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Evaluation of grapevine resistance to downy and powdery mildew in a population segregating for run1 and rpv1 resistance genes.

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Abstract: In this paper we present the behaviour of a population of vine segregating for several sources of resistance to powdery (PM) and downy mildews (DM). Resistance genes from *Muscadinia* and *Vitis* American species were introduced in a *Vitis vinifera* genetic background. Resistance to both pathogens was assessed in bioassays under controlled inoculations with two isolates for PM and three isolates for DM and in greenhouse or field experiments under natural infections. 19 out of 38 segregant genotypes were identified as totally resistant to PM on leaves and partially resistant to DM on both leaves and bunches. All these genotypes presented a level of damage satisfactory in an IPM context. There is no evidence to say that the resistance genes are expressed in bunches. However, the descriptors of resistance used in the bioassay, can be used to select with a good accuracy genotypes with an acceptable level of damage on bunches. The link between resistance to powdery mildew and downy mildew suggest that resistance in harvestable genotypes is coming from *Muscadinia* (*Rpv1* and *Run1*).

Key words: mildew, screening for resistance, bioassay, field assessment, *Erysiphe necator* and *Plasmopara viticola*.

Introduction

Powdery and downy mildew are the most widespread diseases on *Vitis vinifera* worldwide and the main target of fungicide treatments. Different options can be used better in combination to improve the control of these diseases in the respect of IPM (Integrated Pest Management). Among these strategies we can remind i) The use of forecast models to predict the risk of disease. However, none of them are able to predict the level of primary infections. ii) Disease survey at periods known for the high susceptibility of the plant or under favourable climatic conditions iii) The use of global models leading to crop protection decision integrating knowledge on epidemiology, period and conditions of risk, crop management and treatments. iv) Finally, the growth and deployment of partially resistant or moderately susceptible varieties such as hybrids coming from American or Asian species.

In this paper we would focus on the development of host resistance. Our study takes place at an advanced step of a breading program which has for objective to ensure the selection of genotypes most likely to confer durable resistance to both diseases. In this program several sources of resistance coming from *Muscadinia* and *Vitis* American species were introduced in a *Vitis vinifera* genetic background. The selection for resistance was based on phenotype characterisation of the resistance level.

In this paper we would present results of disease assessment of resistance to both pathogens *Erysiphe necator* and *Plasmopara viticola*, in bioassays under controlled inoculations and in greenhouse or field experiments under natural infections. Finally, we would test the

ability of the bioassays to predict resistance for these varieties to downy mildew in the field on leaves and on bunches.

Material and methods

Plant material and field experimental design

The population used in this paper came from a cross between 3082-1-42 (BC4 from *Muscadinia rotondifolia*) x Regent (Chambourcin (12.417 SV x 7053 seibel) x Diana (Sylvaner x Muller Thurgau)). Resistance to powdery mildew and downy mildew originates from two linked resistance genes in *Muscadinia*, *Run1* (PM resistance) (Pauquet et al., 2001, Barker et al., 2005) and *Rpv1* (DM resistance) (Merdinoglu et al., 2003) and also from the partially resistant cultivar Regent which inheritates its resistance to both pathogen from different *Vitis American* parents (1 QTL of resistance to PM and 1 QTL for endurance to DM) (Fisher et al., 2004). 200 genotypes were screened in 2001-2002 at the Geilweilerhof Institute (Germany) for their resistance to powdery mildew in greenhouse under natural infection, and for their resistance to downy mildew in a laboratory bioassay on leaf disks and on leaves after artificial inoculation, at INRA Colmar (France). 38 genotypes with different combinations of PM and DM resistance were selected and planted in Bordeaux, Colmar and Montpellier in 2004. The experimental design was made of 4 consecutive vines per genotype planted in a randomized design. Parents and other susceptible or partially resistant cultivars are included in the design.

Bioassays

In 2007, bioassays were done on leaves coming from the field material to select the genotypes resistant to both pathogens.

