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Accepted Version

Relationships between Oak powdery mildew incidence and severity and commensal fungi.

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Keywords: powdery mildew, commensal fungi, interacting fungi, *Erysiphe alphitoides*

Summary

Oak (*Quercus robur*) powdery mildew is a common and damaging fungal disease. In a local survey at Reading, UK, oak powdery mildew was common on trees of all height-classes but was most common on trees of 3-9m. A variety of other fungal species were commonly found growing in association with oak powdery mildew colonies. The abundance of such fungi was estimated through stratified sample surveys for 2.5 years. The taxa most commonly associated with oak powdery mildew were *Acremonium* sp*., Trichoderma* sp*., Ampelomyces/Phoma* sp. and *Leptosphaerulina australis*. Nearly 90% of mildew colonies were associated with *L. australis,* which is not generally considered as a mycoparasite or antagonist, in contrast with the other three fungi. Abundance varied between June and October surveys. *Acremonium* sp. abundance was greater in summer samplings whereas *L. australis* and *Trichoderma* sp. abundances were greater in autumn samplings. *Ampelomyces/Phoma* sp. was never observed in the absence of powdery mildew. Relationships between the mildew-associated fungi and oak powdery mildew appeared curved and differed significantly between sampling years. *L. australis* was positively correlated with the other three associated fungi studied when powdery mildew was also present**.** The variety and high population densities of the mildew-associated fungi suggest that they may be important in determining the final density of oak mildew and the damage caused by it.

1. Introduction

Oak powdery mildew affects oaks of all species, ages and geographical origins, but the impact of the disease differs depending on the species. Three species are responsible for oak powdery mildew in Europe. The most common is *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam.. Far less frequent are *E. hypophylla* (Nevod.) U. Braun & Cunningt. and *Phyllactinia guttata* (Wallr.) Lév. (sensu lato, including *P. roboris* (Gachet) S. Blumer) (Mougou et al., 2008; Mougou-Hamdane et al., 2010). A fourth species, not previously reported in Europe, was recently found in France under the name *E. quercicola* (Mougou-Hamdane et al., 2010). *E. alphitoides* is the most prevalent and damaging species. The host range of *E. alphitoides* lies mainly in the genus *Quercus*, with *Q. robur* being the most susceptible species (Ayres, 1976; Ufnalski and Przybyl, 2004), and oak powdery mildew is ubiquitous and abundant on *Q. robur* in the British Isles. Infection by powdery mildew seriously reduces the life-span of leaves (Hajji et al. 2009). Seedlings and young oak trees in forests (as well as in nurseries and plantations) are very susceptible to the disease (Ufnalski and Przybyl, 2004); growth of young stands is substantially retarded; and infection may cause severe seedling mortality (Soutrenon, 1998; Desprez-Loustau et al, 2014). Repeated attacks by *E. alphitoides* in combination with attacks by other pathogens and/or insects can reduce the vigour of mature trees (Hajji et al., 2009). In most European areas where oaks are grown, the combined effects of powdery mildew with other fungal and insect infestations are implicated in oak decline (Ufnalski and Przybyl, 2004; Marcais and Desprez-Loustau, 2014).

In Europe, oak mildew epidemics usually start late in spring, as the spring shoots develop (Marcais et al., 2009). Young, expanding and developing leaves are very susceptible to the disease but their susceptibility decreases as leaves mature (Edwards

and Ayres, 1982; Marcais and Desprez-Loustau, 2014). Consequently, the pathogen has a nearly monocyclic infection cycle. The disease can occasionally be severe if epidemics occur early in spring (Marcais et al., 2009) when high levels of inoculum coincide with the presence of susceptible leaf tissues. Host-pathogen synchrony in spring appears to regulate disease severity (Desprez-Loustau et al., 2010). Disease prevalence is greater on the second and the third leaf flushes produced between the end of June and August (Marcais et al., 2009); these leaves tend to be ~~very~~ severely infected, presumably because inoculum is abundant as they expand. Host-pathogen synchrony is probably responsible for the lower disease severity observed on large trees since the second and third flush leaves are a smaller proportion of the total leaf area produced during the season (Bréda et al., 1995; Marcais et al., 2009). Chasmothecia, the sexual fruiting bodies which contain asci, are abundant on falling leaves in the autumn. However, the role of ascospores in the epidemiology of the disease has been controversial~~. For a long time~~ ~~i~~Initial infections were previously believed to start from overwintering mycelium in buds (Kerling, 1966; Nef and Perrin, 1999). However, Marcais et al. (2009) found such initial infections to be ~~very~~ infrequent in spring, and ascospores, which are widely dispersed by wind, to be the most common source of inoculum for the primary spring infections.

