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Airborne versus soilborne inoculum: white mould, where do you come from?

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Identifying the sources of fungal inoculum that induces epidemics is instrumental to managing crop health in a more efficient way by implementing prophylactic methods and by using better targeted biocontrol and fungicide applications. For some phytopathogenic fungi this identification is hampered by the multiplicity of inoculum types. This is the case for *Sclerotinia sclerotiorum*, the agent of white mould, which can produce ascospores disseminated via the atmosphere, sclerotia stored in the soil and mycelium in plant debris of a large variety of hosts. The present study aims to assess the relative importance of airborne and soilborne inoculum in white mould disease development and its persistence across two crop seasons. *S. sclerotiorum* isolates were collected from air, soil and carrot plants during two crop seasons in south-western France and genotyped with 16 microsatellite markers. Among the 490 isolates, 162 clonal lineages were identified. The genetic characteristics did not differ significantly among isolates collected from air, soil and carrots. The epidemics of white mould on carrots were initiated either by airborne or soilborne inoculum. It also appears that some isolates from airborne inoculum persisted from year to year.

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1 Introduction

Many plant diseases are caused by fungi *sensu lato* (including fungal-like organisms such as Oomycetes), causing billions of euros of losses worldwide each year. The use of chemical pesticides as the primary method for crop protection has not only a financial, but also an environmental and health cost. In temperate environments, epidemics are cyclical and develop on an annual basis depending on the plant growth period. In favourable environmental conditions, the outbreak of a fungal disease is initiated by the encounter between a susceptible plant and the matching fungal inoculum, generally in the form of a spore or a free-living mycelium. The fungal inoculum that starts the epidemic is often referred to as the primary inoculum. A better knowledge of the nature and localization of this primary inoculum could allow the scope of surveillance, both in terms of space and time, to expand and to diversify opportunities for prophylactic plant protection methods and therefore use less or even no fungicide (Morris et al., 2022).

Among the phytopathogenic fungi, two kinds of primary inocula can be distinguished, being either airborne or soilborne. Some phytopathogens are well known for their ability to produce numerous spores that are released and dispersed in the air (e.g., the agents of rust), while others are well known for their persistence over years in the soil (e.g., *Rhizoctonia* spp., *Pythium* spp.). Beyond this simple dichotomy, many fungal species may produce both airborne and soilborne inocula. *Sclerotinia sclerotiorum* is one of them. This ascomycete can produce a compact mass of hyphae, called sclerotia, that can be stored and stay viable in the soil for several years. Sclerotia lead either to direct plant infection through myceliogenic germination or to the production of ascospores through carpogenic germination (Abawi & Grogan 1979; Steadman, 1983). Ascospores can be detected in the air during most of the crop season as, for

example, in witloof chicory (Leyronas et al., 2019). They can be dispersed by wind and thus transport the pathogen within and among fields (Kora et al., 2003).

Direct observation of spore movement is quite difficult for such minute organisms. In this respect, population genetics can help unravel the origin of an outbreak (Xhaard et al. 2012). Population genetics analyses the genetic diversity and its apportionment within and among sampling units (the so-called populations) (Saubin et al., 2022). Different levels of biological organization can therefore be considered to study the origin of the inoculum. At the population level, for example, between sampling units, the apportionment of genetic diversity (e.g., by means of the F_{ST} index) would be indicative of the magnitude of gene flow, which can be related to the extent of movement between different compartments. At the individual level, for clonal and partially clonal organisms, the occurrence of the same genotype in multiple sampling units can indicate past or present movement among compartments. *S. sclerotiorum* is a homothallic species which reproduces primarily by haploid selfing, which is equivalent to clonal reproduction (Billiard et al., 2011). In accordance, populations of *S. sclerotiorum* show some genetic diversity through outcrossing but reveal a predominantly clonal reproduction (Aban et al., 2021; Atallah et al., 2004; Leyronas et al., 2018a; Sexton et al., 2006). This clonal population structure is the result of both sexual reproduction by self-fertilization and asexual reproduction by means of sclerotia (Attanayake et al., 2019).

