

COMPARISON OF GRAPE SEEDLINGS POPULATION AGAINST DOWNY MILDEW BY USING DIFFERENT PROVOCATION METHODS

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Abstract. This research was conducted to compare a laboratory technique leaf disk with hotbed and field evaluations in the screening of large populations of grape seedlings (*Vitis* spp.), towards downy mildew (*Plasmopara viticola* Berk. et Curt.) resistance. Seedlings produced by crossing two hybrid families, in (*Vitis amurensis* Rupr. x *Vitis vinifera* L.) x (Franco-American hybrid x *Vitis vinifera* L.) and the antipodal crossing of the Franco-American hybrid x *Vitis vinifera* L. x (Franco-American hybrid x *Vitis vinifera* BC) combinations. The ‘Bianca’, ‘Merzling’, ‘Kunleany’, ‘Hibernal’ and ‘Hibia’ cultivars were used. These varieties were rated for sporulation on five levels, from 0 to 80 percent. Sporulation ratings were assessed at the first and second rating. Significant differences were signalled between field evaluations and the leaf disk technique. Furthermore, a highly significant correlation was found between the leaf disk technique and hotbed evaluation. The leaf disk procedure appeared to be a good predictor of field resistance and is more practical than the hotbed provocation method for the screening of large populations.

Key words: Downy mildew, interspecific hybrid, resistance, sporulation, leaf disk technique

INTRODUCTION

Downy mildew is one of the most widespread and destructive vineyard diseases in the whole world. Symptoms appear as yellowish, oily spots on the bottom of the leaves, resulted in chlorophyll dissociation and the rise of a square necrotic spot, which can possibly join in bigger necrotic areas. Other characteristics consist in whites’ sporangiophor and sporangium, growing up from the hundred-stalemate (Emmet et al., 1992). Strong infection results in strong plant defoliation. If sufficient chemical protection isn't provided, overall vitality may be markedly reduced (immaturity of shoots), while during winter frost plant death can also be induced in sensitive varieties. Therefore, practices are increasingly starting to use new resistant varieties, the so-called Interspecific. The Interspecific variety of grapevine is a variety, which originates in a crossing between the species *Vitis vinifera* L. ‘European grapevine’ and another *Vitis* spp. “American or Asiatic species” (Becker, 1989). The degree of resistance or, on the contrary, the susceptibility to fungal disease is different in these varieties, according to climatic conditions, location, annual weather condition, growing shapes and vineyard treatment (Dunkel et al., 1980). Attempts of breeding grapevine to resist against fungal diseases go back to the middle of the 19th century. At that time, the first hybrid between American species was developed in America. The next wave of resistant breeding begins in the late 19 century and early 20 century, in France. The so-called “old French hybrids” was created here, with breeders such as: Seibel, Couderc, Millardet, Oberlin, Baco, etc. The 20th century begins with the breeding of Seyve Villard with hybrids which are now

mostly used for resistance breeding. Its hybrid, SV 12375 and other such hybrids exhibit a very good tolerance against downy mildew, and thus transfer this character to the next offspring (Cypko, 1982).

Hybrid populations may be classified in field conditions (Eibach et al., 1989; Sestras et al., 2006), according to the provoking method in glasshouse (Denzer et al., 1995a) and in laboratory conditions in vitro on leaf disks (Denzer et al., 1995b; Eibach et al. 1989, Brown et al., 1999).

MATERIAL AND METHODS

A reciprocal crossing between interspecific varieties was conducted: 'Bianca', 'Merzling', 'Kunleany', 'Hibernal' and 'Hibia'. The 'Bianca' variety (Hungarian) came from the crossing of SV 12375 x Bouvier and displays a good resistance to downy mildew, while wine quality is relatively good (Kozma and Dula, 2003). The 'Merzling' (German) variety came from crossing SV 5276 x (Rhone Riesling x Pinot gris), with very good wine quality and quite good tolerance to downy mildew (Basler and Pefnninger, 2000). The 'Kunleany' (Hungarian) variety came from crossing (*Vitis amurensis* x *Vitis vinifera*) x Afuz Ali. It is resistant to downy mildew, but wine quality is rather low (Korbuly, 2002). The 'Hibernal' (German) variety came from crossing (Seibel 7053 x Rhone Riesling Gm 239)F₂ as it is resistant to downy mildew and wine quality is very good (Kraus, 2005). The 'Hibia' variety (Czech Republic) came from crossing Hibernal x Bianca variety and is quite resistant to downy mildew and wine shows good quality (Sotolář, 2004).

