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1 **Comparison of *Botrytis cinerea* populations collected from tomato greenhouses**  
2 **in Northern Algeria**

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11  
12 **Abstract**

13 To estimate the genetic diversity and population structure for a better  
14 understanding of the spread of *Botrytis cinerea*, we genotyped with nine  
15 microsatellite markers 174 isolates collected from four greenhouses during three  
16 growing seasons in the region of Bejaia. Four of these isolates were detected as *B.*  
17 *pseudocinerea* according to the allele size at locus Bc6. For all other isolates further studied,  
18 all loci were polymorphic, with the mean number of alleles per locus ranging from 2.77 to  
19 5.22. Considerable genetic variability was detected in all sub-populations ( $D^* > 0.87$ ;  
20  $H_{nb} > 0.40$ ). Based on the standardized index of association analysis, significant but low level  
21 of clonality occurred, not excluding the possibility of recombination. ( $r_D = 0.07$ ,  $P < 0.001$ ). A  
22 total of 109 haplotypes were characterized among the isolates, few of which were shared  
23 between sub-populations. This, together with moderate genetic differentiation among sub-  
24 populations according to the geographic origin ( $0.080 < F_{ST} < 0.167$ ), suggested a low level of  
25 inoculum exchange among greenhouses and little carryover of inoculum from one sampling  
26 season to the next. The importance of genetic structure of *B. cinerea* populations is discussed  
27 and should be taken into consideration for the management of gray mold.

28 **Key words:** Microsatellite makers / Gray mold / Genetic diversity/ Inoculum exchange

29  
30 **Introduction**

31 Dispersion is a key process in the dynamics and evolution of natural populations. In  
32 addition to its primary role in the colonization process, dispersion also affects the process of  
33 adaptation of organisms. In plant pathogens, a better understanding of the dispersion process  
34 thus appears to be a major issue for better control. The pace at which microbes spread to new  
35 niches is a critical determinant of the emergence or re-emergence of infectious diseases in  
36 plants (Anderson et al. 2004). Plant pathogens are dispersed by contact, wind, water, animal  
37 vectors (e.g. insects and birds) and by humans through seed and infected plant material  
38 (Nagarajan and Singh 1990). Dispersal can occur over a distance from a few centimeters or  
39 less between roots in soil to hundreds of kilometers between susceptible crops. The transport  
40 of a small number of pathogenic spores can result in the eventual infection of entire fields  
41 (Mims and Mims 2004; Viljanen-Rollinson et al. 2007).

42 For many fungal plant pathogens, long-distance dispersal is an important strategy  
43 enabling them to colonize new areas or to survive between different seasons (Brown and  
44 Hovmøller 2002; Isard et al. 2005).

45 Local spread of inoculum is also of interest for day-to-day disease management and  
46 much effort has been dedicated to studying short-distance gradients of dispersion for various  
47 plant pathogenic fungi, including *B. cinerea* (Agrios 2005; Allard and Soubeyrand 2012;  
48 Mfegue et al. 2012; Johnson and Powelson 1983). Gradients of dispersion are considered to  
49 be influenced by many factors, including the type of cropping system (Qandah and Del Rio  
50 Mendoza 2012). Greenhouses, for example, are often regarded as quasi-closed systems, which  
51 generate short distance dispersal (Campen and Kempkes 2009). However, depending on the  
52 season and the type of greenhouse, substantial exchange of air can occur between the inside of  
53 a greenhouse and its outside environment (Boulard et al. 1997; Fatnassi et al. 2009), which  
54 could thus result in concomitant exchange of airborne inoculum.

55           While direct observation of the movements of airborne inoculum is a difficult  
56 experimental task, other approaches have been used to study the spread of inoculum within a  
57 greenhouse and possible exchanges with the outside environment. This is the case for *Botrytis*  
58 *cinerea*, a fungus known for its capacity to produce massive amounts of anemophilic spores  
59 on diseased plant tissue, and considered as a key pathogen on many greenhouse crops (Dik  
60 and Wubben 2004). Using selenate-resistant mutants, Korolev *et al.* (2006) clearly  
61 demonstrated the exchange of airborne inoculum in both directions (in- and outbound)  
62 between a glasshouse and its close environment. Modelling approaches have also been  
63 developed for the quantification of such exchanges (Leyronas and Nicot 2013; Leyronas *et al.*  
64 2011).

