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

Ahmed Adjebli, Christel Leyronas, Kamel Aissat, Philippe C. Nicot

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

Ahmed Adjebli[1*], Christel Leyronas[2], Kamel Aissat[1], Philippe Nicot[2]

[1]Université Abderrahmane Mira, Faculté des Sciences de la Nature et de la Vie, Laboratoire d'écologie microbienne (L.E.M), Bejaia, 06000, Algérie.
[2]INRA-UR407 Pathologie végétale, Domaine St Maurice CS 60094, F-84143 Montfavet cedex, France.

*Corresponding author: philippe.nicot@avignon.inra.fr

Abstract

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

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

Introduction

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

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

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

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

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

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

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

Materials and methods

Fungal sampling and isolation

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

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

DNA extraction

The DNA of all field isolates of *B. cinerea* was extracted from 15 mg aliquots of lyophilized fungal material in 96-well plates, using a DNeasy Plant extraction maxi Kit (Qiagen®). DNA quality and quantity were assessed using agarose gel (1%) electrophoresis. The plates containing DNA were then stored at –20°C until used for 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).

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

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

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

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

Genetic differentiation between sub-populations

Estimation of genetic differentiation by pairwise comparison between the sub-populations collected at different locations in a given season was performed using the software ARLEQUIN 3.0 (Excoffier et al. 2005), without repeated MLGs, after 3024 permutations and progressive Bonferroni correction as described by Rice (1989). According to Wright (1978), the genetic differentiation between two populations is low when $0<F_{ST}\leq0.05$, moderate when $0.05<F_{ST}\leq0.15$, substantial when $0.15<F_{ST}\leq0.25$ and very high when $F_{ST}>0.25$.

Results

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

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

Global genetic diversity

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

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

Genetic diversity among sub-populations

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

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

Genetic differentiation between sampling sites

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

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

Discussion

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

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

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

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

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

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

Acknowledgments

This work was supported partially by a grant of the Ministry of Higher Education and Scientific Research of Algeria, and INRA, UR407 Pathologie végétale, Domaine Saint

279 Maurice Montfavet France. We are grateful to Magali Duffaud and Claire Troulet for their
280 technical participation in the experiments.

281 **References**

282 Agapow PM, Burt A. (2001) Indices of multilocus linkage disequilibrium. Mol Ecol Notes. 1:
283 101-102.

284 Agrios, GN. 2005. Plant Pathology. Elsevier Academic Press.

285 Aissat K, Nicot PC, Guechi A, Bardin M, Chibane M. (2008) Grey mould development in
286 greenhouse tomatoes under drip and furrow irrigation. Agron Sustain Dev 28: 403-409.

287 Alkama R, Adjabi S, Abbaci F, Mouaci K.(2007) Atmospheric pollution from industrial and
288 automobile source emissions in the region of Bejaia. (ed) proc 10th intern conference on
289 environmental science and technology, 5-7 September 2007. Kos island, Greece, pp 16-23.

290 Allard D, Soubeyrand S. (2012) Skew-normality for climatic data and dispersal models for
291 plant epidemiology: When application fields drive spatial statistics. Spatial Statistics 1: 50-64.

292 Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P. (2004)
293 Emerging infectious diseases of plants: pathogen pollution, climate change and
294 agrotechnology drivers. Trends Ecol Evol 19: 535-544.

295 Arnaud-Haond S, Duarte CM, Alberto F, Serrao EA. (2007) Standardizing methods to address
296 clonality in population studies. Mol Ecol 16: 5115-5139.

297 Beever RE, Weeds PL. (2004) Taxonomy and genetic variation of Botrytis and Botryotinia.
298 In: Botrytis: Biology, Pathology and Control. (Elad, Y., Williamson, B., Tudzynski, P. and
299 Delen, N., eds), pp. 29–52. Dordrecht, The Netherlands: Kluwer Academic Press.

300 Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. (1996) Genetix 4.05, logiciel sous
301 Windows pour la génétique des populations. Laboratoire génome, populations, interactions.
302 CNRS UMR 5000, Université de Montpellier II, Montpellier, France.Boulard T, Feuilloley P,
303 Kittas C. (1997) Natural ventilation performance of six greenhouse and tunnel types. J Agri
304 Eng Res 67: 249-266.

305 Brown AHD, Feldman MW, Nevo E. (1980) Multilocus structure of natural-populations of
306 *Hordeum spontaneum*. Genetics 96: 523-536.

307 Brown JKM, Hovmøller MS. (2002) Aerial dispersal of pathogens on the global and
308 continental scales and its impact on plant disease. Science 297: 537-541.

