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# 1 **Airborne versus soilborne inoculum: white mould, where do you** 2 **come from?**

3

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12

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15

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

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## 33 1 Introduction

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

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

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

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

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

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

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

92

## 93 2 Materials and methods

### 94 2.1 Collection of *S. sclerotiorum* isolates

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

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

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

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

122         Prior to their entry into the laboratory fungal collection, all isolates (from soil, carrots  
123 and the air) were subjected to a step of single hypha isolation. For this, single pieces of hyphal  
124 tip were excised from the growing margin of a colony after 2 days of incubation on PDA and  
125 transferred to fresh medium as described by Lehner et al. (2016). When forming sclerotia, the  
126 single-hypha isolates were stored as sclerotia at –20°C.

## 127 **2.2 Isolate genotyping**

128 Sclerotia were taken out of the freezer and spread on PDA to germinate and produce mycelium.  
129 Genomic DNA was extracted in 96-well plates from aliquots of 100 mg of mycelium, following  
130 the Dneasy Plant extraction kit protocol (Qiagen). Sixteen microsatellite markers designed for  
131 *S. sclerotiorum* by Sirjusingh and Kohn (2001) were amplified with forward primers  
132 conjugated with fluorescent dyes following the protocol described by Leyronas et al. (2018a).  
133 To determine the size of the microsatellites, the PCR products were diluted and multiplexed  
134 prior to scanning with an ABI 3730 sequencer (Applied Biosystems). GeneMapper software v.  
135 4.1 (Applied Biosystems) was then used to assess the size of microsatellite markers on each  
136 isolate. Complete microsatellite profiles (referred to as haplotypes hereafter) were obtained for  
137 all isolates.

## 138 **2.3 Identification of multilocus lineages**

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

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

#### 156 **2.4 Genetic characterization and comparison of *S. sclerotiorum* isolates**

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

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



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

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

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

182

## 183 3 Results

### 184 3.1 Genetic diversity of *S. sclerotiorum* isolates

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

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

192 In total, we identified 158 distinct genotypes. Of these, 108 occurred only once. The  
193 number of replicates (among repeated genotypes) ranged from 2 to 47 and the  $P_{sex}$  values  
194 ranged from  $1.42 \times 10^{-185}$  to  $1.18 \times 10^{-6}$ . Random simulations of genotypes (considering the

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

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

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

### 212 **3.1.1 Season 1**

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

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

### 221 **3.1.2 Season 2**

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

### 234 **3.2 Temporal persistence of multilocus lineages**

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

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

242 depending on the dataset) (Table 1). *S. sclerotiorum* isolates collected in the air during Season  
243 2 showed a higher clonal diversity than those isolated during Season 1. Eighteen MLLs were  
244 shared by airborne isolates collected during Season 1 (distributed among 93 MLLs) and isolates  
245 collected during Season 2 (distributed among 54 MLLs). There was no genetic differentiation  
246 between *S. sclerotiorum* isolates from the air of the two seasons ( $F_{ST} = 0.004$ ,  $p = 0.87$ ).

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

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

256

## 257 4 Discussion

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

265 First, we compared the abundance of clonal lineages of *S. sclerotiorum* among air, soil  
266 and plant compartments across years. Overall, our results showed a low level of clonal lineage  
267 diversity of *S. sclerotiorum* isolates in carrot fields in southwestern France. The isolates  
268 collected specifically on carrots (standing or buried) showed a clonal diversity ranging from  
269 0.44 to 0.80. These values are consistent with previous studies on various host plants showing  
270 a predominant clonal mode of reproduction (Aban et al., 2021; Atallah et al., 2004; Leyronas  
271 et al., 2018a, 2018b; Sexton et al., 2006). In addition, the gene diversity and linkage  
272 disequilibrium values are consistent with those found by Clarkson et al. (2013) on *S.*  
273 *sclerotiorum* isolates collected from carrots in England.

