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ORIGINAL ARTICLE

Genetic structure of Italian population of the grapevine downy mildew agent, *Plasmopara viticola*

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Abstract

Downy mildew, caused by the Oomycete *Plasmopara viticola*, is one of the most important diseases affecting the Eurasian grapevine, *Vitis vinifera*. The pathogen originated in Northern America and its presence was signalled for the first time into Europe in 1878. In this study, the genetic variability and structure of Italian *P. viticola* population was investigated by genotyping 106 *P. viticola* strains belonging to 12 different regions, at 31 microsatellite markers. As a result of the high percentage of missing data, 96 strains and 19 *loci* were retained for the data analysis. The overall Italian population presents absence of clones, evidence of sexual and asexual reproduction and a low genetic diversity, as expected for an introduced pathogen, but a slightly higher genetic diversity than that found in other European populations, based on allelic diversity at the investigated microsatellite *loci*. Out of 19 *loci*, half shows deviation from Hardy–Weinberg equilibrium and, indeed, structure analysis indicates the presence of two separate genetic clusters, with little but significantly different distribution according to geography (west–east gradient) and climatic conditions. Overall, the analysis of the *P. viticola* population, 140 years after its appearance in Italy, provides indication on the pathogen adaptability. This should be taken into consideration in the future for preserving the durability of disease resistant varieties in open field. In this view, all the disease control methods available should be integrated in order to reduce the selection of pathogen strains able to overcome plant resistance.

KEYWORDS

genetic characterisation, Oomycete, plant disease management, plant pathogen, plant pathology, resistance to pathogen, SSR analysis

1 | INTRODUCTION

Grapevine is one of the most extensively cultivated plants with a global economic importance. The grapevine varieties cultivated worldwide belong to the Eurasian species, *Vitis vinifera* L. (McGovern et al., 2017), that is, susceptible to different pathogens, responsible for serious crop losses. The most important fungal pathogens of grapevine are the Oomycete *Plasmopara viticola* (Berk. *et* Curt.) Berl. and de Toni and the Ascomycete *Erysiphe necator* Schwein., both originated in North America and introduced into Europe during the second half of 19th century (Delye, Laigret, & Corio-Costet, 1997). Downy mildew, in particular, has the highest incidence on the reduction of grape production that can reach up to 75% (Buonassisi et al., 2017). *P. viticola* is an obligate biotrophic parasite and a polycyclic pathogen infecting all green parts of the plant: leaves, inflorescences and bunches (Gessler, Pertot, & Perazzolli, 2011). The seriousness of the damages caused by

P. viticola is influenced by weather conditions: frequent rainfall, high humidity and moderate temperatures in late spring/summer lead to numerous infection cycles, that cause severe quantitative and qualitative yield reductions if the pathogen is not adequately controlled (Toffolatti, Russo, et al., 2018).

P. viticola was introduced into Europe (Millardet, 1881) with the American grapevine species imported to be used as rootstocks for *V. vinifera* that was experiencing great damage because of phylloxera. The American grapes possess, in fact, natural resistance to the grape parasite *Daktulosphaira vitifoliae* Fitch that causes phylloxera. *P. viticola* was first signalled during 1878 in France, in the Bordeaux area, and 1 year later it spread out in different French areas and was detected also in Northern Italy, in the South-West of Lombardy (Ferraris, 1913). The disease diffused all over Northern Italy, reaching the North-Eastern region Veneto (Galet, 1977) and the Central region Tuscany during 1880 and was signalled in the Southern regions and islands between 1881 and 1882. Downy mildew progressively caused severe damages to the plants and a consistent reduction in European wine production that returned to its former levels only after the discovery that copper was active against the pathogen (Gessler et al., 2011). The potential harm of the pathogen, combined with the low efficacy of the agronomic practices in contrasting *P. viticola*, makes the use of chemical control necessary for disease control (Armijo et al., 2016).

The pathogen actively grows only in presence of susceptible tissues of grapevine and survives to the absence of the host by differentiating resting structures, the oospores, originated by sexual reproduction (Vercesi, Toffolatti, Zocchi, Guglielmann, & Ironi, 2010; Vercesi, Tornaghi, Sant, Burruano, & Faoro, 1999). The mating system in *P. viticola* is heterothallic (Scherer & Gisi, 2006) and requires distinct sexual compatibility or mating types (Lamour & Kamoun, 2009) that in the case of *P. viticola* are called P1 and P2. The existence of other mating systems is unlikely, but cannot be completely excluded (Scherer & Gisi, 2006). The occurrence of sexual reproduction allows to recombine alleles in genotypes.

P. viticola is a pathogen characterised by high mutation rate and high asexual sporulation efficiency, factors that probably contribute to the rapid adaptation to single-site fungicides (Chen et al., 2007; Delmas et al., 2017; Toffolatti et al., 2011; Toffolatti, Russo, et al., 2018; Toffolatti & Vercesi, 2012) and to grapevine resistance genes (Delmas et al., 2016; Delmotte et al., 2014; Peressotti et al., 2010; Toffolatti, Venturini, Maffi, & Vercesi, 2012). These studies also indicate that the rapid evolution of *P. viticola* involved soft sweeps, that is, the recurrent evolution of adapted alleles from standing variation (Chen et al., 2007; Delmas et al., 2017).

Genetic characterisation of *P. viticola* is essential in order to investigate the epidemic development and the evolution of the pathogen in the field. The first studies on the genetic structure of *P. viticola* population, carried out using SSR markers, highlighted the role of sexual and asexual reproduction in grapevine downy mildew epidemics on a fine spatial scale (vineyard) in Italy (Gobbin, Pertot, & Gessler, 2003; Gobbin, Rumbou, Linde, & Gessler, 2006). These studies pointed out, in particular, that the inoculum produced by the oospores contributes to the epidemics until late in the season. These and subsequent studies, moreover showed that *P. viticola* populations are panmictic not only at field scale, but also at regional scale (Delmas et al., 2016, 2017; Gobbin et al., 2003; Rouxel et al., 2012). Large-scale population genetic studies increased our knowledge on the worldwide genetic structure of this invasive plant pathogen. They showed that the European populations possess a rather low genetic diversity, suggesting that the isolates introduced into Europe in the 1870s came from a single source population of North America (Fontaine et al., 2013). Interestingly, a weak, but significant, continental-wide population structure, with two geographically and genetically distinct clusters in Western and Eastern European vineyards, was also identified (Fontaine et al., 2013).

To date, the information available on the genetic structure of *P. viticola* population in Italy is limited to either a very restricted spatial scale, consisting of a low number of regions (four) located in the Northern part of the country (Gobbin et al., 2003, 2006), or to a low number of isolates (four) over a large (European) spatial scale (Fontaine et al., 2013). Moreover, previous studies were based on a very limited number of markers (5 to 8 SSR). In this study, we used 31 SSR to describe the genetic structure of Italian *P. viticola* population by sampling a large number of strains (106) from twelve different wine-growing regions, going from North to South and including the main islands. Aim of the study is to collect information on the genetic variability of the pathogen population almost 140 years after its first appearance in the country, taking into consideration the hypothesis that Italy is the country where the two European clusters (Western and Eastern) are admixed (Fontaine et al., 2013). Finally, several factors (geographic origin, weather conditions, disease management strategy and host cultivar) that could contribute in shaping the pathogen population, were investigated.