309 Campen JB, Kempkes FLK. (2009) Climatic evaluation of semi-closed greenhouses. *In* Acta
310 Horticulturae pp. 495-501.

311 Decognet V, Bardin M, Trottin-Caudal Y, Nicot PC. (2009) Rapid change in the genetic
312 diversity of *Botrytis cinerea* populations after the introduction of strains in a tomato
313 glasshouse. Phytopathol 99: 185-193.

Dik AJ, Wubben JP. (2004) Epidemiology of *Botrytis cinerea* diseases in greenhouses. *In* Botrytis: biology, pathology and control, pp319-333.

Dorken ME, Eckert, CG. (2001) Severely reduced sexual reproduction in northern populations of a clonal plant, Decodon verticillatus (Lythraceae). J Ecol 89: 339-350.

Esterio M, Munoz G, Ramos C, Cofre G, Estevez R, Salinas A, Auger J. (2011) Characterization of *Botrytis cinerea* isolates present in Thompson seedless table grapes in the central valley of Chile. Plant Dis 95: 683-690.

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

Fatnassi H, Leyronas C, Boulard T, Bardin M, Nicot P. (2009) Dependence of greenhouse tunnel ventilation on wind direction and crop height. Biosys Eng 103: 338-343.

Fekete É, Fekete E, Irinyi L, Karaffa L, Arnyasi M, Asadollahi M, Sandor E. (2012) Genetic diversity of a *Botrytis cinerea* cryptic species complex in Hungary. Microbiol Res 167: 283-291.

Fournier E, Giraud T. (2008) Sympatric genetic differentiation of a generalist pathogenic fungus, *Botrytis cinerea*, on two different host plants, grapevine and bramble. J Evol Biol 21: 122-132.

Fournier E, Giraud T, Albertini C, Brygoo Y.(2005) Partition of the *Botrytis cinerea* complex in France using multiple gene genealogies. Mycologia 97: 1251-1267.

Fournier E, Giraud T, Loiseau A, Vautrin D, Estoup A, Solignac M, Cornuet JM, Brygoo Y. (2002) Characterization of nine polymorphic microsatellite loci in the fungus *Botrytis cinerea* (Ascomycota). Mol Ecol Notes 2: 253-255.

Fournier E, Levis C, Fortini D, Leroux P, Giraud T, Brygoo Y. (2003) Characterization of Bc-hch, the *Botrytis cinerea* homolog of the *Neurospora crassa* het-c vegetative incompatibility locus, and its use as a population marker. Mycologia 95: 251-261.

Haubold B, Travisano M, Rainey PB, Hudson RR. (1998) Detecting linkage disequilibrium in bacterial populations. Genetics 150: 1341-1348.

Isard SA, Gage SH, Comtois P, Russo JM. (2005) Principles of the atmospheric pathway for invasive species applied to soybean rust. BioScience 55: 851-861.

Isenegger DA, Ades PK, Ford R, Taylor PWJ. (2008a) Status of the *Botrytis cinerea* species complex and microsatellite analysis of transposon types in South Asia and Australia. Fungal Divers 29: 17-26.

Isenegger DA, Macleod WJ, Ford R, Taylor PWJ. (2008b) Genotypic diversity and migration of clonal lineages of *Botrytis cinerea* from chickpea fields of Bangladesh inferred by microsatellite markers. Plant Pathol 57: 967-973.

Johnson KB, Powelson, ML. (1983) Analysis of spore dispersal gradients of *Botrytis cinerea* and grey mold disease gradients in snap beans. Phytopathol 73: 741-746.

Karchani-Balma S, Gautier A, Raies A, Fournier E. (2008) Geography, plants, and growing systems shape the genetic structure of Tunisian *Botrytis cinerea* populations. Phytopathol 98: 1271-1279.

Khalfa D, Benretem A, Herous L, Meghlaoui I. (2013) Establishment of the wind speed model for the installation of wind turbines in the algerian offshore. JECE 3: 13-19.

Korolev N, Katan T, Elad Y. (2006) Use of selenate-resistant strains as markers for the spread and survival of *Botrytis cinerea* under greenhouse conditions. Phytopathol 96: 1195-1203.

Kushta J, Solomos S, Kallos G. (2012) Contribution of aviation emissions on the air pollution levels of the Mediterranean region with the use of an online coupled, fully integrated modeling system In: Air Pollution Modeling and its Application XXI. Springer, Netherlands, pp 327-332.

