Fruit Growing Research, Vol. XXXIV, 2018 DOI 10.33045/fgr.v34.2018.07
http://publications.icdp.ro/index.php

COMPORTAREA LA ATACUL DE RAPĂN ŞI FĂINARE A HIBRIZILOR F_1 DE MĂR PROVENIŢI DIN ÎNCRUCIŞĂRI DIRECTE ŞI RECIPROCE

RESPONSE TO APPLE SCAB AND POWDERY MILDEW ATTACK OF DIRECT AND RECIPROCAL F1 APPLE HYBRIDS

Militaru Mădălina[1], Călinescu Mirela[1], Butac Mădălina[1], Chivu Mihai[1], Sestraș Adriana[2]
[1]Research Institute for Fruit Growing Pitesti, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania

Abstract

One of the most important methods to create variability in apple breeding, in order to obtain new valuable cultivars, remains intraspecific hybridizations and, the success of creating new apple cultivars depends on the availability of sufficient genetic diversity. The response to apple scab and powdery mildew attack of 469 F_1 hybrids (in 2^{nd}-3^{th} year of age) belonging to six combinations were analyzed. The response of hybrids to diseases attack was assessed depending of the genitors and type of hybridization (direct and reciprocal crosses). Under natural conditions of infection, without fungicide treatments, a high variability was recorded inside the cross combination. For apple scab, the coefficient of variability (CV%) had values between 12.2% ('Crimson Crisp' × 'Topaz') and 55.5% ('Ariane' × 'Gala'), and for powdery mildew attack between 68.3% ('Crimson Crisp' × 'Topaz') and 85.6 % ('Gala' × 'Ariane'). For some apple varieties (i.e. 'Topaz', 'Ariane'), the immunity or the monogenic resistance to scab has not been confirmed, and the heterosis in the hybrid progeny was calculated, being a hypothetical model of quantitative heredity, in order to identifying the progeny with high heterosis effect on scab and powdery mildew resistance.

Cuvinte cheie: hibridare, puieți hibrizi, rapăn, făinare
Key words: hybridization, seedlings, scab, powdery mildew

1. Introduction

Apple scab, caused by *Venturia inaequalis* (Cke.) Wint., and powdery mildew, caused by *Podosphaera leucotricha* (Ell. et Everh.) Salm., are the main fungal diseases which are controlled with considerable difficulties and costs, but with long-term consequences and negative effects on the environment and on the fruit quality (Berriea and Xua, 2003; Holb, 2009; Sestraș et al., 2011)

Intraspecific hybridization is one of the most used method to create variability in apple breeding. Due to its extensively practice and especially as the result of choosing a confined base for genitors, as there were tendency to use the most known apple cultivars for fruit quality, meaning a relatively small number of options, and thus leading to a narrow genetic basis (Kellerhals et al., 2004), in the process of apple selection the threat of losing genetic diversity is a continuous concern (Sestras, 2004). Obtaining new resistant and valuable genotypes is even more challenging as most of the economical traits of interest in apple are of quantitative nature determined by polymorphism (Dan et al., 2015). In addition, resistant apple cultivars are necessary for organic and integrated fruit growing system, since many of the highly appreciated commercial cultivars are very susceptible to apple scab and powdery mildew attack (Sestraș et al., 2011). This study aimed to analyze the resistance response to apple scab and powdery mildew attack of F_1 hybrids obtained inside artificial crosses (direct and reciprocal) using donors for fruit quality and for diseases resistance.

2. Material and methods

The research was released at Research Institute for Fruit Growing Pitești - Mărăcineni, Romania and the biological material used in this study was represented by 468 F_1 apple hybrids (in 2^{nd}-3^{th} year of age), belonging to following, direct and reciprocal, 6 cross combinations: 'Enterprise' x 'Fuji', 'Fuji' x 'Enterprise', 'Ariane' x 'Gala', 'Gala' x 'Ariane', 'Topaz' x 'Crimson Crisp', 'Crimson Crisp' x 'Topaz'. Four apple cultivars known as monogenic source of resistance to apple scab (*Vf* gene) were used as male and female genitors: 'Enterprise', 'Ariane', 'Crimson Crisp', 'Topaz'. The number of progenies per combinations varied from 26 ('Fuji' x 'Enterprise') up to 131 ('Topaz' x 'Crimson Crisp') (Table 1).

