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The Response to Natural Infections with *Erwinia amylovora* Burrill of Five Quince (*Cydonia oblonga* Mill.) Cultivars in Bistrița Area, Romania

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RESEARCH ARTICLE

Abstract
Erwinia amylovora is the most damaging bacterial disease of quince, causing a lot of damage to this crop. In cases of severe infections, this disease could even cause the death of the tree. The climatic conditions in Bistrita region were favourable for fire blight outbreak in 2022, and gave us the opportunity to study the differences manifested by five quince cultivars ('Aromate', 'Aurii' 'Bereczki', 'Adonia' and 'Cedonia') when naturally infected with *Erwinia amylovora* Burrill. The second wave of infections, in September, was significantly less damaging than the first one which occurred in June. Overall, after assessing the frequency of the blighted shoots of the mentioned quince cultivars, in June and in September of the year 2022, we concluded that there were significant differences between responses to natural infection *Erwinia amylovora* of these cultivars. The data showed that the cultivars 'Aromate' and 'Aurii' have a better behaviour than the Hungarian cultivar 'Bereczki', while the Romanian cultivars 'Adonia' and 'Cedonia' appeared to be very susceptible under the climatic conditions of the year 2022 in Bistrita area.

Keywords: bacterial disease, climatic conditions, frequency of infection, infected shoots.

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INTRODUCTION

Quince (*Cydonia oblonga* Mill.) is a species within the genus *Cydonia*, the Rosaceae family, subfamily *Spiraeoidaea* (Bassil et al., 2015). The history of quince cultivation dates back at least 2,500 years and indicates that it originated from Transcaucasia (the center of origin) and spread to Northern Iran and Western America (Naémi, 2017), where quince was found in the wild. The first attempts to domesticate quince were most likely made in Mesopotamia, through the selection of valuable local genotypes. This initial attempt to cultivate quince laid the foundation for its introduction and cultivation in Europe, including in the ancient Greek empire (Moradi et al., 2017). Although the exact year of its cultivation in Europe is not known, it is believed to have come from the area of Cydon (on the island of Crete), which is where the genus name of quince is thought to originate. The exchange of quince trees between populations led to the establishment of the first germplasm collection in this region. In a subsequent stage, germplasm collections expanded to Northern Europe, including France, Germany, Hungary, and the United Kingdom (Abdollahi, 2021). This allowed the study of morphological and horticultural characteristics of quince, which provided opportunities to improve various aspects, such as breeding varieties with smaller tree sizes, enhancing fruit quality, and, most importantly, increasing resistance to major diseases like fire blight (Abdollahi, 2019).

Quince requirements for the environmental conditions recommends it for the mediterranian and temperate climate. Regarding temperature, it needs areas with mild winters and warm summers, preferring cultivation areas with a southern exposure, it grows and bears fruit poorly in insufficient light. Water requirements are relatively high due to its shallow root system preferring irrigation for proper development and fruiting (Mitre, 2002). As for the soil, quince thrives on soluble soils. Quince is often used as a rootstock for pear species, resulting in reduced tree vigor, early fruiting, and high productivity (Machando et al., 2014).

The fire blight of rosaceaous plants is among the oldest diseases mentioned in the specialized literature, Coxe used this terminology in his fruit growing book in 1817 (Severin and Iliescu, 2006). Later, in 1878, Thomas J. Burrill from the University of Illinois, USA, proposed the new and revolutionary ideea, for plant protection at that time, that the pathogen organism causing this disease is bacterial. Almost 150 years of research followed because of the high value losses caused by *Erwinia amylovora* in apple, pear and quince.

