

EVALUATION OF BACTERIAL PATHOGEN *AGROBACTERIUM TUMEFACIENS* ATTACK ON SOME APPLE VARIETIES

Mihaela NECREALĂ¹, Alexandra ROTARU¹, R. CHIFAN¹, A. CĂRĂBET¹, Ramona ȘTEF¹

¹ Banat's University of Agricultural Sciences and Veterinary Medicine "Regele Mihai I al României" from Timisoara

Corresponding author: chirita_ramona@yahoo.com, alin70599@yahoo.co.uk

Abstract. *Agrobacterium tumefaciens* cause severe damage, often causing the loss of infected plants. The pathogen has a wide range of hosts, with a high aggressiveness, attacks over 140 grassy and woody species. In recent decades, the spread of the disease has greatly increased due to the intensive exchange and marketing of latently infected propagating material. The aim of the study was to determine the aggressiveness, virulence and degree of attack of the bacterium *Agrobacterium tumefaciens* as well as to observe the varieties that are sensitive and tolerant to the attack of this pathogen, in the climatic conditions of 2021. Between March and May 2021 observations were made on the presence and attack of the bacterium *Agrobacterium tumefaciens* on the apple varieties: *Generos*, *Delicios de Voinești* and *Florina* from the plantation of the Didactic and Experimental Resort Lugoj Herindești. The attack of *Agrobacterium tumefaciens* was easily reported due to visible hypertrophies. The observations showed that the pathogen *Agrobacterium tumefaciens* attacked the three apple varieties studied. The *Florina* variety was the most sensitive to the attack of bacterial cancer, presenting the highest values of aggression, virulence and degree of attack. The frequency of attack (40%), the lowest, was registered in the *Delicios de Voinești* variety, but the intensity of the attack was higher compared to the one manifested in the *Generos* variety. As a result of the study, the *Generos* variety proved to be the most resistant to this bacterium, the degree of attack being significantly lower compared to the control (average variety).

Keywords: *Agrobacterium tumefaciens*, attack, apple varieties

INTRODUCTION

Agrobacterium tumefaciens (sin *Rhizobium radiobacter*) is the pathogen that causes the formation of cancer in over 140 eukaryotic species (ex. pear trees, apple trees etc.), being a Gram negative bacterium. *Agrobacterium tumefaciens* is an *Alpha-preteobacterium* belonging to *Rhizobiaceae* family, and unlike nitrogen-fixing symbionts, *Agrobacterium* species are pathogenic and are not beneficial for the plant (YOUNG ET AL., 2001, 2003; Barbu et al., 2019, SIMERIA ET AL., 2004, BORCEAN ET AL., 2021, BORCEAN ET AL., 2021).

The pathogen *Agrobacterium tumefaciens* is present on the continents: *Africa*, *Asia*, *Europe*, *North America*, *Australia*, *South America* (figure 1). In Europe it is present in about 29 countries (<https://www.cabi.org>, <https://www.cabi.org/>). It affects a wide variety of plants, which makes it very important for the agricultural industry (SMITH AND TOWNSEND, 1907, quoted by SIMERIA ET AL., 2004). *Agrobacterium tumefaciens* is a pathogen for vines, fruit trees, rhubarb, sugar beet and perennial crops (PAMFIL M. ET AL., 2008, POPESCU., 2005).

Agrobacterium tumefaciens causes severe damage, often causing the loss of infected plants. In recent decades, the spread of the disease has greatly increased due to the intensive exchange and marketing of latently infected propagating material (BURR ET AL., 1998; PIONNAT ET AL., 1999). It is an important pathogen producing high losses in respect of quality and quantity. The frequency of *Agrobacterium tumefaciens* attack is generally higher in trees planted on alkaline soils than on acid soils (VISIT D. E., 2016). In addition, the disease leads to the decline of the plantation. More and more researchers are reporting the presence of the bacterium on young trees. Studies conducted by BARBU ET AL. (2019), in Călărași County - Romania, highlights the presence of the bacterium *Agrobacterium tumefaciens* on young

cherries, a similar report is made in Turkey by AYSAN ET AL. (2003), which reports the pathogen on apricots (*Prunus armeniaca*) for two years, an explanation for the development of the pathogen on young fruit trees can be attributed to the planting of infected material. *Agrobacterium tumefaciens*, has a very wide range of host plants and occurs frequently in nurseries and orchards. The pathogen causes greater damage in apple plantations (EPPO - <https://gd.eppo.int/taxon/AGRBTU/documents>), its action leads to the appearance of tumors (hypertrophies), at the base of the trunk.

