# Characterization of Sclerotinia sclerotiorum airborne inoculum, the widespread agent of white mould disease

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**Abstract**

A means to rationalize the use of fungicides for crop protection and to make agriculture friendlier to environment and human health is to develop disease-risk forecasting systems based on the assessment of airborne inoculum abundance. *Sclerotinia sclerotiorum*, the pandemic agent of white mould disease, is disseminated via the atmosphere in the form of ascospores. These airborne spores are the primary source of inoculum initiating the majority of epidemics. However for witloof chicory (*Cichorium intybus* var. *foliosum* L.), there is no data about airborne inoculum of *S. sclerotiorum*, which makes it difficult to develop a forecasting model. In the present study we characterized the temporal evolution of the abundance and of the genetic characteristics of *S. sclerotiorum* inoculum on a witloof chicory field located in Northern France over a three-year period. To our knowledge, this study provides the first quantification of viable airborne populations of this fungus in witloof chicory crops. Moreover it provides the first genetic characterization of *S. sclerotiorum* airborne inoculum. The results show that viable ascospores were present through 80% of the sampling dates. A significant correlation between abundance of airborne ascospores and local relative humidity suggests a local origin of inoculum. However, the existence of a slight genetic differentiation between isolates carried by air masses coming from the West and from the North-West is compatible with the hypothesis of a distant origin of *S. sclerotiorum* inoculum. We discuss the additional studies that are envisioned to clarify the origin of *S. sclerotiorum* airborne inoculum in witloof chicory fields.

**Keywords** air mass trajectory, ascospores, quantification, microsatellite markers, witloof chicory

## 1. Introduction

Many phytopathogenic fungi are disseminated via the atmosphere from micro- to macro-geographical scales in the form of spores (Brown and Hovmøller 2002; Prospero et al. 2005). The distance of their dissemination determines the extent to which plant diseases can spread and the degree to which novel genotypes of pathogens can invade new territories. For example, it has been demonstrated that rust fungi can be disseminated across continents and oceans (Purdy et al. 1985; Brown and Hovmøller 2002). In order to manage sustainable crop health, predictive tools can help growers in rationalizing their practices, particularly chemical

control, and thus can lead to a reduction in the number of fungicide applications and their environmental impact. These tools often include models that forecast the arrival of spores in areas where susceptible crops are grown (Pan et al. 2006; Tao et al. 2009). Such models are particularly difficult to establish for fungi with broad host ranges because sources of inoculum cannot be readily identified.

*Sclerotinia sclerotiorum* (Lib) de Bary, the pandemic agent of white mould disease, is one of these polyphagous fungi. It can attack more than four hundred plant species (Purdy 1979; Boland and Hall 1994) and generates yearly losses estimated to several millions of dollars worldwide (Petrofeza and Nasser 2012). *S. sclerotiorum* can survive several years in the soil as sclerotia. When conditions are suitable, apothecia are formed on sclerotia and release ascospores. Active release of ascospores needs high humidity for cells to become turgid (asci) (Meredith 1973; Elbert et al. 2007). Ascospores are the primary source of inoculum initiating the majority of white mould epidemics (Abawi and Grogan 1979; Mila and Yang 2008). In certain crops such as canola, it has been shown that airborne inoculum originated from within the field under study (Qandah and Del Rio Mendoza 2012), whereas for other crops such as beans, lettuce, or potato, there was evidence that ascospores came from external sources (Abawi and Grogan 1979; Ben-Yephet and Bitton 1985; Hammond et al. 2008). Indeed, most of the ascospores are thought to be deposited within hundred meters from the source (Abawi and Grogan 1979; Ben-Yephet and Bitton 1985). However the synchronized ejection of thousands of spores creates a minute flow of air that enables some of them to escape the still air layer surrounding the apothecium and then reach air currents that may allow long distance dispersal (Roper et al. 2010; Savage et al. 2012). An evidence for long distance dispersal is the reported presence of viable ascopores of *Sclerotinia* at altitudes up to 5000m (Meier et al. 1933).

In canola fields, the relationship between abundance of airborne inoculum of *S. sclerotiorum* and white mould incidence has been shown (Qandah and Del Rio Mendoza 2012). In carrot plots the scheduling of fungicide treatments according to a disease forecast model, based on predicted inoculum abundance, resulted in equivalent protection efficiency as a calendar-based scheduling, with an 80% decrease in the number of treatments (Foster et al. 2011). However for some other crops, like witloof chicory, also called Belgian endive (*Cichorium intybus* var. *foliosum* L.), there are no data about airborne inoculum of *S. sclerotiorum*, which makes it difficult to develop a disease forecast model. The task is rendered even more difficult by the peculiarity of the witloof chicory production system. In a first step, seeds are sown in the field (typically between mid-April and mid-June in northern France) for the production of

roots. In the field, white mould symptoms appear as subcollar necrosis and losses are usually quite limited (Benigni and Bompeix 2010). In the autumn (typically October-November) the roots are harvested from the field, stored in cold rooms and then transferred to hydroponic systems in the dark (forcing period) for the production of etiolated buds harvested as white chicory hearts. The sanitary status of roots harvested from the field is usually examined and those showing symptoms of white mould are eliminated before their transfer to forcing trays. However, during the forcing period, *S. sclerotiorum* may induce severe damage with losses up to 32% (Benigni and Bompeix 2010).

