Evidence for sexual reproduction and fertile oospore production by *Plasmopara viticola* on the leaves of partially resistant grapevine cultivars

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Abstract

Downy mildew, caused by *Plasmopara viticola*, is a highly destructive disease of grapevine. In recent decades, European breeding programs for disease resistance have led to the creation of new cultivars resistant to downy mildew. This resistance limits mycelium growth and the sporulation of *P. viticola,* thereby decreasing disease epidemics due to the asexual reproduction of the pathogen. However, we still lack biological insight into the effects of partial host resistance on pathogen survival during the sexual stage of its lifecycle. We present here the results of a two-year experiment in which we assessed the production of sexual forms of *P. viticola* (oospores) and the success of subsequent infections on resistant cultivars with the *Rpv1* and *Rpv3* loci. We found that disease resistance had little effect on the sexual cycle of the pathogen. *Plasmopara viticola* produced four times more oospores on susceptible *Vitis vinifera* cultivars than on disease-resistant cultivars. Macrosporangia resulting from the germination of oospores were found on both resistant and susceptible genotypes, and were produced in surprisingly large numbers on some *Rpv1* genotypes. Rates of primary contamination due to macrosporangia were lower on *Rpv3* than on conventional cultivars, but higher on *Rpv1* genotypes. Thus, *P. viticola* can complete its lifecycle (both asexual and sexual phases) on partially resistant grapevine cultivars. The maintenance of downy mildew populations from one year to the next presents a significant challenge to the sustainability of genetic resistance in grapevine.

Keywords: grapevine downy mildew, disease-resistant variety, *Rpv1*, *Rpv3*

INTRODUCTION

Plasmopara viticola, the causal agent of grapevine downy mildew, is an obligate biotrophic oomycete that attacks *Vitis vinifera* (Viennot-Bourgin, 1949; Lafon and Clerjeau, 1988; Burruano, 2000; Luis et al., 2013). All major *V. vinifera* cultivars are highly susceptible to this pathogen (Yin et al., 2017). It was introduced into Europe from North America in the 1870s (Millardet, 1881) and subsequently spread to all major wine-growing regions of the world (Galet, 1977; Lafon and Clerjeau, 1988; Gessler et al., 2011). European *P. viticola* populations have a low level of genetic diversity, consistent with the occurrence of a bottleneck at the time of introduction (Fontaine et al., 2013).

P. viticola has direct quantitative (Savary et al., 2009; Fermaud et al., 2016) and qualitative (Pons et al., 2018) effects on yield. Fungicide treatment is currently the only available method for controlling this pathogen on *V. vinifera* (Caffi et al., 2010; Gessler et al., 2011). However, the systematic use of chemicals has led to fungicide resistance in *P. viticola* populations (Chen et al., 2007; Delmas et al., 2017), decreasing the efficiency of a growing number of products (Gessler et al., 2011). Rational fungicide management approaches based on decision support systems are now being implemented (Delière et al., 2015).

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Acta Hortic. 1248. ISHS 2019. DOI 10.17660/ActaHortic.2019.1248.82
Proc. XII International Conference on Grapevine Breeding and Genetics
Eds.: S. Delrot et al.

The use of cultivars with natural disease resistance is a cost-effective and environment-friendly alternative to fungicide use for the control of plant disease (Nelson et al., 2018). At the beginning of the 20th century, French breeders produced a large number of disease-resistant hybrids, using American species of the genus *Vitis* as sources of resistance. The classification principle introduced in 1955 resulted in the retention of only about 20 "hybrid" grape cultivars from the several hundred that were circulating at the time. Their use was limited to table wine production, mostly because the wine produced from these cultivars was of insufficiently high quality, with the notable exception of the 'Baco Blanc' cultivar used to produce spirits in Armagnac. These hybrids were gradually eliminated from French vineyards, and they account for less than 1% of French vines today (Delière et al., 2017). Breeding work continued in Europe, culminating in the breeding of disease-resistant grape cultivars with agronomic and organoleptic qualities compatible with current production standards. In 1974, INRA launched a conventional breeding program in France with the aim of incorporating original resistance factors carried by *Vitis rotundifolia* into European cultivars (*V. vinifera*) (Bouquet, 1980, 1983). After 25 years of effort, this program culminated in a series of genotypes known as the "Bouquet" genotypes. Since 2000, the INRA station at Colmar has been engaged in the "ResDur" varietal breeding program focusing on the combination of resistances from "Bouquet" genotypes on the one hand with cultivars registered in the German official catalog, such as 'Regent' or 'Bronner', on the other. The pyramiding of resistance factors has been achieved by crossing, with the goal of achieving more durable resistance (Bouquet, 2000; Merdinoglu et al., 2009; Merdinoglu and Caranta, 2013). Marker-assisted selection has been used to sort the progenies and to ensure exclusively the retention of cultivars with pyramided resistance. This strategy has led to the development of candidate cultivars bearing two or three genes to control each disease. Four new resistant cultivars with polygenic resistance ('Artaban', 'Floreal', 'Vidoc' and 'Voltis') are already registered, and about 10 more should be released by 2024 (Schneider et al., 2019).

Durability is one of the principal challenges in breeding for disease resistance in grapevine (Merdinoglu et al., 2014). The durability of resistance is particularly important in this species, because grapevines are planted for decades. Durable resistance cannot be achieved without a sound knowledge of the pathogen adaptation to the resistance of the cultivar at all stages of its lifecycle. Casagrande et al. (2011) and Peressotti et al. (2010) showed that the *Rpv3* gene present in the cultivar 'Bianca' was overcome by a Czech isolate and an Italian isolate of *P. viticola* (race-specific interaction). Delmotte et al. (2014) later provided evidence of the recurrent emergence of "aggressive" *P. viticola* isolates displaying high levels of sporulation on the partially resistant cultivar 'Regent' (*Rpv3* locus). Multisite sampling in three remote wine-growing areas suggested that adaptation to this cultivar may have occurred independently, at least three times. Delmas et al. (2016) extended these results by showing that partial resistance selects for greater aggressiveness in *P. viticola* populations.