Erwinia amylovora is a gram-negative bacterium that thrives in temperatures between 21 and 27 degrees Celsius (Boucher et al., 2021) with high humidity, between 60-95 % (Severin and Iliescu, 2006). It infects easily wounded tissues, flowers and young organs of the host plant when these conditions are met. Difficulties of fire blight control, in Europe, consist in: variable pattern of appearance due to climatic variability, small number of chemical control substances accepted in Europe, low availability of biological products for fire blight control, interdiction to use of antibiotics in European agriculture, limited efficacy of control agents. New methods of control are being explored by scientist with promising results. For example, very good results on apple trunk injection of different control agents were reported (Aćimović et al., 2015). Still there are very few data on quince and the fire blight control or resistance genes. There have been some experiments regarding the fire blight resistance in flowering quince – *Chenomeles* spp. (Bell et al., 2004) regarding the behavior of different genotypes in field or in special greenhouses. Recent data about the behavior of different quince cultivars, was reported in Turkey (Şahin et al., 2020) and Iran (Abdollahi et al., 2008, none of them referring to the cultivars we studied.

The first recorded infection with *Erwinia amylovora* in Romania was in 1992 in the southern part of the country and from there it spread across all regions (Severin and Iliescu, 2006). In Romania, there have been other studies regarding the behavior of fruit trees to fire blight, mostly regarding to apple (Militaru et al., 2012) and pear (Sestras et al., 2008) species.

The aim of this study was to evaluate the behavior of five quince cultivars when naturally infected with *Erwinia amylovora*, to highlight the differences manifested by them in Bistrita region, in the specific climatic conditions of the year 2022 and to select the most suitable cultivar for this region.

MATERIALS AND METHODS

Experimental setting

The biological material was represented by the following quinces cultivars: 'Aurii', 'Adonia', 'Cedonia', 'Bereczki' and 'Aromate'. Trees were planted in 2021 at Fruit Research & Development Station Bistrita (FRDS Bistrita). Planting distances are 2.25 x 4 m (1111trees/ha) cultivated in three replicates with two plants per replicate. The observations were performed in June and September of the year 2022 after each infection that occurred naturally. We assessed the frequency of blighted shoots as a percentage from the total number of shoots in each replicate, given the fact that the trees were young and counting was possible. First round of visual observations was in the last decade of June. After the data was recorded all infected shoots were cut 20 centimeters into healthy wood with the garden shears being disinfected after each cut. For disinfection we used a solution of chlorine in sodium hypochlorite (1%). We spayed the trees with copper hydroxide after the pruning of the blighted shoots for preventing the spread of the disease.

In September, another wave of infection affected the trees. Consequently, we collected the second set of data using the same method.

The field trial was performed under conventional pest management program. A total of ten sprays were applied, out of which five against bacterial diseases. The first antibacterial treatment was in winter when we used copper oxycloride (1140g/ha). During de the vegetation period we sprayed once with aluminum fosetyl (2400g/ha) during bloom and then the other three treatments, performed in June and July, we used copper hydroxide (200g/ha).

Statistical Analysis

The data was analyzed using the XLSTAT - Addinsoft software (Addinsoft, 2022), which utilizes the MS Office Excel platform version 2019. All data collected from the field were arranged in completely randomized blocks and the XLSTAT program was used to perform the analysis of variance (ANOVA) (Fisher, 1925). Afterwards, the Duncan's Multiple Rage Test was used to analyze the degree of significance between the different variants (Duncan, 1955) at $p<0.0001$. To compare the means of the infections in June and September was used t-test for two paired samples (Two-tailed test). Correlations were calculated for the damage degree in June and the number of shoots per tree.

RESULTS AND DISCUSSIONS

Climate conditions

The visible damages were expressed in the orchard during the intense growing period of the shoots, in June respectively. It was manifested by the appearance of wilted and then necrotic shoots, in the characteristic form of a shepherd's crutch in the canopy (Figure 1). In the morning, bacterial exudate was visible withit unaided eye on the affected shoots.

