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Comparative study of some Romanian and foreign apple cultivars response to natural infections with *Erwinia amylovora* (Burrill.)

Studiu comparativ al răspunsului unor soiuri românești și străine de măr, la infecțiile naturale cu *Erwinia amylovora* (Burrill.)

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ABSTRACT

The bacterium *Erwinia amylovora* is one of the most important pathogens of apples, causing significant damage. During the experimental years, 2021 and 2022, both the temperature and humidity in summer months, were favourable for the occurrence of fire blight in areas with a temperate climate. The absence of major genes for resistance to fire blight makes it difficult to improve apple cultivars in terms of this characteristic. Therefore, studies regarding the behaviour of apple cultivars, in certain environmental conditions, have kept their relevance. The behaviour of the cultivars studied was compared with that of 'Auriu de Bistrița', previously known to be susceptible to fire blight. Ten of the twelve cultivars performed well in 2021 and 2022 The results showed that 'Jonaprim' was not affected by *Erwinia amylovora* while other Romanian or foreign cultivars were sporadically affected. 'Auriu de Bistrița' presented a higher frequency of specific symptoms, than all the other cultivars.

Keywords: bacterial disease, fire blight, temperate climate, necrotic shoots

REZUMAT

Bacteria *Erwinia amylovora* este unul dintre cei mai importanți patogeni ai mărului cauzand pagube importante. În timpul anilor experimentali, 2021 și 2022, în care s-a desfășurat studiul, atât temperatura cât și umiditatea, din lunile de vară, au fost propice pentru apariția de foc bacterian în zonele cu climat temperat. Dată fiind absența genelor majore de rezistență la focul bacterian, este dificil de ameliorat această caracteristică a soiurilor de măr. Prin urmare, studiile privitoare la comportamentul soiurilor, în anumite condiții de mediu, și-au păstrat relevanța. Comportamentul soiurilor studiate a fost comparat cu cel al soiului Auriu de Bistrița, cunoscut ca fiind sensibil la foc bacterian, iar zece din cele douăsprezece soiuri s-au comportat bine în 2021 și 2022. Soiul Jonaprim nu a fost afectat în timp ce celelalte soiuri românești sau străine au fost afectate doar sporadic. Soiul Auriu de Bistrița a prezentat procente mai ridicate ale frecvenței simptomelor specifice decât toate celelalte soiuri.

Cuvinte cheie: boală bacteriană, foc bacterian, climat temperat, lăstari necrozați

INTRODUCTION

Rosaceous fire blight affects more than 180 species of fruit trees and shrubs that belong to the *Rosaceae* family around the world (Vanneste et al., 2000). It can cause severe damage to flowers, fruits, young shoots, leaves, branches, trunks, and root collars (Aktepe and Aysan, 2022). Fire blight distinguishes itself among fruit tree diseases by its destructiveness (Gaganidze et al., 2020), causing significant economic loss in apple orchards (*Malus domestica* Borkh.). The first mention of its occurrence was in 1780 in the State of New York (Van der Zwet and Beer, 1999).

The disease fire blight affects not only the current production, but it can also have a subsequent negative effect on next year's production by affecting fruit spurs, debilitating branches and, in the end, the whole tree (Van der Zwet and Beer, 1999). The bacteria *E. amylovora* infects over 75 species of trees and shrubs, including ornamental plants as well: crabapple (*Malus sylvestris* (L.) Mill., *Rubus* spp., (*Photinia* spp., ornamental pear (*Pyrus calleryana* Decne.), mountain ash (*Sorbus* spp.), *Pyracantha* spp., *Cotoneaster* spp., loquat (*Mespilus japonica* Thunb.), hawthorn (*Crataegus* spp.), quince (*Cydonia oblonga* Mill. and *Chaenomeles x superba* Fahm.). Raspberry (*Rubus idaeus* L.) is also susceptible to specific races of *E. amylovora* (Burrill.) but those races do not infect apples (*Malus domestica* Bork.) or pears (*Pyrus communis* L.) (Severin and Iliescu, 2006).

All these facts make the disease widespread, leading to very serious consequences for all Rosaceaous plants cultivation, in many countries of the world. Especially in the 20th century, the country of origin, the United States, has repeatedly suffered significant losses. The total value of those losses is very difficult to estimate but certainly being millions of dollars in the years in which fire blight occurs (Van der Zwet and Beer, 1999).

