APARIȚIA VIRULENȚEI ÎN AGROSISTEMUL POMICOL: STUDIU DE CAZ *VENTURIA INAEQUALIS* LA SOIURILE *VF* DE MĂR CULTIVATE ÎN ROMÂNIA

EMERGE OF VIRULENCES IN AGROSYSTEMS: *VENTURIA INAEQUALIS* AT *VF* APPLE CULTIVARS GROWN IN ROMANIA AS A CASE STUDY

Militaru Mădălina, Călinescu Mirela, Butac Mădălina
Research Institute for Fruit Growing Pitesti, Romania

Abstract

Scab symptoms were found for the first time in 2016 in Arges District, Romania on leaves and fruits of apple cultivars ('Ariane', 'Florina', 'GoldRush', 'Enterprise', 'Topaz' and 'Rubinola'), carrying the *Vf* (= *Rvi6*) major gene to *Venturia inaequalis*. An F_1 progeny derived from six different crosses: (1) 'Generos' x 'Crimson Crisp', (2) 'Topaz' x 'Crimson Crisp', (3) 'GoldRush' x 'Luca', (4) 'Gala' x 'Ariane', (5) 'Fuji' x 'Ariane' and (6) 'Enterprise' x 'Fuji' was screening for the resistance genes study. For inoculum was used isolates derived from one resistant genotype (*Vf* gene) and two sensitive varieties. The seedlings were inoculated in the glasshouse and classified according to the reaction type. A clear effect of the susceptible parent on the resistance of the progeny was observed.

Cuvinte cheie: măr, gena *Vf*, rapăn, inocul.
Key words: apple, *Vf* gene, scab, inoculum.

1. Introduction

It has been admitted for almost a century that plant pathogens evolve rapidly in agrosystems and gee-for-genes resistances only control diseases for a few years (Johnson, 1961; Brown, 1994; Crute and Pink, 1996; McDonald, 2010; Lemaire and al., 2016).

Apple scab also known as black spot, caused by an ascomycetes fungus *Venturia inaequalis* [Cooke] *Wint.*, is one of the most serious disease of apple (*Malus x domestica*) in many apple-growing areas. Annually, scab caused premature leaf loss of trees and 100% fruit lesion in susceptible cultivars if chemical protection is not used. In Romania, to control this disease in the commercial orchards are require more of 16 fungicide sprays per season in order to avoid losses. For this reason, the main objective of apple program breeding was to create new varieties with durable resistance.

The genetic background of apple scab resistance started in 1926 in the USA, when Charles S. Crandall observed that clone 821 of the wild species *Malus floribunda* was completely resistant to apple scab (Crandall, 1926). Later, in 1947, in the PRI breeding for resistance program between the Purdue, Rutgers and Illinois universities was registered 'Prima' cv., the first cultivar which has been incorporated *Vf* (*Rvi_6*) gene and also, big fruit size and quality (Dayton and Mowry, 1970; Janick, 2006). Most of the PRI cultivars ripen early. For example, the harvest time for 'Prima' cv. is 1 month before 'Red Delicious' and has a short storage life (Williams and al., 1972). Analyzing the segregation of scab resistance provided from *M. prunifolia, M. atrosanguinea, M. baccata, M. prunifolia microcarpa*, Williams and al. (1966) observed that the gene involved in resistance to apple scab is the same or allelic with *Vf* resistance of *M. floribunda* 821 (Gessler et al., 2006).

Starting with 1970s, a lot of breeding programs developed to bred for apple scab resistance around the world, using different source of resistance: *Va* (*Rvi_{10}*) from 'Antonovka' cv., *Vb* (*Rvi_{12}*) from *M. baccata*, *Vbj* (*Rvi_{11}*) from *M. baccata jackii*, *Vr* from *M. pumila* (Russian seedling) R12740-7A, *Vm* from *M. x micromalus* and *M. x atrosanguinea 804, Vg* (*Rvi_1*) from 'Golden Delicious' cv., *Vr_2* (*Rvi_{15}*) from GMAL 2473, *Vd* (*Rvi_{13}*) from 'Durello di Forli', *Vd_3* from 1980-015-25, *Vmis* from MIS (Mildew Immune Selection), *Vh_2* (*Rvi_2*) and *Vh_4* (*Rvi_4*) from differential host derived from *M. pumila* R12740-7A, *Vh_8* (*Rvi_8*) from *M. sylvestris* W193b (Gessler et al., 2006).