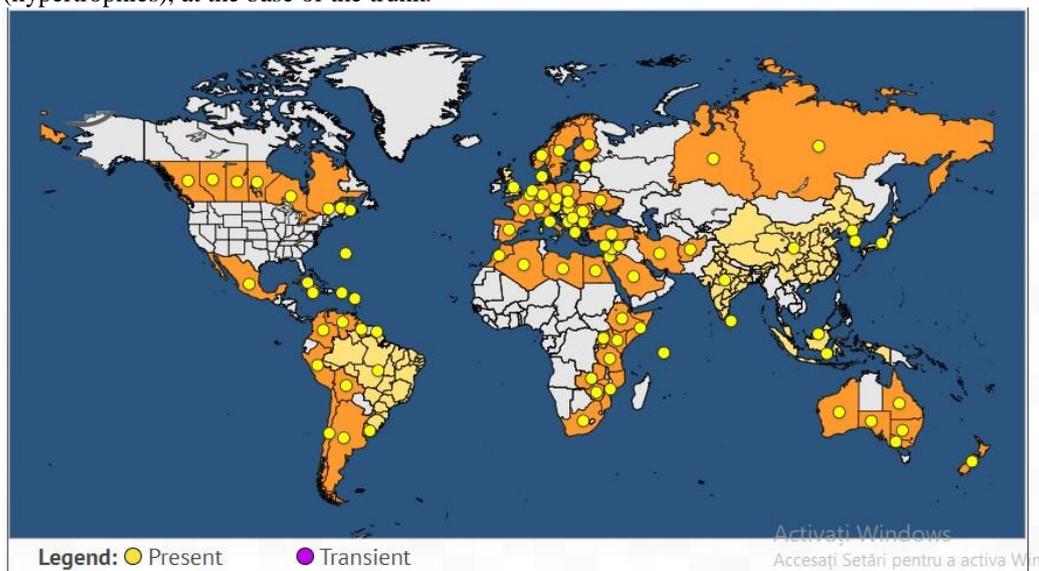


Fig. 1 – Presence of *Agrobacterium tumefaciens* (<https://gd.eppo.int/taxon/AGRBTU/distribution>)

Agrobacterium tumefaciens infection is easily recognized due to tumors that form in the area of the roots and the package of fruit trees (SIMERIA ET. AL., 2004). They can have different sizes, shapes and consistency. It initially manifests as small swellings on the root or stem at the base of the soil and occasionally on aerial parts of the plant. The young tumors are soft, somewhat spherical and white to cream. As the disease progresses, the tumors lignify, their shape becomes quite irregular and they become brown or black (POPESCU ET. AL., 2006).

The pathogen can be controlled by applying the following prophylactic measures, tough good results: long rotations (4 - 5 years); use of resistant varieties; destruction of all infected plants; avoid planting seedlings on contaminated soils; immersion of seedling roots in copper solutions before transplanting; the planting material to be certified, free of *Agrobacterium tumefaciens*; the location of nurseries on light lands, which were previously cultivated with grass or alfalfa; soil disinfection; soil acidification - this measure is supported by some specialists although it is not economical and in the long run does not completely solve the problem; pest control in the soil; removal of seedlings from tumors with planting; removal of tumors from the secondary roots of trees before planting (remove with roots) (TERRY A. TATTAR, 1989).

The study aimed to determine the aggressiveness, virulence and degree of attack of the bacterium *Agrobacterium tumefaciens* on sensitive and tolerant varieties to the attack of this pathogen, in the climatic conditions of 2021.

MATERIAL AND METHODS

The research was carried out within the Orchard and Experimental Facility - Lugoj Herindești, the study being carried out on 3 apple varieties: Generos, Delicios de Voinești, Florina. The experience included 3 variants, each variant was represented by a variety, in three repetitions.

The method of calculating monofactorial experiments was used to determine the degree of attack produced by the bacterium *Agrobacterium tumefaciens*.

The entire tree (trunk, frame) was analyzed. In all five trees in the replicate, the frequency of attack was noted. The intensity of the attack was determined by the number of tumors and their diameter.

The degree of attack was calculated following, in order, the frequency and intensity of the attack.

The diameter of tumors and tumors produced by the pathogen *Agrobacterium tumefaciens* was determined using a digital caliper.



RESULTS AND DISCUSSIONS

Between March and May 2021, observations were made on the presence and attack of the bacterium *Agrobacterium tumefaciens* on the apple varieties studied: Generos, Delicios de Voinești and Florina, from the orchard within Didactic and Experimental Facility Lugoj Herindești. The attack of *Agrobacterium tumefaciens* was easily reported due to visible hypertrophies.