Plants can become infected by *E. amylovora* in specific ways. This pathogen can exist in the orchard without showing any symptoms in the trees. The infection occurs when the bacteria is transported by bees and enters in flowers during pollination or through surface injuries caused by insect feeding, hail or mechanical damage. The bacterial exudate can be dispersed locally by wind, rain, insects and birds.

The infection involves the penetration of the bacteria into the tissues of the plants and the transition from an epiphytic lifestyle to an endophytic one, inside the plant (Zeng et al., 2021). This event occurs when the environmental conditions become favourable: air temperature above 18 °C and relative humidity over 80%. In the following period the infection begins to manifest itself on the organs of the tree. Not only environmental factors contribute to the occurrence of infections, but also the characteristics of the trees such as the age of a tree, growth stage, or cultivar. Given the multiple factors involved in the disease occurrence, the patterns are very variable.

The response of the trees is determined by the very complex genetic mechanism of resistance to *E. amylovora*. Previous studies suggest there are no major genes involved in the genetic mechanism of resistance to *E. amylovora* (Khan et al., 2011), but it involves several regulatory or structural genes and different pathways. Some of the resistant cultivars have a hypersensitive reaction, which is a form of programmed cell death in response to the pathogen. The structural genes linked to *E. amylovora* resistance are involved in the synthesis of different proteins. Those proteins seem to be part of the defense against this bacterium. The structural genes are also coding the enzymes involved in the synthesis of terpenoids and flavonoids, known to be effective antimicrobial agents, etc. (Markiewicz and Michalczuk, 2015). Apparently, the characteristics of the cultivars related to the suberization of the leaf cuticle are also of great importance in the manifestation of resistance to *E. amylovora*.

Considering all these aspects related to the genetic source of resistance to *E. amylovora* it is much more difficult to obtain resistant cultivars to *E. amylovora* than to other pathogens such as apple scab caused by *Venturia inaequalis*. The major genes that induce the resistance to apple scab are determined by major genes that are known and have been used in the breeding of apple cultivars (Guzu et al., 2022; Rosu-Mares et al., 2023).

However, there have been identified certain loci, in the apple genome, that are related to resistance to fire blight. The molecular markers that help to identify valuable genotypes from this point of view are available. Furthermore, these sources of resistance can be used in marker-assisted breeding programs. The cultivars obtained in this program, after inoculation with *E. amylovora*, show lower sensitivity than cultivars without resistance such as 'Gala' (Kellerhals et al., 2014). Unfortunately, these facts do not ensure complete resistance to fire blight.

Therefore, performing experiments that involve on-field observations of the behavior of apple cultivars remains indispensable and a valuable tool for selecting those valuable for apple breeding programs or cultivation. Several previous researches, aimed at evaluating the behavior of some apple cultivars to fire blight infections through artificial inoculation with the bacteria *E. amylovora* in the laboratory (Markiewicz and Michalczuk, 2015) or in-field (Sillernova et al., 2014). Our study aimed to observe the behavior of some Romanian and foreign apple cultivars, in field conditions, under natural *E. amylovora* infection in order to test and select the most appropriate Romanian apple cultivars for large-scale cultivation.

MATERIAL AND METHODS

Experimental setting

The Fruit Research and Development Station Bistrița is situated in the northern part of Romania on the hills of Bistrița, in the proximity of the Carpathian Mountains. The climate is temperate-continental with hot summers and cold winters. In this area, apples are the main fruit species grown, according to the Romanian National Institute of Statistics (INSSE, 2023).

Fifteen apple cultivars have been studied in this research, ten of them are of Romanian origin: 'Auriu de Bistrița', 'Generos', 'Salva', 'Bistrițean', 'Starkprim', 'Jonaprim', 'Goldprim', 'Alex', 'Dany', 'Doina' and five of them are well-known foreign cultivars: 'Jonathan', 'Florina' 'Golden Delicious', 'Idared' and 'Starkrimson'. The trees were planted in 2000, covering 6.15 ha, at a planting distance of 1.5 m x 4 m, resulting in a density of 1667 trees/ha.

In the experimental lot, a conventional pest management program was applied every year. Four or five treatments, respectively, were applied against plant pathogen bacteria in 2021 and 2022. The bactericide substances used were: copper oxycloride (1140 g/ha) in winter sprays, copper hydroxide (200 g/ha) in spring or summer and aluminium fosetyl (2400 g/ha) during bloom.

