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Evaluation of the impact of natural infections with *Erwinia amylovora* in quince (*Cydonia oblonga*) on some quality characteristics of fruits

Călin BACIU[1], Carmen BEINȘAN[1], Mariana POIANĂ[2], Marinela COSTEA[1], Radu ȘUMĂLAN[1*]

[1] University of Life Sciences "King Mihai I" from Timisoara, Faculty of Engineering and Applied Technologies, Department of Genetic Engineering, e-mail: calin.baciu@certisbelchim.com, carmenbeinsan@usvt.ro, costeamarinela21@yahoo.com, radusumalan@usvt.ro
[2] University of Life Sciences "King Mihai I" from Timisoara, Faculty of Food Processing Technology, Food Technology Department, e-mail: marianapoiană@usvt.ro

* Corresponding author: radusumalan@usvt.ro

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Abstract
Quince (*Cydonia oblonga* Mill.) is a monospecies of the genus Cydonia, a member of the subfamily Spiraeoideae, family Rosaceae, being related to several other fruit and ornamental species such as *Pseudocydonia sinensis* (Thouin) C. K. Schneid. (Chinese quince), *Chaenomeles spp*., (Japanese quince), *Crataegus spp*. (hawthorn), *Mespilus sp*. (medlar), precum și cu *Pyrus spp*. (pear) and *Malus spp*. (apple).
Erwinia amylovora (Burrill, 1882), is the agent that causes fire blight in some fruit and ornamental species. The disease was initially identified in the U.S. in wild species of the Rosaceae family, and with the introduction of breeded cultivars of apples and pears it spread rapidly.
The objective of this study was to determine the impact of natural fire blight infection on fruit quality in two quince cultivars (Bereczky and Aurii), by morphological (size, mass, shape index, etc.), senzorial (firmness) and biochemical (titratable acidity, soluble sugars) traits. Methods of determination for morphological parameters were based on the dimensions measurement (electronic caliper) and weights (precision balance), fruit firmness on penetrometry, titratable acidity by titration of intrinsic acids with a standard base and soluble sugars by refractometric method.
The obtained results showed that fruits harvested from infected plants have smaller size and lower weight by 29,7 % in Bereczky, and 37,2 % in Aurii, compared to fruits harvested from healthy plants. Regarding the fruits qualitysenzorial and bichemical indices, the results showed higher firmness levels in infected variants in both varieties (8,71 $\pm$ 0,28 kgf/cm^2 -infected, compared to 7,22 $\pm$ 0,28 kgf/cm^2 -uninfected, in cv. Bereczky, and 8,08 $\pm$ 0,09 kgf/cm^2- infected, compared to 7,02 $\pm$ 0,22 kgf/cm^2 -uninfected in the cv. Aurii).
Therefore, natural infection with fire blight causes important changes in the morphological and biochemical quality characteristics of quince fruits, in particular by reducing the mass and size, the dry substance content and increasing firmness.

Keywords: fire blight, natural infection, fruit morphology, biochemical indices

Introduction

The history of quince cultivation is at least 2500 years old, having origins in the Roman Empire, Persia and ancient Greece. This fruit species belongs to the genus Cydonia, being specific to temperate climate areas and representing a valuable source of phenolic compounds. Quince is native to northern Iran, Turkmenistan and the Transcaucasian region, and its local varieties have been obtained by growers mainly through selections of genotypes with valuable characteristics [1]

Quinces are consumed both fresh but especially processed and industrialized in the form of jams, compotes, pellets, marmalade, etc. Fruits have a high degree of gelling, maintain their aroma, acidity and firmness even after boiling. They keep well for a long time, allowing to extend the processing period. For therapeutic purposes, seeds, fruits and flowers are used from quince. Due to its aromatic and functional properties, quince is fortified in various products such as beer and yogurts. Quince seed mucilages and hydrocolloids can be used as thickeners and bulking agents in many food products, making this fruit a viable alternative in the food industry [7].

