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## Niche partitioning of the genetic lineages of the oak powdery mildew complex

Nicolas FEAU<sup>a,\*</sup>, Aurélien LAURON-MOREAU<sup>a</sup>, Dominique PIOU<sup>a,b</sup>, Benoît MARÇAIS<sup>c</sup>, Cyril DUTECH<sup>a</sup>, Marie-Laure DESPREZ-LOUSTAU<sup>a</sup>

<sup>a</sup>INRA, UMR 1202 BIOGECO, 69 route d'Arcachon, 33612 Cestas Cedex, France

<sup>b</sup>Ministère de l'Agriculture, de l'Alimentation et de la Pêche, DGAL-SDQPV, Département de la Santé des Forêts, Paris Cedex, France

<sup>c</sup>INRA, Nancy Université, UMR 1136 Interactions Arbres/Microorganismes, IFR110, 54280 Champenoux, France

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### ABSTRACT

Recent molecular studies have shown that four genetic lineages, probably cryptic species, cause oak powdery mildew in Europe. We tested the hypothesis of niche differentiation between the two most frequent species (*Erysiphe alphitoides* and *Erysiphe quercicola*), by determining their relative prevalences at various times during the growing season. *E. quercicola* was strictly associated with the flag-shoot symptom in a 2-yr sampling campaign of 35 natural oak seedling populations. Additional sampling during Aug.–Sep. in the same stands showed that *E. alphitoides* predominated in most (70 %) cases. Time-course monitoring of powdery mildew in two natural regenerating oak populations confirmed the inverse patterns of relative abundance for these two species in early and late season. The coexistence of these two closely related species may be due to the use of different strategies, resulting from a trade-off between overwintering (flag-shoots vs. chasmothecia) and late-season performance.

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### Introduction

In plant pathology studies, it is frequently found that a single plant species may be infected with several (sometimes closely related) pathogens, apparently contradicting the ecological niche theory (Fitt *et al.* 2006). According to this theory, identical competitors should not coexist (Gause 1934; Fitt *et al.* 2006), and the number of coexisting species cannot exceed the number of limiting resources (Sommer 1999). However, studies on plant-pathogen species complexes have shown that cryptic species frequently occur on the same host but generally in slightly different niches (Brasier 1987; Gudelj *et al.* 2004; Fitt *et al.* 2006). These niche differences may be engendered by subtle differences in the biological/epidemiological

features of the related species, leading to a separation in space, time and/or on resource use (Chesson 2000; Amarasekare 2003; Fitt *et al.* 2006).

The Erysiphales (Ascomycota) is an important group of obligate biotrophic plant pathogenic fungi that cause “powdery mildew” on about 10 000 species of angiosperms (Amano 1986; Glawe 2008). Recent molecular taxonomy studies have shown that powdery mildews are more diverse and have a more complex biology than previously suspected. Several examples of complexes of morphologically indistinguishable taxa forming genetically differentiated clusters and coexisting on the same host have been described for this group of organisms (Amrani & Corio-Costet 2006; Seko *et al.* 2008; Takamatsu *et al.* 2009). For instance, molecular data for

\* Corresponding author.

E-mail address: [feaunico@mail.ubc.ca](mailto:feaunico@mail.ubc.ca) (N. Feau).

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nuclear ribosomal internal transcribed sequences (*ITS*) have suggested that at least four genetic lineages are involved in oak powdery mildew in European forests (Mougou *et al.* 2008; Desprez-Loustau *et al.* 2011). High levels of similarity were found between these *ITS* sequences and those obtained from three morphologically defined species found on oaks in Europe—*Erysiphe alphitoides*, *Erysiphe hypophylla* and *Phyllactinia guttata*—and a fourth species, *Erysiphe quercicola*, recently described in Asia (Takamatsu *et al.* 2007; Mougou *et al.* 2008; Desprez-Loustau *et al.* 2011). Furthermore, multigene phylogeny studies have shown that two of these taxa, *E. alphitoides* and *E. quercicola*, belong to different phylogenetic species (Feau *et al.* 2011).

Powdery mildew is a very common disease of pedunculate (*Quercus robur*) and sessile (*Quercus petraea*) oaks in Western Europe. This disease can be very severe, particularly in seedlings growing in naturally regenerating forest, decreasing the growth of the plant and causing its death in some cases (Butin 1995). Investigations of the distribution of the four taxa of the oak powdery mildew complex in France have revealed no clear pattern of spatial niche differentiation (Mougou-Hamdane *et al.* 2010). The most frequent species (*E. alphitoides*, with a prevalence of approximately 80%), generally coexists with the other three lineages, regardless of the scale considered (i.e. region, altitude, tree, or even lesion) (Mougou-Hamdane *et al.* 2010). Furthermore, no preferential association was found between any of these species and the three different oak species sampled—pedunculate, sessile and Pyrenean (*Quercus pyrenaica*) oak—in France (Mougou-Hamdane *et al.* 2010). The occurrence of these different sibling species of *Erysiphe/Phyllactinia* together raises questions about the mechanisms underlying their coexistence on oaks.

