

Spatio-temporal distribution of *Erysiphe necator* genetic groups and their relationship with disease levels in vineyards

Josselin Montarry · Philippe Cartolaro ·
Sylvie Richard-Cervera · François Delmotte

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Abstract The discovery of genetically distinct *Erysiphe necator* groups (A or B), with high phenotypic similarities, raises important questions about their coexistence. For plant pathogens, niche partitioning, allowing the coexistence on the same host (*i.e.* the same resource), might result from separation in space and/or time. We used a landscape genetic approach to study the geographic distribution of genetic groups of *E. necator* (distinguished by a SNP in the β -tubulin gene) at the spatial scale of the Languedoc-Roussillon region (southern France) and to assess the temporal succession of groups along the course of the 2007 epidemic. Spatial distribution revealed a high heterogeneity between vineyards: from 100% B to 100% A, with 62% and 38% of vineyards showing a majority of A and B isolates, respectively. Temporal isolation seems to be the major mechanism in the coexistence of the two genetic groups: all isolates collected towards the end of the epidemic belonged to group B, whatever the initial frequency of genetic groups. Our results confirm that both A or B isolates can lead to flag-shoot symptoms, and showed that group A isolates tend to disappear during the course of the epidemic, whereas group B isolates may be active during the entire

epidemic and involved in further production of cleistothecia, when recombination takes place. For the first time, the relationship between the frequency of genetic groups and disease levels on leaves and clusters at the end of the epidemic was evaluated. We showed a strong relationship between the disease severity and the genetic composition of *E. necator* populations: the damage was more important when the epidemic was initiated by B isolates.

Keywords Coexistence · Cryptic species · Grapevine powdery mildew · Landscape epidemiology · Plant pathogen · *Vitis vinifera*

Introduction

A growing number of genetic studies of plant pathogens demonstrate that species harbour hidden genetical diversity in the form of cryptic species. There are now several well documented examples of plant pathogen species, such as *Leptosphaeria maculans* (Williams and Fitt 1999), *Gaeumannomyces graminis* var. *tritici* (Lebreton et al. 2004), *Botrytis cinerea* (Fournier et al. 2005) and *Erysiphe syringae* (Seko et al. 2008), which are indeed composed of genetically differentiated clades that have led to the description of new groups or even new species. The discovery of genetically differentiated sibling species, with high phenotypic similarities, raises important questions about their coexistence. Since the competi-

J. Montarry (✉) · P. Cartolaro · S. Richard-Cervera ·
F. Delmotte
INRA, Institut des Sciences de la Vigne et Vin de
Bordeaux, UMR1065 Santé Végétale (INRA-ENITAB),
F-33883 Villenave d'Ornon, France
e-mail: Josselin.Montarry@bordeaux.inra.fr

tion theory states that two species occupying the same ecological niche cannot coexist indefinitely (Gause 1934; Chesson 2000), sibling plant pathogen species might coexist on the same host through niche partitioning despite their high phenotypic similarity. Ecological differences that lead to niche partitioning can occur in three basic ways: resource partitioning, temporal niche partitioning, and spatial niche partitioning (Wilson and Lindow 1994; Chesson 2000; Amarasekare 2003). For plant pathogenic species coexisting on the same host, *i.e.* the same resource, the separation in space and/or time might better explain niche partitioning (e.g. Fitt et al. 2006).

Grapevine powdery mildew, caused by the biotrophic ascomycete *Erysiphe necator* (syn. *Uncinula necator*), is one example of a plant pathogen showing two genetically differentiated groups of isolates coexisting on the same host, *Vitis vinifera* (Délye et al. 1997; Evans et al. 1997; Miazzi et al. 2003; Nuñez et al. 2006). Several studies have suggested that genetic *E. necator* groups (A and B) correlated with ecological features of the pathogen; Délye et al. (1997) proposed that group A isolates over-winter as resting mycelium within dormant buds that reinitiate growth after budbreak and colonise young flag-shoots (Pearson and Gärtel 1985), while group B isolates would survive as ascospores released from over-wintering cleistothecia (Gadoury and Pearson 1988). Indeed, an association between flag-shoot symptoms and infection by group A isolates has been found in earlier studies in France (Délye and Corio-Costet 1998; Amrani and Corio-Costet 2006) and Italy (Miazzi et al. 2003). Due to this association, these authors proposed that group A isolates may be responsible for early infections in the season while group B isolates may be responsible for late infections (Délye and Corio-Costet 1998; Miazzi et al. 2003). However, the association between genetic groups and over-wintering survival has been challenged by recent studies reporting that flag-shoot symptoms may harbour both group A and B isolates (Cortesi et al. 2005; Nuñez et al. 2006; Péros et al. 2005; Willocquet et al. 2007). Moreover, the hypothesis of a temporal succession of genetic groups was based on genetic studies that suffered from sampling strategies confounding time during the epidemic with over-wintering mode and source of inoculum.

