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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

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ABSTRACT

Crop pathogens evolve rapidly to adapt to their hosts. The use of crops with quantitative disease resistance is expected to alter selection of pathogen life-history traits. This may result in differential adaptation of the pathogen to host cultivars and, sometimes, to the erosion of quantitative resistance. Here, we assessed the level of host adaptation in an oomycete plant pathogenic species. We analysed the phenotypic and genetic variability of 17 *Plasmopara viticola* isolates collected on *Vitis vinifera* and 35 isolates from partially resistant varieties (Regent and genotypes carrying the *Rpv1* gene). Cross-inoculation experiments assessed two components of aggressiveness and a life-history trait of the pathogen: disease severity, sporangial production and sporangia size. The results contribute evidence to the emergence of *P. viticola* aggressive isolates presenting a high level of sporulation on the partially resistant Regent. By contrast, no adaptation to the *Rpv1* gene was found in this study. The erosion of Regent resistance may have occurred in less than 5 years and at least three times independently in three distant wine-producing areas. Populations from resistant varieties showed a significant increase in sporangia production capacity, indicating an absence of fitness costs for this adaptation. The increase in the number of sporangia was correlated with a reduction in sporangia size, a result which illustrates how partial plant disease resistance can impact selection of the pathogen's life-history traits. This case study on grapevine downy mildew shows how new plant pathogen populations emerge in agro-ecosystems by adapting to partial host resistance. This adaptive pattern highlights the need for wise management of plant partial disease resistance to ensure its sustainability over time.

1. Introduction

Plants and pathogens evolve in response to each other. In the host–parasite co-evolutionary arms race, it has been argued that parasites have an advantage because they evolve faster than hosts on account of shorter generation times and higher mobility (Hamilton et al., 1990). Confirming this view, local adaptation of parasites has been found to occur in most of the wild pathosystems that have been investigated (Kaltz and Shykoff, 1998). This is all the more true in agro-ecosystems where the high densities and the genetic homogeneity of hosts resulting from human-guided selection impose strong directional selection on pathogen populations (Stukenbrock and McDonald, 2008; Thrall et al., 2010). In many crops, the use of resistant cultivars to control crop diseases further increased the selection pressure on targeted pathogen populations, often leading to the breakdown or erosion of plant resistances (Pariaud et al., 2009; Parlevliet, 2002). Indeed, breeding for disease resistance during the 20th century has recurrently led to the rapid emergence of new virulence profiles in fungal plant pathogens able to overcome newly deployed crop resistance (Ahmed et al., 2012; Johnson, 1961).

Two categories of disease resistance have long been recognised in plants, e.g. qualitative and quantitative resistance. Qualitative resistance is based on gene-for-gene interactions often associated with a hypersensitive response of the host (Flor, 1971). By contrast,

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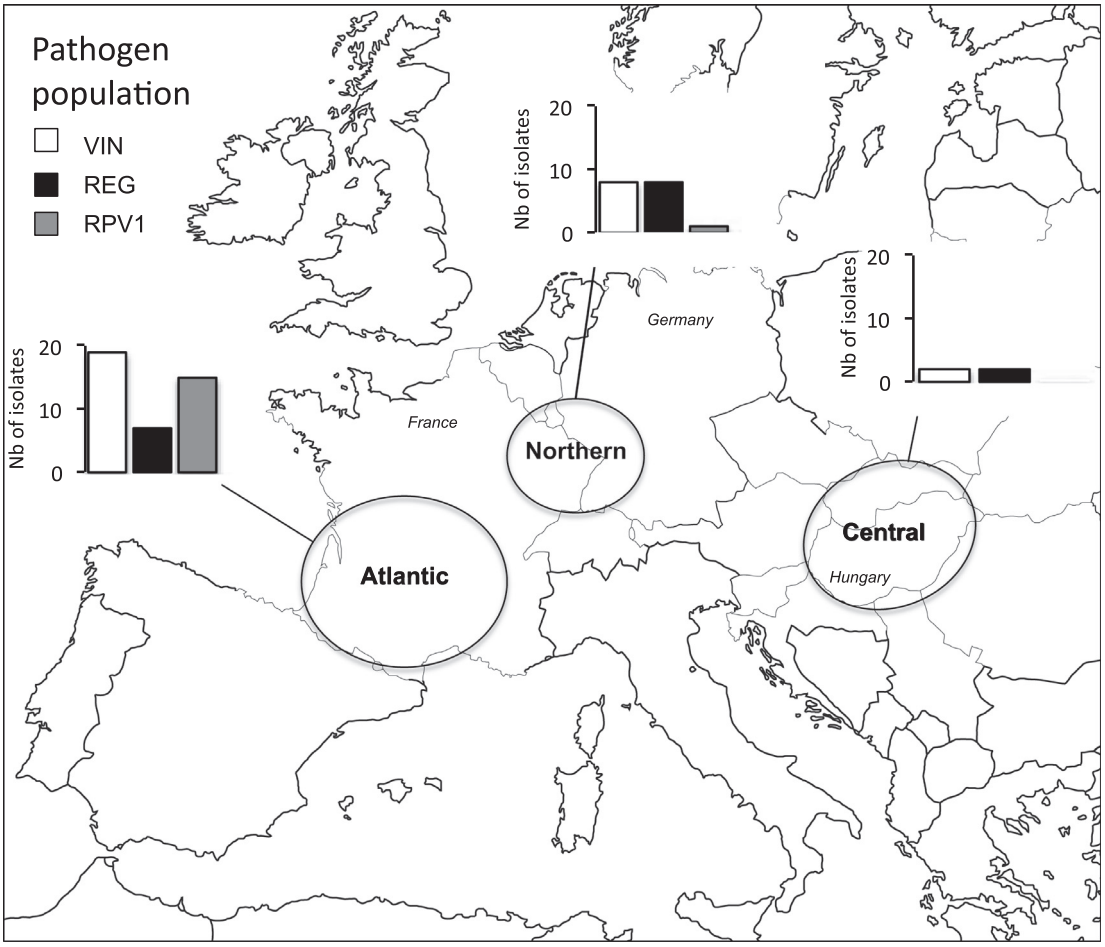