P. viticola has a mixed reproduction system based on multiple cycles of asexual reproduction and a single sexual generation each year. Previous studies of *P. viticola* adaptation to grapevine resistance have focused on the asexual phase of the pathogen's biological cycle (Peressotti et al., 2010; Gómez-Zeledón et al., 2013; Delmas et al., 2016; Foria et al., 2018). This concentration on the asexual phase probably reflects the difficulties involved in experimental studies on the sexual structures of the pathogen (Vercesi et al., 1999). However, *P. viticola* is a heterothallic species (Wong et al., 2001) that must undergo sexual reproduction to complete its annual lifecycle. Sexual reproduction begins in the summer with the mating of strains on grapevine leaves. Oospores are produced by the fusion of an antheridium and an oogonium (Lafon and Clerjeau, 1988; Gessler et al., 2011). These oospores germinate in free water the following spring (Lafon and Clerjeau, 1988; Rossi and Caffi, 2012), giving rise to one, or occasionally two, macrosporangia containing the zoospores that serve as the infectious stage of the parasite (Lafon and Clerjeau, 1988; Vercesi et al., 1999; Burruano, 2000). In temperate climates, oospores resulting from sexual reproduction are the only stage of the parasite capable of surviving the winter and are the initial source of inoculum for the next growing season (Lafon and Bulit, 1981; Burruano,

2000). High germination rates under laboratory conditions are positively correlated with the appearance of numerous primary infections in the vineyard (Pertot and Zulini, 2003). Population genetic studies have demonstrated that grapevine downy mildew epidemics result principally from the germination of oospores, also referred to as the "primary inoculum" (Gobbin et al., 2005; but see Gobbin et al., 2007). Assessments of *P. viticola* oospore production during the sexual phase are, therefore, highly relevant to the management of downy mildew epidemics on partially resistant cultivars.

The present study investigates the various sexual stages of *P. viticola* on resistant grapevine cultivars. We assessed the presence and germination of oospores and the success of infection from macrosporangia.

MATERIALS AND METHODS

Plant material

The pseudo-F_1 progeny studied (50,001) resulted from a cross between Mtp3082-1-42 and 'Regent'. Mtp3082-1-42 is a genotype derived from a cross between *V. rotundifolia* and *V. vinifera* followed by four backcrosses with *V. vinifera* (Merdinoglu et al., 2003; Bouquet, 2009). 'Regent' is the offspring of a cross between 'Chambourcin' (12.417 SV × 7053 Seibel) and 'Diana' ('Sylvaner' × 'Müller Thurgau'). The Mtp3082-1-42 parent transmitted the *Rpv1* locus, which confers partial resistance to downy mildew, and the 'Regent' parent transmitted the *Rpv3* locus, which also confers partial resistance to downy mildew (Fischer et al., 2004). The *Rpv1* and *Rpv3* loci provide moderate resistance (decrease in sporulation of about 75% under laboratory conditions), and *Rpv1* is slightly more effective than *Rpv3* (Guimier et al., 2018).

We analyzed oospore germination for the two parents ('Regent', 3082-1-42), seven genotypes from the segregating population and the 'Merlot' cultivar previously identified as a good susceptible control (Calonnec et al., 2007). The seven progenies included one genotype with no downy mildew resistance locus (38), one genotype with the *Rpv3* locus (151), one genotype with the *Rpv1* locus (170), and four genotypes with both *Rpv3* and *Rpv1* loci (159, 119, 97, 163). Genotypes were classified into four possible locus combinations: [-/-], [*Rpv1*/-], [-/*Rpv3*] and [*Rpv1*/*Rpv3*]. All these genotypes were planted at the INRA experimental site in Latresne (Bordeaux region: 44°46'52.2"N 0°29'00.8"W) in 2004, in a randomized experimental design in which four consecutive vines for each genotype were studied. Plants were left untreated for the two years of the study. Natural infestations of grapevine downy mildew were observed during the two years of the experiment.

Field disease assessment

Assessments of downy mildew were performed in the field on the four vinestocks of each genotype in 2008. The foliar severity of downy mildew was assessed by determining the percentage of the tissue area covered by lesions, categorized in 5% steps, but with the inclusion of a 1% category: 0, 1, 5, 10%, etc. Defoliation due to disease expression was defined as the percentage of fallen leaves, assessed for each vine with the scale described above.

Oospore maturation and germination

The method for manipulating and assessing oospore maturation and germination was adapted from a published method (Ronzon-Tran Manh Sung and Clerjeau, 1988). Briefly, oospores were isolated from "mosaic" leaves collected directly from the field in the fall, and the germination of the sexual forms of the pathogen from these spores was then assessed the following spring.

1. Sample collection and oospore maturation.

Samples were collected from the INRA experimental vineyard at the start of October (8 October 2008; 5 October 2009). For each genotype, we collected leaves displaying the "mosaic" symptoms typical of sexual reproduction resulting from natural infection in the

field. In 2008, a severe grapevine downy mildew outbreak was observed throughout French vineyards (Grosman et al., 2008), whereas disease severity was moderate in 2009 due to drier conditions less favorable for downy mildew (Anonymous, 2010). We were able to sample infected leaves of all genotypes in both years.