In Romania, the apple breeding activity was initiated at Research Station for Fruit Growing Voinești in 1950 and then it was extended in the other research centers: Cluj (1953), Fălticeni (1957), Târgu Jiu (1959), Pitești (1967) and Bistrița (1967) going through more steps with different objectives related to the trends market. The first success of scab resistance breeding was 'Generos' cv., with horizontal resistance, registered in 1983, by Moruju Gh. and Șerboiu L. from ('Parmain d'or' x '*M. kaido*') x ('Jonathan' x 'Frumos de Voinești') cross combination. Useful for Romanian breeding program was the special collaboration with L. F. Hough, from Rutgers University, USA finalized with registered, in 1984, of 'Romus 3' cv., keeping it still in assortment as a good cultivar with early ripening time. About 20 new

varieties were created using 'Prima' cv., like genitor in cross hybridizations or irradiation of open pollination seeds. In 1979, Braniște N. introduced at Research Institute for Fruit Growing Pitesti the French variety 'Florina' ('Querina') cv. developed in 1970s at INRA Angers. Due scab resistance, moderately resistance to powdery mildew, tolerance to fireblight and good storage capacity, after few testing years, it was propagate about 150,000 trees/year and became the most spread *Vf* cultivar in Romanian orchards.

The aim of this study is screening for scab resistance in the progeny of 6 cross combinations using different sources of inoculum to show the specific virulences or avirulences. The inoculum was released after observed first scab symptoms on leaves and fruits on *Vf*-cultivars like 'Florina', 'Ariane', 'Enterprise' and 'GoldRush' .

2. Material and methods

Obtaining and cultivation of *Venturia inaequalis* monosporic isolates

The monosporic isolates were derived from naturally infected leaves of three apple varieties: 'Ariane', 'Gala' and 'Golden Delicious' cvs. from demonstrative apple plots of Research Institute for Fruit Growing Pitesti, Romania. The leaves were collected in 2016, from August till September, dried and keep in storage. The inoculum was each adjusted to 2-3 x 10^5conidia per mililiter and the conidial suspensions applied till runoff with a manual atomizer. The potted seedlings were inoculated for each isolate and incubated at 20.3^{0}C with 88% relative humidity. To ensure constant leaf wetness, the potted seedlings were placed in polythene covered structure and incubated for 48 h in dark. Symptoms were assessed 14 days after inoculation.

Plant material

Six different crosses: (1) 'Generos' x 'Crimson Crisp', (2) 'Topaz' x 'Crimson Crisp', (3) 'GoldRush' x 'Luca', (4) 'Gala' x 'Ariane', (5) 'Fuji' x 'Ariane' and (6) 'Enterprise' x 'Fuji', provided from Genetic and Breeding Department were used for the resistance genes study. The list and background information about parental progeny are presented in table 1. Hybrid seeds, obtaining from the crosses released in 2016, were after stratification sowed in greenhouse. 60 seedlings from each cross combination were selected for inoculation. A third of each progeny was sprayed with the inoculum from leaves of 'Ariane', second third with inoculum from 'Gala' leaves and last third with inoculum obtained from 'Golden Delicious' leaves.

Reading of symptoms

The reading of the symptoms was made 14 d after the inoculations using the following reaction classes (adapted from Gessler et al., 2006): A = no macroscopic evidence of infection; B = pit-point symptom. Depression of 100-500 µm where the epidermal cells have collapsed. No subcuticular stroma; 2 = wide but shallow depressions. Limited stroma formation. No sporulation; C = epidermal cells collapsed over large areas. Close to the centre the abundant mycelial stromata could produce conidiophores with a limited number of conidia; D = lesions are a network of mycelial strands. Aborted conidiophores are mixed with normal conidiophores. Sporulating chlorosis and sporulating necrosis occur; E = numerous conidiophores are often grouped in clusters and sporulate abundantly. The mycelial stroma forms a dense subcuticular network. The seedlings assigned to reaction class E were considered complete susceptible (Fig. 1).

3. Results and discussions

The breakdown of *Vf* resistance

The *Vf* (*Rvi6*) gene, isolated from wild crab apple *Malus floribunda* 821, is the most studied *R* gene of apple and, also, has been extensively used for breeding scab resistance (MacHardy, 1996; Gessler et al., 2006).

