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Christel Leyronas, Fabien Halkett, Philippe C. Nicot. Relationship between the genetic characteristics of *Botrytis* sp. airborne inoculum and meteorological parameters, seasons and the origin of air masses. *Aerobiologia*, 2015, 31 (3), pp.367-380. 10.1007/s10453-015-9370-x . hal-01268891

**HAL Id: hal-01268891**

**<https://hal.science/hal-01268891>**

Submitted on 8 Feb 2022

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# **Relationship between the genetic characteristics of *Botrytis* sp. airborne inoculum and meteorological parameters, seasons and the origin of air masses.**

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## **Abstract**

Grey mould is a worldwide disease on many economically important crops. It is caused by two fungal species, *Botrytis cinerea* and *B. pseudocinerea*, which are mainly airborne dispersed. Although several studies have considered the abundance of airborne inoculum of *B. cinerea* in models forecasting the risk of grey mould epidemics, the genetic characteristics of this inoculum are poorly known. In the present study, airborne inoculum of *B. cinerea* and *B. pseudocinerea* was collected on 29 dates over a 2.5-year period on a site in south-eastern France. The 683 sampled isolates were genotyped with 9 microsatellites markers and 616 were identified as *B. cinerea*. The genetic structure of *B. cinerea* airborne inoculum was inferred with Bayesian assignment tests. Eight genetic clusters were identified. Cluster abundance showed temporal variation and was statistically linked to the season ( $P=0.0009$ ) and the origin of air masses ( $P<0.0001$ ). The proportion of isolates belonging to the species *B. pseudocinerea* was equal to 9.8% on average, but it showed temporal variation; it tended to be higher in winter. This study is the first to provide information about the genetic characteristics of airborne inoculum of *B. cinerea* and *B. pseudocinerea* and to bring evidence of relationship with seasons, meteorological parameters and with the origin of air masses.

**Keywords:** spores, grey mould, genetic clusters, diversity, *Botrytis*

## 1. Introduction

*Botrytis cinerea* causes grey mould on more than two hundred species, among which some are cultivated worldwide and have a great economic importance (grapevine, lettuce, tomato for example). On its host plants, when conditions are favourable, this fungus may produce several millions of spores in a few days per square centimetre of lesion surface (Nicot et al. 1996) and this may result in rapid epidemic development on the crop (Decognet et al. 2009). Spores are predominantly wind dispersed (Jarvis 1962; Harrison and Lowe 1987). Recently, *Botrytis pseudocinerea*, a new species causing grey mould, living in sympatry with *B. cinerea* has been described (Walker et al. 2011). Both species are morphologically identical. *B. pseudocinerea* is found principally in Europe, though it has recently been reported on grapevine in New Zealand (Johnston et al. 2013) and on tomato in Algeria (Adjebli et al. 2014). Its frequency on plants was reported to be the highest in spring (Fekete et al. 2012; Walker et al. 2013).

Predictive tools could help growers to rationalize their crop health management practices, particularly the timing for chemical control. This reduction in the number of fungicide applications has the twofold advantage of lowering the cost of crop protection and of preserving the environment while maintaining the efficacy of disease control. The majority of models described in the literature for predicting the risk of grey mould are based on local climatic variables (Broome et al. 1995; Shtienberg and Elad 1997; Berrie et al. 2002; Rasiukevičiūtė et al. 2013). Indeed many studies have been conducted for several major susceptible crops to find the relationship between local meteorological parameters during the growing season, the abundance of airborne inoculum and levels of grey mould. Examples include studies on grapes in North-West Portugal (Oliveira et al. 2009) and North-West Spain (Diaz et al. 1997 and 1998; Rodriguez-Rajo et al. 2010; Fernández-González et al. 2011) and on strawberries in South-West Spain (Blanco et al. 2006).

Integrating information about genetic characteristics of *B. cinerea* and *B. pseudocinerea* airborne inoculum in forecasting models could improve their efficiency. For example, *B. pseudocinerea* is naturally resistant to the hydroxylanilide fungicide fenhexamid (Leroux et al. 1999; 2002; Walker et al. 2013). Thus, taking into account the proportion of *B. pseudocinerea* isolates among the airborne inoculum of *Botrytis* in models could provide more precise recommendations about the best fungicide to use to prevent grey mould outbreaks. But to our knowledge none of the studies about airborne inoculum of *B. cinerea* take into account its genetic characteristics.

In the present study we propose to examine the genetic characteristics of *B. cinerea* and *B. pseudocinerea* airborne inoculum. As the abundance of airborne inoculum of *B. cinerea* is variable overtime and linked to local meteorological parameters and to the origin of air masses (Leyronas and Nicot, 2013), we hypothesise that the genetic characteristics of this inoculum are also variable and linked to the meteorological parameters. Hence, we conducted a study over a 2.5-year period to determine the temporal variation of the genetic characteristics of these fungi on a non-cultivated site. Our specific objectives were to i)- quantify the proportion of *B. pseudocinerea* and *B. cinerea* in the airborne inoculum, ii)- determine the genetic characteristics of isolates of both species and to iii)- determine the relationships between these genetic characteristics and meteorological parameters (namely, local meteorological conditions, season and origin of air masses).