Niche partitioning due to a temporal separation of related plant pathogenic fungi could potentially account for the coexistence of these species on the same host plant (Gudelj *et al.* 2004; Fitt *et al.* 2006). Differences in the ecological strategies used to reinitiate the infection cycle after overwintering may be responsible for this temporal succession of genetically differentiated groups. For example, several studies have shown that different genetic groups of *Erysiphe necator* (biotypes A and B), the causal agent of grapevine powdery mildew, are associated with different ecological features (Delye *et al.* 1997; Delye & Corio-Costet 1998; Miazzi *et al.* 2003) resulting in the temporal niche partitioning of these groups (Cortesi *et al.* 2005; Péros *et al.* 2005; Núñez *et al.* 2006; Montarry *et al.* 2008). Biotype A strains overwinter in buds more frequently than biotype B strains and are active only at the start of the growing season, whereas biotype B strains are found on leaves or berries in late summer (Fitt *et al.* 2006; Montarry *et al.* 2008; Montarry *et al.* 2009). Oak powdery mildew fungi are widely thought to survive from one growing season to the next by generating chasmothecia, the sexual fruiting bodies (Marçais *et al.* 2009), or by mycelia infecting shoot primordia under bud scales (Neger 1915; Yarwood 1957; Kerling 1966). Although not clearly demonstrated, it is widely accepted that the resting mycelium within dormant buds reinitiates growth after bud break, colonizing young “flag-shoots” with a dense sporulating mycelium (Kerling 1966).

Based on these observations, we hypothesized that the different genetic lineages causing oak powdery mildew might

have different preferential overwintering modes associated with different strategies for the colonization and use of the same resource. For example, some species may overwinter as conidia, with the mycelium in the buds infecting shoots and leaves early in the season, whereas others may overwinter as chasmothecia and infect oak leaves later. Differences in winter survival would then result in differences in the temporal dynamics of epidemics, given that flag-shoot infections occur early in spring and sexual ascospores are generally released from chasmothecia later, after bud-burst (Marçais *et al.* 2009; Desprez-Loustau *et al.* 2010). The susceptibility of oak leaves is restricted to a few days after bud break (Woodward *et al.* 1929; Edwards & Ayres 1982; Butin 1995), so the phenological synchrony of host and pathogen is critical in this system (Desprez-Loustau *et al.* 2010). A species overwintering in oak buds would have the advantage of being perfectly synchronized with the susceptible leaves of the first growth unit. A better late-season performance of the species colonizing the plant later would account for the coexistence of the two species despite the competitive advantage of the species overwintering in the buds.

We tested these hypotheses, by monitoring the occurrence of oak powdery mildew lineages with specific PCR–RFLP markers during the time-course of oak powdery mildew epidemics, in a large sampling campaign of oak seedling populations from various French regions. We specifically investigated the relationships between the species of the oak powdery mildew complex and symptom types, including flag-shoot symptoms in particular.

## Materials and methods

### Field survey and fungal material

**Regional sampling:** Naturally regenerating oak stands (seedling height from 10 to 200 cm; mean 33 cm  $\pm$  16 cm) in various regions of France were monitored by technicians of the DSF (Département de la Santé des Forêts, – Ministry of Agriculture, France) for the presence of flag-shoot symptoms (most of the oaks sampled were *Q. robur* or *Q. petraea*). In 2008, 24 plots were monitored for the presence of flag-shoot symptoms. In 2009, the survey was increased to 35 plots, including all those monitored in 2008. Each plot was observed once, shortly after bud-burst. The sampling dates therefore differed between the different regions, ranging from Apr. 27 to May 16 in 2008, and from Apr. 27 to May 20 in 2009. We scored 100 m transects, recording the number of flag-shoot symptoms and the mean density of regeneration (number of seedlings m<sup>-2</sup>).

In 2008, flag-shoots were collected from four of the 24 regenerating stands monitored (Grandpré P168, Belles Forêts, Villy le Moutier and Vitry aux Loges). In 2009, flag-shoots were systematically collected in the 35 regenerations monitored. We collected up to 10 flag-shoots per transect (when present) and sent them to the laboratory for analysis. A second sampling was performed in Jul./Aug. in all these plots, with five to 10 plants (one to three diseased leaves per plant) selected at random and collected from the three transects. On arrival at the laboratory, each leaf sample was examined and the quality and extent of infection were assessed with a scale

based on leaf surface darkening (which was not unusual in these young leaves after a few days of transport) and infection. Leaves were dried for subsequent analysis.

