Four years of monitoring of disease-resistant grapevine varieties in French vineyards

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1 Introduction

Resistant cultivars are widely used for arable crops, but remain rarely considered for perennial ones, such as apple and grapevine. This is all the more surprising as these crops often received numerous pesticide treatments. For example, in France, the mean treatment frequency index in viticulture is 15.3 (Agreste, 2019). In this context, the deployment of disease-resistant grapevine varieties is a promising innovation paving the ways toward sustainable viticulture. Various European breeding programs led to the registration of several INRAE and other foreign resistant varieties of grapevine in France. They can now be grown in the vineyards.

The adoption of new disease-resistant grapevine varieties remains challenging for farmers. Indeed, as for any newly registered variety, many agronomic parameters must be assessed in the vineyard. More specifically, disease protection goals and strategies must be adapted to complement the partial genetic resistance conferred by resistant cultivar but also to control the secondary diseases that might re-emerge following the reduction of fungicide use. Long-term cropping system experiments have already been conducted with disease-resistant grape varieties at INRAE in Bordeaux vineyard (Delière *et al*., 2018), but further evaluations are now required to address these issues in more diverse agro-climatic production situations.

Another major challenge raised by the deployment of disease-resistant grapevine varieties is the management of resistance durability *i.e.* the maintenance of resistance efficacy in the long term. Resistance genes do not remain effective forever (McDonald and Linde, 2002). Accordingly, given their scarcity in genetic resources and the time required for breeding new cultivars, resistance genes must be careful manage to ensure that they will remain effective as long as possible (Merdinoglu *et al.* 2018). Pathogens evolve to adapt to resistant varieties (McDonald and Linde, 2002), through evolutionary mechanisms resulting in the breakdown of qualitative resistances and/or a gradual erosion of the efficiency of quantitative resistances. Several cases of resistance breakdowns have already been reported even though resistant grapevine varieties are not widely cultivated. In Europe, the Rpv3.1 resistance factor present in Bianca and Regent has been overcome by virulent strains of *Plasmopara viticola*, the causal agent of downy mildew (DM) (Peressotti *et al*., 2010; Delmotte *et al*., 2014; Delmas *et al*., 2016). Similarly, strains of *Erysiphe necator* from *Vitis rotundifolia* can breakdown the total resistance to powdery mildew (PM) conferred by the Run1 gene (Feechan *et al*., 2013).

Since 2018, several resistant varieties have been authorised and can be planted by wine growers. They have been obtained by French, Swiss, German or Italian breeding programs. The new French disease-resistant varieties currently developed aim to combine into the same cultivar several resistance factors for each targeted disease (PM and DM), a breeding strategy known as gene pyramiding. This approach is expected to slow down the erosion and limit the breakdown of grape resistances (McDonald and Linde, 2002). Several experimental plots of resistant varieties were planted by winegrowers in the early 2010s and these plantings have accelerated since 2018 with the registration of several varieties.

Here, we addressed four main issues related to the deployment of disease-resistant varieties in French vineyards:

- Which level of fungicide treatments reduction can be achieved using disease resistant varieties?
- How effective are grapevine resistances toward DM and PM?
- What are the sensitivity of grapevine resistant varieties to other grapevine pest and diseases?
- Are DM populations becoming more virulent in response to the deployment of disease-resistant varieties?

These four issues have been addressed since 2017 in a network of commercial vineyards plots.

2 Material & Methods

Network of plots
INRAE has set up in 2017 a network of plots planted with powdery and downy mildew resistant varieties. The network is named the OSCAR Observatory (https://observatoire-cepages-resistants.fr/en/). Plots are located in various agro-climatic conditions. They are planted and managed by the winegrowers according to their own objectives**.** To join the network, plots must cover an area of at least 0.2 ha (700 to 1500 vines stock), be planted with a single variety and be cultivated with similar cropping practices as those used elsewhere in the estate (pruning, cropping operations, harvest). OSCAR integrates new plots every year. Each plot is described in terms of location, size, variety, year of plantation, density, rootstock, pruning method, and target yield. In 2017, OSCAR consisted of 34 plots located in 14 locations (Guimier *et al*., 2019). The network has grown and, in 2021, OSCAR is composed of 116 plots (for a total area of 80 ha) distributed in 63 locations. The average size of a plot is 0.7 ha. Twenty-six downy mildew and powdery mildew resistant varieties are monitored, including 14 INRAE varieties and 12 varieties from other European breeding institutes (Table 1).