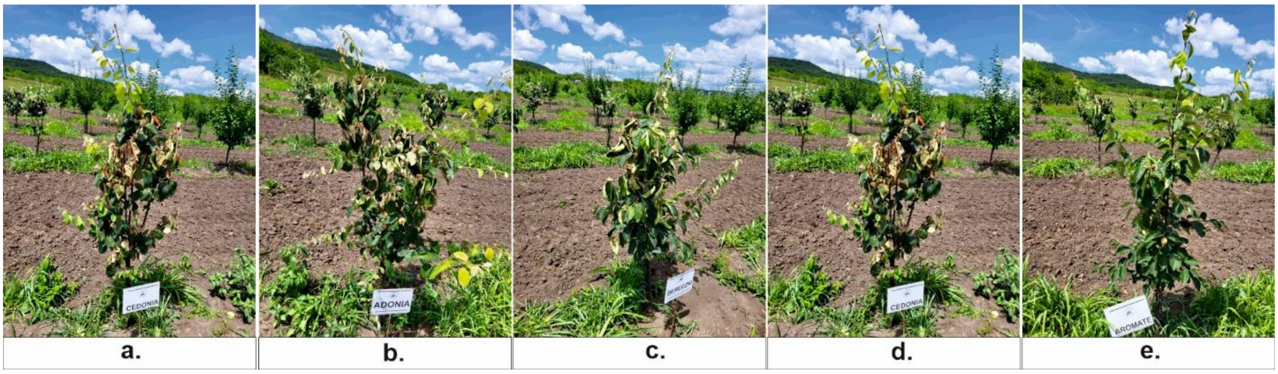


Figure 1. *Erwinia amylovora* infection in 2022, (a) 'Cedonia', (b) 'Adonia', (c) 'Bereczki', (d) 'Cedonia', (e) Aromate

In the proximity of the experimental plot there is an old pear (*Pyrus communis* L.) orchard that was most probably the source of the primary inoculum with *Erwinia amylovora*. Most likely, the infection took place after the flowering since there were no symptoms along blooming period of May.

In June, the average temperatures were close to 20˚C with daily maximum situated in the optimum range for the development of *Erwinia amylovora* (Figure 2) and higher average of humidity in the first half of the month (Figure 3b).

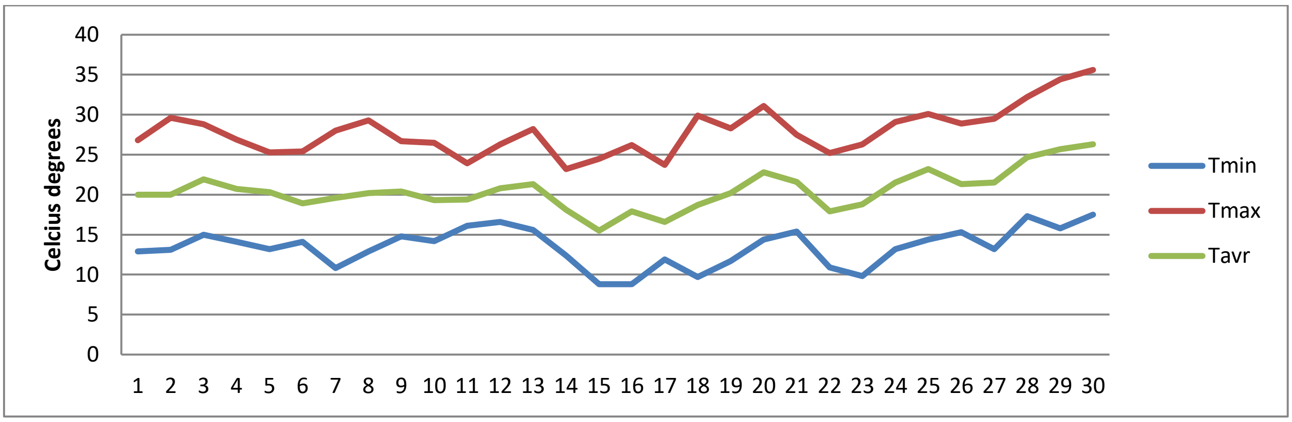


Figure 2. The minimum, maximum and average daily temperatures in June 2022, Bistrita

The infection stopped after the pruning of the affected shoots, although July and August were rainiest than June (Figure 3a), and the average of humidity was mostly higher especially than the last half of June (Figure 3b). September was the rainiest (Figure 3a) and the most humid of all the months analyzed and this might have contributed to the second infection of the year 2022. Symptoms were similar to the ones recorded in June, expressed by the appearance of wilted and then necrotic shoots, in the characteristic form of a shepherd's crutch on the remaining shoots.

