative great variability in the response of the varieties to the attack of the pathogen, this aspect being confirmed by other researches too. The age of the trees is very important. There was noticed that the younger trees have better resistance to the attack than the older ones. The most attacked varieties from the research were Idared, Mutsu and Golden Spur. The varieties Granny Smith, Florina and Generos were manifested as medium resistant (MR). The lowest attack intensity was registered in the variety Granny Smith (between 1% and 2% attacked shoots). The genetic resistance of the varieties from this research is supported by the obtained results, being very important in the management of the infections produced by Erwinia amylovora.*

Key words: *Erwinia amylovora, fire blight, varieties, genetic resistance, apple, intensity.*

INTRODUCTION

Fire blight (*Erwinia amylovora)* is a bacterial disease extremely harmful, being declared as phytosanitary quarantine organism. The bacterium is often found in the apple and pear orchards from worldwide, being reported until nowadays in European countries, North Africa, Mediterranean eastern side, North and Central America, New Zeeland. This pathogen hasn't been reported yet in Sount America and some areas from Asia and South Africa [VANESSTE, 2000; VAN DER ZWET *et al*., 2012]. In present the bacterium *Erwinia amylovora* is widely spread at global level in all the big fruit tree cultivating areas with few exceptions. In Europe is found in the following countries: Austria, Belgium, Bulgaria, Cyprus, Greece, Romania, Netherlands, Croatia, Czech Republic, Denmark, France, Germany, Hungary, Ireland, Italy, Leetonia,

Lithuania, Luxemburg, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden and Great Britain. According with SLETTEN and RAFOSS (2007), in Norway *E. amylovora* is present in few coastal locations, but hasn't reached yet the fruit tree cultivation areas.

Generally, the coordinated efforts for the limiting of the spread of *E. amylovora* bacterium done at the level of the member states and at regional level in the member states have succeeded to limit the present distribution or at least to delay the spread of the pathogen. Having in view the data regarding the present distribution of *E. amylovora* it can be assumed that this pathogen has a great spreading potential on the territory of EU [EFSA JOURNAL, 2014].

In Romania this bacterium has appeared after the year 1990 and has spread in all the fruit tree cultivation areas. In present this pathogen is declared phytosanitary quarantine organism and there are applied great efforts to prevent the appearance of the bacterium in the areas where the disease isn't still present. According with all of these, there was noticed that the bacterium conquer new areas in every year, being now present in the managed orchards, not only in the abandoned ones. During the last years was observed an increase of the attack frequency and intensity in apple-trees, pear-trees and quince-trees, mostly in the areas with abandoned orchards. According with VAN DER ZWET *et al.* (2012) the abandoned orchards are an important infection source.

Other researchers as is V. SEVERIN (1985) assumed that *Erwinia amylovora* is able to attack all the aerial parts of the host plants. The first symptom is usually the inflorescences burn mark and it appears early in spring. The disease progresses from flower to peduncle that appear as hydrozed and finally is turning to black. On the infected offshoots can appear small ulcerations and lesions. The leaves are fading and the entire offshoot is turning to brown in apple. After the inflorescences, the offshoots and small branches are the most sensitive organs of the plants to fire blight attack. Usually, the infection on offshoots progresses much faster, especially when the conditions are favourable to the development of the disease. There was noticed that in few days the infection can progress 10 – 30 cm or even more. The attacked offshoots are curving in top as a crook, being easily recognisable [PARASCHIVU M. *et al*., 2015]. In humid weather, except the flowering period, on the infected offshoots appear usually drops of bacterial exudates. The exudates can have variable colour from pure white to dark red, presenting different nuances of brown-yellow or orange. The infected offshoots with necrosed leaves appear as scorched by fire, as the disease name fire blight shows. In fruits the disease appears occasionally, mostly in the choky fruits and only exceptionally after harvesting. The diseased fruits in the case of apples are turning to black and are shrinking, remaining attached by branches, sometimes mummifying them.