Carrot (*Daucus carota*) is one of the several hundred plant species that are susceptible to white mould caused by *S. sclerotiorum* (Boland & Hall, 1994; Purdy, 1979). In France, carrot is a major vegetable crop, with half of its production located in the south-west of the country. During the crop season, white mycelium develops on foliage and on the collar. After six months of growth, carrots are buried and stored in the soil for up to three months. When they are removed for the commercial market, the tuberous roots may show rot with typical white mycelium bearing sclerotia that can make up to 40% of carrots unmarketable. Identifying the

type of inoculum that causes symptoms may help to better target plant protection methods against white mould, that is, to apply protection methods either to the aerial or the underground part of the plant.

The objectives of the present study were to clarify the relative importance of airborne and soilborne inoculum in white mould disease development on carrot and to determine whether the inoculum can persist from one season to another. To achieve these goals, we (a) characterized *S. sclerotiorum* isolates collected from the air, soil and carrots during two seasons using microsatellite markers, (b) set up a method to determine the true clones among the isolates, (c) compared the abundance of clonal lineages of *S. sclerotiorum* among compartments and years, and (d) examined the genetic structure, or the lack thereof, as an indication of the level of exchange among compartments.

2 Materials and methods

2.1 Collection of *S. sclerotiorum* isolates

Isolates of *S. sclerotiorum* from air, soil and plants were collected from carrot crops located in two fields separated by 5 km and situated in south-western France (Gironde district). Each field was sampled once during a given crop season. The crops were managed as follows (Figure 1): (a) seeds were sown in July, (b) carrots grew until December when they were harvested (the 'standing carrots' period hereafter), (c) just after harvest, carrots were buried in the sandy soil until March (the 'buried carrots' period hereafter). In the first field, the crop ran from July 2014 to March 2015 (Season 1) and in the second field, it ran from July 2015 to March 2016 (Season 2) (Table 1). Sclerotia were collected from white mycelium developing on the collar of standing

carrots from July to December and on buried carrots when they were taken out of the soil to be marketed in March.

The soilborne isolates originated from sclerotia collected after elutriating soil samples. Ten samples were collected in each field, at a depth of 0 to 30 cm, for a total of 1.5 kg of soil. We used a method described for the elutriation of nematodes (Seinhorst, 1962), in which the flow of water was adapted to the density of *S. sclerotiorum* sclerotia. Sclerotia from both soil and plant samples were surface-disinfested for 3 min in 5% sodium hypochlorite solution, rinsed in sterile water and plated on potato dextrose agar (PDA) to obtain a pure culture.

The airborne isolates were collected between sowing and harvesting the carrot crop (i.e., from July to end of December). These were obtained from Petri plates filled with a semiselective medium amended with bromophenol blue (Steadman et al., 1994). The plates were placed above the crop canopy in carrot fields and the medium was either exposed directly (plates open for 1 h) or inserted in portable air samplers (Burkard; throughput 20 L/min) set to run for 9 min. After exposure, the plates were incubated in the laboratory at room temperature (c.22°C) and the presence of *S. sclerotiorum* on the selective medium was assessed by the development of yellow halos, presumably caused by the production of oxalic acid (Steadman et al., 1994). Mycelial colonies associated with yellow halos were transferred to fresh PDA to obtain pure cultures. Isolates showing the typical morphological features of *S. sclerotiorum* were kept.

Prior to their entry into the laboratory fungal collection, all isolates (from soil, carrots and the air) 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 2 days of incubation on PDA and transferred to fresh medium as described by Lehner et al. (2016). When forming sclerotia, the single-hypha isolates were stored as sclerotia at −20°C.

2.2 Isolate genotyping

Sclerotia were taken out of the freezer and spread on PDA to germinate and produce mycelium. Genomic DNA was extracted in 96-well plates from aliquots of 100 mg of mycelium, 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 fluorescent dyes following the protocol described by Leyronas et al. (2018a). To determine the size of the microsatellites, the PCR products were diluted and multiplexed prior to scanning with an ABI 3730 sequencer (Applied Biosystems). GeneMapper software v. 4.1 (Applied Biosystems) was then used to assess the size of microsatellite markers on each isolate. Complete microsatellite profiles (referred to as haplotypes hereafter) were obtained for all isolates.