In assay 1, resistance was tested with one isolate of powdery mildew on all the 38 genotypes. The 25th of April (average grape phenology 7 leaves/shoot), 1 young leaf per vine (first to second leaf under the last expended leaf) was sampled for each genotype (4 leaves= 4 repetitions/genotype), as well as for each of the control varieties randomized within the plot (Merlot, Chardonnay, Chambourcin, Villard blanc) and for both parents Regent and 3082-1-42. Leaves were disinfected 10 min by a hypochlorite solution (50g/l), and a sample of 1 disk of 16 mm is taken from each leaf and placed in Petri dishes (Pd) on 20g/l of agar medium (upper surface on the top). One Pd contains 5 genotypes. In each Pd one control of infection success (Cabernet-Sauvignon leaf disk coming from cuttings) was included. The day after, the 38 genotypes plus the controls were inoculated in settling towers (4 towers to infect the 4 repetitions/genotype) by blowing 600 to 800 spores/cm² of the isolate S7 (biotype B). 13 days after inoculation, disks were briefly observed at the stereomicroscope for an assessment of mycelium and / or sporulation and sporulation was assessed for each disk by a Coulter cell counter (Multisizer III) after shaking the disk in a solution of isoton with non ionic dispersant (Nacconol *90F*) (particles between 17 µm et 37 µm are counted). For the totally resistant varieties an adhesive tape test was performed on one repetition to control at which stage the spores were stopped.

In assay 2, the genotypes resistant to powdery mildew at bioassay 1 were tested using three isolates of downy mildew and two more isolates of powdery mildew. 14 days and 7 days before the test, leaves just expanded were marked on different shoots. The 26 of June, the marked shoots are sampled and leaves aged of 7 days are tested against powdery mildew, whereas those aged of 14 days are tested against three isolates of downy mildew.

For the **DM assay**, 3 disks of 16 mm are taken from each leaf and placed each in one Petri dishes (Pd) (one/isolate) containing a filter paper leaks out soaks (4 ml of water/Pd),

lower side of the leaf up. One Pd contains 5 genotypes plus a control of Cabernet-Sauvignon from cuttings. The day after, each disk was inoculated with 3 droplets of 10 µl each, with a cold solution of 19000 sporangia/ml. The three isolates used were selected the week before the test in the same field either on the susceptible variety Merlot or on the two partially resistant genotypes 70 and 134, and multiplied on Cabernet Sauvignon. Isolates selected on partially resistant varieties may have acquire a higher level of aggressiveness. Two repetitions (leaves) per genotypes and pathogen are used. 7 days after inoculation, the disease was scored on a visual scale similar to that of OIV-452 (IPGRI, 1997) taken into account the sporulation intensity and the necroses. Sporulation was also assessed for each disk by the Coulter cell counter (particles between 10 µm and 25 µm are counted).

For the **PM assay**, 2 disks of 16 mm were taken from each leaf and placed in two Pd (one /isolate) and prepared as before. The two mono-spores isolates used were coming from the two genetic groups of powdery mildew biotype B (S7) and biotype A (PVR38) (Delye et al., 1997).

Field or greenhouse assessments

Whereas this year was exceptionally favorable for downy mildew, there was almost no powdery mildew observed in the field except few colonies on Chardonnay. Then for powdery mildew, results of bioassays were compared to previous greenhouse assessments performed on cuttings under natural infection and based on OIV scale 455 (IPGRI, 1997). For downy mildew, disease was assessed in the vineyard on the 30th of June. Each vine was characterised for its susceptibility to DM by a visual assessment of severity (% of area diseased) on leaves and on bunches. Severity data of every genotype on leaves corresponds to an average of the scores of four vines whereas severity on bunches results on the average of severity scores of all bunches of four vines. Genotypes were also scored for black-rot disease with the same direct severity assessment. At beginning of September, genotypes were lastly observed on bunches and classified as "harvestable" or not. The genotypes called "harvestable" could be visually classified 5 on the OIV 453 scale (20 to 30% of bunches attacked with distinct consequences for the vintage).