Lelieveld J, Berresheim H, Borrmann S, Crutzen PJ, Dentener FJ, Fischer H, Feichter J, Flatau PJ, Heland J, Holzinger R, Korrmann R, Lawrence MG, Levin Z, Markowicz KM, Mihalopoulos N, Minikin A, Ramanathan V, de Reus M, Roelofs GJ, Scheeren HA, Sciare J, Schlager H, Schultz M, Siegmund P, Steil B, Stephanou EG, Stier P, Traub M, Warneke C, Williams J, Ziereis H. (2007) Global air pollution crossroads over the Mediterranean. Science 298: 794-799.

Leyronas C, Fatnassi H, Bardin M, Boulard T, Nicot PC. (2011) Modelling *Botrytis cinerea* spore exchanges and production in unheated greenhouses. J Plant Pathol 93:407-414.

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

Leyronas C, Duffaud M, Nicot PC. (2012) Compared efficiency of the isolation methods for *Botrytis cinerea*, Mycology: Int J Fungal Biol 4: 221-225.

Ma Z, Michailides TJ. (2005) Genetic structure of *Botrytis cinerea* populations from different host plants in California. Phytopathol 89. 1083-1089.

Martinez F, Dubos B, Fermaud M. (2005) The role of saprotrophy and virulence in the population dynamics of *Botrytis cinerea* in vineyards. Phytopathol 95: 692-700.

Mfegue CV, Virginie MC, Tharrreau D, Michel D. (2012) Origine et mécanismes de dispersion des populations de *Phytophthora Megakarya*, pathogène du cacaoyer du Cameroun. PAF.

Mims SA, Mims FM. (2004) Fungal spores are transported long distances in smoke from biomass fires. Atmos Environ 38: 651-655.

Mirzaei S, Goltapeh EM, Shams-Bakhsh M, Safaie N, Chaichi M. (2009) Genetic and phenotypic diversity among *Botrytis cinerea* isolates in Iran. J Phytopathol 157: 474-482.

Moyano C, Alfonso C, Gallego J, Raposo R, Melgarejo P. (2003) Comparison of RAPD and AFLP marker analysis as a means to study the genetic structure of *Botrytis cinerea* populations. Eur J Plant Pathol 109: 515-522.

Nagarajan S, Singh DV. (1990) Long-distance dispersion of Rust pathogens. Ann Rev Phytopathol 28: 139-153.

Qandah IS, Del Rio Mendoza LE. (2012) Modelling inoculum dispersal and Sclerotinia stem rot gradients in canola fields. Can J Plant Pathol 34: 390-400.

Rice WR. (1989) Analyzing tables of statistical tests. Evolution 43: 223-225.

Scagnelli S, Moretti M, Vercesi A. (2010) Phenotypic and genotypic variability of populations of *Botrytis cinerea* Pers. isolated in northern Italy. Micologia Italiana 39: 57-65.

Staats M. (2007) Botrytis species on flower bulb crops: phylogeny, genetic variation and host specificity. Netherland, Wageningen University, Ph D thesis.

Van Der Vlugt-Bergmans CJB, Brandwagt BF, Vantt Klooster JW, Wagemakers CAM, Van Kan JAL. (1993) Genetic variation and segregation of DNA polymorphisms in *Botrytis cinerea*. Mycol Res 97: 1193-1200.

Viljanen-Rollinson SLH, Parr EL, Marroni MV. (2007) Monitoring long-distance spore dispersal by wind. Rev New Zeal Plant Protect 60: 291-296.

Walker A-S, Gautier AL, Confais J, Martinho D, Viaud M, Le P Cheur P, Dupont J, Fournier E. (2011) *Botrytis pseudocinerea*, a new cryptic species causing gray mold in French vineyards in sympatry with *Botrytis cinerea*. Phytopathol 101: 1433-1445.

Wissels AB. (2012) Genetic characterization and fungicide resistance profiles of *Botrytis cinerea* in rooibos nurseries and pear orchards in the Western Cape of South Africa. Clanwilliam, South Africa, Stellenbosch University, Thesis.

Wright S. (1978) Evolution and the Genetics of Populations: Variability within and among natural populations. USA, University of Chicago Press.