The assessment of the response to apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha)* has been performed under natural infection environment, in the absence of phytosanitary treatments, every hybrid from the hybrid nursery being rated regarding the attack on leaves.

For scab infection on leaves, the used assessment system has comprised scores (1-9) corresponding to the following levels: 1 = no visible symptom; 2 = a few small scab spots are detectable on close scrutiny of the tree; 3 = scab immediately apparent, with lesions very thinly scattered over the tree; 5 = infection widespread over the tree, majority of leaves with at least one lesion; 7 = heavy infection, multiple lesions or more large surfaces covered by scab on mostly leaves; 9 = maximum infection, leaves black with scab (Lateur and Populer, 1996). Response to primary infection in the spring with powdery mildew on top of twigs, was assessed using 1-9 scale, using following marks: 1 = no visible symptom; 2 = one or few organs affected, detectable on close scrutiny of the tree; 3 = infected organs readily apparent, but without important consequences for the tree; 5 = primary mildew widespread over the branches, inducing the infection of a substantial part of the crown; 7 = heavy infection, half of the organs are badly affected; 9 = crown completely affected, nearly all top of the organs are infected by primary mildew (Lateur, 1999).

The assessment was individually performed for each hybrid per each F1 combination, for a period of two consecutive years (on spring for primary infection of powdery mildew and in the middle of July for apple scab), the hybrids being in their 2^{nd}-3^{th} year of age (since their obtaining from seeds). The genitor's response to the diseases attack was assessed in similar conditions with their hybrids, respectively those two-year of observations, under natural infections with no fungicide treatments, the same scale of assessment etc. (the notes for apple scab and powdery mildew attack are presented in Figure 1). For hybrids' combinations, the means of the marks for attack response were compared using one-way ANOVA, followed by Duncan Multiple Range Test.

The level of heterosis (absolute heterosis, relative heterosis and heterobeltiosis) for the marks accorded to diseases attack was calculated. Absolute heterosis values were determined for apple scab and powdery mildew attack in two ways (Baciu et al., 2012):

-By comparing the average response of F1 hybrids with parental mean of notes for attack response, using formula: H = F1 - (P1 + P2) / 2.

-By comparing average of F1 hybrids with mean of trait (notes for attack) to the best parent (the parents with lower attack, in this case), using formula: $H = F1 - P_{min}$.

To calculate relative heterosis (RH), the formula used the average values of F1 hybrids and parents (genitors): $H = (\overline{F}1 - \overline{P}) / \overline{P} \times 100$; there was determined also heterobeltiosis (BH) as the percentage of heterosis reported to the best parent according to: $BH = (\overline{F}1 - \overline{P}_{Min}) / \overline{P}_{Min} \times 100$; using the average of F1 hybrids and the best genitor (also, with lower value for the reaction to the disease, because the interest is for resistance or tolerance to diseases attack).

3. Results and discussions

Response of F_1 hybrids to apple scab attack

The hybrid combinations with the lowest average of notes for apple scab attack derived from the following crosses: 'Crimson Crisp' x 'Topaz' and 'Topaz' x 'Crimson Crisp' (Figure 2). The higher mean of rates for scab attack were registered in 'Ariane' x 'Gala' and 'Gala' x 'Ariane' combinations. The mean of marks for apple scab attack on leaves was dispersing, with limits ranging from 1.02 ('Crimson Crisp' used as maternal genitor) to 2.25 ('Ariane' used as maternal genitor). The coefficient of variability (CV%) had values between 12.2% ('Crimson Crisp' × 'Topaz') and 55.5% ('Ariane' × 'Gala') (Figure 3). The results showed that 'Topaz' and 'Crimson Crisp' cvs. transmitted the best response to apple scab attack to their seedlings (both in direct and reciprocal crosses).