Aerial leaf surfaces of all plants are naturally inhabited by numerous micro-organisms (Gowdu and Balasubramanian, 1988; Jumpponen and Jones, 2010; Cordier et al., 2012) which not infrequently provide partial control of some plant pathogens (Rishbeth et al., 1988). Powdery mildews are ectotrophic pathogens and therefore in close contact with and potentially attacked by the phylloplane microflora (Bélanger et al., 1998; Bélanger and Labbé, 2002). Organisms from diverse biological groups

(bacteria, fungi, arthropods) have been reported to actively antagonise powdery mildews (Bélanger and Labbé, 2002) by parasitism, antibiotic production, or induction of host-resistance (Blakeman and Fokkema, 1982; Bélanger et al., 1998; Kiss, 2003; Cortes-Penagos et al., 2006). These modes of action are not mutually exclusive (Kiss, 2003). The most common fungal antagonists reported in natural association with mildew species are *Ampelomyces* spp., *Tilletiopsis* spp., *Cephalosporium* spp., *Cladosporium* spp. (Kiss, 2003), *Acremonium alternatum* (Malathrakis, 1985), *Dissoconium aciculare*, *Aphanocladium album* and *Acremonium byssoides*, *Pseudomyza flocculosa* (Kiss, 2003). Species of *Trichoderma*, *Fusarium* and *Penicillium chrysogenum* have not been found to be mycoparasites of any powdery mildew species in nature but have been successfully tested for anti-powdery mildew activity in field and glasshouse trials (Kiss, 2003). *Ampelomyces quisqualis* is the only mycoparasite which has been reported from oak specimens (*Q. robur* and *Q. petrea*) infected with *E. alphitoides* (Kiss, 1998). There are no effective methods to manage oak powdery mildew within forest environments and therefore, biological control is an attractive option. In order to explore this option, it is essential to understand the biology and ecological setting of the phyllosphere organisms which naturally co-exist with powdery mildew and how they vary throughout the season (Heuser and Zimmer, 2002; Kaneko et al. 2003; Osono, 2009; Jumpponen and Jones, 2010).

Our underlying hypotheses were that the abundance of mildew on oak leaves would form a substantial resource for other fungi, and that these would limit the abundance of mildew. Therefore the primary purpose of the present study was to identify the main fungi which are naturally associated with oak powdery mildew, to record their abundance and the appropriate scale of study. A secondary purpose was to examine how

the abundance of mildew-commensal fungi was related to the abundance of powdery mildew and environmental factors.

2. Materials and methods

2. 1 Sampling

Samples were collected from two areas on the campus of the University of Reading (Fig. 1) in areas where oak trees of various species (mainly *Quercus robur* but also *Q. cerris*, *Q. canariensis, Q. × turneri, Q. × hispanica "Lucombeana", Q. nigra, Q. dentata, , Q. castanifolia*, *Q. ilex*,) and other tree species are frequent. Leaf samples were collected from 11 trees (*Q. robur*) in summer (04/07/2005, 15/06/2006 depending on the appearance of powdery mildew symptoms on leaves) and autumn (12/10/2004, 03/10/2005, 28/09/2006). The trees were arbitrarily selected within each of the two areas so as to include trees falling into each of three height-classes (1-3m, 3-9m and 9-12m). On each tree, powdery mildew severity was assessed on a total of 50 leaves, as follows: From each tree, 25 branches (of 2-3cm in diameter and located 1-4 m above the ground) were collected arbitrarily from the periphery of the canopy and a single leaf was sampled at random from two shoots of each branch; one from the right of the branch and one from the left. Leaves were assessed on both the upper and the lower surface. For practical reasons it was not possible to sample the upper canopy of the large trees. To study associations between mildew and other organisms, two out of the 25 previously assessed branches (each bearing about 10 leaves) were selected and sticky tape impressions were prepared as described in the next section. From each of the 10 leaves on a branch, two sticky tape impressions were prepared within 24 h of collection, one from the upper and one from the lower leaf surface. The impressions were made under