In the control of the fire blight the most important are the prophylaxis measures. The use of fire blight resistant varieties is a very good strategy for the prevention of this disease. Unfortunately there aren't available apple varieties completely resistant to *Erwinia amylovora*. The most resistant varieties available in present have moderate sensitivity to low to the pathogen [THIBAULT *et* LE LEZEC, 1990; VAN DER ZWET *et* BEER, 1995]. In present there have been created transgenic apple varieties with high resistance level to fire blight as is the transgenic apple variety Royal Gala [NORELLI *et al.*, 2003; MALNOY *et al.*, 2004]. The transgenic apple varieties haven't been approved for cultivation in EU. The multiplication material must to be compulsory certified. The management strategy for the control of fire blight has as purpose the interferation with the key evolution stages from the disease cycle and the consideration of the inoculus sources, respectively the spreading mechanisms and the dynamics of the disease progress [JOHNSON *et* STOCKWELL, 1998].

The methods of cultural hygiene have in view the removal of the infected tissues by cutting during the vegetative repose and during the growing season to reduce the infection sources. Extra, there is compulsory the removal of the infected trees and of the wild host plants from the vicinity of the orchard [VAN TEYLINGEN, 2002].

Unfortunately, in present aren't known curative chemicals that control *E. amylovora* from the infected plants. The methods for the diminishing of the pathogen inoculus in the vegetative material have limitations: thermotherapy demands to destroy the bacterium, affecting the buds survival and the disinfectant treatments doesn't affect the endophyte inoculus [KECK e*t al.*, 1995; RUZ *et al*., 2008].

The main objective of this research was the monitoring of six apple varieties from an orchard from Banat area during two years where the bacterium *Erwinia amylovora* is present by several years. There was analysed the response to the pathogen attack. The observed apple varieties were: Florina, Granny Smith, Generos, Mutsu, Idared and Golden Spur. The analysed varieties have different resistance to the bacterium attack.

MATERIAL AND METHODS

The biological material consisted in six apple varieties that have been monitored during two years. During this period there was monitored their response to the attack of *Erwinia amylovora* bacterium in conditions of natural infection. The observed varieties are cultivated in fruit tree farm from western Romania, respectively Banat. The orchard is infected by several years with this bacterium because in the vicinity is an abandoned apple orchard. The monitored apple varieties were the following: Florina, Granny Smith, Generos, Mutsu, Idared and Golden Spur.

The attack frequency (F%) was set by examining 100 threes for every for every monitored variety using the classical formula from plant pathology. ***The virulence of the bacterium*** (I%) was set using the 0 - 9 scale. There have been analysed 10 trees for every variety from different sides of the orchard where the attack was greater. For every analysed tree have be numbered the offshoots turned to black and the fruits with symptoms (Figure 1). There wasn't registered attack to the inflorescences, but it is possible to be present but not obviously visible. The infection has been evolved considering the frequency of the trees with symptoms using the following scale: low attack -< 3% trees with symptoms; average attack among 3-10% trees with symptoms; strong attack among 10-30% trees with symptoms; very strong attack between 30-60% trees with symptoms; and extremely strong attack at more than 60% trees with symptoms.

The climatic data have been received from the Meteorological Station Sânnicolau Mare. The obtained results were processed using ANOVA.

RESULTS AND DISCUSSION

The attack of the bacterium *Erwinia amylovora* has been monitored in a fruit tree farm from Banat region in conditions of natural infection during the years 2017 and 2018. During this period there have been monitored six apple varieties (Granny Smith, Florina, Generos, Mutsu, Idared and Golden Spur) with different resistance to the attack of this bacterium. From the analysed varieties had been evidenced the varieties Golden Spur and Mutsu with low sensitivity to *Erwinia amylovora*. Average sensitivity was determined in the variety Granny Smith. The variety Idared is highly sensitive to this pathogen. This evaluation was done by other researchers during a longer period of time too [THIBAULT *et* LE LEZEC, 1990; MARTÍNEZ -

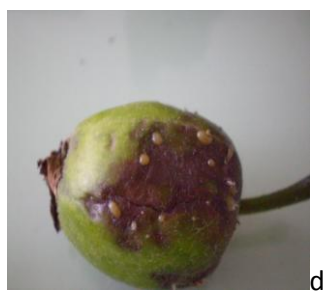
BILBAO *et al.*, 2009; VAN DER ZWET *et al.*, 2012]. Thus, in literature are numerous apple varieties classifications referring to the resistance to the attack of the bacterium.