Geographical distribution that could lead to spatial niche differentiation of the *E. necator* genetic groups

has always been addressed on a small number of populations, impeding tests for spatial structure. Data available showed that the frequencies of the groups could vary greatly from one field to another, suggesting a high level of spatial heterogeneity at the vineyard scale (Cortesi et al. 2005; Amrani and Corio-Costet 2006; Bouscaut and Corio-Costet 2007; Willocquet et al. 2007).

Here, our aim is to study the regional dynamics of *E. necator* genetic groups at a large spatial scale. This work is part of the recent development using genetic tools to study the influence of habitat heterogeneity in space and time on plant pathogen epidemics (e.g. Plantegenest et al. 2007). We conducted a landscape genetic approach combining landscape epidemiology and population genetics (Manel et al. 2003) in order to explore the geographic distribution of *E. necator* genetic groups in southern France vineyards, and to assess the temporal succession of groups along the course of the epidemics. Moreover, we have evaluated the relationship between the frequency of genetic groups and disease level on leaves and clusters at the end of the epidemics. This study therefore addressed three questions: (1) what is the genetic variability (A or B) of *E. necator* populations on flag-shoots at a regional scale? (2) are there changes in the frequency of genetic groups between the start and the end of the epidemic? and, (3) is there a relationship between the frequency of genetic groups assessed early in the season and disease levels at the end of the growing season?

Materials and methods

Isolate collection

Diseased leaves of cv. Carignan (*Vitis vinifera*) were randomly sampled twice during the 2007 growing season in commercial vineyards of the Languedoc-Roussillon region. The first sampling was performed in 32 vineyards early in the growing season (end of April) and the second sampling in 16 of those 32 vineyards at the end of the growing season (early September). At the first sampling, diseased leaves were collected only on flag-shoots; at the second sampling, diseased leaves were randomly collected within each vineyard. Depending on the disease pressure, up to 40 leaves were collected per vineyard.

This led to a total of 1,253 leaves infected with *E. necator*, of which 769 were sampled in April and 484 in September. On each leaf, a 1 cm diam disc was taken out with a punch and frozen for DNA extraction. The location of each vineyard was recorded using GPS (Table 1).

Molecular characterisation

The molecular method used to differentiate genetic groups was the amplification of the β -tubulin gene of *E. necator* (tub2, accession number AY074934)

exhibiting a T/C single nucleotide polymorphism (SNP) between group A and group B isolates (Amrani and Corio-Costet 2006). SNP creates a recognition site of restriction endonuclease *AccI* that allows the characterisation of A or B isolates by Cleaved Amplified Polymorphic Sequence (CAPS) analysis (e.g. Baudoin et al. 2008).

Total genomic DNA was extracted directly from lesions (infected leaf discs) collected in the vineyards without any prior subculture of the fungus. Lesions were crushed in 400 μ l CTAB buffer and then heated at 65°C for 1 h; 400 μ l isoamyl alcohol/chloroform

Table 1 GPS position, number of isolates sampled (N_s), number of isolates yielding a PCR amplicon of the β -tubulin gene (N) and percentage of group A isolates and group B

isolates for each of the 32 fields of cv. Carignan at the start of the epidemic, and for 16 out of the 32 fields at the end of the epidemic