In order to better understand white mould outbreaks on witloof chicory and to consider the development of a forecasting model, the present study aimed to acquire knowledge on the abundance and the characteristics of airborne inoculum of *S. sclerotiorum* in the field. Hence, we conducted a study over a three-year period to characterize the temporal evolution of the abundance and of the genetic characteristics of *S. sclerotiorum* inoculum on a witloof chicory field located in Northern France. The specific objectives were to determine (i) if the concentration of airborne spores of *S. sclerotiorum* follows a seasonal pattern, (ii) if local climatic parameters influence the quantity of spores *S. sclerotiorum* observed in the air and (iii) if the genetic characteristics and abundance of this airborne inoculum can be related to the origin of air masses arriving on the sampling site.

## 2. Materials and methods

### *2.1 Sampling and quantification of airborne propagules in the field*

The air sampling was carried out in northern France, in the area of Arras (lat. 50.28 N; long. 2.78 E) at the experimental station of French producers of witloof chicory (APEF). This region is subjected to a temperate and humid climate. Air samples were collected in the field during 3 successive witloof chicory cropping seasons. The quantification of airborne inoculum of *S. sclerotiorum* was carried out over 100 sampling dates spread out from July 2014 to October 2016 (Figure 1). In 2014, the sampling period corresponded to the presence of witloof plants in the field, from July until the harvest of roots in late October. In 2015 and 2016, sampling was initiated earlier to cover a period that preceded the sowing of the crop. A portable air sampler for agar plates (Burkard manufacturing, Rickmansworth, UK) with a flow rate of 20 L $min^{-1}$ was used. Petri plates were filled with a semi-selective medium amended with bromophenol blue (Steadman et al. 1994). Growth of *S. sclerotiorum* on this medium causes the development of a yellow halo, corresponding to a drop in pH related to the

production of oxalic acid. On each sampling date, the samplers were set to run for three periods of 9 minutes, distributed between 10:00 and 16:00.

After exposure, the plates were incubated in the lab at room temperature (ca 22°C ± 1°C) and mycelial colonies associated with yellow halos were transferred to fresh PDA to obtain pure cultures. Isolates showing the typical morphological features of *Sclerotinia* sp. were kept and the number of *S. sclerotiorum* colony-forming units (CFU) was recorded. These counts were then used to estimate the number of viable spores that had been collected in each Petri plate. The number of viable spores were reported to the numbers of cubic meters of sampled air, taking into account the sampler's throughput and the sampling duration. Concentrations of viable spores per cubic meter of air were then obtained.

Prior to their entry in the fungal collection of the laboratory, all airborne isolates were subjected to a step of single hypha isolation. For this, single pieces of hyphal tip were excised from the growing margin of a colony after two days of incubation on PDA and transferred to fresh medium as described by Lehner et al. (2016). The single-hypha isolates were then stored as sclerotia at -20°C.

*2.2 Isolate genotyping*

Genomic DNA was extracted in 96-well plates from aliquots of 100 mg (fresh weight) of frozen fungal material, following the Dneasy Plant extraction Kit protocol (Qiagen). Sixteen microsatellite markers designed for *S. sclerotiorum* by Sirjusingh and Kohn (2001) were amplified with forward primers conjugated with the following fluorescent dyes: FAM for loci 5-3, 7-2, 7-3, 8-3, 12-2, 13-2, 17-3, 36-4, 42-4, 55-4, 92-4, 106-4 and HEX for loci 5-2, 9-2, 110-4 and 114-4 (MWG). Reverse primers did not carry any fluorescent dye. All markers were amplified at a hybridization temperature of 55°C, except 8-3, 42-4, 55-4 and 92-4 which were amplified at 60°C. To determine the size of the microsatellites, the PCR products were diluted and multiplexed prior to scanning with the help of an ABI 3730 sequencer (Applied Biosystems). The multiplexing consisted of mixing in a same well the PCR products of up to six markers. The markers were separated in three mixes: one for markers 7-2, 12-2, 13-2, 36-4, 106-4 and 114-4, a second one for markers 7-3, 8-3, 9-2, 17-3 and 42-4 and a third one for 5-2, 5-3, 55-4 92-4, and 110-4. In each well, 600LIZ was used as a standard size marker. GeneMapper software version 4.1 (Applied Biosystems) was then used for the microsatellite size analysis. Complete microsatellite size profiles (referred to as "haplotypes" hereafter) were obtained for 105 isolates.