One surprising results was obtained especially for 'Ariane' (which was registered with a high note for sensitivity to apple scab), because the cultivar carries the *Vf* gene. It is know that it is resistant to the five most common races of *Venturia inaequalis* (races 1 to 5), but tests in the greenhouse showed that it is highly susceptibility to different strains of race 6 (Laurens et al., 2005). Based on two-year field observations of scab symptoms (leaves and fruits) in cv. 'Ariane', the mechanism of *Vf* resistance has been overcome in Romania (Militaru et al., 2017).

Response of F_1 hybrids to powdery mildew attack

The smallest average value of the marks, identifying the hybrids with the best response to the powdery mildew attack, was recorded for 'Gala' x 'Ariane' cross (Figure 4). The highest average value was 4.13 and was recorded for 'Crimson Crisp' x 'Topaz' cross. This sensitive response could be explained by the genitors' influences, 'Crimson Crisp' being known as sensitive to powdery mildew (Biggs et al., 2009). For four cross combinations, the mean of marks was quite close, ranging from 3.16 ('Enterprise' x 'Fuji') to 3.68 ('Topaz' x 'Crimson Crisp'). For powdery mildew attack, the coefficient of variability (CV%) had values between 68.3% ('Crimson Crisp' × 'Topaz') and 85.6 % ('Gala' × 'Ariane') (Figure 5).

Heterosis for hybrids response to apple scab and powdery mildew attack

Based on the response of cultivars used as genitors to apple scab and powdery mildew attack (Figure 1), in similar conditions with their hybrids (two-year observations, natural infections, the same scale etc.), the level of heterosis calculated in F1 descendants are presented in Table 2 (absolute heterosis, using two of the most common formulas), relative heterosis (Figure 6), and heterobeltiosis (Figure 7).

As can be seen in Table 2, the most valuable absolute heterosis was obtained in combinations 'Ariane' x 'Gala' and 'Gala x Ariane' for apple scab. As stated, because for some apple varieties (i.e. 'Ariane'), the immunity or the monogenic resistance to scab has not been confirmed, the heterosis in the hybrid progeny was calculated, being considered a hypothetical model of quantitative heredity. The result is explained by the unexpectedly high values of the attack at both genitors, and consequently by the transgressive values of the hybrids compared with them.

Based on parental and hybrids values and heterosis percentage calculated from P_{max}/P_{min} (here there was used P_{min}), major genetic effects involved in the development of heterosis response to apple scab and powdery mildew attack had highlighted, as follows (Baciu et al., 2012):

a) $F_1 > P_{max}$ or $F_1 < P_{min}$, heterosis (probably overdominance): 'Ariane' x 'Gala' and 'Gala x Ariane' for apple scab, as has been shown previously.

b) $F_1 = P_{max}$ or P_{min}, complete dominance: 'Crimson Crisp' x 'Topaz' with 'Crimson Crisp' as genitors, and eventually also in reciprocal combination, for apple scab. However, probably in this cross the result is not due to the polygens resistance, but to the *Vf* gene and the Mendelian transmission of this monogenic resistance to the descendants.

c) $P_{max} > F_1 > \overline{P}$ and $P_{min} < F_1 < \overline{P}$, partial dominance; e.g. 'Enterprise' x 'Fuji' and 'Fuji' x 'Enterprise', 'Topaz' x 'Crimson Crisp' and 'Crimson Crisp' x 'Topaz' for apple scab.

d) $F_1 = \overline{P}$, absence of dominance: 'Crimson Crisp' x 'Topaz' for apple scab, respectively 'Topaz' x 'Crimson Crisp' both for apple scab and powdery mildew.

Both for relative heterosis and heterobeltioses, the most favourable results were noticed in 'Ariane' x 'Gala' and 'Gala x Ariane' crosses for apple scab (Figure 6 and Table 3). The coefficient of correlations between response to apple scab and powdery mildew attack inside the hybrid combinations were negative in five of six crosses, but only in two the value of 'r' was significant (Table 3). Specifically, in 'Enterprise' x 'Fuji' and 'Gala' x 'Ariane' hybrids families, the relation between apple scab and powdery mildew response of the hybrids was inversely proportional ('r' significant), consequently when the resistance of hybrids to one disease decreases, to the other one increases.