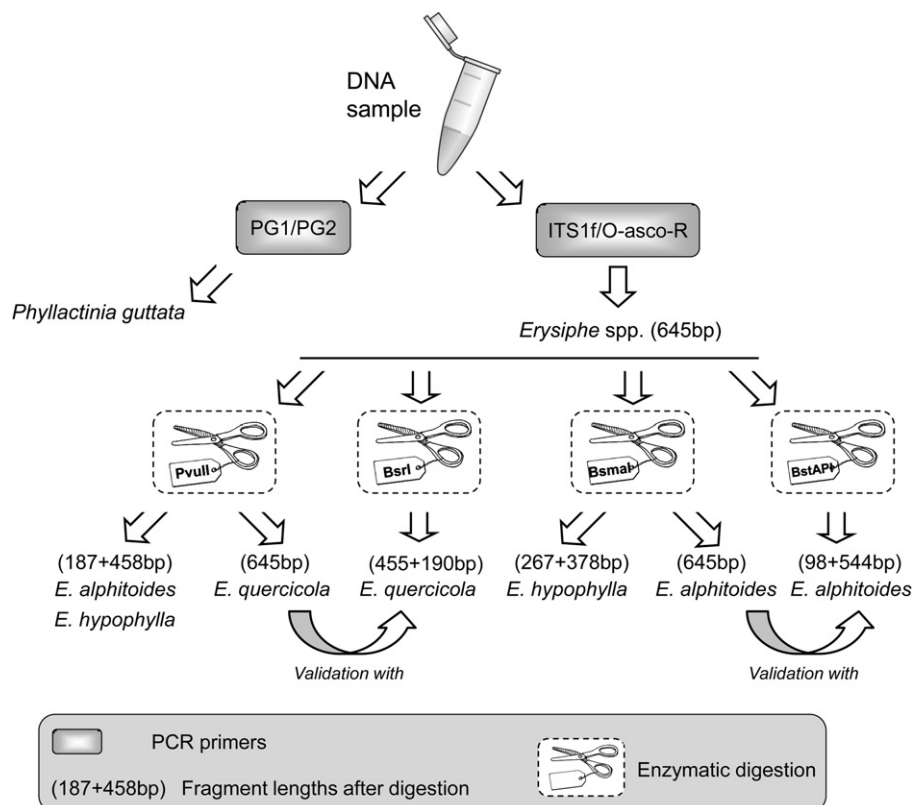
**Local epidemic time-course monitoring:** In 2009, we performed epidemic time-course surveys in two naturally regenerating oak stands at Pierroton, South–West France (latitude 44°44′13″ N, longitude 00°46′35″ E) and Vierzon, Central France (latitude 47°15′45″ N, longitude 02°06′02″ E), by sampling one leaf on each of 10–50 infected plants on five to 10 different dates during the course of an oak powdery mildew epidemic. Infected plants were selected at random in these ~800–1 000 m<sup>2</sup> plots. Both time-courses began with the collection of flag-shoots (Apr.–May), the first visible sign of the disease, immediately after bud-burst, followed by lesions on leaves, and ending with the sampling of chasmothecia in Sep.–Oct. The stand at Pierroton is a regenerating plot of *Q. robur* with a mean density of 20 oak saplings m<sup>-2</sup>. In this stand, at each sampling date, we collected only freshly infected leaves, to ensure that only recent infections were targeted. Plant density at Vierzon was 90–100 saplings per m<sup>-2</sup>, with a species composition of 10–40 % *Q. robur* and 60–90 % *Q. petraea*. In this second stand, we sampled recent and more long-standing infections at random on the various sampling dates.

### Molecular identification

DNA was extracted directly from the lesions on infected leaves, without prior subculture of the fungus. For each leaf

sample, fungal material (mostly mycelia and conidia [anamorphic stage]) was scraped from a single lesion (~0.5 cm<sup>2</sup>) with a scalpel; most isolates contained some host tissue (e.g. cuticle tissues and trichomes). The material collected was then freeze-dried overnight and homogenized in a steel ball mill (TissueLyser II, Retsch, Qiagen®). Genomic DNA was extracted by the hexadecyltrimethylammonium bromide (CTAB) method, as described by Zolan & Pukkila (1986). Total DNA was then diluted 50-fold in Nanopure water and used as a template for polymerase chain reaction (PCR) amplifications.

We distinguished between the *Phyllactinia* and *Erysiphe* internal transcribed spacer (ITS) sequences by amplification with genus-specific oligonucleotide primers (Fig 1). For *Phyllactinia* sp., we used the PG1/PG2 primers described elsewhere (Mmbaga 2000; Mougou et al. 2008). The ITS1f and O-asco-R primers were used for the amplification of sequences from *Erysiphe* sp. by PCR (Gardes & Bruns 1993; Heuser & Zimmer 2002). In both amplifications, the PCR mixture contained 2 µl of DNA added to 20 mmol l<sup>-1</sup> Tris–HCl, 50 mmol l<sup>-1</sup> KCl, 2 mmol l<sup>-1</sup> MgCl<sub>2</sub>, 0.2 mM dNTP, 0.2 µmol l<sup>-1</sup> of each primer and 0.07 U of SilverStar Taq DNA polymerase (Eurogentec). PCR amplification was carried out in a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems), under the following conditions: initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 70 s, and a final extension at 72 °C for 5 min. PCR products were visualized by electrophoresis in 1 % agarose gels stained with ethidium bromide.