After February stratification was conducted, and seeds were sowed to multi plates in greenhouses. Subsequently, seedlings were transferred to containers, namely Jiffy-Pots (10 cm in diameter) and further cultivated. In the late May, a part of the seedlings were planted to the hot-bed, while another part was planted to the field trial and the remaining part was kept in the greenhouse for sampling control disks.

To classification of seedlings in the greenhouse employed the so-called *Leaf disk technique* (Brown et al., 1999). The sample was taken from fully mature, uninfected leaves (out of nodes 4-6 from the base), i. e. a minimum. 2 leaf disk from each leaf, a cork knife 2.5 cm in diameter. These leaves usually provide a more reliable classification than older leaves (Staudt and Kassemeyer, 1995). Leaf disks are laid on an abaxial surface, on filter paper saturated with distilled water and contained in Petri dishes. Foliage fragments are further infected with pathogen spores (inoculum = cca. 5 x 10 sporangium in 1 ml of suspension). The samples of downy mildew extracted from oil spots, either exsiccate (as we can store the dry sporangium for approximately 2 months at -20°C) or further multiply on the green leaves relevant variety within the laboratory. Cultivation further proceeds in the sterile environment of a climabox at 22°C. An evaluation was made every fifth and seventh day after infection. Hybrids were ranged on 5 levels (disease index):

1. 0 - 0.5% symptomless or hypersensitive reactions, tiny spots, no sporulation
2. 0.5 - 10% hypersensitive reactions, very light sporulation
3. 10 - 30% hypersensitive reactions, light sporulation
4. 30 - 50% oil-spots, strong sporulation
5. > 50% oil-spots, very strong sporulation, sporangias, necrosis.

This technique serves as an easy-applicable provoking method, which is quite independent from season, as most disks are easily infected with downy mildew, whereas even during the classification period (4-6 days) the leaves are mostly fresh and green enough

(Brown et al., 1999). As a control the sensitive Müller-Thurgau variety and mother plants were chosen.

Classification of Seedlings from the Hot-Bed

Plants used for classification were inoculated on the bottom part of leaves 5 x 10 sporangium/mls. Inoculum was prepared employing the leaf disk method. Cold fog moistener (blower sprayer) was closed in the hot-bed, in order to hold 100% relative humidity after 24 hours, to guarantee infection. After 24 hours, the hot bed was opened and let dry. The flow of water and transpiration held relative humidity near 100%. For the reduction of sunshine, a shade was used (sunburn, high transpiration and temperature) and a temperature of 21±2°C, to help infection and the development of fungi. The daily duration was 12 hours and no supplementary lighting was used. Each inoculation was evaluated on an identical scale from 0 – 5, as with the leaf disk method. Plants were observed and classified, before the 7 days' period. Nevertheless, they did not show attack symptoms, as they did after 7 days.

Classification of Field Trial

Seedlings were first hardened (with the reduction of greenhouse temperature), and were transplanted to the experimental vineyard in May. Seedlings and parental plants, i. e. one parent per block, were planted in blocks. Plantation spacing was 0.3 x 0.5 m according to the Vertico system. The vineyard was irrigated by trickling irrigation, in order to exclude stress from drought. No fungicide or insecticide was applied. Seedlings and mother plants were classified, according to their exposition to natural inoculation, the same method as with disk and hot-bed. Plants were evaluated on 25 August and 8 September of the year 2004.

RESULTS AND DISCUSSION

The two different groups of seedlings were tested for resistance against downy mildew. The total number of seedlings was 573. For the first group, which contained 218 seedlings, the crossing was obtained with *V. amurensis* Rupr.