*2.3 Genetic characterization of* S. sclerotiorum *isolates*

The number of different multilocus haplotypes (MLH) was computed with GenClone 1.0 software (Arnaud-Haond and Belkhir 2007). We used the index of haplotypic diversity (based on the number of individuals and the number of distinct MLH), which estimates the proportion of haplotypes present in a population and takes a value of 1 when a population is composed exclusively of unique haplotypes (Arnaud-Haond et al. 2007). The level of genetic differentiation between the identified clusters was assessed by computing $R_{ST}$ values using the software Arlequin version 3.5 (Excoffier et al. 2005)

The Bayesian clustering software Structure version 2.3.4 (Falush et al. 2003) was used to determine the genetic structure of *S. sclerotiorum* isolates. The analysis was carried out using 59 MLH (a single exemplary of each clone was kept for the analysis). The tested numbers of clusters (K) varied from 1 to 7, with 7 replicates for each value of K. Each simulation consisted in 300,000 Monte-Carlo Markov Chain iterations preceded by a burn-in period of 200,000 iterations. The most probable structure was determined by computing the posterior probability for each K using the distribution of maximum likelihoods. The Structure Harvester program v0.6 (Earl and vonHoldt 2012) was used to visualize Structure software output. When the probability of ancestry of an isolate in a cluster was greater than the arbitrary threshold of 0.75, this isolate was considered to be unambiguously assigned to this cluster.

*2.4 Acquisition of meteorological data*

Meteorological data (air temperature, wind speed, relative humidity and precipitation) were acquired continuously on a climatic station located at Arras (lat. 50.28 N; long. 2.78 E) during the period from March 20$^{th}$ to November1$^{st}$, common to the 3 years of sampling.

*2.5 Backward trajectories of air masses*

To evaluate the impact of the origin of air masses on the abundance and on the genetic characteristics of *S. sclerotiorum* inoculum, backward trajectories of air masses arriving in Arras 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 500, 1500 and 2500 meters above ground level.

*2.6 Statistical analysis*

Statistical analyses were performed with Statistica (version 12, Statsoft). Statistical inferences were made at the 5% level of significance, unless indicated otherwise. Non parametric tests were used to determine correlations (Spearman test) and significant differences (Kruskal-Wallis) between data. In order to test the significance of the association between airborne spore concentrations and air masses trajectories an exact test of Fisher was realized.

## 3. Results

### *3.1 Abundance of* S. sclerotiorum *airborne inoculum and climatic parameters.*

Viable inoculum of *S. sclerotiorum* was detected in the air during 80 of the 100 sampling days at Arras (Figure 1). Failure to detect viable airborne inoculum occurred for 13 sampling dates in 2015 (22 May, 11 and 19 June, 3 and 30 July, 10 and 14 August, 1, 3 11, 18, 25 and 28 September) and 7 sampling dates in 2016 (24 May, 16 and 31 August, 7 and 26 September, 1 and 8 October).

When airborne inoculum was detected, estimated daily concentrations varied between 14.8 and 183.3 spores per cubic meter of air in 2014 and between 1.9 and 22.2 spores per cubic meter of air in 2015 and 2016. The highest values of inoculum abundance where found in spring, summer and autumn depending on the year of sampling. There was no significant difference of inoculum abundance between seasons for 2015 and 2016 ($P_{Kruskall\text{-}Wallis} = 0.19$). Data for 2014 were not included in this analysis as no sampling was carried out in spring 2014. During the sampling period of 2014, the observed concentrations of viable airborne inoculum of *S. sclerotiorum* were substantially higher than those observed in the sampling periods of 2015 and 2016 ($P_{Kruskall\text{-}Wallis} < 0.01$) (Figure 1). The cumulative precipitation and the numbers of days with mean relative humidity > 90% were also higher in 2014 than in 2015 and 2016 (Table 1). This is consistent with the fact that, based on Spearman's tests, there was a significant positive correlation ($\rho$ up to 0.52) between inoculum concentration and mean, minimum and maximum humidity (from the sampling date back to 10 days earlier) (Table 2). Abundance of airborne inoculum was positively correlated with mean wind speed and minimum temperature measured on the sampling dates and 1, 2, and 5 days earlier. In addition a significant correlation was also observed with wind speed values 7 days before spore sampling.

*3.2 Relation between inoculum abundance and air mass trajectory*

Backward trajectories of air masses arriving at the sampling site on the sampling dates were determined. For the 100 sampling dates, 35% of the air masses arrived from the North-West (after passing over the UK or Ireland) (Figure 2), 33% from the West (over the Atlantic Ocean and passing over Britany), 18 % from the North-East (after passing over Scandinavia or Eastern Europe), 9% from the South West (passing over Southwestern France and Spain) and the rest (5%) were from the South east (only once) or from variable origin.