**Fig 1** – Cleaved amplified polymorphic sequence [CAPS] markers used in this study to identify strains of *Erysiphe alphitoides*, *E. quercicola*, *E. hypophylla* and *Phyllactinia* sp.

We then used cleaved amplified polymorphic sequence (CAPS) markers to discriminate between the ITS types associated with *E. alphitoides*, *E. quercicola* and *E. hypophylla* (Fig 1). The PCR products obtained with the ITS1f/o-asc-rev primers were digested with three restriction enzymes: *PvuII*, *BsmAI* and *BsrI* (New England Biolabs). When the ITS type associated with *E. hypophylla* was detected (digestion with both *PvuII* and *BsmAI*), the *BstAPI* enzyme (SibEnzyme) was used to detect the possible co-occurrence of *E. alphitoides*. Restriction reactions were performed at 37 °C (*PvuII*), 55 °C (*BsmAI*), 65 °C (*BsrI*) or 60 °C (*BstAPI*), in a total volume of 10 µl consisting of 4 µl of PCR product, 1× reaction buffer, 4.8 µl Nanopure water, and 0.2 U of enzyme. The digestion products were then resolved by electrophoresis in a 2 % agarose gel. Restriction fragment length polymorphism (RFLP) fragment sizes were estimated by comparison with the GeneRuler™ 1 kb DNA ladder (Fermentas).

## Results

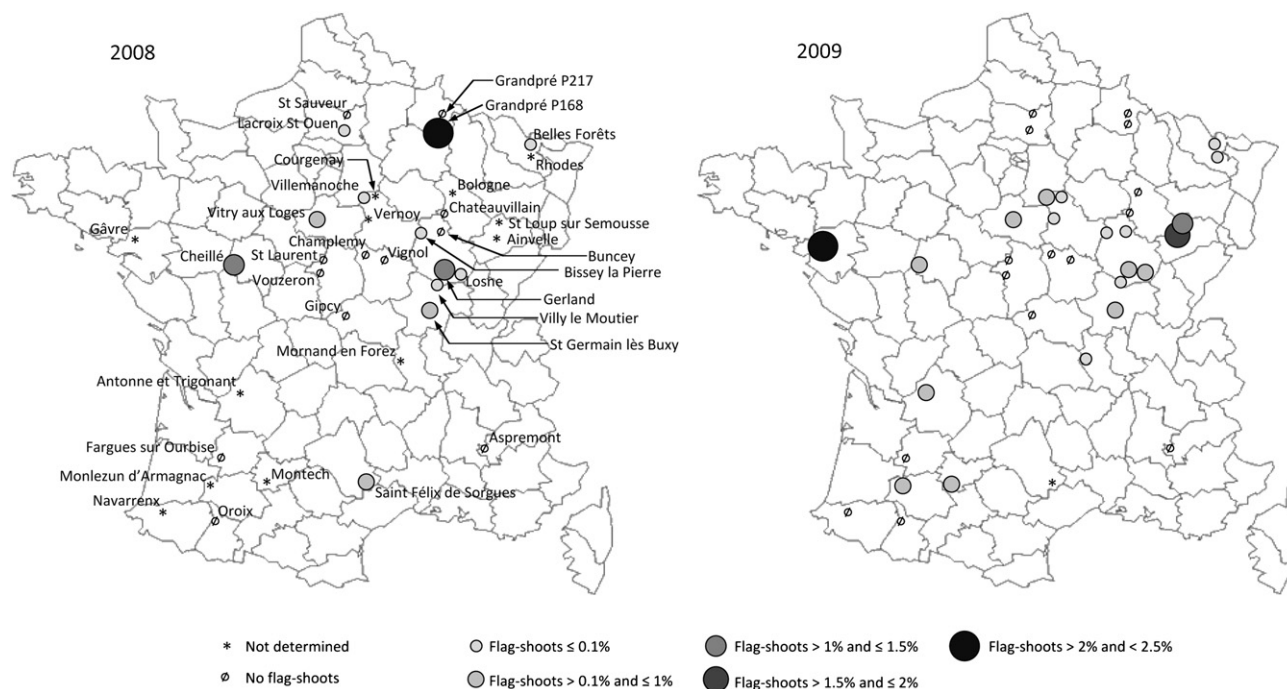
### Flag-shoot survey

Flag-shoot symptoms were observed in 12 of the 24 (50.0 %) oak plots surveyed in 2008 and 20 of the 35 (57.1 %) oak plots surveyed in 2009 (Fig 2). The weighted frequency of this type of infection (i.e. the number of flag-shoots divided by the number of seedlings in the plot) was  $0.30 \pm 0.61$  in 2008 and  $0.24 \pm 0.48$  in 2009. The maximum frequency of flag-shoots was that in Cheillé in 2008 (408 flag-shoots, corresponding to a frequency of 1.42 %) and Gâvre in 2009 (339 flag-shoots; 2.19 %). The frequency of flag-shoots at plot level remained stable from year-to-year (Spearman's rank correlation:

$r = 0.70$ ,  $t = 4.49$ ,  $df = 22$ ,  $P$ -value  $< 0.001$ ), although strong differences were found on the Grandpré P168 plot (2.46 % in 2008 vs. 0 % in 2009) and at Vitry aux Loges (0.97 % in 2008 vs. 0.15 % in 2009) (Fig 2). No clear geographic pattern of flag-shoot occurrence was observed at the national scale, in either of the 2 yr (Fig 2). At the local scale (i.e. within each plot), the frequencies of flag-shoots were generally similar in the three transects studied. ANOVA (with "plot" and "transect" considered as random effects) showed a significant plot effect ( $df = 34$ ,  $F = 9.69$ ,  $P < 0.0001$ ) but no significant transect effect within plots ( $df = 86$ ,  $F = 0.39$ ,  $P = 1.0$ ).

