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## Compared dynamics of grey mould incidence and genetic characteristics of *Botrytis cinerea* in neighbouring vegetable greenhouses

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1 **COMPARED DYNAMICS OF GREY MOULD INCIDENCE AND**  
2 **GENETIC CHARACTERISTICS OF *BOTRYTIS CINEREA* IN**  
3 **NEIGHBOURING VEGETABLE GREENHOUSES.**

4

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7

8 Running title: Dynamics of grey mould in greenhouses.

9

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13

14 **SUMMARY**

15 Production of vegetables in southern France often relies on groups of greenhouses located in  
16 close vicinity. These crops are commonly affected by grey mould caused by *Botrytis cinerea*, a  
17 fungus known for its ability to produce abundant airborne inoculum. Possible exchange of  
18 inoculum could affect the epidemics developing in neighbouring greenhouses. To test this  
19 hypothesis, grey mould incidence was assessed in four successive crops in six unheated  
20 polyethylene tunnels located in the region of Avignon. On lettuce, the incidence was similar for  
21 a given harvest date in two tunnels where this crop was grown four times consecutively. In the  
22 four other tunnels, lettuce was grown in alternation with tomato. No grey mould developed on  
23 tomato and disease incidence was low on lettuce. One hundred and seventy four strains  
24 collected from lettuce in two tunnels were investigated for their genetic diversity, genetic  
25 structure and their mating type. Both known mating types of *B. cinerea* were observed in the

26 tunnels but MAT1-1 was prevalent. The gene diversity of *B. cinerea* strains was similar in both  
27 tunnels. However haplotypic diversity and linkage disequilibrium were substantially higher in  
28 one tunnel. We hypothesize that this situation is related to differences in microclimatic  
29 conditions in the tunnels. It highlights a possible interest in individualizing disease management  
30 in the different tunnels of a given farm.

31

32 *Keywords: tunnel, inoculum, tomato, lettuce*

33

## 34 INTRODUCTION

35 In south-eastern France many vegetables, berries and flowers are grown under greenhouse.  
36 According to the 'Agreste' statistics of the French Ministry of Agriculture (Anonymous, 2014)  
37 for the 'Provence-Alpes-Côte-d'Azur' region, located on the east side of the Rhône Valley and  
38 bordered by the Mediterranean Sea, there are 2500 hectares of greenhouses. The greenhouses  
39 constitute shelters in which climatic conditions foster plant growth but also the development of  
40 diseases. One of the most damageable plant pathogens for crops grown under greenhouse  
41 (lettuce, tomato, strawberry among others) is *Botrytis cinerea*. This ascomycete fungus is the  
42 causal agent of grey mould. The symptoms of this disease are necrotic lesions on stems, leaves  
43 and fruits that make the products unmarketable and that may even lead to plant death.

44 Grey mould epidemics can be induced by soilborne or airborne inoculum. *B. cinerea*  
45 inoculum can be present in the soil in the form of sclerotia, conidia (mitotic spores) or mycelium  
46 on plant debris (Coley-Smith, 1980; Raposo *et al.*, 2001; Strømeng *et al.*, 2009; Leyronas *et al.*,  
47 2014a). *B. cinerea* is considered to lack host specialization (Leyronas *et al.*, 2014b) and  
48 soilborne propagules are likely to carry over the inoculum to successive susceptible crops.  
49 However *B. cinerea* is considered to be predominantly airborne disseminated. Indeed, when  
50 climatic conditions are favourable, the lesions formed on aerial parts of plants become covered

51 with large amounts of conidia (Nicot *et al.*, 1996) that can be easily dispersed by wind (Jarvis,  
52 1962; Harrison and Lowe, 1987). These conidia can serve as primary inoculum since they are  
53 present almost all the time in the air (Leyronas and Nicot, 2013). They also play a major role in  
54 the rapid colonization of neighbouring plants when conditions are favourable (Holz *et al.*, 2004)  
55 and they can spread outside of the greenhouse. Although greenhouses are often regarded as  
56 quasi-closed systems, exchange of *B. cinerea* inoculum can occur between the inside and the  
57 outside of a greenhouse through vents (Korolev *et al.*, 2006; Leyronas *et al.*, 2011). The  
58 dissemination of *B. cinerea* airborne inoculum is also likely to occur on a larger scale. Strains  
59 of *B. cinerea* showing an identical haplotype (based on 9 microsatellites markers) were  
60 collected in tomato greenhouses located 23 km apart in northern Algeria (Adjebli *et al.*, 2014)  
61 and 220 km apart in southern France (Bardin *et al.*, 2014). The hypothesis that airborne conidia  
62 have been carried by air masses between these greenhouses appears relevant since it is known  
63 that long distant dispersal of fungal spores can spread diseases on regional and continental  
64 scales (Brown and Hovmøller, 2002). The absence of differentiation of *B. cinerea* populations  
65 between several vegetable greenhouses reported by Alfonso *et al.* (2000) in southern Spain  
66 could be due in part to such dissemination, resulting in the homogenisation of airborne  
67 inoculum.

