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ASSESSMENT OF THE BACTERIUM *ERWINIA AMYLOVORA* ATTACK ON SEVERAL PEAR VARIETIES (*PYRUS COMMUNIS* L.) AND THE INFLUENCE ON FRUITS SUGAR CONTENT

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Abstract

Proven to be the first pathogenic bacterium of plants, Erwinia amylovora causes Fire Blight which is nowadays one of the most devastating disease of apples and pears in many parts of the world, especially in temperate ones. The current paper assessed in natural conditions of infection in 2016 year, the impact of fire blight on four pear genotypes (cv. Napoca, Red Bartlett, Beurre Bosc, Curè) within a plantation located in the proximity of Craiova city, using Area under the Disease Progress Curve (AUDPC) and the impact of pathogen attack on fruits yield and sugars content. The response of tested pear genotypes to the fire blight attack ranged on a large scale of variability depending on the genotype resistance or sensitivity to disease and environmental conditions. The most susceptible pear variety to fire blight was Curè, which also proved the lowest yielding capacity and sugar content in fruits under fire blight impact. AUDPC values ranged from 164 to 376 with unfavorable impact on fruits yield and sugar content (R^2 = 0.9799; R^2 = 0.9557).

Key words*: Erwinia amylovora, AUDPC, Fire Blight, pear, sugar content.*

INTRODUCTION

Belonging to Rosaceae family, European pear (*Pyrus communis* L.) has been enjoyed since centuries worldwide for its desirable taste and highly nutritious value, being a rich source of important vitamins and minerals, flavonoid antioxidants, soluble and insoluble fibre, including prebiotics which promote digestive and heart health (Gayer et al., 2019; Navaei et al., 2019). Nowadays is one of the most economically important fruit species grown in Europe, North America and temperate regions of both two hemispheres of the Earth on different soils and environmental conditions (Cichi et al., 2008).

In 2018 world pear production has been reported as 23.733.772 tons (FAO 2018). Despite their economically importance, health benefits and costumers preference for fresh fruits, the most important factor that limits pear cultivation worldwide is the bacterium *Erwinia amylovora*, which develops the disease known as Fire Blight (FB). The pathogen is considered quarantine pest on the list of European and Mediterranean Plant Protection Organization (EPPO, http://www.eppo.org/QUARANTINE/quarante.htm). Currently phytosanitary control and early eradication of any Fire Blight are the best measures to delay disease spread and avoid losses.

Since first report about Fire Blight in the USA in the late 1700s, the disease has spread in more than 46 countries from America, Australia, Europe, Middle East, Africa and other regions where pear tree is gown, despite the control measures adopted (Denning, 1794; Jock et al., 2000; Bonn and Van der Zwet, 2000; Sestras et al., 2008; Peil et al., 2009; Braun-Kiewnick et al., 2011; Jock et al., 2013; Gaaliche et al., 2018). In Romania, Fire Blight symptoms were first observed in 1992 in the south region of the country (Severin et al., 1999).

Affecting pear, apple, quince and other rosaceous plants, Fire Blight causes serious fruits losses and even whole tree dieback,

especially in young orchards (Yom Din et al., 2007; Johnson et al., 2016; Gaaliche et al., 2018; Gaganidze et al., 2018). This epiphytic bacteria attacks parts or whole tree affecting blossoms, leaves, shoots, branches, fruits, and roots, being able to devastate pear trees within one season, especially on sensitive genotypes (Kuflik et al., 2008; Braun-Kiewnick et al., 2011; Gaganidze et al., 2018). The injuries have long-term impact because sometimes it is necessary to be removed large portions of the tree increasing the dieback risk of the whole tree. It can be spread easily by vectors (wind, rain, insects, birds), but also by contaminated pruning tools and infected plant material.

Many genetically studies have been done on different crop species about the impact of management measures and climate change on different plants traits and their ability to face the stress produced by different biotic and abiotic constrainers (Duncan and Howard, 2000; Loarie et al., 2009; Wittenberg et al., 2009; Burger at al., 2012; Johnson et al., 2012; Bonciu, 2018; Bonciu et al., 2018; Bonciu, 2019). However, despite biological, chemical and cultural methods, the use of resistant genotypes remains the most efficient way to control the disease (Aysan et al., 1999; Durel et al., 2003; Bell et al., 2005; Dondini et al., 2005; Stockwell et al., 2011; Montanari et al., 2016; Calis et al., 2017; Hashman et al., 2017; Kellerhals et al., 2017; Mertoğlu and Evrenosoglu, 2017).

The aim of the present study was to determine the response of four pear varieties to the attack of the bacteria *Erwinia amylovora* under natural infection in terms of the relationship between weather conditions, varieties susceptibility to Fire Blight and pathogen impact on pear fruits yield and sugar content. However, little research has been done on the impact of Fire Blight on the affected fruits quality.