## **2. Materials & Methods**

### *2.1 Collection of airborne isolates*

Air sampling was carried out at a research unit of the French National Institute of Agricultural Research (INRA) in Avignon (lat. 43.95N; long. 4.81E), located in the South East of France. The region of Avignon is subjected to a Mediterranean climate with hot and dry summers and temperate winters. It is predominantly scoured by a strong northern wind (Mistral) blowing between 5 and 15 days per month (Salameh et al. 2007). Sporadically, southern winds can also blow in the region, mostly from spring to autumn.

The protocol for collecting *B. cinerea* isolates from the air was previously detailed by Leyronas and Nicot (2013). All air samples were collected from a single site, mostly surrounded by prairies and urban dwellings and removed from any commercial crop or wild plants likely to be a significant source of *B. cinerea* inoculum within 5 kilometres. Air sampling was carried out, using a high throughput jet sampler (Burkard manufacturing, Rickmansworth, UK), on 29 days distributed between September 2007 and February 2010. Airborne inoculum was collected on Petri plates filled with the *Botrytis* spore trap medium (BSTM) described by Edwards and Seddon (2001). Every colony showing radially-branched hyphae and producing a dark brown halo in the medium was transferred to Potato Dextrose Agar medium (PDA) for further identification. Fungus identity was confirmed a few days later by observing forms of sporulation characteristic of *B. cinerea* and *B. pseudocinerea* (conidiophore shape) as both species cannot be distinguished on the basis of morphological characteristics. Finally, spores of all isolates were collected from those colonies with a sterile

cotton swab and then stored at -20 °C until use for single-spore isolation as described by Leyronas et al. (2012).

## 2.2 Genetic characteristics of airborne inoculum

Six hundred and eighty-three isolates were genotyped. Genomic DNA was extracted from 15 mg aliquots of lyophilized fungal material (harvested from two-week old cultures on PDA) in 96-well plates, following the Dneasy Plant extraction Kit protocol (Qiagen). The nine microsatellite markers designed for *B. cinerea* by Fournier et al. (2002) were amplified as described by Leyronas et al. (2014a). To determine the size of the microsatellites, the PCR products were diluted and multiplexed prior to scanning with the help of a Megabace sequencer (Amersham Pharmacia). Genetic Profiler software (Amersham Biosciences) was then used for the microsatellite size analysis. Multilocus microsatellite profiles are referred to as "haplotypes" hereafter.

Presence of the private allele associated with the cryptic species *B. pseudocinerea* at microsatellite locus BC6 (Walker et al. 2011) was controlled for the 683 isolates and the proportion of *B. pseudocinerea* isolates was computed for each date.

The number of different haplotypes among *B. cinerea* isolates was computed with an *ad hoc* visual basic script (Goyeau et al. 2007) and was used to calculate the haplotypic richness (computed as  $\frac{\text{number of distinct haplotypes}-1}{\text{sample size}-1}$ ) which estimates the proportion of haplotypes present in a population and takes a value of 1 if all individuals bear a different haplotype and 0 if the population is composed of a single haplotype (Arnaud-Haond et al. 2007).

## 2.3 Clustering analysis of *B. cinerea* isolates

The population structure was investigated using the Bayesian clustering method implemented in GENELAND (Guillot et al. 2005; Guillot 2008). A major strength of GENELAND is that it infers the best number of clusters, *K*, in a single step unlike all others clustering methods. This is enabled by the specific Reverse Jump MCMC (Markov chain Monte Carlo) algorithm, which allows the Markov chain to explore the whole range of putative cluster numbers at each step of the simulation run. Hence, there is no need to compare models assuming different numbers of parameters (here the *K* clusters). The number of clusters that best explain the structure of the genetic data is given by the value of *K* in which the Markov chain spent most iterations (Guillot et al. 2005). The number of clusters, *K*, was allowed to

vary from 1 to 30 and initialized at 15. Seven independent runs were performed to check for convergence of Markov chains. This first batch of runs with varying  $K$  enabled the best number of clusters to be assessed. Once determined, three additional runs were performed, with the corresponding value of  $K$  fixed, to better infer the assignment level of each individual to the  $K$  clusters (Guillot et al. 2005). For the three runs with varying  $K$ , we used the uncorrelated allele frequency model, and for those with fixed  $K$ , we used the correlated allele frequency model according to Guillot (2008) with default settings, except for the number of nuclei, which was increased to 800, given the number of individuals. Run length was set to 550 000 iterations with a burn-in period of 50 000 iterations. Assignment tests were conducted without taking into account information on the geographical origin of individuals. The threshold of assignment coefficient ( $q$ ) for individual membership to a cluster was set to 0.8.

The level of genetic differentiation between the identified clusters was assessed by computing pairwise  $F_{ST}$  (estimate of Weir & Cockerham, 1984) with the software Arlequin version 3.5 (Excoffier et al., 2005).

#### *2.4 Meteorological data acquisition and backward trajectories of air masses*

Meteorological data were acquired continuously on a climatic platform (CLIMINRA platform, US1116, AGROCLIM, F-84000 Avignon) located on a site representative of regional climate, 5 kilometres from our sampling site. We recorded solar radiation, mean, maximal and minimal air temperature, mean and maximal wind speed, mean, maximal and minimal relative humidity and precipitations.

To evaluate a possible link between the origin of air masses and the genetic characteristics of *B. cinerea* inoculum, backward trajectories of air masses arriving in Avignon on the sampling days were calculated using HYSPLIT (Hybrid Single Particle Lagrangian Integrated Trajectory Model) (Rolph 2011; Draxler and Rolph 2011). All trajectories were calculated up to 72 hours before sampling time. Focus was placed on air masses arriving at 1000, 1500 and 2000 meters above ground level.