68         In horticultural farms of south-eastern France, several vegetable greenhouses are usually  
69 located in close vicinity. Since this region is predominantly scoured by a strong northern wind  
70 (Mistral), the greenhouses are aligned, side by side, in a north-south direction. Groups of  
71 greenhouses are often protected by a high cypress hedge to attenuate strong air currents that  
72 may damage the structures. Species susceptible to grey mould are often cultivated  
73 concomitantly in these adjacent greenhouses or in rotation in the same greenhouse. Control  
74 methods are mainly based on fungicidal treatments applied on aerial parts of plants but this can  
75 be complicated by the appearance of resistant strains (Leroux, 2004). Moreover the cryptic

76 species *B. pseudocinerea* (Walker *et al.*, 2011) causing the same symptoms of grey mould is  
77 naturally resistant to the hydroxyanilide fungicide fenhexamid (Leroux *et al.*, 1999; 2002).  
78 Climate management inside greenhouses, particularly the control of temperature coupled to  
79 relative humidity, also constitutes a tool to reduce disease outbreaks (Jewett and Jarvis 2001;  
80 Dik and Wubben, 2004). In the Mediterranean climate, there may be wide thermic amplitude  
81 between night and day, inducing dew formation on plants and enhancing grey mould  
82 development. Heating can prevent dew formation in glasshouses equipped with heating system.  
83 In non-heated polyethylene greenhouses (tunnels), climate is only minimally controlled through  
84 the opening of lateral and top vents.

85         In order to reduce the risk of fungicide resistance appearance and to reduce the cost of  
86 chemical and energy inputs in greenhouses, the cultural practices to control grey mould may be  
87 enhanced. The objective of this study was to test the hypothesis that *B. cinerea* inoculum and  
88 grey mould epidemics are similar in neighbouring greenhouses where a same crop is grown. To  
89 this aim, we monitored grey mould incidence in six neighbouring tunnels in south-eastern  
90 France over four successive crops grown during a year-and-a-half-period. A collection of *B.*  
91 *cinerea* isolates sampled from diseased lettuce was genetically characterized and its diversity  
92 was assessed over time and space.

93

## 94 **MATERIALS AND METHODS**

### 95 **Experimental set up**

96 From 2006 to 2008, vegetable crops were grown in six experimental unheated polyethylene  
97 greenhouses (tunnels numbered from T1 to T6). These tunnels were built in 2006 specifically  
98 for this experiment, on a plot located at the Plant Pathology research unit of the French National  
99 Institute of Agricultural Research (INRA) in southern France (lat. 43.93N; long. 4.88E). All

100 the tunnels were oriented north-south. Tunnel T1 was the most eastern and T6 was the most  
101 western of the group. The tunnels each covered an area of 128 m<sup>2</sup> and were located 4 m apart.

102 In T1 and T6 four successive butterhead lettuce crops (cv. Faustina and Leandra) were  
103 grown (Table 1). In T2, T3, T4 and T5, two lettuce crops (cv. Leandra) were grown in rotation  
104 with two tomato crops (cv. Swanson). During lettuce cropping the tunnels each contained 14  
105 rows of 93 plants with a density of 12 lettuces per square meter. The crops were conducted on  
106 a micro-perforated polyethylene mulch mat. Irrigation was realised by sprinkling. The plants  
107 were efficiently protected against downy mildew with fungicide treatments applied until the  
108 18-leaf stage according to common practice in commercial production. During tomato  
109 cropping, the tunnels each contained 8 rows of 29 plants that were drip irrigated. Throughout  
110 all experiments, the climate inside the tunnels was minimally controlled through the opening of  
111 lateral vents. The vents and doors of all tunnels were kept fully open during the warm months  
112 (from early May to mid-September) and closed during the cold season (from early November  
113 to end of February). During the intermediate periods, the opening of the vents was adjusted  
114 daily according to the inside temperature.

115 Throughout the growing seasons, the crops were examined daily for the presence of  
116 pests and diseases. In the tomato crop, axillary shoots were removed twice a month and fruits  
117 were harvested once a week starting in late May. Lettuce harvest was completed within one day  
118 at the end of each cropping season (Table 1). After each lettuce and tomato culture, the plant  
119 residues were removed with the mulch mat and the soil in the tunnels was tilled and left fallow  
120 until the next crop.

121

## 122 **Grey mould incidence at harvest and isolate collection**

123 On lettuce crops, grey mould incidence was evaluated at harvest by recording the presence of  
124 grey mould symptoms on every plant (1302 per tunnel). To account for possible spatial

125 variability, each tunnel was divided in four sectors. Mean grey mould incidence and its  
126 associated standard error were calculated for each tunnel. On tomato crops, the presence of grey  
127 mould was monitored once-a-week during the growing season.

128 Isolates of *B. cinerea* were sampled on lettuce plants at harvest time in T1 and T6, by  
129 rubbing dry sterile cotton buds on sporulating lesions. A total of 40 samples were collected  
130 throughout each tunnel. The cotton buds were then stored at -20°C until isolate purification. All  
131 isolates used in this study were purified and single-spored in a classical way (Leyronas *et al.*  
132 2012) prior to their genotypic characterization.