For each genotype, 20 detached leaves were collected. The presence of oospores in the necrotic part of each leaf was assessed with a stereoscopic microscope at ×50 to 100 magnification, with lighting from below. Disks of 6-mm diameter were excised with a cork-borer from the parts of the leaf producing oospores. We assessed the presence of oospores for 10 genotypes in 2008, but for only six genotypes in 2009 because mold development on the detached leaves prevented assessment for the remaining four genotypes: 'Regent', 170, 97 and 163. However, leaf disks could be selected randomly from the leaf samples of these genotypes despite their high moisture content, and oospores germinated successfully from these leaf disks, causing primary infections as observed in 2008.

At least 50 leaf disks were sampled for each genotype studied. Leaf disks were stored in plaster modeling tubes (plaster, 1.33 g mL^{-1}; length, 40 mm; external diameter, 30 mm; internal diameter, 12 mm; interior volume, 3 mL) closed with a silicone plug. Each plaster tube contained 50 leaf disks and was buried at a depth of 5 cm in a gravelly soil at the INRA La Grande-Ferrade research station, Villenave d'Ornon (Bordeaux region: 44°47'24.9"N 0°34'34.8"W).

2. Oospore maturation.

Oospores were allowed to mature under natural climatic conditions from October (year *n*) to April (year *n*+1). Climatic conditions were recorded at the standard weather station 50 m from the site. Monthly rainfall during the winter varied from year to year (December to March). In 2008, the fall was rainy and the winter was characterized by an alternation of dry and rainy conditions. In 2009, a rainy winter followed an already very rainy November. Rainy conditions during oospore formation (September-December) accelerate the maturation process (Tran Manh Sung et al., 1990). We used oospore germination data (Rouzet and Jacquin, 2003) provided by the French Plant Protection Services in Villenave d'Ornon, which were collected for technical alerts, to determine the optimal maturation date for oospores in the 2008/2009 and 2009/2010 experiments. Oospore germination was therefore assessed on 6 April (date 1) and 20 April (date 2) in 2009 and 30 March (date 1) and 13 April (date 2) in 2010.

3. Bioassay of oospore germination.

On each of the four dates and for each genotype, 15 leaf disks were removed from the plaster tubes and brought back to the laboratory to evaluate macrosporangium production. The leaf disks were placed in Petri dishes (five per dish) containing 10 mL medium solidified with 1% agar, to maximize humidity, thereby preventing desiccation and promoting germination. The dishes were placed in a controlled environment chamber at 22°C in the dark. Twice weekly for 14 days, the number of macrosporangia formed was counted on each disk under a binocular microscope at ×50 to 100 magnification, with lighting from above.

4. Infection test.

Disks from which oospores germinated were used to assess the ability of macrosporangia to cause primary infections under controlled conditions. The leaf disks were used to inoculate a sensitive cultivar, 'Cabernet Sauvignon'. Inoculations were performed overnight (in the dark) by placing the adaxial side of a disk against the abaxial side of a 'Cabernet Sauvignon' leaf, with a drop of sterile water between the disk and the leaf. After 12 h, the water was removed by aspiration and the infected 'Cabernet Sauvignon' leaves were placed on filter paper soaked in deionized water in a separate Petri dish. The Petri dishes were wrapped in Parafilm® and placed in a growth chamber at 22°C, under a 12-h photoperiod, for up to 14 days.

Data analysis

As described by Southwood (1978), a square-root transformation was applied to count data and a log transformation was applied to percentages. Differences between genotypes or loci were assessed by analysis of variance (ANOVA), followed when required by pairwise comparisons of mean values. All tests were performed with a type I error of 5%, with SYSTAT®11 software.

RESULTS

Field assessment

1. Severity and defoliation.

The severity of downy mildew infection on leaves (Table 1) differed between genotypes (P<0.0001). More than 75% of the leaf area presented signs of downy mildew for the 'Regent', 151 and 'Merlot' genotypes. A significant effect of genotype was also detected for defoliation (P<0.0001), with more than 50% defoliation for 'Regent'.

Table 1. Field assessment of downy mildew severity and oospore number for partially resistant grapevine genotypes. Dates are given in the format MM/DD/YYYY. Values in parentheses are SEM. na, No data available. Letters, when present, represent statistical groupings of the data defined by analysis of variance of the effects of genotype and followed by pairwise differences in least-squares means at the 95% confidence level.

Date of collection	Loci	Genotype	Field severity (%)[1]	Vinestock defoliation (%)[1]	Leaves with oospores (%)[2]	Mean number oospores per leaf (SEM)[2]
10/08/2008	[-/-]	Merlot	76.3 (1.3)b	20.0 (0.0)c	90	807.8 (82.1)
		38	42.5 (6.3)c	27.5 (5.2)c	100	887.5 (44.7)
	[*Rpv1*/-]	3082-1-42	25.0 (0.0)d	5.0 (0.0)d	90	260.3 (75.8)
		170	11.8 (2.0)e	0.5 (0.3)d	70	177.5 (72.5)
	[-/*Rpv3*]	Regent	90.0 (0.0)a	80.0 (0.0)a	65	276.5 (84.3)
		151	85.0 (0.0)ab	60.0 (0.0)b	80	232.2 (67.9)
	[*Rpv1*/*Rpv3*]	97	11.3 (1.3)e	0.0 (0.0)d	85	165.3 (51.2)
		119	40.0 (0.0)c	1.0 (0.0)d	85	406.3 (86.3)
		159	9.3 (0.8)e	0.8 (0.3)d	90	298.8 (68.1)
		163	8.5 (0.9)e	1.5 (0.3)d	85	409.0 (85.7)
10/05/2009	[-/-]	Merlot	na	na	100	887.5 (44.7)
		38	na	na	95	573.5 (86.5)
	[*Rpv1*/-]	3082-1-42	na	na	95	163.8 (60.8)
		170	na	na	na	na
	[-/*Rpv3*]	Regent	na	na	na	na
		151	na	na	65	181.8 (55.5)
	[*Rpv1*/*Rpv3*]	97	na	na	na	na
		119	na	na	75	274.8 (90.6)
		159	na	na	90	282.8 (83.3)
		163	na	na	na	na

[1]Assessed on the foliage of four vinestocks per genotype.