Fig. 1. Geographical origin and source host plants of *P. viticola* isolates used in this study.

quantitative resistance is usually controlled by multiple genetic factors and leads to a reduction in symptom severity (Poland et al., 2009). Quantitative resistance allows the pathogen to infect and multiply, but it limits the pathogen's development, growth and reproduction (Parlevliet, 1978). Recent studies have demonstrated that quantitative resistance is able to significantly increase the sustainability of a combination of qualitative and quantitative resistance (Brun et al., 2010; Palloix et al., 2009). While the role of gene-for-gene interactions in shaping the genetic and phenotypic structure of pathogens in crop systems is well known, the impact of partial resistance on the evolution of quantitative traits of the pathogen is much less documented. The use of crops showing partial resistance to fungal diseases is indeed expected to exert selection pressure on life-history traits and modify the adaptive strategy of the pathogens. Confirming this viewpoint, plant pathogens have been shown to undergo differential adaptation to host cultivars, sometimes leading to erosion of partial resistance (Andrivon et al., 2007; Krenz et al., 2008; Pariaud et al., 2009).

Plasmopara viticola, the causal agent of grapevine downy mildew, is an obligate biotrophic oomycete that attacks *Vitis vinifera* (Viennot-Bourgin, 1949). This pathogen was first introduced into European vineyards from North America in the 1870s (Millardet, 1881) before spreading to all major grape-producing regions of the world (Galet, 1977; Gessler et al., 2011). The Eurasian wine grape *V. vinifera* is sensitive to downy mildew and genetic resistance has to be introgressed from American and Asiatic *Vitis* spp. In Europe, conventional breeding programs for resistance to grapevine downy mildew have resulted in the creation of several partially resistant varieties that are currently grown on limited acreages. Grapevine downy mildew is thus a prime candidate for studying pathogen adaptation to partial host plant resistance because the main cultivated grape (*V. vinifera*) is susceptible and resistant varieties resulting from breeding are yet to be deployed on a large geographical scale. This particular situation creates a unique opportunity to monitor the evolution of pathogen populations responding to this new host–plant selective pressure. *P. viticola* is known to have a high evolutionary potential, as proven by the appearance of fungicide resistance (Blum et al., 2010; Chen et al., 2007) and the report of a breakdown of resistance for the cv. Bianca despite its limited deployment (Peressotti et al., 2010). It is therefore essential to determine to what extent populations of *P. viticola* are being selected by these new grapevine cultivars showing different levels of resistance. This is particularly important for woody species such as grapevine because the cultivated varieties are planted for decades.

In this study, we combined phenotypic and genetic data to assess the level of adaptation of *P. viticola* to partially resistant grapevine varieties. We have addressed this question by determining (i) whether populations of *P. viticola* infecting resistant varieties have adapted to their hosts and (ii) whether the deployment of resistance can modify the genetic architecture of *P. viticola* populations.

2. Material and methods

2.1. Plant material

The plant genotypes used in this study were Regent, Mtp3082-1-42 and *V. vinifera* cv. Cabernet Sauvignon. Regent is a commercial

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grape cultivar that was created in 1967 at the Geilweilerhof Institute and that has been deployed in Germany over the last 20 years. Regent is an offspring of cv. Chambourcin and cv. Diana carrying different resistance factors to downy mildew (Fisher et al., 2004). It has been suggested that the main gene for Regent is *Rpv3*, which has been described in cv. Bianca and causes partial resistance to downy mildew but which has been overcome by new aggressive isolates (Di Gaspero et al., 2012; Peressotti et al., 2010). Mtp3082-1-42 is an INRA selection derived from a cross between *Muscadinia rotundifolia* and *V. vinifera* followed by four backcrosses with *V. vinifera* (Bouquet et al., 2000). It carries the *Rpv1* gene that confers partial resistance to downy mildew (Merdinoglu et al., 2003). Cabernet Sauvignon is a *V. vinifera* cultivar grown worldwide and known for its susceptibility to downy mildew.

For each cultivar, 1-year-old woody canes were collected in 2009 in Bordeaux experimental vineyards and cut into one-bud

Table 1
Characteristics of the *Plasmopara viticola* isolates used in the cross-inoculation experiments. Experiment 1: VIN-REG comparison; Experiment 2: VIN-RPV1 comparison.