133

#### 134 **Isolate genotyping**

135 The isolates were genotyped following the protocol described by Leyronas *et al.* (2014b). In  
136 short, genomic DNA was extracted from aliquots of 15 mg lyophilized fungal material  
137 following the Dneasy Plant extraction Kit protocole (Qiagen). The nine microsatellite markers  
138 designed for *B. cinerea* by Fournier *et al.* (2002) were amplified following the protocol  
139 described by Leyronas *et al.* (2014b). The size of the microsatellites were determined with a  
140 Megabace sequencer (Amersham Pharmacia) and Genetic Profiler software (Amersham  
141 Biosciences) was used for the microsatellite size analysis. Complete microsatellite size profiles  
142 (referred to as "haplotypes" hereafter) were obtained for 174 isolates. Hereafter, characterized  
143 isolates from tunnels T1 and T6 will be referred to as "T1 strains" and "T6 strains", respectively.

144

#### 145 **Genetic diversity of *B. cinerea* strains**

146 Several indices of genetic diversity were used to compare *B. cinerea* strains collected from  
147 tunnels T1 and T6 at different dates. Unbiased gene diversity (Hnb) and allelic richness were  
148 computed with the Genetix software (Nei, 1978). The number of different multilocus  
149 haplotypes (MLH) was computed with GenClone 1.0 software (Arnaud-Haond and Belkhir,

150 2007) and was used to calculate the haplotypic diversity (computed as the ratio of (number of  
151 distinct MLH - 1) over (sample size - 1)) which estimates the proportion of haplotypes present  
152 in a group and takes a value of 1 when a group is composed exclusively of unique haplotypes  
153 (Arnaud-Haond *et al.*, 2007). The program Multilocus 1.3b was used to calculate the  
154 standardized version of index of association ( $r_D$ ) that is a measure of the multilocus linkage  
155 disequilibrium (Agapow and Burt, 2001). It varied between 0 (complete panmixia) and 1  
156 (complete clonality).

157

### 158 **Mating types of *B. cinerea* strains**

159 Mating types were utilized as an additional marker to compare the strains collected from tunnels  
160 T1 and T6. We used the primers BcMAT1alpha-for and BcMAT1alpha-rev, and  
161 BcMAT2HMG-for and BcMAT2HMG-rev described by Duarte (2008). PCR amplification  
162 was carried out in a 10  $\mu$ L reaction mixture, containing 10 ng of genomic DNA, 5  $\mu$ L of master  
163 mix (Qiagen), 1  $\mu$ L of solution Q, 1.6  $\mu$ L of water and 0.2  $\mu$ L of each primer (10  $\mu$ M). The  
164 targeted fragments were amplified as follows: activation of Taq polymerase at 94°C for 15 min;  
165 40 cycles of denaturation at 94°C for 30 sec, hybridization at 51°C for 1.5 min and extension at  
166 72°C for 1.5 min. A final extension phase was performed at 72°C for 10 min. PCR products  
167 were observed under UV light on a 1% agarose gel electrophoresis containing 0.5  $\mu$ g/L of  
168 ethidium bromide). Strains SAS56 and SAS405 of *B. cinerea* were used as reference for MAT1-  
169 1 and MAT1-2, respectively.

170

### 171 **Genetic differentiation between strains from tunnels T1 and T6**

172 The genetic differentiation between strains collected from the two tunnels at different harvest  
173 times was assessed with pairwise Weir and Cockerham's  $F_{ST}$  indices and with a hierarchical



174 analysis of molecular variance (AMOVA) performed with version 3.0 of Arlequin software  
175 (Excoffier *et al.*, 2005).

176

### 177 **Genetic structure of *B. cinerea* populations**

178 The genetic structure of the populations was determined with version 2.3.4 of Structure  
179 software (Falush *et al.*, 2003). Since there was no geographical barrier between tunnels T1 and  
180 T6, the model with admixture was chosen. We tested values of clusters (K) between 1 and 9,  
181 with 10 replicates for each K. Each simulation consisted of 100,000 Monte-Carlo Markov Chain  
182 iterations preceded by a burn-in period of 200,000 iterations. The most probable structure was  
183 determined by computing the posterior probability for each K using the distribution of  
184 maximum likelihoods. When the probability of ancestry of a strain in a cluster was greater than  
185 the arbitrary threshold of 0.80, this strain was considered to be unambiguously assigned to this  
186 cluster.

187

### 188 **Statistical analyses**

189 Statistical analyses were performed with StatView (version 5, SAS Institute). Non-parametric  
190 tests were used to determine significant differences between tunnels for disease incidence,  
191 unbiased gene diversity and allelic richness (Mann-Whitney) and between sampling dates for  
192 the distribution of strains among genetic clusters in each tunnel (Kruskal-Wallis). Exact tests  
193 of Fisher were realised to determine if there was a link between the distribution of *B. cinerea*  
194 isolates among mating types and the tunnel they had been collected from at each harvest date.  
195 The tests were computed with the GENEPOP version 3.1c, Prog. STRUC with 500,000  
196 iterations (Raymond and Rousset, 1995). Statistical inferences were made at the 5 % level of  
197 significance, unless indicated otherwise.