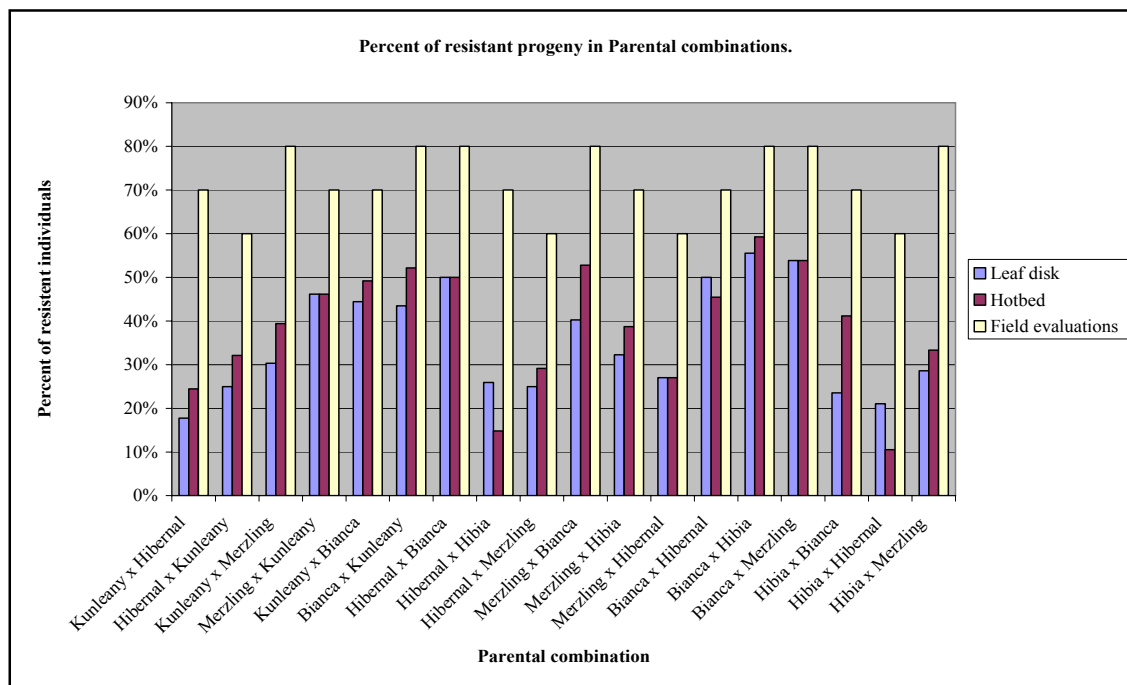


Figure 1. Percent of resistant progeny in parental combinations

The second group included crossing of Franco-American hybrids with each other and contained 355 seedlings. The number of obtained seedlings was lower in the case of the crossing with ‘Bianca’ and ‘Hibia’ varieties, because both of them have problems with pollination due to late flowering time and long stamen (Figure 1).

Resistance of new seedlings against downy mildew

Crossing with *V. amurensis* Rupr.

For this group the ‘Kunleany’ variety was chosen, which displays the characters of *V. amurensis* Rupr. This variety is tolerant against downy mildew and resistant to grey fungi and also frost tolerant. Unfortunately, the expected strong effect of a higher resistance against downy mildew in progeny was not confirmed. An exception in this case was the crossing with the ‘Bianca’ variety, which proves good resistance. A second combination with the tolerant ‘Merzling’ variety, is also very important, but only when the ‘Kunleany’ variety was used as father (Table 1).

Table 1

Resistance of seedlings derived from crossing with *V. amurensis* Rupr.
against *Plasmopara viticola* Berk. Et Toni

Techniques	Level of resistance					Ratio
	Resistant	Res/Tol	Tolerant	Sensitive	Susceptible	
	1	2	3	4	5	
<u>Kunleany x Hiberna</u>						
Leaf disk techn.	0	2	6	20	17	8:37
Hotbed screening	2	4	5	15	22	11:34
Field screening	4	1	2	2	1	7:3
<u>Hiberna x Kunleany</u>						
Leaf disk techn.	0	2	5	10	11	7:21
Hotbed screening	0	3	6	11	8	9:19
Field screening	2	2	2	3	1	6:4
<u>Kunleany x Merzling</u>						
Leaf disk techn.	1	2	7	15	8	10:23
Hotbed screening	2	3	8	14	6	13:20
Field screening	6	0	2	1	1	8:2
<u>Merzling x Kunleany</u>						
Leaf disk techn.	1	2	9	10	4	12:14
Hotbed screening	2	3	7	11	3	12:14
Field screening	5	1	1	3	0	7:3
<u>Kunleany x Bianca</u>						
Leaf disk techn.	1	3	24	26	9	28:35
Hotbed screening	3	6	22	17	15	31:32
Field screening	6	0	1	3	0	7:3
<u>Bianca x Kunleany</u>						
Leaf disk techn.	0	3	7	10	3	10:13
Hotbed screening	1	3	8	9	2	12:11
Field screening	6	1	1	2	0	8:2

Crossing Franco-American hybrids

This group includes crossing varieties chosen mainly due to wine quality. But this fact affects the level of the resistance, because these varieties are tolerant or highly resistant against downy mildew. A higher level of resistance was encountered in this group of seedlings, when ‘Bianca’ variety was used as a parent.