The first breakdown of *Vf* resistance was reported in 1983 on 'Prima' cv. growing in Moldavia (Fischer et al., 1983). Scab on Vf-resistant cultivars has been observed at Ahrensburg, N-Germany since 1984 (Krüger, 1999). Later, in 1988, in various other European countries were reported apple scab symptoms on resistant cultivars. Apple scab symptoms on *M. floribunda* were reported in a private English garden as early as 1989, even before plantation of *Rvi6* apple varieties in English orchards (Roberts and Crute, 1994). At other location near the Baltic Sea scab was observed in 2000 only on 'Prima' and 'Ecolette' and in 2001 on other resistant cultivars (Höhne, 2001). In fact, the situation is very variable but not hopeless because of different results in each year according to the actual environmental situation (Dierend, 2003).

A study released by Sestraș (2003), between 1990 and 1996, in Central part of Romania (Transylvania) shows not scab symptoms neither on leaves or fruits on 'Florina' and 'Priam ' - French cvs., with vertical resistance and, also, another Romanian varieties like: 'Voinea', 'Romus 3', 'Pionier', with vertical resistance and 'Generos', with horizontal resistance, derived from *Malus kaido*. He reported a

significantly phenotypic correlation between the attack degree on leaves and average yearly temperature, total yearly rainfall.

In 2016 and 2017, for the first time, in several commercial orchards from Arges District, Romania, were found scab symptoms on leaves and fruits on *Vf*-cultivars like 'Florina' (Fig. 2), 'Ariane' (Fig. 3), 'Enterprise' (Fig. 4) and 'GoldRush' (Fig. 5). The symptoms were generally most noticeable and serious on leaves and fruit. The first lesions seen in the spring were usually on the underside of expanding leaves. Once the leaves open, the upper surfaces also became vulnerable to infection. A lesion first was appeared as an area which was a lighter shade of green than the surrounding leaf. The lesion was circular and step by step it becomes olive-colored and velvety due to production of asexual spores (conidia). The fruit epidermis became distorted and puckered, often cracked and torn. Lesions on the leaves and fruit were generally blistered and "scabby" in appearance, with a distinct margin. Infections of young fruit were cause fruit distortion, especial of 'Ariane' fruits. (Fig. 3, b_2)

The effect of the scab inoculation of the progenies

The overall effect of the 3 scab inoculum sources on the distribution of the progenies to the different classes of symptoms is illustrated in figure 6. The percentage of resistant seedlings (class A) was high for all inoculum sources. Also, the results show that inoculum from 'Ariane' is more virulent than the other once. This result confirms that the resistance to scab of apple progenies is influenced by the susceptible parent. In this study, we show that parent varieties which are high susceptible to scab like 'Fuji' and 'Gala' influenced the susceptibility of the progeny (Fig. 7). From genetic and breeding work, the conclusion is: the susceptible parent can significantly improve the resistance of the progeny.

4. Conclusions

For the durability of scab resistance in the field we recommend:

- no 'monoculture' with Vf-cultivars;
- three - five fungicide spray applications per year are required to control spring infection;
- the breeding of cultivars with two or more resistance sources by pyramiding different resistance genes and using more cultivars with polygenic scab resistance.