198

## 199 **RESULTS**

### 200 **Grey mould incidence on crops**

201 No symptom of grey mould developed on tomato plants during the two growing seasons of the  
202 study. In contrast, the incidence of grey mould on lettuce in the six tunnels ranged from 0.2 to  
203 25.9% (Fig. 1). Lower incidence values were recorded at harvest time in tunnels with an  
204 alternation of lettuce and tomato crops (T2 to T5) compared to tunnels with a succession of  
205 lettuce crops (T1 and T6). However, such comparison can only be made in February 2008  
206 because in 2007 the harvest time of T2, T3, T4 and T5 occurred one month before that of T1  
207 and T6. Disease incidence was never significantly different ( $P>0.99$ ) between T1 and T6. Its  
208 variation followed the same pattern in both tunnels ( $\rho=1.0$ ,  $P=0.08$ ) over the four lettuce crops,  
209 with a noticeable peak in March 2007.

210

### 211 **Spatial and temporal variability of *B. cinerea* genetic characteristics**

212 Among the 174 isolates fully genotyped, four (three collected from T1 and one from T6) carried  
213 a private allele on microsatellite locus BC6 and were identified as *B. pseudocinerea* (Walker *et*  
214 *al.*, 2011). They were removed from further analysis. We thus compared the indices of genetic  
215 diversity based on the haplotypes obtained for the 170 remaining strains considered to be *B.*  
216 *cinerea* (85 in each tunnel). There was no significant difference between strains collected from  
217 T1 and those from T6 when considering unbiased gene diversity and allelic richness ( $P=0.38$   
218 and  $P=0.79$ , respectively) for each of the nine microsatellite loci (Table 2). When considering  
219 the four sampling dates taken together, the strains collected from T1 and T6 had similar gene  
220 diversity and mean number of alleles per locus (Table 3). However, T1 strains had higher  
221 haplotypic diversity and lower linkage disequilibrium than T6 strains.

222 When considering the four sampling dates successively, a different variation pattern was  
223 observed in the two tunnels for global unbiased gene diversity (Fig. 2A) but not for haplotypic

224 diversity (Fig. 2B). Only 2 MLHs were shared by T1 strains and T6 strains (accounting for  
225 2.3% of the total number of strains) with a single copy of these MLHs observed in each tunnel:  
226 one associated with 2 strains collected in March 2007 in T1 and T6; one associated with two  
227 strains collected in May 2007 in T1 and in June 2006 in T6.

228         Inside a given tunnel certain MLHs were observed repeatedly at different dates (Table  
229 4). The number of such strains was higher in T6 (5 MLHs representing 31.7 % of all T6 strains)  
230 than in T1 (2 MLHs representing 10.5 % of all T1 strains).

231         Linkage disequilibrium decreased over the study period in T1 and reached a value close  
232 to zero indicating the occurrence of substantial level of recombination (Fig. 2C). In contrast, it  
233 increased to 0.26 (March 2007) in T6, reflecting a substantial level of clonality.

234         The *B. cinerea* strains collected in T1 and T6 had predominantly only the MAT1-1 allele  
235 (51.7 and 59.1% respectively). Some strains had both alleles MAT1-1 and MAT1-2 (10.8 and  
236 9.4% respectively in T1 and T6). The distribution of strains among mating types varied over  
237 time in both tunnels (Fig. 3). There was no relationship between the distribution of strains  
238 among mating types and the tunnel of origin on June 2006, March and May 2007 ( $P>0.5$ ).  
239 However in February 2008, the distribution of strains among mating types was not independent  
240 of their tunnel of origin ( $P=0.0004$ ).

241

#### 242 **Spatial and temporal genetic differentiation between *B. cinerea* strains in tunnels**

243 The global degree of genetic differentiation between T1 strains and T6 strains was very low  
244 and not significant ( $F_{ST} < 0.001$ ,  $P=0.99$ ). When considering each date,  $F_{ST}$  values between T1  
245 and T6 strains were also very low ( $<0.01$ ) and not significant ( $P>0.40$ ). These results are  
246 consistent with those of AMOVA analysis showing that only 0.21% ( $P=0.40$ ) of genetic  
247 variation occurred between T1 and T6 strains (Table 5). In addition to this absence of spatial  
248 genetic differentiation, no temporal genetic differentiation was observed between sampling

249 dates for strains inside a given tunnel ( $F_{ST}$  values low  $<0.05$  and not significant). These results  
250 are consistent with those of AMOVA analysis showing that no genetic variation occurred  
251 between dates in the tunnels (Table 5).

252

### 253 **Spatial and temporal variation of *B. cinerea* genetic structure**

254 Bayesian clustering was carried out using 114 MLH (clones were removed from each date in  
255 each tunnel for the analysis) in order to assess the genetic structure of *B. cinerea* strains sampled  
256 from T1 and from T6. The highest probability given by the maximum likelihood distribution was  
257 obtained for  $K=5$ . The percentage of strains assigned to the 5 genetic clusters and of strains not  
258 assigned ( $P<0.80$ ) at each date is shown in Figure 3. The differences in the distribution of strains  
259 in the genetic clusters between T1 and T6 at each sampling date were not statistically significant  
260 ( $P=0.93$ ;  $0.57$ ;  $0.68$ ;  $0.41$  for June 2006, March 2007, May 2007 and February 2008,  
261 respectively). Inside each tunnel, the distribution of strains among the five genetic clusters  
262 seemed to vary over time but there was no significant difference between sampling dates in T1  
263 ( $P=0.34$ ) nor in T6 ( $P=0.78$ ).