4. Conclusions

The artificial intraspecific hybridisations offered the chance to obtain a population of hybrids that have a large variability for response to apple scab and powdery mildew attack.

For some apple varieties (i.e. 'Ariane', 'Topaz'), the immunity or monogenic resistance to apple scab has not been confirmed. Heterosis in all hybrid families was calculated, following a hypothetical model of quantitative heredity of response to the disease in order to identify progenies with an adequate response to apple scab and powdery mildew attack.

References

1. Baciu A., Pamfil D., Sestras A., Mihalte L., Sestras R., 2012. Heterosis Studies for Response to Aphis fabae Attack in Calendula. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture 69(1): 40-47.
2. Berriea A.M., Xua X.M., 2003. Managing apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) using Adem™. International Journal of Pest Management 49(3): 243-249.
3. Biggs A. R., Keith S.Y., David A. R., 2009. Relative susceptibility of selected apple cultivars to powdery mildew caused by *Podosphaera leucotricha*. Plant Health Progress.
4. Dan C., Sestras A. F., Bozdog C., Sestras R.E., 2015. Estimation of genetic effects implied in apple inheritance of quantitative traits. Journal of Horticulture, Forestry and Biotechnology, Vol. 19 (1):85-90.
5. Holb I.J., 2009. Fungal disease management in organic apple orchards: epidemiological aspects and management approaches. Recent Developments in Management of Plant Diseases 1: 163-177.
6. Kellerhals M., Bertschinger L., Gessler C., 2004. Use of genetic resources in apple breeding and for sustainable fruit production. Journal of Fruit Ornamental Plant Research 12: 53-62.

7. Lateur M., 1999. Evaluation et caractérisation des ressources génétiques d'arbres fruitiers. In M. Chauvet, compiler. Le patrimoine fruitier. Hier, aujourd'hui, demain. AFCEV/BRG/INRA, Paris, France: 167-177.
8. Lateur M., Populer C., 1996. Evaluation and identification methods used for apple genetic resources at the State Plant Pathology Station in Gembloux, Belgium. In: HJ Case, editor. European Malus Germplasm. Proceedings of a workshop, 21-24 June 1995, Wye College, University of London. International Plant Genetic Resources Institute, Rome: 78-87.
9. Laurens F., Lespinasse Y., Fouillet A., 2005. A new scab-resistant apple: Ariane. HortScience 40(2): 484-485.
10. Militaru M., Calinescu M.F., Butac M., 2017. Emerge of virulences on agrosystems: *Venturia inaequalis* at *Vf* apple cultivars grown in Romania as a case study. Fruit Growing Research, Vol. XXXIII: 60-66.
11. Sestras A.F., Pamfil D., Dan C., Bolboaca S.D., Jantschi L., Sestras R.E., 2011. Possibilities to improve apple scab (*Venturia inaequlis* (Cke.) Wint) and powdery mildew (*Podosphaera leucotricha* (Ell. et Everh.) Salm) resistance on apple by increasing genetic diversity using potentials of wild species. Australian Journal of Crop Science 5(6): 748-755;.
12. Sestras R.E., 2004. Ameliorarea speciilor horticole. Editura AcademicPres Cluj Napoca.

Tables and figures

Table 1. Hybrid combinations and number of F_1 hybrids

♀/♂	Enterprise	Fuji	Ariane	Gala	Topaz	Crimson Crisp
Enterprise	-	49	-	-	-	-
Fuji	26	-	-	-	-	-
Ariane	-	-	-	55	-	-
Gala	-	-	78	-	-	-
Topaz	-	-	-	-	-	131
Crimson Crisp	-	-	-	-	130	-

Table 2. Absolute heterosis for hybrids response to apple scab and powdery mildew, calculated by two most common formulas