#### *2.5 Statistical analyses*

Fisher's exact tests of independence were realised to determine if there was a link between the distribution of *B. cinerea* isolates among genetic clusters and either the origin of air masses or the season when samples were collected. The same analysis was performed with *B. pseudocinerea* airborne inoculum. The tests were computed with the GENEPOP version

3.1c, Prog. STRUC with 500,000 iterations (Raymond and Rousset 1995). The non-parametric rank test of Spearman was performed with StatView (version 5, SAS Institute) to evaluate the correlation between local meteorological parameters and proportion of *B. cinerea* isolates assigned to each genetic cluster, and between local meteorological parameters and the proportions of *B. pseudocinerea* isolates. Statistical tests were carried out at the 5 % level of significance, unless otherwise indicated.

### 3. Results

#### 3.1 Genetic characteristics of *B. cinerea* airborne inoculum

Among all isolates tested in this study, 616 (90.2% of the total) were identified as *B. cinerea*. All microsatellite loci were highly polymorphic showing 14 to 36 alleles, with the exception of BC4 which only had 3 alleles (Table 1). The global haplotypic richness was high (0.72) (Table 2). For six of the 29 sampling dates, it reached the value of 1, indicating that all isolates had different haplotypes (Figure 1).

Bayesian assignment tests identified a strong genetic structure among airborne inoculum of *B. cinerea*. Depending on the simulation run, the variability among isolates was partitioned into 7 or 8 genetic clusters. Three runs converged to  $K=8$ ; two runs converged to  $K=7$  and two runs were not conclusive (the Markov Chains explored both  $K=7$  and  $K=8$  dimensions). Looking into more details at the assignment coefficients of individuals to the 7 or 8 clusters, it appeared that the eighth cluster derived from cluster 2. Indeed, this cluster split into two under  $K=8$ ; with some individuals formerly assigned to cluster 2 (under  $K=7$ ) forming cluster 8. All other assignment coefficients of individuals were consistent across simulation runs. Considering that log likelihood was higher for the simulation runs converging to  $K=8$  (data not shown), we concluded that the genetic data were best explained assuming 8 genetic clusters. It is noteworthy that this eighth cluster consisted of few individuals, a fact which may explain the inconsistent sorting across runs.

Applying an assignment threshold of  $q>0.80$ , more than 94% of the isolates could be assigned to a genetic group. The number of isolates in each cluster varied from 15 to 257 (Table 2). These 8 clusters were significantly differentiated ( $P<0.001$ ) with high pairwise  $F_{ST}$  values ranging from 0.17 to 0.46 (clone-corrected data set) (Table 3). Cluster 5 was the most abundant, comprising 41.7% of total airborne inoculum. Interestingly it displayed the smallest value of haplotypic richness and reduced gene diversity, maybe as a result of a predominantly asexual reproductive mode (Table 2). At the opposite, cluster 2 showed the highest haplotypic

richness (with nearly all individuals bearing a distinct haplotype) and large gene diversity, a situation consistent with frequent episodes of sexual reproduction.

### *3.2 Relationship between B. cinerea genetic clusters and local meteorological parameters.*

Based on Spearman's tests, the proportions of *B. cinerea* airborne isolates assigned to cluster 4 and to cluster 6 were negatively correlated with mean, minimum and maximum air temperatures recorded up to ten days before the sampling date (Table 4). The proportion of isolates assigned to cluster 4 was also correlated with mean and minimum humidity recorded ten days before sampling, and with maximum humidity on the sampling day and that of isolates assigned to cluster 6 was also correlated with mean and maximum wind speed recorded 5 days before sampling. In contrast, correlations for clusters 1, 2 and 5 were limited to a few parameters and none was significant for clusters 3, 7 and 8 with any of the meteorological parameters. Finally, no correlation was observed with solar radiation for any of the clusters.

### *3.3 Variation of B. cinerea genetic clusters according to the origin of air masses and seasons.*

The airborne isolates of *B. cinerea* were distinguished according to the air mass trajectory that may have brought them in the air of Avignon. Three typical trajectories were distinguished according to their origin: South, North and West (Leyronas and Nicot 2013) including respectively 9, 11 and 5 sampling dates. Four dates were removed because the air mass trajectories were not clear (varying at different altitudes or rotating in different directions) and thus could not be classified. The distributions of isolates among genetic clusters varied significantly (Fisher's exact test,  $P < 0.001$ ) according to the origin of air masses (Figure 2). The proportion of isolates in each cluster when air masses originated from the North was similar to that when air masses originated from the South ( $P = 0.99$ ). In contrast, the distribution of isolates among clusters was different when considering air masses originating from the West ( $P < 0.001$  and  $P = 0.002$  in comparisons with North and South, respectively).

The isolates of *B. cinerea* were then distinguished according to the season when they were collected. The distributions of isolates among genetic clusters differed significantly across seasons (Fisher's exact test,  $P < 0.001$ ). It was similar in autumn and summer ( $P = 0.74$ ) and it was similar in winter and spring ( $P = 0.20$ ). The predominant cluster (cluster 5) was equally abundant in autumn and summer, with respectively 56% and 59% of isolates (Figure 3A). However this cluster collapsed in winter and spring while other clusters (especially



clusters 4 and 6) increased in frequency (Figure 3B). This pattern of co-variation in cluster abundance was confirmed by the values of correlation coefficients (Spearman's tests) between the proportions of isolates assigned to each of the eight clusters (Table 5). Some of these proportions were significantly negatively correlated (clusters 5 and 6 for example) and others were positively correlated (clusters 1 and 3, clusters 2 and 6).