the binocular microscope from sporulating zones of the leaves where these were present; since the leaves on each branch exhibited different levels of powdery mildew infection, some impressions were also made from leaves which had no visible powdery mildew colonies.

2.2 Assessment

Powdery mildew was assessed visually by estimating the percentage of the leaf area which was covered by *E. alphitoides* mycelium on a scale 0-100%: 0 for no visible mildew spots and 100% for full coverage of the leaves by mildew. The assessment of fungi growing on *E. alphitoides* colonies was made by examining one transect (25mm x 0.2mm) across the long axis of each slide under a light microscope with a 10x or 40x objective and scoring the abundance of characteristic features of each commensal fungus on a four-point scale, 0 (none), 1 (rare), 2 (many) and 3 (abundant) (Fig 2) in each-transect. Fungi were visually assigned to morphological groupings. Isolation of representatives of the most common fungi seen was attempted on Potato Dextrose Agar (PDA) and/or Tap Water Agar (TWA), both including 0.1 g/L streptomycin and 0.1g/L penicillin. Spores, mycelium and other fungal structures (e.g. pseudothecia) were picked up with a fine needle and deposited either directly on the medium or into a small droplet of sterile water on the medium, which was then spread over the surface with a glass spreader. Plates were incubated in darkness at 18°-20° C and checked at 2-3day intervals. Fungal colonies seen were sub-cultured repeatedly on PDA until they appeared to be visually stable. Multiple morphological types on a plate were separated by serial dilutions of spores. For long term storage the commensal fungi were sub-cultured onto malt extract agar (MEA) slopes and kept under oil at 3°C. Distinct groups of fungi were identified as far as possible to genus level based on original appearance

and culture morphological characteristics, using literature keys and descriptions (Gams, 1971; Ellis and Ellis, 1997; Barnett and Hunter, 1998; Gams and Meyer, 1998). Selected samples were further identified by using the ITS sequence of ribosomal DNA (Schorch et al., 2012). Mycelium and stroma were scraped from cultures, ground in liquid nitrogen and DNA extracted using a DNEasy Plant kit (Qiagen, Crawley, West Sussex, UK). ITS 5.8S sequences were amplified from two independent isolates of typical morphology using ITS4 and ITS5 universal primers according to the methods of Gardes and Bruns (1993), White et al. (1990) and Abler (2003). The amplicons were sequenced commercially (Macrogen, Seoul, Korea).

2.3 Statistical analysis

The effects of environmental and host factors on mildew severity, the abundance of commensal fungi, and their associations with powdery mildew severity were evaluated by REML models using the facilities in Genstat v11 (VSN International Ltd, Hemel Hempsted-Hertfordshire, UK). In the overall REML analysis, the season, height-class of trees, and leaf-surface were specified as fixed factors, with sampling year and location as random factors. Estimates of the percentage of powdery mildew severity were transformed to logits and conidia counts were transformed into $\log_e$ for analysis. Zero values were regarded as 0.001 for conversion to the log scale.

Relationships between the fungi associated with *E. alphitoides* were investigated by linear regression analysis. Powdery mildew severity was related to the score of each associated fungi by conventional stepwise regression analysis. The response (dependent) variable was the mean score of each commensal fungus on a tree in each survey, so n =

55, and powdery mildew severity was set as an explanatory variate. Parallel curve analysis (Mead et al., 2002, chap. 10) was used to determine whether separate regression models were needed in each surveyed year. Relationships between the commensal fungi surveyed were also investigated by parallel curve analysis.