### Molecular identification

The oligonucleotide primer pairs ITS1f/O-asco-R and PG1/PG2 amplified a single DNA fragment (597 bp for *Phyllactinia* sp. and 645 bp for *Erysiphe* sp.) for 60.2 % of the 1 335 powdery mildew isolates studied. The quality of the DNA extracted and the success of the PCR subsequently carried out depended strongly on both the quality and the level of infection of the sample (Supplementary Fig 1). Based on enzymatic digestions of the PCR product obtained with ITS1f/O-asco-R, 54 % of the strains contained ITS types corresponding to *E. alphitoides*, 33 % to *E. quercicola*, 12 % contained mixed types corresponding to *E. alphitoides*/*E. quercicola*, *E. hypophylla* and *P. guttata* ITS types (less than 1 %, each) were extremely rare and always detected in mixed infections with *E. alphitoides* and/or *E. quercicola*. The identifications based on these digestion profiles were confirmed by sequencing 14 pure samples for *E. quercicola* and *E. alphitoides* (GenBank Acc. nos. JN564751–JN564765) and two mixed samples for *E. hypophylla*/*E. alphitoides*. Blastn searches with the sequences obtained for the *E. quercicola* profiles revealed matches to the ITS sequence

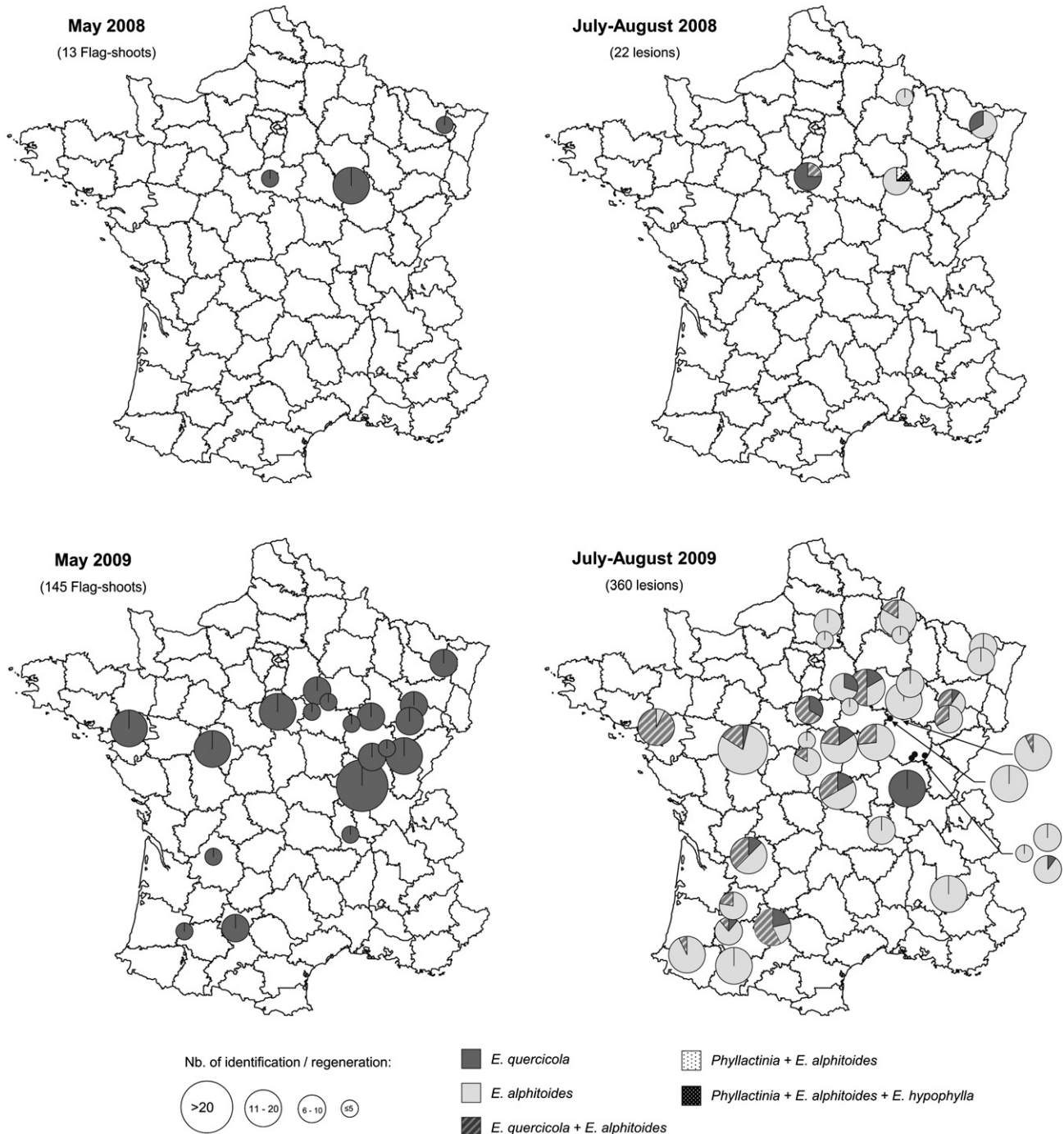


**Fig 2 – Mean frequencies of the flag-shoot symptom observed in the regenerating oak plots surveyed in 2008 (24 plots) and 2009 (35 plots). All the plots are indicated, together with their full name, on the 2008 map, to facilitate their localization.**

of *E. quercicola* holotype MUMH885 (Takamatsu et al. 2007) (Acc. No. AB193591; 100 % identity over the entire length of the alignment, E-value = 0.0). Similarly, searches carried out with the sequences obtained for *E. alphitoides* profiles perfectly matched the ITS sequence of the *E. alphitoides* neotype MUMH631 (Acc. No. AB193591; 100 % identity over the entire length of the alignment, E-value = 0.0).