2 | MATERIALS AND METHODS

2.1 | *P. viticola* sampling

Leaves showing downy mildew symptoms were randomly collected between June and July 2016 from 106 vineyards located in 12 different geographic regions of Italy (Table S1; Figure 1): Abruzzo (one vineyard), Campania (two vineyards), Friuli (three vineyards), Lazio (one vineyard), Liguria (four vineyards), Lombardy (29 vineyards), Piedmont (10 vineyards), Sardinia (four vineyards), Sicily (two vineyards), Tuscany (34 vineyards), Umbria (six vineyards) and Veneto (10 vineyards). The characteristics of the individual vineyards, in terms of location, cultivar, disease management and meteorological conditions during the sampling period (Spring), are reported in Table S1. Overall, 30 different cultivars of international (e.g., Chardonnay, Merlot, Pinot noir), national (e.g., Barbera, Sangiovese) and local (e.g., Pigato, Canaiolo, Nebbiolo, Dolcetto) diffusion, cultivated in 25 different provinces, were sampled. The disease management strategy adopted in vineyard was organic farming in 55 cases and integrated pest management (IPM) in 51 cases (Table S1).

FIGURE 1 Localization of the samples at regional level

2.2 | Meteorological conditions in the sampling regions

Meteorological conditions of the 2016 Spring period largely varied at the national scale in terms of precipitation anomaly (PA), measured as deviation of precipitation ratio of spring 2016 from the mean precipitation rate of 1971–2000 and expressed in percentage (Brunetti, Maugeri, Monti, & Nanni, 2006). Meteorological conditions strongly influence the pathogen epidemic. For each location, PA values were recorded from the website (http://www.isac.cnr.it/climstor/climate_news.html; access in September 2016) of the Institute of Atmospheric Sciences and Climate (CNR-ISAC). Each sample was assigned to a different PA category (Table S1): drought stress, associated to PA values ranging from −75 to −10% (category 1); no variation (PA = 0; category 2); and precipitation excess, when PA values ranged from +10 to +100% (category 3). Temperature anomalies were not considered because no important differences occurred in the period at the national level (Desiato et al., 2017).

2.3 | Isolation, DNA extraction and microsatellite amplification

In laboratory, a single leaf per vineyard was randomly chosen. A single oilspot was excised from the leaf with a 1 cm diameter cork borer, as described by Rouxel et al. (2013), and placed in a 1.5 mL sterile tube (Eppendorf, Germany). A single oilspot (sample) was therefore analysed per vineyard. The samples were stored at −20°C, lyophilized and then kept at room temperature until DNA extraction. Morphological criteria (shape of sporangiophores, sporangia, mycelium and haustoria) were used for confirming the identification of the samples at the species level (Lafon & Bulit, 1981).

Total DNA was extracted from single lyophilized leaf disc according to the standard cetyl-trimethyl-ammonium-bromide (CTAB) and phenol–chloroform methods described by Delmotte et al. (2006). The DNA quantity was determined by NanoDrop Spectrophotometer (Thermo Scientific, Fremont, CA).

P. viticola isolates were genotyped with 31 species-specific microsatellite *loci*: ISA (Gobbin et al., 2003); PV7, PV14, PV16, PV17, PV31 and PV39 (Delmotte et al., 2006); PV65, PV67, PV74, PV76, PV83, PV87, PV88, PV91, PV93, PV101, PV103, PV104, PV126, PV127, PV134, PV137, PV138, PV139, PV140, PV141, PV142, PV143, PV147 and PV148 (Rouxel et al., 2012). Primer sequences, annealing temperatures and amplicon sizes are reported in Table S2. The 5′ ends of the forward primers were conjugated with fluorescent dyes (-Table S2) and multiplex PCR was carried out by mixing the following primer pairs: (a) PV14, ISA, PV17, PV39, PV31, PV16, PV7; (b) PV138, PV140, PV143, PV147, PV101, PV103, PV74); (c) PV135, PV137, PV141, PV93, PV65, PV148, PV104; (d) PV139, PV76, PV87, PV126, PV88, PV83; and (e) PV127, PV134, PV67, PV142.

PCR mix (6 μL) contained 4 ng DNA template, ×0.5 QIAGEN Multiplex PCR master mix (Qiagen, Courtaboeuf Cedex, France), all primers at a final concentration of 0.8 μM and Nuclease-Free water (Qiagen) to adjust the volume. Cycling conditions were: 15 minutes at 95°C, 35 cycles of 30 seconds at 94°C, 1 minutes at 55°C, 45 seconds at 72°C and final elongation step of 30 minutes at 60°C. PCR runs were performed in an Eppendorf Mastercycler Ep (Eppendorf) and the fragment size analysis was performed in an automated capillary genetic analyser-sequencer 3130 (Applied Biosystems, Thermo Fisher Scientific, Merelbeke, Belgium). Chromatograms were analysed using the software GENEMAPPER 4.0 (Applied Biosystems) and allele sizes were recorded in bp.

2.4 | Data analysis

GenAlEx v. 6.501 software (Peakall & Smouse, 2006, 2012) was used to calculate the following parameters: (a) number of alleles, allele frequencies, observed (H_o) and expected (H_E) heterozygosity, Fixation index (F_{is}) and deviation from Hardy–Weinberg Equilibrium (HWE) for each microsatellite; (2) genetic differentiation (F_{ST} measured via analysis of molecular variance—AMOVA; Peakall, Smouse, & Huff, 1995) among *P. viticola* samples divided according to geographic region, precipitation anomaly, disease management strategy (organic vs integrated pest management), and cultivar.

The existence of a population structure in the total dataset was further investigated using the Bayesian approach implemented in *tess3* (Caye, Deist, Martins, Michel, & Francois, 2016) R package. This clustering algorithm assumes a model in which there are *K* populations (where *K* may be unknown), each of which is characterised by a set of allele frequencies at each *locus*. Individuals in the sample are assigned probabilistically to these *K* populations, or jointly to two or more populations if their genotypes indicate that

they are admixed, without consideration of their region of sampling. *K* varied from 1 to 10, each with 10 independent simulations to check the consistency of the results. Lambda value for the spatial regularisation parameter was 1, the method chosen was "projected.ls" (alternating projected least squares algorithm), with a maximum number of iterations of the optimization algorithm up to 200 and a tolerance (value corresponding to the stopping criteria of the optimization algorithm) of $1e^{-05}$. The most likely *K* value was estimated inspecting the cross-validation curve and the membership proportion for each genotype at each *K* was plotted in a barplot. The threshold for membership to a group was set at 80%.

Discriminant analysis of principal components (DAPC, Jombart, Devillard, & Balloux, 2010) was moreover performed to identify genetic clusters using the package *adegenet* (Jombart, 2008) of R software. DAPC approach was used because it does not need the assumption of a panmictic population as Bayesian structuring does. The maximum number of clusters to be tried was set to 10 and number of axes retained in the principal component analysis step was set to 60. The densities of individuals on a given discriminant function were plotted.

Individuals belonging to the groups discriminated by DAPC analysis were further investigated to evaluate which factors could be acting on the Italian *P. viticola* population: (a) geographic origin; (b) precipitation anomaly-PA; (c) disease management strategy; d) host cultivar. As a result of the low frequency (eight strains in total), samples isolated from vineyards with PA excess were discarded from this analysis. Only the cultivars with a number of isolates equal or greater than five were analysed: Chardonnay (14 isolates), Merlot (five isolates), Pinot noir (six isolates) and Sangiovese (22 isolates).