264

## 265 **DISCUSSION**

266 This study is the first attempt to assess the variability of grey mould incidence and the variability  
267 of *B. cinerea* strains genetic diversity in six neighbouring tunnels in which lettuce and tomato  
268 were cultivated. Based on monitoring of grey mould over four successive crops and on the  
269 genetic characteristics of 170 *B. cinerea* strains collected from lettuce, our results supported the  
270 hypothesis that grey mould epidemics and the genetic characteristics of *B. cinerea* strains were  
271 similar in neighbouring greenhouses where a same plant species was grown. The similar  
272 variation of disease incidence in the tunnels monitored in the present study is consistent with  
273 results recently reported in another region (Leyronas *et al.*, 2014a). The absence of genetic

274 differentiation of *B. cinerea* strains between both tunnels is consistent with the weak association  
275 between population structure and geography, even when assessed on large spatial scales  
276 (Fournier and Giraud, 2008; Walker *et al.*, 2014). The absence of temporal genetic  
277 differentiation of *B. cinerea* strains in each tunnel of the present study is also consistent with  
278 the absence of genetic differentiation over time reported by Alfonso *et al.* (2000) in vegetable  
279 greenhouses in southern Spain and the stability of genetic structure reported by Walker *et al.*  
280 (2014) for strains collected from crops grown in open fields and in greenhouses. The higher  
281 abundance of mating type MAT1-1 in both tunnels and the existence of strains showing both  
282 mating types are consistent with results previously reported (Beever and Weeds, 2004; Wessels  
283 *et al.*, 2013).

284         However, the results of the present study also raise several questions. The first intriguing  
285 result is the lower haplotypic diversity observed in the present study (0.55 and 0.65; Table 3)  
286 compared to that of strains collected from four successive lettuce crops in another region (0.80;  
287 Leyronas *et al.*, 2014a). Since the values of gene diversity indices and the numbers of alleles  
288 per locus were in the same range in both regions, we expected similar values of haplotypic  
289 diversity. The differences found between the two regions may have been induced by differences  
290 in local climatic conditions. Indeed, humid periods known to be favourable to *B. cinerea*  
291 development (Eden *et al.*, 1996) often occur in the Alenya region (on the Mediterranean coast  
292 near the Spanish border) due to winds blowing from the sea, while they are much less frequent  
293 in Avignon (Rhône Valley). These climatic differences are likely to be the cause of (i) the lower  
294 disease incidence observed in this study for lettuce grown in Avignon compared to that in the  
295 region of Alenya (Leyronas *et al.*, 2014a), and (ii) the absence of disease on tomato in the  
296 present study whereas, in similar experimental set up in Alenya, tomato crops are regularly  
297 attacked (Leyronas *et al.*, 2014b). It could be further speculated that the generally less  
298 favourable conditions of Avignon may have allowed only a few strains to multiply, thus

299 reducing haplotypic diversity. Microclimatic differences might also explain the differences  
300 observed between tunnels T1 and T6 in the present study. Although no specific climatic data  
301 were collected inside the tunnels, we suspect that conditions were drier in tunnel T6 where  
302 lower haplotypic diversity and higher linkage disequilibrium were observed. During the spring  
303 of 2006 and of 2007, the averages of the daily relative humidity recorded at the local  
304 meteorological station of the Meteo France network (located at INRA-Avignon) were 57.3%  
305 and 68.6%, respectively, with minimal values of 34% and 41%, respectively. Tunnel T6  
306 benefited of the longest daily exposure to sunshine and was noticeably warmer than T1, which  
307 was rapidly shaded by neighbouring tunnels. These differences in temperature, combined with  
308 overall low values of ambient relative humidity, are believed to have created drier conditions  
309 inside T6. Further work is needed to investigate possible links between local climatic  
310 conditions and the genetic diversity of *B. cinerea*.

311 Another surprising result of the present study is that tunnels T1 and T6 shared only two  
312 haplotypes despite their spatial proximity, the long periods when all vents and doors were kept  
313 open and the daily passage of workers inside each tunnel. A higher number of shared haplotypes  
314 was expected since Adjebli *et al.* (2014) reported 9 haplotypes shared by tomato greenhouses  
315 located 15-23 km apart. The low number of shared haplotypes in the present study may have  
316 been caused by limited exchange of air between the tunnels due to their north-south orientation  
317 (parallel to the dominant winds) and to the presence of cypress hedges. Another explanation for  
318 the differences between the present study and that of Adjebli *et al.* (2014) may originate from  
319 differences in plant architecture and sites of *Botrytis* sporulation between the lettuce and tomato  
320 crops. In the tomato study, sporulating lesions were observed mostly on stems, enabling the  
321 pathogen to be easily dispersed by air currents toward other greenhouses (Adjebli *et al.*, 2014).  
322 In contrast, sporulating lesions on lettuce were observed under older leaves, near the ground,  
323 suggesting that release of inoculum from diseased plants into the air may have been limited

324 until harvest time. Evidence for this phenomenon is the peak in disease incidence observed in  
325 T1 and T6 in March 2007 following the harvest four weeks earlier in tunnels T2-T5 (Fig. 1) for  
326 which up to 5% of the plants showed sporulating lesions. Possible exchange of airborne  
327 inoculum between tunnels at that particular time was fostered by the circulation of workers and  
328 the opening of doors to facilitate harvest operations (Hausbeck and Pennypacker, 1991).