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Fungicide use

Phytosanitary practices are collected from the wine growers. The data collected are dates of treatment, target, product name, active ingredients, dose and decision rules used. The level of pesticide used is estimated by the treatment frequency index (TFI). For each treatment, the amount of fungicide used is calculated as follows: TFI per treatment = ((Dose sprayed / Dose recommended) * (Area sprayed / Area total)). Over the whole cropping season, we then have TFI = Σ TFI per treatment (Pingault *et al.*, 2009 ; OECD, 2013).

Varieties	*QTL conferring downy mildew resistance*	*QTL conferring powdery mildew resistance*	*Breeding institute*	*Nb of plots*
1D10 B	Rpv1	Run1	INRAE	1
2E5 B	Rpv1	Run1	INRAE	2
3B12 B	Rpv1	Run1	INRAE	2
3159-2-12 B	Rpv1	Run1	INRAE	1
3160-11-3 N	Rpv1	Run1	INRAE	3
3176-21-11 N	Rpv1	Run1	INRAE	7
3184-1-9 N	Rpv1	Run1	INRAE	6
3196-57 B	Rpv1	Run1	INRAE	3
3197-81 B	Rpv1	Run1	INRAE	5
Artaban	Rpv1/Rpv3.1	Run1/Ren1	INRAE	12
Floreal	Rpv1/ Rpv3.1	Run1/Ren1	INRAE	20
Vidoc	Rpv1/Rpv3.1	Run1/Ren1	INRAE	9
Voltis	Rpv1/ Rpv3.1	Run1/Ren1	INRAE	5
Col-50#83	Rpv1/ Rpv10	Run1/Ren3.2	INRAE	2
Cabernet Cortis	Rpv3.3/ Rpv10	Ren3/Ren9	WBI Freiburg	5
Monarch	Rpv3.3/Rpv10	Ren3/Ren9	WBI Freiburg	2
Muscaris	Rpv10	Ren3/Ren9	WBI Freiburg	9
Pinotin	Rpv3.1	Ren3/Ren9	Volker Freytag	1
Prior	Rpv3.1/ Rpv3.3	Ren3/Ren9	WBI Freiburg	1
Sauvignac	Rpv3.1/ Rpv12	Ren3/Ren9	Blattner	3
Soreli	Rpv3.1/Rpv12	unknown	IGA Udine	3
Souvignier gris	Rpv3.2	Ren3/Ren9	WBI Fribourg	10
Other varieties	-	-	-	4
Total (26 varieties)				116

Table 1: Characteristics of the 26 grapevine resistant varieties monitored in OSCAR in 2021 along with their resistant genes, breeding institute origin and number of plots. In 2021, the network OSCAR monitors 116 commercial plots covering a total area of 80 ha. The average plot size is 0.7 ha.

Monitoring of resistant varieties and resistance efficacy

In each plot of OSCAR, the epidemic dynamics of the pests and diseases targeted by the resistance (downy and powdery mildew) but also of black-rot, anthracnose, erineum mite and phylloxera are monitored. Five visual observations were carried out at key phenological stages: pre-bloom, flowering, bunch closure, veraison and harvest. The occurrences of the various pests and diseases on leaves and bunches are assessed using a qualitative scoring grid.

In addition to field monitoring, the evolution of the aggressiveness of DM populations is monitored in the laboratory. Isolates of *P. viticola* are collected annually across the observatory from the plots planted with resistant varieties and from adjacent plots planted with susceptible *V. vinifera* cultivars. These isolates are stored in liquid nitrogen, to constitute a collection of *P. viticola* populations that have faced resistant grape varieties. Since 2018, more than 4,000 isolates have been collected and stored. Based on these collections, cross-inoculation bioassays have been carried out in the laboratory in 2019 following the protocol described by Delmas *et al.* (2016).