### 3.4 Variation of *B. pseudocinerea* airborne inoculum.

Sixty-seven isolates carried the private allele at microsatellite locus BC6 associated with the cryptic species *B. pseudocinerea* (Walker et al. 2011). The *B. pseudocinerea* isolates showed less polymorphism in the 9 loci than the *B. cinerea* isolates (Table 1). In average they represented 9.8% of the total number of isolates but their daily proportion varied among the 29 sampling dates (Figure 1). No isolate of *B. pseudocinerea* was observed on ten of the sampling dates. For the 19 other dates, the proportion of *B. pseudocinerea* isolates was up to 50% (Figure 4). Contrary to *B. cinerea* isolates, there was no significant correlation (Spearman's rank coefficient) between these proportions and any of the ten local meteorological parameters measured during the sampling day and up to ten days before (data not shown).

There was no relationship between the number of days when *B. pseudocinerea* isolates were observed and either the origins of air masses or the seasons (Fisher's exact test,  $P=0.44$  and  $P=0.14$ , respectively). However when considering the daily proportions among all isolates collected, there were substantially more airborne *B. pseudocinerea* isolates during winter than during autumn, spring and summer (Figure 5A). The origin of air masses did not seem to influence this proportion (Figure 5B).

## Discussion

This study is the first attempt to investigate the genetic characteristics of airborne inoculum of *B. cinerea* and *B. pseudocinerea* on a site located away from any susceptible crop, and their temporal variation. Based on the genotypic characterisation of 683 isolates, collected in the air in south-eastern France at 29 sampling dates over a period of 2.5 years, it provided evidence for an influence of the season, the meteorological parameters and the origin of air masses on the genetic structure of airborne *B. cinerea* inoculum.

The first key result of the present study is the strong genetic structure of *B. cinerea* airborne isolates. Eight genetic clusters were identified. To our knowledge, this is the highest number of such clusters ever reported for *B. cinerea*. The highest number of clusters reported

before was in a study of 3546 isolates sampled from cultivated and wild host plants in France (Walker et al. 2014). For isolates collected from plants, two types of factors have been considered to affect the genetic structure: the host plant and the geographical origin. Genetic differentiation was adequately explained by differences in the host of origin in certain studies (Muñoz et al. 2002; Fournier and Giraud 2008; Rajaguru and Shaw 2010; Mirzaei et al. 2009; Walker et al. 2014) but not in others (Ma and Michailides 2005; Isenegger et al. 2008; Karchani-Balma et al. 2008; Leyronas et al. 2014a). The geographical origin of isolates has also been reported as a structuring factor in some cases (Karchani-Balma et al. 2008; Esterio et al. 2011; Bardin et al. 2014) but not in others (Fournier and Giraud 2008; Mirzaei et al. 2009). A strength of the present study, conducted on airborne inoculum, is that the analysis of the genetic structure of this highly polyphagous and cosmopolitan plant pathogen was not biased by sampling isolates from specific host plants. This study thus provided a more comprehensive and wider view of the genetic diversity of *B. cinerea*. However, in the absence of reference isolates in this study, we cannot link this genetic structure to any host plant or geographic area of origin. A dedicated work is needed to realise a meta-analyse of *B. cinerea* isolates collected in the air, and from different plant species and geographical locations in order to further assess the ecological characteristics and the spatial distributions of each cluster. Eventually, such data could lead to better comprehend whether some clusters are identical or admixed between plant and air populations, and thus how the airborne inoculum can be (re)distributed and influence population structures on plants. Despite their narrow host range, the occasional presence of airborne inoculum of other species of *Botrytis* should also be considered, in case some of the microsatellite marker designed for *B. cinerea* would also be amplified for certain of these isolates.

Beyond the strong genetic structure among isolates, we found a large haplotypic and gene diversity of airborne inoculum of *B. cinerea* and *B. pseudocinerea*. The haplotypic richness of *B. cinerea* isolates collected away from any diseased culture reported in the present study (0.72) was comparable to that reported by Decognet et al. (2009) for isolates collected in the air of a glasshouse prior to the development of any disease symptoms. These authors observed 63 distinct haplotypes among 80 *B. cinerea* isolates, which amounts to a haplotypic richness value of 0.78. To our knowledge there is no other published work about the genetic diversity of *B. cinerea* airborne inoculum. Here we also found different levels of haplotypic richness and gene diversity among genetic clusters which may be related to different modes of reproduction (sexual vs asexual) or to different evolutionary histories (such as different selection pressures exerted by the use of resistant plants or application of

fungicides). Again, in the absence of reference isolates or biological data, it is not possible to conclude. Further genetic and phenotypic characterization of the isolates would be necessary to expand our knowledge of the ecology of each genetic cluster.