Isolate	Experiment	Population name	Source host	Location	Wine-producing area	Country of origin	Year
330	1	REG	Regent	Pfaffenweiler	Northern	Germany	2010
331	1	REG	Regent	Pfaffenweiler	Northern	Germany	2010
332	1	REG	Regent	Pfaffenweiler	Northern	Germany	2010
91A	1	REG	Regent	Pfaffenweiler	Northern	Germany	2008
124	1	REG	Regent	Pècs	Central	Hungary	2008
125	1	REG	Regent	Pècs	Central	Hungary	2008
115	1	REG	Regent	Colmar	Northern	France	2008
293	1	REG	Regent	Colmar	Northern	France	2009
294	1	REG	Regent	Colmar	Northern	France	2009
295A	1	REG	Regent	Colmar	Northern	France	2009
13	1	REG	Regent	Latresne	Atlantic	France	2008
272	1	REG	Regent	Latresne	Atlantic	France	2009
273	1	REG	Regent	Latresne	Atlantic	France	2009
274	1	REG	Regent	Latresne	Atlantic	France	2009
276	1	REG	Regent	Latresne	Atlantic	France	2009
278	1	REG	Regent	Latresne	Atlantic	France	2009
280	1	REG	Regent	Latresne	Atlantic	France	2009
321	1	VIN	*V. vinifera*	Krör	Northern	Germany	NA
334	1	VIN	*V. vinifera* cv. Chassselas	Ehrenkirchen	Northern	Germany	2010
335	1	VIN	*V. vinifera* cv. Chassselas	Ehrenkirchen	Northern	Germany	2010
328	1	VIN	*V. vinifera* cv. Muller Thurgau	Freiburg	Northern	Germany	2010
340	1	VIN	*V. vinifera* cv. Furmint	Tolcsva	Central	Hungary	2010
336	1	VIN	*V. vinifera* cv. Kefrankos	Eger	Central	Hungary	2010
209	1	VIN	*V. vinifera* cv. Cabernet Franc	Monsegur	Atlantic	France	2009
256	1	VIN	*V. vinifera* cv. Cabernet Sauvignon	Ludon	Atlantic	France	2009
113	1	VIN	*V. vinifera* cv. Gamay	Villefranche-sur-Saône	Northern	France	2008
327	1	VIN	*V. vinifera* cv. Pinot Noir	Rouffach	Northern	France	2010
326	1	VIN	*V. vinifera* cv. Riesling	Guebwiller	Northern	France	2010
245	1	VIN	*V. vinifera* cv. Sauvignon Blanc	Blanquefort	Atlantic	France	2009
241	1; 2	VIN	*V. vinifera* cv Merlot	Parempuyre	Atlantic	France	2009
257	1; 2	VIN	*V. vinifera* cv Muscadelle	Listrac	Atlantic	France	2009
243	1; 2	VIN	*V. vinifera* cv. Merlot	Pessac	Atlantic	France	2009
258	1; 2	VIN	*V. vinifera* cv. Petit Verdot	Margaux	Atlantic	France	2009
222	1; 2	VIN	*V. vinifera* cv. Sauvignon Blanc	Blanquefort	Atlantic	France	2009
216	2	VIN	*V. vinifera*	Talence	Atlantic	France	2009
217	2	VIN	*V. vinifera*	Virelade	Atlantic	France	2009
208	2	VIN	*V. vinifera* cv. Cabernet Sauvignon	Pujols sur Ciron	Atlantic	France	2009
319	2	VIN	*V. vinifera* cv. Chardonnay	Beaune	Northern	France	NA
250	2	VIN	*V. vinifera* cv. Chasselas	Arveyres	Atlantic	France	2009
261	2	VIN	*V. vinifera* cv. Gamay	Villefranche de Lonchat	Atlantic	France	2009
218	2	VIN	*V. vinifera* cv. Merlot	Cadaujac	Atlantic	France	2009
210	2	VIN	*V. vinifera* cv. Merlot	Mauriac	Atlantic	France	2009
215	2	VIN	*V. vinifera* cv. Merlot	Montagne	Atlantic	France	2009
221	2	VIN	*V. vinifera* cv. Sauvignon Blanc	Blanquefort	Atlantic	France	2009
207	2	VIN	*V. vinifera* cv. Semillon	Pujols sur Ciron	Atlantic	France	2009
239	2	VIN	*V. vinifera* cv. Ugni Blanc	Parlebosq	Atlantic	France	2009
317	2	RPV1	Mtp3082-1-42	Latresne	Atlantic	France	2009
B11	2	RPV1	Mtp3159	Carcassonne	Atlantic	France	2008
B6	2	RPV1	INRA-JKI co-obtention	Colmar	Northern	France	2008
B10	2	RPV1	Mtp3082-1-49	Piolenc	Atlantic	France	2008
B1	2	RPV1	INRA-JKI co-obtention	Latresne	Atlantic	France	2008
B2	2	RPV1	INRA-JKI co-obtention	Latresne	Atlantic	France	2008
B3	2	RPV1	INRA-JKI co-obtention	Latresne	Atlantic	France	2008
B9	2	RPV1	INRA-JKI co-obtention	Latresne	Atlantic	France	2008
231	2	RPV1	INRA-JKI co-obtention	Latresne	Atlantic	France	2009
230	2	RPV1	INRA-JKI co-obtention	Latresne	Atlantic	France	2009
309	2	RPV1	INRA-JKI co-obtention	Latresne	Atlantic	France	2009
312	2	RPV1	INRA-JKI co-obtention	Latresne	Atlantic	France	2009
236	2	RPV1	INRA-JKI co-obtention	Latresne	Atlantic	France	2009
235	2	RPV1	INRA-JKI co-obtention	Latresne	Atlantic	France	2009
308	2	RPV1	INRA-JKI co-obtention	Latresne	Atlantic	France	2009
307	2	RPV1	INRA-JKI co-obtention	Latresne	Atlantic	France	2009