Downy mildew isolates were collected in 2018 from resistant varieties carrying Rpv1 (the so-called 'Bouquet' variety), from varieties carrying Rpv1 and Rpv3.1 (ResDur1) as well as from susceptible varieties. Sixty isolates originating from resistant varieties and 60 originating from susceptible varieties were included in the bioassay conducted in 2019. They were inoculated onto leaf discs of four varieties: a susceptible variety (Cabernet Sauvignon), a Bouquet variety (3176-21-11N; Rpv1), a German variety (Regent; Rpv3.1) and a Resdur1 variety (Artaban; Rpv1 and Rpv3.1). After incubation, the quantity of sporangia emitted per unit leaf area was measured by image analysis. We defined the efficacies of the resistance factors Rpv1 and Rpv3.1, alone or cumulated, as a function of the plants where isolates were collected. For each resistance factors QTLi (3 levels : Rpv1, Rpv3-1 and Rpv1-Rpv3.1) and each isolate origin ORj (3 levels, isolates from Bouquet, Resdur1 and susceptible varieties), we calculated the efficacy Ei,j of QTLi on isolates originating from ORj as 1- (average percentage of sporulation on QTLi of the isolates with origin ORj /average percentage of sporulation on Cabernet sauvignon of the isolates with origin ORj).

3 Results

Fungicide use

The average fungicide TFI obtained on 30 plots in 2017, 32 plots in 2018, 36 plots in 2019 and 61 plots in 2020 amounted to 1.4, 1.6, 0.6 and 1.3, respectively, compared to an average fungicide TFI of 12.7 for the 2016 national reference (Agreste, 2019) (Figure 1). Thus, the reduction in fungicide TFI on plots planted with resistant varieties range, depending on the year, from 87 to 95%.

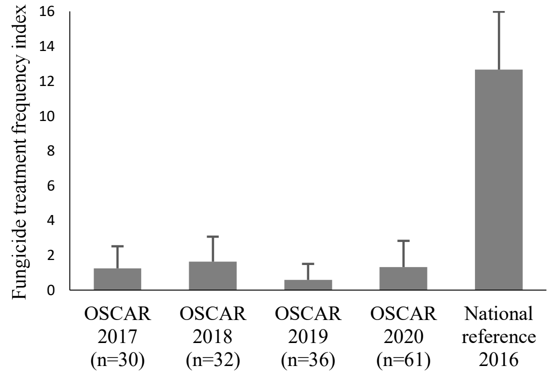

Figure 1: Fungicide treatment frequency index (TFI) in the OSCAR network in 2017, 2018, 2019 and 2020 along with the national reference 2016. All OSCAR plots are planted with grapevine resistant varieties.

Field monitoring over three cropping seasons.
Since 2017, all the pests and disease surveyed have been satisfactorily controlled on the plots monitored. For DM, symptoms appeared on the plots from 2018 to 2020 and especially during the cropping season 2018 which suffered an high epidemic pressure. However, although disease incidence may have been high in 2018, severities remained low and did not lead to significant crop losses. For PM, no symptoms were detected in the field on varieties carrying the Run1 gene conferring total resistance. Finally, neither significant impact of the diseases usually controlled by fungicides (anthracnose, black rot), nor of pests such as erineum mites and phylloxera, were observed on the plots, except for black rot, which led to significant losses for one plot in 2018 and 2 plots in 2020 (Table 3).