The bacterium *Erwinia amylovora* is present by short time in the analysed apple orchard. The infections are favoured due to the inoculus source from the vicinity of the plantation, respectively an unmanaged apple orchard, which is almost totally damaged by the bacterium. To the realisation of the infection contribute the climatic factors too, mainly the rainfalls. The monitored years were very different from climatic point of view. Referring to rainfalls, the fallen amount during 2017 was 33.5 mm compared with the multiannual average of 541.4 mm from the area Sânnicolau Mare. The rainfall deficit registered during this year was 210.9 mm. Analysing the rainfall amount for Sânnicolau Mare area in 2017 we can assume that the deficit was greater than 25% (39%). According with the meteorologists, when the rainfall deficit is greater than 25% the classification of the year is **"exceptionally dry year"**. Thus, the spring, summer and winter were very dry in 2017. The temperature regime of the year 2017 has registered quite great positive deviations compared with the multiannual average. The calculated annual average was 12°C, greater with 1.5°C compared with the multiannual average of the area of 10.5°C.


a)


b)


c)


d)


e)

Figure 1. a), b) offshoots curved in top as a crook; c) mummified fruits due to the bacterium *Erwinia amylovora*; d), e) fruits with bacterial exudates [photo: Cotuna Otilia]

The rainfall amount from the first months of the year 2018 were greater compared with the multiannual averages. From the multiannual rainfall amount for Sânnicolau Mare (541.4 mm), have been fallen 235.80 mm during spring and winter. The temperature regime of the 2018 spring have been characterised by monthly average temperatures that have surpassed

strongly the multiannual averages except March when the average temperature registered was lower compared with the multiannual average with 1.7°C. The maximal monthly temperatures registered in the spring of 2018 had historical values. The monthly maximal value registered in March was 21.6°C on 31th March 2018. In April, the highest temperature registered was 29.4°C on 29th April 2018. The highest temperature registered in May 2018 was 30.4°C on 31th May 2018. Due to very high temperatures registered during the flowering period, the early flowering varieties have aborted the flowers and the pollen hasn't germinated. Thus, in the case of temperature minimal values have been registered uncommon values, much lower compared with the normal values. On 1st March 2018 was registered an exceptional minimal vale of the temperature, respectively -19.7°C. Due to the very low temperatures from March 2018 the flowers of several fruit tree species were frozen (peach, apricot). From the same reason the start in vegetation was delayed with about two weeks. Thus, *Erwinia amylovora* is quite resistant to drought and sunrays.

From the meteorological factors, rainfall seems to be the most important in the dissemination of the pathogen. The raindrops and mainly the windy rain realizes both the spread of the primary inoculus and the secondary inoculus too [SEVERIN V., 1985].

Table 1

Attack rate (GA%) of the bacterium *Erwinia amylovora* in apple during the years 2017-2018

Variety	GA% offshoots		GA% fruits		F% attacked trees	
	2017	2018	2017	2018	2017	2018
Florina	1.7	2.6	4.5	7.6	3	8
Granny Smith	1.2	1.9	7.2	5.4	2	6
Generos	2.6	3	14.7	22	5	11
Mutsu	2.8	3.5	18.9	24.5	4	10
Idared	3.2	4	15.68	28	8	16
Golden Spur	2.9	3.8	19	33.6	7	12

Table 2

Response of apple varieties to the attack of the bacterium *Erwinia amylovora* in conditions of application of chemical treatments (2017-2018)

Variety	GA% offshoots	Difference compared with control	Significance
Year 2017			
Idared (M)	3.2		
Granny Smith	1.2	-2	000
Generos	2.6	-0.6	00
Mutsu	2.8	-0.4	0
Golden Spur	2.9	-0.3	-
Florina	1.7	-1.5	000
LSD 5% = 0.3165; LSD 1% = 0.4499; LSD 0.1% = 0.6515 *0 – low significant; 00 - significant; 000 – highly significant; - non-significant*			
Year 2018			
Idared (M)	**4**		
Granny Smith	1.9	-2.1	000
Generos	3	-1	00
Mutsu	3.5	-0.5	-
Golden Spur	**3.8**	-0.2	-
Florina	2.6	-1.4	00
LSD 5% = 0.8409; LSD 1% = 1.1954; LSD 0.1% = 1.7309 *0 – low significant; 00 - significant; 000 – highly significant; - non-significant*			

