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# INFECTION OF *ERWINIA AMYLOVORA* ON DIFFERENT APPLE VARIETIES AND THE IMPACT ON FRUITS QUALITY

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***Abstract***

*Fire Blight (FB) caused by Erwinia amylovora is the most feared bacterial disease in the genus Malus and other genera belonging to Rosaceae family, causing severe losses in orchards in favourable years, unmarketable fruits quality and even completely trees dieback, especially in highly susceptible genotypes. Currently E. amylovora is of quarantine concern in many countries all over the world and continue to conquer new territories. During 2018-year, six apple genotypes ('Golden Delicious', 'Idared', 'Gala', 'Jonagold', 'Fuji' and 'Jonathan'), within an orchard located in the proximity of Craiova city, Romania, have been assessed for their response to the infection to Erwinia amylovora and the impact of the pathogen attack on fruits yield and quality. The response of apple genotypes to the Fire Blight (FB) attack ranged from moderately resistant/susceptible ('Golden Delicious' and 'Fuji') to highly susceptible ('Idared', 'Gala', 'Jonagold' and 'Jonathan') depending on genetic background and environmental conditions. Fruits chemical parameters Total Soluble Solids (TSS), Total acidity (TA), Total Sugar (TS) were significantly affected by the pathogen attack in all apple varieties.*

***Key words***: *apple, AUDPC, Erwinia amylovora, Fire Blight, fruits quality.*

## INTRODUCTION

Originated in Central Asia, apple trees (*Rosaceae* family, genus *Malus*, species *Malus domestica* Borkh.) have been grown for thousands of years in Europe and Asia and are the most widely grown species in the genus *Malus* worldwide. Nowadays, aside other spontaneous and cultivated plants and trees in temperate regions, apples are in the first place among fruits in Eastern Europe, where they meet favourable climatic conditions for thousands of varieties, resulting in a range of colours, tastes, textures and chemical properties (Wilton, 2001; Blažek and Hlušičková, 2003; Sansavini et al., 2004; Rosculete and Rosculete, 2017; Bushal et al., 2018; Viškelis et al., 2019; Łysiak et al., 2020; Prundeanu et al., 2020; Răduțoiu, 2019; Răduțoiu, 2020; Răduțoiu and Cătuțoiu, 2020; Răduțoiu and Cosmulescu, 2020).

Considered to be a major functional food resource, apples are one of the most desirable fruits, being rich in vitamins, fibre, antioxidants, phytochemicals associated with human health benefits (Wolfe et al., 2003; Charde et al., 2011; Hyson, 2011; Ahmad et al., 2020; Bondonno et al., 2020; Li et al., 2020).
Rapid progress in breeding, in biotechnology in functional food and nutraceuticals has provided insight into disease resistance, genetic basis of food quality, improving favourable traits in various crops and products (Gardiner et al., 2007; Marić et al., 2010; Gardiner et al., 2012; Lusser et al., 2012; Broggini et al., 2014; Celton et al., 2014; Bonciu, 2020).
In 2019 world apple production has been reported as 87.236.221 tons (FAO 2019). Factors that contribute to increased apple demand include new varieties, population growth, products that meet healthy lifestyle, food diversification and rising incomes.

Despite their health benefits and costumer's preference for fresh fruits, the most important factor that limits apple cultivation worldwide is the necrogenic and highly infectious Gram-negative bacterium *Erwinia amylovora*, which develops the disease known as Fire Blight (FB) (Winslow et al., 1920).
The pathogen is considered quarantine pest on the "black" list of European and Mediterranean Plant Protection Organization (EPPO, http://www.eppo.org/QUARANTINE/quarantine.htm). Currently phytosanitary control and early eradication of any Fire Blight are the best measures to delay disease spread and avoid losses (Braun-Kiewnick et al., 2011).
Since its discovery in the USA in the late 1700s, despite the control measures adopted, Fire Blight has spread in more than 50 countries from America, Australia, Europe, Middle East, Africa and Asia recently (Denning, 1794; Jock et al., 2000; Bonn and Van der Zwet, 2000; Jock et al., 2013; Gagnidze et al., 2018; Zhao et al., 2019), affecting pear, apple, quince and other rosaceous plants. In Romania, Fire Blight symptoms were first observed in 1992 in the south region of the country, affecting apple, pear, quince and ornamental shrubs such as *Cotoneaster*, *Crataegus* and *Pyracantha* (Severin et al., 1999; Amzăr and Ivănescu, 2003).
Fire Blight affects blossoms, leaves, shoots, branches, causing serious fruits losses and even whole tree dieback, especially on sensitive genotypes (Kuflik et al.,2008; Braun-Kiewnick et al., 2011; Gaganidze et al., 2018). When fruits are not lost due to the pathogen attack, they have reduced size and quality (Đorđević et al., 2019; Emeriewen et al., 2019; Paraschivu et al., 2020).
The bacterium can be spread easily by vectors (wind, rain, insects, birds), but also by contaminated pruning tools and infected plant material.
Despite management, phytosanitary control, biological, chemical and cultural methods, the use of resistant genotypes remains one of the most efficient method to limit losses in fruit trees and other cultivated crops (Peil et al., 2009; Matei, 2011; Matei and Rosculete, 2011; Partal et al., 2013; Popa et al., 2013; Paraschivu et al., 2014; Partal et al.,2014; Calis et al., 2017; Hashman et al., 2017; Kellerhals et al., 2017; Marin et al., 2018; Cotuna et al., 2020a; Cotuna et al., 2020b; Partal and Paraschivu, 2020; Pompili et al., 2020; Tegtmeier et al., 2020).
The present study aimed the response of six apple varieties to the attack of the bacterium *Erwinia amylovora* under natural infection in terms of the relationship between weather conditions, varieties susceptibility to Fire Blight and the bacterium impact on apple fruits yield and quality.

