RJH Vol. V, 2024: 77-84
10.51258/RJH.2024.09

Results of some apple hybrid populations to the attack of scab and powdery mildew under natural conditions

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ABSTRACT

Apple scab, caused by the fungus *Venturia inaequalis*, and powdery mildew, caused by the fungus *Podosphaera leucotricha*, are two of the most significant diseases affecting apple production worldwide. These diseases can lead to substantial crop losses and often require frequent fungicide applications, with negative environmental impacts and the development of fungicide resistance. Apple breeding programs frequently aim to develop cultivars exhibiting resistance to scab and powdery mildew. This is also the case of a breeding program initiated at FRDS Bistrita to obtain hybrids resistant/tolerant to scab and powdery mildew, accompanied by superior fruit qualities and a later harvest period. Thus, the pollinations were performed on apple trees in 2023. The experiment was organized into two hybridization schemes involving six apple cultivars. Scheme 1 consisted of five maternal genitors ('Auriu de Bistriţa', 'Jonathan', 'Idared', 'William's Pride', and 'Bistriţean') and one paternal genitor ('Generos'). Scheme 2 consisted of one maternal genitor ('Generos') and five paternal genitors ('Auriu de Bistriţa', 'Jonathan', 'Idared', 'William's Pride', and 'Bistriţean'). The aim of this study was to evaluate the hybrids of these crosses in terms of early response to natural infection with apple scab and powdery mildew. Results showed no symptoms of scab infection on the leaves of the hybrids, while 83.8% of the total showed no symptoms of powdery mildew infection. The hybrid combination with the lowest percentage of infected hybrids was 'William's Pride x Generos' (10.4%), closely followed by the 'Auriu de Bistriţa x Generos' hybrid combination (12.5%). The highest percentage of infected hybrids was observed in the 'Jonathan x Generos' hybrid combination (20.0%).

Keywords: controlled pollination, monogenic resistance, *Podosphaera leuchotricha*, polygenic resistance, *Venturia inaequalis*

INTRODUCTION

Apple scab, caused by the fungal pathogen *Venturia inaequalis*, remains one of the most economically significant diseases affecting apple (*Malus domestica*) production worldwide. The disease can cause considerable crop losses due to the infection of leaves, fruit, and shoots, making resistance to *V. inaequalis* a major focus in modern apple breeding programs (Parisi *et al.,* 1993). Traditional management of apple scabs often relies on frequent applications of fungicides; however, the increasing concerns regarding environmental impact and the development of fungicide resistance highlight the need for durable genetic resistance as a more sustainable solution (MacHardy, 1996).

The two primary forms of genetic resistance to apple scab are vertical (monogenic) and horizontal (polygenic) resistance (Švara *et al*., 2024). Vertical resistance, typically conferred by single resistance (R) genes, such as *Vf* derived from *Malus floribunda* 821, provides strong and specific resistance to certain races of *V. inaequalis*. However, this form of resistance is often short-lived due to the ability of the pathogen to rapidly evolve virulent strains that overcome single-gene resistance, as has been observed with the breakdown of *Vf* resistance in several apple-growing regions (Bus *et al.,* 2011). As a result, the durability of vertical resistance is limited, making it less effective in the long term (Calenge & Durel, 2006). In contrast, horizontal (polygenic) resistance is generally regarded as more durable and robust. This form of resistance is controlled by multiple genes, each contributing to a partly but cumulative resistance effect. Polygenic resistance does not completely prevent infection but significantly reduces the severity of the disease, slowing down the pathogen's ability to adapt and evolve virulence (Parisi *et al.,* 1993). Because polygenic resistance is less specific to individual pathogen races, it provides a broader and more stable defense over time, making it an attractive target for sustainable apple breeding programs (Gessler, Pertot & Perazzolli, 2014).