Fields	Location	Epidemic start (April)				Epidemic end (September)			
		N_s	N	%A	%B	N_s	N	%A	%B
AZI	N43 17 41.7 E2 38 36.3	32	31	87.1	12.9				
BRU ₁	N43 08 13.6 E2 09 07.7	21	21	100.0	0.0	31	26	0.0	100.0
BRU ₂	N43 08 09.6 E2 09 08.0	25	23	26.1	73.9	26	5	0.0	100.0
CAF	N42 48 35.9 E2 23 12.3	29	26	0.0	100.0	30	15	0.0	100.0
CAN	N42 37 00.2 E2 50 17.7	36	34	44.1	55.9				
CAV	N42 55 56.3 E2 59 16.5	27	23	65.2	34.8	34	11	0.0	100.0
CRL ₁	N43 02 13.6 E2 13 44.8	14	14	35.7	64.3				
CRL ₂	N43 02 10.1 E2 13 59.4	15	15	13.3	86.7				
ESL	N42 45 57.4 E2 40 45.1	30	29	65.5	34.5	31	12	0.0	100.0
FIT	N42 54 01.3 E2 56 09.0	36	36	100.0	0.0	12	6	0.0	100.0
GIN ₁	N43 16 38.1 E2 51 56.9	28	24	0.0	100.0	18	5	0.0	100.0
GIN ₂	N43 16 38.1 E2 51 56.9	20	13	0.0	100.0				
LAM ₁	N43 16 58.1 E2 32 28.2	32	26	88.5	11.5				
LAM ₂	N43 16 58.1 E2 32 28.2	30	20	75.0	25.0				
LAP	N42 57 28.2 E2 59 09.6	31	29	86.2	13.8				
LBO	N42 32 50.4 E2 51 14.8	6	6	100.0	0.0				
LDA ₁	N43 02 36.9 E2 11 09.2	17	17	47.1	52.9	33	31	0.0	100.0
LDA ₂	N43 02 42.8 E2 11 04.9	8	7	100.0	0.0	28	6	0.0	100.0
LEU ₁	N42 55 01.8 E3 02 02.3	20	20	100.0	0.0				
LEU ₂	N42 55 05.2 E3 02 05.5	16	14	100.0	0.0				
NAR	N43 10 26.8 E2 59 30.2	13	10	100.0	0.0				
POR	N43 18 37.2 E3 19 15.3	20	8	12.5	87.5				
POV	N42 30 23.7 E3 06 55.3	29	29	100.0	0.0	32	14	0.0	100.0
PYM ₁	N43 17 49.1 E2 33 27.9	36	20	55.0	45.0	40	17	0.0	100.0
PYM ₂	N43 17 55.0 E2 33 26.0	20	11	18.2	81.8				
PYM ₃	N43 17 55.0 E2 33 26.0	21	17	5.9	94.1				
RXM	N43 16 28.7 E2 36 30.8	32	32	96.9	3.1	40	13	0.0	100.0
SPF	N42 49 17.1 E2 30 08.0	7	5	0.0	100.0	28	12	0.0	100.0
THU	N42 39 00.2 E2 46 11.1	37	37	100.0	0.0	39	8	0.0	100.0
TRE	N42 34 10.3 E2 51 04.8	36	34	100.0	0.0	22	9	0.0	100.0
VLZ	N43 16 46.5 E2 27 53.7	25	21	71.4	28.6	40	15	0.0	100.0
VMS	N43 15 13.1 E2 22 09.3	20	7	71.4	28.6				

All isolates collected at the beginning of the 2007 growing season originate from flag-shoot symptoms.

(24:1) were then added and a centrifugation was performed at 3,700 rpm at 4°C for 30 min. The aqueous phase was collected and 200 µl of isopropanol were added. DNA precipitation was performed at -20°C for 2 h. After centrifugation at 3,700 rpm at 4°C for 20 min, the pellet was rinsed with 500 µl of 70% ethanol and centrifugated at 3,700 rpm for 10 min. The DNA pellet was finally dissolved in 50 µl of water.

Polymerase chain reactions (PCR) were performed in a 16.5 µl volume containing 1.5 µl of the stock genomic DNA solutions diluted $\times 3$, 1.5 µl of 10X PCR Buffer (Eurogentec), 1.5 mM MgCl₂, 0.5 mM of each dNTP, 0.2 µM of each primer (EnTub-F: 5'-GCGA GATCGTAAGCTTGACAC-3' and EnTub-R: 5'-GGCACGAGGAACGTATTTGT-3') and 0.25 U *Taq* SilverStar DNA polymerase (Eurogentec). The PCR programme was performed as follows: a first denaturation step of 3 min at 96°C, followed by 38 cycles of 40 s at 96°C, 55 s at 58°C, 55 s at 72°C, and a final elongation step of 5 min at 72°C. Cleaving reactions were realised in 10 µl volume with 2 µl of PCR product, 1 µl of 10 \times -buffer no. 4 (BioLabs) and 1.5 U of *AccI* enzyme (BioLabs) at a temperature of 37°C with an incubation period of 1.5 h. Restriction fragments were visualised on 2% agarose after staining with ethidium bromide.