Following assessments results that in the Generos variety, it was observed that 9 trees had an attack of *Agrobacterium tumefaciens*, and at six the attack was absent. The bacterium produces as a characteristic symptom - the tumor or small tumors. The attacked trees showed between 2 and 7 tumors/tree, most of them showing 2 hypertrophies (figure 2). The pathogenesis of tumors is determined by a plasmid that the bacterium transfers to the plant cells, which causes the formation of opines and cytokines in the host plant cells that lead to tissue growth due to a chaotic division (POPESCU, 2005). Bacterial cancer also causes decreases in the concentration of chlorophyll and iron in apple leaves (POPESCU, 1998).

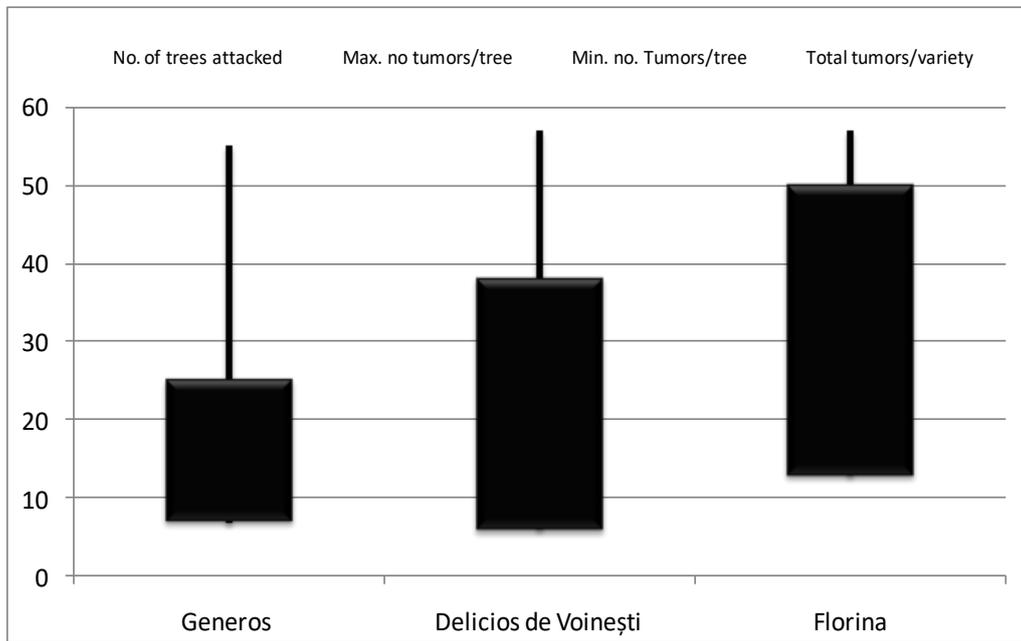


Fig. 2 – Graphical representation of the number of tumors determined by AGRBTU of the investigated varieties