The second key result of the present study is the influence of seasons on the distribution of *B. cinerea* isolates among genetic clusters. The proportions of isolates assigned to the eight clusters were similar in autumn and in summer, with one cluster (cluster 5) comprising most of the isolates. In contrast, a lower proportion of isolates were assigned to cluster 5 during spring and winter and other clusters were then more represented, among which cluster 4 and cluster 6 formed the major contribution. Although the reason for this temporal variation is not known, one possible hypothesis could be that the isolates assigned to these two clusters tend to be more sensitive to heat than the others. This hypothesis is consistent with the observation that the proportions of isolates assigned to these clusters were negatively correlated with local air temperature. This hypothesis is also consistent with the putative asexual reproductive mode of cluster 5, as it will be more sensitive to frost in the absence of resistant forms produced by sexual reproduction. Phenotypic characterisation is needed to assess life history traits (such as mycelial growth, sporulation) of isolates from clusters 4 and 6 at high temperatures.

The present study also showed an influence of the origin of air masses on the genetic structure of *B. cinerea* isolates collected at the sampling site. Air masses originating from the West appeared to carry inoculum that was genetically different from those coming from the North or from the South. It may be due to a differential loading of air masses with *B. cinerea* isolates as western masses spend more time above the Atlantic Ocean and less time above the continent (where there are potentially more sources of *B. cinerea* than above the ocean) than northern and southern masses. An element pointing to this direction is that western air masses tend to bring less *B. cinerea* airborne inoculum in the region of Avignon than southern and northern air masses (Leyronas and Nicot, 2013). We may also hypothesise that western air masses may have different characteristics than southern and northern air masses, thus influencing the microbial content and its viability. This observation is consistent with results of Celle-Jeanton et al. (2009) showing that air masses arriving in Avignon from the West, North and South differed in their compositions in terrestrial, marine and anthropogenic components. Furthermore Amato et al. (2007) reported an influence of cloud chemistry on microbial content and its viability in samples collected at Puy-de-Dôme (in central France). Indeed, increasing concentrations of bacteria and fungi were observed with increasing levels of sodium in the air masses. We can hypothesise that some *B. cinerea* isolates can survive

better than others in air masses with high levels of sodium. Phenotypic characterisation will be needed to test this hypothesis.

Noticeably, the presence of *B. pseudocinerea* isolates in the air was variable but non negligible. In the present study, the average proportion of *B. pseudocinerea* isolates among the total of airborne *cinerea*-like isolates was equal to 9.8%. This result is consistent with the proportions reported in previous studies dealing with inoculum collected on various plant species. *B. pseudocinerea* represented less than 10% and 14.5% of isolates sampled on grapevine in France and New Zealand, respectively (Walker et al. 2013; Johnston et al. 2013), 0-2.9% of those sampled on lettuce (Leyronas et al. 2014a, 2014b), 2.2% on tomato (Adjebli et al. 2014) and 14% of isolates collected from strawberry, raspberry and oilseed rape in Hungary (Fekete et al. 2012). However in our study the daily proportions of *B. pseudocinerea* airborne isolates were quite variable and could reach high levels, with two noticeable peaks in February 2008 and February 2010 (50% and 47%, respectively). Although these variations could not be linked to any local meteorological parameters or to the origin of air masses, we noticed that *B. pseudocinerea* was more abundant in the air during winter. On plants, the frequency of this cryptic species was reported to be the highest in spring (Fekete et al. 2012; Walker et al. 2013). We may hypothesise that in France, the peaks of *B. pseudocinerea* isolates observed in the air in February could induce the presence of this species on grapevine in spring. But we wonder about the sources releasing *B. pseudocinerea* isolates at the time when peaks were observed in the present study. Although the specific host range of *B. pseudocinerea* is not known, it could be hypothesized that this inoculum was emitted by local environmental reservoirs (including plant debris) or early agricultural crops located in the warmer environment of southern latitudes. Indeed, among the more than 200 botanical species that *B. cinerea* can attack, some are wild plant species (Elad et al. 2004). As *B. pseudocinerea* does not show any morphological difference with *B. cinerea* (Walker et al. 2011), some isolates observed on wild plants may belong to this recently identified cryptic species. So far, the resistance of *B. pseudocinerea* and some strains of *B. cinerea* to fenhexamid does not appear to be linked to significant failure of chemical control in the field, but it is considered as a subject of concern (Walker et al. 2013). Therefore, more attention should be paid to wild plants and debris that could harbour and release this type of isolates.

## Conclusion

The present study provided data about the genetic diversity of airborne inoculum of *B. cinerea* and *B. pseudocinerea* in the region of Avignon. It provided evidence about an

influence of the origin of air masses, seasons and local meteorological parameters on the genetic structure of airborne isolates. Additional data for other sampling sites would be useful to consolidate these observations and explore the existence of such relationships in other regions. Further work is also needed to investigate the relationships between genetic clusters of airborne inoculum and genetic clusters in isolates collected from plants. Work is also currently conducted to characterize biological traits of *B. cinerea* airborne isolates potentially important for the development of grey mould epidemics and traits of ecological interest such as tolerance to temperature and sodium stresses. Much more data are also needed on *B. pseudocinerea* before its ecology and epidemiological relevance can be precisely understood. All this knowledge would help to improve models aiming to forecast grey mould epidemics.

### **Acknowledgements**

This study was partially supported by a grant of the INRA department of Plant Health and Environment. The authors gratefully acknowledge the National Oceanic and Atmospheric Administration (NOAA) Air Resources Laboratory (ARL) for the provision of the HYSPLIT transport and dispersion model and READY website (<http://www.arl.noaa.gov/ready.php>) used in this publication. The authors also thank M. Duffaud for her excellent technical support.