Hybrid combination ♀/♂	Apple scab		Powdery mildew	
	H=F1-(P1+P2)/2	H = F1 - P_{max}	H=F1-(P1+P2)/2	H = F1 - P_{max}
Enterprise x Fuji	-2.68	0.82	0.66	2.16
Fuji x Enterprise	-2.65	0.85	0.81	2.31
Ariane x Gala	-5.75	-5.75	2.15	2.15
Gala x Ariane	-5.86	-5.86	1.99	1.99
Topaz x Crimson Crisp	-0.39	0.11	0.18	1.68
Crimson Crisp x Topaz	-0.48	0.02	0.63	2.13

Table 3. Heterobeltiosis for response to *Venturia inaequalis* and *Podosphaera leucotricha* attack of F_1 hybrid combinations

Hybrid combination ♀/♂	Heterobeltiosis ($\bar{F}1 - \bar{P}_{Min}$) / $\bar{P}_{Min}$ × 100)		Correlations (marks scab-powdery m.)
	Apple scab	Powdery mildew	
Enterprise x Fuji	81.6	216.3	-0.284*
Fuji x Enterprise	84.6	230.8	-0.335
Ariane x Gala	-71.8	214.5	0.195
Gala x Ariane	-73.2	198.7	-0.220*
Topaz x Crimson Crisp	10.7	84.0	-0.080
Crimson Crisp x Topaz	1.5	106.5	-0.123

*,**,***: Significant at P≤0.05, 0.01 and 0.001

DOI 10.33045/fgr.v34.2018.07

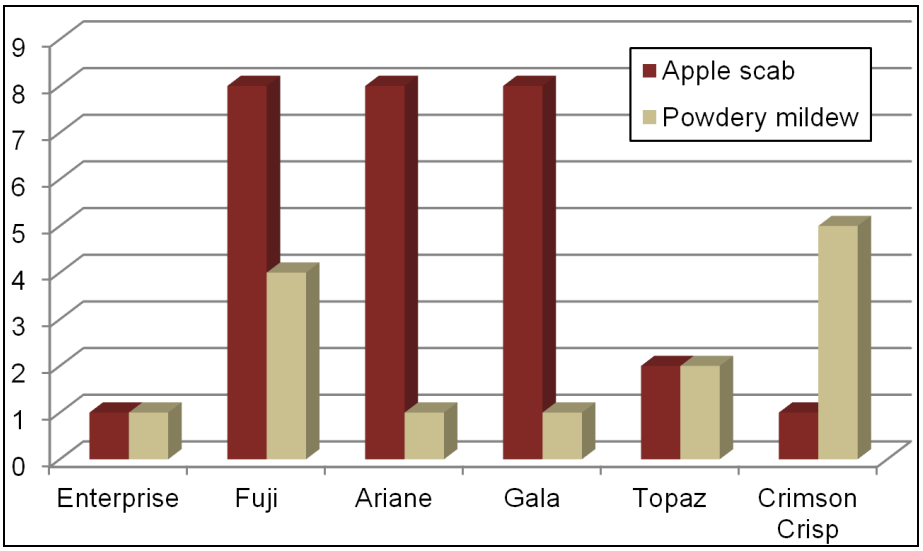


Fig. 1. The response of cultivars used as genitors to apple scab and powdery mildew attack in similar conditions with their hybrids

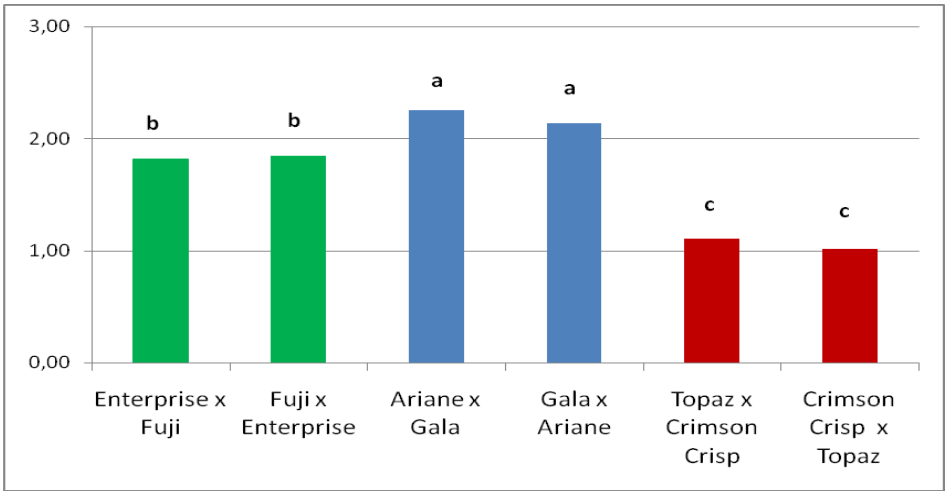


Fig. 2. Response to apple scab attack of F1 hybrid combinations (mean values of the marks on the scale 0-9)