In recent decades, the main attempts to breed and select new varieties and genotypes of quince have focused on several main objectives, including fire blight tolerance [12;18], spotting leaves and fruits [20], increased tolerance to leaf chlorosis [3] etc.

Due to the high fire blight sensitivity of quince, attempts at improvement for this purpose have not yielded particular results [9;19;27]. Fire blight caused by *Erwinia amylovora* (Burrill) Winslow *et al*. is one of the most catastrophic bacterial diseases. Among the various host plants, quince (*Cydonia oblonga*) is one of the most susceptible species, and the use of tolerant genotypes is the most effective and economical method of controlling these diseases [12].

Erwinia amylovora can survive as an endophyte or epiphyte in infected or asymptomatic parts of plants; therefore, its spread is extremely difficult to control [28], and the generated economic losses are huge [21]. Even though copper-based substances, bactericides and some antibiotics are used to control fire blight. The results obtained attest that there are no effective chemical treatments against this disease. Cultural practices such as pruning, preventive chemical treatments, soil and environmental control have been shown to be partially effective in preventing the occurrence and limiting the spread of disease [26]. However, so far, it has not been possible to identify an effective method of fighting this real scourge. However, sets of cultural, genetic and technological measures are used that can control the infection process of plants and the extent of the disease.

Early detection and control methods have paid increased attention to disease management through integrated control strategies based on the use of tolerant genotypes in combination with sanitation, cultural, physical, chemical and biological control practices [5].

The use of tolerant genetic resources, intermediate rootstocks is essential in disease control and obtaining highly tolerant cultivars is a priority of quince breeding processes [8;26]. Identifying accessible and disease-tolerant quince genetic resources is the first step in obtaining varieties and rootstocks to solve the fire blight problem [26].

In Romania, the first fire blight infection was reported in 1992 in the southern area, then spreading rapidly to the other regions [24]. The fire blight affects cultivated stone species, impact studies in pear species [23] and apple [15] being published over time.

The main objective of the study is to determine the tolerance capacity to natural infections with *Erwinia amylovora* of two varieties of quince, Bereczky and Aurii, cultivated on important areas in Romania and the impact of the disease on some morphological and biochemical traits of fruits.

Material and Method

Biological material

Bereczky - an old quince variety dating back to the time of the Habsburgs, named after Hungarian pomologist Máté Bereczki (1824-1895). It is a famous quince with fragrant and juicy fruits of limony-yellow color, covered with a grayish-brown fluff before maturity. At maturity the tree reaches a height of 2-3m and is self-fertile. It blooms in April and begins to bear fruit in about 2-3 years after planting, with the harvest period in September. The plant loves the sun and well-drained fertile soils, and the ideal pH is around 6.5.

Aurii - Romanian quince variety, clone of Bereczky, with large, aromatic, globular fruits with wide ribs and thin pericarp, yellow-orange colour, crispy, yellowish and dense pulp. The flowering period is April, and ripening in September - October. The tree is of medium vigour, reaching at maturity 3-4 m in height.

Experimental conditions

The fruits were harvested from healthy plants (control - C) and naturally infected (I) with fire blight from an organic orchard in the Cluj area. The intensity of infection was severe, with more than 50% of the plant canopy volume affected. The evaluation of the infection intensity was carried out from flowering to harvesting, each tree was visually inspected for the presence of fire blight symptoms by the same person almost weekly by going through the rows for both varieties (Maryblyt model prediction method). The fruit was taken at commercial maturity for both varieties at the beginning of October.

The climatic data specific to 2022 in Jucu, Cluj county show that favourable conditions were met for the appearance of fire blight in the organic quince plantation as follows; the daily maximums in March and April were 7 and 15 ^{0C} respectively, and the rain was 75 and 95 mm, with a relatively high degree of cloudiness of 15 and 13 days with cloudy skies, respectively.