**Regional sampling:** All flag-shoot samples collected in May 2008 and 2009 presented ITS digestion profiles corresponding to *E. quercicola* (19 plots, 145 flag-shoots) (Fig 3). *E. alphitoides*,

*E. hypophylla* and *Phyllactinia* sp. were never found in these flag-shoot samples, even in mixtures with *E. quercicola*. By contrast, both *E. alphitoides* and *E. quercicola* ITS profiles were found when sampling was performed on the same plots in Jul./Aug./Sep. At this stage of the season, the ITS type associated with *E. alphitoides* was the most frequently detected (in 64 % and 74 % of the sampled lesions in 2008 and 2009, respectively). The ITS type associated with *E. alphitoides* was identified at a frequency of 100 % in 16 of the 36 regenerating plots surveyed in 2009 (44 %), despite the detection of flag-



**Fig 3 – Frequencies of the various oak powdery mildew lineages determined for the sampling campaigns performed in May and Jul./Aug. 2008 (four plots sampled) and 2009 (35 plots).**

shoot symptoms (associated with the *E. quercicola* ITS type) in half of these stands early in the growing season. At most of the remaining sites (18 of 20), we detected both *E. quercicola* and *E. alphitoides*, with *E. alphitoides* the more frequent of the two. Mixed lesions of *E. alphitoides* and *E. quercicola* were found in 39 % of the 171 samples analyzed at these 20 sites. The composition of oak powdery mildew populations at the end of the season seems to depend on the initial frequency of flag-shoot symptoms early in the season, because the frequencies of the *E. quercicola* ITS type in Jul./Aug./Sep. were generally higher on the plots with the highest frequencies of flag-shoot symptoms (Spearman's rank correlation = 0.42, two-tailed P-value < 0.02). The frequency of lesions associated with *E. quercicola* was greater than that of lesions associated with *E. alphitoides* on only one plot in 2008 (Vitry aux Loges, *E. quercicola* in 100 % of lesions and *E. alphitoides* in only 25 %) and two plots in 2009 (Vitry aux Loges [100 % of lesions with *E. quercicola* and 67 % with *E. alphitoides*] and St Germain lès Buxy [100 % *E. quercicola*]). In these last two plots, we also looked for leaves carrying sexual fruit bodies in Oct. 2009. The six chasmothecia collected were all identified as being of the *E. alphitoides* type, with the molecular method described above.