3. Results

3.1 Identification

Specimens of the powdery mildew population from three out of the eleven sampled trees were sequenced (S. Takamatsu, Mie University, Tsu, 514-8507, Japan, unpublished data). The sequences indicated that only *E. alphitoides* s. str. (Takamatsu et al., 2007) Not included in Refs. was present.

Most powdery mildew colonies examined were associated with other fungi, even when first visible, during the summer assessment. Five morphological types were common (Fig. 2): *Trichoderma* sp.; *Ampelomyces/Phoma* sp.; *Acremonium* sp.; a *Tilletiopsis*-like group, and *Leptosphaerulina sp.*. Patches of powdery mildew colonies containing *Leptosphaerulina sp.* were often visibly browned. A minority of undetermined spores of various types were recorded but were insufficiently common or distinctive for meaningful analysis.

Isolations from the *Trichoderma*-like spores produced two distinct types of culture, differing in colour and growth-rate (Genbank KM406100, KM406101). Their highest match was at 98% similarity with Genbank sequences (AY605750, AY605742, AY605733, AY222349, AB297802) identified as *Hypocrea lixii* (= *Trichoderma harzianum*) and *Trichoderma* sp.. The *Acremonium*-like sequences (Genbank

KM406102, KM406103, KM406104) had ~~a~~ the highest match with Genbank sequences identified as *Acremonium* sp. (Genbank AM262391, AM262392) at up to 98% similarity. Identification to species level was not possible. *Leptosphaerulina sp.* isolates were identified by Dr B. Spooner of CAB IMI as *L. australis.* Sequences (Genbank KF275143, KF275144) matched at 99% similarity with? Genbank sequences identified as *L. trifolii* (GenBank AY131203), which is considered a synonym of *L. australis* (Irwin and Davis, 1985 Not in Refs.; Abler 2003).

Repeated attempts to isolate *Ampelomyces quisqualis* on artificial media were fruitless. Consequently, its presence in our study was based on the characteristic development of the pycnidial forms. However, distinction of *A. quisqualis* from *Phoma* species is difficult under light microscopy and therefore, this morphological group was recorded as *Ampelomyces/Phoma*.

3.2 Powdery mildew incidence and severity

Powdery mildew incidence varied substantially between the sampled years but considerable variation was also attributed on tree specific factors (Table 1). Powdery mildew severity varied substantially between the sampled years but substantial differences were also spotted in the disease levels within the canopy of single trees. The extra variance among trees within a location was negligible (Table 1). The two locations studied differed no more than expected from differences among trees within the same location (Table 1). However, both powdery mildew incidence and severity were highest in autumn (Fig. 3, 4; $P<0.001$, Fisher exact test) and were also affected significantly by the tree height-class; 3-9m trees were more diseased than the other two height classes (Fig. 3, 4; $P<0.001$, Fisher exact test).

3.3 Abundance of commensal fungi

Powdery mildew colonies were already associated with a range of other fungi (Fig. 5). *L. australis*, *Trichoderma* sp., *Ampelomyces/Phoma* sp. and *Acremonium* sp. were commonly associated with powdery mildew on all sampling occasions (Fig. 5, 6). Variance between trees was very low whereas there was substantial variation in the abundance scores of all commensal fungi between the different occasions of specimen collection and within the canopies of the trees (Table 2).

Acremonium sp. was common in all samples but abundance varied between years (Fig. 6). The incidence and abundance score of *Acremonium* sp. differed between seasons ($P<0.001$, Fisher exact test): abundance scores were always higher in the early summer samplings (Fig. 6). *Acremonium* sp. abundance scores greater than 2 were rare but associated with lower powdery mildew densities on the same trees (Fig. 7).

Ampelomyces/Phoma sp. abundance scores differed between years (Figs. 6). *Ampelomyces/Phoma* scores were larger in autumn samplings but not significantly so compared to the summer samplings. The taxon was never observed in the absence of powdery mildew (Table 3) and was never observed at high population densities (score>2) even when powdery mildew severity was high (Fig. 7). Abundance score did not differ significantly between the two leaf-surfaces ($P>0.05$, Fisher exact test).