Disease assessment on leaves and clusters

At the end of the 2007 growing season, prior to the grape harvest (mid-September), the disease levels on leaves and clusters were visually estimated in 13 out of the 32 vineyards sampled at the beginning of the epidemic for genetic analysis. That estimation, based on the observation of five areas (composed at least of 100 vines) randomly distributed in the field, took into account incidence of diseased vines (*i.e.* an estimation of the percentage of diseased vines) and global symptom severity (*i.e.* an estimation of the percentage of leaf area infected), using the following category scale: 0=severity <5% and incidence 0–5%; 1=severity <5% and incidence 5–20%; 2=severity <5% and incidence 20–50%; 3=severity <5% and incidence >50%; 4=severity 5–30% and incidence >50%; 5=severity >30% and incidence >50%.

Statistical data analyses

The spatial autocorrelation structure for the frequency of genetic groups was analysed using the Spatial

Analysis in Macroecology v2.0 software (Rangel et al. 2006). Spatial autocorrelation measures the similarity between samples for a given variable as a function of spatial distance. The Moran's *I* coefficient, which is the most commonly used coefficient in univariate autocorrelation analyses, was calculated for seven distance classes, each 15 km wide. Moran's *I* compares the value of the variable at any one location with the value at all other locations. *I* ranges from -1 to 1, for maximum negative and positive autocorrelation, respectively. To estimate *P*-values, tests of significance were performed using 1,000 permutations. If no Moran's *I* coefficients are significant, there is no spatial pattern in the data (*i.e.* absence of autocorrelation).

Correlations between the frequency of genetic groups and the altitude or the distance to the sea, and also between the initial frequency of group B isolates and the final disease levels on leaves or clusters, were carried out by Spearman's rank correlation rho using the statistical freeware R, version 2.6.1 (R Development Core Team 2007).

Results

From the 769 lesions sampled at the beginning of the season, 659 (85.7%) yielded a PCR amplicon of the β -tubulin gene; from the 484 lesions sampled at the end of the season, only 205 (42.4%) did so. Genotyping failures could result either from an increased level in PCR inhibitors (as polyphenolic and polysaccharide compounds) in leaves along the epidemics (*e.g.* Tattersall et al. 2005), or from the sampling of inactive lesions towards the end of the epidemic. Both reasons may explain the higher success of genotyping on isolates collected at the start of the epidemics.

Among the 659 *E. necator* isolates collected at the beginning of the season from flag shoots, 440 (67%) belonged to group A and 219 (33%) to group B. This confirmed that both group A and group B isolates can over-winter as resting mycelium within dormant buds and lead to flag-shoot symptoms. The frequencies of the genetic groups per field varied greatly, from 100% group A (in ten fields) to 100% group B (in four fields). From the 18 fields showing a mix of A and B isolates, ten contained a majority of group A isolates and eight a majority of group B isolates (Table 1 and Fig. 1).