65           In contrast, little is known about the spread of inoculum beyond the vicinity of a  
66 greenhouse despite its potential impact on disease management practices, possibly because of  
67 experimental difficulties. Nevertheless, molecular typing of isolates and tools of population  
68 genetics offer the possibility of an indirect approach to this subject through the comparison of  
69 distant sub-populations of the pathogen. In the case of *B. cinerea*, microsatellite markers are  
70 available (Fournier *et al.* 2002) and previous studies have shown a high level of diversity  
71 among isolates of this fungus on a variety of crops, allowing for comparison of population  
72 structures (Fournier and Giraud 2008; Isenegger *et al.* 2008a; Karchani-Balma *et al.* 2008).  
73 While populations of *B. cinerea* have been well characterized in various regions of the world  
74 (Fekete *et al.* 2012; Fournier and Giraud 2008; Decognet *et al.* 2009; Esterio *et al.* 2011; Ma  
75 and Michailides 2005; Mirzaei *et al.* 2009), information is scant for northern African  
76 countries (Karchani-Balma *et al.* 2008) and lacking for Algeria.

77           The main objectives of the present study were thus to establish a first detailed record  
78 of the genetic characteristics of the *B. cinerea* population in a vegetable growing area of  
79 Northern Algeria. A second objective was to identify traits that are compatible with the

80 hypothesis that exchange of inoculum occurred between farms. For this, isolates were  
81 collected from diseased plants in greenhouses of the Bejaia region during three growing  
82 seasons, and their genetic features were examined to test the hypothesis of a geographical  
83 effect on the structure of the population.

84

## 85 **Materials and methods**

### 86 **Fungal sampling and isolation**

87 A total of 264 *B. cinerea* isolates were collected from commercial tomato  
88 greenhouses in May-June of 2007, 2008, and 2010, in the Bejaia region of Algeria  
89 (Fig. 1, Table 1). Access to one of the sampling sites (greenhouse in Souk El  
90 Tenine) was not possible after 2007. Using sterile cotton swabs, isolates were taken from  
91 diseased plant organs (fruits, leaves, flowers and stems) showing abundant sporulation,  
92 brought back to the laboratory and stored at  $-20^{\circ}\text{C}$  until use for isolate purification and single  
93 spore isolation as described by Leyronas et al. (2012).

94 To produce mycelial tissue for DNA extraction, the single spore isolates were  
95 cultured on malt extract agar medium at  $25^{\circ}\text{C}$  in the dark. After 10-14 days of incubation,  
96 mycelia and conidia were collected by adding 1 ml of sterile distilled water in the Petri  
97 plates, and by scraping the surface of the colony with a sterile cotton pad. The fungal  
98 material was then lyophilized and stored at  $-20^{\circ}\text{C}$  until used for DNA extraction.

### 99 **DNA extraction**

100 The DNA of all field isolates of *B. cinerea* was extracted from 15 mg aliquots  
101 of lyophilized fungal material in 96-well plates, using a DNeasy Plant extraction maxi Kit  
102 (Qiagen®). DNA quality and quantity were assessed using agarose gel (1%)  
103 electrophoresis. The plates containing DNA were then stored at  $-20^{\circ}\text{C}$  until used for  
104 microsatellite amplification.

## 105 **Microsatellite amplification and size analysis**

106       Amplification reactions for the nine microsatellite loci described by Fournier et al.  
107 (2002) were carried out using a QIAGEN® Amplification Multiplex PCR kit. Some of the  
108 primers had the same annealing temperature, which allowed the amplifications to be  
109 multiplexed. The reaction mixture consisted of 552 µl of PCR Master mix (containing DNA  
110 polymerase, MgCl Multiplex PCR buffer and triphosphate-deoxyribonucleotides), 242µl of  
111 MilliQ water and 22µl of each multiplexed primer. Each well of a PCR plate contained 8 µl of  
112 reaction mixture and 2 µl of DNA from a *Botrytis* isolate.

113       The amplifications were carried out using an Eppendorf thermocycler. They entailed an  
114 initial preheat for 15 min at 95°C, followed by 25-40 cycles comprising 0.5 min of  
115 denaturation at 94°C, 1.5 min of annealing at 50°C (for the microsatellite loci Bc1, Bc3, Bc6,  
116 Bc9), 53°C (for Bc2 and Bc5) or 59°C (for Bc4, Bc7, Bc10) and 1 min of extension at  
117 72°C. Amplifications were ended by a final extension step of 30 min at 60°C. In order to  
118 verify that the amplification occurred, sub-samples were then taken from each well and  
119 deposited on 1% agarose gels for electrophoresis.