The trajectories of the ten sampling days of each year with the highest airborne inoculum concentrations were compared with the ten sampling days of each year with the lowest concentrations. Air masses with the highest concentrations came 12 times from the West, 11 times from the North West, 7 times from the South West and 4 times from the North East. The air masses with the lowest concentrations arrived 12 times from the North West, 10 times from the North East, 6 times from the West and 3 times from the South West. Fisher's exact test showed no significant association between inoculum concentration and the origin of air masses (P value = 0.11).

*3.3 Genetic characteristics of* S. sclerotiorum *airborne inoculum*

Fifty nine different MLH were found among the 105 airborne isolates of *S. sclerotiorum* with complete haplotypes. Sixteen of these MLH were shared by several isolates (Figure 3). The most abundant MLH was shared by 13 isolates all sampled on the same date. The second most abundant MLH was shared by 12 isolates collected during the 3 years of sampling. In total, 8 of the shared MLH were represented by isolates collected during different years. This result is supported by the absence of genetic differentiation between isolates collected during the 3 different years (non-significant $R_{ST}$ values).

The relationship between air masses origin and genetic characteristics was not clear. Indeed, there was no significant genetic differentiation between isolates potentially carried by different air masses except a slight one between those carried by air masses coming from the West and from the North-West ($R_{ST}$=0.08, P=0.037).

The software Structure was run 5 times. The number of clusters was not consistent from one run to another (it was equal to 4 twice, and equal to 5 three times). Moreover in average over the 5 runs, 20 % of isolates were not clearly assigned to a cluster. It showed that there was no clear genetic clusters structuring the airborne inoculum of *S. sclerotiorum* collected in the Arras area.

## 4. Discussion

The aim of the present work was to determine when and in which quantity viable inoculum of *S. sclerotiorum* was present in the air above a witloof chicory field and to determine if this presence was linked to local climatic parameters or to the origin of air masses. To our knowledge, this study provides the first quantification of viable airborne populations of this fungus in witloof chicory crops. Moreover it provides the first genetic characterization of *S. sclerotiorum* airborne inoculum.

The air sampling carried out in the present study showed that viable airborne ascospores of *S. sclerotiorum* were present most of the sampling dates (80%). Over the three-year study, airborne inoculum of the pathogen was observed during all months when sampling was carried out, without any seasonal patterns of particularly high or low abundance. This leads to the conclusion that susceptible crops in the Arras region may be at risk all the time, at least between April and November. As apothecia were never observed in the field where the sampler was located, our results raise the question of the origin of ascospores. *S. sclerotiorum* apothecia may have been present in neighboring fields on crops or weeds as reported in previous studies for bean, lettuce and potato crops (Abawi and Grogan 1979; Ben-Yephet and Bitton 1985; Hammond et al. 2008).

The significant correlation between abundance of airborne ascospores and local relative humidity suggests a local origin of inoculum. This hypothesis is supported by results of the genetic characterization of *S. sclerotiorum* isolates which suggest that the same local sources are emitting ascospores year after year. This evidence includes the absence of a clear genetic structure of airborne isolates, the lack of genetic differentiation between isolates collected in different years and the several identical haplotypes encountered during the 3 years of air sampling. This convergence of evidence is compatible with a predominantly local origin of inoculum. However, occasional influx of inoculum from distant origins cannot be excluded, as we observed a slight genetic differentiation between isolates carried by air masses coming from the West and from the North-West. A similar situation has already been observed for *Botrytis cinerea*, another ascomycete with a large host range (Leyronas and Nicot 2013; Leyronas et al. 2015).

Additional studies will be needed to identify the specific origins of those occasional introductions of distant inoculum. For example, the genetic characteristics of French isolates could be compared with those of isolates previously examined in neighbouring countries (Lewartowska et al. 1996; Pascual et al. 2010; Clarkson et al. 2017). Recently, the species

*Sclerotinia subarctica* (agent of white mould, morphologically indistinguishable from *S. sclerotiorum*) has been reported on witloof chicory in the Arras area (Leyronas et al. 2018). Formerly, this species was observed in rather northern countries. Many isolates have been found in England (Clarkson et al. 2017), in a region located approximately 500 km from the site of the present study in northern France. Since air masses often travel over the United Kingdom before arriving in the Arras area (35 % of the time in the present study), introductions of airborne inoculum of *S. sclerotiorum* and *S. subarctica* from those regions should not be excluded.

Furthermore, data on local isolates could be complemented by a systematic surveillance of the occurrence of *S. sclerotiorum* apothecia in the Arras area and the characterization of isolates, not only from witloof chicory fields but also from other potential agricultural and non-agricultural reservoirs. Indeed it has been shown that populations of microorganisms from non-agricultural reservoirs may impact the evolution of populations from agricultural reservoirs. (Monteil et al. 2013; Leroy et al. 2016).