L. australis was abundant each year and was associated with 91% of powdery mildew colonies examined. Although, rarely, *L. australis* ascospores were observed on leaf areas free of powdery mildew colonies, the fungus was more frequent on leaves with powdery mildew ($P<0.001$, Fisher exact test; Table 3). *L. australis* incidence on mildewed leaves was greater on 3-9 m trees (interaction $P=0.006$, Fisher exact test). *L.*

australis scores were higher in autumn ($P<0.001$, Fisher exact test; Fig. 6) and increased over the observation period (Fig. 6). The mean powdery mildew severity was slightly greater with greater *L. australis* scores in the 11 tree samples (Fig. 7), but the variation in powdery mildew severity was reduced in samples with greater *L. australis* scores (Fig. 7).

About 80% of powdery mildew colonies examined were associated with *Trichoderma* sp. Its incidence and population score were larger in autumn ($P<0.001$, Fisher exact test; Fig. 6). The upper leaf surface was more intensively colonised by *Trichoderma* sp. than the lower ($P<0.001$, Fisher exact test; Table 3). Powdery mildew severity was greatest and most variable at intermediate *Trichoderma* scores (Fig. 7).

3.4 Correlations among powdery mildew and commensal fungi

Our hypothesis was that commensals would be more common when powdery mildew severity was greater and so provided a larger resource. Both slope and intercept of the relationship between powdery mildew severity and commensal fungus score varied between years for all commensals ($P\leq0.001$; Fig. 8). Season, height-class and leaf surface did not significantly improve the model fit. Higher powdery mildew severities were associated with intermediate *Acremonium* sp. and *Trichoderma* sp. scores (Fig. 7, 8). The slope of regressions of *Trichoderma* sp. score on powdery mildew severity were strong and positive in 2005, 2006 and negative but not strong in 2004 (Fig. 8). However, the overall interaction was strong (Wald-test interaction $P<0.001$). The relationships of *Trichoderma* sp. and *Acremonium* sp. scores to powdery mildew severity appeared curved (Fig. 7, 8), with very high scores associated with moderate powdery mildew severity (>60%). The slope and intercept of *Acremonium* sp. score were negative in 2004 but positive in 2005 and 2006 (Wald-test interaction $P\leq0.05$; Fig.

8). *Ampelomyces/Phoma* sp. scores were low in the years studied (Fig. 6, 8) and although the association to powdery mildew severity was positive in all years (Fig. 8), the overall interaction was not significant ($P>0.1$). The intercept and slope for *L. australis* score were positive in the years studied (Fig. 8) and higher *L. australis* scores were associated with lower powdery mildew severity (>40%) (Fig. 7). Such association was strong in 2006 (Fig. 8) and the overall interaction was also strong (Wald-test interaction $P<0.001$).

There were weak but significant associations between *L. australis* and the other three fungi associated with powdery mildew (Fig. 9). Separate regression lines for cases where powdery mildew was present and where it was absent greatly improved the fit ($P<0.001$). *Acremonium* sp., *Ampelomyces/Phoma* sp. and *Trichoderma* sp. were positively associated with *L. australis* when powdery mildew was also present (Fig. 9). No statistically significant linear dependence of the mean scores of *Acremonium* sp., *Ampelomyces/Phoma* sp. or *Trichoderma* sp. on *L. australis* scores was detected when powdery mildew colonies were not present (Fig. 9). The three-way interaction between *Ampelomyces/Phoma sp., L. australis* score and powdery mildew presence was significant (Wald-test $P<0.05$). The same interaction was also significant for *Trichoderma* sp. (Wald-test $P<0.001$).

4. Discussion

Powdery mildew severity varied much more across years than among trees, and there was only slightly more variation among trees than expected from the variation between leaves from the same tree. Powdery mildew severity is critically influenced by the time

between flushing of shoots and maturation of the leaves (Edwards and Ayres, 1982; Marcais et al. 2009) because oak leaves greatly increase their resistance to powdery mildew infections after a few weeks (Edwards and Ayres 1981; Marcais et al. 2009). Competition between powdery mildew species and competition, parasitism or resistance induction by other phylloplane fungi may also affect powdery mildew severity (Mougou et al., 2010). Along with ~~as~~ *E. alphitoides*, various other powdery mildew species have a host range including *Quercus* spp. (Takamatsu et al., 2007) Not in Refs.. The co-existence of visually similar but distinct species of powdery mildew on oak could confuse study of the population dynamics, but our limited sample suggests at least a strong preponderance of *E. alphitoides* in this study area.