In-field observations

Observations were performed each year at the main phenological stages of apple that are critical for the infection and the development of symptoms. The first set of data was collected at bloom (BBCH 61 according to Meier et al., 2001), then on different stages of fruit growth (BBCH 72 – fruit size up to 20 mm, BBCH 76 – fruit size 60% final size). All specific symptoms known to appear on infected apple plants were assessed: flower blight, wilting or necrosis of shoots in the specific form of 'shepherd's crook', presence of bacterial exudate on organs, colouring of tissues, and bark necrosis. The main symptoms observed were wilting and necrosis of young shoots and the frequency of this symptom was used to assess the susceptibility of the cultivars. The frequency was determined by observing 300 shoots randomly taken from each cultivar, at every phenological stage mentioned above in every year. In most of the cases, the affected shoots had bacterial ooze on them when observations were performed.

Statistical analysis

The data was analysed using the software XLSTAT by Addinsoft (version 2019.3.2) (Addinsoft, 2022), which utilizes the Microsoft Office Excel platform. The XLSTAT program was used to perform the analysis of variance (ANOVA) (Fisher, 1925) of the fire blight frequency detected in the experimental plot.

Afterwards, Duncan's Multiple Rage Test (Duncan, 1955) was used to determine significant differences between the cultivars at $P < 0.0001$ in terms of fire blight damage frequency.

To test whether there were differences between the data from the two years in terms of fire blight damage degree, a two-sample two-tail t-test (William, 1908), was conducted using the XLSTAT application, with a significance level alpha of 0.05.

RESULTS AND DISCUSSION

Climate conditions usually have a great influence on the evolution of plant diseases and fire blight is not an exception. Although, in the scientific literature (Severin and Iliescu, 2006; Zeng et al., 2021) flowers are considered to be the most susceptible organs of the tree and the first ones on which *E. amylovora* damage manifests, in this case, the flowers have not presented disease symptoms.

The low average temperatures in May, in both years (Table 1) delayed the onset of symptoms until the phenological stage of rapid growth of shoots in June (Figure 1).

In both years, the damage began to manifest in the third decade of June. This might be explained by the average temperatures in June which were above the minimum temperature for the development of fire blight symptoms, ranging between 16-18 °C (Severin and Iliescu, 2006).

As it can be seen in Table 1 both the number of rainy days and the amount of rainfall were higher in 2021 than in 2022. In both analysed months the average daily temperatures were higher by almost 2 °C in 2022 than in 2021 so it can be noted that the two years were climatically different.

Figure 1. Wilting with bacterial exudate and necrotic shoots, caused by *E. amylovora* on 'Auriu de Bistrița', 24 June 2022 (Photo: author's collection)

Table 1. Main meteorological parameters in May and June 2021 and 2022

Year of study	Rainfall (l/m^2)		Number of rainy days		Average temperature (°C)	
	May	June	May	June	May	June
2021	98.90	99.00	18	17	13.44	18.75
2022	70.50	18.00	9	7	15.24	20.17

The damage consisted of wilted (Figure 1) or necrotic shoots presenting an orange ooze of bacterial exudate. After assessing the data regarding the frequency of the damaged shoots at BBCH 72 all of them were removed from the trees and then another series of observations were performed at BBCH 76 and BBCH 78 – fruit size 80% final size.

It appears that the higher temperatures in 2022 from May to June (Table 1) favoured the occurrence of fire blight symptoms more frequently and on more cultivars (Table 2). The most sensitive cultivar in both 2021 and 2022 years was the Romanian cultivar 'Auriu de Bistrița'. This cultivar exhibited the highest damage with significant differences from all of the other cultivars, in both years (Table 2).

Table 2. Means for the frequency of fire blight symptoms on shoots of apple cultivars in 2022

Cultivar	2021	Cultivar	2022
Auriu de Bistrița	5.500 ± 0.557[a]	Cultivar	10.000 ± 0.889[a]
Idared	0.100 ± 0.018[b]	Auriu de Bistrița	5.000 ± 0.523[b]
Salva	0.050 ± 0.017[b]	Florina	3.000 ± 0.346[c]
Florina	0.030 ± 0.008[b]	Dany	2.003 ± 0.222[d]
Bistrițean	0.010 ± 0.000[b]	Doina	0.500 ± 0.026[e]
Alex	0.000 ± 0.000[b]	Salva	0.300 ± 0.072[e]
Dany	0.000 ± 0.000[b]	Starkprim	0.200 ± 0.346[e]
Doina	0.000 ± 0.000[b]	Bistrițean	0.200 ± 0.044[e]
Generos	0.000 ± 0.000[b]	Generos	0.200 ± 0.026[e]
Golden Delicious	0.000 ± 0.000[b]	Golden Delicious	0.200 ± 0.080[e]
Goldprim	0.000 ± 0.000[b]	Goldprim	0.200 ± 0.030[e]
Jonaprim	0.000 ± 0.000[b]	Idared	0.200 ± 0.075[e]
Jonathan	0.000 ± 0.000[b]	Jonathan	0.197 ± 0.021[e]
Starkprim	0.000 ± 0.000[b]	Alex	0.103 ± 0.072[e]
Starkrimson	0.000 ± 0.000[b]	Starkrimson	0.000 ± 0.000[e]
Pr > F(Model)	< 0.0001	Jonaprim	< 0.0001
Significant	Yes		Yes