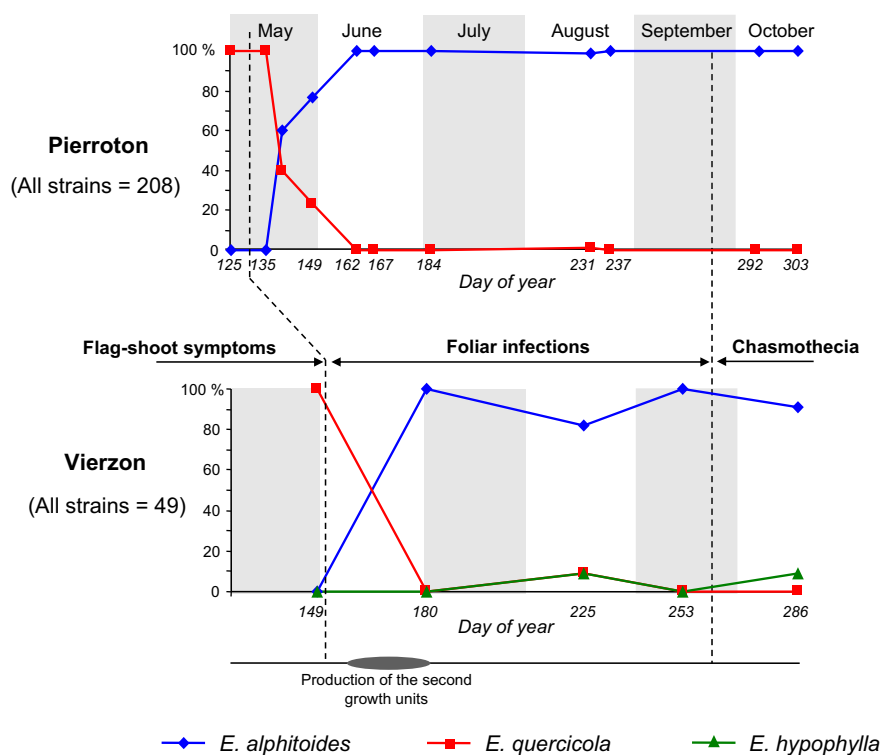
**Local epidemic time-course monitoring:** Time-course studies of the Vierzon and Pierroton stands (49 and 208 powdery mildew isolates yielding an ITS amplicon, respectively) indicated that the frequencies of oak powdery mildew species gradually changed during the course of the epidemic (from May to Oct.; Fig 4). At the first sampling (5 May at Pierroton and 24 d later at Vierzon), the only symptoms of powdery mildew present were flag-shoots, which were randomly distributed within plots

and affected 0.05 % of the seedlings at Pierroton and 1.0 % at Vierzon. All shoots sampled at both sites yielded ITS sequences of the *E. quercicola* type. The relative frequency of this fungus in the infected leaves sampled rapidly decreased, this decrease being accompanied by an increase in the relative frequency of *E. alphitoides* (five sampling dates between May and Jun. in Pierroton), which reached a frequency of almost 100 % in Jul. All but one (identified as *E. hypophylla*) of the 31 chasmothecia harvested in Sep./Oct. from these two regenerating stands were identified as belonging to *E. alphitoides*.

## Discussion

Our results suggest that niche partitioning may account for the coexistence of the two species most frequently causing oak powdery mildew in France, *E. alphitoides* and *E. quercicola*. Although very closely related (less than 2 % divergence in ITS sequences; Takamatsu et al. 2007), these two species differed phenologically, probably due to differences in their modes of overwintering.

One striking result of our study is the strict association between flag-shoot symptoms and *E. quercicola*. We assessed the robustness of this finding by monitoring several stands in various regions in two different years (2008 and 2009). *E. quercicola* was systematically found to be associated with flag-shoots. It has been suggested that oak powdery mildew can survive from one growing season to the next as resting mycelium or conidia within dormant buds (van Peeteren 1912; Neger 1915). Woodward et al. (1929) and Kerling (1966)



**Fig 4 – Epidemic time-course surveys (May–Oct. 2009) on regenerating plots at Pierroton (latitude 44°44'13" N, longitude 00°46'35" E.) and Vierzon (latitude 47°15'45" N, longitude 02°06'02" E). The time period given for the production of the second growth units of oaks is that estimated in Marçais et al. (2009).**

observed the overwintering of mycelia in infested buds, from which malformed shoots developed in spring, the stem and leaves being covered with mycelium and conidia.

Previous surveys (Marçais et al. 2009; Mougou-Hamdane et al. 2010) and additional observations in this study suggest that the sexual stage of *E. quercicola* (initially described on *Quercus phillyraeoides* in Asia; Takamatsu et al. 2007) is absent or rare in European populations, consistent with the survival of this species principally through the completion of an asexual cycle involving overwintering in dormant buds. The biological cycle of *E. alphitoides* seems to be different from that of *E. quercicola*, with chasmothecia playing a major role, through the release of sexual ascospores in spring. This primary inoculum infects leaves, leading to the production of conidia and complete asexual cycles involving the re-infection of oak leaves. Recent advances in the development of intra-specific markers, such as microsatellite markers (Malausa et al. 2011), should make it possible to assess the degree of clonality in *E. alphitoides* and *E. quercicola* populations and will improve our understanding of the contribution of the sexual and asexual modes of overwintering described here for the two species.