329 In conclusion, in tunnels, often regarded as quasi-closed systems, some exchange of *B.*  
330 *cinerea* airborne inoculum may happen, and particularly at harvest time due to the handling of  
331 diseased plants. Thus it is likely that inoculum released at harvest time may impact the  
332 susceptible crops in neighbouring tunnels. Nevertheless, this study also showed that each tunnel  
333 harboured a high proportion of strains with specific characteristics and that there were  
334 differences in the level of haplotypic diversity, a situation which could have implications for  
335 disease management such as the choice of anti-*Botrytis* fungicides and the timing of their  
336 application. A low level of haplotypic diversity results from the multiplication of specific strains  
337 in a tunnel. If some of these strains happen to be resistant to certain fungicides, a treatment  
338 against grey mould may be adequately efficient in one tunnel but less in another. Furthermore,  
339 a possible difference in aggressiveness between haplotypes (a point which remains hypothetical  
340 at this time) could result in differences in disease severity in tunnels with similar microclimatic  
341 environment, with consequences on the timing of intervention by the grower. Altogether, these  
342 considerations point to a possible interest in individualizing disease management in the different  
343 tunnels of a given farm.

344

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349

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**Table 1.** Successions of lettuce and tomato crops in the six experimental tunnels.

Dates	T1 and T6	T2, T3, T4 and T5
5 April to 8 June 2006	Lettuce (cv. Faustina)	
20 April to 5 October 2006		Tomato (cv. Swanson)
30 November to 22 February (T2-T5) or 19 March 2007 (T1, T6)	Lettuce (cv. Leandra)	
3 April to 31 May 2007	Lettuce (cv. Leandra)	
26 April to 19 July 2007		Tomato (cv. Swanson)
15 November 2007 to 19 February 2008	Lettuce (cv. Leandra)	

**Table 2.** Allelic richness (AR) and unbiased gene diversity (Hnb) of *B. cinerea* strains collected from tunnels T1 and T6, at each of 9 microsatellite loci.

	BC1		BC2		BC3		BC4		BC5		BC6		BC7		BC9		BC10	
	AR	Hnb	AR	Hnb	AR	Hnb	AR	Hnb	AR	Hnb	AR	Hnb	AR	Hnb	AR	Hnb	AR	Hnb
T1	15	0.90	12	0.88	13	0.87	3	0.25	11	0.88	18	0.83	10	0.85	8	0.50	14	0.89
T6	16	0.92	14	0.91	13	0.87	4	0.34	12	0.83	14	0.88	9	0.80	7	0.46	13	0.90

**Table 3.** Genetic characteristics of *B. cinerea* strains collected from tunnels T1 and T6.

	Sample size	Gene diversity	Mean number of alleles per locus	Number of distinct haplotypes	Haplotypic diversity	$r_d^a$
T1	85	0.76 (0.22)	11.60	56	0.65	0.09 **
T6	85	0.77 (0.21)	11.33	48	0.55	0.12 **

a: linkage disequilibrium

\*\* : statistically highly significant (P<0.01)

**Table 4.** Frequency distribution of *B. cinerea* haplotypes common to several dates in T1 and T6.

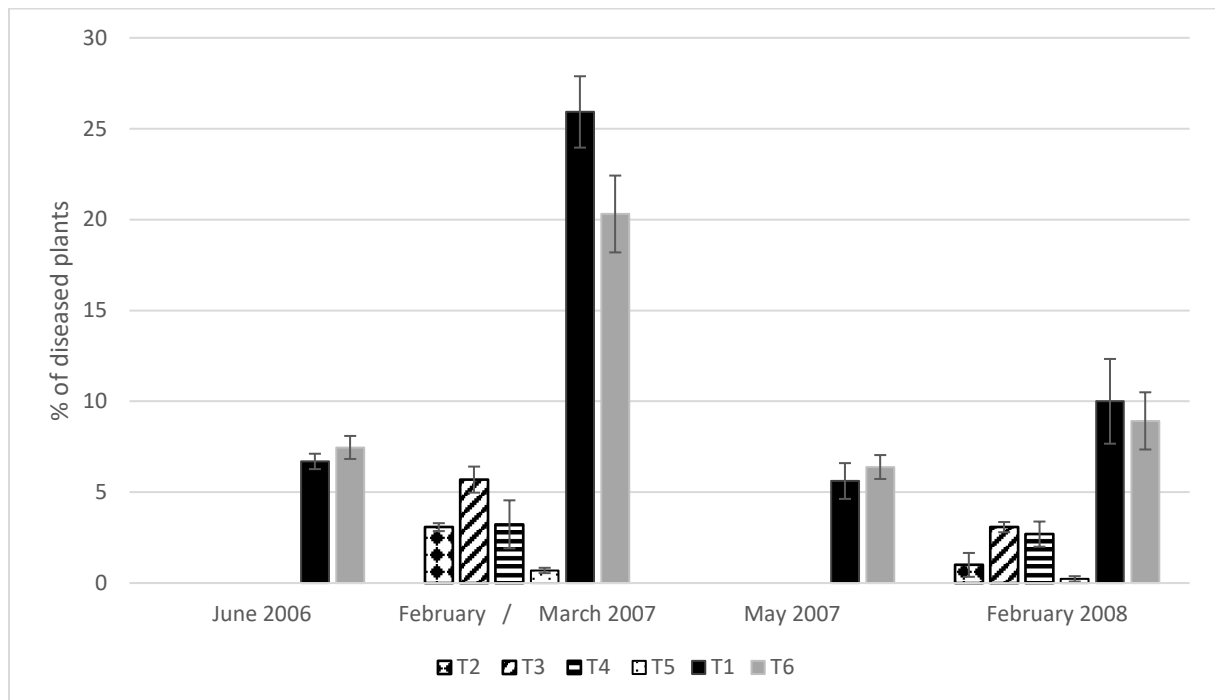
	MLH code	Total number of copies	June 2006	March 2007	May 2007	February 2008
T1	a	2	1		1	
	b	7		5	1	1
T6	c	2		1	1	
	d	3	1			2
	e	3			1	2
	f	8	3	4		1
	g	11		3	2	6