The average temperatures in the first half of September was below the optimum range for propagation of *Erwinia amylovora*, varying between 11.2 ˚C and 18.4 ˚C. However, the maximum temperature had values between 14.4 and 25.8 ˚C, in 13 days the maximum temerature being over 21 ˚C (Figure 4). Consequently, the day temperatures in this period of time along, in combination with the high humidity created the optimum development conditions for the bacteria.

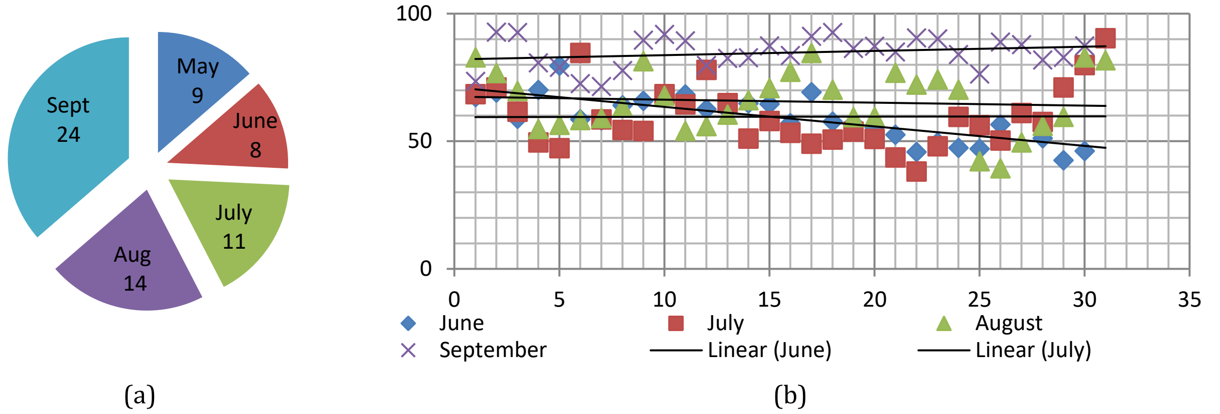


Figure 3. (a) The number of rainy days in 2022 season; (b) the average of daily humidity from June to September