2.3 Identification of multilocus lineages

As this study primarily deals with the extent and impact of clonality on the population structure of *S. sclerotiorum*, we paid particular attention to the delineation of clonal lineages, defined as a group of isolates that bear the same haplotype and result truly from asexual reproduction (or from haploid selfing). Two common pitfalls arise in such analysis. First, the lack of differentiating power in the set of molecular markers used can lead to an overestimate of clonality, because some individuals have the same combination of alleles by chance (Arnaud-Haond et al., 2007; Halkett et al., 2005). We thus used MLGsim (Stenberg et al., 2003) to identify haplotypes and to test among identical haplotypes those that were significantly over-represented (i.e., those that indeed result from asexual multiplication) (Barrès et al., 2012). Secondly, small differences in allelic profiles among haplotypes can result in a spurious split of clonal lineages (Arnaud-Haond et al., 2007). This is especially true if clonal lineages accumulate somatic mutations through time (Becheler et al., 2010; Rozenfeld et al., 2007). To

merge clonal lineages that only differed by one allele, we thus conducted the same MLGsim analysis according to a jackknife procedure in omitting each locus one by one. Note that only haplotype variants that were significantly over-represented (and thus result from asexual multiplication or haploid selfing) were merged. Clonal lineages are called multilocus lineages (MLL) hereafter.

2.4 Genetic characterization and comparison of *S. sclerotiorum* isolates

According to Arnaud-Haond et al. (2007) and Dorken and Eckert (2001), we calculated the index of clonal richness, $R = (G - 1)/(N - 1)$, where G is the number of MLL and N the number of isolates tested. This index estimates the proportion of clonal lineages present in a population and is equal to 1 when a population is composed exclusively of a single lineage and 0 if all isolates have a distinct haplotype.

The software FSTAT v. 2.9.3 (Goudet, 1995) was used to compute the unbiased gene diversity (Hnb) for total dataset and clone corrected data. It was also used to compute the number of alleles (Ra) after a rarefaction procedure based on a minimal sample size of 12 isolates (total dataset) and 10 isolates (clone corrected data). Mean and standard deviation of Hnb and Ra were calculated over the 15 loci (the monomorphic locus 36_4 was removed for this analysis). The program Multilocus v. 1.3b (Agapow & Burt, 2001) was used to calculate the standardized version of the index of association ($\bar{r}_D$). This index is a measure of the multilocus linkage disequilibrium, which varies between 0 (complete panmixia) and 1 (no recombination). The null hypothesis of complete panmixia was tested by permuting alleles (microsatellite sizes) among isolates, independently for each microsatellite marker (500 permutations).

Genetic differentiation between years and among compartments (air, soil, carrots) was estimated using Arlequin (Excoffier et al., 2005). Moreover, we used GENEPOP software (Rousset, 2008) to calculate pairwise F_{ST} between groups of isolates, at each locus.

Venn diagrams were used to compare groups of isolates having different origins (air, soil, plants) on the basis of haplotype lists, using the website Jvenn (http://jvenn.toulouse.inra.fr/app/index.html; Bardou et al., 2014).

Fisher's exact tests of independence were used to determine if there was a difference in the abundance of haplotypes among compartments. The tests were computed with the GENEPOP v. 3.1c, Prog. STRUC with 500,000 iterations (Raymond & Rousset, 1995).

3 Results

3.1 Genetic diversity of *S. sclerotiorum* isolates

In total, 490 *S. sclerotiorum* isolates were collected during the two carrot crop seasons. During both seasons, isolates were sampled from standing and buried carrots with symptoms and from the air. However, soilborne isolates were collected during Season 2 only, and no sclerotia were found in the soil samples collected during Season 1.

Among the 16 markers used to characterize the 490 isolates, one (locus 36-4) was monomorphic, showing a single 411 bp allele, while all others were polymorphic with a number of alleles ranging from three (locus 5-2) to 24 (locus 106-4).

In total, we identified 158 distinct genotypes. Of these, 108 occurred only once. The number of replicates (among repeated genotypes) ranged from 2 to 47 and the *Psex* values ranged from 1.42×10^{-185} to 1.18×10^{-6}. Random simulations of genotypes (considering the

same sample size as the observed data) generated by chance 17 repeated genotypes. The distribution of the simulated *Psex* values allowed us to set the threshold for significant over-representation of genotypes at 1.72×10^{-9}. Four repeated genotypes (all occurring twice) had *Psex* values above this threshold and were therefore considered distinct multilocus lineages (MLLs). This resulted in 162 MLLs (Table S1).