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Table 1: Allele size range and numbers of alleles observed for the nine microsatellite loci among airborne isolates of *Botrytis cinerea* and *B. pseudocinerea* collected in Avignon.

Locus	Locus motif repeat	<i>B. cinerea</i>		<i>B. pseudocinerea</i>	
		Allele size range	Number of alleles	Allele size range	Number of alleles
BC1	(CTTT) <sub>10-...</sub> - (CA) <sub>2</sub> CG(CA) <sub>10</sub>	214-276	26	222-228	6
BC2	(AC) <sub>12</sub> AT(AC) <sub>4</sub>	139-197	25	159-177	6
BC3	(GA) <sub>10</sub>	204-240	16	204	1
BC4	(TTC) <sub>6</sub> TTATC(TTC) <sub>2</sub>	119-131	3	119-128	2
BC5	(AT) <sub>12</sub>	140-174	14	150-166	5
BC6	(CA) <sub>10</sub>	80-208	36	88	1
BC7	(TA) <sub>9</sub>	114-150	14	122-130	3
BC9	(CT) <sub>11</sub>	143-169	14	155-161	7
BC10	(AC) <sub>13</sub>	144-194	20	160-180	4

Table 2: Genetic diversity of the eight clusters identified among 616 airborne isolates of *B. cinerea*.

	Number of isolates	Number of different haplotypes	Haplotypic richness	Mean number of alleles per locus	Gene diversity <sup>a</sup>
<b>Total</b>	<b>616</b>	<b>443</b>	<b>0.72</b>	<b>19.11</b>	<b>0.71</b>
cluster 1	47	40	0.85	6.00	0.51
cluster 2	24	23	0.96	7.66	0.78
cluster 3	85	66	0.77	7.66	0.60
cluster 4	98	80	0.81	6.11	0.59
cluster 5	257	151	0.58	4.55	0.44
cluster 6	40	30	0.74	6.00	0.42
cluster 7	15	12	0.78	6.33	0.63
cluster 8	18	16	0.88	2.77	0.63
<i>Not assigned</i>	32	25	-	-	-

a: Hnb nonbiased genetic diversity

Table 3: Genetic differentiation of *B. cinerea* airborne isolates assessed with Weir & Cockerham's  $F_{ST}$  (clonally corrected data: a single strain retained per haplotype). All  $F_{ST}$  values were highly significant ( $P < 0.001$ ).

	cluster 1 <sup>a</sup>	cluster 2	cluster 3	cluster 4	cluster 5	cluster 6	cluster 7
cluster 2	0.25						
cluster 3	0.29	0.24					
cluster 4	0.33	0.20	0.32				
cluster 5	0.37	0.31	0.38	0.18			
cluster 6	0.36	0.30	0.37	0.32	0.41		
cluster 7	0.42	0.27	0.38	0.24	0.38	0.46	
cluster 8	0.30	0.17	0.25	0.18	0.30	0.24	0.32

a: Eight genetic clusters were identified among 616 isolates

Table 4: Spearman's rank correlation between daily proportions of *B. cinerea* isolates in genetic clusters and meteorological parameters measured on CLIMINRA platform during the sampling day and several days before. Only data for which the Rho value was significant ( $P < 0.05$ ) are shown.

Meteorological parameters	Spearman's rank correlation <sup>a</sup>	Genetic clusters <sup>b</sup>				
		1	2	4	5	6
Mean Air Temperature	$\rho_D$					-0.43
	$\rho_{D-1}$			-0.39		-0.45
	$\rho_{D-2}$					-0.47
	$\rho_{D-5}$					-0.38
	$\rho_{D-7}$					-0.44
	$\rho_{D-10}$			-0.39		-0.45
Minimum Air Temperature	$\rho_D$					-0.45
	$\rho_{D-1}$			-0.37		-0.46
	$\rho_{D-2}$					-0.55
	$\rho_{D-5}$					-0.40
	$\rho_{D-7}$			-0.37		-0.55
	$\rho_{D-10}$					-0.56
Maximum Air Temperature	$\rho_D$					-0.45
	$\rho_{D-1}$					-0.45
	$\rho_{D-5}$					-0.42
	$\rho_{D-7}$			-0.38		-0.43
	$\rho_{D-10}$			-0.41		
Mean Humidity	$\rho_{D-1}$		-0.43			
	$\rho_{D-10}$			0.40		
Minimum Humidity	$\rho_{D-1}$		-0.50			
	$\rho_{D-10}$			0.40		
Maximum Humidity	$\rho_D$			0.39		
Mean Wind Speed	$\rho_{D-5}$					0.41
Maximum Wind Speed	$\rho_D$				-0.42	
	$\rho_{D-5}$					0.38
Rainfall	$\rho_{D-5}$				0.37	
	$\rho_{D-10}$	0.36				

a: The indices "D", "D-1", etc. indicate the time of measurement of meteorological parameters (in days) relative to the sampling date D. Only dates with significant correlations are shown.

b: No significant correlation was observed for clusters 3, 7 and 8.