120       The amplification products were stored at -20°C until used for assessing the sizes of  
121 the microsatellites. After a denaturation phase at 94°C for 3 min and addition of size  
122 markers, amplification products were scanned with an ABI 3730 xl DNA sequencer. Data  
123 were analyzed with GeneMapper software (Applied Biosystem). Based on the sizes of the 9  
124 microsatellite markers, a haplotypic profile could be assigned to each isolate, defining  
125 multilocus genotypes (MLG). Genotyping was repeated for some (but not all) of the isolates  
126 to confirm the results. Isolates belonging to subspecies *B. pseudocinerea* are morphologically  
127 identical to those of *B. cinerea*, but they can be identified on the basis of the allele size at  
128 locus Bc6 (Walker et al. 2011).

129 **Population genetics analysis**The analysis was carried out only for isolates of *B. cinerea*  
130 (excluding *B. pseudocinerea*) whose complete 9-microsatellite haplotypic profile could be  
131 obtained.

132 The software Genetix 4.05 (Belkhir et al. 1996) was used to compute an index of  
133 unbiased allelic diversity (Hnb), the mean number of alleles per locus (based on allele  
134 frequencies) and allelic richness over all loci in the total sample and in each sub-population  
135 collected in the four greenhouses cited previously. Intra-population diversity was  
136 characterized by computing diversity indices using Genclone software (version 2.0). We used  
137 the Simpson diversity index ( $D^*$ ), an equitability index ( $ED^*$ ) and the index of genotypic  
138 richness (R). The  $D^*$  index measures the probability that two randomly selected individuals in  
139 a population have different MLGs (Arnaud-Haond et al. 2007). The  $ED^*$  index is equal to 1  
140 when all MLGs represented in a population have the same abundance (equitable distribution  
141 of clones) (Arnaud-Haond et al. 2007). Finally, we used the R index of genotypic richness,  
142 which varies from 0 when all isolates in a sample possess the same genotype, to 1, when all  
143 isolates possess a different genotype (Dorken and Eckert 2001).

144 In order to test for genetic recombination, the standardized index of association ( $r_D$ ) was  
145 calculated using Multilocus 1.3b (Agapow and Burt 2001). The  $r_D$  index is a measure of the  
146 multilocus linkage disequilibrium (Brown et al. 1980; Haubold et al. 1998), which gives  
147 information on whether two different individuals sharing the same allele at one locus are more  
148 likely to share an allele at another locus. For each pair of isolates, the number of loci with  
149 respect to which they differ is calculated, and the variance of this number is compared with  
150 that expected if there is no linkage disequilibrium. The  $r_D$  index is equal to zero if there is no  
151 linkage disequilibrium, and it increases as linkage disequilibrium increases. The null  
152 hypothesis of complete panmixia ( $r_D= 0$ ) was tested with the procedure implemented in the  
153 software, by comparing the observed data set to 1000 randomized data sets in which infinite

154 recombination has been imposed by randomly shuffling the alleles among individuals,  
155 independently for each locus.

### 156 **Genetic differentiation between sub-populations**

157 Estimation of genetic differentiation by pairwise comparison between the sub-  
158 populations collected at different locations in a given season was performed using the  
159 software ARLEQUIN 3.0 (Excoffier et al. 2005), without repeated MLGs, after 3024  
160 permutations and progressive Bonferroni correction as described by Rice (1989). According  
161 to Wright (1978), the genetic differentiation between two populations is low when  
162  $0 < F_{ST} \leq 0.05$ , moderate when  $0.05 < F_{ST} \leq 0.15$ , substantial when  $0.15 < F_{ST} \leq 0.25$  and very high  
163 when  $F_{ST} > 0.25$ .