Table 2

Resistance of seedlings derived from Franco-American crossing against *Plasmopara viticola* Berk. et Toni

Techniques	Level of resistance					Ratio
	Resistant	Res/Tol	Tolerant	Sensitive	Susceptible	
	1	2	3	4	5	
Leaf disk techn.	2	3	11	10	6	16:16
Hotbed screening	2	4	10	11	5	16:16
Field screening	5	1	2	2	0	8:2
<u>Hibernal x Hibia</u>						
Leaf disk techn.	0	1	6	14	6	7:20
Hotbed screening	0	1	3	13	10	4:23
Field screening	4	1	2	2	1	7:3
<u>Hibernal x Merzling</u>						
Leaf disk techn.	0	2	4	7	11	6:18
Hotbed screening	0	2	5	10	7	7:17
Field screening	4	0	2	3	1	6:4
<u>Merzling x Bianca</u>						
Leaf disk techn.	2	5	22	23	20	29:43
Hotbed screening	3	7	28	24	10	38:34
Field screening	6	1	1	2	0	8:2
<u>Merzling x Hibia</u>						
Leaf disk techn.	0	4	6	13	8	10:21
Hotbed screening	1	2	9	9	10	12:19
Field screening	5	1	1	2	1	7:3
<u>Merzling x Hibernal</u>						
Leaf disk techn.	0	3	7	9	18	10:27
Hotbed screening	1	3	6	12	15	10:27
Field screening	4	2	2	2	2	6:4
<u>Bianca x Merzling</u>						
Leaf disk techn.	2	5	7	7	5	14:12
Hotbed screening	2	4	8	9	3	14:12
Field screening	7	0	1	2	0	8:2
<u>Bianca x Hibia</u>						
Leaf disk techn.	1	6	8	7	5	15:12
Hotbed screening	0	5	11	9	2	16:11
Field screening	5	1	2	1	1	8:2
<u>Bianca x Hibernal</u>						
Leaf disk techn.	1	3	7	9	2	11:11
Hotbed screening	1	3	6	8	4	10:12
Field screening	5	1	1	2	1	7:3
<u>Hibia x Bianca</u>						
Leaf disk techn.	0	1	3	8	5	4:13
Hotbed screening	0	2	5	6	4	7:10
Field screening	5	0	2	3	0	7:3
<u>Hibia x Hibernal</u>						
Leaf disk techn.	0	1	3	8	7	4:15
Hotbed screening	0	1	1	11	6	2:17
Field screening	2	2	2	3	1	6:4
<u>Hibia x Merzling</u>						
Leaf disk techn.	0	2	4	8	7	6:15
Hotbed screening	1	2	4	9	5	7:14
Field screening	6	1	1	2	0	8:2

Interesting results show also for the ‘Hibia’ variety, but the transfer of its resistance property to progenies is larger than in the case of the ‘Bianca’ variety. However, the benefit of higher resistance in progenies was not confirmed. The ‘Hibernal’ variety has lower resistance transmission to progenies except the combinations with ‘Bianca’ variety, when used as mother or father. The transmission of resistance against downy mildew is not significant in the case of a crossing with the ‘Merzling’ variety (Table 2).

Comparison of used methods for resistance detection

Seedlings were tested by three methods: the leaf disk method technique (Figure 2), classical provocation method in hotbed (Figure 3), and field screening.