Determined indices and used methods

Quince is a golden yellow fruit, fleshy, fluffy and with rich flavour and variable number of seeds. It presents different shapes, sizes and weights with a strong genetic determinism, which can be influenced by environmental factors and existing stress conditions.

Morphological parameters of fruits

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A sample size of 8 fruits per cultivar and experimental variant was evaluated according to U.P.O.V. morphological parameters: fruit weight, height and equatorial diameters were determinated, and shape index were calculated. Fruit weight was accounted by a Kern digital bench scale (model BLJ-510 3M, 0.1mg accuracy). Fruit equatorial diameter and height were measured with a Neiko Electronic Digital Caliper (0.01 mm accuracy).

Sensory and biochemical parameters

Pulp firmness was definitively assessed by a Force Gauge PCE-FM 200 penetrometer. Several analysis were conducted to assessed some chemical traits (pH, titratable acidity (TA), total soluble solids (TSS)) and maturity index (TSS/TA) were calculated. Three juice samples of 2 fruits each were used to perform the chemical analysis. Juice pH was determined by a Consort pH-meter (model C 933); total soluble solids (TSS) were measured with an Hanna refractometer (model HI 96801 0.2 % Brix accuracy); titratable acidity values (TA) were determined by acid–base potentiometer (NaOH, 0.1N up to pH 8.1) and expressed as grams per 100 g (%) of malic acid [25], and maturity index (MI) was calculated by the following ratio: TSS/TA.

For all determination were calculated the average and standard deviation using basic function of Microsoft Excel.

Results and Discussion

The impact of fire blight infection on some morphological parameters of fruits.

It is proven that biotic and abiotic stressors affect plant productivity, especially the number of fruits, their size and mass. The analysis of experimental data obtained on the impact of natural infection with fire blight on the diameter and height of fruits in the two quince varieties tested (Fig.1) confirms that the stress generated by the disease causes significant reductions in fruit size. Thus, in Bereczky, the diameter of fruits varies from 65.77 $\pm$ 7.53 mm in control, to 57.93 $\pm$ 4.92 mm in the infected variant (12% - difference), and in Aurii the average diameter of the fruit had values of 68.40 $\pm$ 3.79 mm in control, compared to 57.47 $\pm$ 4.34 mm in infected fruits (14% - difference). Fruit height also varied within fairly wide limits, from 91.18 $\pm$ 10.72 mm in Bereczky-control to 54.32 $\pm$ 7.28 mm in infected Aurii. The differences in fruit height were about 13% in favour of the control variant in Bereczky and only 4.4% in favour of the control variant in Aurii.

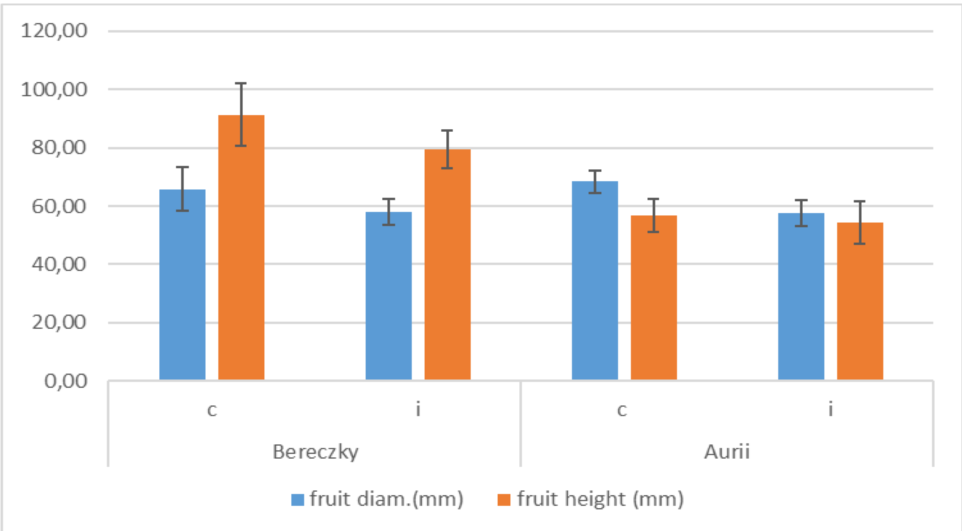

Figure 1. Diameter and height in fruits quince cultivars (c- control; i- infected)