Results and discussion

Resistance to powdery mildew

Among the 38 genotypes, 17 showed a level of resistance as strong as that of the resistant parent 3082-1-42 with no or nearly no germinated spores or sporulation. 12 genotypes had a level of sporulation not significantly different from the other parent Regent and 9 were as susceptible as the control varieties (Cabernet-Sauvignon, Merlot and Chardonnay) (Fig. 1). Regent, the partially resistant parent was surprisingly not very resistant according to the OIV score. Sporulation, was however decreased up to 40% compared to its respective Cabernet-Sauvignon control (cutting leaves), a response closed to that of Chambourcin. From the 16 most resistant genotypes all were previously scored resistant in greenhouse (R. Eibach), however 8 other genotypes scored resistant in Germany had an intermediate or fully susceptible profile in the *in vitro* test. The hypothesis that German isolates may have revealed resistance genes other than *Run1* and *Rpv1* is not likely with European isolates. The most likely hypothesis is that the *in vitro* test, by controlling the inoculation at a susceptible phenological stage, avoids most misclassifications of resistant genotypes. In the test 2, the 17 resistant genotypes were again totally resistant to both tested isolates, with no significant differences between isolates on the control varieties.

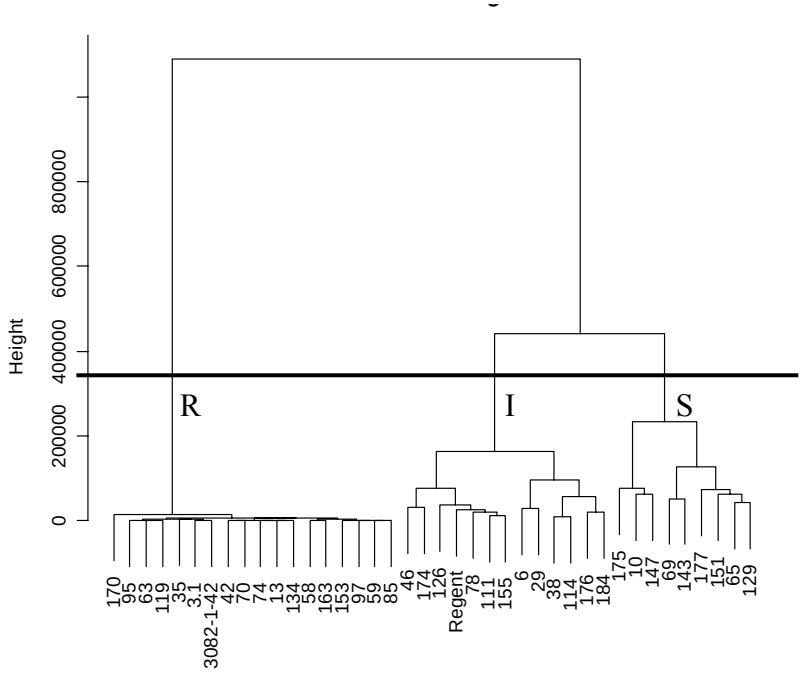

Fig. 1. Result of a hierarchical clustering analysis performed on the sporulation of powdery mildew leaf disks for the 38 genotypes and parent of the population 3082-1-42 x Regent, differentiating three groups of resistant (R), intermediate (I) and susceptible (S) varieties with isolate S7 (test 1).

Resistance to downy mildew in bioassay

No significant difference of sporulation was observed between the three isolates for the different genotypes and controls (Anova, $F_{2,89}$=0.357, P=0.7045) (Figure 2). A good correlation is observed between the score for resistance according to the OIV scale and the sporulation amount (R^2=0.79). Based on the sporulation of the 6 repetitions, 11 genotypes could be distinct for their low level of sporulation (< 14413) with an average OIV score of 5.97 and were closed to the resistant parent 3082-1-42 and to Chambourcin (Figure 3). 9 genotypes were characterised by higher level of sporulation and an average OIV of 4.18. Regent was highly susceptible with an OIV score of 3, and similar to the control varieties Cabernet-Sauvignon and Merlot.