Another widespread disease that affects the apple crop is powdery mildew, one of the most dangerous harmful fungal diseases. Production is reduced, by at least half in the orchards where no treatments are applied, compared to those where only sulphur compounds are applied (Holb and Kunz 2016). Some studies attest to the dramatic effect that powdery mildew has not only on the production of the current year but also on the following year, in the case of sensitive cultivars (Yoder, 2000).

Apple's resistance to powdery mildew (*Podosphaera leucotricha*) is primarily associated with specific genes identified in wild *Malus* species. Key genes include Pl-1 from *Malus robusta*, Pl-2 from *Malus zumi*, and Pl-w from *Malus × micromalus* or ornamental crabapples like "White Angel" (Alston, 1976). These genes have been incorporated into breeding programs for resistant cultivars, often supported by marker-assisted selection methods to improve resistance efficiency and durability in orchards.

The number of genetically resistant cultivars to powdery mildew is much lower than those with scab resistance but there are some examples: 'Ariwa' - *Pl 1* gene, 'Bramleys Seedling' – *Pld* gene, 'Erbachhofer' – *Plm* gene, etc. (Höfer *et al.,* 2021).

The current breeding efforts focus on crossing apple varieties with desirable technological traits, such as improved fruit quality and storage capabilities, with those possessing polygenic resistance to scabs or specific diseases. By focusing on the integration of multiple resistance genes, we aim to develop cultivars that offer long-term resistance to *V. inaequalis* and *Podosphera leucotrichia* without sacrificing fruit quality, ultimately contributing to more sustainable apple production.

MATERIALS AND METHODS

Controlled pollinations were carried out in 2023 at the Fruit Research and Development Station Bistriţa (FRDS Bistriţa), Romania, and the biological material consisted of six apple cultivars (Table 1) and their F1 hybrids. In our experiments, we utilized the Romanian apple cultivar 'Generos', which was registered in 1983 by the Fruit Research Station Voinesti, in Romania. 'Generos' is known for its resistance to *Venturia inaequalis* and has been used in breeding programs due to its favorable agronomic characteristics, including good fruit quality and enhanced disease resistance. The polygenic resistance to apple scab in 'Generos' makes it a valuable resource in the development of new apple cultivars with durable resistance (Militaru *et al.,* 2018). This cultivar's resistance originates from the use of *Malus kaido* in its pedigree, which has contributed to its robust defense against multiple

races of *Venturia inaequalis*, further supporting the importance of polygenic resistance in modern breeding programs.

Table 1. The selection criteria for the apple cultivars used as genitors in the hybrid combinations

Cultivars	**Selection criteria of the genitors**	**The behavior to scab infections (references)**	**The behavior to powdery mildew infections/references**
Auriu de Bistrița	Superior fruit characteristics, ripening time, local cultivar	Tolerant (Guzu *et al.,* 2021; Ștefan *et al*., 2018)	Low susceptible (Roșu-Mareș *et al.,* 2022; Ștefan *et al*., 2018))
Jonathan	Harvest period	Susceptible (Afunian *et al.,* 2004)	High susceptible (Roșu-Mareș *et al.,* 2022)
Idared	Very good storage capacity in the warehouse	Susceptible (Roșu Mares *et al.,* 2022)	High susceptible (Roșu-Mareș *et al.,* 2022)
Bistrițean	The presence of the *Vf* gene, resistance to specific diseases, fruit appearance, local cultivar	Resistant (Militaru *et al.,* 2020; Ștefan *et al*., 2018))	Low susceptible (Roșu-Mareș *et al.,* 2022; Ștefan *et al*., 2018))
Williams Pride	Harvest period, resistance to specific diseases, taste qualities of the fruit, presence of the *Vf* gene	Resistant (Kaymak *et al.,* 2013)	Resistant (Swezey, 2000)
Generos	Polygenic resistance, indigenous cultivar, valued for its taste qualities	Resistant (Militaru *et al.,* 2018)	Medium sensitive (Roșu-Mareș *et al.,* 2022)

For artificial pollination in apple species, classical hybridization steps were followed (Cordea, 2014). The controlled hybridizations in this experiment are organized into two cyclical schemes as detailed in Table 2.