Oak powdery mildew colonies co-existed with several other fungi. Four different morphological types of fungus co-occuring with powdery mildew colonies were commonly observed. Most colonies co-existed with several of them. The least common was an *Ampelomyces/Phoma*-like species. These have been associated with direct mycoparasitism of powdery mildews (Kiss, 1998). Two of the remaining three morphological types identified (*Trichoderma* sp. and *Acremonium* sp.) have often been found to actively antagonise other fungi, including powdery mildews, by antibiosis or direct parasitism (Malathrakis, 1985; Elad, 2000;Not in Refs. Kiss, 2003); however, based on this study no definite inferences can be made regarding *Trichoderma* sp. and *Acremonium* sp. modes of action. The final fungus, identified as *L. australis,* has not been mentioned before in association with powdery mildew. In parallel work with this survey, *L. australis* was shown to reduce infection of oak leaves by powdery mildew in controlled inoculations (Topalidou, 2008) Not in Refs. although the mechanism is unknown. *Leptosphaerulina* sp. are reported as weak pathogens or as saprobes. In some

studies *L. chartarum* has been shown to survive as a symptomless endophyte in its host (Suryanarayaman and Murali, 2006; Salvanathan et al, 2011). The same species (Wu et al, 2013) has been shown to produce xylanotic enzymes which enhanced plant defence against stress and disease. In view of this, it would be interesting to explore the possible endophytic existence of *L. australis* in oak leaves in view of its common association with oak powdery mildew. The incidence and severity of commensal fungi may depend on or influence population densities of powdery mildew. Powdery mildew infection alters the physiological condition of leaves which will in turn influence microbial community structure and growth on the phylloplane. Hyper-parasitic fungi are likely to show density-dependent relations to powdery mildew severity; neutrally associated fungi may depend on powdery mildew without influencing its density. In this study, the fungi associated with powdery mildew, apart from *Ampelomyces/Phoma sp.*, came from taxa not reported to be specialised mycoparasites and having wide host and geographic ranges.

According to Sadaka and Ponge (2003) the appearance of *Acremonium* sp. coincided with the senescence of the leaves of holm oak. In this survey, we found *Acremonium* sp. more abundant in summer. *Trichoderma s*pecies are known as secondary colonisers of various forest litters (Domsch et al, 1980). Six different *Trichoderma* species were reported on leaves of *Q. rotundifolia* at different stages of leaf senescence (Sadaka and Ponge, 2003). In this study *Trichoderma* sp. was recorded on living leaves infected with powdery mildew, but the mean score was higher in autumn (Figs 5, 6), shortly before leaf-fall.

L. australis was very commonly found in association with oak powdery mildew. It was more abundant at high powdery mildew severity (Fig. 7, 8), although the

association was weak. In laboratory tests, inoculation of young oak leaves with *L. australis* reduced powdery mildew colonies and appeared to colonise other powdery mildew species (*P. aphanis, P. xanthii*) (Topalidou 2008, pp 199-205) Not in Refs.. *L. australis* has been recorded repeatedly in co-existence with many other fungal genera on *Q. macrocarpa* leaves (Jumpponen and Jones, 2010) but it has not been reported as a mycoparasite or antagonist of any pathogen. However, several species of *Leptosphaerulina* have been reported in association with diseases of turf-grasses such as anthracnose, *Pythium* blight and *Pyricularia grisea* (Dernoeden, 2002) Not in Refs.. *L. australis* in particular has been regularly found in conjunction with other pathogens such as *Fusarium* spp. and *Sclerotinia homoeocarpa* (Abler, 2003).