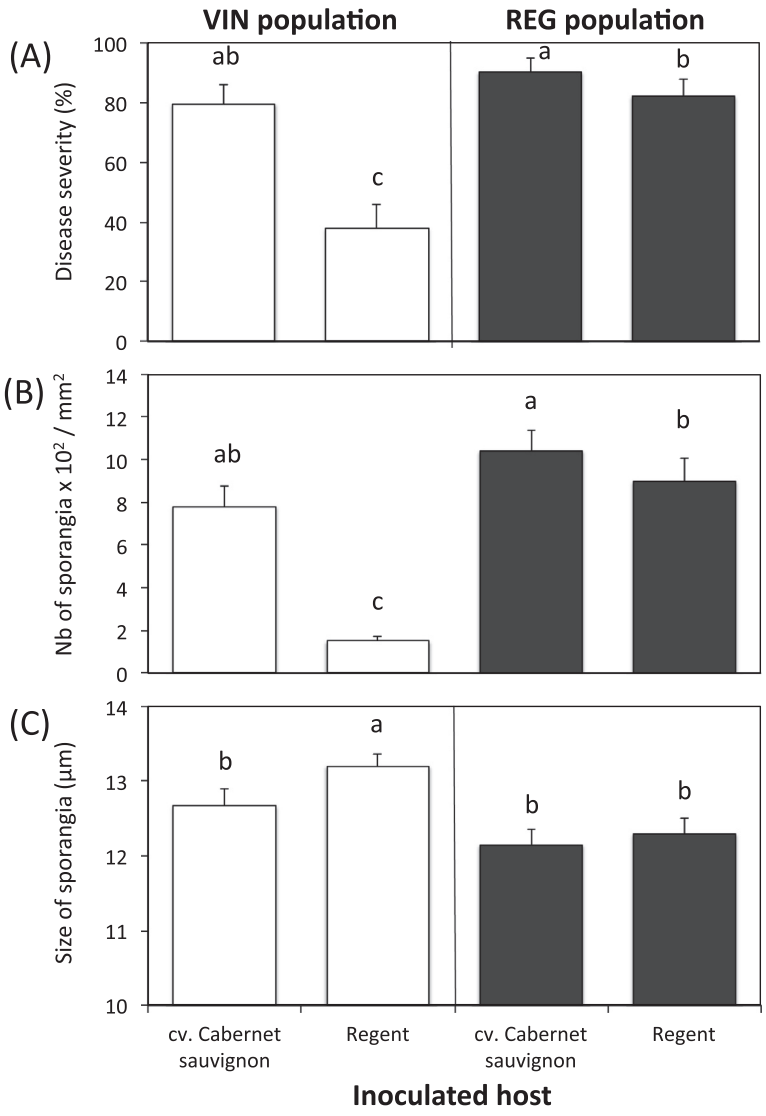

Fig. 2. Comparison of VIN and REG – Aggressiveness components and life-history traits for VIN and REG *P. viticola* populations. (A) Disease severity, (B) sporangia production, (C) mean size of sporangia.

Table 2
Analysis of variance showing the effect of source hosts (REG, VIN) on severity, sporulation and size of sporangia of 34 *P. viticola* isolates.

Source	Disease severity			Sporulation		Size of sporangia	
	DF	F-ratio	*P*-value	F-ratio	*P*-value	F-ratio	*P*-value
Pathogen population	1	34.1493	<0.0001	55.27	<0.0001	13.1343	0.0011
Inoculated host	1	225.3041	<0.0001	471.90	<0.0001	50.9177	<0.0001
Pathogen population*inoculated host	1	81.8952	<0.0001	278.57	<0.0001	15.8892	<0.0001
Origin	2	0.307	0.7379	0.3835	0.6847	1.6414	0.2106

Table 3
Analysis of variance showing the effect of source hosts (RPV1, VIN) on severity, sporulation, size of sporangia of 33 *P. viticola* isolates.

Source	Disease severity			Sporulation		Size of sporangia	
	DF	F-ratio	*P*-value	F-ratio	*P*-value	F-ratio	*P*-value
Pathogen population	1	0.1283	0.7226	0.7749	0.3855	5.0795	0.0314
Inoculated host	1	417.261	<0.0001	1132.144	<0.0001	5.1694	0.0233
Pathogen population*Inoculated host	1	8.8089	0.0031	2.487	0.1153	21.8956	<0.0001

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cuttings. Plants from cuttings were grown in a greenhouse with day/night natural photoperiod. For each experiment, all plants were grown simultaneously in the same climatic conditions. For each cultivar, leaves three and four below the apex of young shoots were taken from the plants at the ten-unfolded-leaf stage. Leaves were rinsed with distilled water. Leaf discs 18 mm in diameter were excised using a cork borer.

2.2. P. viticola isolates

A total of 62 *P. viticola* isolates were collected in vineyards between 2008 and 2010 in different geographic regions in Europe (Fig. 1). Details on the isolates used in the study are given in Table 1. Briefly, each isolate field sample consisted of a single sporulating lesion (oil spot) from which sporangia were collected and resuspended in water. Isolates were propagated on detached leaves of glasshouse-grown *V. vinifera* cv. Cabernet Sauvignon plants. Sporulating lesions of the propagated isolates were stored at −20 °C and further used for cross-inoculation experiments. A first set of isolates consisted of 17 isolates collected on *V. vinifera* (VIN population) and 17 isolates collected on Regent (REG population). These isolates came from three geographic areas where Regent had been planted either in commercial vineyards or for experimental purposes: a central-European wine-growing area (Pecs, Eger and Tolcsva vineyards, Hungary), a northern area (Alsace, Baden and Mosel vineyards in France and Germany) and an Atlantic area (Bordeaux, France). A second set of isolates consisted of 17 isolates collected on *V. vinifera* (VIN population) and 16 isolates from different offsprings carrying the *Rpv1* gene (INRA-JKI co-obtentions; Schneider and Prado, 2012, Personal Communication) (RPV1 population), collected in the Atlantic and Northern areas of France. Detailed information about each isolate and their geographical origin is presented in Table 1 and Fig. 1.

2.3. Cross-inoculation tests

Two cross-inoculation experiments were conducted in 2010. In the first experiment (VIN/REG comparison), a set of *P. viticola* isolates was inoculated onto both Regent and cv. Cabernet Sauvignon; in a second experiment (VIN/RPV1 comparison), a different set of isolates was inoculated on Cabernet Sauvignon cv. and Mtp3082-1-42 (*Rpv1*). Inocula for these experiments were obtained by propagating the 62 *P. viticola* isolates on detached leaves of glasshouse-grown *V. vinifera* cv. Cabernet Sauvignon plants. Sporangia resulting from the sporulation 7 days after inoculation were collected using a sterile brush and resuspended into sterile water. For each isolate, the sporangia concentration was adjusted to 5×10^3 sporangia/ml using a particle counter, and 25 ml of the sporangia suspension was transferred into one Petri dish for each cultivar. Leaf disks were randomised among treatments. Inoculations were performed by floating leaf disks at the surface of the sporangia suspension, adaxial side up, for 4 h at 20 °C.

Inoculated leaf disks were placed abaxial side up in 11 square Petri dishes (23 × 23 cm) containing moistened filter paper. Each Petri dish included all isolate cultivar combinations, thus constituting one replicate of the experiment.