Figure captions:

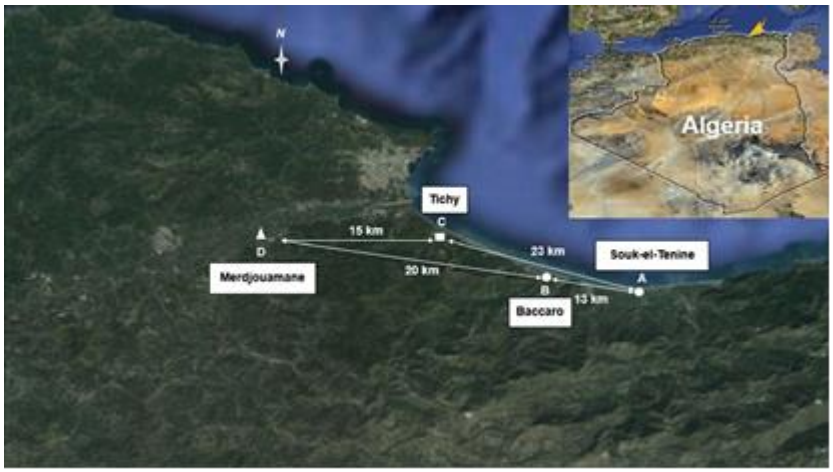

Fig. 1: Location of *Botrytis cinerea* sampling sites in the Bejaia region (North Algeria) (A: Souk-el-Tenine, B: Baccaro, C: Tichy and D: Merdjouamane).

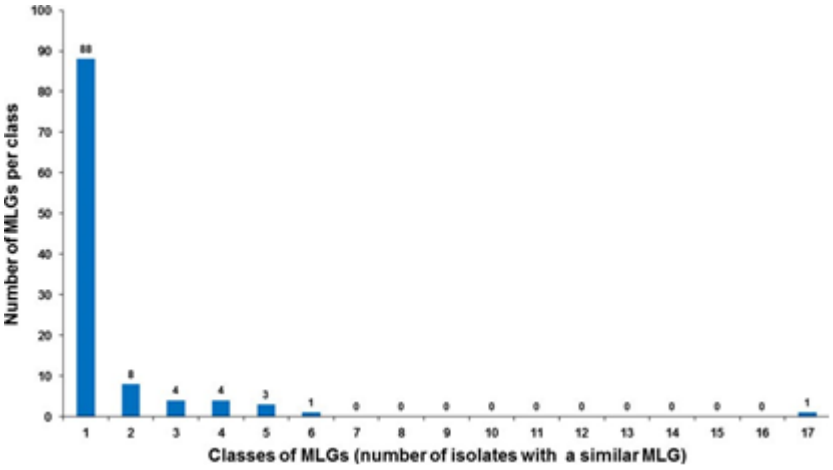

Fig. 2: Frequency distribution of multilocus genotypes (MLGs) among 170 isolates of *B. cinerea* sampled from the Bejaia region.

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

Sampling location	GPS coordinates	Sampling date	Number of genotyped isolates
A: Souk-el-Tenine	Latitude: **36.62958** Longitude: **5.29764**	30 May 2007	23
B: Baccaro	Latitude: **36.64683** Longitude: **5.20958**	30 May 2007 1 June 2008 5 June 2010	21 19 19
C: Tichy	Latitude: **36.6938** Longitude: **5.10217**	30 May 2007 1 June 2008 5 June 2010	05 22 19
D: Merdjouamane	Latitude: **36.67619** Longitude: **4.94483**	1 June 2008 5 June 2010	23 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[a]	Allelic diversity[b]		Haplotypic diversity[c]			Index of multilocus linkage disequilibrium[d]	
			Hnb	number of alleles per locus	D*	ED*	R	r_D	P value
Total sample	170	109	0.57 (0.17)	8.22	0.98	0.90	0.63	0.07	<0.001
2007 samples									
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
all sites	**49**	**35**	**0.54 (0.18)**	**5.33**	**0.97**	**0.71**	**0.71**	**0.12**	**<0.001**
2008 samples									
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
all sites	**64**	**39**	**0.54 (0.14)**	**4.66**	**0.97**	**0.85**	**0.60**	**0.08**	**<0.001**
2010 samples									
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
all sites	**58**	**45**	**0.60 (0.20)**	**6.55**	**0.99**	**0.86**	**0.77**	**0.10**	**<0.001**

[a] MLG: multilocus genotypes.

[b] Hnb: nonbiased genic diversity, calculated with repeated genotypes (standard deviations between brackets),

ND: undefined.

[c] D*: genotypic diversity index; E: equitability index; R: Richness index.

[d] r_D: 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**[a] (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[b]	0,167	0,115	ND[d]	ND
2008	ND	ND	0,095	0,058	-0,019
2010	ND	ND	0,011	0,016	0,050

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

[b] 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.

[c] ND: not determined.

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

[a] 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[a]	D*[b]	r_D[d]	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

[b] D*: genotypic diversity index; E: equitability index; R: Richness index.

[c] r_D: standardized index of association.