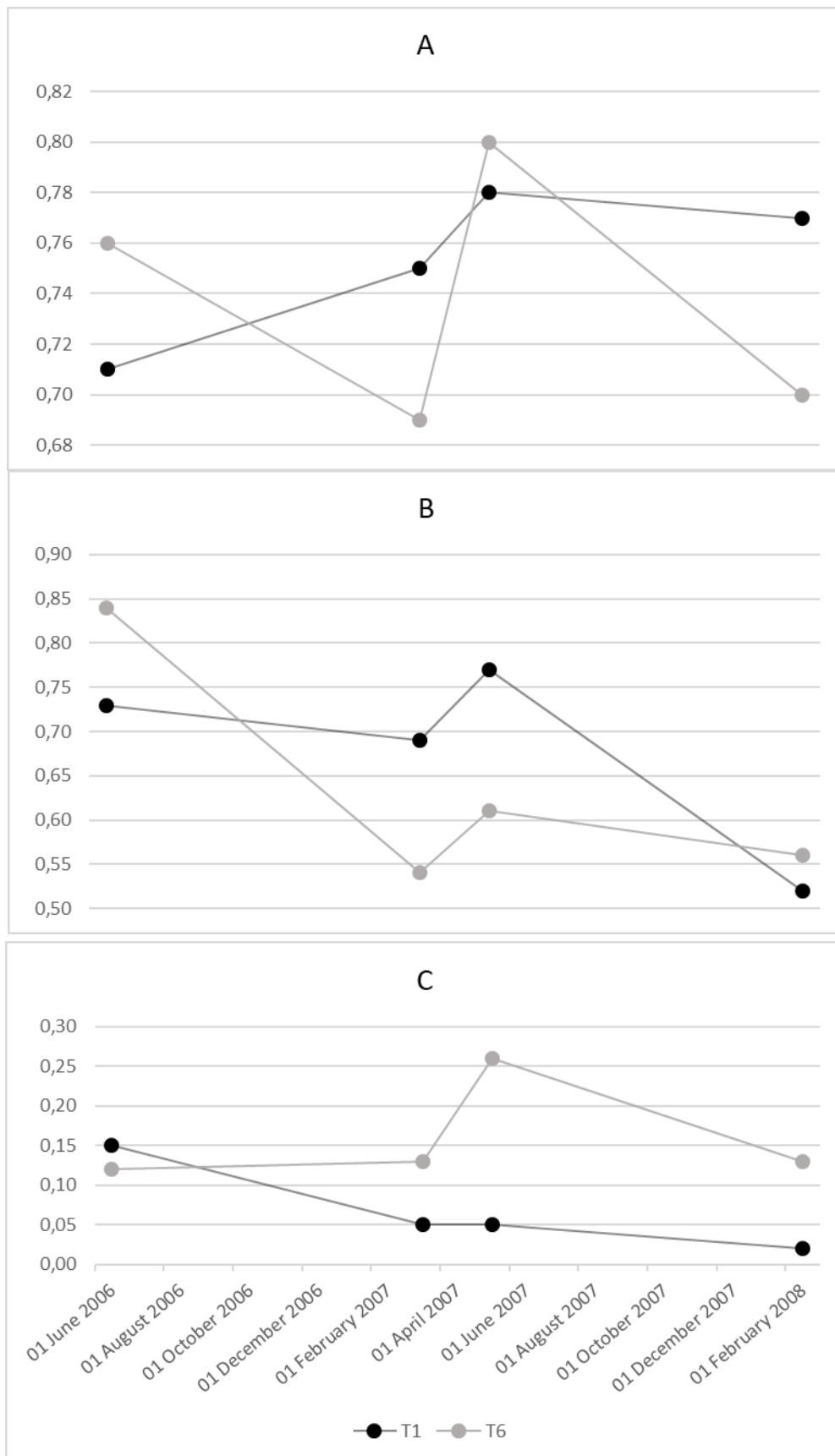
**Table 5.** Hierarchical analysis of molecular variance (AMOVA) with the origin of strains (T1 vs T6) as grouping factor.