In the monitored apple orchard the chemical treatments were done using chemicals with wide spectre of action. The used fungicides have been the following: Folicur SOLO 250 EW (*tebuconazole*), Chorus 50 (WG) - *ciprodinil*, Score 250 E (*difenoconazole*), Embrelia (SC) - *izopirazam* and *difenoconazole* and Luna Experience 400 SC (*fluopiram* and *tebuconazole*). During the vegetative repose period there were applied treatments with copper sulphate and calcium solution with a concentration rate of 3%. Many of the used fungicides have action mainly on apple scab and powdery mildew and less on *Erwiniei amylovora*.

The evolution of the fire blight in the monitored apple orchard is slow. The attack degree on offshoots registered in the analysed varieties was low and the differences between the two years were non-significant. The amplitude of the attack degree was comprised between 1.2% - 3.2% in 2017 and 1.9% - 4% in 2018. The lowest degree attack was registered in the variety Granny Smith and the highest in the variety Idared (Table 1). If in the case of the damaged offshoots hasn't been registered significant differences between the analysed years, in the case of fruit attack they have existed. In 2017, the varieties Granny Smith, Generos, Florina and Golden Spur have registered decreases of the infection compared with the control Idared in 2018 when the varieties Mutsu and Golden Spur have registered significant increase of the infection rate. These differences were strongly influenced by the different climate conditions from the two years.

The statistical analysis of the obtained results during the two experimental years shows significant differences in comparison with the control Idared (Table 2). The variety Idared was chosen as control because the literature mentions this variety as highly sensitive to the attack of the bacterium *Erwinia amylovora.*

The response of the six varieties to the fire blight attack was evaluated considering the frequency of the trees with symptoms. The frequency of the attacked trees has increased in

2018 compared with 2017. In 2017 the attack rate hasn't passed over 10% of trees with fire blight symptoms, oscillating between 2% in the variety Granny Smith and 8% in Idared. According with the registered frequency, the attack level was medium in five from all the six varieties. In the variety Granny Smith the attack was low (< 3% trees with symptoms). In 2018 the bacterium attack was assessed as medium in the varieties Florina and Granny Smith and strong in the varieties Generos, Mutsu, Idared and Golden Spur (> 10% trees with symptoms) (Table 1). The framing in the category strong attack is done in the interval 10 - 30 % trees with symptoms.

Following this research there was noticed that there is a quite great variability in the response of the varieties to the fire blight attack, this aspect being confirmed by other researches too. The tree age is an important factor for their sensitivity. There was noticed that the younger trees resist better to *Erwinia amylovora* attack in comparison with the older ones.

Fire blight is a very harmful disease of the apple-tree. In severe cases the disease spreads very fast and can kill an apple-tree even in three months. In favourable conditions the infections are spreading strongly, the rainfalls and temperatures of 23 – 27°C being optimal for the development of the disease.

In the monitored orchard during the two years the bacterium is present and spreads even in conditions of chemical treatments applying. There is recommended the identification of the new infections. In the infected trees, the attacked offshoots must to be cut at a distance of about 50 cm from the infection place. Sometimes, when the tree is totally affected it must to be removed and burned. The cuts can be done both in vegetation and in vegetative repose. The tools used for cutting must to be disinfected permanently with sodium hypochloride. The key treatments for the control of the bacterium must to be applied before flowering, during flowering and after flowering with certified chemicals. Usually the copper based products provide a good protection.

CONCLUSIONS

The most attacked varieties from this experience were Idared, Mutsu and Golden Spur, they being very sensitive to the bacterium attack. The less attacked apple varieties from this research were varieties Granny Smith, Florina and Generos. The year 2018 has favoured the attack of the bacterium *Erwinia amylovora.* The attack level in this year has oscillated between medium and very strong, depending by the genetic resistance of the varieties and climatic condition.

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