Laboratory monitoring of resistance efficacy.
The resistance factors Rpv1 and Rpv3.1, alone or cumulated, significantly reduced the sporulation of isolates collected on susceptible varieties (sporulation reduced by 64% for Rpv1; 78% for Rpv3.1; 89% for Rpv1/Rpv3.1 pyramids). They also reduced the sporulation of isolates collected on Bouquet varieties (carrying the Rpv1 factor) by 65% for Rpv1, 74% for Rpv3.1 and 88% for the Rpv1/Rpv3.1 pyramid). This is also the case for the resistance factors Rpv1 and Rpv1/Rpv3.1 pyramided which reduced the sporulation of isolates collected from Resdur1 varieties by 69% for Rpv1 and 78% for Rpv1/Rpv3.1 (Table 2). However, we evidenced a lower efficacy of Rpv3.1 when inoculated with isolates collected from Resdur1 varieties. The decrease of efficacy to 47% can be explained by isolates overcoming the resistance conferred by Rpv3.1 (Figure 3). The presence of such isolates in DM population have been previously reported by Peressotti *et al.* (2010) on the variety Bianca and later by Delmotte *et al* (2014) and Delmas *et al.* (2016) on the varieties Bronner, Prior and Regent. This result also highlights the interest of characterizing DM aggressiveness both at the level of population of isolates but also at the level of individual isolates

Isolates collected from	**Resistance efficacy on**		
	Rpv1	**Rpv3.1**	**Rpv1/Rpv3.1**
Vitis vinifera	65%	78%	89%
Bouquet varieties	65%	74%	88%
Resdur1 varieties	69%	47%	78%

Table 2: Efficacy of the resistance factors Rpv1 and Rpv3.1, alone or pyramided, as a function of the plants where isolates were collected (3 origins : *Vitis vinifera*, Bouquet varieties and ResDur1 varieties).

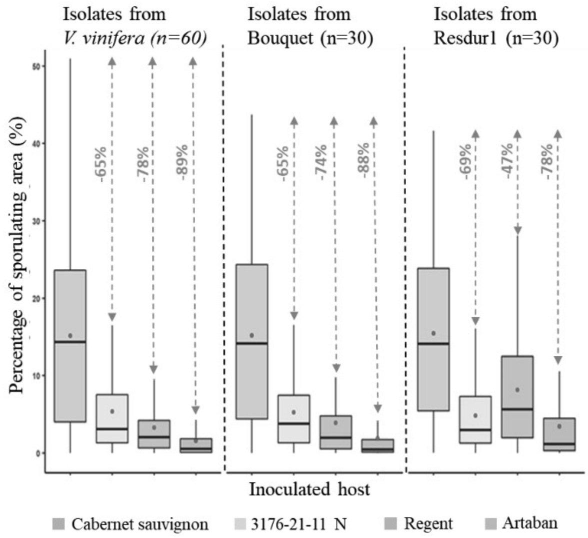

Figure 2: Boxplot of the percentage sporulation of the isolates collected on *V. vinifera* (60 isolates), Bouquet varieties (30 isolates), and Resdur1 varieties (30 isolates). Each isolates was replicated 5 times. The medians are represented by horizontal black lines and the means by red circles. The blue arrows represent the efficacy of resistance measured as the reduction of sporulation obtained with the resistant varieties compared to the susceptible Cabernet Sauvignon.

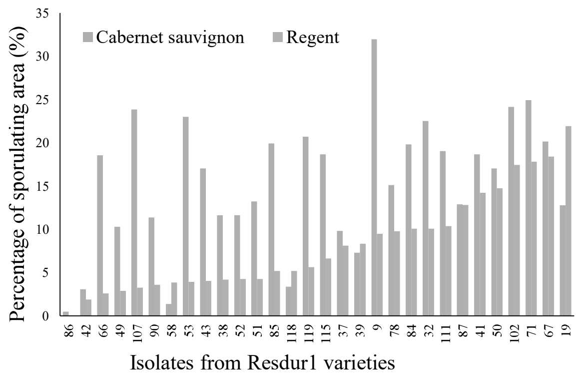

Figure 3: Percentage of sporulating area of the 30 *P. viticola* isolates collected on Resdur1 varieties and inoculated on Cabernet sauvignon (green) and Regent (orange) leaf discs.