References

1. Barbara, D.J., A.L. Roberts and X.M. Xu, 2008. Virulence characteristics of apple scab (*Venturia inaequalis*) isolates from monoculture and mixed orchards. Plant Pathol., 57: 552-561.
2. Brown J.K.M., 1994. Chance and selection in the evolution of barley mildew. Trends in Microbiology 12: 470-475.
3. Crandall C.S., 1926. Apple breeding at the University of Illinois. Illinois Agricultural Experimental Station Bulletin 275: 341-600.
4. Crute I.R., Pink D.A., 1996. Genetics and utilization of pathogen resistance in plants. Plant Cell 8: 1747-1755.
5. Dayton D.F. and Mowry J.B., 1970. Prima - the first commercial scab-resistant apple variety. Fruit Varieties and Horticultural Digest 12: 7.
6. Gessler C., Patocchi A., Sansavini S., Tartarini S., Gianfrancesci L., 2006. *Venturia inaequalis* Resistance in Apple. Critical Reviews in Plant Sciences 25 (6): 473-503.
7. Fischer C., Bukartschuk V.F., Bondarenko A.A., Artamonova E.S., 1983. Erste Ergebnisse zur stabilitat der Schorfresistenz beim Apfel under verschiedenen okologischen Bedingungen in der UdSSR und DDR. Arch Gartenbau 31: 263-264.
8. Janick J., 2006. The PRI apple breeding program. Hortscience 41: 9-10.
9. Johnson T., 1961. Man-guided evolution in plant rusts. Science 133: 357-362.
10. Krüger J., 1988. Bestandigkeit der Schorfresistenz aus Malus floribunda 821 auf dem Versuchsfeld der Bundesforschugsanstalt für gartenbauuliche Pflanzenzüchtung in Ahrensburg. Erwerbsobstban 52(2): 52.
11. Lemaire Ch., De Gracia M., Leroy T., Michalecka M., Lindhard-Pedersen H., Guerin F., Gladieux P., Le Cam B., 2016. Emergence of new virulent populations of apple scab from nonagricultural disease reservoirs. New Phytologist 209: 1220-1229.
12. MacHardy W.E., 1996. Apple scab, biology, epidemiology and management, APS, St. PAul, Minn, USA, 1996.
13. Patzak J., Paprstein F., Henychova A., 2011. Identification of apple scab and powdery mildew resistance genes in Czech apple by PCR molecular markers. Czech J. Genet. Plant Breed 47: 156-165.
14. Roberts A.L., Crute I.R., 1994. A scab resistance from *Malus floribunda* 821 (*Vf*) is rendered ineffective by isolates of *Venturia inaequalis* from *Malus floribunda*. Netherlands Journal of Agricultural Sciences 17: 403-406.

15. Sestraș R., 2003. Response of several apple varieties to apple scab (*Venturia inaequalis*) attack in Central Transylvania conditions. Journal of Central European Agriculture (online) 4: 355-362.
16. Williams E.B., Dayton D.F., Shay J.R., 1966. Allelic genes in *Malus* for resistance to *Venturia inaequalis*. Proc Am Soc Hort Sci 88: 52-56.
17. Williams E.B., Janick J., Emerson F.H. Dayton D.F., Mowry J.B., Hough L.F., Bailey C., 1972. Six scab-resistant apple selections released for grower testing. Research Progress Report, Cooperating Agricultural Experiment Stations, Purdue University, University of Illinois and Rutgers University 399: 4.

Tables and figures

Table 1. List and background information about parental progeny (adapted from *Patzak et. al., 2011, Sturzeanu, 2016, unpublished results*)

No.	Cultivar	Institution	Reported parentage	Scab resistance gene	
				Vf (*Rvi*$_6$)	*Vr* (*Rvi*$_2$)
1	Ariane	INRA Angers, France	(Florina x Prima) x (Golden Delicious x unknown parent)	+	
2	Crimson Crisp Co-op 39	Purdue, Rutgers Illinois (PRI), SUA	PCFW2-134 x PRI 669-205	+	
3	GoldRush Co-op 38		Golden Delicious x Coop 17	+	
4	Enterprise Co-op 30		PRI 1661-2 x PRI 1661-1	+	
5	Topaz	Institute of Experimental Botany Strizovice, Czech Republic	Rubin x Vanda	+	
6	Fuji	Tohoku Research Station, Japan	Red Delicious x Ralls Janet		+
7	Generos	Research Station for Fruit Growing Voinesti, Romania	('Parmain d'or' x '*M. kaido*') x ('Jonathan' x 'Frumos de Voinești')		
8	Luca		Champion x Prima	+	