## 164 **Results**

### 165 **Distinction between *B. cinerea* and *B. pseudocinerea***

166 After isolation, single-spore purification, DNA extraction and genotyping of the 264  
167 isolates, complete genotypic profiles with the nine microsatellites were obtained for only 174  
168 isolates. No variation of the microsatellite sizes was found after genotyping repetitions of  
169 some isolates. Four of them, collected in Tichy in 2007 and 2008, were characterized as *B.*  
170 *pseudocinerea* and excluded from further genetic analysis. We had thus 170 fully genotyped  
171 isolates of *B. cinerea*, distributed in nine groups of approximately 20 isolates per sampling  
172 site and per year (Table 2; one exception in Tichy in 2007 with only 5 isolates). Each of these  
173 groups will be referred to as a "sub-population" in the rest of this paper.

### 174 **Global genetic diversity**

175 A total of 109 multilocus genotypes (MLG) were distinguished among the 170 isolates  
176 of *B. cinerea* and the mean number of alleles per locus was 8.22 (Table 2). The overall level  
177 of genetic diversity in the total population was high, as shown by an unbiased genetic  
178 diversity (Hnb) of 0.57, an index of genotypic diversity ( $D^*$ ) of 0.98 and a Richness index (R)



179 of 0.64. The high index of equitability ( $ED^*= 0.90$ ) indicated an equitable distribution of  
180 clonal haplotypes between the nine sub-populations. Finally, the estimate of linkage  
181 disequilibrium was significant but very low ( $r_D=0.07$ ), suggesting the occurrence of limited  
182 genetic recombination (Table 2). Although the results obtained for the total sample were  
183 interesting in our study, other repetitions in the time for the site of Souk-El-Tenine must be  
184 undertaken to confirm these results.

### 185 **Genetic diversity among sub-populations**

186 Separate examination of the nine sub-populations confirmed the overall high level of  
187 allelic diversity, with an Hnb index ranging from 0.40 to 0.68 (Table 2). This diversity  
188 appeared higher in 2010 than in other years. The average number of alleles per locus was 8.22  
189 for the total sample and ranged from 2.77 to 5.22 within the nine sub-populations, suggesting  
190 that several alleles were not shared among sub-populations. Haplotypic diversity was also  
191 high, with a  $D^*$  index higher than 0.90 in eight of the nine sub-populations. Significant but  
192 low multilocus linkage disequilibrium ( $r_D$ ) was observed in all sub-populations (Table 2; one  
193 exception: Tichy 2007, for which only 5 isolates were available).

194 Among the 109 MLGs observed within the total sample, 88 were represented by a  
195 single isolate and 21 were represented by at least two isolates (Fig. 2). Among those, twelve  
196 were shared between sub-populations collected in different greenhouses and seasons (Table  
197 3). One MLG was detected 17 times in the total sample and was shared between four sub-  
198 populations. The number of shared MLG between greenhouses varies with the year.  
199 Therefore, 3 MLGs were found in 2008, only 2 in 2010 and 1 MLG was found in 2007 (Table  
200 3).

### 201 **Genetic differentiation between sampling sites**

202 Pairwise tests of genetic differentiation among sub-populations yielded generally low  
203 to moderate  $F_{ST}$  values (Table 4). The highest values were obtained in 2007 between Tichy

204 (site C) and the two other coastal sites, showing significant genetic differentiation between  
205 these sub-populations on that year. Moderate but significant differentiation was also observed  
206 between Tichy and Baccaro (site B) in 2008. No significant differences were observed in  
207 2010.

## 208 **Discussion**

209 This study provides the first data on genetic features of *B. cinerea* populations in  
210 Algeria. It is also the first report of the detection of *B. pseudocinerea* in North Africa. This  
211 species, detected at a frequency of 1.5 % among the 264 isolates collected in our study, was  
212 also observed at low frequencies (varying between 0.5 and 15% according to the location and  
213 the season) in other parts of the world, in sympatry with *B. cinerea* populations (Fekete et al.  
214 2012; Fournier et al. 2005; Fournier et al. 2003; Martinez et al. 2005; Scagnelli et al. 2010,  
215 Walker et al. 2011; Wissels 2012; Ma and Michailides 2005).

216 Among the isolates of *B. cinerea*, the high genetic diversity (both allelic and  
217 haplotypic) observed in the present study indicates a highly heterogeneous population. Similar  
218 situations have been described for populations collected from different hosts around the world  
219 (Table 5). Such high levels of diversity could result from genetic recombination, as suggested  
220 by the low value of the standardized index of association  $r_D$  found in the present work and in  
221 several previous studies (Table 5). High levels of recombination have been reported for most  
222 studies of *B. cinerea* populations, with the exception of a study in various field crops in  
223 California (Ma and Michailides 2005). Recombination has been mostly attributed to sexual  
224 reproduction, and could also be related to other mechanisms of variability known for *B.*  
225 *cinerea* (Van Der Vlugt-Bergmans et al. 1993; Beever and Weeds 2004).