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

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

290 release ascospores that are known to be mostly deposited within a few tens of metres from their  
291 source (Kora et al., 2003; Wegulo et al., 2000). However, a part of this airborne inoculum may  
292 have a more distant origin, as *S. sclerotiorum* isolates sharing identical haplotypes had been  
293 found 700 km apart in regions of France with high levels of aerial connectivity. The south-  
294 western region, where the present study was carried out, is regularly connected by air masses  
295 to central-western regions and to north-western regions (Leyronas et al. 2018b) where host  
296 plants of *S. sclerotiorum*, including carrots, melon and beans, are cultivated (Leyronas et al.,  
297 2018a).

298         The multiple types of inoculum and their geographical origins makes the  
299 implementation of reasoned, effective and durable protection of plants complex. The results of  
300 the present study show that we cannot focus on a single type of inoculum to set up control  
301 strategies for white mould epidemics, in particular on carrots. Soilborne inoculum must be  
302 considered, and modifying rotations to include plant species that are non-hosts of *S.*  
303 *sclerotiorum* may help reduce the sclerotia and mycelium in the soil. However, this strategy is  
304 limited because of the extensive host range and duration of survival of sclerotia in the soil.  
305 Another strategy is to reduce the viability of sclerotia and thus the quantity of apothecia and  
306 ascospores released during the following season. This can be achieved by applying biocontrol  
307 agents in the soil, for example, the mycoparasite *Paraphaeosphaeria minitans* (Nicot et al.,  
308 2018).

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

315 favourable, apothecia that produce ascospores. The closer a source is to the ascospore  
316 collection area, the less dilution of individuals, and thus haplotypes, occurs in the air. This  
317 could guide actions towards prophylactic measures aimed at eliminating sclerotia present on  
318 the soil surface in the area considered. When applied to timed samplings, this approach would  
319 also be indicative of the timing of inoculum release (Leyronas et al., 2015). Moreover, if in a  
320 given area the airborne inoculum shows a haplotypic diversity varying over time, this could  
321 indicate one or several distant sources. A study of the aerial interconnectivity (Leyronas et al.,  
322 2018b) between this area and other areas bearing host species of *S. sclerotiorum* could help  
323 manage plant protection via management of the landscape. The tools and analytical methods  
324 to provide aerial interconnectivity data are currently being developed in order to address the  
325 impact of aerial inoculum from long distance sources and the capacity to account for them in  
326 developing strategies of crop protection.

327

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340

341 Conflict of interest

342 The authors have no conflict of interest to disclose.

343

344 Data availability statement

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

347

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448

#### 449 Supporting Information

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

452 **Table S1** Characteristics of the multilocus lineages (MLLs).

453

454

**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.

	Sample size	All data			Haplotype diversity			Clone-corrected data				
		Hnb <sup>a</sup>	Ra <sup>b</sup>	$\bar{r}_D$	MLL <sup>c</sup>	NA <sup>d</sup>	Clonal diversity <sup>e</sup>	Hnb <sup>a</sup>	Ra_cc <sup>f</sup>	$\bar{r}_D$		
<b>Season 1 (July 2014-March 2015)</b>												
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	**
<i>Standing</i>	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	**
<i>Buried</i>	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	**
<b>Total season 1</b>	<b>300</b>	<b>0.59 (0.20)</b>	<b>4.40 (2.15)</b>	<b>0.11</b>	<b>**</b>	<b>105</b>	<b>0</b>	<b>0.35</b>	<b>0.61 (0.20)</b>	<b>4.47 (2.08)</b>	<b>0.06</b>	<b>**</b>
<b>Season 2 (July 2015-March 2016)</b>												
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	**
<i>Standing</i>	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	*
<i>Buried</i>	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	**
<b>Total season 2</b>	<b>190</b>	<b>0.59 (0.21)</b>	<b>4.35 (2.21)</b>	<b>0.09</b>	<b>**</b>	<b>85</b>	<b>7</b>	<b>0.46</b>	<b>0.60 (0.20)</b>	<b>4.34 (2.19)</b>	<b>0.05</b>	<b>**</b>
<b>Total S1&amp;S2</b>	<b>490</b>	<b>0.59 (0.2)</b>	<b>4.46 (2.28)</b>	<b>0.11</b>	<b>**</b>	<b>162</b>	<b>7</b>	<b>0.33</b>	<b>0.61 (0.20)</b>	<b>4.51 (2.19)</b>	<b>0.06</b>	<b>**</b>