The overall value of gene diversity was 0.59 and 0.61 for total dataset and clone corrected data, respectively (Table 1) and the overall allelic richness was 4.46 and 4.51 for total dataset and clone corrected data, respectively. The 490 isolates were distributed among 162 distinct MLLs which is equivalent to a clonal diversity of 0.33. Thirty-five MLLs were represented by more than one isolate. The five most represented MLLs included 31% of the isolates (152 isolates); these MLLs encompassed 24 to 47 isolates each. Linkage disequilibrium after clone correction reached a value close to zero ($\bar{r}_D$=0.06, $p < 0.002$) indicating the occurrence of a substantial level of recombination.

In all, most genetic variability occurred within compartment (more than 93% of total genetic variability, whether considering clonal replicates or not). We observed no significant difference between years but significant F_{ST} values among sampled compartments (Table 2). Below, we detail the population genetics characteristics within each season.

3.1.1 Season 1

Among the 300 *S. sclerotiorum* isolates collected from the air and from standing and buried carrots during Season 1, 105 distinct MLLs were identified. Nineteen MLLs were shared between airborne isolates and the isolates collected from carrots. Airborne isolates shared 10 MLLs with isolates from standing carrots and 14 with isolates from buried carrots (Figure 2). Isolates from standing and buried carrots shared five MLLs. There was no evidence of genetic differentiation between *S. sclerotiorum* isolates from the air and from standing and buried

carrots, with the F_{ST} index statistic not significant ($p > 0.05$) for pairwise combinations (clone-corrected data).

3.1.2 Season 2

Among the 190 *S. sclerotiorum* isolates collected from the air, from standing and buried carrots and from the soil during Season 2, 85 distinct MLLs were identified. The isolates collected from carrots shared nine and seven MLLs with airborne and soilborne isolates, respectively (Figure 3). Airborne isolates shared six MLLs with isolates from standing carrots and five MLLs with isolates from buried carrots. Soilborne isolates shared four MLLs with isolates from standing carrots and five MLLs with isolates from buried carrots. The distribution of shared MLLs among isolates collected from the air and from the soil did not differ significantly (Fisher's exact test, P=1) according to the part of the carrot they were collected from (aerial or underground part). Airborne and soilborne isolates shared nine MLLs. There was evidence of low but significant genetic differentiation between *S. sclerotiorum* isolates sampled from air and soil compartments (F_{ST} = 0.017, p = 0.044, clone-corrected data). There was no evidence of genetic differentiation for all the other pairwise comparisons.

3.2 Temporal persistence of multilocus lineages

Comparison of the 105 MLLs found in Season 1 with the 85 MLLs found in Season 2 showed that 33 of them were present during both crop seasons. There was no genetic differentiation between *S. sclerotiorum* isolates from the two seasons (F_{ST} = 0.00051, p = 0.36). The values of gene diversity were even across seasons (Table 1). Mean allele number per locus was slightly lower, and clonal diversity was higher in Season 2 (Table 1).

Taking a closer look at airborne isolates shows that isolates from Season 1 (229) and those from Season 2 (106) had a very close level of gene diversity (Hnb between 0.59 and 0.61

depending on the dataset) (Table 1). *S. sclerotiorum* isolates collected in the air during Season 2 showed a higher clonal diversity than those isolated during Season 1. Eighteen MLLs were shared by airborne isolates collected during Season 1 (distributed among 93 MLLs) and isolates collected during Season 2 (distributed among 54 MLLs). There was no genetic differentiation between *S. sclerotiorum* isolates from the air of the two seasons ($F_{ST} = 0.004$, $p = 0.87$).

Isolates collected from carrots from Season 1 and Season 2 showed similar levels of gene diversity (Hnb between 0.58 and 0.61 depending on the dataset). *S. sclerotiorum* isolates collected from carrots during Season 2 showed a higher clonal diversity than those collected during Season 1 but a slightly lower number of alleles per locus (Table 1). Nine MLLs were shared among isolates collected from carrots during Season 1 (distributed among 32 MLLs) and Season 2 (distributed among 20 MLLs). There was no genetic differentiation between *S. sclerotiorum* isolates from carrots of the two seasons ($F_{ST} = 0.009$, $p = 0.77$).