## MATERIALS AND METHODS

The focus of the experiment was to evaluate the response of ninety-six fruit trees including six apple genotypes (cv. 'Golden Delicious', 'Idared', 'Gala', 'Jonagold', 'Fuji' and 'Jonathan'') to the attack of the bacteria *Erwinia amylovora* assessed in natural conditions of infection.
The experiment was conducted during 2018 year to individual trees in a randomized compete block design in four replicate blocks (24 apple trees/block) within a private apple orchard established in 2008 year (3.5 m between rows x 3.5 m between trees on row) in the proximity of Craiova city, Dolj county, Romania. There was calculated the cumulative number of Fire Blight infections per each assessed apple tree.
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 apple 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 apple 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). Then the AD% was used to assess disease severity at

each measurement and three consecutive AD values were used to calculate Area under Disease Progress Curve (AUDPC), which shows the evolution and disease quantity on each apple tree included in the trail, 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 (AD%) at each measurement; ti = time in days of each measurement; n = number of Fire Blight (FB) assessments.

To estimate the response of apple genotypes to Fire Blight attack was used the scale 1 (no attack) to 9 (tree dead), corresponding to AD% classes.

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

The juice from apple fruits with no visible symptoms (but taken from apple trees affected by Fire Blight) was extracted at ambient temperature (29°C ± 1°C) using a domestic juice extractor and was filtered. Total Soluble Solids (TSS) of filtered juice was done using a digital refractometer (WYT-J 0–32% Chong Qing, China) and reported as degrees Brix, which is equivalent in percentage (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 (TS) are 85% of TSS. Total sugars (TS) content was determined by Luff-Schoorl method and presented in percent (%).

Titratable Acidity (TA) was determined in a water extract of a weight amount of homogenized apple tissue using standard titration method, titrating to pH 8.1 with 0.1 NaOH (AOAC, 2019). The results of Titratable Acidity (TA) were expressed as g/100 g apple tissue calculated as malic acid at harvest. All analysis was carried out three times and the results are mean values. The experimental data were calculated and analysed, using MS Office Excel 2010 facilities.

## RESULTS AND DISCUSSIONS

Since its discovery Fire Blight has been considered the most destructive disease of apple and pear fruit trees, being included recently in the top 10 plant pathogenic bacteria worldwide (Akhlaghi et al., 2021).

In Romania the disease has been spread in all regions of the country (mostly in the south and south east) with variable intensity.

Marin et al. (2018) showed that in the south of Romania the years 2016-2018 were very favourable for the Fire Blight attack on apples.

The inspections in the evaluated apple 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 apple genotype during blooming and continue in other three moments on leaves, shoots and fruits. Necrotic symptoms of Fire Blight have been observed on all apple genotypes assessed (Figure 1).

Figure 1. Fire Blight attack symptoms on apple (shoot blight symptoms and affected fruits found on diseased branches)
(original photos Cotuna O. and Paraschivu M., 2018)

For scouting optimization and to predict the disease development, rainfalls and temperatures were taken into account.

Thus, climatic conditions of 2018 year favoured the infection with *Erwinia amylovora* and further Fire Blight development.

Humidity was determined by the amount of rain of 908.5 mm, comparatively with multiannual average rainfall of 585.4 mm, while the average temperature was 12.6°C comparatively with multiannual average temperature of 10.8°C (Figure 2).