Finally, this search for apothecia and sclerotia should be complemented by an interrogation about the role of ascospores, compared to other types of inoculum, in the development of white mould on witloof chicory. It has been shown that ascospores initiate white mould epidemics on many crops (Abawi and Grogan 1979; Clarkson et al. 2007; Mila and Yang 2008; Qandah and Del Rio Mendoza 2011). Since few roots develop symptoms in the field (Benigni and Bompeix 2010), the possible implication of other sources of inoculum should also be addressed in addition to ascospores or mycelium germinating from sclerotia in the soil. Recently, it has been shown that *B. cinerea* can survive as endophyte in plants, especially in different species of *Asteraceae* (Sowley et al. 2010; Shaw et al. 2016). Since *S. sclerotiorum* is genetically close to *B. cinerea*, and since witloof chicory belongs to the *Asteraceae* family, it would be interesting to look for a possible endophytic form of *S. sclerotiorum* in witloof chicory that could explain white mould outbreaks during the forcing period.

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The authors declare no conflict of interest.

**References**

Abawi, G.S., & Grogan, R.G. (1979). Epidemiology of diseases caused by *Sclerotinia* species. *Phytopathology, 69*(8), 899-904.

Arnaud-Haond, S., & Belkhir, K. (2007). Genclone: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Notes, 7*(1), 15-17.

Arnaud-Haond, S., Duarte, C.M., Alberto, F., & Serrao, E.A. (2007). Standardizing methods to address clonality in population studies. *Molecular Ecology, 16*(24), 5115-5139.

Ben-Yephet, Y., & Bitton, S. (1985). Use of a selective medium to study the dispersal of ascospores of *Sclerotinia sclerotiorum*. *Phytoparasitica, 13*(1), 33-40.

Benigni, M., & Bompeix, G. (2010). Chemical and biological control of *Sclerotinia sclerotiorum* in witloof chicory culture. *Pest management Science*, *66*(12), 1332-1336.

Boland, G. J., & Hall, R. (1994). Index of plant hosts of *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology*, *16*(2), 93-108.

Brown, J.K.M., & Hovmøller, M.S. (2002) Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science*, *297*(5581), 537-541.

Clarkson, J.P., Phelps, K., Whipps, J.M., Young, C.S., Smith, J.A., & Watling, M. (2007). Forecasting sclerotinia disease on lettuce, a predictive model for carpogenic germination of *Sclerotinia sclerotiorum* sclerotia. *Phytopathology, 97*(5), 621-631.

Clarkson, J.P., Warmington, R., Walley, P.G., Denton-Giles, M., Barbetti, M.J., Brodal, G., & Nordskog, B. (2017). Population structure of *Sclerotinia subarctica* and *Sclerotinia sclerotiorum* in England, Scotland and Norway. *Frontiers in Microbiology*, doi: 10.3389/fmicb.2017.00490

Draxler, R.R., & Rolph, G.D. (2011). HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory) Model access via NOAA ARL READY Website

(http://ready.arl.noaa.gov/HYSPLIT.php). NOAA Air Resources Laboratory, Silver Spring, MD.

Earl, D.A., & vonHoldt, B.M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources, 4*(2), 359-361.

Elbert, W., Taylor, P.E., Andreae, M.O., & Pöschl, U. (2007). Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions. *Atmospheric Chemistry and Physics, 7*, 4569-4588.

Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online, 1,* 47-50.

Falush, D., Stephens, M., & Pritchard, J.K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics, 164*(4), 1567-87.

Foster, A.J., Kora, C., McDonald, M.R., & Boland, G.J. (2011). Devlopment and validation of a disease forecast model for *Sclerotinia* rot of carrot. *Canadian Journal of Plant Pathology, 33*(2), 187-201.

Hammond, C.N., Cummings, T.F, & Johnson, D.A. (2008). Deposition of ascospores of *Sclerotinia sclerotiorum* in and near potato fields and the potential to impact efficacy of a biocontrol agent in the Columbia basin. *American Journal of Potato Research, 85*(5), 353-360.

Lehner, M.S., Trazilbo, J.P. & Mizubuti, E.S.G. (2016). Does hyphal-tip ensure the same allelic composition at SSR loci as monosporic isolates of *Sclerotinia sclerotiorum*? *Journal of Phytopathology, 164*(6), 417-420.

Lewartowska, E., Jedryczka, M., & Frencel, I. (1996). Pathogenicity of *Sclerotinia sclerotiorum* (Lib.) de Bary isolates from different localities of rapeseed in Poland.

Proceedings Scientific Symposium on Plant Diseases and the Environment, Poznan, Poland (Jun 27-28, 1996)

Leroy, T., Caffier, V., Celton, J.M., Anger, N., Durel, C.E., Lemaire, C., & Le Cam, B. (2016). When virulence originates from nonagricultural hosts: evolutionary and epidemiological consequences of introgressions following secondary contacts in *Venturia inaequalis*. *New Phytologist*, *210*(4), 1443–1452.

Leyronas, C., & Nicot, P.C. (2013). Monitoring viable airborne inoculum of *Botrytis cinerea* in the South-East of France over 3 years: relation with climatic parameters and the origin of air masses. *Aerobiologia, 29*(2), 291-299.