With the partial exception of *L. australis,* populations of powdery mildew-associated fungi fluctuated greatly over the study period (Fig. 6). Low levels of *Ampelomyces/Phoma sp.* in 2006 (Fig. 6) were presumably related to climatic factors, since *Ampelomyces/Phoma sp.* is favoured by high RH (Jarvis and Slingsby, 1977; Schweigkofler, 2006) and the summer of 2006 was extremely hot and dry.

This survey showed seasonal and within-tree effects on powdery mildew severity to be more important than variation between *Q. robur* specimens. It indicates that powdery mildew colonies on oak leaves are, from their first appearance, very frequently associated with other fungi, some of which either attack the powdery mildew directly (eg. *Ampelomyces/Phoma* sp.), or may render leaves less susceptible to attack. Therefore, they are important elements in the phylloplane community. *Acremonium* sp. and *Trichoderma* sp. were rarer when powdery mildew was more severe in the early season, but commoner when powdery mildew was more severe in the late season, although the shifts were not always significant. A hypothesis to explain this would be

that the initial association was driven by factors independent of or restricting powdery mildew severity, such as surface wetness, but that later they were either consuming powdery mildew. *Ampelomyces/Phoma* and *L. australis* populations were always positively correlated with powdery mildew severity, although again not always significantly. It is possible that powdery mildew represented a nutrient resource for both organisms, though *L. australis* is known purely as a saprophyte or weak plant pathogen.

Acknowledgements

Financial support by IKY, the Greek State Scholarship Foundation, is duly acknowledged for the first author. Dr. Glynn Percival is duly acknowledged for kindly providing the oak seedlings. We also thank Levente Kiss, Hungarian Academy of Sciences for advice and comment during the work. Professor Susumu Takamatsu, Mie University, is also duly acknowledged for his help in identifying the species of powdery mildew samples. Dr. Giannis Meliadis, Hellenic Agricultural Organisation Demeter, Forest Research Institute of Thessaloniki, is duly acknowledged for helping in mapping the trees in Fig. 1.

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FIGURE CAPTIONS

Fig. 1: Map of the sampled trees within the two examined locations (Harris Garden and Earley Gate) within the campus of the University of Reading (aerophotgraph was downloaded from Google Earth, 2008). Each of the trees was classified into one of three height-classes as follows: 3, 6 and 9 into 1-3m; 4, 5, 8 and 11 into 3-9m; 1, 2, 7 and 10 into 9-12m.

Fig. 2: Panel of photos from the assessment of each morphological group of the commensal fungi. (a) *Ampelomyces/Phoma* sp.: (i) conidia exuded from pycnidia, (ii), (iii) pycnidia which grow superficially in/on or around powdery mildew hyphae. Scale bars equal to 10 μm. (b) *Trichoderma* sp.: (i) *Trichoderma* sp. spores and (ii), (iii) arrows pointing at *Trichoderma* sp. spores which were observed around powdery mildew conidia. Scale bars equal to 10 μm, 20 μm and 20 μm respectively for (i), (ii) and (iii). (c) *Acremonium* sp.: (i) *Acremonium* sp. spores and (ii) arrows pointing at *Acremoium* sp. phialides and spores, emerging throughout a cluster of powdery mildew conidia. Scale bars equal to 10 μm. (d) *L. australis*: (i) ascospores emerging from the asci, (ii) ascospore attached on a powdery mildew conium and (iii) an *L. australis* ascospore close to powdery mildew mycelium. Scale bars equal to 10 μm.

Fig. 3 : Incidence of powdery mildew (%) on oak leaves, averaged within each height-class of tree sampled on each sampling occasion.

Fig. 4: Powdery mildew visual severity (%) on oak leaves from trees in three height-classes on five sampling occasions. Data are averaged over both surfaces of all leaves

from a tree. Error bars indicate the standard error of the mean (SEM) based on between leaves variance.

Fig. 5**:** Total incidence of colonies of other fungi in oak powdery mildew colonies over the period of study. Gray areas denote the leafless period; initial sample was taken as soon as powdery mildew colonies were visible.