4 Conclusions

The field monitoring carried out within OSCAR observatory during these first four years did not reveal any resistance erosion or resistance breaking of the varieties deployed. These field observations were supplemented by a laboratory monitoring of the aggressiveness of DM populations collected from the network of plots. No breakdown of Rpv1 factor nor of the pyramid Rpv1/Rpv3.1 were observed in this monitoring. However, the results confirmed the presence of DM isolates breaking down the Rpv3.1 factor. Importantly, these first four years of monitoring also confirmed the high potential of grapevine resistant varieties to reduce phytosanitary treatments. In the plots planted with these varieties, all the pests remained well controlled in most cases despite drastic reductions of around 90% in the fungicide TFI compared to the national reference. Accordingly, the deployment of resistant varieties in the vineyard should be increasingly important in forthcoming years, confirming its role as a key player toward sustainable viticulture. In this context, it will be essential to monitor the durability of the resistance factors deployed. In this respect, the OSCAR observatory is a warning system allowing to estimate the effectiveness of the resistance genes deployed in the vineyard and to rapidly identify the emergence of adapted DM populations.

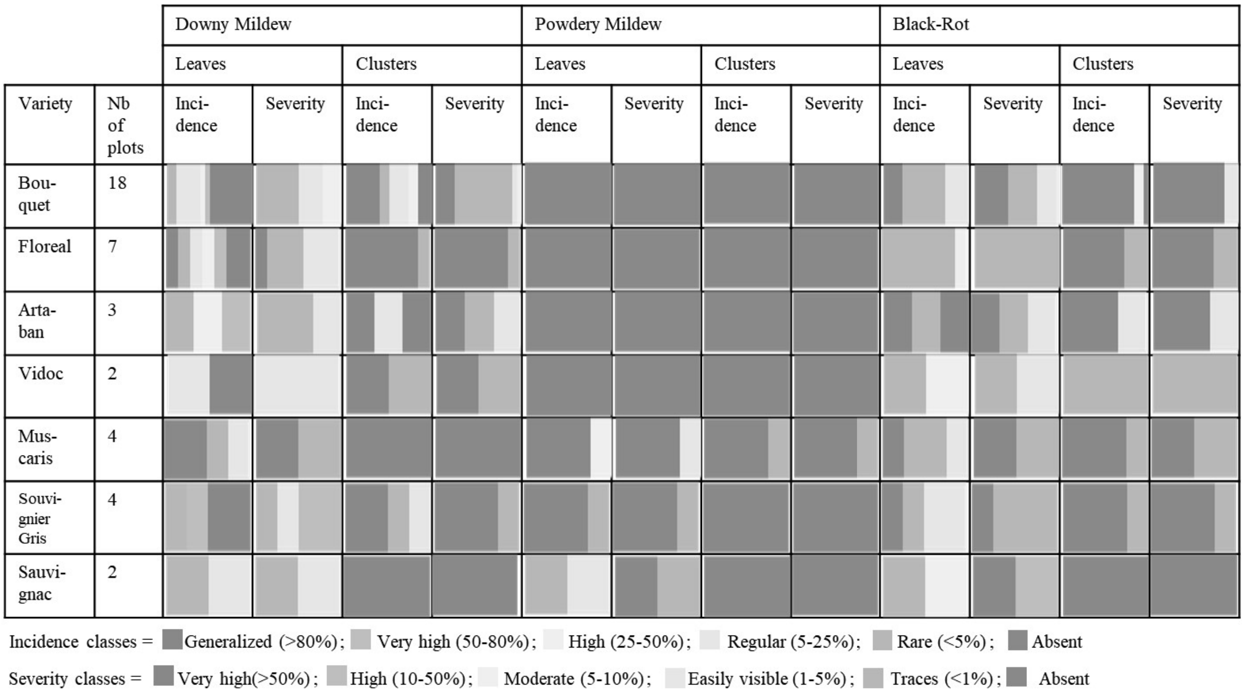

Table 3 : Incidence and severity of downy mildew, powdery mildew and black rot on leaves and clusters in 40 plots of the OSCAR network sampled at harvest stage in 2020. The plots are classified by variety. The varieties represented here are those with the highest number of data collected at the harvest in 2020. Disease incidence on leaves: frequency of vines with at least one infected leaf. Disease incidence on clusters: frequency of vines displaying at least one infected cluster. Disease severity : percentage of tissue area covered by lesions. Incidence and severity are classified into five classes. The table shows the distribution of plots in each rating class.

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