[2]Assessed on 20 leaves per genotype.

ANOVA to assess the effect of locus showed that the severity of downy mildew on leaves did not differ between susceptible [-/-] and [-/*Rpv3*] cultivars, whereas a significant difference was found between [*Rpv1*/-] and [*Rpv1*/*Rpv3*] (P<0.0001). There was also a

significant locus effect for defoliation (P<0.0001), with [-/*Rpv3*] presenting higher levels of defoliation than [-/-].

2. Percentage of leaves with oospores.

In the fall, oospores were found on the leaves of all genotypes, regardless of the year considered (Table 1). The percentage of leaves displaying oospores was highest for 'Merlot' and 38.

ANOVA revealed significant differences between locus classes (P=0.022): [-/*Rpv3*] had fewer leaves with oospores than [-/-], [*Rpv1*/-] and [*Rpv1*/*Rpv3*].

3. Number of oospores per leaf.

'Merlot' and [-/-] had the highest numbers of oospores per leaf, at more than 500. Both genotype and locus had significant effects (P<0.0001). 'Merlot' and 38 clearly produced more oospores than [*Rpv1*/-], [*Rpv1*/*Rpv3*] and [-/*Rpv3*]. We also found a significant effect of year on oospore production on leaves (P=0.042), with a higher frequency of sexual stages in 2008 than in 2009, a trend observed for all genotypes (P=0.185 for the genotype × year interaction).

Laboratory assessments

1. Macrosporangium germination.

Oospore germination was detected on 183 of the 600 leaf disks assessed (30.5%). ANOVA revealed no significant differences between genotypes (P=0.237). There was also no significant difference in oospore germination between loci (P=0.124).

The mean number of germinating macrosporangia per cm^2 of leaf area differed significantly between genotypes for the first test date for 2008 collection (P<0.001) and for both dates for 2009 collection (P=0.029 and P=0.002, respectively), but not for the second test date for 2008 collection (P=0.091). Germination levels in the second test for 2008 collection were lower than in any other test, and genotype 170 was generally the most infested overall.

We found no significant effect of locus on the number of macrosporangia (P=0.263). Genotype 170 was excluded from ANOVA analyzing the effect of time, due to outliers. We found no effect of year (P=0.684), but both test date within a year (repetition) (P<0.0001) and the year × repetition effect (P=0.009) were significant. Values were higher for the first test in a year than for the second.

2. Percentage of successful infections.

For material collected in 2008, infection tests with a subset of 37 disks were conducted (Table 2). For 2009, all 94 disks available were used for infection tests. On average, successful infections were obtained with 23.2% (±29.9%) of the disks, with no significant difference between years or repetitions. Infection rates were high for the resistant genotype 97, with a large number of primary infections. High levels of infection were also observed for the resistant genotype 170. Despite the variation displayed by genotypes, no statistical difference in the percentage of successful infections was found between genotypes (P=0.237) or loci (P=0.111).

Table 2. Macrosporangium counts and downy mildew infection tests for partially resistant grapevine genotypes at each date. Dates are given as MM/DD/YYYY. Letters, when present, represent statistical groupings of data defined by analysis of variance of the effects of genotype followed by pairwise differences in least-squares means at the 95% confidence level. na, Not available/not applicable.

Collection date	Germination test date	Loci	Genotype	Disks with macrosporangia (%)[1]	Mean number of macrosporangia cm^{-2} (SEM)	Number of disks tested in infection test	Disks with infection (%)
10/08/2008	04/06/2009	[-/-]	Merlot	33.3c	4.72 (2.26)	2	0
			38	6.7c	0.24 (0.24)	na	na
		[*Rpv1*/-]	3082-1-42	60.0b	12.5 (4.63)	3	0
			170	80.0a	51.4 (17.21)	4	75
		[-/*Rpv3*]	Regent	46.7c	5.19 (2.65)	3	0
			151	46.7c	6.13 (2.71)	2	50
		[*Rpv1*/*Rpv3*]	97	53.3c	7.55 (3.57)	4	50
			119	53.3c	3.30 (1.27)	2	50
			159	46.7c	4.72 (2.36)	na	na
			163	40.0c	2.83 (1.10)	2	0
10/08/2008	04/20/2009	[-/-]	Merlot	20.0	1.89 (1.03)	2	10
			38	6.7	0.24 (0.24)	1	0
		[*Rpv1*/-]	3082-1-42	13.3	0.71 (0.51)	2	50
			170	20.0	0.94 (0.54)	2	50
		[-/*Rpv3*]	Regent	0.0	na	na	na
			151	13.3	0.47 (0.32)	2	0
		[*Rpv1*/*Rpv3*]	97	6.7	0.24 (0.24)	1	0
			119	13.3	0.47 (0.32)	1	0
			159	33.3	2.12 (0.96)	4	25
			163	0.0	na	na	na
10/05/2009	03/30/2010	[-/-]	Merlot	60.0a	5.19 (1.67)	9	11.1
			38	13.3c	0.71 (0.51)	2	0
		[*Rpv1*/-]	3082-1-42	53.3a	5.66 (0.95)	8	0
			170	53.3a	6.37 (2.54)	8	25
		[-/*Rpv3*]	Regent	20.0c	1.18 (0.75)	3	0
			151	33.3abc	3.07 (0.54)	5	0
		[*Rpv1*/*Rpv3*]	97	53.3a	4.95 (1.46)	8	75
			119	40.0bc	1.65 (0.58)	6	0
			159	53.3a	6.13 (2.20)	8	0
			163	20.0abc	2.12 (1.65)	3	33.3

Table 2. Continued.