Comparison between seasons was not possible for the *S. sclerotiorum* inoculum in the soil compartment because no soilborne isolates were collected during Season 1.

4 Discussion

The present study was initiated to assess the relative importance of airborne and soilborne inoculum in white mould of carrot. As a surrogate to direct observation of spore movement, we used tools from population genetics to quantify the level of exchange among compartments using two kinds of metrics, gene flow and shared occurrence of clonal lineages. Results from these two methods are congruent and provide evidence for a large level of exchange among compartments, hence a joint contribution of both airborne and soilborne inoculum to the white mould epidemics.

First, we compared the abundance of clonal lineages of *S. sclerotiorum* among air, soil and plant compartments across years. Overall, our results showed a low level of clonal lineage diversity of *S. sclerotiorum* isolates in carrot fields in southwestern France. The isolates collected specifically on carrots (standing or buried) showed a clonal diversity ranging from 0.44 to 0.80. These values are consistent with previous studies on various host plants showing a predominant clonal mode of reproduction (Aban et al., 2021; Atallah et al., 2004; Leyronas et al., 2018a, 2018b; Sexton et al., 2006). In addition, the gene diversity and linkage disequilibrium values are consistent with those found by Clarkson et al. (2013) on *S. sclerotiorum* isolates collected from carrots in England.

The genetic characteristics did not differ significantly between isolates collected from air, soil and carrots. The isolates from the three different compartments shared MLLs, which indicates exchange across compartments. In particular, some isolates collected from lesions on carrots shared MLLs with isolates from the air or from the soil. These results provide direct evidence that white mould on carrot can be initiated both by airborne or soilborne inoculum. We found low levels of genetic differentiation between airborne and soilborne samples of *S. sclerotiorum*. These results are comparable to those obtained on sunflower and oilseed rape, which showed that there was no significant genetic differentiation between the ascospores and stem lesion populations (Ekins et al., 2007; Sexton et al., 2006).

The genetic characteristics did not differ significantly between isolates collected from Season 1 and Season 2. Concerning airborne inoculum, some isolates persisted from one year to the next, consistent with results of *S. sclerotiorum* airborne inoculum on witloof chicory where isolates with identical haplotypes were found during three successive crop seasons (Leyronas et al., 2019). This indicates survival of clonal lineages over crop seasons. In addition, this persistence points to a local origin of the inoculum, probably as mycelium in crop debris or as sclerotia that can survive for several years. These sclerotia can produce apothecia and

release ascospores that are known to be mostly deposited within a few tens of metres from their source (Kora et al., 2003; Wegulo et al., 2000). However, a part of this airborne inoculum may have a more distant origin, as *S. sclerotiorum* isolates sharing identical haplotypes had been found 700 km apart in regions of France with high levels of aerial connectivity. The south-western region, where the present study was carried out, is regularly connected by air masses to central-western regions and to north-western regions (Leyronas et al. 2018b) where host plants of *S. sclerotiorum*, including carrots, melon and beans, are cultivated (Leyronas et al., 2018a).

The multiple types of inoculum and their geographical origins makes the implementation of reasoned, effective and durable protection of plants complex. The results of the present study show that we cannot focus on a single type of inoculum to set up control strategies for white mould epidemics, in particular on carrots. Soilborne inoculum must be considered, and modifying rotations to include plant species that are non-hosts of *S. sclerotiorum* may help reduce the sclerotia and mycelium in the soil. However, this strategy is limited because of the extensive host range and duration of survival of sclerotia in the soil. Another strategy is to reduce the viability of sclerotia and thus the quantity of apothecia and ascospores released during the following season. This can be achieved by applying biocontrol agents in the soil, for example, the mycoparasite *Paraphaeosphaeria minitans* (Nicot et al., 2018).