Chi-square test was performed on the frequencies of strains grouped according to DAPC clusters, PA and disease management strategy for testing if these variables are related (SPSS v. 24, IBM Analytics Italia, Milano, Italy).

3 | RESULTS

3.1 | Genotyping and diversity statistics of the Italian *P. viticola* population

One hundred and six *P. viticola* strains were genotyped with 31 microsatellites *loci*. As a result of the high percentage of missing data (>14%), 10 microsatellites (Pv7, Pv67, Pv74, Pv76, Pv103, Pv126, Pv137, Pv138, Pv140 and Pv143) and 10 strains (from no. 97 to 106) were excluded from further analyses. Out of the 21 microsatellites examined, two (PV87 and PV134) resulted monomorphic (PV87 = 154 bp; PV134 = 224 bp) and were furthermore excluded. The DNA profiles of the 96 strains at 19 SSR *loci* are shown in Table S3.

The percentage of missing data, allele size range, observed heterozygosity (H_o), expected heterozygosity (H_E), Fixation index (F_{IS}) and the deviation from Hardy–Weinberg Equilibrium (HWE) of the 19 polymorphic microsatellites are listed in Table 1. No strains showed identical allelic profiles, therefore all the strains represent a

TABLE 1 Mean proportion of missing data over *loci* (MD%), number of alleles (Na), allele size range (bp), observed (H_o) and expected (H_E) heterozygosity, Fixation index (F_{IS}) and deviation from the Hardy–Weinberg equilibrium (HWE) of the 19 polymorphic microsatellites estimated for the 96 *Plasmopara viticola* strains

Locus	MD%	N_a	Allele size range (bp)	H_o	H_E	F_{IS}	HWE[a]
PV14	3.0	3	120-124	0.82	0.66	−0.242	*
ISA	1.0	6	112–138	0.76	0.60	−0.283	*
PV17	2.0	4	142–148	0.80	0.63	−0.274	**
PV39	3.0	2	175–177	0.08	0.08	−0.044	Ns
PV31	3.0	6	237–242	0.52	0.44	−0.182	***
PV16	2.0	4	245–250	0.47	0.39	−0.213	***
PV91	12.1	3	142–146	0.59	0.50	−0.172	Ns
PV147	7.1	5	195–219	0.57	0.47	−0.216	Ns
PV148	6.1	3	126–137	0.20	0.22	0.078	Ns
PV93	8.1	3	148–152	0.49	0.42	−0.186	Ns
PV141	6.1	3	189–192	0.57	0.45	−0.265	*
PV65	7.1	3	194–198	0.08	0.51	0.848	***
PV104	10.1	2	322–324	0.01	0.10	0.883	***
PV88	11.1	2	204–206	0.16	0.19	0.130	Ns
PV83	11.1	3	238–242	0.09	0.09	−0.040	Ns
PV142	7.1	2	209–211	0.68	0.46	−0.499	***
PV139	10.1	3	131–135	0.07	0.07	−0.031	Ns
PV127	13.1	4	216–221	0.22	0.22	−0.005	***
PV101	9.1	3	262–266	0.41	0.45	0.069	Ns
All *loci*	7.0	3.4	-	0.40	0.37	−0.034	–

[a]Level of significance: Ns, not significant; *p < .05; **p < .01; ***p < .001.

distinct multilocus genotype. Most of the *loci* (13) showed a percentage of missing data lower than 10%, and only six (PV91, PV104, PV88, PV83, PV139 and PV127) had values ranging from 10.1 to 13.1 (Table 1). The microsatellites exhibited a number of alleles ranging from two (PV39, PV104, PV88 and PV142) to six (ISA and PV31) (Table 1). H_E, also known as Nei's genetic diversity (Nei, 1973), ranged from 0.07 (PV139) to 0.66 (PV14) and was equal to 0.37 on average (Table 1). Most of the *loci* showed F_{IS} values close to zero or lower than zero, and only two *loci* (PV65 and PV104) displayed F_{IS} values higher than 0.8 (Table 1). Globally, 10 *loci* (PV14, ISA, PV17, PV31, PV16, PV141, PV65, PV104, PV142 and PV127) showed a significant deviation from HWE ($p < .05$) and the remaining nine showed no significant deviation ($p > .05$) (Table 1).

3.2 | Genetic structure of the Italian *P. viticola* population

In order to infer the relationship among genotypes, the structuring algorithm was used. The hierarchical population structure was uncovered exploring different numbers of *K* populations, from 1 to 10. Cross-validation test estimated the most likely number of populations at $K = 2$ (Figure 2), suggesting that the Italian population could be divided in two ancestral groups. The plot of *K* vs Δ*K*, shows, in fact, that Δ*K* has a rapid reduction, normally regarded as a signal that the true value of *K* has been reached, between $K = 1$ and 2 (Figure 2a). Looking at the ancestry coefficients of $K = 2$, most of the strains were admixed and only seven strains can be divided in two genetically distinct groups: strains n. 95 and 96 in group 1 and strains n. 63, 64, 76, 79 and 91 in group 2 (Figure 2b). Members of group 1 were isolated in Sardinia island, from an area with drought stress in 2016 (Table S1). Those of group 2 were found in areas with no precipitation anomaly (PA = 0) or excess of precipitation located in Lombardy, Piedmont, Umbria and Sicily. Strains from the two main Italian islands (Sardinia and Sicily) grouped in two different clusters. Members of group 1 were isolated from IPM treated vineyards, whereas those of group two belonged to IPM (two strains) or organic treated farms (three strains). The plot for *K* values ranging from three to five is reported in Figure S1. Taking into account higher values of ancestral populations (with *K* values ranging from three to five), the plotting of ancestral coefficients confirmed the cross-validation results. Indeed, no additional populations can be observed as a result of the lower number of individuals reaching high values of membership to a population.

DAPC, used to infer the number of clusters of genetically related individuals, confirmed the existence of two distinct clusters in the Italian *P. viticola* population (Figure 3): the first and the larger group is composed of 63 strains; the second one of 33 strains. Almost all the

TABLE 2 Number of *Plasmopara viticola* isolates (N), number of alleles (Na), observed (H_o) and expected (H_E) heterozygosity, Fixation index (F_{IS}) and deviation from the Hardy–Weinberg equilibrium (HWE) calculated for each *locus* dividing *P. viticola* genotypes according to DAPC clusters