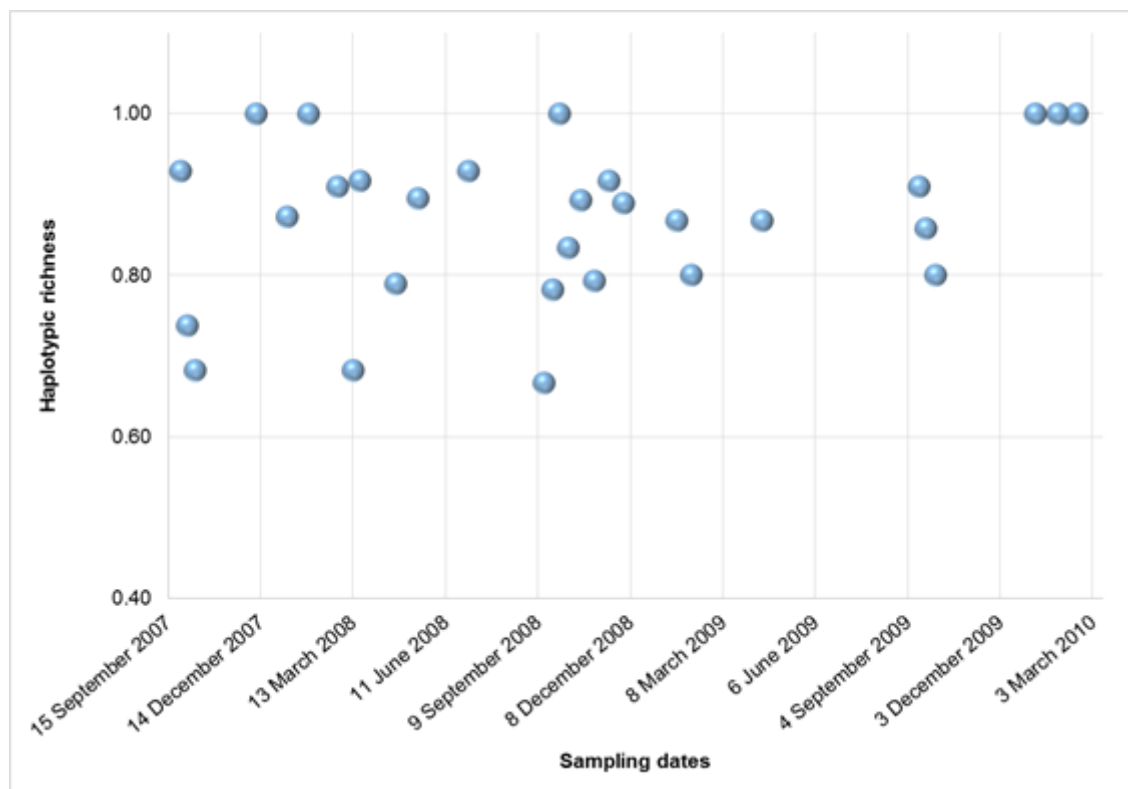
Table 5: Correlation assessed with Spearman's rank correlation  $\rho$  between daily proportions of *B. cinerea* isolates assigned to each of the 8 genetic clusters. Shaded cells indicate significant values ( $P < 0.05$ ) of  $\rho$ .

	cluster 1	cluster 2	cluster 3	cluster 4	cluster 5	cluster 6	cluster 7
cluster 2	-0.18						
cluster 3	0.42	-0.06					
cluster 4	-0.07	-0.12	-0.08				
cluster 5	-0.44	-0.23	-0.61	-0.28			
cluster 6	0.06	0.40	0.03	0.04	-0.48		
cluster 7	-0.19	0.05	-0.08	0.25	-0.29	0.21	
cluster 8	0.17	-0.06	0.03	0.14	-0.30	0.02	0.27