Microevolutionary approaches focusing on airborne inoculum could provide decisive elements for plant protection management. Establishing a link between variations in genetic indices and epidemic processes could allow better monitoring of *S. sclerotiorum* inoculum within the cropping system. For example, temporal persistence of certain haplotypes in airborne isolates collected in a given area could indicate a local origin of the inoculum; sclerotia generated by a single individual remain in the same field and produce, when conditions are

favourable, apothecia that produce ascospores. The closer a source is to the ascospore collection area, the less dilution of individuals, and thus haplotypes, occurs in the air. This could guide actions towards prophylactic measures aimed at eliminating sclerotia present on the soil surface in the area considered. When applied to timed samplings, this approach would also be indicative of the timing of inoculum release (Leyronas et al., 2015). Moreover, if in a given area the airborne inoculum shows a haplotypic diversity varying over time, this could indicate one or several distant sources. A study of the aerial interconnectivity (Leyronas et al., 2018b) between this area and other areas bearing host species of *S. sclerotiorum* could help manage plant protection via management of the landscape. The tools and analytical methods to provide aerial interconnectivity data are currently being developed in order to address the impact of aerial inoculum from long distance sources and the capacity to account for them in developing strategies of crop protection.

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Conflict of interest

The authors have no conflict of interest to disclose.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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Supporting Information

Additional Supporting Information can be found in the online version of this article at the publisher's website.

Table S1 Characteristics of the multilocus lineages (MLLs).

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Table 1: Number, origin and genetic diversity of *Sclerotinia sclerotiorum* isolates collected from soil, from air and from carrot during two crop seasons in France.

		All data				Haplotype diversity			Clone-corrected data			
	Sample size	Hnb[a]	Ra[b]	$\bar{r}_D$		MLL[c]	NA[d]	Clonal diversity[e]	Hnb[a]	Ra_cc[f]	$\bar{r}_D$	
Season 1 (July 2014-March 2015)												
Air	229	0.59 (0.21)	4.38 (2.16)	0.11	**	93	0	0.4	0.61 (0.20)	4.42 (2.08)	0.06	**
Total carrots	71	0.59 (0.19)	4.30 (1.99)	0.12	**	32	0	0.44	0.61 (0.18)	4.24 (1.87)	0.05	**
Standing	*30*	*0.58 (0.21)*	*4.01 (1.94)*	*0.12*	**	*17*	*0*	*0.55*	*0.58 (0.20)*	*3.91 (1.74)*	*0.05*	**
Buried	*41*	*0.59 (0.19)*	*4.07 (1.80)*	*0.14*	**	*20*	*0*	*0.48*	*0.62 (0.18)*	*4.15 (1.83)*	*0.05*	**
Total season 1	**300**	**0.59 (0.20)**	**4.40 (2.15)**	**0.11**	**	**105**	**0**	**0.35**	**0.61 (0.20)**	**4.47 (2.08)**	**0.06**	**
Season 2 (July 2015-March 2016)												
Air	106	0.60 (0.23)	4.32 (2.19)	0.12	**	54	2	0.51	0.60 (0.22)	4.18 (2.06)	0.07	**
Total carrots	36	0.58 (0.19)	3.73 (1.65)	0.12	**	20	3	0.59	0.61 (0.19)	3.83 (1.75)	0.04	**
Standing	*18*	*0.59 (0.20)*	*3.74 (1.68)*	*0.10*	**	*13*	*2*	*0.80*	*0.63 (0.20)*	*3.72 (1.67)*	*0.03*	*
Buried	*18*	*0.57 (0.21)*	*3.26 (1.32)*	*0.16*	**	*10*	*1*	*0.56*	*0.61 (0.22)*	*3.33 (1.39)*	*0.05*	*
Soil	48	0.57 (0.20)	4.09 (2.03)	0.06	**	33	2	0.71	0.58 (0.18)	3.93 (1.87)	0.05	**
Total season 2	**190**	**0.59 (0.21)**	**4.35 (2.21)**	**0.09**	**	**85**	**7**	**0.46**	**0.60 (0.20)**	**4.34 (2.19)**	**0.05**	**
Total S1&S2	**490**	**0.59 (0.2)**	**4.46 (2.28)**	**0.11**	**	**162**	**7**	**0.33**	**0.61 (0.20)**	**4.51 (2.19)**	**0.06**	**

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[a] unbiased gene diversity (standard deviation in brackets)
[b] Number of alleles after a rarefaction procedure based on a minimal sample size of 12 isolates (standard deviation in brackets)
[c] Number of distinct multilocus lineages after MLGsim analysis
[d] Number of isolates not assigned to a MLL (MLL= clonal lineage)
[e] amounts to haplotype diversity ((number of MLL-1)/ (total number of isolates- NA-1))
[f] Number of alleles after a rarefaction procedure based on a minimal sample size of 10 isolates calculated on the clone corrected data set (standard deviation in brackets)
* P<0.05 ; ** P<0.01

Table 2: Results of the hierarchical analysis of molecular variance (AMOVA) performed between crops season (different years) and among compartment (air, carrots and soil) within season. Analysis was performed considering the whole data set or only one replicate for each MLL.