	Cluster 1						Cluster 2					
Locus	N	Na	H_o	H_E	F_{IS}	HWE[a]	N	Na	H_o	H_E	F_{IS}	HWE[a]
PV14	61	3	0.85	0.66	−0.30	*	33	3	0.76	0.64	−0.18	Ns
ISA	63	6	0.78	0.60	−0.29	*	33	5	0.73	0.57	−0.27	Ns
PV17	62	4	0.82	0.65	−0.27	*	33	3	0.76	0.59	−0.29	Ns
PV39	62	2	0.06	0.06	−0.03	Ns	32	2	0.13	0.12	−0.07	Ns
PV31	62	3	0.50	0.40	−0.24	Ns	32	6	0.56	0.51	−0.10	***
PV16	62	3	0.47	0.38	−0.23	Ns	33	3	0.48	0.41	−0.18	***
PV91	56	2	0.54	0.50	−0.08	Ns	29	3	0.69	0.51	−0.35	Ns
PV147	60	5	0.62	0.49	−0.26	Ns	30	5	0.47	0.42	−0.12	Ns
PV148	57	3	0.21	0.22	0.04	Ns	33	2	0.18	0.21	0.15	Ns
PV93	57	3	0.53	0.41	−0.29	Ns	32	2	0.44	0.43	−0.02	Ns
PV141	57	2	0.58	0.44	−0.32	*	33	3	0.55	0.46	−0.18	Ns
PV65	57	2	0.12	0.12	−0.07	Ns	33	1	0	0	–	–
PV104	55	2	0.02	0.12	0.85	***	32	2	0	0.06	1	***
PV88	53	2	0.15	0.20	0.25	Ns	32	2	0.19	0.17	−0.10	Ns
PV83	53	3	0.11	0.11	−0.05	Ns	32	2	0.06	0.06	−0.03	Ns
PV142	59	2	0.66	0.44	−0.49	***	30	2	0.73	0.48	−0.53	**
PV139	58	3	0.09	0.08	−0.04	Ns	28	2	0.04	0.04	−0.02	Ns
PV127	55	3	0.16	0.18	0.11	***	28	3	0.32	0.27	−0.17	Ns
PV101	58	3	0.40	0.45	0.11	Ns	29	2	0.45	0.44	−0.02	Ns

[a]Level of significance: Ns, not significant; *$p < .05$; **$p < .01$; ***$p < .001$.

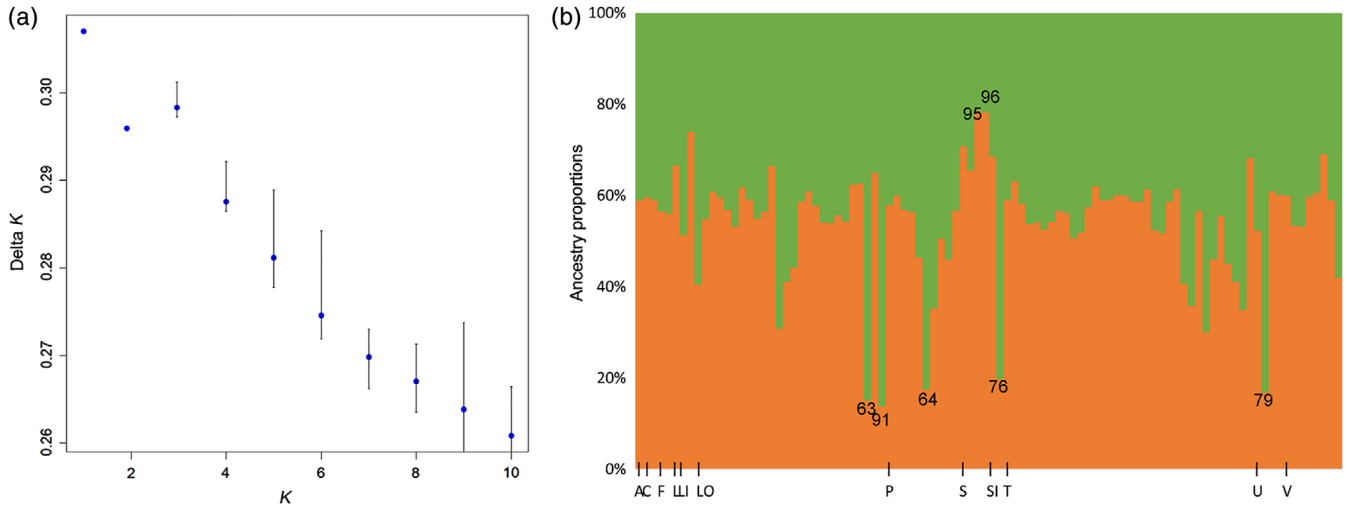

FIGURE 2 Genetic structure of *Plasmopara viticola* population in Italy. (a) Plot of ΔK (Delta K) analysis, at values of K from 1 to 10, used to infer the true value of K. (b) Individual ancestry within population clusters in *Plasmopara viticola* inferred using Bayesian approach implemented in tess3 R package; vertical bars represent individual assignment probability into different genetic clusters inferred under the K = 2 model from the STRUCTURE analysis (based on results from the ad hoc statistic ΔK) depicted with colours; colours represent individual groups: group 1 (orange) and group 2 (green). Letters on the *x*-axis represent the region of origin of the strain: A, Abruzzo; C, Campania; F, Friuli; L, Lazio; Li, Liguria; Lo, Lombardy; P, Piedmont; S, Sardinia; Si, Sicily; T, Tuscany; U, Umbria; V, Veneto. Strain numbers of individuals univocally belonging to groups 1 and 2 are indicated

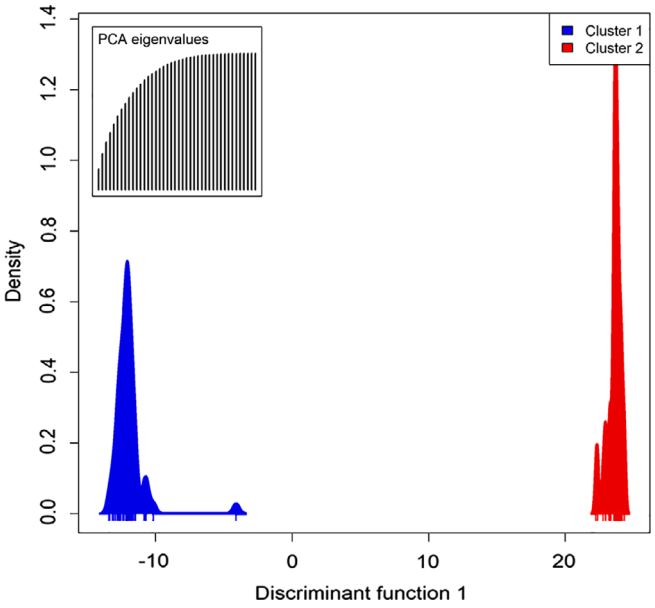

FIGURE 3 Plot of the individual densities against the first discriminant function retained by Discriminant Analysis of Principal Components (DAPC) analysis carried out on *Plasmopara viticola* strains genotyped at 19 *loci*. DAPC analysis shows that the Italian population is divided in two clusters (subpopulations) separated over a single discriminant function

strains belonging to groups 1 and 2 identified by Bayesian clustering analysis grouped in DAPC group 1, apart from strain 64.

In Table 2 the number of alleles, heterozygosity indexes, fixation index (F_{IS}) values and deviation from HWE of the 19 polymorphic *loci* divided according to DAPC cluster are reported. Considering the two clusters, it is possible to notice a significant deviation from HWE for seven *loci* out of 19 in cluster 1, and for four *loci* out of 19 for cluster 2 (Table 2). In the latter case, PV65 *locus* resulted monomorphic. *Locus* PV104 displayed a significantly high level of heterozygote deficiency in both clusters (with F_{IS} values of 0.85 and 1, respectively).