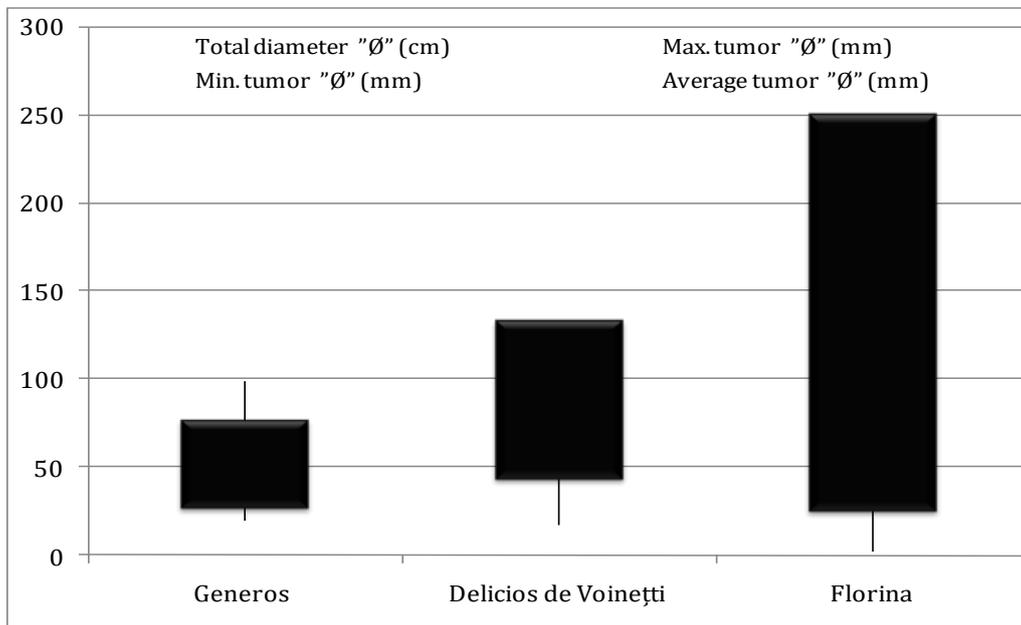
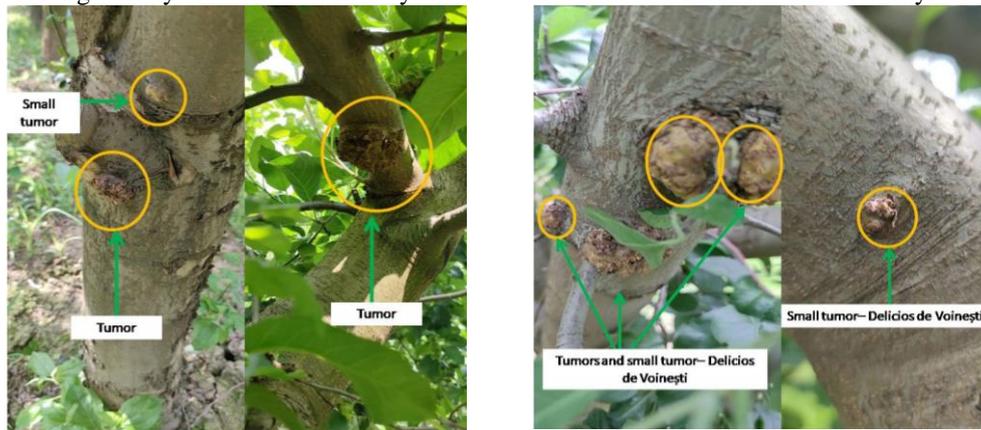


Fig. 3 - Diameter of tumors produced by AGRBTU * of studied varieties (* AGRBTU EPPO Code - *Agrobacterium tumefaciens*)

Tumor diameter values ranged from 19.3 mm to 98.63 mm. The sum of the tumor diameters, in the Generos variety, was 765, 19 mm (figure 3).

The results obtained, as a result of the evaluation of the apple variety, Delicios de Voinești, at the attack of the AGRBTU bacterium, showed an average aggressiveness of 40%. The attack was reported on 6 of the 15 trees analyzed (figure 2). The number of tumors and small tumors varied between 1 and 6/tree with diameters between 17.15 - 87.14 mm. The tumors and small tumors from the Delicios de Voinești variant totaled 1333.95 mm (figure 3). The tumors and small tumors present on the frames were greenish with a spongy appearance. Tumors generally rot at the end of the year and fall off. New ones will be formed next year.



In the Florina variety, the AGRBTU pathogen produced 69 tumors and small tumors in 13 of the 15 analyzed. The number of tumors/tree varied between 1 and 14. The Florina variety presented large, hard, dark brown tumors with a spongy consistency. The appearance of tumors can be explained by the faster manifestation of the disease, compared to other varieties experienced.



The results obtained regarding the attack of the AGRBTU pathogen in the three investigated apple varieties are shown in figure 4. The Florina variety presented an attack frequency of 86.67% with a significantly negative difference compared to the average variety (24.45). The Delicious de Voinești variety presented the least aggressiveness, the difference compared to the average experience being significantly positive.

The aggressiveness of the attack, determined by the eubacterium AGRBTU, in the Generos variety (60%) did not show differences compared to the control.

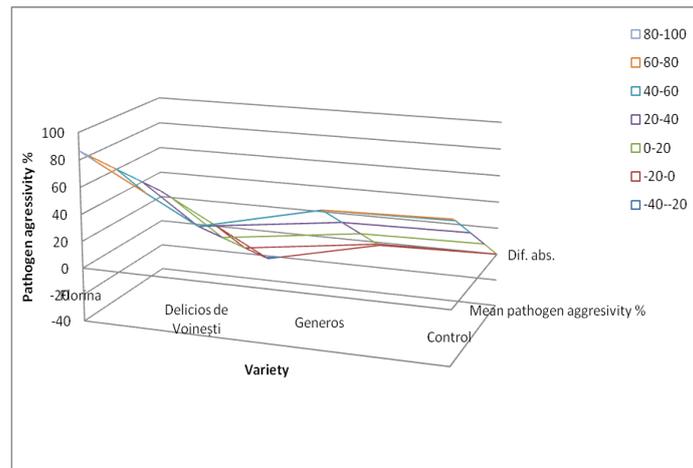


Fig. 4 – Results regarding the aggressiveness of the AGRBTU pathogen in the studied varieties

The Florina variety showed the highest sensitivity to the attack of bacterial cancer, the intensity of the attack being 5.30% (figure 5).

