Assessing host specialization of Botrytis cinerea on lettuce and tomato by genotypic and phenotypic characterization

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Short title: Host specialization of *B. cinerea*

Informative title: Assessing host specialization of *Botrytis cinerea* on lettuce and tomato by genotypic and phenotypic characterization.

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Abstract

This study tested the hypothesis that *B. cinerea* shows host specialization on tomato and lettuce, using phenotypic and genotypic tools. Strains were isolated from tomato and lettuce grown together in a same greenhouse. Forty-four lettuce strains and forty-two tomato strains were investigated for their genetic diversity and their aggressiveness. Both gene diversity and allelic richness were significantly higher in lettuce strains than in tomato strains (P=0.01). Cluster analysis revealed a clear structure of the strains under study in two clusters. However, this structure did not separate the strains according to their host of origin. Tomato strains were significantly more aggressive than lettuce strains when inoculated on tomatoes (P=0.001). But no significant differences in aggressiveness were observed when the strains were inoculated on lettuce (P=0.17) or on apple (P=0.87). Our results suggest an absence of clear host specialization of *B. cinerea* on tomato and lettuce.

Introduction

Botrytis cinerea is an Ascomycete (teleomorph *Botryotina fuckeliana*) responsible for grey mould on cultivated and wild plants in temperate regions worldwide (Jarvis 1980). Some of the plant species susceptible to *B. cinerea* are of economic importance (lettuce, tomato, grapevine among others). When a crop is attacked by *B. cinerea* its yield may be reduced and products may become unmarketable. This fungus may produce several million spores in a few days on diseased plants when conditions are favourable (Nicot *et al.*, 1996). These spores are easily dispersed by air currents (Jarvis, 1962; Harrison & Lowe, 1987) and may cause rapid development of epidemics (Decognet *et al*., 2009; Bardin *et al*., 2014).

It has long been considered that *B. cinerea* lacks host specificity, as it was reported to attack more than 200 plant species (Jarvis, 1980) and it is also known to develop as a saprophyte on numerous types of substrates (Holtz *et al*., 2004). However, several studies have revealed genetic differentiation among isolates collected from different host plants. In France, Diolez *et al*. (1995) showed that strains of *B. cinerea* containing the transposable element *Boty* were present on grapes and tomatoes but not on lentil. Giraud *et al.* (1999) reported that the frequency of transposable elements *Boty* and *Flipper* was significantly different in populations of *B. cinerea* collected on different host plants. In Chile, Muñoz *et al.* (2002) showed that strains collected from tomato and grapes were genetically differentiated on the basis of PCR-RFLP markers. Using microsatellite markers, Rajaguru & Shaw (2010) and Fournier & Giraud (2008) reported similar situations, respectively between strains sampled from raspberries and blackberries in England, and between strains sampled from grapevine and bramble in France. Genetic differentiation of fungal populations through host shift speciation has been hypothesized to result from several possible mechanisms, including the cessation of gene flow between two populations (Giraud *et al.*, 2010). Thus, former studies reporting genetic

differentiation on different hosts may challenge the initial assumptions and indicate that *B. cinerea* presents a certain degree of host specialization in certain situations.

Comparisons of aggressiveness have also revealed differences between *B. cinerea* isolates depending on their host of origin. For example, strains isolated from tomato were more aggressive on tomato leaves than strains isolated from grapevine (Cotoras & Silva, 2005) and strains collected on grapevine were more aggressive on this plant than strains from other host plants (Derckel *et al*., 1999). Pie & Brouwer (1993) also reported that strains of *B. cinerea* collected on roses had higher aggressiveness on rose petals than strains collected on gerbera flowers or pea leaves. Such differences in aggressiveness may provide a limitation in gene flow among strains of the pathogen present on different hosts, as successive generations of inoculum (possibly numerous in one growing season, depending on the crop) result in the gradual selection of the most aggressive strains (Decognet *et al.*, 2009).