Based on the determinations regarding the diameter and height of the fruits, the shape index was calculated, which represents the ratio between the two parameters (Fig.2.). In the case of the Bereczky variety, there were no changes in the shape index in the infected variants compared to the control, the calculated value being 0.73, i.e. oblong, priform-looking fruits with small diameter and high height.

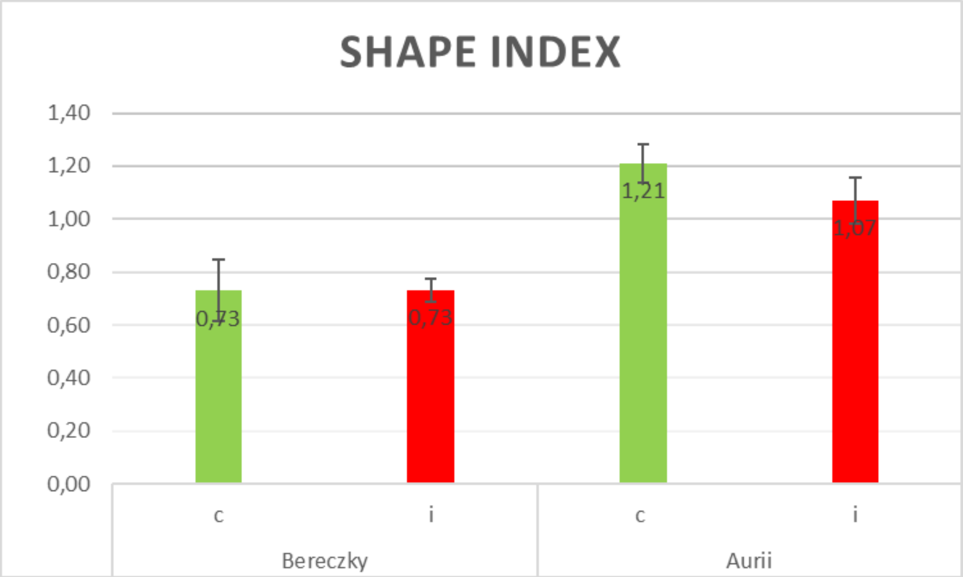

Figure 2. Fruit form index of quince varieties (c-control; i-infected)

In the Aurii variety, the fruits are strongly flattened, and biotic stress conditions have caused changes in the shape index, by strongly reducing the diameter and less the height. The values of this indicator were 1.21 + 0.07 at the control and 1.07 + 0.09 for fruits of the infected variant.

The fruit mass was also negatively affected by the stress induced by the fire blight attack, so in the Bereczky variety, the average weight of a fruit in control was 189.63 + 55.36 g, compared to 133.00 + 28.00 g in infected (difference -30%). The Aurii variety showed a similar trend, the average weight of fruits in control was 161.43 + 24.44 g, compared to 101.83 + 22.03 g in infected, with a difference of 37%, in favour of the control variant. These results attest that the fruits of the Bereczky quince variety are heavier, but those of the Aurii variety appear to be more strongly affected by the stress induced by fire blight infection.