MATERIALS AND METHODS

The experiment was conducted during 2016 year to individual trees in a randomized compete block design in four replicate blocks (10 pear trees/block) within a private pear orchard established in 2006 year (3.5 m between rows x 3.5 m between trees on row) in the proximity of Craiova city, Dolj county, Romania. A total of forty pear trees including four pear genotypes (cv. Napoca, Red Bartlett, Beurre Bosc, Curè) were assessed in natural conditions of infection for their response to the attack of the bacteria *Erwinia amylovora*. There was calculated the cumulative number of Fire Blight infections per each assessed pear tree. The quantitative determination of sugars content in fruits was done using digital refractometer (WYT-J 0-32% Chong Qing, China) and reported as degrees Brix, which is equivalent in percentage (Ball, 2006; Wei and Wang, 2013; Dongare et al., 2014). Total soluble solids (TSS) values obtained from the digital refractometer have been adjusted using the factor 0.85 which means that sugars are 85% of TSS. For the pathogen isolation and identification have been taken samples of diseased young shoots, flower clusters, leaves and fruits with visible symptoms of Fire Blight (necrosis, wilting, bacterial ooze), taken after symptoms were visible for each assessed pear tree from all genotypes.

Isolation of the pathogen was made from fresh samples (symptomatic shoots, flowers, leaves, fruits) according to the EPPO protocol (EPPO, 2013). Detection of the bacterium was done using PCR assays and MALDI-TOF mass spectroscopy protocols (Sauer et al., 2008; Wensing et al., 2012). For all assessed pear trees were determined Frequency (F%) and Intensity (I%) of Fire Blight attack. These parameters were used to calculate Attack Degree (AD%) using the formula: AD% = (F% x I%)/100 (Cociu and Oprea,1989). To estimate the response of pear genotypes to Fire Blight attack was used the scale 1 (no attack) to 9 (tree dead), corresponding to AD% classes. Also, for assessing the Fire Blight evolution and disease quantity on each pear tree included in the trail was used the Area under Disease Progress Curve (AUDPC), following the formula (Campbell and Madden, 1990):

$$\text{AUDPC} = \sum_{i=1}^{a}\left[\left\{\frac{Yi + Y(i+1)}{2}\right\} x(t(i+1) - ti)\right]$$

where, Yi = disease severity at each measurement; ti = time in days of each measurement; n = number of Fire Blight (FB) assessments. AD% was used to assess disease severity at each measurement.

The fruits yield for each assessed pear tree was calculated using the formula: number of fruits/tree x average weight of the fruit.

RESULTS AND DISCUSSIONS

Since the first report regarding the occurrence of Fire Blight in Romania in 1992 (Severin et al., 1999), the disease has been spread in all regions of the country (mostly in the south and south east) with variable intensity. In the climatically conditions of 2016 year inspections in the pear orchard have been performed periodically during the growing season in order to identify typical symptoms of fire blight, assuming an infection occurred. Scouting of the disease has started for each pear genotype during blooming and continue in other three moments on leaves, shoots and fruits, because the meteorological conditions were favourable to Fire Blight development. Necrotic symptoms of Fire Blight have been observed on all pear genotypes assessed (Figure 1).

Figure 1. Fire Blight attack symptoms on pear (dry and necrotic leaves, blight, affected fruits found on diseased branches) (original photo)

For scouting optimization and to predict the disease development, rainfalls and temperatures were taken into account. Thus, climatic conditions of 2016 year favoured the infection with *Erwinia amylovora* and further Fire Blight development. Humidity was determined by the amount of rain of 825.8 mm, comparatively with multiannual average rainfall of 585.4 mm, while the average temperature was 12.4ºC comparatively with multiannual average temperature of 10.8ºC (Figure 2).

During periods of high humidity and warm temperature affected tissues of leaves, shoots and fruits became water soaked and dull, covered with small droplets of bacterial ooze rich in polysaccharide, which creates a matrix that protects the pathogen on plant surfaces and attracts insects that disseminate the pathogen.

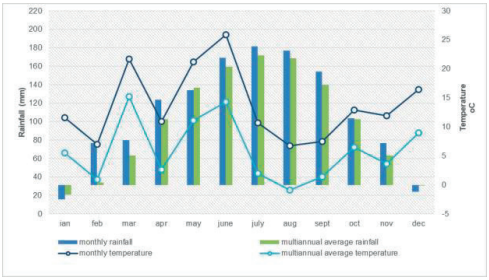

Figure 2. Weather conditions during the study period (2016 year)

The severity of the disease was noticed by Attack Degree (%) which was calculated for each scouting and introduced in the formula of AUDPC. All pear genotypes correspond to different classes for their response to Fire Blight attack. The response of tested pear genotypes to the fire blight attack ranged on a large scale of variability depending on the genotype resistance or sensitivity to disease and environmental conditions. Thus, appreciation scale indicates class 3 (low attack) for Beurre Bosc, class 5 (supra medium attack) for Napoca, class 6 (strong attack) for Red Bartlett and class 7 (very strong attack) for Curè (Table 1).