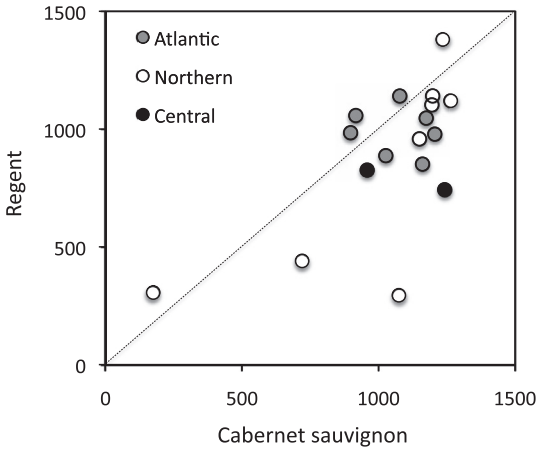

Fig. 3. Sporulation (number of sporangia/mm^2) of individual REG isolates from the three geographic areas (Atlantic, central, northern) when inoculated on Regent and cv. Cabernet Sauvignon. Each circle represents an individual isolate.

Petri dishes were sealed with Parafilm and placed in growth chambers at 22 °C with a 12-h photoperiod for 7 days. Ten replicates were used to measure the aggressiveness and one for the molecular characterisation of isolates.

2.4. Measurement of aggressiveness and life-history traits

Disease severity (hereafter called severity), sporangial production (called sporulation) and sporangia size were assessed for each leaf disk at 7 days post-inoculation (dpi). Severity corresponds to the disc area covered by sporulation and was visually assessed with a dissecting microscope at × 10 magnification. The number and size of sporangia was assessed using a particle counter (Coulter Counter® Multisizer™ 3; Beckman Coulter). Briefly, sporulating disks were placed in a vial with 20 mL Isoton with one drop of non-ionic dispersant (Nacconol 90F) and shaken. For each leaf disc, the number of sporangia was assessed by counting the number of particles between 8 and 20 µm in diameter in a 500-µl sample, and sporangia size was calculated as the weighted average of the sporangia size distribution.

2.5. Data analysis

The two cross-inoculation experiments were analysed separately. For the analysis, data for sporulation were log transformed to improve homogeneity of variance. Analyses of variance were conducted using JMP 9 software (SAS Institute Inc., Cary, NC, USA). For each experiment, we used a mixed-model with 'host source', 'inoculated host' and 'geographical origin' as fixed effects and 'isolates' as the random effect. Means were compared with Tukey–Kramer honestly significant differences (HSD). We used simple linear regression to analyse the relationships between number and size of sporangia in the two experiments.

Table 4
Mean and standard deviation of aggressiveness components and life-history traits for the two comparisons of *P. viticola* populations (VIN/REG, VIN/RPV1) on the susceptible cv. Cabernet Sauvignon. The *P*-value results from an analysis of variance with pathogen population as fixed effect and isolates as random effect.

Aggressiveness components and life history traits	VIN-REG comparison			VIN-RPV1 comparison		
	VIN population	REG population	*P*-value	VIN population	RPV1 population	*P*-value
Disease severity	79.4 ± 3.3	89.9 ± 3.5	0.0182	64.2 ± 5.2	71.5 ± 4.9	0.2503
Sporulation	780 ± 53	1031 ± 65	0.0336	918 ± 71	1097 ± 76	0.1
Size of sporangia	12.7 ± 0.13	12.1 ± 0.18	0.0261	12.4 ± 0.19	11.7 ± 0.2	0.0088

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For genetic data, 20 microsatellite markers were used to study the genetic relationship between the 62 isolates used in this study and two additional isolates of American (Michigan, USA) origin used as the outgroup. DNA extractions were performed, as described by Delmotte et al. (2006), on one infected Cabernet Sauvignon leaf disk per isolate. Following the protocol reported by Delmotte et al. (2006), Gobbin et al. (2003) and Rouxel et al. (2012), pathogen isolates were genotyped at the following 20 microsatellite loci: ISA, Pv7, Pv14, Pv13, Pv16, Pv17, Pv31, Pv39, Pv61, Pv65, Pv67, Pv76, Pv91, Pv93, Pv100, Pv101, Pv103, Pv137, Pv138, Pv139, Pv140, Pv143, Pv144 and Pv147. PCR amplifications were carried out in a 15-μL reaction volume including 1.5 μL of 10× Buffer, 0.45 μL of 50 mM MgCl2, 0.4 μL of 10 mM dNTPs, 0.3 μL of a dye-labelled forward primer and an unlabelled reverse primer (10 mM), 0.2 U of Taq Silverstar DNA Polymerase (Eurogentec). PCR was performed in an Eppendorf Mastercycler Gradient with the conditions as follows: an initial denaturation at 94 °C for 4 min, 38 cycles of 30 s at 94 °C, 30 s at the appropriate annealing temperature and 35 s at 72 °C, ending with a 5-min extension at 72 °C. PCR products were diluted at 1:100 and 1 μL was analysed in an ABI 3130 capillary sequencer. Alleles were scored using the GeneMapper v4.0 software (Applied Biosystems). In order to investigate the relationships between isolates, a matrix of pairwise allele-shared distances (DAS) between all genotypes was calculated using the microsatellite data, and a neighbour-joining tree was constructed. POPULATION v. 1.2.01 was used to calculate DAS and to construct the neighbour-joining trees. The bootstrap support of nodes for the microsatellite tree was calculated with 1000 replicates.

3. Results

All 1340 interactions performed (comparison VIN-REG: two host-plants, 34 isolates, 10 leaf disks; comparison VIN-RPV1: two host-plants, 28 isolates, 10 leaf disks) gave a sporulating lesion allowing us to measure aggressiveness components (disease severity, sporulation) and a life-history trait (sporangia size). The analyses focus on the comparison of *P. viticola* populations defined according to their source hosts (VIN-REG, VIN-RPV1) without addressing the differences between isolates within populations.