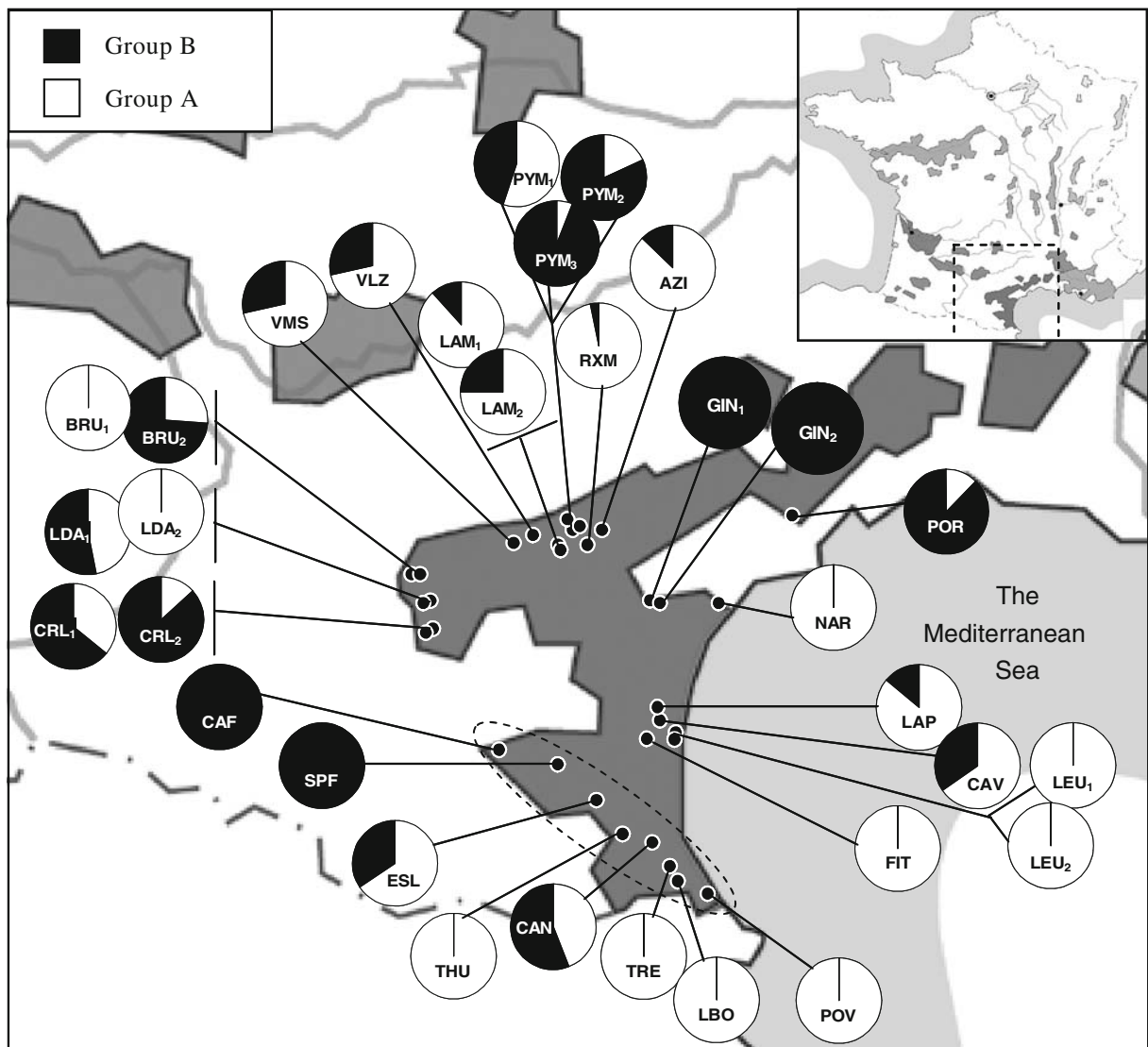


Fig. 1 Spatial distribution of the 32 fields sampled in the south of France, and frequency of *Erysiphe necator* isolates belonging to group A (white) and B (black) for each field. The dotted line shows the geographical position of the Agly's Valley

The autocorrelation analysis performed on our data did not allow the detection of the spatial structure of the two genetic groups, which appeared to be randomly distributed at the spatial scale of vineyards (Fig. 2). This is illustrated by the fact that neighbouring fields sometimes showed very different frequencies of *E. necator* genetic groups (Table 1 and Fig. 1). For instance, bru₁ and bru₂ which are 120 m away from one another, showed 100% and 26.1% of A isolates, respectively; similarly, the frequency of group A isolates in populations from vineyards lda₁ and lda₂ (200 m away from each other) were 47.4% and 100%, respectively. It is noteworthy that the eight

vineyards situated along the Agly Valley (*i.e.* CAF at an altitude of 334 m, SPF, ESL, THU, CAN, TRE, LBO and POV at an altitude of 32 m) showed an altitudinal gradient related to the genetic structure of populations: *E. necator* isolates collected in the uppermost vineyards belonged to group B, vineyards at an intermediate altitude showed a mix of A and B isolates, while populations from the lowest vineyards included exclusively group A isolates (Fig. 1). Nevertheless, neither the altitude nor the distance to the sea were significantly associated to genetic group frequency in the complete dataset (Fig. 3, Spearman's rank correlation $\rho=0.288$, $P=0.110$ for the altitude

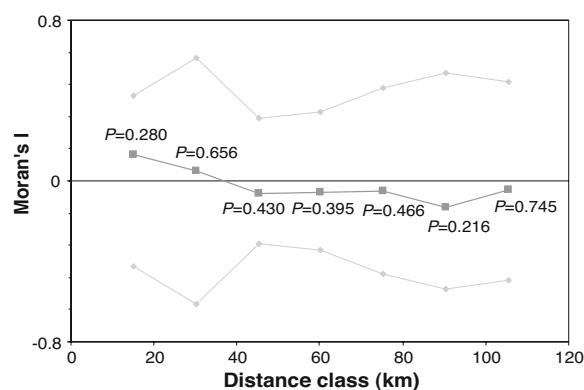


Fig. 2 Correlogram showing Moran's autocorrelation coefficient I in relation to seven distance classes (class width 15 km). Dotted curves showed the Max-Moran's I . P -values indicate each distance class

and Spearman's correlation $\rho=0.305$, $P=0.090$ for the distance to the sea).