Leyronas, C., Halkett, F., & Nicot, P.C. (2015). Relationship between the genetic characteristics of *Botrytis sp.* airborne inoculum and meteorological parameters, seasons and the origin of air masses. *Aerobiologia*, *31*(3), 367-380.

Leyronas, C., Troulet, C., Duffaud, M., Villeneuve, F., Benigni, M., Leignez, S., & Nicot, P.C. (2018). First report of *Sclerotinia subarctica* in France detected with a rapid PCR-based test. *Canadian Journal of Plant Pathology*, https://doi.org/10.1080/07060661.2018.1438515

Meier, F.C., Stevenson, J.A., & Charles, V.K. (1933). Spores in the upper air. *Phytopathology*, *23*, 23-23.

Meredith, D.S. (1973). Significance of spore release and dispersal mechanisms in plant disease epidemiology. *Annual review of Phytopathology*, *11*, 313-342.

Mila, A.L., & Yang, X.B. (2008). Effects of fluctuating soil temperature and water potential on sclerotia germination and apothecial production of *Sclerotinia sclerotiorum*. *Plant Disease*, *92*(1), 78-82.

Monteil, C.L., Cai, R., Liu, H., Llontop, M.E., Leman, S., Studholme, D.J., Morris, C. E., & Vinatzer, B.A. (2013). Nonagricultural reservoirs contribute to emergence and evolution of *Pseudomonas syringae* crop pathogens. *New Phytologist*, *199*(3), 800-811.

Pan, Z., Yang, X.B., Pivonia, S., Xue, L., Pasken, R., & Roads, J. (2006). Long-term prediction of soybean rust entry into the continental United States. *Plant Disease*, *90*(7), 840-846.

Pascual, A., Campa, A., Perez-Vega, E., Giraldez, R., Miklas, P.N., & Ferreira, J.J. (2010). Screening common bean for resistance to four *Sclerotinia sclerotiorum* isolates collected in Northern Spain. *Plant Disease*, *94*(7), 885-890.

Petrofeza, S., & Nasser, L.C.B. (2012). Case Study: *Sclerotinia sclerotiorum* : Genetic diversity and disease control. In M. Caliskan (Ed.) *The Molecular Basis of Plant Genetic Diversity*. InTech, doi: 10.5772/33780. https://www.intechopen.com/books/the-molecular-basis-of-plant-genetic-diversity/case-study-sclerotinia-sclerotiorum-genetic-diversity-and-disease-control

Prospero, J.M., Blades, E., Mathison, G., & Naidu, R. (2005) Interhemispheric transport of viable fungi and bacteria from Africa to the Caribbean with soil dust. *Aerobiologia*, *21*(1), 1-19.

Purdy, L.H. (1979). *Sclerotinia sclerotiorum*: history, diseases and symptomatology, host range, geographic distribution, and impact. *Phytopathology*, *69*(8), 875-880.

Purdy, L.J., Krupa, S.V., & Dean, J.L. (1985). Introduction of sugarcane rust into the Americas and its spread to Florida. *Plant Disease*, *69*(8), 689-693.

Qandah, I.S., & Del Rio Mendoza, L.E. (2011) Temporal dispersal patterns of *Sclerotinia sclerotiorum* ascospores during canola flowering, *Canadian Journal of Plant Pathology*, *33*(2), 159-167.

Qandah, I.S., & Del Rio Mendoza, L.E. (2012). Modelling inoculum dispersal and *Sclerotinia* stem rot gradients in canola fields. *Canadian Journal of Plant Pathology*, *34*(3), 390-400.

Rolph, G.D. (2011) Real-time Environmental Applications and Display sYstem (READY) Website (http://ready.arl.noaa.gov). NOAA Air Resources Laboratory, Silver Spring, MD.

Roper, M., Seminara, A., Bandi, M.M., Ann Cobb, A., Dillard, H.R., & Pringle, A. (2010). Dispersal of fungal spores on a cooperatively generated wind. *PNAS*, *107*(41), 17474-17479.

Savage, D., Barbetti, M.J., MacLeod, W.J., Salam, M.U., & Renton, M. (2012). Seasonal and diurnal patterns of spore release can significantly affect the proportion of spores expected to undergo long-distance dispersal. *Microbial Ecology*, *63*(3), 578-585.

Shaw, M.W, Emmanuel, C.J., Emilda, D., Terhem, R.B., Shafia, A., Tsamaidi, D., Emblow, M., and van Kan, J.A.L. (2016). Analysis of cryptic, systemic *Botrytis* infections in symptomless hosts. *Frontiers in Plant Science*, https://doi.org/10.3389/fpls.2016.00625

Sowley, E.N.K., Dewey, F.M., & Shaw, M.W. (2010). Persistent, symptomless, systemic, and seedborne infection of lettuce by *Botrytis cinerea*. *European Journal of Plant Pathology*, *126*(1), 61-71.