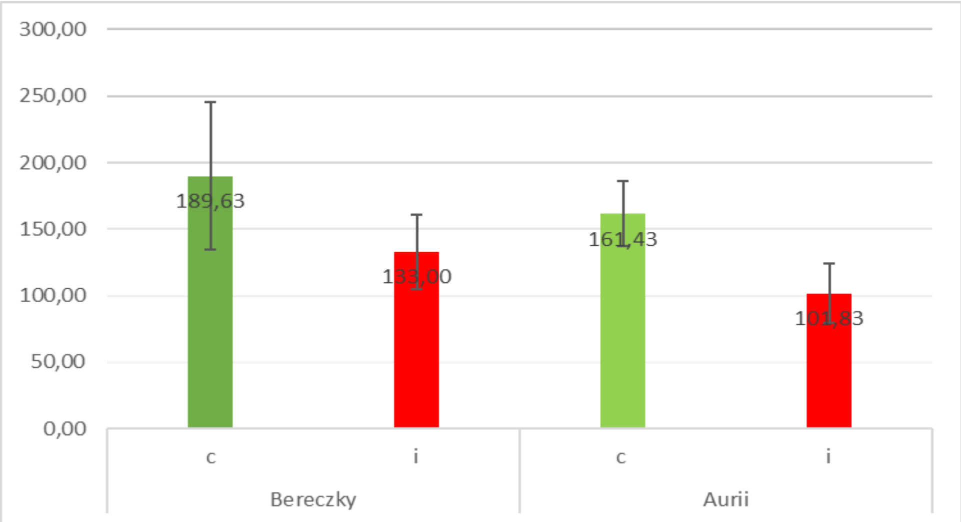

Figure 3. Fruit mass (g) of quince varieties (c-control; i-infected)

Impact of fire blight infection on some sensory and biochemical parameters

The fruits firmness is an important quality indicator with implications for handling, transport and storage capacity. Firmness is a primary indicator in characterizing fruit texture and a key determinant of quality (Saei, et al., 2011).

The results obtained show that fruits from infected plants with fire blight show a higher firmness compared to those from non-infected plants (fig.3). Thus, in the case of the Bereczky cv., the determined firmness was 8.71 + 0.28 kgf/cm2 in infected fruits, compared to 7.22 + 0.28 kgf/cm^2 in uninfected fruits, and

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in the Aurii cv., values between 8.09 ± 0.09 kgf/cm^2 were recorded in the infected and 7.02 ± 0.22 kgf/cm^2 in the control.

The increase in fruit firmness in the case of infected variants of the two cultivars can be attributed to the increase in the share of petrified tissues in the fruit pulp and also to the reduction in the amount of water in the pulp.

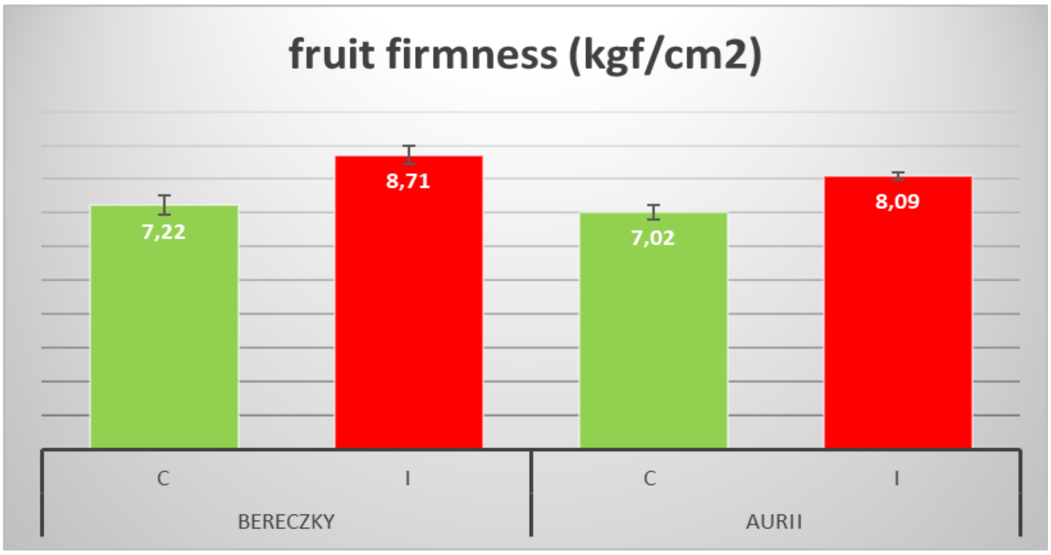

Figure 4. Fruit firmness in quince cultivars (c-control; i-infected)