All isolates collected at the end of the growing season belonged to group B, whatever the initial

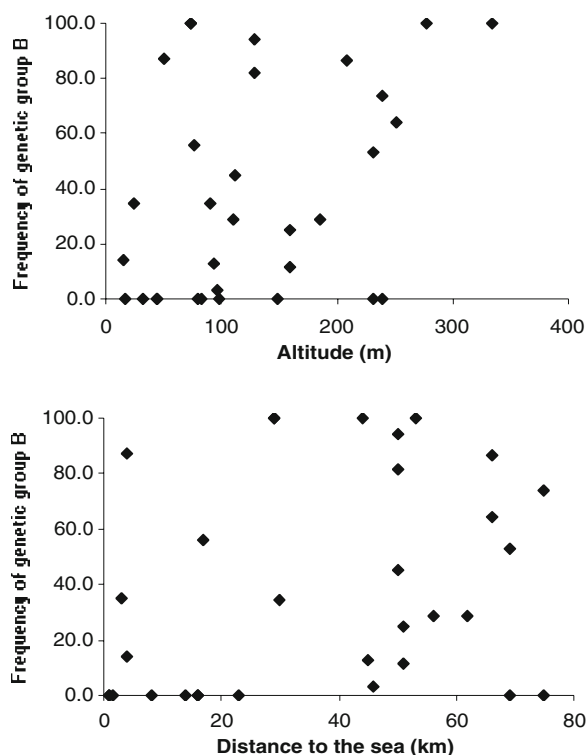


Fig. 3 Scatter-plots showing the relationship between frequency of *E. necator* genetic group B and altitude (at the top of the figure) or distance to the sea (at the bottom of the figure). Spearman's rank correlation $\rho=0.288$, $P=0.110$ for altitude and Spearman's correlation $\rho=0.305$, $P=0.090$ for distance to the sea

frequencies of group A; thus, even populations composed of 100% A at the start (BRU₁, FIT, LDA₂, POV, THU and TRE) were 100% B at the end of the epidemic (Table 1 and Fig. 4).

A strong relationship was observed between the disease levels on leaves and clusters, estimated at the end of the growing season in 13 fields, and the initial frequency of genetic group B (Spearman's rank correlation $\rho=0.905$, $P<0.001$ for damage on clusters and Spearman's correlation $\rho=0.756$, $P=0.003$ for damage on leaves). Every vineyard from which only B isolates were detected at the onset of the epidemic had a high final severity of disease (disease score >2); whereas vineyards infected by *E. necator* populations including group A isolates (from 26.1% to 100%) had a low final disease severity (disease scores 0 or 1; Table 1 and Fig. 5).

Discussion

The spatial genetic analysis of flag-shoot symptoms sampled early in the season revealed the absence of aggregation of genetic groups at the vineyard scale in southern France. This result indicates that a genetic group was not more likely to occur in a vineyard if it was close to other fields including *E. necator*

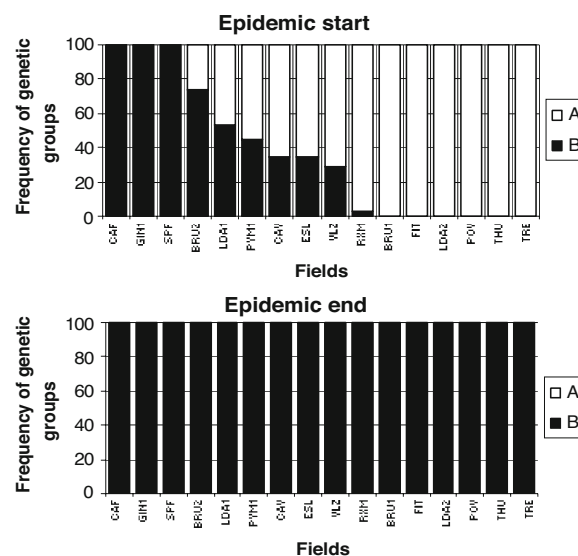


Fig. 4 Frequency of *E. necator* genetic groups, in the 16 fields sampled twice over the growing season, at the epidemic start (at the top of the figure) and at the epidemic end (at the bottom of the figure)