	Variance components		**Fstatistics**		**P (rand. value < obs. value)[a]**
All data					
Between years	Va	0,28	FCT	0,003	0.2092 (ns)
Among compartments within year	Vb	6,06	FST	0,063	0.0000 ****
Within compartment	Vc	93,65	-	na	na
Clone-corrected data					
Between years	Va	0,63	FCT	0,006	0.2131 (ns)
Among compartments within year	Vb	6,08	FST	0,067	0.0000 ****
Within compartment	Vc	93,29	-	na	na

[a] based on 1023 permutations

**** $p<0.0001$

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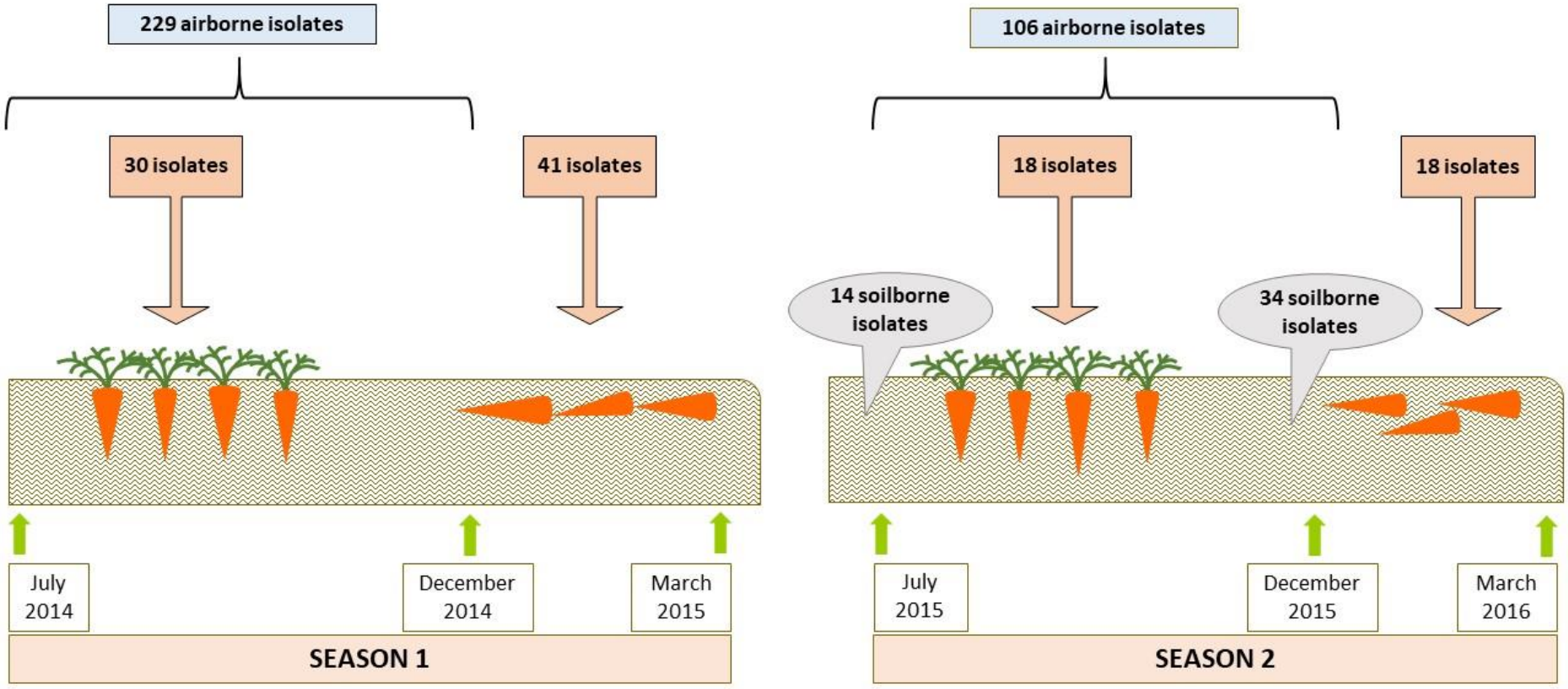
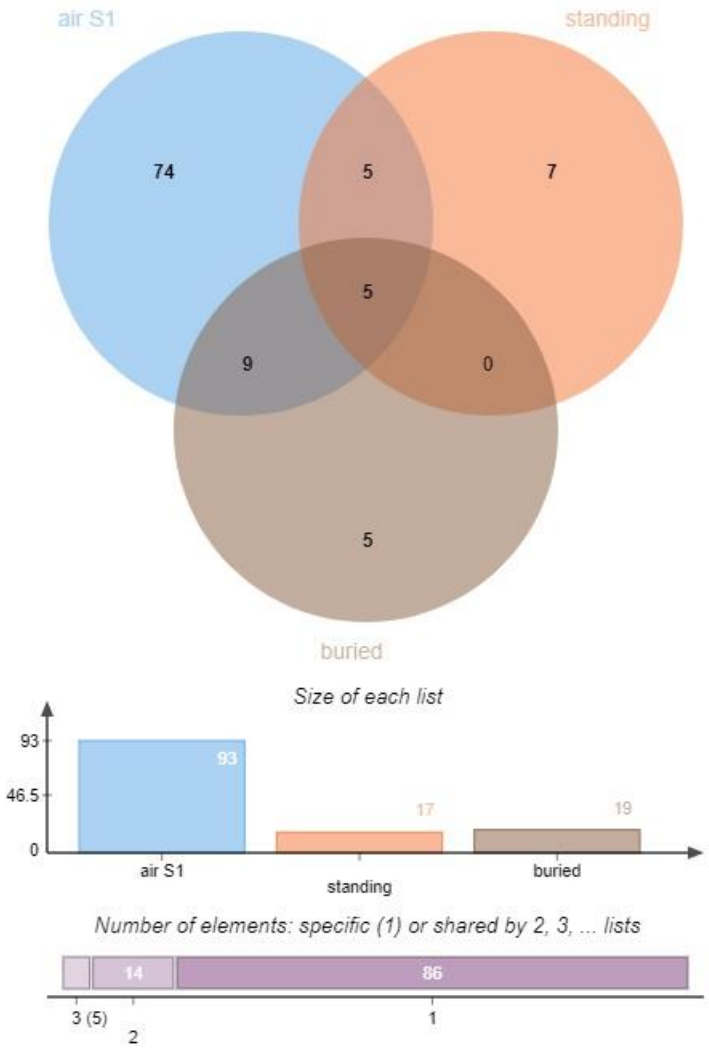
Figure 1 Collection of *Sclerotinia sclerotiorum* isolates in the air, in soil and on carrots (standing and buried) during two successive crop seasons in south-western France.



Leyronas, C., Halkett, F. & Faloya, V. (2023) Airborne versus soilborne inoculum: White mould, where do you come from? *Plant Pathology*, 00, 1– 9. Available from: https://doi.org/10.1111/ppa.13699

Figure 2 Top : Venn diagram showing the number of multilocus lineages (MLLs) in *Sclerotinia sclerotiorum* isolates collected from each compartment (air and standing and buried carrots) and shared between compartments during Season 1 (S1) from July 2014 to March 2015. Below: the number of MLLs in each compartment and the number of MLLs specific to one compartment or shared by two or three different compartments.



Leyronas, C., Halkett, F. & Faloya, V. (2023) Airborne versus soilborne inoculum: White mould, where do you come from? *Plant Pathology*, 00, 1– 9. Available from: https://doi.org/10.1111/ppa.13699

Figure 3 Top : Venn diagram showing the number of multilocus lineages (MLLs) in *Sclerotinia sclerotiorum* isolates collected from each compartment (air, soil, and standing and buried carrots) during Season 2 (S2) from July 2015 to March 2016. Below: the number of MLLs in each compartment and the number of MLLs specific to one compartment or shared by two or more different compartments.