Knowledge of host specialization among strains of *B. cinerea* could be of great importance for disease control as crops considered to be susceptible are often grown simultaneously in close vicinity or successively in rotation schemes. This is often the case in vegetable production. Transfer of inoculum from one crop to the other is likely through dissemination of airborne spores (Jarvis, 1980). Then, in case of a lack of host specialization, airborne inoculum produced on a species, easily released and disseminated by air currents, may induce symptoms on another species located in a close vicinity. Thus, the management of greenhouse vents will have to take into account not only the climate inside the greenhouse but also possible entry of inoculum from the outside. Limiting the exposure of crop to airborne inoculum can be achieved by separating crops in time. But *B. cinerea* produces also survival structures such as sclerotia and mycelium in plant debris (Coley-Smith, 1980). Then, in case of a lack of host specialization, the inoculum left in the soil by one susceptible species may serve as primary inoculum in a following susceptible different species and thus provoke a grey mould outbreak. In such a case,

soil disinfestation would be useful. On the contrary, if *B. cinerea* shows clear host specialization on tomato and lettuce less attention will be required on a possible role as inoculum source for crops grown in close vicinity or in rotation.

The objective of our work was thus to assess host specialization of *B. cinerea* on two vegetable crops, lettuce and tomato, which are usually cultivated in close vicinity or in rotation in southern France and sustain particularly high risks of yield loss from grey mould. To this aim, we used genetic and phenotypic tools to characterize a collection of isolates sampled from diseased lettuce and tomato plants. The strains were also inoculated on a third host plant (apple) to assess if their level of aggressiveness was host-dependant or conversely, consistent across host species.

Materials and methods

Isolate collection

Lettuce (cv Zendria, Rijk Zwaan) and tomato (cv Brenda, Gautier Semences) plants were grown at the Alenya-Roussillon experimental domain of the French National Institute of Agricultural Research (INRA) in southern France (lat. 42.64N; long. 2.98E). In order to minimize geographical and temporal factors that may interfere with the detection of host specialization, both crops were grown in the same greenhouse (Fig. 1) with a two-month overlapping period.

Following the development of grey mould on both crops in the greenhouse, isolates were collected on diseased lettuce and tomato plants. Sample collection consisted of rubbing sterile cotton buds on sporulating lesions. The cotton buds were then stored at -20°C until isolate purification. All isolates used in this study were purified and single spored in a classical way (Leyronas *et al*., 2012) prior to their genotypic and phenotypic characterization. Hereafter, characterized single spore isolates will be referred to as ‘strains’. Those originating from tomato

will be referred to as “tomato strains” while those from lettuce will be referred to as ‘lettuce strains’.

Isolate genotyping

Genomic DNA was extracted from aliquots of 15 mg lyophilized fungal material (harvested from two-week old cultures on Potato Dextrose Agar) in 96-well plates, following the Dneasy Plant extraction Kit protocole (Qiagen). The nine microsatellite markers designed for *B. cinerea* by Fournier *et al.* (2002) were amplified with forward primers conjugated with the following fluorescent dyes: Fam for BC1, BC4, BC9 and BC10; Hex for BC2 and BC6; Tamra for BC3, BC5 and BC7 (MWG). Reverse primers did not carry any fluorescent dye. Primers were multiplexed for amplification of several microsatellites in a single PCR as follows. Four pairs of markers were amplified together at the following hybridization temperatures: BC1 and BC9 at 50°C; BC2 and BC5 at 53°C; BC3 and BC6 at 50°C; BC4 and BC10 at 59°C. Marker BC7 was amplified singly at 59°C. To determine the size of the microsatellites, the PCR products were diluted and multiplexed prior to scanning with the help of a Megabace sequencer (Amersham Pharmacia). The multiplexing consisted of mixing in a same well the PCR products of several markers (either BC1, BC2, BC5 and BC9 or BC3, BC6, BC4, BC7 and BC10). In each well, ET-400 labelled with Rox dye (Amersham Pharmacia) was used as a size marker. Genetic Profiler software (Amersham Biosciences) was then used for the microsatellite size analysis.

Complete microsatellite size profiles (referred to as "haplotypes" hereafter) were obtained for 44 lettuce strains and 42 tomato strains.

Genetic diversity of tomato and lettuce strains

Unbiased gene diversity (Hnb) and allelic richness were computed separately for the tomato and for the lettuce strains with the Genetix software (Nei, 1978). The software FSTAT version 2.9.3 (Goudet, 1995) was used to compute allelic richness per locus corrected for minimum sample size of 16 isolates. Several other diversity indices (all taking values between 0 and 1; 1 indicating the maximum level of diversity) were also computed with GenClone 1.0 software (Arnaud-Haond & Belkhir, 2007) as follows. We used the Simpson diversity index (D*), which measures the probability that two randomly selected individuals in a population have different genotypes. We also used the evenness index, whose value tends to 1 when genotypes have a similar abundance (equitable distribution of clones) (Arnaud-Haond *et al*., 2007). The number of different multilocus haplotype (MLH) was also computed with GenClone. Finally, we used the index of haplotypic diversity (based on the number of individuals and the number of distinct MLH), which estimates the proportion of haplotypes present in a population and takes a value of 1 when a population is composed exclusively of unique haplotypes (Arnaud-Haond *et al*., 2007).