Table 1. Attack appreciation scale to Fire Blight (*Erwinia amylovora*) (Sestras et al., 2008)

Class	Attack appreciation	Attack degree (AD%)
1	No attack	0
2	Very low attack	0.1-5.0
3	Low attack	5.1-10.0
4	Medium attack	10.1-20.0
5	Supra medium attack	20.1-40.0
6	Strong attack	40.1-60.0
7	Very strong attack	60.1-80.0
8	Extreme strong attack	80.1-99.9
9	Complete scorching (trees dead)	100

The results confirms that all four pear genotypes included into the study are susceptible to *Erwinia amylovora* attack, which confirms the previous research (Zwet and Beer, 1995; Sestras, 2004; Montanari et al., 2016; Calis et al., 2017; Hashman et al., 2017; Kellerhals et al., 2017; Mertoğlu and Evrenosoglu, 2017). The most susceptible to Fire Blight (FB) was Curè, which also proved the lowest yielding capacity and sugar content

in fruits under Fire Blight impact (Table 2). The genotype Beurre Bosc has recorded lowest AUDPC value and the highest yield and fruits sugars content.

Table 2. The response of pear genotypes to Fire Blight (FB) attack and the impact on fruits yield (t/ha) and sugars content (%)

Pear Genotype	2016		
	AUDPC	Yield (t/ha)	Sugars (%)*
Napoca	210	9.75	17.8
Red Bartlett	312	6.4	16.7
Beurre Bosc	167	10.2	18.1
Curè	376	5.15	15.3

*sugars (sucrose, glucose and fructose)

For all pear genotypes assessed for their behaviour to Fire Blight attack it was noticed a very significant negative correlation between AUDPC values and fruits yield (R^2 = 0.9799) (Figure 3).

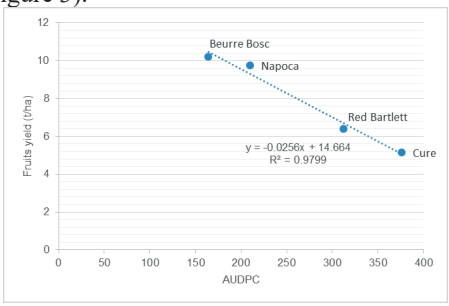

Figure 3. Relationship between Fire Blight AUDPC and pear fruits yield in 2016 year

Also it was found a very significant negative correlation between AUDPC values and fruits sugars content (R^2 = 0.9557) (Figure 4).

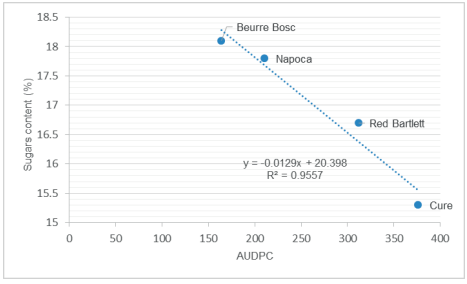

Figure 4. Relationship between Fire Blight AUDPC and pear fruits sugars content in 2016 year

However, effective management of Fire Blight is complex and largely preventative. It requires a combination of sanitation, cultural practices and chemical or biological control to keep the disease in check.

CONCLUSIONS

The present study was carried out to assess the response of four different pear genotypes to the attack of Fire Blight (*Erwinia amylovora*) in natural infections and to evaluate the impact of the pathogen attack on pear fruits and sugar contents. The response of tested pear genotypes to Fire Blight attack ranged on a large scale of variability depending on the genotype resistance or sensitivity to disease and environmental conditions. The most susceptible pear variety to Fire Blight was Curè, which also proved the lowest yielding capacity and sugar content in fruits under fire blight impact. AUDPC values ranged from 164 to 376 with unfavourable impact on fruits yield and sugar content (R^2 = 0.9799; R^2 = 0.9557).

Beside breeding programs focused on identifying sources with durable resistance to Fire Blight, severe quarantine measures attempt to reduce the disease in pear orchards and especially in private gardens. Also, monitoring of Fire Blight on ornamental plants such as *Crataegus* sp., *Sorbus* sp., *Amelanchter*, wild *Malus* and *Pyrus* sp., during the growing season when the symptoms can be visible, is effective to avoid a subsequent spread of the disease.

The obvious conclusion is that the most effective methods to control Fire Blight are section for resistance and optimization of scouting in pear orchards during growing season in order to reduce yield losses and impairment of fruit quality.

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