Table 2. The cyclical schemes of hybrid combinations implemented in the experiments conducted in 2023

Scheme 1	**Scheme 2**
23.01 - ♀Auriu de Bistrița x ♂Generos	23.06 – ♀Generos x ♂Auriu de Bistrița
23.02 – ♀Jontahan x ♂Generos	23.07- ♀Generos x ♂Jonathan
23.03 – ♀Idared x ♂Generos	23.08 – ♀Generos x ♂Idared
23.04 – ♀Williams Pride x ♂Generos	23.09 – ♀Generos x ♂Williams Pride
23.05 – ♀Bistrițean x ♂Generos	23.10 – ♀Generos x ♂Bistrițean

After refrigeration, in February 2024, the seeds were sown in a mixture of soil and sand in a 2:2 ratio and the germinated seedlings were then raised in a greenhouse. During the seedling stage, hybrids were selected based on their development.
After assessing the number of hybrids suitable for planting from both hybridization schemes, the selected plants were transferred in the field (in April, 2024) by establishing

an experimental plot surrounded by large apple orchards infected by *Venturia inaequalis* and/or *Podosphaera leucotricha*. Thus, an appropriate natural infection pressure was secured. Then the behavior of the hybrids to the two specific apple diseases (scab and powdery mildew) was assessed. In order to classify the hybrids according to their response to the two diseases, the frequency of attack (F%) for the target pathogen was determined, according to phytosanitary methodologies.
To uniquely identify each hybrid, a six- or seven-digit code was assigned. The first two digits indicate the year of artificial pollination, the next two represent the hybrid combination number, and the final two or three digits denote the individual hybrid's sequential number.
The statistical determination of the results was conducted using the XLSTAT - Addinsoft software (Addinsoft, 2022), which runs on the Microsoft Office Excel platform. ANOVA analysis was used to assess variance, followed by a Duncan's test with a significance level of $p<0.0001$.

RESULTS AND DISCUSSIONS

From the cyclic hybridization schemes 1 ('Generos' cv. used as the paternal parent) and 2 'Generos' cv. used as the maternal parent), a total of 1,203 seedlings were obtained as follow in table 3:

Table 3. The total number of hybrids obtained from each hybrid combination

Hybrid combination	No. of hybrids
Auriu de Bistriţa x Generos	72
Jonathan x Generos	43
Idared x Generos	412
Williams Pride x Generos	161
Bistriţean x Generos	35
Generos x Auriu de Bistriţa	231
Generos x Jonathan	50
Generos x Idared	112
Generos x Williams Pride	38
Generos x Bistriţean	42

From the two schemes, only the plants suitable for planting were selected, so from the over 1,200 hybrids obtained, only 31.84% (383 hybrids) fulfilled the requirements for transplantation.
Following the analysis of scheme 1 (Figure 1), it can be observed that the hybrids from four hybrid combinations were suitable for planting. The best percentages of hybrids suitable for planting in pots were obtained in two combinations: ultimately 'Auriu de Bistriţa' x 'Generos' with 38.5% and 'Jonathan' x 'Generos' with 37.2%. Statistical analysis of the results shows that there are significant differences in the number of hybrids suitable for pot planting depending on the hybrid combination. Thus, three significant classes were identified. The number of hybrids suitable for planting was significantly higher in the combinations 'Auriu de Bistriţa' x 'Generos' and 'Jonathan x Generos', compared to the other combinations.