Linkage disequilibrium

The existence of linkage disequilibrium among the lettuce strains and among the tomato strains was estimated by computing the r_d index with Multilocus software version 1.3 (Agapow & Burt, 2001). The null hypothesis of complete panmixia ($r_d = 0$; absence of clonality) was tested by permuting alleles (microsatellite sizes) among strains, independently for each microsatellite marker (1000 permutations).

Genetic structure

To test the hypothesis of host specialization in *B. cinerea*, we used the discriminant analysis of principal components (DAPC) implemented in the R package adegenet (Jombart, 2008). In this

analysis, we fixed the number of clusters to two, with one cluster including all strains from tomato and the other one including all strains sampled from lettuce. We used the optimisation procedure implemented in adegenet to select the optimal number of principal components to be retained in the analysis (Jombart *et al*., 2010). In a second step, the DAPC was also performed without any *a priori* on the genetic structure. In this case, we first identified the optimal number of clusters in our data set, using the k mean clustering approach (function 'find.clusters' in adegenet). The best clustering solution was determined, using Bayesian Information Criterion (BIC). The distribution of tomato and lettuce strains among clusters and their probability of assignation were analysed. A strain was considered as accurately assigned to a cluster when its membership probability was greater than 0.8. The level of genetic differentiation between the identified clusters was assessed by computing Weir & Cockerham's F_{ST} values with the software Arlequin version 3.5 (Excoffier *et al.*, 2005).

Finally, to better understand the relationships between the different strains, we computed a neighbor-joining (NJ) tree with the program POPULATIONS (version 1.2.32 provided by Olivier Langella, UMR de génétique végétale, Gif-sur-Yvette, France). The NJ-tree was based on the distances of Cavalli-Sforza & Edwards (1967), computed from our microsatellite loci. The tree was visualized and edited under the program TREEVIEW (Page, 1996). All analyses were conducted on data sets excluding clone replicates.

Evaluation of aggressiveness on tomato

The aggressiveness of tomato strains and lettuce strains was compared on 8-week old tomato plants cv. Swanson (De Ruiter Seeds). Plants were grown in a greenhouse in individual pots containing a horticultural mix (De Baat) and watered daily with a nutrient solution as described by Decognet *et al*. (2009). Each strain was inoculated on three plants. On each plant, three leaves were removed, leaving 1cm petiole stubs on the stems and the wounds were inoculated

with 10 µL aliquots of spore suspension. The spore suspensions were prepared from two-week old cultures on Potato Dextrose Agar (PDA) and were adjusted to 10^6 spores mL^{-1}. All plants were incubated in a growth chamber with a photoperiod of 14 h, with a light intensity of 162 µmol. m^{-2}. s^{-1}, maintained at 21°C with a relative humidity above 90%. The length of resulting stem lesions was monitored daily from the 3rd to the 7th day after inoculation and these data were used to compute the area under the disease progression curves (AUDPC). Based on prior work, strain BC1 was used as a reference for aggressiveness (Decognet *et al*., 2009). For each strain, an index of aggressiveness (IA) was calculated, relative to that of strain BC1, as IA = 100* ($AUDPC_{strain}$ / $AUDPC_{BC1}$). The test was conducted twice independently.

Evaluation of aggressiveness on lettuce leaves

The aggressiveness of the strains was also compared on detached lettuce leaves (cv Mantilia). Leaves were taken from 8-week old lettuce plants and placed in clear polystyrene boxes over water-saturated filter paper in order to keep high humidity. Each leaf was inoculated in its centre with a mycelial plug (diameter 5 mm) excised from a 3-day old colony on PDA. Care was taken to position the mycelium in direct contact with the plant tissue. The inoculated leaves were incubated in a growth chamber at 21°C with a light intensity of 162 µmol. m^{-2}. s^{-1} and a photoperiod of 10h. The leaves were photographed at 48, 72 and 96 hours after inoculation and the surface of lesions was measured with image analysis software Assess 2.0 (APS Press). The AUDPC and IA were calculated as described above. The test was conducted twice independently and nine leaves were inoculated for each strain in a given test.