3.3 | Factors affecting the genetic structure of the Italian *P. viticola* population

Based on AMOVA, the overall *P. viticola* genotypes did not differ according to the region of origin (Table S4). No differences were found among the strains isolated from Lombardy and Tuscany (Table S5), the regions with the highest number of isolates available (25 and 34, respectively). However, looking at the frequency of distribution of the strains grouped in clusters 1 and 2 by DAPC analysis, slight differences can be observed: the frequency of strains belonging to group 2 showed a progressive reduction going from Western to Eastern Italy (Figure 4). AMOVA, indeed, showed the presence of little (percentage of molecular variance = 1%) but significant differentiation between isolates of Eastern and Western Italy (F_{st} = 0.009; p = .04).

In spring 2016 precipitation anomalies indicating drought stress or water excess were observed in the Western and Eastern regions, respectively (Figure 4). AMOVA showed no significant differentiation among individuals grouped according to the type of stress (no precipitation anomaly, drought, precipitation excess) as reported in Table S6. While equal frequencies of strains belonging to DAPC groups 1 and 2 are visible where drought stress occurred in spring 2016, the frequency of group 2 strains was significantly lower than that of group 1 where no precipitation anomalies occurred (Figure 4).

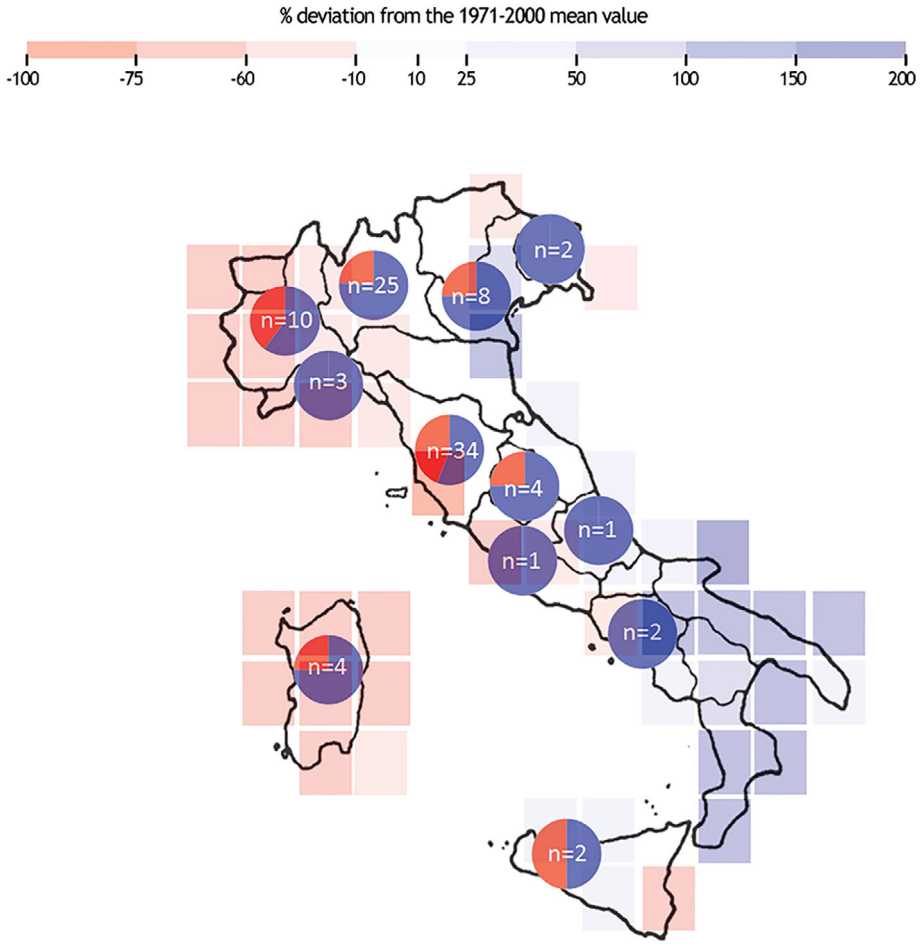

FIGURE 4 Precipitation anomaly occurring in Italy during Spring 2016. Rectangles in red represent drought stress, rectangles in white no stress and rectangles in blue water excess (adapted from: http://www.isac.cnr.it/climstor/climate_news.html; access in September 2016). Percentages of deviation from the 1971 to 2000 mean values are indicated in the bar above the map. Pie charts represent the frequency of isolates clustered in DAPC group 1 (in blue) and group 2 (in red)

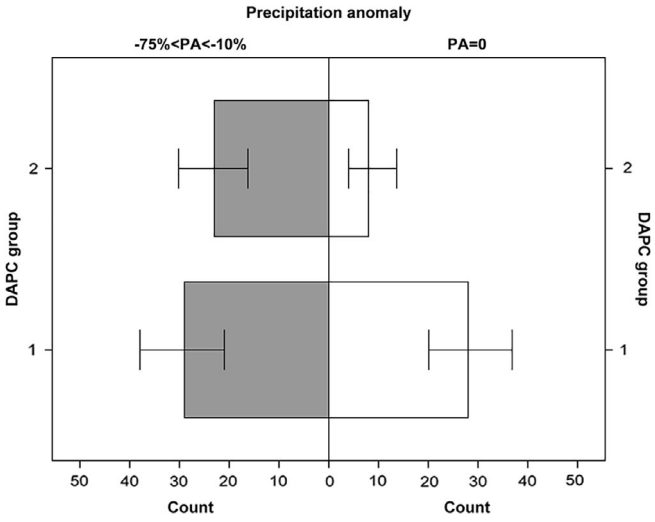

FIGURE 5 Frequency of strains belonging to DAPC groups 1 and 2 in relation to precipitation anomaly (PA), measured as deviation of precipitation ratio of spring 2016 from the mean value recorded in 1971–2000 period expressed in percentage. PA = 0 indicates no variation from the mean value. PA < 0 indicates lower precipitation ratios. Bars represent confidence interval (p = 95%)

Chi-square test performed on the frequencies of strains belonging to DAPC and PA groups, showed a significant, even if weak, relationship among the two variables (χ^2 = 3.842; df = 1; p = .049) (Figure 5).

The strains did not group according to disease management strategies (organic vs integrated pest management) applied in vineyard (Table S7) and no significant relationship was found among DAPC groups and disease management strategy frequencies (χ^2 = 3.192; df = 1; p = .074). Finally, the cultivar from which *P. viticola* strains were sampled did not contribute to structuring of the Italian population (Table S8).

4 | DISCUSSION

In this paper, the genetic structure of the Italian *P. viticola* population was investigated by analysing 96 strains isolated from vineyards located in most of the major wine-producing regions of the country. *P. viticola* European population shows little genetic diversity as a consequence of the bottleneck originated at pathogen introduction, when a single population was transferred from North America to the Old Continent (Rouxel et al., 2012, 2013). Our results indicate that the

Italian *P. viticola* population also presents little genetic variability, with heterozygosity estimates (H_E = 0.37) similar to those found in other European populations (H_E = 0.39), but slightly lower than those of isolates from West Europe (H_E = 0.44) (Fontaine et al., 2013). The difference found between heterozygosity estimates of Western European and Italian populations could be associated to the dynamics of a spatially expanding pathogen population, that lead to a reduced genetic diversity (Rouxel et al., 2013).

Compared to a study on European isolates of *P. viticola* (Rouxel et al., 2012), the Italian population has equal or greater number of alleles at 15 *loci*. Compared to North American isolates (Rouxel et al., 2012), Italian population shows equal or greater number of alleles at 8 *loci*, and lower number of alleles at seven *loci*. H_o of the Italian population is more often greater than lower than that of European isolates at the 19 *loci* examined (Fontaine et al., 2013; Rouxel et al., 2012). Out of 13 comparable *loci*, the Italian population shows greater H_o at six *loci* and lower H_o at seven *loci*.