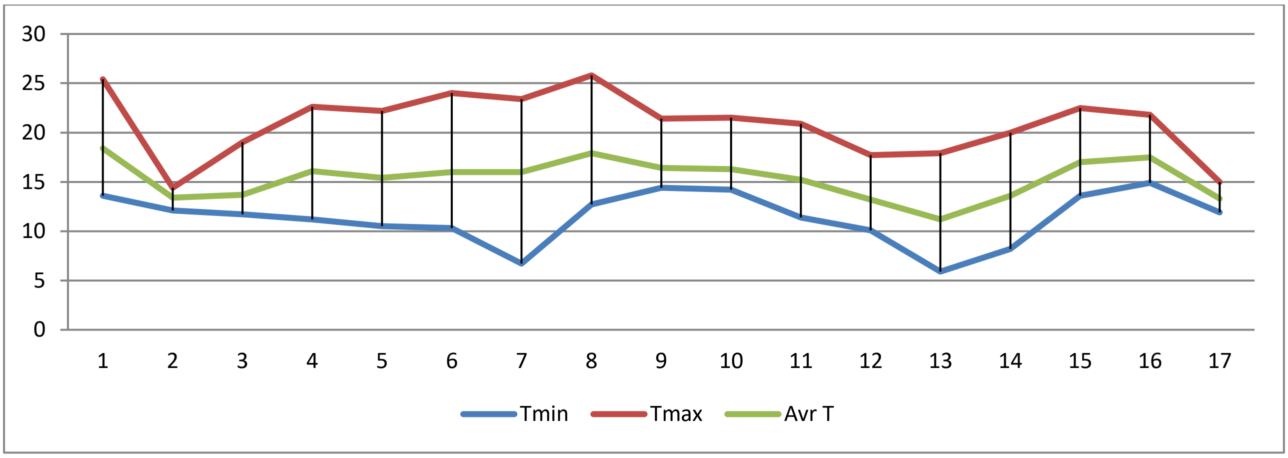


Figure 4. The temperatures in the first half of September 2022 in Celsius degrees (vertical axy), from 1st to 17th (horizontal axys)

Cultivars behavior

The year 2022 provided favorable climate conditions for *Erwinia amylovora* propagation, unlike the precedent years when no infections were spotted in the area (unpublished results).

After collecting the data from all three replicates of each cultivar, we counted and compared the least squares means for all, and found that each of the cultivar differed significantly from all the others after infection occurred in June (Table 1).

Table 1. The level of fire blight damage degree on five quince cultivars in the experimental plot of FRDS Bistrita, June 2022.

Cultivar	Damage degree
Cedonia	68.937 ± 3.86 [e]
Adonia	57.510 ± 4.65 [d]
Bereczki	31.273 ± 3.79 [c]
Aurii	19.650 ± 0.90 [b]
Aromate	10.237 ± 2.00 [a]
Pr > F(Model)	< 0.0001
Significant	Yes

Note: The values presented in the table are averages of damage degree in June for every quince cultivar. Averages followed by different letters indicate differences at p < 0.0001 according to Duncan`s Multiple Range Test.

Thus, the highest damage degree was recorded in 'Cedonia' (68.9%) while the smallest damage degree was noted in 'Aromate' (10.2%). Although 'Aurii' and 'Aromate' cultivars are clones of the 'Bereczki', selected in the eighty's (Ghena and Braniste, 2003), they revealed a significantly better behavior to naturally infection by *Erwinia amylovora* than 'Bereczki'. The cultivars have different types of growing, the Romanian cultivars 'Adonia' and 'Cedonia' have lower vigor but higher number of shoots per tree.

In order to check if there is a correlation between the number of shoots and the damage degree, we found that there is a strong correlation between these two characteristics, given by the correlation factor (r^2=0.8011 (Figure 5).

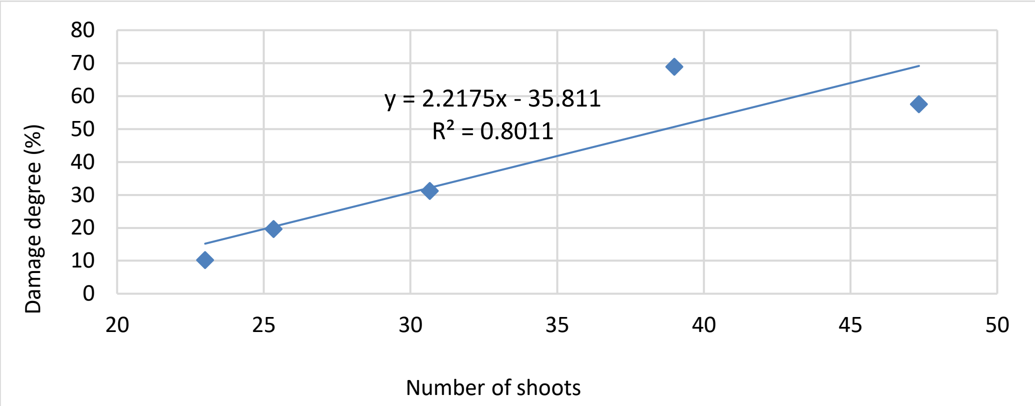


Figure 5. The correlation between the average number of shoots per repetition and the average damage degree caused by *Erwinia amylovora* in quince cultivars