Collection date	Germination test date	Loci	Genotype	Disks with macrosporangia (%)[1]	Mean number of macrosporangia cm^{-2} (SEM)	Number of disks tested in infection test	Disks with infection (%)
10/05/2009	04/13/2010	[-/-]	Merlot	33.3ab	4.24 (1.70)	5	20
			38	0.0c	na	na	na
		[*Rpv1*/-]	3082-1-42	20.0b	1.65 (1.19)	3	66.7
			170	46.7ab	5.42 (2.41)	7	14.3
		[-/*Rpv3*]	Regent	33.3abc	2.83 (1.51)	5	0
			151	26.7bc	1.41 (0.76)	4	0
		[*Rpv1*/*Rpv3*]	97	46.7a	9.43 (4.84)	7	71.4
			119	0.0c	na	na	na
			159	20.0b	2.59 (1.56)	3	0
			163	0.0c	na	na	na

[1]Evaluated on 15 leaf disks per genotype.

DISCUSSION

This is the first quantitative study to our knowledge to address the question of the sexual reproduction of *P. viticola* on partially resistant grapevine cultivars. Under laboratory conditions, the *Rpv1* and *Rpv3* loci limit mycelium growth without totally inhibiting the sporulation of *P. viticola* (Merdinoglu et al., 2014). This finding was confirmed under natural conditions, and disease severity on both leaves and bunches was higher for *Rpv3* genotypes than for *Rpv1* genotypes (Calonnec et al., 2013). Regardless of the resistance locus considered, mycelia of compatible *P. viticola* isolates can, therefore, potentially settle on the leaves of resistant genotypes and cross at the end of the season to produce oospores. Our study confirmed the presence of sporulation and "mosaic" symptoms typical of sexual reproduction on aged leaves of these resistant genotypes. We also found that, regardless of the resistance locus considered (*Rpv1*, *Rpv3*), *P. viticola* produced oospores leading to the release of fertile macrosporangia the following year. *P. viticola* colonization may also be favored by a decrease in the efficacy of resistance, with the senescence of leaf tissues in the fall. However, this has never been demonstrated experimentally.

For genotypes with only the *Rpv3* resistance locus, the production of fertile oospores may be further facilitated by the emergence of pathogen strains able to overcome plant resistance. Indeed, *Rpv3* has been shown to act by effector-mediated immunity (ETI) (Casagrande et al., 2011), and the resistance conferred by this locus can be broken down by virulent *P. viticola* strains (Peressotti et al., 2010; Casagrande et al., 2011; Delmotte et al., 2014). The resistant genotypes studied had only recently been planted, but the evolution of virulence is not unlikely, because it has been shown that resistance can be broken down within 4 years of deployment (Delmotte et al., 2014). The rapid adaptation of *P. viticola* may be facilitated by its two-speed genome architecture, allowing pathogenicity factors to evolve more rapidly (Dussert et al., 2019).

Oospore germination and macrosporangium production were more limited on plants bearing the *Rpv3* locus than on plants bearing the *Rpv1* or *Rpv1*/*Rpv3* loci. This difference may be due to biochemical differences between the leaves of these cultivars. Calcium has been shown to play an important role in oospore germination (Vercesi et al., 1999), and calcium concentration in vine leaves is three times higher at the end than at the start of the season (Lanning, 1977). This chemical element is the main mediator of the immune response in plants (Boudsocq and Sheen, 2013). It is found in CDPK proteins, which are present in the genus *Vitis* (Kiselev et al., 2013). Differences in calcium concentration are observed between grape cultivars, particularly for some hybrids (Miklós et al., 2000). Studies of the biochemical composition and calcium content of the leaves of resistant cultivars should therefore be performed in the long term.

Our study also highlights the considerable differences in oospore germination rates between genotypes with the same combination of resistance loci. Similar results have already been reported for secondary downy mildew infestations on genotypes carrying the *Rpv1* or *Rpv3* loci (Calonnec et al., 2013) and in the descendants of a line carrying the *Rpv3* locus (Foria et al., 2018) under natural field conditions. For this line, considerable variability in the intensity of attacks on leaves was reported, and the level of resistance of genotypes carrying the *Rpv3* locus was found to decrease with the number of backcrosses with *V. vinifera*. The differences in germination rates within genotypes are, therefore, probably due to the presence/absence of minor additive genetic factors not detected by assessments of the genetic markers associated with resistance loci.

Resistant cultivars can limit the pathogen's ability to multiply during asexual cycles, but they do not prevent the sexual reproduction phase. In terms of epidemics, such partial resistance provides effective disease control during the season. From an evolutionary point of view, the small size of populations during asexual cycles reduces the chances of developing and fixing a favorable mutation, such as those breaking down resistance (virulence alleles) in populations. However, the sexual phase still provides opportunities for the recombination of favorable alleles in many genetic backgrounds or the formation of new combinations of virulence alleles. The durability of resistance is, therefore, a major issue in

the deployment of these resistant cultivars, and a new framework is required to monitor this aspect: the observatory for the deployment of disease-resistant grape cultivars. We need to monitor the deployment of cultivars to follow the evolution of downy mildew populations. An observatory-type system has already been set up in France (Delière et al., 2017; Guimier et al., 2019). This observatory is based on plots planted in production situations and allows the collection of epidemiological data in the vineyard and the constitution of a collection of downy mildew isolates. Vine-growers will also need to implement complementary epidemic management strategies, with the objective of limiting the size of downy mildew populations and preventing completion of the sexual cycle. These management strategies may include a limited number of fungicide treatments. Other levers can also be exploited, including the use of plant defense stimulators or the implementation of prophylactic measures (i.e., removal of senescent leaves).