The overall Italian *P. viticola* population is characterised by the absence of clones (i.e., genotypes sharing the same allelic profile) and most of the examined *loci* display high levels of heterozygosity, an indication that the population could be randomly mating. This is not surprising, because both mating types are present in Italy and sexual spores (oospores) are regularly found in vineyard (Vercesi et al., 1999, 2010; Wong, Burr, & Wilcox, 2001). Most of the loci showed F_{IS} values close to zero or lower than zero, and only two loci (PV65 and PV104) displayed F_{IS} values higher than 0.8. Negative F_{IS} values indicate an excess of heterozygotes. The F_{IS} trend observed in the Italian *P. viticola* population, with a high number of negative F_{IS} values among loci, is typical of partially asexual populations (population that can reproduce both through sexual and asexual events), such as *P. viticola* (Gessler et al., 2011). Populations reproducing using high rates of asexuality strongly shifted their F_{IS} distribution values into negative values with a tail of over high values of positive F_{IS} (Reichel, Masson, Malrieu, Arnaud-Haond, & Stoeckel, 2016). Asexual reproduction can act in both maintaining and increasing heterozygosity through the accumulation of mutations over generations. Previous studies carried out on *P. viticola* strains isolated from different European countries showed that the populations are predominantly panmictic (Delmas et al., 2016, 2017; Fontaine et al., 2013; Gobbin et al., 2003, 2006). In our study, however, 10 *loci* out of 19 display a significant deviation from HWE. This is in contrast with what reported by other authors on European and Italian *P. viticola* populations, where most of the microsatellite *loci* are in HWE (Fontaine et al., 2013; Gobbin et al., 2006). This suggests that the analysed Italian population could be deviating from panmixia.

Structure analyses, indeed, highlight the presence of two distinct subpopulations in the Italian *P. viticola* population as previously described for European vineyards, where two genetically distinct clusters, with a different distribution over a longitudinal gradient going from Western to Eastern Europe, were found (Fontaine et al., 2013). According to the most probable scenario, *P. viticola* isolates have been most likely introduced first in Western Europe (Bordeaux area) from North America and then diffused toward Eastern Europe following a leap frog pattern, leading to the formation of two genetic clusters (Fontaine et al., 2013). In the study carried out by Fontaine et al. (2013), the analysis of a few Italian isolates suggested that both groups were present in Italy. Here, the analysis of a large number of Italian *P. viticola* isolates corroborates the hypothesis that two slightly different subpopulations, with a weak geographic differentiation between Eastern and Western vineyards, are present in Italy. Genetic variation parameters strengthen the existence of a difference among the two clusters. While the number of *loci* under HW disequilibrium is 10 out of 19 in the overall *P. viticola* Italian population, this number decreases in the two subpopulations identified by DAPC analysis, that also differ for the *loci* under HW disequilibrium: cluster 1 shows a higher frequency of *loci* under HW disequilibrium (seven *loci*) than cluster 2 (four *loci*). Only two *loci* under HW disequilibrium are shared among the two clusters (PV104 and PV142). Overall, these results indicate that the Italian *P. viticola* population is either dividing in two subpopulations or a combination of two different populations. According to the large scale analysis of *P. viticola* population in Europe, the first scenario is the more likely (Fontaine et al., 2013).

When a phytopathogen is newly introduced in an area, it must have adaptive capacity to both abiotic conditions (range of temperature, humidity and precipitation) and biotic interaction with the host plant (Ghatak, 2017). The genetic structure of the Italian *P. viticola* population was put in relation with abiotic (geography, climatic conditions and disease management strategy) and biotic factors (host cultivar), to investigate if they are involved in substructuring the pathogen population.

The lack of a genetic structure related to the regional origin of strains suggests that the Italian population is admixed at large spatial scale. However, both Bayesian clustering and DAPC analyses showed the existence of two subpopulations in the Italian *P. viticola* population. Based on ancestry coefficients, Bayesian clustering shows that most of the samples are admixed and only seven samples univocally group in the two clusters. The high genetic differentiation existing in the Sardinian samples encompassed in group 1, that is, separated from group 2, could be as a result of a geographic isolation of the strains that should be more deeply investigated. DAPC analysis confirmed the existence of two groups in the Italian *P. viticola* population that are characterised by a differentiation over a geographical (longitudinal) gradient as observed by Fontaine et al. (2013). However, the sample size of Western and Southern *P. viticola* isolates should be increased to confirm this result.

P. viticola is strongly dependent on the presence of water on the surface of susceptible tissues for the host infection, therefore rainfall plays a key role in the pathogen epidemics (Rossi, Giosuè, & Caffi, 2009), especially in Spring, when the primary infections occur. During the first years since its introduction in Italy, downy mildew was mainly detected on leaves late in the season, when the bunches were not susceptible, causing limited damages (Ferraris, 1913). Starting from 1890s the infection period anticipated to late spring, causing brown rot of grapes, and only from 1905 attacks were reported on early phenological stages of grapevine, causing dramatic yield losses (Gessler et al., 2011). This trend could indicate an adaptation of the pathogen

to the Italian climate. Starting from the end of XIX century, climatic conditions in Italy showed a positive trend, uniformly distributed all over Italy, for the temperatures, with an increase of 1°C per century on a yearly basis (Brunetti et al., 2006). On the contrary, precipitation showed a tendency toward a decrease in the frequency and an increase in the intensity of the rainy events, in this case with marked differences among regions and seasons (Brunetti, Maugeri, Monti, & Nanni, 2004). DAPC analysis highlighted a different distribution of the Italian *P. viticola* strains in terms of climatic conditions, precipitation changes in particular. Members of DAPC group 2 were more frequently found in areas with drought stress than where no precipitation anomalies occurred in the year of sampling. Evidence of substructuring based on climate has been found also in Chinese *P. viticola* populations characterised by both SSR markers and polymorphisms at four gene regions (Yin, Zhang, Hao, & Lu, 2014; Zhang et al., 2017). Further studies are however needed to investigate if members of the two groups are characterised by an adaptation to different climates, taking into account traits influenced by the environment, such as infection efficiency, latent period and spore production rate, that are linked to pathogen evolution in the agricultural system (Lannou, 2012).

Disease management could also contribute in shaping the pathogen population. It has been reported that fungal communities in vineyard can vary according to the disease management practices adopted (Morrison-Whittle, Lee, & Goddard, 2017). However, the results obtained in the analysis of microbial communities of grapevine are not conclusive in discriminating among disease management strategies (Kecskeméti, Berkelmann-Löhnertz, & Reineke, 2016). Disease management strategies on grapevine in IPM and organic farming strategies mainly differ for the fungicides that can be applied: while the multisite fungicide copper is the only active ingredient that can be adopted in organic farming against *P. viticola*, single-site fungicides are frequently used in IPM. Resistance to single-site fungicides is one of the main issues in downy mildew management, because *P. viticola* is at high risk of developing resistance to fungicides (FRAC pathogen risk list, www.frac.info) and resistance against several fungicide classes has been reported (Gisi & Sierotzki, 2008; Toffolatti, Russo, et al., 2018). A reduction in genetic diversity of *P. viticola* population, as a consequence of directional selection toward higher resistance, has been observed in a population sprayed with a single-site fungicide (Matasci, Gobbin, Schärer, Tamm, & Gessler, 2008). In this paper, the absence of a genetic structure of *P. viticola* population sampled from vineyards treated with different disease management strategies suggests that, at present, this factor is not shaping the Italian pathogen population.