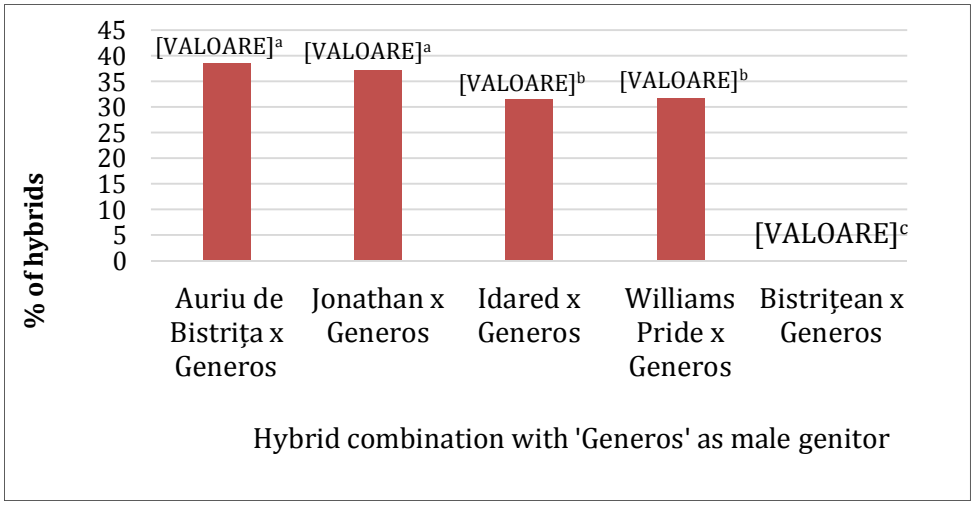

Figure 1. The percentage of hybrids suitable for planting in individual pots (scheme 1)

From cyclic scheme number 2, out of the five hybrid combinations, only three produced hybrids suitable for planting in individual pots (Figure 2). Leading the ranking is the combination 'Generos' x 'Idared' (53.6%), closely followed by the combination 'Generos' x 'Jonathan' (46.0%).

According to statistical results, the hybrid combinations from scheme 2 were classified into four significance classes.

The hybrids resulting from the combinations 'Generos' x 'Bistrițean' and 'Bistrițean x 'Generos' did not survive.

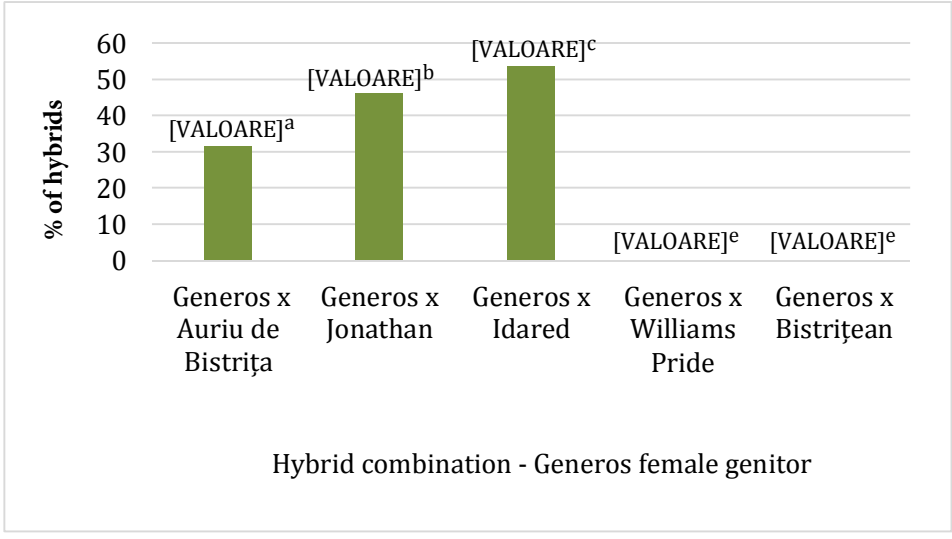

Figure 2. The percentage of hybrids suitable for planting in individual pots (scheme 2)

Following the second objective of this study, the early behavior of hybrids in terms of scab and powdery mildew infections, the results were encouraging. Regarding scabs, no typical symptoms were observed on the hybrids obtained in the year of monitoring (2024).