226 The overall level of clonality in the *B. cinerea* population, measured by the ratio of the  
227 number of distinct MLGs present among the total number of isolates characterized in the  
228 present study was 0.64. When considering the sub-populations of individual greenhouses, it

229 was consistently higher than 0.56. These numbers are overall similar to those reported for  
230 studies of *B. cinerea* in various crops and locations (Table 5). However, they contrast sharply  
231 with results of a study conducted in tomato greenhouses in experimental conditions (Decognet  
232 et al. 2009). In that study, the ratio of the number of distinct MLGs identified among the total  
233 number of characterized isolates was 0.70 among isolates sampled from the air spores before  
234 the development of the disease in the greenhouses. It decreased sharply to a value of 0.23  
235 among isolates sampled from the air 15 days after the inoculation of plants with two known  
236 strains of *B. cinerea*, and decreased further to 0.03 among isolates collected from diseased  
237 plants 60 days after inoculation. Under the hypothesis that two isolates sharing an identical  
238 genotypic profile were clones, these authors concluded that abundant sporulation by the two  
239 introduced strains of *B. cinerea* resulted in a displacement of the initially present population  
240 (Decognet et al. 2009).

241 In the present study, we observed identical MLGs in different greenhouses during a  
242 given year (Table 3). This observation is compatible with the hypothesis that inoculum  
243 exchange may have occurred between greenhouses. This hypothesis is supported by the fact  
244 that genetic differentiation ( $F_{st}$  values) was not significant on a given year for greenhouses  
245 which shared MLGs (one exception in 2008 for Baccaro and Tichy; Table 4). The possibility  
246 of inoculum exchange between the greenhouses is also supported by meteorological data  
247 showing that prevailing winds in the Bejaia region are west-bound between April and  
248 September (Alkama et al. 2007; [http://fr.windfinder.com/windstats/windstatistic\\_bejaia\\_aeroport.htm](http://fr.windfinder.com/windstats/windstatistic_bejaia_aeroport.htm)), a period that covers the time when *Botrytis* epidemics occur in the tomato  
250 greenhouses of that region (Aissat et al. 2008). Considering the dominant wind direction  
251 during our study and the distance between the sampling sites, genotypic data are generally  
252 (but not systematically) in agreement with possible contribution of inoculum between  
253 greenhouses from the most eastern to the most western site of a given year.

254 Earlier studies have reported genotype flows between regions for *B. cinerea*  
255 populations examined in chickpea fields in Bangladesh (Isenegger et al. 2008b) and for two  
256 other species of *Botrytis* in field crops in the Netherlands (Staats 2007). In our study, the  
257 small number of shared MLGs between sampling sites could be due to the fact that  
258 greenhouses are somewhat confined environments, although some level of inoculum  
259 exchange between the inside and outside of a greenhouse has previously been demonstrated  
260 (Korolev *et al.* 2006). However, the occasional presence of shared MLGs in different  
261 greenhouses of the Bejaia region could also result from their presence in multiple copies in  
262 global air masses arriving to that region. A consideration of the trajectories of air masses  
263 across the Mediterranean (Kushta et al. 2012; Khalfa et al. 2013; Lelieveld et al. 2007) makes  
264 it impossible to exclude occasional influx of *B. cinerea* inoculum from more distant sources,  
265 such as agricultural regions in southern France and Spain where *Botrytis* is known to be  
266 abundant (Leyronas and Nicot 2013; Moyano et al. 2003). This hypothesis is further  
267 supported by the detection in Bejaia of *B. pseudocinerea*, a new species formerly described in  
268 France and considered to be migrating to other parts of the world (Isenegger et al. 2008b).  
269 More conclusive evidence for direct exchange of inoculum among farms of the Bejaia region  
270 would thus require additional information on short term evolution of the population structures  
271 during a growing season, together with concomitant information on dominant haplotypes  
272 present in distant potential sources of occasional inoculum. In the meantime, gray mold  
273 management strategies in the Bejaia region should not rule out the possible exchange of  
274 inoculum between farms and thus the spread of particularly aggressive strains or fungicide-  
275 resistant strains.