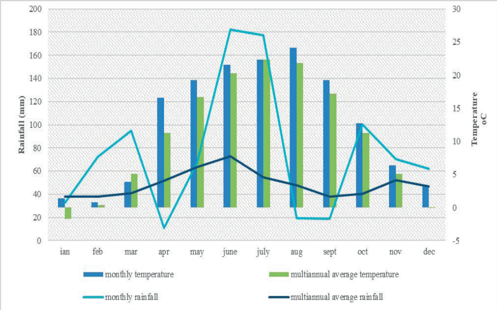


Figure 2. Weather conditions during the study period (2018 year)

These high humidity and warm temperature make young apple fruitlets to 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 3).

Figure 3. Apple fruitlets with creamy amber small droplets of bacterial ooze under humid conditions (original photos Cotuna O. and Paraschivu M., 2018)

The severity of the disease was noticed by Attack Degree (AD%) which was calculated for each scouting and introduced in the formula of AUDPC.
All apple genotypes correspond to different classes for their response to Fire Blight attack.
The response of tested apple 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 ('Golden Delicious'), class 4 ('Fuji'), class 5 ('Jonagold') class 6 ('Idared', 'Gala' and 'Jonathan') (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 susceptibility to Fire Blight of the six apple genotypes included in the study confirm previous research which emphasized that some cultivars may appear highly or moderately susceptible in one year and moderately resistant in the other (Lee et al., 2010; Kostick et al., 2019; Pompili et al., 2020).
The most susceptible genotypes to Fire Blight (FB) were 'Jonathan', 'Idared' and ''Gala' which also proved the lowest yielding capacity under Fire Blight impact (Table 2).
The best response to Fire Blight was recorded by 'Golden Delicious' and 'Fuji'.

Table 2. Classification of apple genotypes according with their resistance/susceptibility to Fire Blight (FB) attack and the impact on fruits yield (t/ha)

| Apple Genotype | 2018 | | |
|---|---|---|---|
| | Class* | AUDPC | Yield (t/ha) |
| Golden Delicious | 3 MS/MR | 176 | 22.4 |
| Fuji | 4 MS/MS | 158 | 21.6 |
| Jonagold | 5 MS/MS | 264 | 20.1 |
| Idared | 6 HS/MS | 361 | 15.8 |
| Gala | 6 MS/HS | 327 | 19.2 |
| Jonathan | 6 HS/HS | 438 | 16.1 |

* MS = moderate susceptible; MR = moderate resistant; HS = high susceptible.

The value of determination coefficient ($R^2$ = 0.8757), for all apple genotypes assessed, indicated that up to 87% of variation in apple yield could be explained by AUDPC variability.
It was noticed a significant correlation between AUDPC values and fruits yield (r = -0.9358*). (Figure 4).
Yield loses due to Fire Blight in apples were also reported previously by different authors (Longstroth, 2001; Norelli et al., 2003; Zwet et al., 2012)

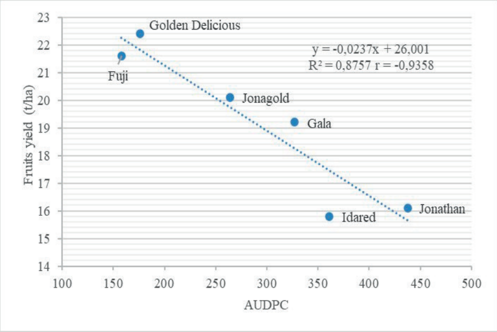


Figure 4. Relationship between Fire Blight AUDPC and apple fruits yield in 2018 year

The AUDPC had significant impact on the variation in Total Soluble Solids (TTS), Total Sugars (TS) and Titratable Acidity of apple fruits provided from the trees affected by Fire Blight. Thus, up to 82% of variation in TTS could be explained by AUDPC variability ($R^2$ = 0.8209), being recorded also significant correlation between TTS and AUDPC (r = -0.9060*) (Figure 5).

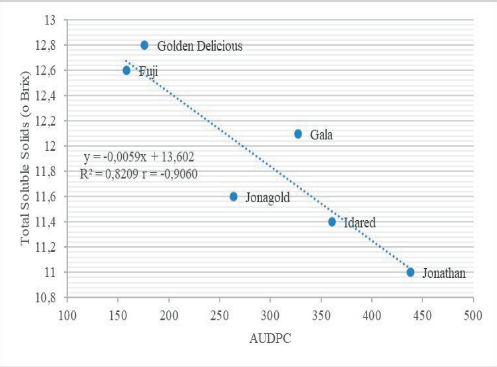


Figure 5. Relationship between Fire Blight AUDPC and Total Soluble Solids (TTS) in apple fruits in 2018 year

It was found that up to 78% of variation in Total Sugars (TS) content ($R^2$ = 0.7826) could be explained by AUDPC variability, being reported also significant correlation between TS and AUDPC (r = -0.8847*) (Figure 6).
Together with sugars the acidity has a profound effect on the perception of apple fruits quality and therefore a proper balance between these two quality parameters makes a desirable apple variety.