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<sup>a</sup> unbiased gene diversity (standard deviation in brackets)

<sup>b</sup> Number of alleles after a rarefaction procedure based on a minimal sample size of 12 isolates (standard deviation in brackets)

<sup>c</sup> Number of distinct multilocus lineages after MLGsim analysis

<sup>d</sup> Number of isolates not assigned to a MLL (MLL= clonal lineage)

<sup>e</sup> amounts to haplotype diversity  $((\text{number of MLL}-1) / (\text{total number of isolates}- \text{NA}-1))$

<sup>f</sup> 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.

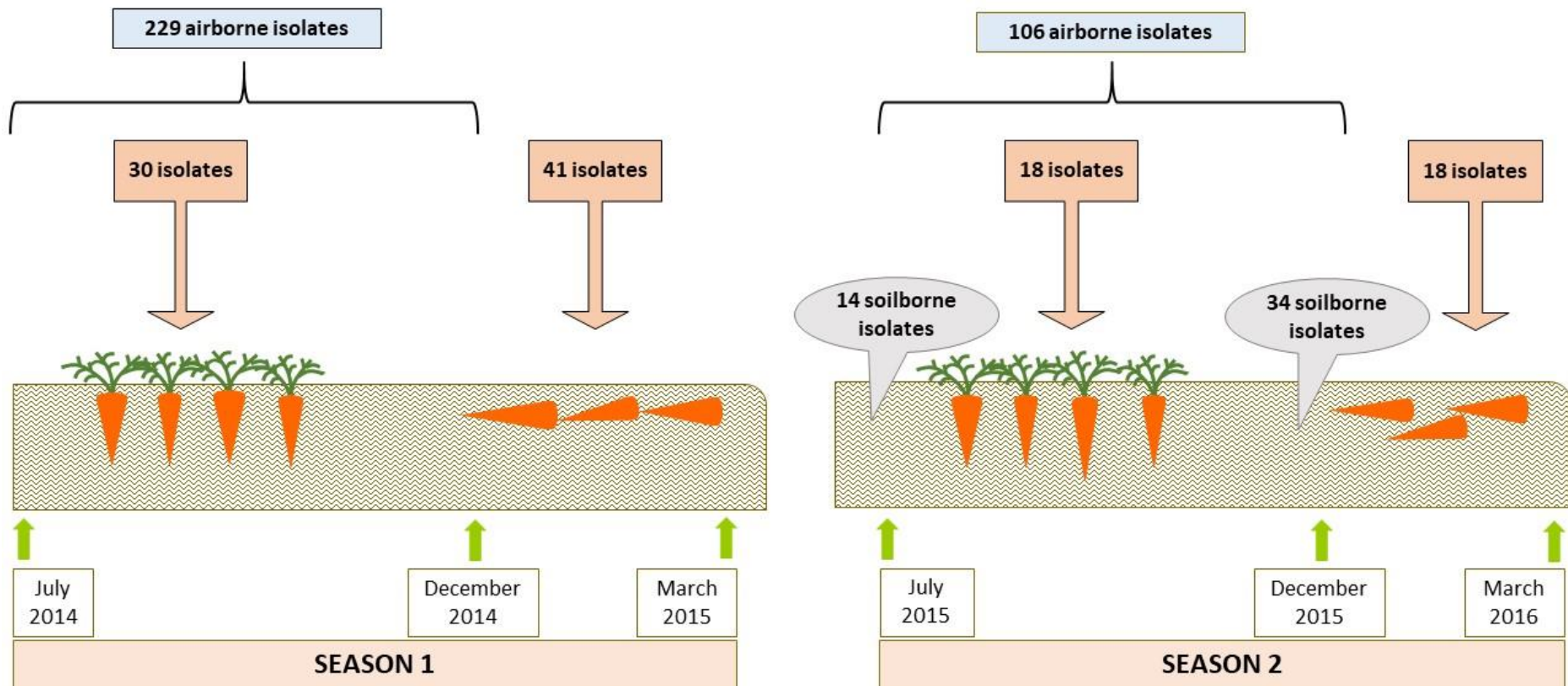
	Variance components		Fstatistics		P (rand. value < obs. value) <sup>a</sup>
<b>All data</b>					
Between years	Va	0,28	FCT	0,003	0.2092 (ns)
Among compartments within year	Vb	6,06	FST	0,063	0.0000 ****
Within compartment	Vc	93,65	-	na	na
<b>Clone-corrected data</b>					
Between years	Va	0,63	FCT	0,006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