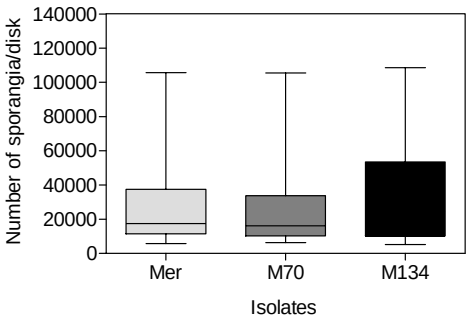

Fig. 2: Variability of sporulation (number of sporangia per disk) for the different genotypes and control varieties for the three isolates of downy mildew

Some discrepancies were observed between these results and those previously obtained at INRA- Colmar, with for example genotypes resistant at INRA-Bordeaux (OIV > 4.5, sporulation ~11500) (70-153) giving at Colmar either intermediate level of resistance (OIV = 4 for

genotypes 70 and 153) or a fully susceptibility (OIV = 3, for genotype 35). The reverse was also observed with 4 genotypes (58-74-95) expressing an intermediate level of resistance at Bordeaux (OIV < 4.5, sporulation ~ 28450) and a resistant level at Colmar (OIV > 5). These differences are probably due to variation of the expression of the resistance, however we cannot exclude the hypothesis that the diversity of isolates used allowed to reveal minor resistance genes or to reveal virulence on these genes.

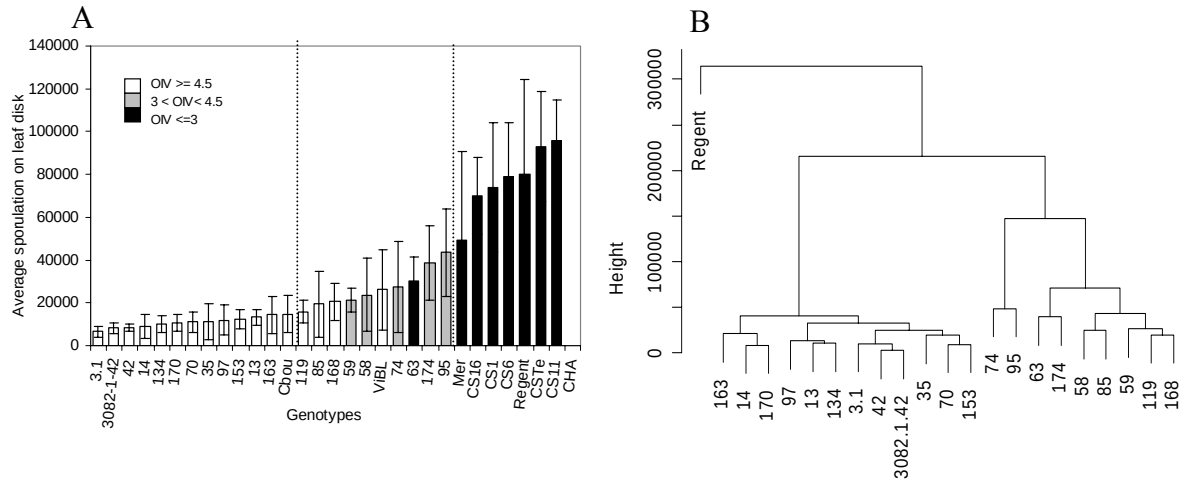

Fig. 3. Downy mildew sporulation for the different genotypes and control varieties with their corresponding OIV score (A) and classification of the different genotypes based on the sporulation of the six repetitions (B) (3 isolates x 1 leaves) .

Resistance to downy mildew in the field.

The correlation between the resistance on leaf disk (OIV score) and the leaf severity in the field is not so strong (R^2=0.54). It is not as surprising as the leaf severity in the field is more complex as it results of several cycles of natural infections with probably a mixture of primary and secondary inoculum. However, the *in vitro* test can be used with a reasonable likelihood to select genotypes potentially resistant on leaves in the field. If we consider as satisfying (resistant) a severity in the field with less than 20% (for untreated vines), a threshold of more than 3 on the OIV scale allowed to select 92% of the resistant genotypes in the field with 30% of misclassified (individuals that would be susceptible in the field) (accuracy of a Response Operating Curve test =0.82).