ACKNOWLEDGMENTS

We would like to thank Dominique Forget (INRA Bordeaux) for technical assistance in the cultivation of the plants. We would like to thank Sabine Wiedemann-Merdinoglu (INRA Colmar) for her scientific contributions concerning the mechanisms of downy mildew resistance. We also thank the FREDON technicians, and the French Plant Protection Services in Villenave d'Ornon for sharing information about the dynamics of oospore germination.

Literature cited

Anonymous (2010). Bilan de Campagne 2010. Bulletin de Santé du Végétal Aquitaine – Viticulture, No. 22 (Bordeaux, France: Chambre Régionale d'Agriculture d'Aquitaine), pp.15.

Boudsocq, M., and Sheen, J. (2013). CDPKs in immune and stress signaling. Trends Plant Sci. *18* (*1*), 30–40 https://doi.org/10.1016/j.tplants.2012.08.008. PubMed

Bouquet, A. (1980). *Vitis* × *Muscadinia* hybridization: a new way in grape breeding for disease resistance in France. Paper presented at: 3rd International Symposium on Grape Breeding (Davis, CA, USA).

Bouquet, A. (1983). Contribution à l'étude de l'espèce *Muscadinia rotundifolia* (Michx) Small et de ses bybrides avec *Vitis vinifera* L. Applications en sélection. PhD thesis (Bordeaux, France: University of Bordeaux II).

Bouquet, A. (2000). Vers l'obtention de variètès de vigne résistantes a l'oïdium et au mildiou par les métohodes conventionnelles et biotechnologiques. Prog. Agric. Vitic. *117*, 383–389.

Bouquet, A. (2009). Réduction des intrants phytosanitaires : les variétées de vigne résistantes aux parasites. Vieille histoire ou nouvelles perspectives? Prog. Agric. Vitic. *126*, 234–243.

Burruano, S. (2000). The life-cycle of *Plasmopara viticola*, cause of downy mildew of vine. Mycologist *14* (*4*), 179–182 https://doi.org/10.1016/S0269-915X(00)80040-3.

Caffi, T., Rossi, V., and Bugiani, R. (2010). Evaluation of a warning system for controlling primary infections of grapevine downy mildew. Plant Dis. *94* (*6*), 709–716 https://doi.org/10.1094/PDIS-94-6-0709. PubMed

Calonnec, A., Delière, L., Cartolaro, P., Delmotte, F., Forget, D., Wiedemann-Merdinoglu, S., Merdinoglu, D., and Schneider, C. (2007). Evaluation of grapevine resistance to downy and powdery mildew in a population segregating for *run1* and *rpv1* resistance genes. Paper presented at: European Meeting of the IOBC/WPRS Working Group "Integrated Protection in Viticulture" "Biological and Integrated Control in Modern Viticulture (Marsala, Italy).

Calonnec, A., Wiedemann-Merdinoglu, S., Delière, L., Cartolaro, P., Schneider, C., and Delmotte, F. (2013). The reliability of leaf bioassays for predicting disease resistance on fruit: a case study on grapevine resistance to downy and powdery mildew. Plant Pathol. *62* (*3*), 533–544 https://doi.org/10.1111/j.1365-3059.2012.02667.x.

Casagrande, K., Falginella, L., Castellarin, S.D., Testolin, R., and Di Gaspero, G. (2011). Defence responses in Rpv3-dependent resistance to grapevine downy mildew. Planta *234* (*6*), 1097–1109 https://doi.org/10.1007/s00425-011-1461-5. PubMed

Chen, W.-J., Delmotte, F., Richard Cervera, S., Douence, L., Greif, C., and Corio-Costet, M.-F. (2007). At least two origins of fungicide resistance in grapevine downy mildew populations. Appl. Environ. Microbiol. *73* (*16*), 5162–5172 https://doi.org/10.1128/AEM.00507-07. PubMed

Delière, L., Cartolaro, P., Léger, B., and Naud, O. (2015). Field evaluation of an expertise-based formal decision system for fungicide management of grapevine downy and powdery mildews. Pest Manag. Sci. *71* (*9*), 1247–1257 https://doi.org/10.1002/ps.3917. PubMed

Delière, L., Schneider, C., Audeguin, L., Cunff, L.L., Cailliatte, R., Prado, E., Onimus, C., Demeaux, I., Guimier, S., and Fabre, F. (2017). Cépages résistants: la vigne contre-attaque! Phytoma *708*, 34–37.

Delmas, C.E.L., Fabre, F., Jolivet, J., Mazet, I.D., Richart Cervera, S., Delière, L., and Delmotte, F. (2016). Adaptation of a plant pathogen to partial host resistance: selection for greater aggressiveness in grapevine downy mildew. Evol. Appl. *9* (*5*), 709–725 https://doi.org/10.1111/eva.12368. PubMed

Delmas, C.E.L., Dussert, Y., Delière, L., Couture, C., Mazet, I.D., Richart Cervera, S., and Delmotte, F. (2017). Soft selective sweeps in fungicide resistance evolution: recurrent mutations without fitness costs in grapevine downy mildew. Mol. Ecol. *26* (*7*), 1936–1951 https://doi.org/10.1111/mec.14006. PubMed

Delmotte, F., Mestre, P., Schneider, C., Kassemeyer, H.-H., Kozma, P., Richart-Cervera, S., Rouxel, M., and Delière, L. (2014). Rapid and multiregional adaptation to host partial resistance in a plant pathogenic oomycete: evidence from European populations of *Plasmopara viticola*, the causal agent of grapevine downy mildew. Infect. Genet. Evol. *27*, 500–508 https://doi.org/10.1016/j.meegid.2013.10.017. PubMed

Dussert, Y., Mazet, I.D., Couture, C., Gouzy, J., Piron, M.-C., Kuchly, C., Bouchez, O., Rispe, C., Mestre, P., and Delmotte, F. (2019). A high-quality grapevine downy mildew genome assembly reveals rapidly evolving and lineage-specific putative host adaptation genes. Genome Biol. Evol. *11* (*3*), 954–969 https://doi.org/10.1093/gbe/evz048. PubMed

Fermaud, M., Smits, N., Merot, A., Roudet, J., Thiéry, D., Wery, J., and Delbac, L. (2016). New multipest damage indicator to assess protection strategies in grapevine cropping systems: an indicator of multipest damage in grapevine. Aust. J. Grape Wine Res. *22* (*3*), 450–461 https://doi.org/10.1111/ajgw.12238.