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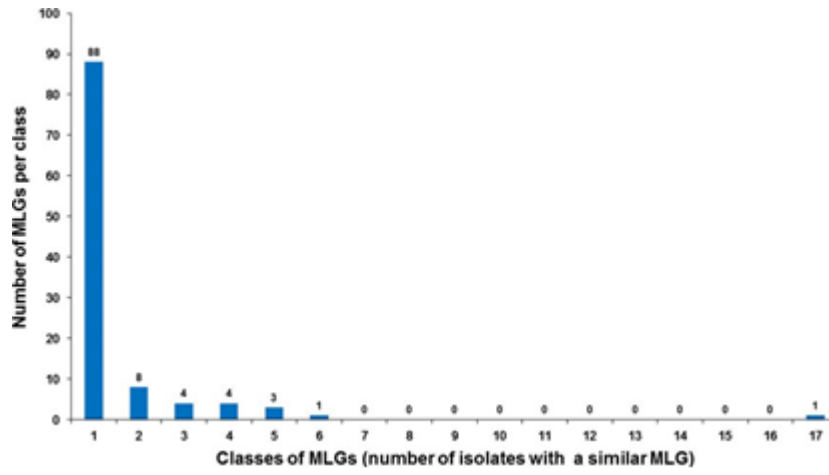
414 **Figure captions:**



415  
 416 **Fig. 1:** Location of *Botrytis cinerea* sampling sites in the Bejaia region (North Algeria) (A:  
 417 Souk-el-Tenine, B: Baccaro, C: Tichy and D: Merdjouamane).

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419

420 **Fig. 2:** Frequency distribution of multilocus genotypes (MLGs) among 170 isolates of *B.*  
 421 *cinerea* sampled from the Bejaia region.

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424

425 **Table 1.** Origin of *Botrytis cinerea* isolates collected from commercial tomato greenhouses in  
 426 Bejaia region (North Algeria) from 2007 to 2010.

427

<b>Sampling location</b>	<b>GPS coordinates</b>	<b>Sampling date</b>	<b>Number of genotyped isolates</b>
<b>A:</b> Souk-el-Tenine	Latitude: <b>36.62958</b> Longitude: <b>5.29764</b>	30 May 2007	23
<b>B:</b> Baccaro	Latitude: <b>36.64683</b> Longitude: <b>5.20958</b>	30 May 2007	21
		1 June 2008	19
		5 June 2010	19
<b>C:</b> Tichy	Latitude: <b>36.6938</b> Longitude: <b>5.10217</b>	30 May 2007	05
		1 June 2008	22
		5 June 2010	19
<b>D:</b> Merdjouamane	Latitude: <b>36.67619</b> Longitude: <b>4.94483</b>	1 June 2008	23
		5 June 2010	19

**Table 2.** Genetic diversity in nine *B. cinerea* sub- populations sampled from commercial tomato greenhouses.

Sampling year and location	Number of isolates	Number of MLG <sup>a</sup>	Allelic diversity <sup>b</sup>		Haplotypic diversity <sup>c</sup>			Index of multilocus linkage disequilibrium <sup>d</sup>	
			Hnb	number of alleles per locus	D*	ED*	R	r <sub>D</sub>	P value
<b>Total sample</b>	170	109	0.57 (0.17)	8.22	0.98	0.90	0.63	0.07	<0.001
<b>2007 samples</b>									
Souk-el-Tenine	23	14	0.45 (0.25)	3.33	0.90	0.53	0.59	0.28	<0.001
Baccaro	21	17	0.47 (0.21)	2.77	0.97	0.66	0.80	0.13	<0.001
Tichy	5	5	0.68 (0.14)	3.33	1.00	ND	1.00	-0.01	0.587
<b>all sites</b>	<b>49</b>	<b>35</b>	<b>0.54 (0.18)</b>	<b>5.33</b>	<b>0.97</b>	<b>0.71</b>	<b>0.71</b>	<b>0.12</b>	<b>&lt;0.001</b>
<b>2008 samples</b>									
Baccaro	19	15	0.56 (0.11)	3.44	0.96	0.40	0.78	0.11	<0.001
Tichy	22	14	0.50 (0.17)	3.44	0.91	0.54	0.62	0.16	<0.001
Merdjouamane	23	13	0.40 (0.20)	3.55	0.87	0.50	0.55	0.21	<0.001
<b>all sites</b>	<b>64</b>	<b>39</b>	<b>0.54 (0.14)</b>	<b>4.66</b>	<b>0.97</b>	<b>0.85</b>	<b>0.60</b>	<b>0.08</b>	<b>&lt;0.001</b>
<b>2010 samples</b>									
Baccaro	19	16	0.57 (0.25)	4.44	0.97	0.47	0.83	0.12	<0.001
Tichy	19	17	0.65 (0.13)	5.22	0.98	0.53	0.89	0.12	<0.001
Merdjouamane	20	15	0.50 (0.21)	3.55	0.95	0.60	0.74	0.15	<0.001
<b>all sites</b>	<b>58</b>	<b>45</b>	<b>0.60 (0.20)</b>	<b>6.55</b>	<b>0.99</b>	<b>0.86</b>	<b>0.77</b>	<b>0.10</b>	<b>&lt;0.001</b>