Our threshold of acceptable damage on bunches was quite high considering the high pressure of downy mildew. Genotypes were classified as harvestable or non harvestable. Harvestable genotypes had an average severity at the end of June of 20% with a max of 50% for some of them (Incidence of 100%). All 19 harvestable genotypes (13,168,97,119,95,3-1,59,74,14,134,58,85,153,163,42,170,63,35,70) had a very low level of severity on leaves at the end of June (Fig. 4). Some genotypes with a moderate severity on bunch at this date but a high level of severity on leaf were not harvestable in September (175, 176, 114, 65, 38). All the harvestable genotypes fitted exactly with those highly resistant to powdery mildew (except 168 and 14 which were not tested against powdery mildew). Both parents, 3082-1-42 and Regent, as well as Villard-blanc and Chambourcin were also harvestable. Except Regent and genotype 63, all harvestable genotypes showed in the bioassay test an OIV > 3 with an average of 5.3 (Fig. 5). Therefore, the bioassay on leaf disk can be used to select genotypes resistant on bunches with an accuracy of 0.76. A threshold > 5 on the OIV scale allowed to

select 62% of the genotypes resistant on bunches among the resistant genotypes with 13% of misclassified individuals. However, in some cases harvestable genotypes can be susceptible to downy mildew on leaves in the bioassay whereas non harvestable genotypes can be resistant. This raises the question of the expression of the resistance genes in bunches.

Some of the harvestable genotypes were also highly susceptible to other diseases like botrytis (63, 70, 134, 97, 3-1) or black rot (13).

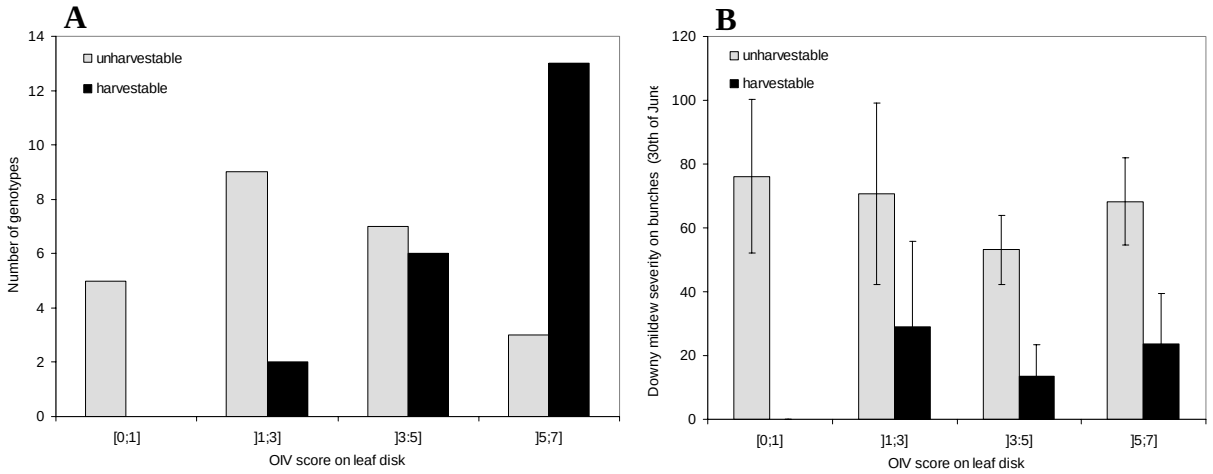

Fig. 5: Distribution of genotypes (A) and average severity on bunches at the end of June (B) function of their OIV scoring on leaf disk.