In contrast, the behavior of hybrids to powdery mildew infections, highlights the fact that some of them showed typical symptoms of powdery mildew (Figure 3).

Figure 3. Powdery mildew symptoms observed in the developed apple hybrids

Interpretation of the obtained results highlights that the infection rate of hybrids with powdery mildew, in the case of scheme 1 ('Generos' cultivar as paternal parent), ranged between approximately 10% and 20% (Figure 4). The hybrid combination with the lowest percentage of infected hybrids was 'William's Pride' x 'Generos' (10.4%), followed by the hybrid combination 'Auriu de Bistrița' x 'Generos' (12.5%). The highest number of infected hybrids was found in the hybrid combination 'Jonathan' x 'Generos' (20.0%), followed by 'Idared' x 'Generos' (16.5%). The statistical interpretation of the obtained results revealed that the four hybrid combinations are classified into three significance classes. Thus, there are significant differences between the 'Jonathan' x 'Generos' respectively 'Idared' x 'Generos' combinations and the other hybrid combinations in terms of the infection rate by powdery mildew of the hybrids. The combinations 'Auriu de Bistrița' x 'Generos' and 'William's Pride' x 'Generos' are classified in the same significance class, therefore there are no statistically significant differences.
In the case of the second scheme ('Generos' cultivar as a maternal parent), the percentages ranged between 13.6% ('Generos' x 'Jonathan' hybrid combination) and 19.1% ('Generos' x 'Idared') (Figure 5).

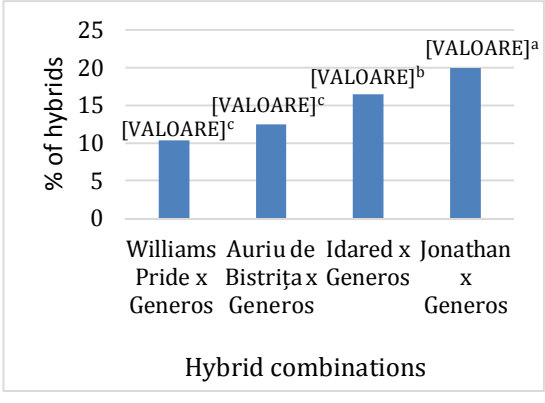

Figure 4. Infection rate of hybrids with powdery mildew (scheme 1)

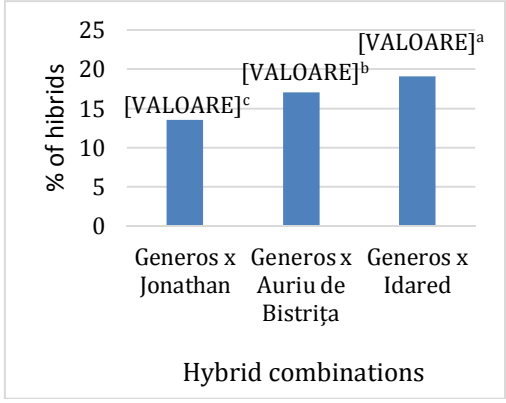

Figure 5. Infection rate of hybrids with powdery mildew (scheme 2)

The results were categorized into three levels of significance, with each hybrid combination belonging to a distinct level. The Duncan test revealed statistically significant differences among all hybrid combinations.

Overall, out of the total of 383 hybrids obtained and monitored, 83.8% did not exhibit powdery mildew symptoms, while the remaining 16.2% were infected with powdery mildew in various percentages.

CONCLUSIONS

Following the selection process and disease monitoring the hybrids did not phenotypically display any symptoms characteristic of apple scab. Regarding the response to powdery mildew, over 80% of the hybrids exhibited typical symptoms of this disease during the experimental year (2024).

ACKNOWLEDGEMENTS

We are grateful to Fruit Research & Development Station Bistrita for the generous help and assistance, as well as to The University of Agricultural Science and Veterinary Medicine of Cluj-Napoca.

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