Sirjusingh, C., & Kohn, L.M. (2001). Characterization of microsatellites in the fungal plant pathogen, *Sclerotinia sclerotiorum*. *Molecular Ecology Notes*, *1*(4), 267-69.

Steadman, J.R., Marcinkowska, J., & Rutledge, S. (1994). A semi-selective medium for isolation of *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology*, *16*(1), 68-70.

Tao, Z., Malvick, D., Claybrooke, R., Floyd, C., Bernacchi, C. J., Spoden, G., Kurle, J., Gay, D., Bowersox, V., & Krupa, S. (2009) Predicting the risk of soybean rust in Minnesota based on an integrated atmospheric model. *International Journal of Biometeorology*, *53*(6), 509-521.

**Table 1** Climatic characteristics of the period between March 20th and November 1st of years 2014, 2015 and 2016 in Arras.

| Year | Daily mean temperature (°C) | Number of days with precipitation | Cumulative precipitation (mm) | Number of days with mean RH>90%[a] |
|---|---|---|---|---|
| 2014 | 14,82 | 98 | 459,7 | 50 |
| 2015 | 13,60 | 122 | 373,3 | 28 |
| 2016 | 14,31 | 125 | 413,3 | 30 |

[a] RH: relative humidity

**Table 2** Spearman's rank correlation between airborne inoculum concentrations and climatic parameters measured on Arras climatic station during the sampling day (D) and up to 10 days before.

| | Precipitations | Mean air temperature | Minimum air temperature | Maximum air temperature | Mean humidity | Minimum humidity | Maximum humidity | Mean wind speed | Solar radiation |
|---|---|---|---|---|---|---|---|---|---|
| $\rho_{D}$ | 0.02 NS | 0.10 NS | 0.20 * | 0.02 NS | 0.4 *** | 0.26 ** | 0.52 *** | 0.24 ** | -0.05 NS |
| $\rho_{D-1}$ | 0.06 NS | 0.03 NS | 0.20 * | -0.08 NS | 0.4 *** | 0.29 ** | 0.37 *** | 0.23 * | -0.09 NS |
| $\rho_{D-2}$ | 0.12 NS | 0.005 NS | 0.10 NS | -0.02 NS | 0.43 *** | 0.28 ** | 0.51 *** | 0.10 NS | -0.14 NS |
| $\rho_{D-3}$ | 0.08 NS | -0.03 NS | 0.19 NS | -0.09 NS | 0.46 *** | 0.35 *** | 0.50 *** | 0.11 NS | -0.25 *** |
| $\rho_{D-5}$ | -0.04 NS | 0.20 * | 0.35 *** | 0.09 NS | 0.28 ** | 0.25 * | 0.29 ** | 0.37 *** | -0.14 NS |
| $\rho_{D-7}$ | -0.07 NS | 0.07 NS | 0.16 NS | 0.04 NS | 0.32 *** | 0.23 * | 0.40 *** | 0.33 *** | -0.02 NS |
| $\rho_{D-10}$ | -0.02 NS | 0.01 NS | 0.15 NS | -0.04 NS | 0.46 *** | 0.30 ** | 0.60 *** | 0.15 NS | -0.13 NS |

Significance levels: NS, non significant; * significant at P=0.05; ** significant at P=0.01; *** significant at P=0.001

D, D-1 indicates the day of measurement of climatic parameters: D is the day of sampling, D-1 is the day before sampling etc.

**Fig. 1** Temporal variation of the abundance of *S. sclerotiorum* viable airborne inoculum in the area of Arras during 3 cropping seasons of witloof chicory (the black lines indicate the beginning and the end of the sampling period each year)