Fig. 6: Distribution across trees of average abundance scores of fungi seen in sticky tape impressions of oak powdery mildew colonies. The score for a tree is the average of 2 sticky tape impressions taken from 20 leaves (2 branches, each bearing 10 leaves) each scored on a 0-3 scale. Boxplots show the range, medians and quartiles of the average spore abundance score in a tree.

Fig. 7: Distribution of powdery mildew severity over trees, categorized by mean abundance scores of commensal fungi in the same tree on the same sampling occasion. Powdery mildew severity is the mean of the assessed leaves. Individual abundance scores are means from 2 sticky tape impressions (one on the upper leaf surface and one on the lower) taken from 20 leaves per tree (2 branches, bearing 10 leaves) on a single sampling occasion. Boxplots show the range, medians and quartiles of the powdery mildew severity. In all cases, means and medians are similar.

Fig. 8: Relationship between mean abundance score of fungi seen in sticky tape impressions of the assessed leaves, averaged over all impressions from each branch of each sampled tree, and mean powdery mildew severity on the same trees. Data from summer and autumn surveys are plotted together. Regression equations are shown for each year separately (a) *Acremonium* sp. (Percentage of variance accounted for by regression, R^{*2} = 12.5, standard error of observations, s.e.=0.65). (b)

Ampelomyces/Phoma sp. (R^{*2} =48.4, s.e.=0.34). (c) *L. australis* (R^{*2} = 28.4, s.e.=0.6). (d) *Trichoderma* sp. (R^{*2} =39.7. s.e.=0.62).

Fig. 9: Relationships between *L. australis* abundance (La) score and the scores of other fungi seen in sticky tape impressions of oak leaves. Each point represents the mean score from a branch, averaging over replicate slides and bot leaf surfaces. Separate lines are fitted for the cases where powdery mildew was present and absent. (a) *Acremonium* sp. (A): powdery mildew absent, *Acremonium* score = 0.24+0.03 La (*P*=0.09); leaves with powdery mildew present A=0.73+0.17 La (*P*=0.002). (b) *Ampelomyces/Phoma* sp. (Ph): never found in the absence of powdery mildew; powdery mildew present, Ph=0.19+0.12 La (*P*=0.001). (c) *Trichoderma* sp.: powdery mildew absent, T = 0.21+0.41La (*P*=0.4); powdery mildew present T=0.34+0.46 La (*P*<0.001). pm, powdery mildew.

Table 1: Estimated variance of the random components which contributed to the observed differences in powdery mildew incidence and severity within the study area at Reading during 2005-7. Fixed effects were season, location and leaf surface.

	Variance component	
Random term	**Incidence**	**Severity**
Between year	0.07±0.07	13.2 ±13.3
Between trees within location	0.03± 0.002	0.48 ±0.35
Leaves within the canopy of each tree	0.14±0.004	16.9 ±0.46

Table 2: Estimated variance of the random components which contributed to the observed differences in incidence and score of fungi associated with oak powdery mildew within the study area at Reading during 2005-2007. Fixed effects were season, location and leaf surface.

	Variance component			
Random term	***Ampelomyces/ Phoma sp.***	***Acremonium sp.***	***L. australis***	***Trichoderma sp.***
Year	0.18 ±0.20	0.27 ±0.28	0.09 ±0.09	0.49 ±0.50
Tree	0.003 ± 0.002	0.01 ±0.01	0.001±0.002	0.007 ±0.001
Leaves within the canopy of each tree	0.11 ±0.01	0.2 ±0.02	0.23 ±0.02	0.26 ±0.020

Table 3: Incidence of the most common commensal fungi in sticky tape impressions of leaf surfaces of *Quercus robur* in leaf surfaces with and without powdery mildew.

		Incidence of the fungal group (% of samples)	
Leaf surface	Fungal group	Leaves without powdery mildew	Leaves with powdery mildew
Upper	Sample size	0	104
	Acremonium sp.	-	90.4
	L. australis	-	100
	Trichoderma sp.	-	84.6
	A*mpelomyces*/*Phoma* sp.	-	52.9
Lower	Sample size	18	86
	Acremonium sp.	78	93
	L. australis	88	100
	Trichoderma sp.	83	80
	A*mpelomyces*/*Phoma* sp.	0	69