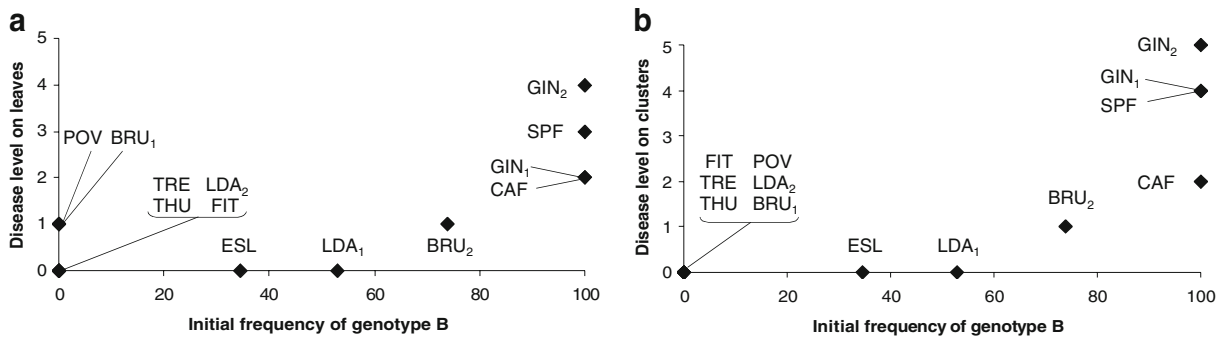


Fig. 5 Relationship between the powdery mildew levels on leaves (A) and clusters (B), estimated (using a 0–5 category scale that takes into account incidence of vine stocks diseased and global symptom severity) at the end of the growing season in 13 fields, and the initial frequency of genotype A, observed

at the beginning of the growing season. Field names (*three-letter code*) are indicated on the graph. Spearman's rank correlation $\rho=0.905$, $P<0.001$ for damage on clusters and Spearman's correlation $\rho=0.756$, $P=0.003$ for damage on leaves

populations of the same group. It is worth noting that our results do not exclude the possibility of within-field aggregation of genetic groups. For instance, Cortesi et al. (2004, 2008) showed that flag-shoot symptoms were aggregated between consecutive years at the within-field scale, as a result of short distance dispersal of conidia from flag-shoots. At the spatial scale studied here, the two genetic groups of *E. necator* appeared randomly distributed, and neither the altitude nor the distance to the sea correlated with their spatial distribution. These results confirm previous data showing inter-vineyard heterogeneity in southern France on a smaller number of populations (Amrani and Corio-Costet 2006; Bouscaut and Corio-Costet 2007), and invalidate the hypothesis of a niche partitioning due to a geographic separation of the groups. Mechanisms underlying such spatial heterogeneity of genetic group distribution have not yet been identified: they could include effects of fungicide treatments, pruning modes, microbial antagonists, or local environmental conditions (micro-climate, soil) that could differentially favour genetic groups of *E. necator*. Since all samples were collected on cv. Carignan, our results indicate that this pattern cannot be associated with an effect of the host cultivar. The spatial heterogeneity of genetic group proportions between vineyards could also reflect genetic drift operating through extinction–recolonisation dynamics characterising plant–pathogen metapopulations (Thrall and Burdon 1997; Laine and Hanski 2006). However, Cortesi et al. (2004, 2008) showed that flag-shoot symptoms are aggregated between consecutive years, suggesting that *E. necator* flag-shoot populations are

not characterised by strong extinction events. Since flag-shoot symptoms are aggregated from year to year, we can hypothesise that the within-field frequency of genetic groups estimated from flag-shoot symptoms could be stable over seasons.