(+) plus indicates the presence of a marker for resistance genes

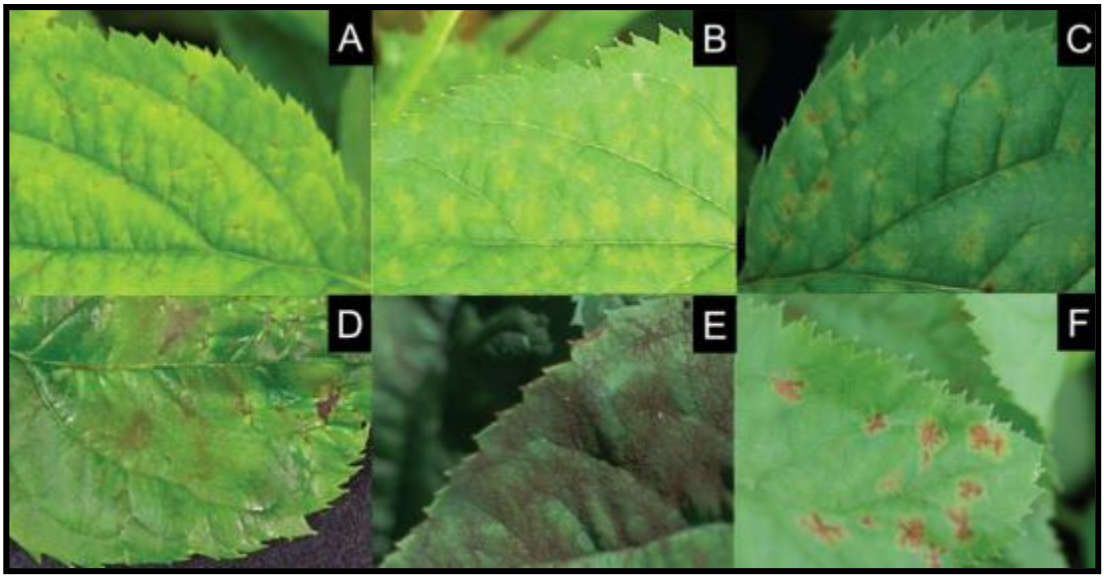

Fig. 1. Macroscopic scab reactions on apple leaves (adapted from Gessler et al., 2006)
A - class 1 with HR; B - class 2 with chlorotic lesions; C - class 3a and D - class 3b, with chlorotic and necrotic with slight sporulation and cleary sporulating lesions; E - class 4 complete susceptibility, F - stellate necrotic reaction

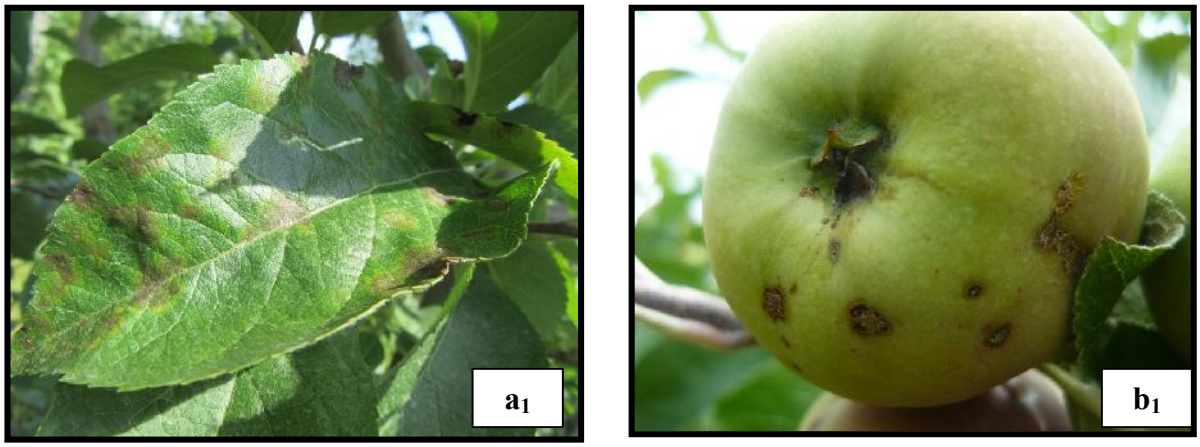

Fig. 2. Field scab symptoms on leaves (a) and fruits (b) of 'Florina' cv. in different phenological stages at Pitesti, Romania (a_1 - on August, b_2 - on June, 2016)