Figures 2 (left). Comparison of two different progenies by the leaf disk method

Figure 3 (right). Infected plants in hotbed frame of provocation method

The level of resistance detected by the leaf disks method corresponds to the hot-bed provocation method, in contrast to the field screening method. Field screening methods show a higher level of resistance, due to climatic influence. As for the observed years, occurrence of downy mildew in nature was lower due to dry conditions. For the screening of larger populations, it is better to use leaf disk techniques in comparison to the classical provocation method. The percent of seedlings assessed as resistant against downy mildew was significantly higher in field trials than in the hotbed provocation method and the leaf disk method, which do not show significant difference among each other. Results of resistance screening will be verified in practice and also another selection of the best candidate for a new variety with best agronomical properties will be conducted.

CONCLUSIONS

This research was provided in order to detect whether the leaf disk method can be used for resistance screening against downy mildew, within larger seedling populations. Screening according to the hot-bed method was as forcible as the leaf disk method. The field screening of seedling populations requires large plots and more time for complete results. Hot-bed classification and field trial classification were significantly different, as well as the leaf disk method, using a number of seedlings for each degree of resistance. On the contrary, differences between the hot-bed and leaf disk methods were not significant. Field trial evaluation displays small variability, which shows that this method is not so strong for the discrimination of resistant and susceptible seedlings. On the other hand, this result shows that the leaf disk method is more practical and faster for the evaluation of larger seedling populations than the hotbed test. Results of this study reflect that both procedures, namely leaf

disk and hotbed tests, may be used for screening of seedlings, in order to detect downy mildew resistance. However, the leaf disk method is more practical and faster for the evaluation of larger seedling populations than the hotbed test.

Many results confirm the generally accepted opinion that the 'Bianca' variety is responsible for total downy mildew resistance (concrete crossing 'Bianca' x 'Merzling', 'Bianca' x 'Hibernal', 'Bianca' x 'Hibia'). But its utility value, namely wine quality, is questionable. Also, there is a danger of breaking immunity for narrow vertical resistance, often using SV 12375 (Villard blanc) as immunity donor. Other varieties do not give such a high immunity to seedlings, though interesting results show the crossing of 'Merzling' x 'Bianca' and 'Kunleany' x 'Merzling', 'Merzling' x 'Kunleany', 'Kunleany' x 'Bianca' upon the crossing with 'amurensis' grapevine. The 'Kunleany' variety proves to be a good resistance porter against downy mildew, in these combinations. Korbuly (2002) says, that this variety is valuable for its donor resistance against grey fungi. The 'Hibernal' variety shows a low resistance against the downy mildew and conclusively, transmission to the offspring is poor, as well.

In this case, it is apparent, that donor resistance S 7053 (Chancellor) already evidently lost its immunity, the transmission to the offspring is insignificant and it is the only one tolerant to downy mildew.

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REZUMAT

COMPARAȚII PRIVIND COMPORTAREA UNOR POPULAȚII HIBRIDE LA VIȚA DE VIE FAȚĂ DE ATACUL DE MANĂ, PROVOCAT PRIN DIFERITE METODE ARTIFICIALE

Cercetările au fost efectuate cu scopul comparării unor populații hibride de viță de vie față de atacul de mană (*Plasmopara viticola* Berk. et Curt.), testările fiind efectuate în laborator (folosindu-se tehnica discurilor de frunze supuse infecției) și în câmp. Descendenții seminali testați au provenit din două familii hibride (*Vitis amurensis* Rupr. x *Vitis vinifera* L.) x (hibrid franco-american x *Vitis vinifera* L.) și din combinații provenite din încrucișări antipodale între hibridul franco-american x *Vitis vinifera* L. x (hibrid franco-american x *Vitis vinifera* BC). Din specia *vinifera* s-au utilizat cultivarele 'Bianca', 'Merzling', 'Kunleany', 'Hibernal' și 'Hibia'. Soiurile respective au fost încadrate în funcție de sporularea după prima și a doua infecție cu mană în cinci clase, cu procente cuprinse între 0 și 80%. S-au identificat diferențe semnificative între variante, date de modul de testare, prin evaluarea în câmp și în laborator, iar între rezultatele obținute prin infecții artificiale prin tehnica discurilor de frunze și evaluarea în „paturi calde” s-a identificat o corelație strânsă. Rezultatele obținute prin metoda discurilor de frunze infectate pot reprezenta un bun indicator pentru rezistența în câmp a plantelor și metoda este mai practică decât infecția artificială în „paturi calde”, cel puțin pentru evaluarea rezistenței la mană a unor populații hibride ample.