Can early population structure of *Erysiphe necator* inform about the disease level on bunches?

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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, while group B isolates would survive as ascospores released from overwintering cleistothecia. 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, Montarry *et al.* 2008). 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. 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; Montarry *et al.* 2008).

Here, our aim is to study the regional dynamics of *E. necator* genetic groups at a large spatial scale. We conducted a landscape genetic approach combining landscape epidemiology and population genetics 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?

Material and Methods

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. This led to a total of 1,253 leaves infected with *E. necator* , of which 769 were sampled in April and 484 in September.

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, Montarry *et al.* 2008, Montarry *et al.* 2009).

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%.

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. 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 (Figure 1).

Figure 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.

All isolates collected at the end of the growing season belonged to group B, whatever the initial 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 (Figure 2).
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) (Figure 3).

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* populations of the same group. 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), and invalidate the hypothesis of a niche partitioning due to a geographic separation of the groups.

Figure 2: Temporal distribution of genetic groups of *E. necator* in the 16 fields sampled in the south of France.

Figure 3: the disease levels on leaves and clusters, estimated at the end of the growing season in 13 fields, and the initial frequency of *E. necator* genetic group B.

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.
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 flagshoot symptoms, confirmed that both genetic groups are able to overwinter asexually in buds (Cortesi *et al.* 2005; Péros *et al.* 2005; Nuñez *et al.* 2006). 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.
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 flagshoot 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, 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.

Interestingly, the ontogenic resistance of leaves 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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