Sources of variation	d.f	Sum of squares	Variance components	Percentage of variation	<i>P</i> value
Between T1 and T6 strains	1	3.89	0.007	0.21	0.40
Between dates	6	20.90	-0.0003	-0.01	0.47
Within dates	106	369.78	3.48	99.8	0.29

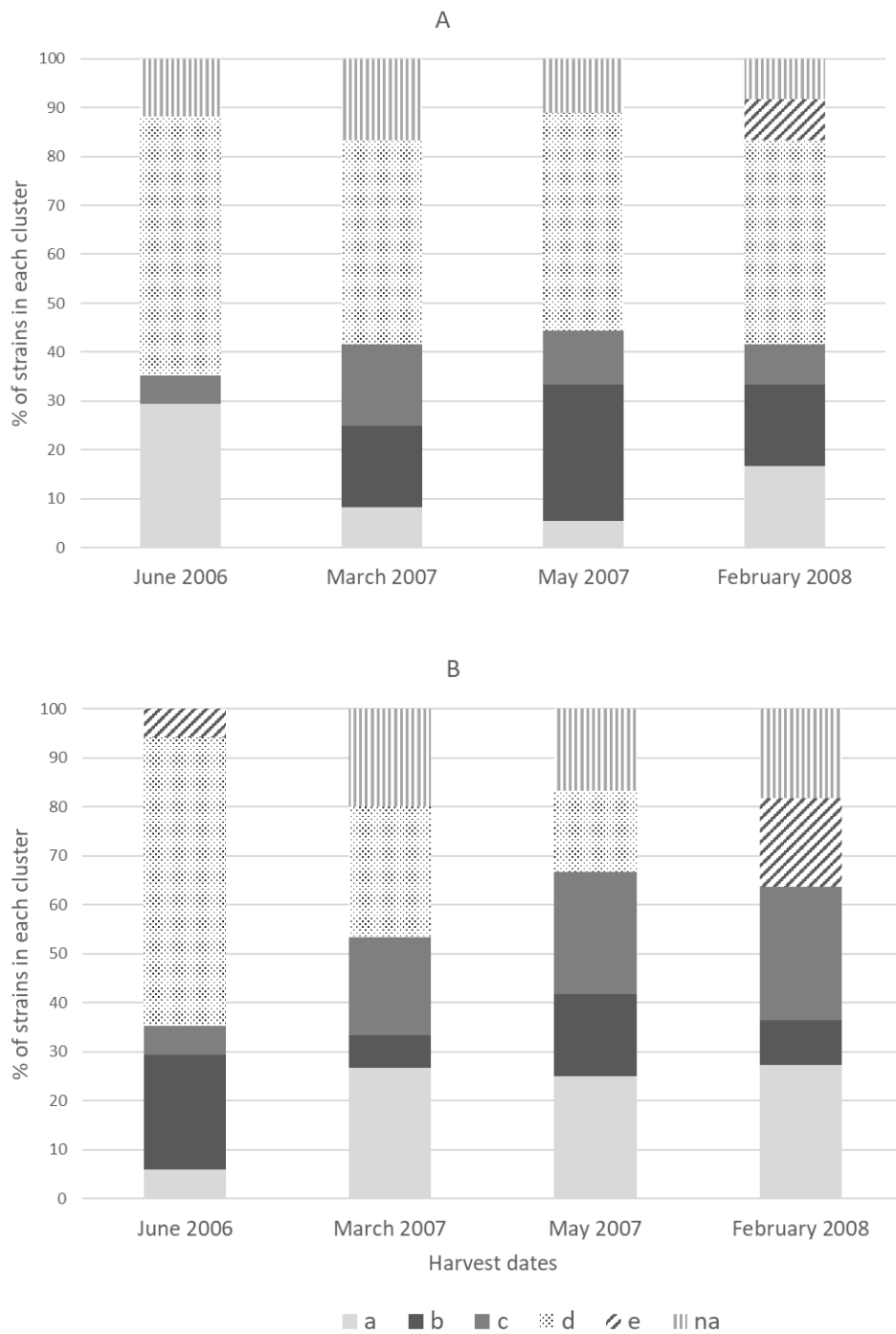
**Fig. 1.** Incidence of grey mould in Tunnels 1 to 6. Four successive lettuce crops were grown in T1 and T6 (plain black and grey bars); two lettuce crops in rotation with two tomato crops were grown in the four other tunnels (bars with patterns). Error bars indicate the standard error of the mean



**Fig. 2.** Temporal variability of genetic characteristics (A: unbiased gene diversity, B: haplotypic diversity, C: linkage disequilibrium) of *B. cinerea* inoculum over four successive lettuce crops in tunnels T1 (black points) and T6 (light grey points).



**Fig. 3.** Distribution of *B. cinerea* strains among 5 genetic clusters (a to e; na : not assigned) at each harvest date in tunnels T1 (A) and T6 (B).



**Fig. 4.** Distribution of *B. cinerea* strains collected in Tunnels T1 (A) and T6 (B) among mating types MAT1-1 (light grey), MAT1-2 (dark grey) and MAT1/1-2 (checkered pattern).