The two most damaged cultivars 'Adonia' and 'Cedonia' had bigger number of shoots than the other cultivars and abundant flowering even though they were just two years old, fruits with thin skin and very low pubescence. The possibility that there is a causality between the number of shoots per tree, their vigor, other trees and fruit tissue characteristics and the susceptibility of the cultivar to fire blight remains to be investigated in further experiments.

In September (Table 2) 'Cedonia' (31.10%) was the most damaged cultivar followed by 'Adonia' (24.01%). The cultivars 'Aromate' and 'Aurii' were not infected this time, probably due to a better maturation of the tissues in the shoots.

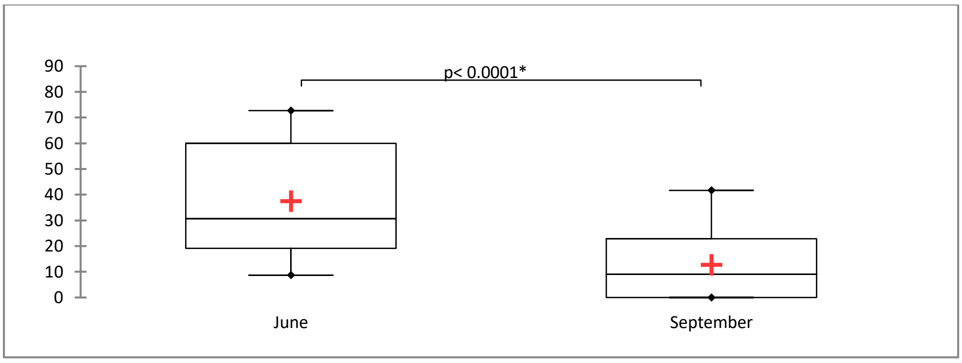
Table 2. The damage degree registered in September 2022 with the significance of differences between cultivars

Cultivar	Damage degree
Cedonia	31.107 ± 12.95 ^{c}
Adonia	24.017 ± 2.09 ^{c}
Bereczki	8.157 ± 7.73 ^{b}
Aromate	0.000 ± 0.00 ^{a}
Aurii	0.000 ± 0.00 ^{a}
Pr > F(Model)	0,001
Significant	Yes

Note: The values presented in the table are averages of damage degree in September for every quince cultivar. Averages followed by different letters indicate differences at p < 0.0001 according to Duncan`s Multiple Range Test.

Epidemiology

All the values of the damage degree in September were lower than those in June with significant differences between the means (Figure 6). This helped us to conclude that the most critical period of the year for *Erwinia amylovora* infections is in the beginning of the summer.



Note: *: significant at level alpha=0.05

Figure 6. The *t* test results when comparing the means of the June and September data (95% confidence interval on the difference between the means)

However, the possibility of infections to appear again in the beginning of autumn, when the climatic conditions favor this disease, should also be taken into account.

CONCLUSIONS

The results revealed that 'Aromate' and 'Aurii'. had a better behavior to natural infection with *Erwinia amylovora* than 'Bereczki' ., while 'Adonia' and 'Cedonia' appeared to be the most susceptible quince cultivars under the climatic conditions of the year 2022 in Bistrita area. A special attention for treatments against *Erwinia amylovora* must be paid during the first part of vegetative period when a significantly higher damaging was recorded.

Author Contributions: S.D.R.M. Conceived and designed the analysis; S.D.R.M. and C.M. Collected the data; L.Z. and Z.J.I. Contributed with the data analysis tools; S.D.R.M. and C.M. Performed the analysis; S.D.R.M., A.M.C, G.G, L.Z. I.Z and V.F. Wrote the paper.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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