Note: The values presented in the table are the average fire blight frequency per apple cultivar. Averages followed by different letters indicate differences at $P < 0.0001$ according to Duncan`s Multiple Range Test

In 2021 all other cultivars were included in a single group of significance when the data was statistically analysed. In the field, these results were observed as sporadic damage or absence of damage on shoots on all cultivars except 'Auriu de Bistrița'. Based on the data obtained in 2022 the cultivars were grouped into five groups according to the means of the frequency of symptoms of fire blight (Table 2). 'Auriu de Bistrița' suffered the most fire blight damage (10%) confirming the results obtained by Jakab-Ilyefalvi and Platon (2012).

The damage on 'Florina' in 2022 was much higher than in 2021 (Table 1) and significantly different from all other cultivars. Although the damage degree of 'Dany' and 'Doina' in 2022 can be categorized in two distinct groups according to Duncan`s Multiple Range Test, they presented quite low levels of damage 3% respectively 2%.

All the remaining cultivars were included in the category of those that presented sporadic symptoms (e). This category included some of the previously described ones which proved to be susceptible such as 'Idared' (Sobieczewski et al., 2014), 'Jonathan' or medium susceptible such as 'Golden Delicious' (Van der Zwet and Beer, 1999). In addition, all the other Romanian cultivars were grouped in one class, except for 'Auriu de Bistrița'.

Cultivar 'Jonaprim' displayed no symptoms of fire blight in the experimental years. Ten out of twelve cultivars responded well to the natural infection with *E. amylovora* in the specific conditions of 2022 (Figure 2) even if that year the climate conditions were highly favourable to fire blight, not only on apple trees but also on quince (Rosu-Mares et al., 2023).

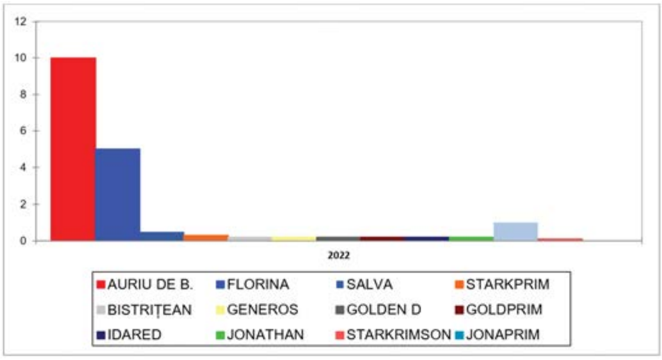

Figure 2. Frequency of fire blight symptoms in 2022

In order to see if there are differences between the means of the damage in the years 2021 and 2022 we performed the two-tailed t-test (William, 1908) and the results statistically confirm the differences (Table 3).

Table 3. Results of the two-tailed t-test in case of the difference between the means of the year 2021 and 2022 fire blight frequency

Difference	1.407
t (Observed value)	2.799
|t| (Critical value)	2.145
DF (Degrees of freedom)	14
p-value (Two-tailed)	**0.014**
alpha	0.05

Note: values for 95% confidence interval on the difference between the means. The number of degrees of freedom is approximated by the Welch-Satterthwaite formula.

Overall, the results show that not only the characteristics of the cultivar influence the frequency of fire blight symptoms, but also the environmental conditions of the experimental years.

CONCLUSIONS

The present study confirms the existing data about the susceptibility of cultivar 'Auriu de Bistrița' and places cultivar 'Florina' in the category of medium to low susceptibility. All the other Romanian cultivars performed well in conventional orchard management practices by displaying low, sporadic damage degree or even the absence of the infection in both years. As a consequence, we can recommend them for growing in areas with a temperate climate similar to those in Transylvania as well as for future breeding programs to improve resistance to *E. amylovora* in apples.

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