Once introduced into Europe at the end of XIX century, *P. viticola* found a susceptible host and suitable climatic conditions for its development. *P. viticola* affects members of the *Vitaceae* family and *V. vinifera*, the Eurasian grapevine species (McGovern et al., 2017), proved to be particularly susceptible as a result of the absence of co-evolution with the pathogen. As a consequence, the main *V. vinifera* varieties cultivated worldwide are extremely susceptible to the pathogen, and only recently the existence of resistant cultivars, such as the Georgian (Caucasus) Mgaloblishvili, has been reported (Toffolatti, De Lorenzis, et al., 2018; Toffolatti et al., 2016). In our study, no evidence of host adaptation has been found, as demonstrated by the absence of structuring according to grapevine cultivar.

To conclude, the Italian *P. viticola* population is characterised by a low genetic variability and signatures of deviation from panmixia, but the occurrence of sexual reproduction in vineyard can contribute to variability and adaptability. The pathogen adaptability must be taken into consideration in the implementation of the disease control methods. The evolutionary potential of *P. viticola* was confirmed by the capability to overcome partial disease resistance (Delmotte et al., 2014; Toffolatti et al., 2012). In this view, the cultivation of the resistant grapevine varieties should be integrated with fungicide treatments, to slow down the selection of virulent strains, able to overcome plant defences, in order to obtain a durable resistance. The different frequency of members DAPC groups 1 and 2 on a longitudinal gradient and in presence of different precipitation rates, indicates that the pathogen population could be changing according to different forces acting simultaneously, among which are geographic isolation and climatic conditions. However, the presence of two distinct clusters in the population could be a consequence of several reasons, that cannot be completely elucidated in the present study and need further investigation. The availability of genome sequences, opening the way to population genomics (Dussert et al., 2016, 2018), could be a powerful tool to be employed in this regard. Of particular importance will also be increasing the number of samples per region to obtain an even sample size and gathering of data on genetic variability of *P. viticola* in other countries (Taylor, Lazar, White, & Burgess, 2018), to understand the patterns of distribution and evolution of the pathogen.

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REFERENCES

Armijo, G., Schlechter, R., Agurto, M., Muñoz, D., Nuñez, C., & Arce-Johnson, P. (2016). Grapevine pathogenic microorganisms: Understanding infection strategies and host response scenarios. *Frontiers in Plant Science*, *7*, 382.

Brunetti, M., Maugeri, M., Monti, F., & Nanni, T. (2004). Changes in daily precipitation frequency and distribution in Italy over the last 120 years. *Journal of Geophysical Research*, *109*, D05102.

Brunetti, M., Maugeri, M., Monti, F., & Nanni, T. (2006). Temperature and precipitation variability in Italy in the last two centuries from homogenised instrumental time series. *International Journal of Climatology*, *26*, 345–381.

Buonassisi, D., Colombo, M., Migliaro, D., Dolzani, C., Peressotti, E., Mizzotti, C., … Vezzulli, S. (2017). Breeding for grapevine downy mildew resistance: A review of "omics" approaches. *Euphytica*, *213*, 103.

Caye, K., Deist, T. M., Martins, H., Michel, O., & Francois, O. (2016). TESS3: Fast inference of spatial population structure and genome scans for selection. *Molecular Ecology Research*, *62*, 540–548.

Chen, W. J., Delmotte, F., Richard-Cervera, S., Douence, L., Greif, C., & Corio-Costet, M. F. (2007). At least two origins of fungicide resistance in grapevine downy mildew populations. *Applied and Environmental Microbiology*, *73*, 5162–5172.

Delmas, C. E. L., Fabre, F., Jolivet, J., Mazet, I. D., Richart Cervera, S., Delière, L., & Delmotte, F. (2016). Adaptation of a plant pathogen to partial host resistance: Selection for greater aggressiveness in grapevine downy mildew. *Evolutionary Applications*, *9*, 709–725.

Delmas, C. E. L., Dussert, Y., Deliere, L., Couture, C., Mazet, I. D., Cervera, S. R., & Delmotte, F. (2017). Soft selective sweeps in fungicide resistance evolution: Recurrent mutations without fitness costs in grapevine downy mildew. *Molecular Ecology*, *26*, 1936–1951.

Delmotte, F., Chen, W. J., Richard-Cervera, S., Greif, C., Papura, D., Giresse, X., … Corio-Costet, M. F. (2006). Microsatellite DNA markers for *Plasmopara viticola*, the causal agent of downy mildew of grapes. *Molecular Ecology Notes*, *6*, 379–381.

Delmotte, F., Mestre, P., Schneider, C., Kassemeyer, H. H., Kozma, P., Cervera, S., … 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. *Infection, Genetics and Evolution*, *27*, 500–508.

Delye, C., Laigret, F., & Corio-Costet, M. F. (1997). RAPD analysis provides insight into the biology and epidemiology of *Uncinula necator*. *Phytopathology*, *87*, 670–677.

Desiato, F., Fioravanti, G., Fraschetti, P., Perconti, W., Piervitali, E., & Pavan, V. (2017). Gli indicatori del clima in Italia nel 2016. *ISPRA, Stato dell'Ambiente*, *72*, 76.

Dussert, Y., Gouzy, J., Richart-Cervera, S., Mazet, I. D., Delière, L., Couture, C., … Delmotte, F. (2016). Draft genome sequence of *Plasmopara viticola*, the grapevine downy mildew pathogen. *Genome Announcements*, *4*, e00987 -16.

Dussert, Y., Mazet, I. D., Couture, C., Gouzy, J., Piron, M.-C., Kuchly, C., … Delmotte, F. (2018). A high-quality grapevine downy mildew genome assembly reveals rapidly evolving and lineage-specific putative host adaptation genes. *Genome Biology and Evolution*, *11*, 954–969. https://doi.org/10.1093/gbe/evz048.

Ferraris, T. (1913). *Trattato di Patologia e Terapia vegetale: I parassiti vegetali delle piante coltivate od utili*. Milano, Italy: Ulrico Hoepli.

Fontaine, M., Austerlitz, F., Giraud, T., Labbé, F., Papura, D., Richard-Cervera, S., & 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*. *Molecular Ecology*, *22*, 2771–2786.

Galet, P. (1977). *Les maladies et les parasites de la vigne, Tome I*. Montpellier, France: Imprimerie du Paysan du Midi.

Gessler, C., Pertot, I., & Perazzolli, M. (2011). *Plasmopara viticola*: A review of knowledge on downy mildew of grapevine and effective disease management. *Phytopathologia Mediterranea*, *50*, 23–25.

Ghatak, A. (2017). Evolution and adaptation in phytopathosystems. In A. Ghatak & M. Ansar (Eds.), *The Phytopathogen – Evolution and adaptation* (pp. 3–20). Oakville, Canada: Apple Academic Press.

Gisi, U., & Sierotzki, H. (2008). Fungicide modes of action and resistance in downy mildews. *European Journal of Plant Pathology*, *122*, 157–167.