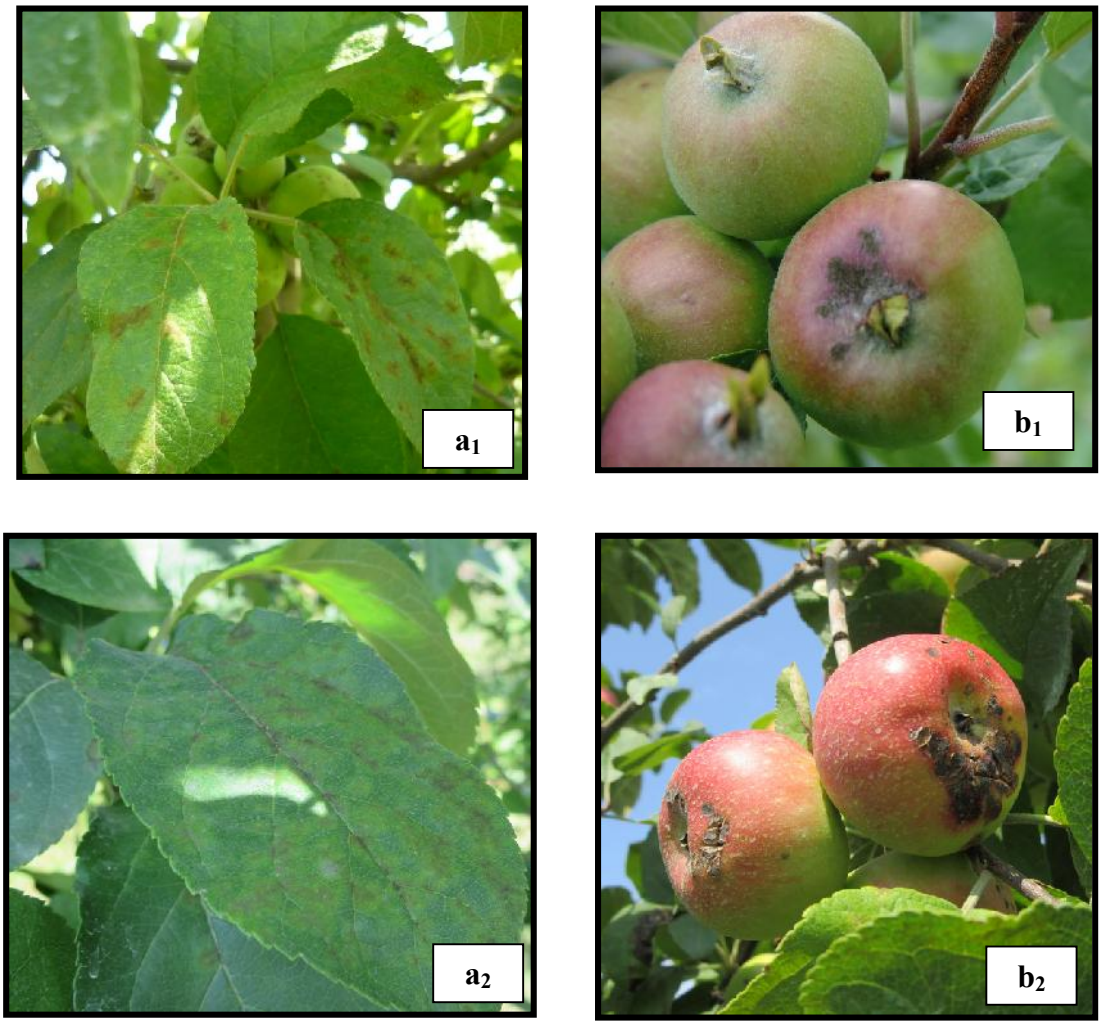

Fig. 3. Field scab symptoms on leaves (a) and fruits (b) of 'Ariane' cv. in different phenological stages at Pitesti, Romania (a_1, b_1 - on June, a_2, b_2 - on August, 2016)