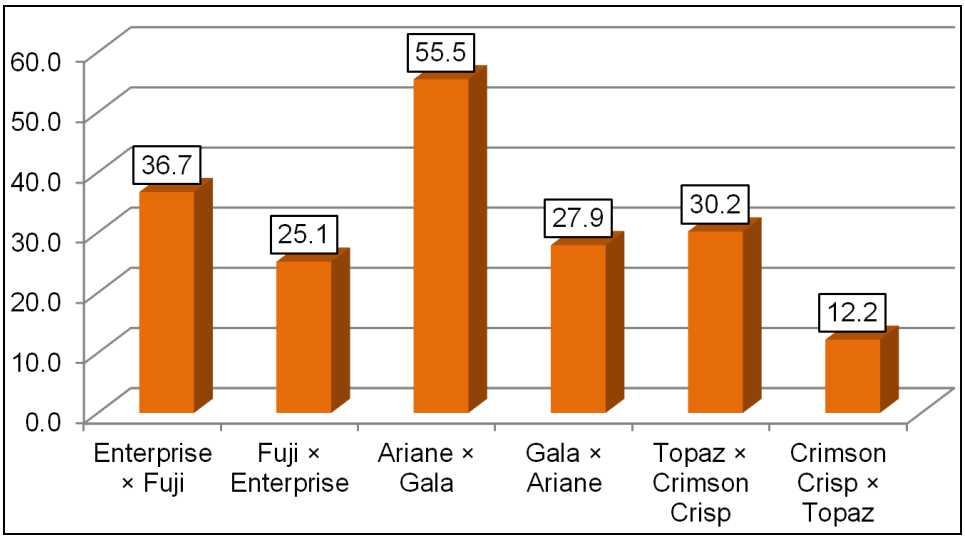


Fig. 3. Coefficient of Variability (CV%) for apple scab attack of F1 hybrid combinations

DOI 10.33045/fgr.v34.2018.07

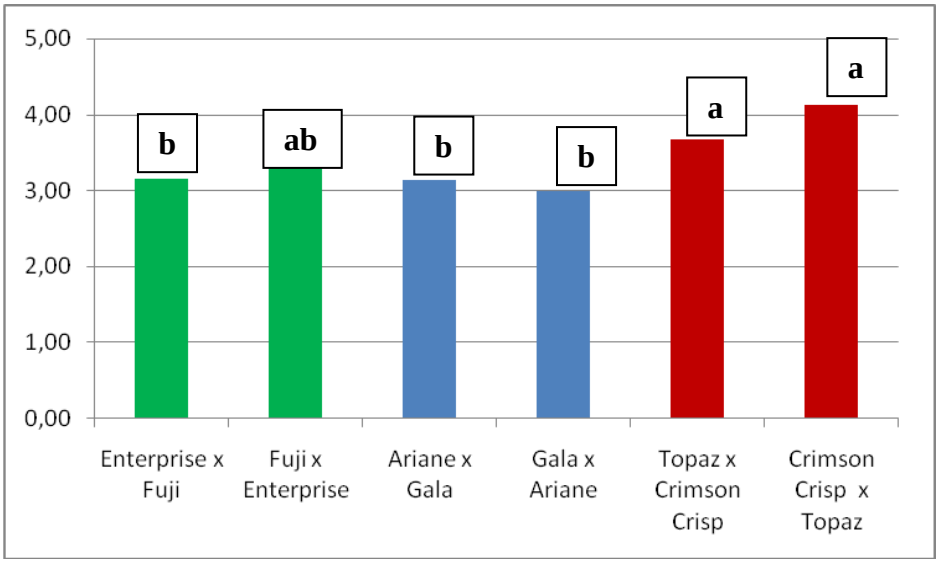


Fig. 4. Response to powdery mildew attack of F1 hybrid combinations (mean values of the marks on the scale 0-9)