Evaluation of aggressiveness on apple

In order to test a third host, the aggressiveness of both tomato and lettuce strains was investigated on apples (cv Golden delicious) purchased from an organic farm. Apple was

198 selected because *B. cinerea* can induce economic losses in postharvest storage. Wounds (2 mm
199 in diameter and 10 mm deep) were made with the help of a sterile pipet tip on the equator of
200 the fruits. Each apple received two wounds on opposite sides. Each wound was inoculated with
201 10 µL of a spore suspension (10^6 spores.mL^{-1}) prepared as described above. The fruit were then
202 incubated at 21°C and lesion diameter was measured from the 3rd to the 7th day after inoculation.
203 The AUDPC and IA were calculated as described above. The test was conducted twice
204 independently and three apples (6 wounds) were inoculated for each strain in a given test.
205
206 *Statistical analyses*
207 Statistical analyses were performed with StatView (version 5, SAS Institute). Non-parametric
208 Mann-Whitney tests were used to determine significant differences between gene diversity,
209 allelic richness, aggressiveness of tomato and lettuce strains. Correlation between
210 aggressiveness on tomato and on lettuce was estimated using the non-parametric Spearman's
211 rank correlation coefficient (Rho). Statistical inferences were made at the 5 % level of
212 significance, unless indicated otherwise.
213
214 **Results**
215 *Genetic diversity*
216 Eighty-six isolates sampled from tomato and lettuce were genotyped through amplification of
217 9 microsatellites. None of these carried the private allele at microsatellite locus BC6 associated
218 with the cryptic species *B. pseudocinerea* (Walker *et al.*, 2011). Thus the 86 strains (44
219 collected on lettuce and 42 collected on tomato) were considered to belong to *B. cinerea* and
220 were kept for further genetic analyses.
221 The lettuce strains showed higher genetic diversity than tomato strains when considering the
222 unbiased gene diversity and the allelic richness (Table 1). The number of alleles in each

223 microsatellite locus and the gene diversity per locus were significantly higher in lettuce strains
224 than in tomato strains (respectively $P_{Mann\text{-}Whitney}$=0.0089, $P_{Mann\text{-}Whitney}$ =0.0149) (Table 2). Some
225 alleles were not shared between tomato and lettuce strains. Among a total of 89 alleles observed
226 within the 9 microsatellites, 5 alleles were only present in tomato strains and 46 alleles were
227 only present in lettuce strains.
228 Differences between tomato and lettuce strains were also observed when examining haplotypic
229 diversity. Forty-three different multilocus haplotypes (MLH) were found among the 86 strains,
230 of which four were shared by tomato and lettuce strains (Table 1). These MLHs appeared in 2,
231 4, 12 and 14 copies among the 86 isolates. The haplotypic diversity of lettuce strains was much
232 higher than that of tomato strains (Table 1). Haplotypic diversity was also assessed with
233 Simpson's index (D). This index was overall high for the two groups, although slightly higher
234 for lettuce strains than for tomato strains (Table 1). Evenness values (ED) reflected a more
235 equitable distribution of haplotypes within tomato strains. Linkage disequilibrium was equally
236 low and highly significant in both tomato and lettuce strains thus rejecting the null hypothesis
237 of random mating in both groups of strains (Table 1).
238
239 *Genetic structure*
240 A first DAPC was performed by fixing the number of clusters (K=2) to test the hypothesis that
241 the genetic structure of *B. cinerea* strains corresponded to their host of origin. The scatter plot
242 of the analysis showed large overlap between tomato and lettuce strains (Fig. 2a). Moreover, a
243 low percentage of strains were clearly assigned to a given cluster (data not shown): only 51%
244 of lettuce and 25% of tomato strains had a membership probability above 0.8. This result is
245 supported by the distribution of the strains in the NJ tree (Fig. 3). Although lettuce strains were
246 more abundant than tomato strains in certain branches, all strains were widely intermixed in the
247 tree regardless to their host of origin.