Fischer, B.M., Salakhutdinov, I., Akkurt, M., Eibach, R., Edwards, K.J., Töpfer, R., and Zyprian, E.M. (2004). Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. Theor. Appl. Genet. *108* (*3*), 501–515 https://doi.org/10.1007/s00122-003-1445-3. PubMed

Fontaine, M.C., Austerlitz, F., Giraud, T., Labbé, F., Papura, D., Richard-Cervera, S., and Delmotte, F. (2013). Genetic signature of a range expansion and leap-frog event after the recent invasion of Europe by the grapevine downy mildew pathogen *Plasmopara viticola.* Mol. Ecol. *22* (*10*), 2771–2786 https://doi.org/10.1111/mec.12293. PubMed

Foria, S., Magris, G., Morgante, M., and Di Gaspero, G. (2018). The genetic background modulates the intensity of Rpv3-dependent downy mildew resistance in grapevine. Plant Breed. *137* (*2*), 220–228 https://doi.org/10.1111/pbr.12564.

Galet, P. (1977). Les Maladies et les Parasites de la Vigne, Vol. 1 (Montpellier, France: Paysan du Midi), pp.1876.

Gessler, C., Pertot, I., and Perazzolli, M. (2011). *Plasmopara viticola*: a review of knowledge on downy mildew of grapevine and effective disease management. Phytopathol. Mediterr. *50*, 3–44.

Gobbin, D., Jermini, M., Loskill, B., Pertot, I., Raynal, M., and Gessler, C. (2005). Importance of secondary inoculum of *Plasmopara viticola* to epidemics of grapevine downy mildew. Plant Pathol. *54* (*4*), 522–534 https://doi.org/10.1111/j.1365-3059.2005.01208.x.

Gobbin, D., Bleyer, G., Keil, S., Kassemeyer, H.H., and Gessler, C. (2007). Evidence for sporangial dispersal leading to a single infection event and a sudden high incidence of grapevine downy mildew. Plant Pathol. *56* (*5*), 843–847 https://doi.org/10.1111/j.1365-3059.2007.01651.x.

Gómez-Zeledón, J., Zipper, R., and Spring, O. (2013). Assessment of phenotypic diversity of *Plasmopara viticola* on *Vitis* genotypes with different resistance. Crop Prot. *54*, 221–228 https://doi.org/10.1016/j.cropro.2013.08.015.

Grosman, J., Magnien, C., and Trespaille-Barrau, J.-M. (2008). Bilan phytosanitaire de la vigne en 2008. Phytoma *621*, 10–15.

Guimier, S., Petitgenet, M., Fabre, F., Demeaux, I., Delmotte, F., Goutouly, J.-P., and Delière, L. (2018). Disease-resistant grapevine cultivars drastically reduce fungicides use: results of a five years multicriteria evaluation of two low-fungicide input cropping systems. Paper presented at: XII International Conference on Grapevine Breeding and Genetics (Bordeaux, France: ISHS).

Guimier, S., Delmotte, F., Miclot, A.S., Fabre, F., Mazet, I., Couture, C., Schneider, C., and Delière, L. (2019). OSCAR, a national observatory to support the durable deployment of disease-resistant grapevine cultivars. Acta Hortic. *XXXX*, 21–34.

Kiselev, K.V., Dubrovina, A.S., Shumakova, O.A., Karetin, Y.A., and Manyakhin, A.Y. (2013). Structure and expression profiling of a novel calcium-dependent protein kinase gene, CDPK3a, in leaves, stems, grapes, and cell cultures of wild-growing grapevine *Vitis amurensis* Rupr. Plant Cell Rep. *32* (*3*), 431–442 https://doi.org/10.1007/s00299-012-1375-0. PubMed

Lafon, R., and Bulit, J. (1981). Downy mildew of the vine. In The Downy Mildews, D.M. Spencer, ed. (London, UK: Academic Press), p.600–614.

Lafon, R., and Clerjeau, M. (1988). Downy mildew. In Compendium of Grape Diseases, H.M. Pearson and A.C. Goheen, eds. (St Paul, MN, USA: APS Press), p.11–13.

Lanning, F.C. (1977). Ash, silicon, calcium and copper in “Concord” grape leaves. HortScience *12*, 121–122.

Luis, P., Gauthier, A., Trouvelot, S., Poinssot, B., and Frettinger, P. (2013). Identification of *Plasmopara viticola* genes potentially involved in pathogenesis on grapevine suggests new similarities between oomycetes and true fungi. Phytopathology *103* (*10*), 1035–1044 https://doi.org/10.1094/PHYTO-06-12-0121-R. PubMed

Merdinoglu, D., and Caranta, C. (2013). Quel déploiement de variétés de vigne résistantes au mildiou et à l’oïdium. In Les Cépages Résistants aux Maladies Cryptogamiques: Panorama Européen (Latte, France: Groupe ICV) p.54–59.