In addition to the firmness of the fruits, the content of dry weight (DW) of fruits, total soluble sugars (TSS) and titratable acidity (TA) of fruit juice was determined, and based on the last two parameters, the maturity index (MI) of the two cultivars was determined in the experimental studied variants (table 1).

The obtained results showed that natural infection with fire blight causes a reduction in the amounts of dry matter in fruits, most likely due to damage to the foliar apparatus of plants and inhibition of photosynthesis. The TSS was higher in infected fruits of the Breczky variety (16.90 ± 1.35 ^{0} Brix - infected and 16.36 ± 0.82 ^{0} Brix - control) and much lower in the case of infected fruits of the Aurii variety (14.03 ± 0.57 ^{0} Brix - infected and 16.20 ± 1.90 ^{0} Brix – control), and TA recorded lower values in juice extracted from infected fruits compared to control (1.56 ± 0.04 % - control / 1.27 ± 0.05 % - infected in Bereczky, respectively 1.54 ± 0.06 % - control / 1.25 ± 0.07% - infected in Aurii).

The level of fruits maturity depends on the amount of soluble sugars, organic acids, vitamins and some inorganic substances representing an important indicator of biochemical quality parameters [13]. The fruit maturity index had significantly higher values in infected variants compared to control in both varieties. These results attest that fire blight infection speeds up fruit ripening by increasing differences between soluble sugar content and acidity of fruit juice.

Table 1. Dry weight (DW), total soluble sugars (TSS), titratable acidity (TA) and maturity index (MA) in quince cultivars

Genotype	exp. var.	DW (%)	TSS (^{0}Brix)	TA (%)	Maturity index (TSS/TA)
Bereczky	control	20,88 ± 1,06	16,36 ± 0,82	1,56 ± 0,04	10,50 ± 0,59
	infected	18,31 ± 1,81	16,90 ± 1,35	1,27 ± 0,05	13,38 ± 1,35
Aurii	control	22,13 ± 0,98	16,20 ± 1,90	1,54 ± 0,06	10,56 ± 1,54
	infected	18,78 ± 0,88	14,03 ± 0,57	1,25 ± 0,07	11,26 ± 0,24

Although the literature is quite poor in information on the impact of the disease on the quality of quince fruits, most studies being focused on the amount of harvest obtained, we tried to analyse the obtained results compared to other species affected by bacterial fire such as apple, pear or medlar. Thus, our results are consistent with those obtained by other authors [4;10;17] regarding the negative impact on the biochemical parameters of fruits, reducing TSS content in juice and TA level. On the other hand, some

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research has shown that there is no major impact of the fire blight on sugar levels [14] or in fruit firmness in pears [6].

Conclusions

Fire blight (*Erwinia amylvora)* is a disease that affects all organs of quince plants throughout the life and annual development cycle, from budding to fruit harvesting. The impact on the fruits main quality traits is extremely strong, causing a reduction in their mass and volume, with irreversible effect on commercial value. Also, this bacterial disease affects the main sensory features, through colour changes of the pericarp, deformation and increased firmness by expanding petrified tissues in the fruit pulp. Some biochemical properties manifested by the reduction of total and percentage of DW, the quantity of TSS and TA are also damaged.

However, detailed research is needed on the impact of fire blight on other biochemical parameters that characterize the quality of quince fruits, such as the content of inorganic compounds, polyphenols, vitamin C and antioxidant and enzymatic activity, for a better understanding of the defence mechanisms against pathogen attack.

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