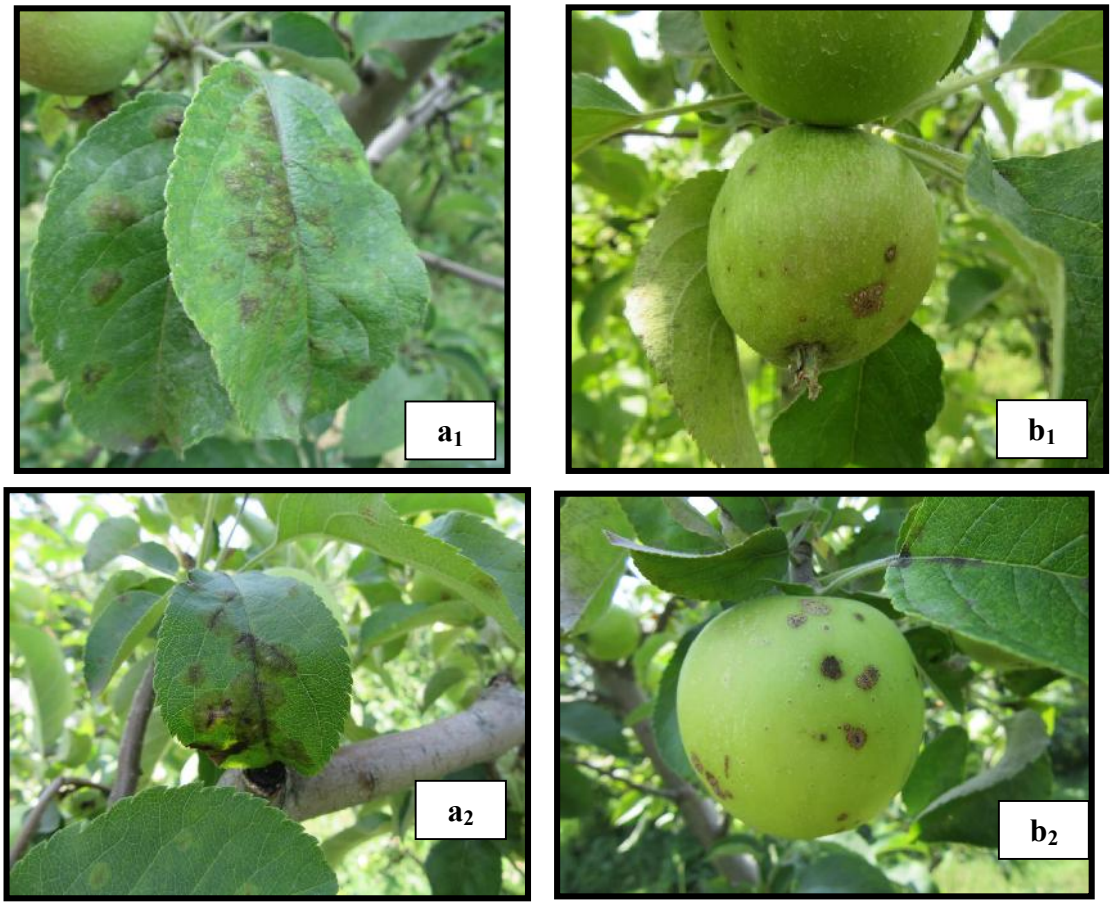

Fig. 4. Field scab symptoms on leaves (a) and fruits (b) of 'GoldRush' cv. in different phenological stages at Pitesti, Romania (a_1, b_1 - on June, a_2, b_2 - on August, 2016)

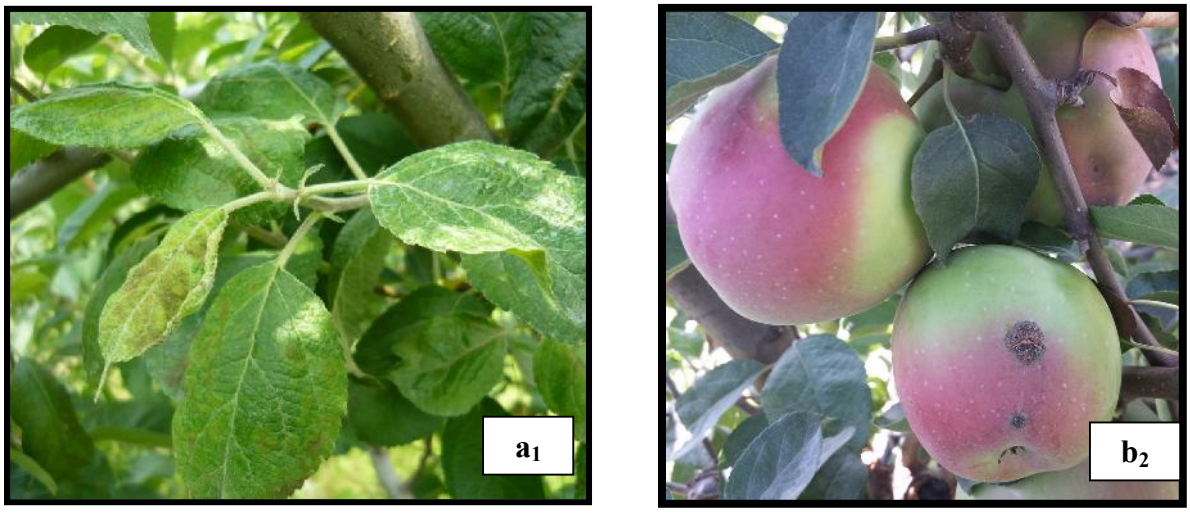

Fig. 5. Field scab symptoms on leaves (a) and fruits (b) of 'Enterprise' cv. in different phenological stages at Pitesti, Romania (a_1 - on June, 2016, b_2 - on August, 2017)