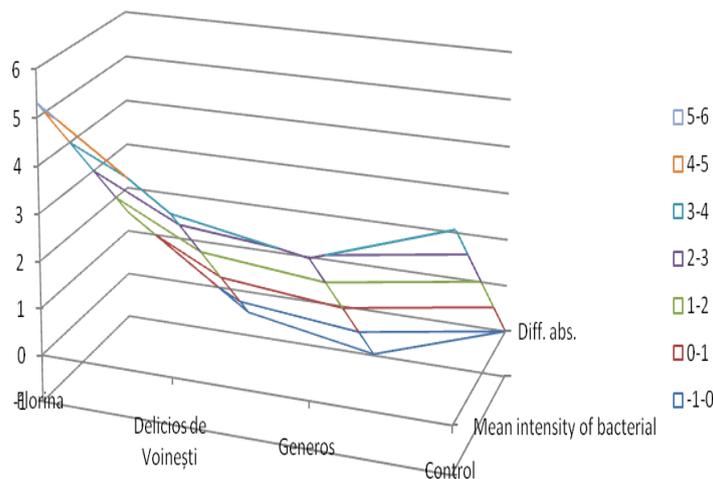


Fig. 5 - Attack intensity of AGRBTU on the studied apple varieties

The virulence of the attack, recorded in the Florina variety, showed significantly negative differences compared to the control (average of the varieties). The virulence of the

bacterium in the Generos variety was the lowest (2.89%), registering significantly positive differences, from a statistical point of view. In the Delicios de Voinești variety, although the lowest aggressiveness was registered, it was not the same situation when we analyzed the attack virulence (3.39%). The virulence of attack registered for the Delicios de Voinești variety did not show significant differences compared to the control (figure 5).

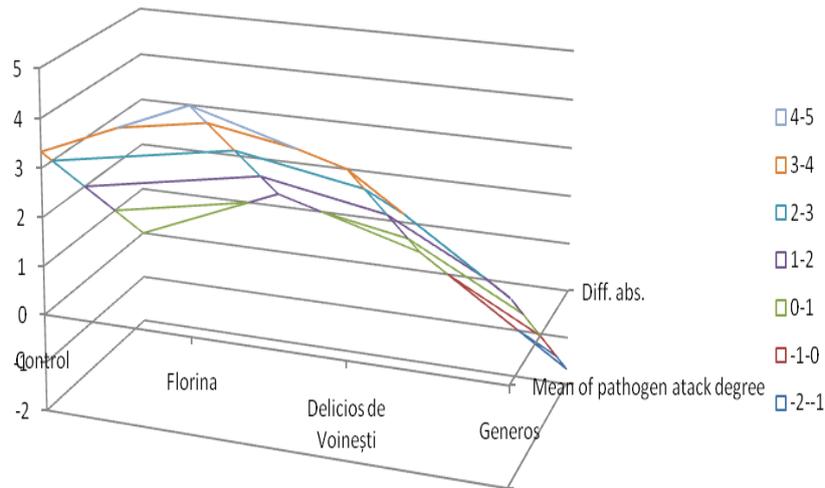


Fig. 6 - The attack degree of the AGRBTU pathogen on the studied apple varieties

Following the calculation of the degree of attack, it is observed that the Generos variety emerged as the most resistant to this bacterium, presenting an absolute difference of -1.67 compared to the average of the varieties. Delicious from Voinești, although it has an absolute difference of +0.40 from the average, from a statistical point of view it is not significant (figure 6).

CONCLUSIONS

The obtained results showed that the species *Agrobacterium tumefaciens* was present in the apple culture, in all three analyzed varieties.

The Florina variety was the most sensitive to the attack of bacterial cancer, presenting the highest values of aggression, virulence and degree of attack.

The Generos variety, although it showed an aggressiveness of 60%, the bacterium did not have a high intensity of attack on it. We can say that the Generos variety is tolerant to the attack of the pathogen that causes bacterial cancer, the degree of attack being 1.67%.

The lowest intensity (40%) was manifested in the Delicios de Voinești variety, but the virulence of the attack was higher compared to that manifested in the Generos variety.

The observations made in the apple orchard of S.D.E. Lugoj - Herindești led us to say that the spread of the bacterium, *Agrobacterium tumefaciens*, was achieved through cutting work with non-disinfected tools. This conclusion was also reached due to the location of the tumors on the twigs.

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