<sup>a</sup> based on 1023 permutations

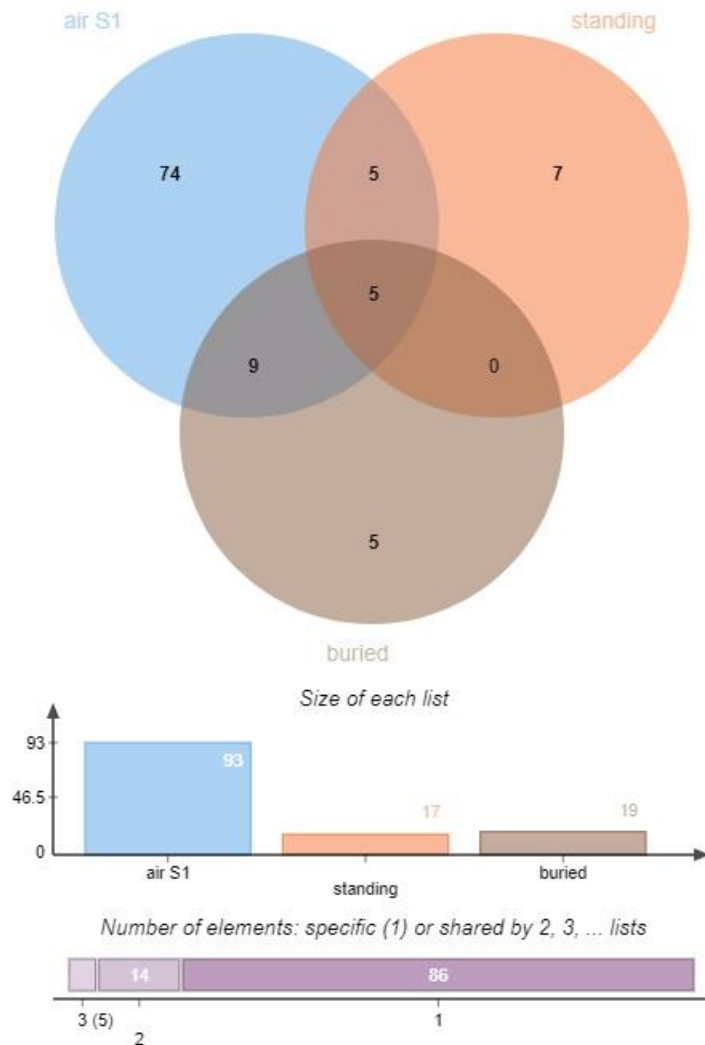
\*\*\*\* p<0.0001



**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.



**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.



**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.