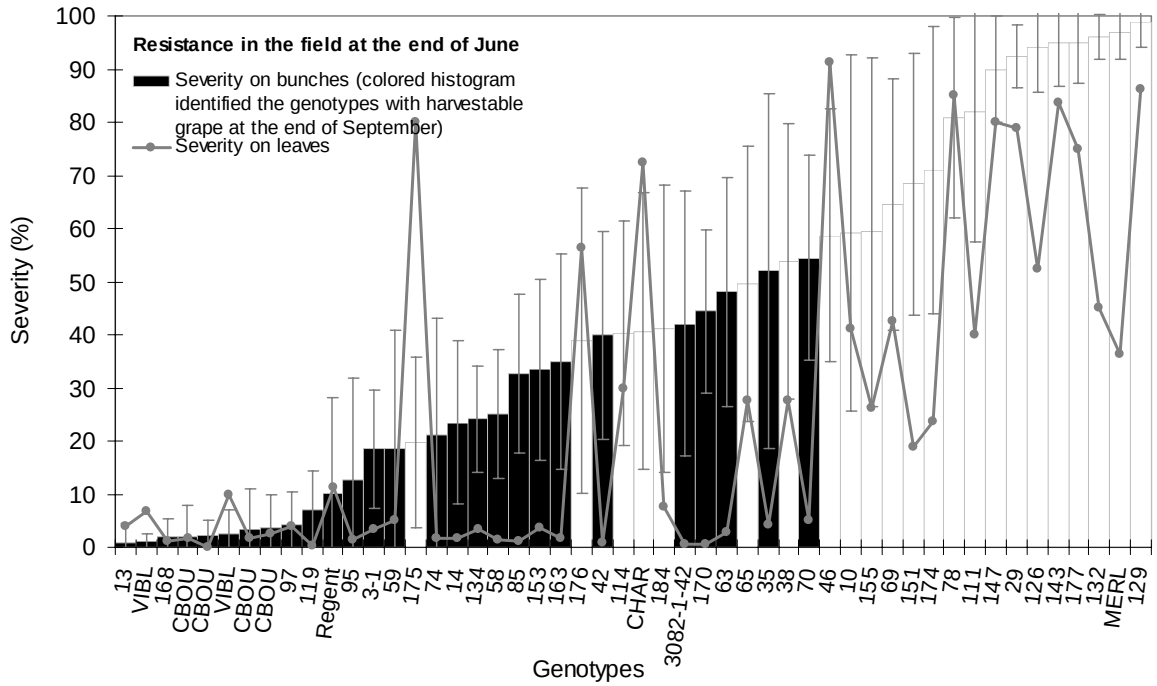

Fig. 4: Average severity on bunches and on leaves in the field at the 29th of June for the 38 genotypes and control varieties. Genotypes considered as harvestable are coloured in black (bars indicate the standard deviation of the mean).

Conclusion

As expected, none of the varieties were totally resistant to DM. Even the genotypes considered as "harvestable" presented this year a high level of damage on bunches. Such levels of damage must be replaced in the context of an outstanding year in term of mildew pressure in Bordeaux and these resistant genotypes could be satisfactory in a context of IPM with fewer treatments at the right time. The low susceptibility of the leaves to DM and PM may be important to delay the epidemics when primary infection is high and to limit the multiplication of inoculum at the end of the season and consequently the damage on bunches. This was evident for some of the varieties with a moderate level of disease on bunch and a high severity on leaf at the end of June that were totally destroy in September. There is however no evidence to conclude that the resistance genes expressed in leaves are also expressed in bunches. Indeed, the field trial shows that some genotypes having a high leaf resistance in bioassay and a moderate leaf resistance in the field were not all harvestable. Final damage depends on the adequacy between the amount of inoculum and the period of bunches susceptibility. As genotypes can be very different in term of precocity, differences in resistance could also be due to delay between the epidemic on leaves and the period of susceptibility of bunches. Further analyses of the phenology and mapping resistance genes data are needed to answer this question. Even, if there is no correlation between the OIV measure of resistance on leaf disk and the disease assessment of severity on bunches in the field, the bioassay can be used to select field resistant genotypes, with a good accuracy, by using a threshold of 5 on the OIV resistance scale.

The link between resistance to powdery mildew and downy mildew suggest that resistance in harvestable genotypes is coming from *Muscadinia* (*Rpv1* and *Run1*). This will be confirmed by QTL analysis of the genotypes. A better understanding of the resistance of Regent would be necessary to understand its low level of resistance in bioassay compare to that in the field and to understand its contribution to genotypes having major resistance genes as *Run1* and *Rpv1*. Field disease assessment for DM but also for the other diseases such as botrytis and black-rot may help us to identify and/or to precise the role of QTL of resistance on other diseases.

Whatever the mechanism of resistance is, these resistant varieties should be tested on larger plot scale and on longer period of time to measure the ability of such strong leaves resistance to improve the control of epidemics and to be durable against new pathotypes.

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