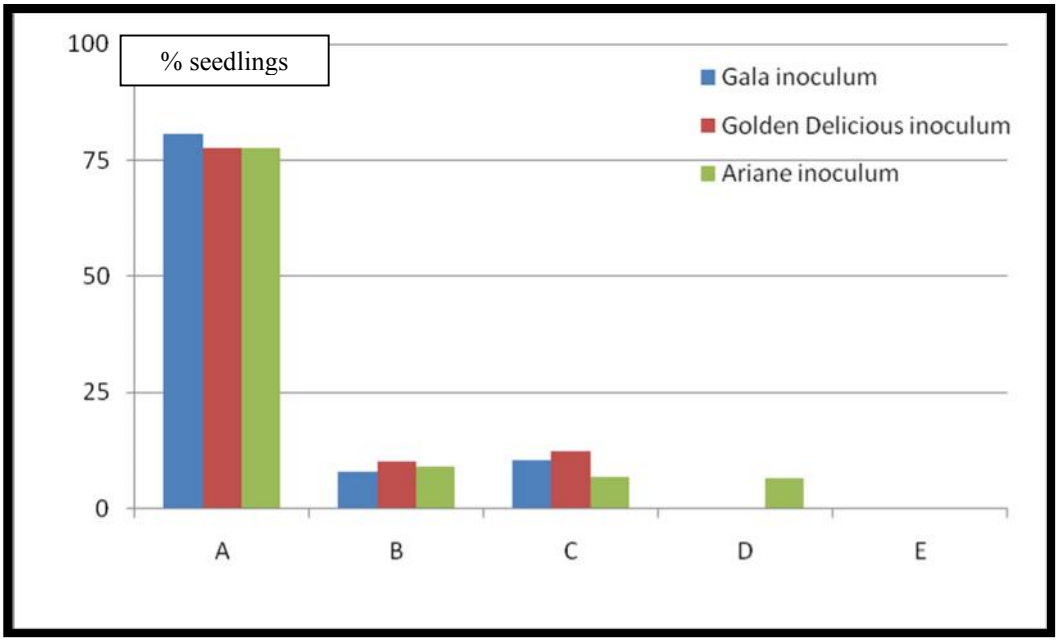

Fig. 6. Effect of the scab inoculum on the distribution to the different reaction classes

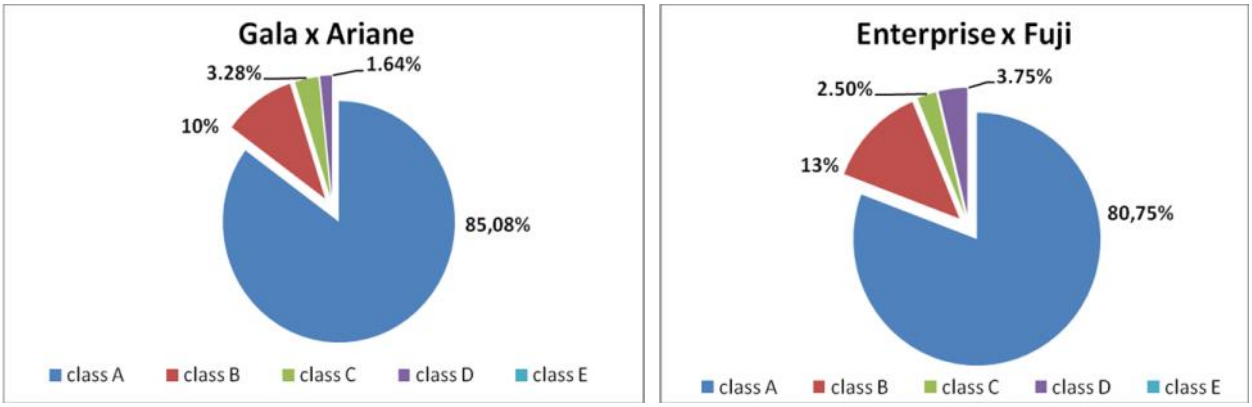

Fig. 7. The distribution of the progeny to the different reaction classes after inoculation with 'Ariane' inoculum