<sup>a</sup>MLG: multilocus genotypes.

<sup>b</sup>Hnb: nonbiased genic diversity, calculated with repeated genotypes (standard deviations between brackets),

ND: undefined.

<sup>c</sup>D\*: genotypic diversity index; E: equitability index; R: Richness index.

<sup>d</sup>r<sub>D</sub>: standardized index of association.

**Table 3.** Frequency distribution of Multilocus Genotypes (MLG) detected in more than one greenhouse.

MLG	2007			2008			2010		
	Souk-el-Tenine	Baccaro	Tichy	Baccaro	Tichy	Merdjouamane	Baccaro	Tichy	Merdjouamane
H20		2		1	1		2		
H32		1		1					
H89				2			3		
H25								2	2
H30	7	1				8		1	
H55		2							
H65					1	2			
H80					1				1
H82		1			1				
H88				1		3			
H100			1		1		1		
H108							1	1	

**Table 4.** Genetic differentiation ( $F_{ST}$ ) of *B. cinerea* sub-populations sampled from four sites in the Bejaia region of Algeria.

Season	Site A-Site B <sup>a</sup> (13 km)	Site A-Site C (23 km)	Site B-Site C (10 km)	Site B-Site D (20 km)	Site C-Site D (15 km)
2007	0,062 <sup>b</sup>	<u>0,167</u>	<u>0,115</u>	ND <sup>d</sup>	ND
2008	ND	ND	<u>0,095</u>	0,058	-0,019
2010	ND	ND	0,011	0,016	0,050

<sup>a</sup> A: Souk-el-Tenine; B: Baccaro; C: Tichy; D: Merdjouamane. Numbers between brackets indicate the distance in km between two sampling sites.

<sup>b</sup> Pairwise  $F_{ST}$  were calculated following Weir and Cockerham, without repeated MLG. Underlined values are significantly different ( $P < 0.01$ ) after 3200 permutations and a Bonferroni correction.

<sup>c</sup> ND: not determined.

**Table 5.** Genetic diversity indices of *B. cinerea* populations characterized with microsatellite makers

<sup>a</sup> Hnb: nonbiased genic diversity, calculated with repeated genotypes (standard deviations between brackets),

Origin of study	Host plant	Number of microsatellites	Number of isolates	Number of MLGs	Number of MLG/Number of isolates	Hnb <sup>a</sup>	D* <sup>b</sup>	r <sub>D</sub> <sup>d</sup>	Reference
Algeria	Tomato	9	170	109	0.64	0.57	0.98	0.07	Present study
France	Grapevine, bramble	8	184	180	0.98	0.75	0.75	-	Fournier et al. 2008
Tunisia	Grapevine, faba bean, tomato, strawberry	9	153	120	0.78	0.79	0.99	0.08	Karchani-Balma et al. 2008
Hungary	Strawberry, rape	5	79	32	0.40	0.66	0.91	0.08	Fekete et al. 2012
Bangladesh	Chickpea fields	9	146	69	0.47	0.25	0.54	-	Isenegger et al.2008
South Africa	Pear orchards	9	181	91	0.50	0.14	0.69	-	Wessels 2012

<sup>b</sup> D\*: genotypic diversity index; E: equitability index; R: Richness index.

<sup>c</sup> r<sub>D</sub>: standardized index of association.