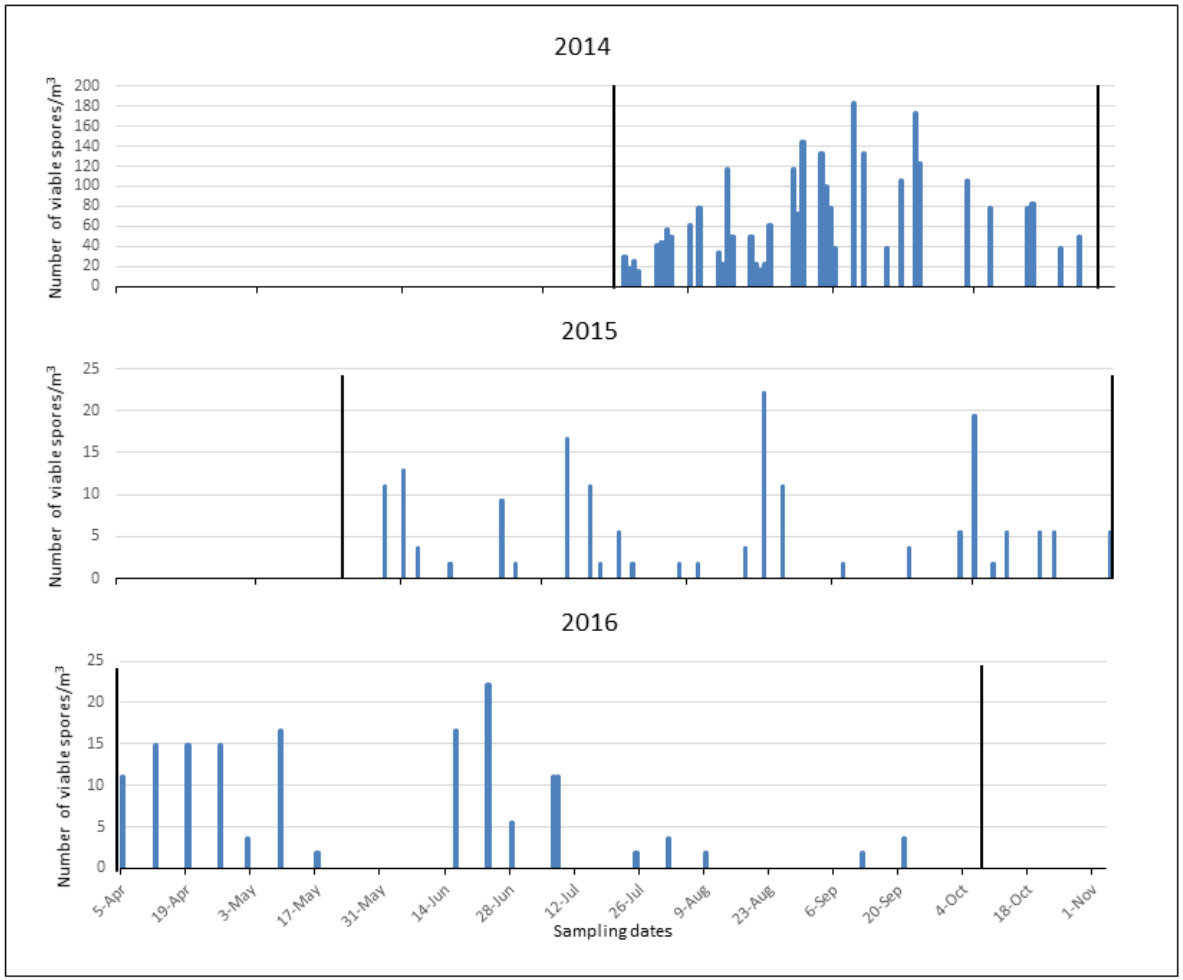

**Fig. 2** Origin of air masses arriving in Arras (data compiled over the 100 days of air sampling)

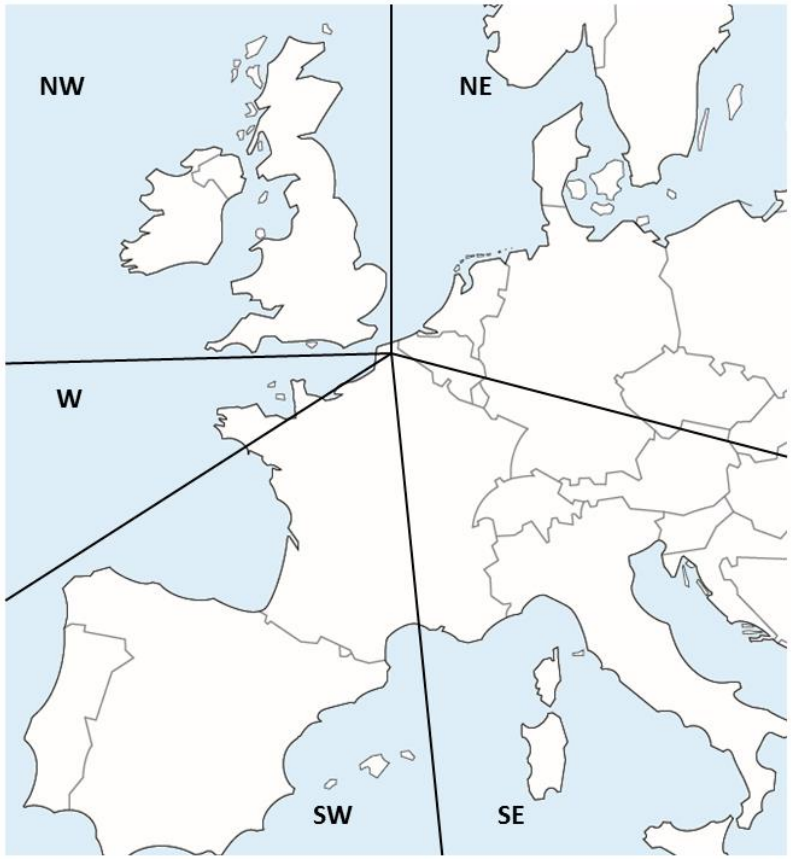

**Fig. 3** Multilocus haplotypes represented by more than one airborne isolate of *S. sclerotiorum* collected in 2014, 2015 or 2016 in the area of Arras