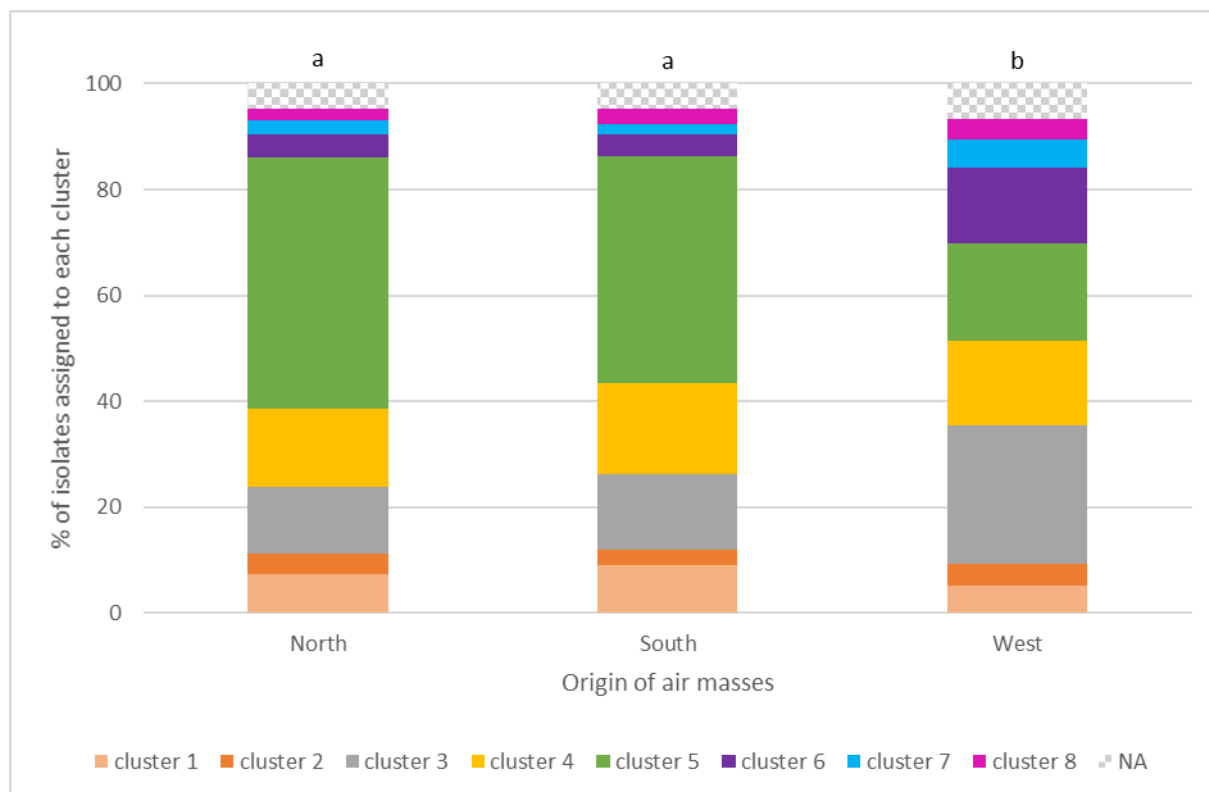


## Figures' captions

**Fig 1** Temporal variation of haplotypic richness of *B. cinerea* airborne inoculum



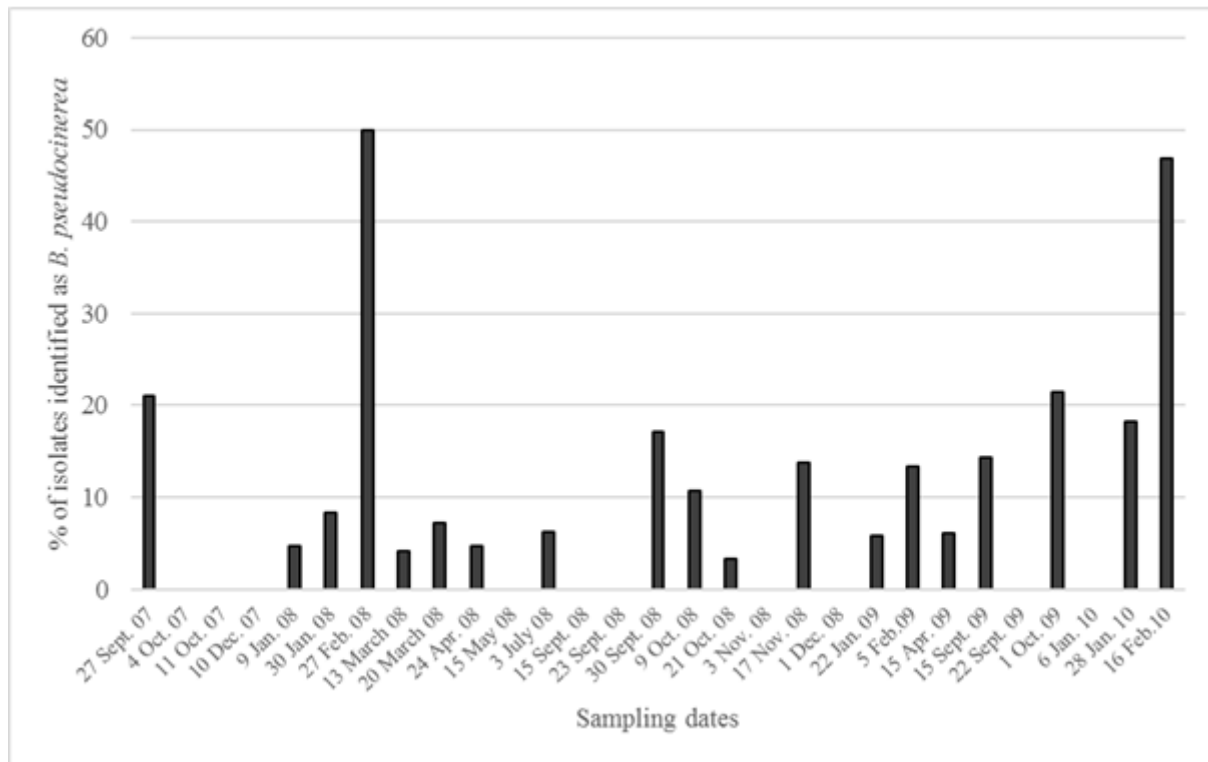
**Fig. 2** Distribution of *B. cinerea* isolates among eight genetic clusters according to the origin of air masses (NA: isolates not assigned to any cluster). Distributions associated with the same letter did not differ significantly at the 5% level (Fisher's exact test)



**Fig. 3** Distribution of *B. cinerea* isolates among eight genetic clusters according to the season (NA: isolates not assigned to any cluster). A: years grouped in four seasons (distributions associated with the same letter did not differ significantly at the 5% level, Fisher's exact test). B: successive seasons along the course of this study



**Fig. 4** Temporal variation of the proportion of *B. pseudocinerea* isolates among the total airborne *B. cinerea*-like isolates on 29 sampling dates between September 2007 and February 2010



**Fig. 5** Proportion of *B. pseudocinerea* isolates among total *cinerea*-like isolates when considering seasons (A) and air mass origin (B) as grouping factors. Bars indicate the standard error of the mean