For the VIN/REG comparison, we found a significant effect of 'pathogen population' (REG, VIN), 'inoculated host' (cv. Cabernet Sauvignon, Regent) and of the interaction of these factors on all

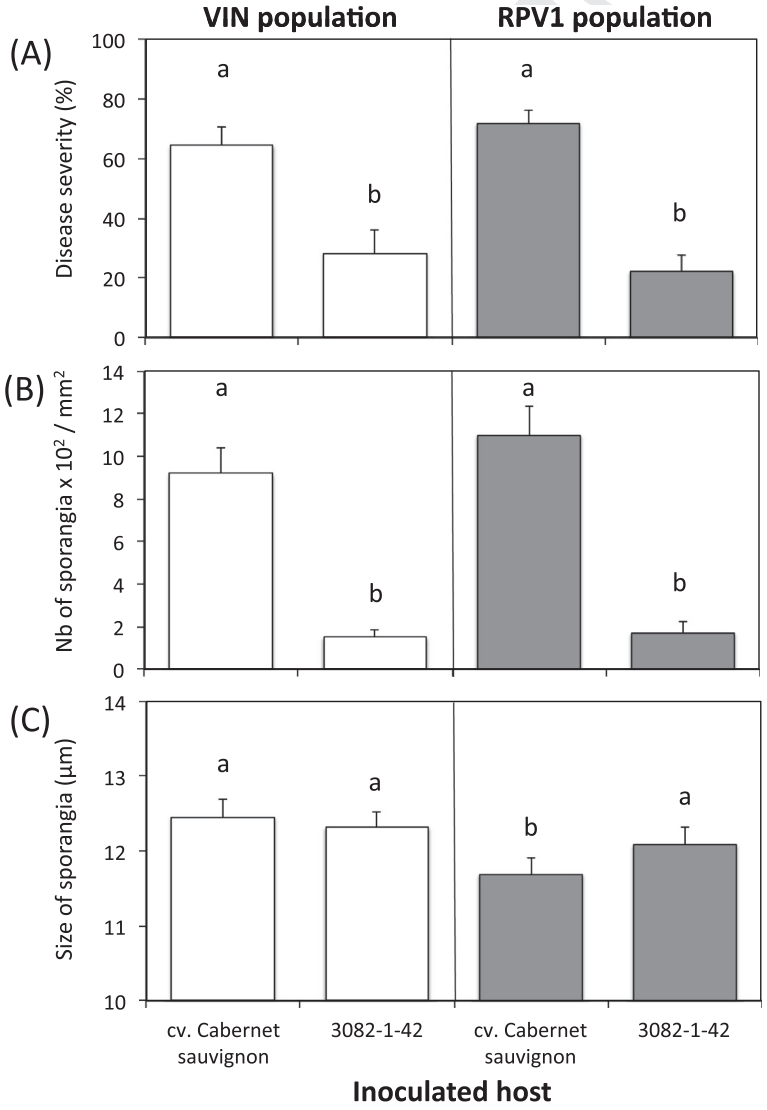

Fig. 4. Comparison of VIN and RPV1 – Aggressiveness components and life-history traits for VIN and RPV1 populations of *P. viticola*. (A) Disease severity, (B) sporangia production, (C) mean size of sporangia.

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components of aggressiveness. No effect of the geographical origin of the populations was found (Table 2). The average severity of the VIN population reached 80% on cv. Cabernet Sauvignon and only 40% on Regent. Conversely, the average severity of the REG population reached 80% whatever host was inoculated (no significant differences). The results of disease severity, sporulation and sporangia size are presented in Fig. 2 and Supplementary Fig. 1. For sporulation, the VIN population produced on average five times fewer sporangia on Regent (160 sporangia/mm^2) than on cv. Cabernet Sauvignon (800 sporangia/mm^2), whilst the REG population produced on average 800 and 1000 sporangia/mm^2 on Regent and cv. Cabernet Sauvignon, respectively. For both VIN and REG populations, sporulation differences between hosts were significant. For the REG population, closer inspection of sporulation of each REG isolate revealed that this difference was due to the presence of two REG isolates that were controlled by Regent (but aggressive on cv. Cabernet Sauvignon) (Fig. 3, and Supplementary Fig. 2). Size of sporangia of the VIN population was significantly higher on Regent than on cv. Cabernet Sauvignon but no difference in sporangia size between inoculated hosts was found for the REG population.

For the VIN/RPV1 comparison, we found a significant 'inoculated host' effect on all aggressiveness components but no effect of pathogen population except on sporangia size (Table 3). The results of disease severity, sporulation and sporangia size are presented in Fig. 4. On cv. Cabernet Sauvignon, VIN and RPV1 populations reached on average 60% and 70% severity and 900 and 1000 sporangia/mm^2, respectively. For both populations considered (RPV1, VIN), severity and sporulation were significantly lower on the resistant (Mtp3082-1-42) than on the susceptible (cv. Cabernet Sauvignon) inoculated host. Sporangia size was not statistically different between inoculated hosts for the VIN population. For the RPV1 population, sporangia collected on cv. Cabernet Sauvignon were significantly smaller than those collected on the resistant cultivar.

To better describe the differences in aggressiveness of *P. viticola* populations on the susceptible host, we conducted additional statistical analyses using reduced data sets including only cv. Cabernet Sauvignon as the inoculated host (Table 4). We found that, compared to the VIN populations, the REG populations showed significantly higher disease severity and sporangia production, with smaller sporangia. Sporangia size was the only life-history trait that differed significantly between the RPV1 and VIN populations (Table 4).

We investigated the relationship between number and size of sporangia on the susceptible cv. Cabernet Sauvignon (Fig. 5). We found a significant negative relationship between number and size of sporangia for each pathogen population in both experiments (REG: $r^2 = 0.42$, $P < 0.0001$; VIN: $r^2 = 0.23$, $P < 0.0001$) and (RPV1: $r^2 = 0.044$, $P < 0.01$; VIN: $r^2 = 0.25$, $P < 0.0001$) (Fig. 5).