Merdinoglu, D., Wiedemann-Merdinoglu, S., Coste, P., Dumas, V., Haetty, S., Butterlin, G., Greif, C., Adam-Blondon, A.-F., Bouquet, A., and Pauquet, J. (2003). Genetic analysis of downy mildew resistance derived from *Muscadinia rotundifolia.* Acta Hortic. *603*, 451–456 https://doi.org/10.17660/ActaHortic.2003.603.57.

Merdinoglu, D., Wiedemann-Merdinoglu, S., Mestre, P., Prado, E., and Schneider, C. (2009). Apport de l’innovation variétale dans la réduction des intrants phytosanitaires au vignoble: exemple de la résistance au mildiou et à l’oïdium. Prog. Agric. Vitic. *126*, 290–293.

Merdinoglu, D., Blasi, P., Wiedemann-Merdinoglu, S., Mestre, P., Peressotti, E., Poutaraud, A., Prado, E., and Schneider, C. (2014). Breeding for durable resistance to downy and powdery mildew in grapevine. Acta Hortic. *1046*, 65–72 https://doi.org/10.17660/ActaHortic.2014.1046.6.

Miklós, E., Szõke, L., Kozma, P., and Erdei, L. (2000). Differences in cell wall binding capacity of grapevine cultivars. Acta Hortic. *526*, 183–186 https://doi.org/10.17660/ActaHortic.2000.526.16.

Millardet, A. (1881). Notes sur les Vignes Américaines et Opuscules Divers sur le Même Sujet (Bordeaux, France: Chez Feret & Fils), pp.62.

Nelson, R., Wiesner-Hanks, T., Wisser, R., and Balint-Kurti, P. (2018). Navigating complexity to breed disease-resistant crops. Nat. Rev. Genet. *19* (*1*), 21–33 https://doi.org/10.1038/nrg.2017.82. PubMed

Peressotti, E., Wiedemann-Merdinoglu, S., Delmotte, F., Bellin, D., Di Gaspero, G., Testolin, R., Merdinoglu, D., and Mestre, P. (2010). Breakdown of resistance to grapevine downy mildew upon limited deployment of a resistant variety. BMC Plant Biol. *10* (*1*), 147 https://doi.org/10.1186/1471-2229-10-147. PubMed

Pertot, I., and Zulini, L. (2003). Studies on *Plasmopara viticola* oospore germination in Trentino, Italy. IOBC-WPRS Bull. *26* (*8*), 43–46.

Pons, A., Mouakka, N., Deliere, L., Crachereau, J.C., Davidou, L., Sauris, P., Guilbault, P., and Darriet, P. (2018). Impact of *Plasmopara viticola* infection of Merlot and Cabernet Sauvignon grapes on wine composition and flavor. Food Chem. *239*, 102–110 https://doi.org/10.1016/j.foodchem.2017.06.087. PubMed

Ronzon-Tran Manh Sung, C., and Clerjeau, M. (1988). Techniques for formation, maturation and germination of *Plasmopara viticola* oospores under control conditions. Plant Dis. *72* (*11*), 938–941 https://doi.org/10.1094/PD-72-0938.

Rossi, V., and Caffi, T. (2012). The role of rain in dispersal of the primary inoculum of *Plasmopara viticola.* Phytopathology *102* (*2*), 158–165 https://doi.org/10.1094/PHYTO-08-11-0223. PubMed

Rouzet, J., and Jacquin, D. (2003). Development of overwintering oospores of *Plasmopara viticola* and severity of primary foci in relation to climate. Bull. OEPP *33* (*3*), 437–442 https://doi.org/10.1111/j.1365-2338.2003.00670.x.

Savary, S., Delbac, L., Rochas, A., Taisant, G., and Willocquet, L. (2009). Analysis of nonlinear relationships in dual epidemics, and its application to the management of grapevine downy and powdery mildews. Phytopathology *99* (*8*), 930–942 https://doi.org/10.1094/PHYTO-99-8-0930. PubMed

Schneider, C., Onimus, C., Prado, E., Dumas, V., Wiedemann-Merdinoglu, S., Dorne, M.A., Lacombe, M.C., Piron, M.C., Umar-Faruk, A., Duchêne, E., et al. (2019). INRA-ResDur: the French grapevine breeding programme for durable resistance to downy and powdery mildew. Acta Hortic. *XXXX*, 207–214.

Southwood, T.R.E. (1978). Ecological Methods – With Particular Reference to the Study of Insect Populations, 2nd edn (Dordrecht, The Netherlands: Springer), pp.548.

Tran Manh Sung, C., Strizyk, S., and Clerjeau, M. (1990). Simulation of the date of maturity of *Plasmopara viticola* oospores to predict the severity of primary infections in grapevine. Plant Dis. *74* (*2*), 120–124 https://doi.org/10.1094/PD-74-0120.

Vercesi, A., Tornaghi, R., Sant, S., Burruano, S., and Faoro, F. (1999). A cytological and ultrastructural study on the maturation and germination of oospores of *Plasmopara viticola* from overwintering vine leaves. Mycol. Res. *103* (*2*), 193–202 https://doi.org/10.1017/S095375629800700X.

Viennot-Bourgin, G. (1949). Les Champignons Parasites de Plantes Cultivées (Paris, France: Masson et Cie.), pp.1850.

Wong, F.P., Burr, H.N., and Wilcox, W.F. (2001). Heterothallism in *Plasmopara viticola.* Plant Pathol. *50* (*4*), 427–432 https://doi.org/10.1046/j.1365-3059.2001.00573.x.

Yin, L., An, Y., Qu, J., Li, X., Zhang, Y., Dry, I., Wu, H., and Lu, J. (2017). Genome sequence of *Plasmopara viticola* and insight into the pathogenic mechanism. Sci. Rep. *7* (*1*), 46553 https://doi.org/10.1038/srep46553. PubMed