Gobbin, D., Pertot, I., & Gessler, C. (2003). Identification of microsatellite markers for *Plasmopara viticola* and establishment of high throughput method for SSR analysis. *European Journal of Plant Pathology*, *109*, 153–164.

Gobbin, D., Rumbou, A., Linde, C. C., & Gessler, C. (2006). Population genetic structure of *Plasmopara viticola* after 125 years of colonization in European vineyards. *Molecular Plant Pathology*, *7*, 519–531.

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*, 403–405.

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*, 94.

Kecskeméti, E., Berkelmann-Löhnertz, B., & Reineke, A. (2016). Are epiphytic microbial communities in the carposphere of ripening grape clusters (*Vitis vinifera* L.) different between conventional, organic, and biodynamic grapes? *PLoS One*, *11*, e0160852.

Lafon, R., & Bulit, J. (1981). Downy mildew of the vine. In D. Spencer (Ed.), *The Downy Mildews* (pp. 601–614). New York, NY: Academic Press.

Lamour, K., & Kamoun, S. (2009). *Oomycete genetics and genomics: Diversity, interactions and research tools*. Hoboken, New Jersey: Wiley-Blackwell.

Lannou, C. (2012). Variation and selection of quantitative traits in plant pathogens. *Annual Review of Phytopathology*, *50*, 319–338.

Matasci, C. L., Gobbin, D., Schärer, H. J., Tamm, L., & Gessler, C. (2008). Selection for fungicide resistance throughout a growing season in populations of *Plasmopara viticola*. *European Journal of Plant Pathology*, *120*, 79–83.

McGovern, P. E., Jalabadze, M., Batiuk, S., Callahan, M. P., Smith, K. E., Hall, G. R., … Lordkipanidze, D. (2017). Early Neolithic wine of Georgia in the South Caucasus. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, E10309–E10318.

Millardet, A. (1881). *Notes sur les vignes américaines et opuscules divers sur le même sujet* (p. 105). Bordeaux: Chez Feret & Fils.

Morrison-Whittle, P., Lee, S. A., & Goddard, M. R. (2017). Fungal communities are differentially affected by conventional and biodynamic agricultural management approaches in vineyard ecosystems. *Agriculture, Ecosystems & Environment*, *246*, 306–313.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences of the United States of America*, *70*, 3321–3323.

Peakall, R., & Smouse, P. E. (2006). GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, *6*, 288–295.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, *28*, 2537–2539.

Peakall, R., Smouse, P. E., & Huff, D. R. (1995). Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalo grass *Buchloë dactyloides*. *Molecular Ecology*, *4*, 135–148.

Peressotti, E., Wiedemann-Merdinoglu, S., Delmotte, F., Bellin, D., Di Gaspero, G., Testolin, R., … Mestre, P. (2010). Breakdown of resistance to grapevine downy mildew upon limited deployment of a resistant variety. *BMC Plant Biology*, *10*, 147.

Reichel, K., Masson, J.-P., Malrieu, F., Arnaud-Haond, S., & Stoeckel, S. (2016). Rare sex or out of reach equilibrium? The dynamics of F_{IS} in partially clonal organisms. *BMC Genetics*, *17*, 76.

Rossi, V., Giosuè, S., & Caffi, T. (2009). Modelling the dynamics of infections caused by sexual and asexual spores during *Plasmopara viticola* epidemics. *Journal of Plant Pathology*, *91*, 615–627.

Rouxel, M., Mestre, P., Comont, G., Lehman, B., Schilder, A., & Delmotte, F. (2013). Phylogenetic and experimental evidence for host-specialized cryptic species in a biotrophic oomycete. *New Phytologist*, *197*, 251–263.

Rouxel, M., Papura, D., Nogueira, M., Machefer, V., Dezette, D., Richard-Cervera, S., … Delmotte, F. (2012). Microsatellite markers for characterization of native and introduced populations of *Plasmopara viticola*, the causal agent of grapevine downy mildew. *Applied and Environmental Microbiology*, *78*, 6337–6340.

Scherer, E., & Gisi, U. (2006). Characterization of genotype and mating type in European isolates of *Plasmopara viticola*. *Journal of Phytopathology*, *154*, 489–495.

Taylor, A. S., Lazar, K., White, D., & Burgess, T. (2018). Evaluation of microsatellite primers developed for grapevine downy mildew, *Plasmopara viticola*, on Australian isolates. *Australasian Plant Pathology*, *47*, 189–193.

Toffolatti, S. L., De Lorenzis, G., Costa, A., Maddalena, G., Passera, A., Bonza, M. C., … Quaglino, F. (2018). Unique resistance traits against downy mildew from the center of origin of grapevine (*Vitis vinifera*). *Scientific Reports*, *8*, 12523.

Toffolatti, S. L., Maddalena, G., Salomoni, D., Maghradze, D., Bianco, P. A., & Failla, O. (2016). Evidence of resistance to the downy mildew agent *Plasmopara viticola* in the Georgian *Vitis vinifera* germplasm. *Vitis*, *55*, 121–128.

Toffolatti, S. L., Prandato, M., Serrati, L., Sierotzky, H., Gisi, U., & Vercesi, A. (2011). Evolution of QoI resistance in *Plasmopara viticola* oospores. *European Journal of Plant Pathology*, *129*, 331–338.

Toffolatti, S. L., Russo, G., Campia, P., Bianco, P. A., Borsa, P., Coatti, M., … Sierotzki, H. (2018). A time-course investigation of resistance to the carboxylic acid amide mandipropamid in field populations of *Plasmopara viticola* treated with anti-resistance strategies. *Pest Management Science*, *74*, 2822–2834.

Toffolatti, S. L., Venturini, G., Maffi, D., & Vercesi, A. (2012). Phenotypic and histochemical traits of the interaction between *Plasmopara viticola* and resistant or susceptible grapevine varieties. *BMC Plant Biology*, *12*, 124.

Toffolatti, S. L., & Vercesi, A. (2012). QoI resistance in *Plasmopara viticola* in Italy: Evolution and management strategies. In T. Thind (Ed.), *Fungicide Resistance in Crop Protection: Risk and Management* (pp. 172–183). Wallingford, UK: CABI.

Vercesi, A., Toffolatti, S. L., Zocchi, G., Guglielmann, R., & Ironi, L. (2010). A new approach to modelling the dynamics of oospore germination in *Plasmopara viticola*. *European Journal of Plant Pathology*, *128*, 113–126.

Vercesi, A., Tornaghi, R., Sant, S., Burruano, S., & Faoro, F. (1999). A cytological and ultrastructural study on the maturation and germination of oospores of *Plasmopara viticola* from overwintering vine leaves. *Mycological Research*, *103*, 193–202.

Wong, F., Burr, H., & Wilcox, W. (2001). Heterothallism in *Plasmopara viticola*. *Plant Pathology*, *50*, 427–432.

Yin, L., Zhang, Y., Hao, Y., & Lu, J. (2014). Genetic diversity and population structure of *Plasmopara viticola* in China. *European Journal of Plant Pathology*, *140*, 365–376.

Zhang, W., Manawasinghe, I. S., Zhao, W., Xu, J., Brooks, S., Zhao, X., … Yan, J. (2017). Multiple gene genealogy reveals high genetic diversity and evidence for multiple origins of Chinese *Plasmopara viticola* population. *Scientific Reports*, *7*, 17304.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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