While the spatial distribution of genetic groups of *E. necator* proved to be random, the temporal dynamics of groups was the same in all fields: the monitoring of 16 different fields showed that group A isolates were active only at the beginning of the growing season and disappeared during the course of the epidemic; by contrast group B isolates are active during the entire epidemic and were responsible for late infections. Our results support a temporal differentiation of niches of the two groups. The temporal succession of *E. necator* genetic groups has already been observed in France (Délye et al. 1999) and Italy (Miazzi et al. 2003), in 1998 and 1999, respectively. Although these studies were based on small sample sizes, these authors did not find any group A isolates after June in their samples. Since results obtained from different regions and different years have evidenced the decline of group A isolates during the course of the epidemics, our finding might not be due to environmental conditions specific for the 2007 growing season. Moreover, our results based on a set of 659 genotyped isolates collected from flag-shoot symptoms, confirmed that both genetic groups are able to over-winter asexually in buds (Cortesi et al. 2005; Péros et al. 2005; Nuñez et al. 2006; Willocquet et al. 2007). Our data suggest that only B isolates could produce cleistothecia *via* sexual reproduction in vineyards, which takes place at the

end of the growing season (Gadoury and Pearson 1988). Thus, at the beginning of the growing season, group A isolates ensure their over-wintering survival by colonising dormant buds for the next year shoots (Pearson and Gärtel 1985), whereas group B isolates may survive during winter both within dormant buds and *via* cleistothecia. The temporal isolation of genetic groups evidenced in our study is a potential mechanism which, by preventing recombination between A and B isolates, can explain the maintenance of the highly differentiated genetic structure in *E. necator* populations. Other studies suggest that recombination between groups of *E. necator* might occur: first, Miazzi et al. (2003) and Stummer and Scott (2003) showed that controlled sexual crosses between A and B genetic groups were viable and fertile; second, Cortesi et al. (2005) have shown the presence of both mating types at 1:1 ratios in natural populations of group A; third, Delmotte et al. (unpublished data) observed some discrepancies between different markers of *E. necator* genetic groups that suggested the presence at low frequency of recombinant isolates between A and B. Finally, we propose that the maintenance of the differentiation between the two genetic groups could result both from the temporal isolation highlighted here (prezygotic isolation), and possibly, from a lower fitness of recombinant isolates (postzygotic isolation). Determining more accurately the temporal dynamics of genetic groups of *E. necator* will require the collection of isolates over the course of an epidemic and a pluriannual monitoring of the same plots in order to also explore the dynamics of *E. necator* genetic groups over seasons.

Our data showed that damage due to *E. necator* on leaves and clusters was less important in commercial vineyards where epidemics started with populations including A isolates than in vineyards showing flag-shoot symptoms caused by group B isolates. The strong association between disease severity at the end of the growing season and the initial composition of the populations raises new questions with both practical and theoretical interests. A hypothesis to explain the association between the initial frequency of A and B groups and damage on clusters at harvest could lie in a difference in aggressiveness on berries between *E. necator* genetic groups. Because the susceptibility period of clusters is restricted to about two weeks after bloom (Gadoury et al. 2003), and

assuming a higher aggressiveness of B isolates on berries, the genetic composition of *E. necator* populations during the susceptibility period of clusters could be the major factor driving damage on berries at harvest. Thus, an initial attack by a population mainly composed of group B isolates (aggressive on berries) would cause severe damage at harvest; conversely, if group B isolates increase in frequency only later (*i.e.*, when the ontogenic, or age-related resistance of clusters is active) then the epidemic will cause little or no damage at harvest. Such a difference in aggressiveness was observed between two genetically distinct groups of the plant pathogen *Gaeumannomyces graminis* var. *tritici* (Ggt). Aggressiveness (defined as the disease severity that reflects the extension of root necrosis) of group G2 (38%) was significantly greater than that of group G1 (29.5%) in controlled laboratory tests (Lebreton et al. 2004). Moreover, a linear relationship between G2 frequency within Ggt populations and disease severity at stem elongation was measured during three cropping seasons (Lebreton et al. 2007). Interestingly, the ontogenic resistance of leaves (Doster and Schnathorst 1985) is less limited in time because of the continuous growth of the vine. This might explain why the association between the frequency of genetic groups and disease levels was slightly stronger on clusters than on leaves. In order to test our hypothesis, further experiments are needed to investigate the aggressiveness of each *E. necator* genetic group on leaves and berries. Moreover, because our observations were based on a limited number of vineyards/populations and did not take into account chemical protection, it will be necessary to follow the epidemic development on leaves and clusters in crops showing different frequencies of genetic *E. necator* groups and with standardised farming methods. A landscape genetic approach will help to determine ecological factors involved in the temporal and spatial genetic variability of *E. necator* populations. The identification of factors favouring one group over another will provide useful information for an integrated crop management with limited fungicide use.

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