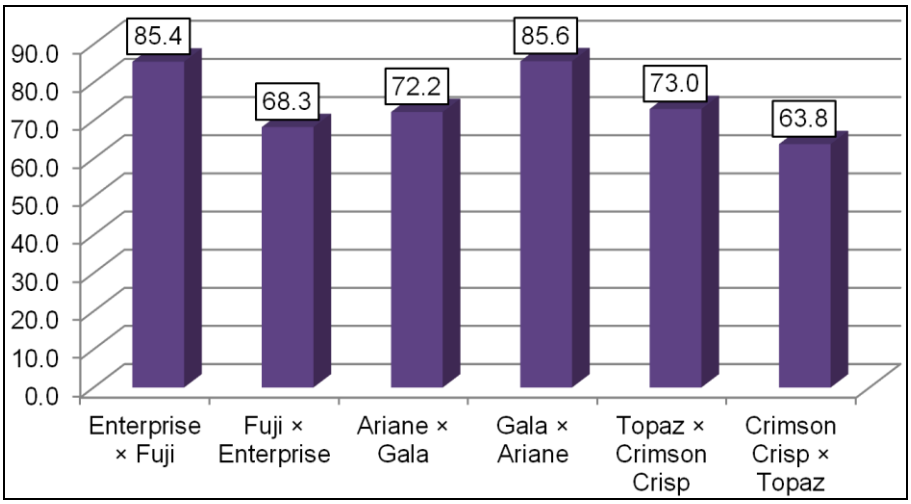
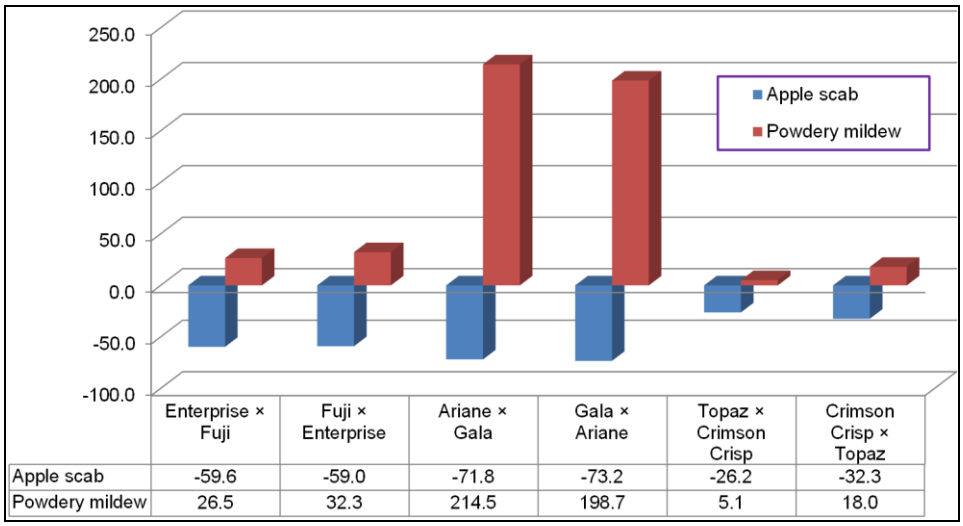


Fig. 5. Coefficient of Variability (CV%) for powdery mildew attack of F1 hybrid combinations



	Enterprise × Fuji	Fuji × Enterprise	Ariane × Gala	Gala × Ariane	Topaz × Crimson Crisp	Crimson Crisp × Topaz
Apple scab	-59.6	-59.0	-71.8	-73.2	-26.2	-32.3
Powdery mildew	26.5	32.3	214.5	198.7	5.1	18.0

Fig. 6. Relative heterosis for response to *Venturia inaequalis* and *Podosphaera leucotricha* attack of F_1 hybrid combinations