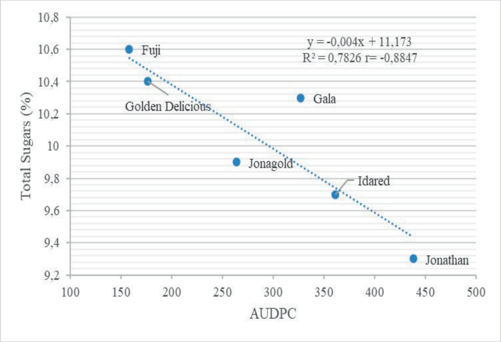


Figure 6. Relationship between Fire Blight AUDPC and Total Sugars (TS) in apple fruits in 2018 year

In apples the predominant acid is malic acid, representing 80-90% of total organic acids and contributes to the sourness of the fruits (Wu et al., 2007; Zhang et al., 2010).
The results from the experiment showed that the Fire Blight had a significant impact on Titratable Acidity (TA) in apples leading to increased values for all genotypes. Approximatively 71% of variation in Titratable Acidity ($R^2$ = 0.7104) could be explained by AUDPC variability and it was noticed also significant correlation between TS and AUDPC (r = 0.8428*) (Figure 7).

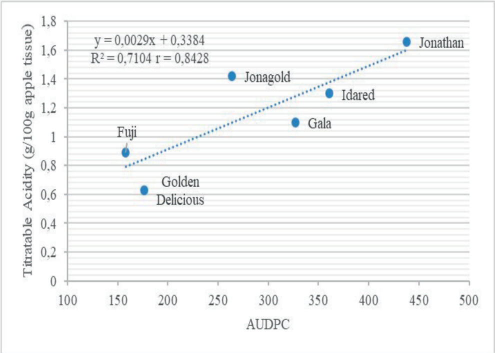


Figure 7. Relationship between Fire Blight AUDPC and Titratable Acidity (TA) in apple fruits in 2018 year

The apple fruits provided from the apple trees with the highest AUDPC values showed higher levels of acidity and lower levels of sugars, being noticed a significant correlation between these two quality parameters (r = -0.8913*) (Figure 8).

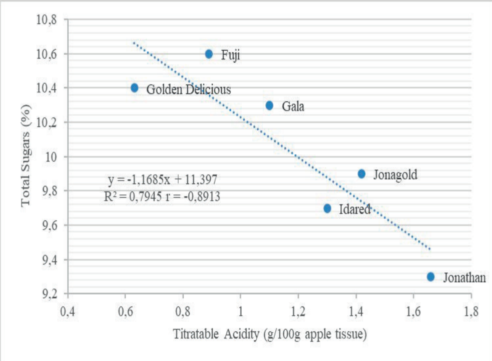


Figure 8. Relationship between Titratable Acidity (TA) and Total Sugars (TS) in apple fruits from the genotypes affected by Fire Blight in 2018 year

However, the results of the experiment show that Fire Blight is a serious concern for yield and fruits quality and even for trees live itself, while effective management of Fire Blight is complex and engage large resources. Therefore, Fire Blight management requires a combination of sanitation, cultural practices chemical or biological control and genetic resistance to keep the disease in check.

## CONCLUSIONS

The present study was carried out to assess the response of six apple genotypes to the attack of Fire Blight (*Erwinia amylovora*) in natural infections and to evaluate the impact of the pathogen attack on yield and apple fruits quality during 2018 year. The response of apple genotypes to the Fire Blight (FB) attack ranged from moderately resistant/susceptible ('Golden Delicious' and 'Fuji') to highly susceptible ('Idared', 'Gala', 'Jonagold' and 'Jonathan') depending on genetic background and environmental conditions.

The values of Area under Disease Progress Curve (AUDPC) ranged from 176 to 438. The best behaviour to Fire Blight was recorded by 'Golden Delicious' and 'Fuji', while the lowest behaviour was recorded by 'Jonathan'. Fruits chemical parameters Total Soluble Solids (TSS), Total acidity (TA), Total Sugar (TS) were significantly affected by the pathogen attack in all apple varieties. The apple genotypes that recorded the highest AUDPC values had the highest acidity values and the lowest sugars content.

The monitoring of Fire Blight and severe quarantine measures may lead to the Fire Blight control especially in private gardens and wild *Malus* and *Pyrus* sp., which will optimize yields and fruits quality beside breeding programs.

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