The results of the genetic relationships between *P. viticola* isolates are presented in Fig. 6. All 62 *P. viticola* isolates analysed presented a distinct multi-locus genotype. A neighbour-joining tree showed no clear grouping of isolates according to either their region or host of origin.

4. Discussion

Oomycetes are known to rapidly overcome plant qualitative resistance, as reported for downy mildew of lettuce (Lebeda and Zinkernagel, 2003), sunflower downy mildew (Ahmed et al., 2012) and potato late blight (Goodwin et al., 1995). Here, we describe a new case of an oomycete species showing adaptation to partial disease resistance, in line with the results obtained for *Phytophthora infestans* (Andrivon et al., 2007). We assessed the evolution of *P. viticola* populations facing grapevine resistance resulting from two different genetic sources. This study contributes evidence for the emergence of *P. viticola* aggressive isolates presenting a high level of sporulation on the partially resistant Regent. Among the 17 isolates collected on Regent in three separate wine-growing areas, 15 were able to overcome the resistance and showed an identical sporulation level on Regent and on the susceptible cv. Cabernet Sauvignon. In a previous study, Kast et al. (2000) also identified an isolate of *P. viticola* presenting a high sporulation level on Regent. This result is comparable to those obtained by Casagrande et al. (2011) and Peressotti et al. (2010), which show that the Rpv3 gene present in the Bianca variety was defeated by a Czech and an Italian isolate of *P. viticola* (race-specific interaction). Regent and Bianca indeed share the common ancestor Villard-Blanc that transmitted Rpv3, which determines a hypersensitive response against *P. viticola* (Bellin et al., 2009; Fisher et al., 2004; Welter et al., 2007). In addition, Regent might also have inherited various minor resistance factors from the resistant grandparent 'Chancellor' (Welter et al., 2007). The data presented here on many different isolates collected across European vineyards indicate that grapevine downy mildew is able to quickly adapt to this type of partial plant resistance.

The data reported herein also provide valuable insights into the mode and rate of grapevine downy mildew evolution. Regent is a cultivar that has been planted in northern and central European vineyards on limited areas but has not yet been planted in France. The isolates collected on Regent in Bordeaux in 2008 and 2009 (Atlantic area) were sampled in an experimental vineyard planted

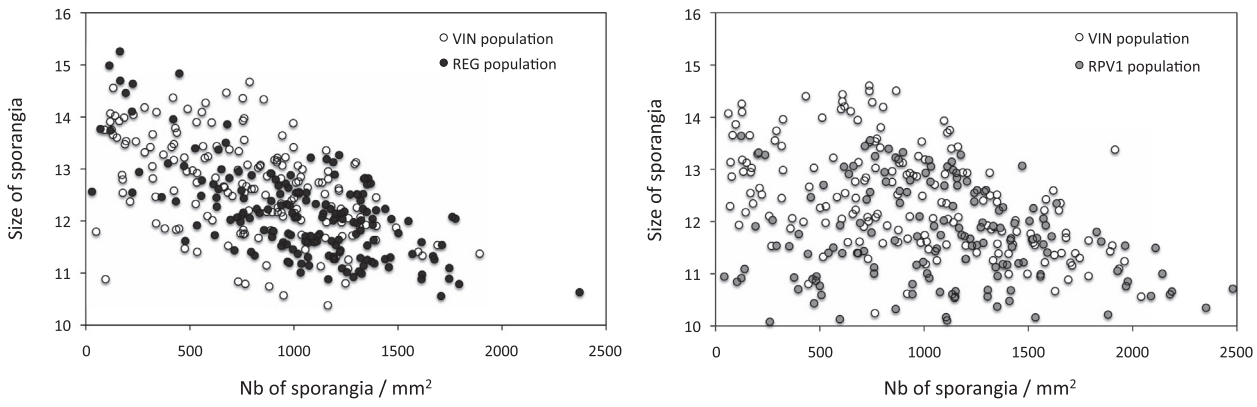

Fig. 5. Correlations between the size and the number of sporangia on cv. Cabernet Sauvignon for the VIN/REG (left) and VIN/RPV1 (right) comparisons. Each data point corresponds to an interaction between host and pathogen (leaf disk).

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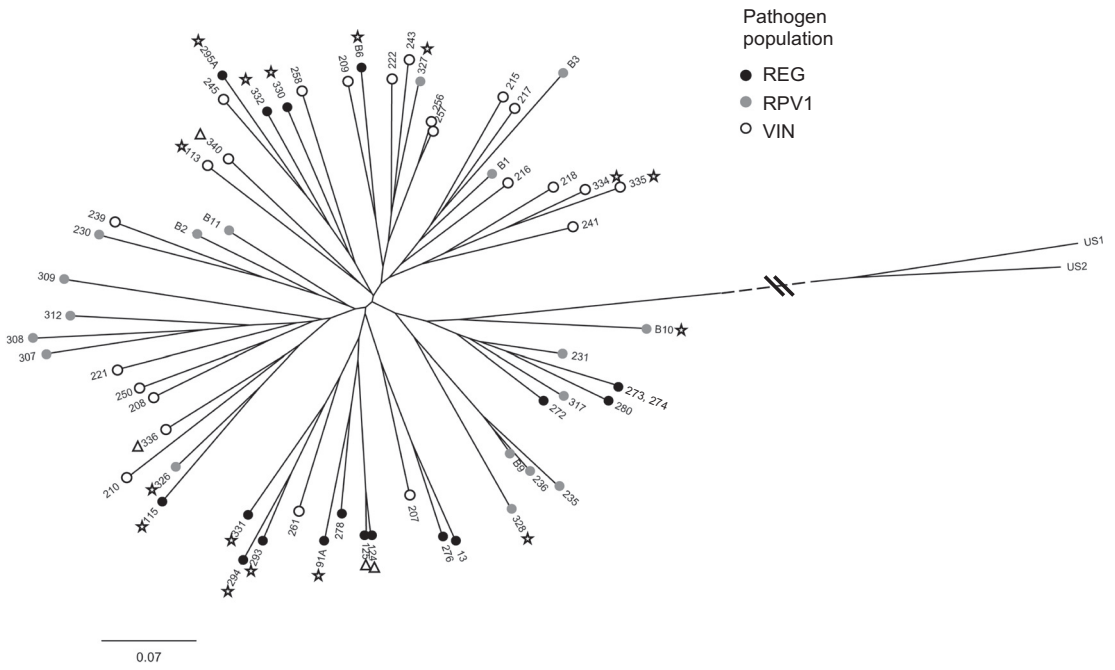