We assumed that these crucial differences in the initiation of the pathogen life cycle would result in temporal differentiation, allowing the two species to coexist on the same host. The coexistence of closely related sympatric genetic lineages adopting different ecological strategies to colonize the same host plant has been observed in other powdery mildew pathosystems. Maple powdery mildew, which is caused by *Podosphaera leucotricha*, forms two major genetically differentiated clusters, probably reflecting differences in the mode of overwintering: as mycelium in dormant buds or sexual chasmothecia (Urbanietz & Dunemann 2005). In the grape powdery mildew pathosystem, an association between flag-shoot symptoms and infection with a specific genetic group of *E. necator* strains (i.e. "A" strains) was initially suggested on the basis of the findings of a regional survey (Delye et al. 1997; Delye & Corio-Costet 1998). However, this hypothesis was subsequently ruled out in more comprehensive studies (Núñez et al. 2006). Both *E. necator* biotypes (i.e. A and B) were found to induce flag-shoot symptoms, although this occurred more frequently with biotype A strains, and a difference was observed in the temporal dynamics of the two biotypes over the course of the epidemic season. Biotype A strains are active only at the beginning of the growing season, gradually disappearing over the course of the epidemic, whereas B strains are found on leaves or berries in late summer (Montarry et al. 2008; Montarry et al. 2009). The oak and grapevine powdery mildew pathosystems are similar in several ways, but there are also key differences between them. In oak, only the young developing leaves produced in successive flushes are susceptible to infection (Woodward et al. 1929; Edwards & Ayres 1982; Doster & Schnathorst 1985; Butin 1995; Marçais et al. 2009; Desprez-Loustau et al. 2010). In this context, phenological synchrony between the host and the pathogen in spring is expected to play a critical role in the initiation of annual epidemics and, therefore, in the severity of the disease (Desprez-Loustau et al. 2010). By contrast, synchrony between grapevine and *E. necator* is not essential, because the

vine grows continually, generating susceptible leaves throughout the growing season.

The importance of phenological synchrony for oak powdery mildews may be crucial for the coexistence of the components of the species complex. Indeed, whereas the primary inoculum produced from flag-shoots ensures temporal synchrony with the development of the first growth units on oak, peaks of ascospore production from chasmothecia generally occur 30–50 d after bud-burst on the first growth unit, resulting in little infection of the first growth unit but providing inoculum for the second growth unit in particular (Marçais et al. 2009; Desprez-Loustau et al. 2010). Thus, predominant overwintering in buds should confer an advantage on *E. quercicola* by allowing this species to colonize susceptible developing leaves early in the growing season in absence of low competition with *E. alphitoides*. However, this early development of *E. quercicola* does not allow this species to outcompete *E. alphitoides* effectively later in the growing season, as *E. alphitoides* establishes itself as the largely predominant species during the summer.

This inversion of the abundance pattern at the end of the season strongly suggests a trade-off between overwintering mode and late-season performance. The potential advantage of a few concentrated sources of inoculum for *E. quercicola* (infrequent flag-shoots) may be more than counterbalanced a few weeks later by the presence of many infection points created by wind-borne ascospores of *E. alphitoides* scattered in the host population. Indeed, we found that the relative frequencies of *E. quercicola* at the end of the growing season seemed to be related to the initial frequency of flag-shoots (and thus to the abundance of subsequent inoculum) early in the season. Alternatively, the observed distribution may reflect the competitiveness of the two species, especially in regards to changing environmental conditions during the season.

Response to temperature may be an important factor, although other climatic components, such as rainfall and humidity, may also play a role in fungal spore survival, dispersion and germination, thereby influencing the balance between two species within a fungal complex (see for example Huang et al. 2001, 2003). Differences in temperature optima for spore germination and/or hyphal growth have already been reported for several species from species complexes, such as the rapeseed pathogens *Leptosphaeria biglosa/Leptosphaeria maculans* (Huang et al. 2001; Huang et al. 2003; Fitt et al. 2006), genetic groups of the entomopathogenic fungus *Metarhizium anisopliae* (Bidochka et al. 2001) and sibling species within the *Ophiostoma piceae* species complex (Brasier & Stephens 1993). Based on the seasonal profiles observed in our survey, *E. alphitoides* strains may be better adapted to high summer temperatures. Conversely, *E. quercicola* strains may be better adapted to lower temperatures, allowing them to overwinter in oak buds and to be active mostly early in the growing season.

We observed flag-shoot symptoms in regions with cold winters, such as North–East France. This contrasts with observations for grapevine powdery mildew, where flag-shoots are much more frequent in Mediterranean (Amrani & Corio-Costet 2006). It is also possible that different species differ in their sensitivity to the biotic pressures exerted by



intracellular mycoparasites, such as *Ampelomyces* species, including *Ampelomyces quisqualis* (synonym *Cicinnobolus cesatii*) and *Ampelomyces quercinus* (Kiss 1997, 1998). Kiss et al. (2011) showed that the timing of the transmission of different *Ampelomyces* species depended on the life cycles of the powdery mildew species they parasitized.

Finally, direct competition between the two species might be restricted if *E. quercicola* exploits a slightly different resource niche, infecting newly formed buds in leaf axils during the season, rather than colonizing more developed leaves. Such colonization of newly formed buds has been documented for apple (Stephan 1991) and grapevine powdery mildew (Rumbolz & Gubler 2005) and was initially advocated by Woodward et al. (1929) for oak powdery mildew. The contamination of oak buds seems to concern mostly the highest buds on the spring shoots, thus occurring during the second period of powdery mildew activity, in Jul. and Aug. (Kerling 1966). Subtle differences in the epidemiological characteristics of these two fungi probably allow them to occupy slightly different niches and to coexist on the same oak resource in Western Europe.

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## Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.funeco.2011.12.003.

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