Fig. 6. Neighbour-joining tree based on allele shared distance calculated with 20 microsatellite loci for 62 isolates of *P. viticola* collected on *V. vinifera* (VIN), Regent (REG) and Rpv1-genotypes (RPV1). △: central region; ★: northern region; no symbol: Atlantic region.

in 2004. This suggests that the Atlantic *P. viticola* REG population has adapted to Regent in only 5 years. It is worth noting that this adaptation has also resulted in the loss of Regent efficacy in the field (Calonnec et al., 2013). The multisite sampling from three remote wine-growing areas (Atlantic, northern, central) suggests that adaptation to the cultivar may have occurred at least three times independently. It seems very unlikely that the same pathogen isolates could have dispersed at such a large spatial scale in such a short time span. The adaptation to Regent stemming from multiple sources is supported by previous genetic studies that have shown a restriction of gene flow in *P. viticola* populations at the European scale (Fontaine et al., 2013; Gobbin et al., 2006). Moreover, our genetic data indicate that REG isolates do not cluster together in a separate group but are randomly distributed in the phylogenetic tree with other isolates, disproving the hypothesis of the dispersion of an aggressive clone of *P. viticola* on Regent.

Bioassays revealed different responses of the pathogen to the two types of grapevine resistance tested. Contrary to Regent, no evidence for cultivar adaptation was found for the RPV1 population, which was controlled by the *Rpv1* gene (Mtp3082-1-42) as efficiently as the VIN population. Several non-exclusive hypotheses can be put forward to explain the differences between Regent and *Rpv1* genotypes. The difference between Regent and Mtp3082-1-42 could lie in the genetic background that has been introgressed in *V. vinifera* to obtain these varieties. It is well documented that the likelihood of parasite emergence is correlated to the phylogenetic distance of hosts, meaning that parasites are more likely to emerge on closely related hosts (Poulin et al., 2011). Similarly, one could hypothesise that the rate of adaptation to a plant resistance may decrease with the phylogenetic distance of the genes that have been introgressed in the focal cultivar. Regent is derived from crosses with American *Vitis*, whilst Mtp3082-1-42 has inherited its resistance from *M. rotundifolia*, a species belonging to a different genus. According to this hypothesis, the distant phylogenetic origin of the genes introgressed in Mtp3082-1-42 could have slowed down the adaptation of *P. viticola* to this cultivar.

Another hypothesis postulates that isolates adapted to Regent actually may have pre-existed at low frequency in the pathogen population, allowing them to be rapidly selected when the cultivar was deployed. The observation that none of the isolates collected on *V. vinifera* (VIN population) was adapted to Regent indicates that the frequency of these mutants is low. The most likely hypothesis has to do with the fact that the *Rpv3* alleles detected in Regent are present in a number of interspecific hybrids that were widely planted in Europe in the early 20th century (Di Gaspero et al., 2012). The selection pressure exerted by these hybrids may have selected *P. viticola* isolates "pre-adapted" to Regent. If there is little or no fitness cost of this adaptation, the "pre-adapted" isolates could have remained in the population despite the disappearance of vineyards planted with these hybrids.

Some authors have shown that adaptation to a resistant cultivar could lead to a decrease in fitness of the pathogen on susceptible varieties (Lehman and Shaner, 2007). The present data contribute evidence for an absence of cost when isolates of the REG population were inoculated on the susceptible cv. Cabernet Sauvignon. On the contrary, these isolates showed a significant increase of sporangia production compared to those of the VIN population, indicating that they are generally more aggressive on *V. vinifera*. The increase in the number of sporangia was correlated with a reduction in sporangia size. This result illustrates how partial plant disease resistance can impact selection of the pathogen's life-history traits. It is also consistent with a general host–parasite model predicting that quantitative host resistance selects for parasites with higher virulence (Gandon and Michalakis, 2000).

We still have little data on the evolution of life-history traits of plant pathogens and especially on the potential trade-off between fitness components that could limit their evolutionary potential (Lannou, 2012). However, a recent study has demonstrated that trade-offs do exist in plant pathogens, as found between latency period and spore production capacity in wheat brown rust (Pariaud et al., 2012). Here, we found evidence for a trade-off between the size and the number of sporangia produced within *P. viticola*. The negative relationship between offspring number and size is a classic example of the role of trade-offs in life-history theory (Stearns, 1992). The theory behind this trade-off is based on the assumption that the amount of energy available to put into offspring is limited;

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hence, parents optimize the investment in individual offspring against the costs to the total number of offspring produced (e.g. Smith and Fretwell, 1974). Although empirical evidence has been found for both plants and animals (e.g. reviews by Roof, 2002; Stearns, 1992), to our knowledge this is the first time that such a trade-off has been evidenced within a plant pathogen species. Since sporangia are the dispersing structure of the pathogen, this could favour dispersal abilities of aggressive isolates providing an ecological advantage to REG populations for the colonisation of new habitats. Nevertheless, it is worth noting that the actual 'offsprings' are not sporangia but zoospores contained in sporangia. Therefore, the relationship between size of sporangia and the number/size of zoospores remains to be explored. More generally, fungal plant pathogens might be good model systems for testing the 'number vs size of offspring' model because they have no parental care and a large spore production (offspring). Further studies on other oomycete or ascomycete pathogenic species are required to assess the generality of this trade-off in plant pathogens.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2013.10.017.

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