A second DAPC was performed to investigate the genetic structure of lettuce and tomato strains without any *a priori* hypothesis about the factors shaping the structure. The BIC showed a rapid decrease and a sharp elbow at K=2 (see supplementary material). The resulting structure visualized with the scatter plot showed almost no overlap between tomato and lettuce strains (Fig. 2b). Moreover 97% and 100% of the lettuce and tomato strains, respectively, were assigned to a cluster with a posterior probability greater than 0.8. The first cluster included 42% and 82% of the lettuce and tomato strains, respectively. The second cluster included 55% and 18% of the lettuce and tomato strains, respectively (3% of lettuce strains were not clearly assigned to a cluster P<0.8). These two clusters were significantly differentiated (P<0.001) with an F_{ST} value of 0.252.

Aggressiveness

All 72 strains were able to infect and cause symptoms on the three plant species tested. For tests on apple, there was no significant difference (P=0.87) between the average aggressiveness of the 33 lettuce (IA=89.9 ± 22) and the 39 tomato strains (IA=89.6 ± 18). When inoculated on lettuce, there was no significant difference (P=0.17) between the average aggressiveness of the lettuce strains (IA=81.6 ±4.9) and the tomato strains (IA=72.8 ± 5.1) (Fig. 4a). In contrast, when inoculated on tomato, tomato strains were significantly more aggressive (P = 0.0012) than lettuce strains (Fig. 4b), with mean IA values of 70.7 (±3.5) and 52.8 (±3.8) respectively.

There was no significant correlation between aggressiveness on tomato and on lettuce ($Rho_{Spearman}$ = 0.033; P=0.78). Strains with the highest level of aggressiveness on one species were not highly aggressive on the other. However, regardless of their host of origin, strains with low aggressiveness on one host tended to have also low aggressiveness on the other plants. Among the 16 strains least aggressive on tomato (IA<40), seven (4 tomato strains and 3 lettuce strains) were also among the 13 least aggressive on lettuce (IA<50). Furthermore, among the

14 strains with the lowest aggressiveness on apple, 10 were also among the least aggressive on tomato and/or on lettuce.

Discussion

This study is the first attempt to address a possible host specialization of *B. cinerea* on tomato and lettuce by genotypic and phenotypic characterization. In addition, it provides the first data on genetic diversity of *B. cinerea* strains collected on lettuce. Based on the examination of 44 lettuce strains and 42 tomato strains, our results suggest an absence of clear host specialization of *B. cinerea* on tomato and lettuce.

The genetic diversity indices of lettuce strains were compared to those reported for populations collected from other crops grown under greenhouses (Table 3). The gene diversity (Hnb and mean number of alleles per locus) of lettuce strains was higher than that of strains from all greenhouse crops (Karnachi-Balma *et al*., 2008). In contrast, the haplotypic diversity of lettuce strains was higher than that of strains from tomato but lower than that of strains from strawberry and grapevine (Karnachi-Balma *et al*., 2008). When comparing to strains from crops grown in open field, all genetic diversity indices for lettuce strains were in the mean range of values (Table 3). All diversity indices for tomato strains in our study (unbiased gene diversity, mean number of allele per locus, haplotypic diversity and Simpson's index of diversity) were systematically lower than those of lettuce strains. They were comparable to those reported for tomato strains in Tunisia by Karchani-Balma *et al.* (2008). As the tomato and lettuce strains of our study were collected from the same greenhouse, an explanation for the differences in diversity between the two groups could come from differences in the epidemiology of the disease on the two crops. On tomato plants, *B. cinerea* sporulates on leaves, stems or fruits and the spores are easily disseminated by air currents and by the frequent technical interventions necessitated by this crop. As this often coincides with favourable microclimatic conditions for

disease development, this allows for successive cycles of inoculum production to occur in the greenhouse (Dik & Wubben, 2004; Decognet *et al.*, 2009; Bardin *et al*., 2014). Indeed, in our experimental greenhouse, sporulating lesions were observed on aerial parts of about 11% of tomato plants during the growing period. Considering that *B. cinerea* can produce spores within one week (Nicot *et al*., 1996), during the two-month overlapping period with tomato and lettuce grown in the same tunnel, 8 cycles of spore production may have occurred on tomato. This in turn may have fostered a gradual selection of strains with the highest fitness on tomato. Decognet *et al.* (2009) showed that two strains of *B. cinerea* became rapidly predominant after their introduction in four experimental tomato greenhouses. On lettuce plants in contrast, the disease mostly develops on the underside of older leaves which are in contact with the soil, and cropping practices do not include frequent manipulation of the plants. As a consequence, sporulation occurs in a confined environment (between the soil and the oldest leaves covering it) and secondary inoculum is essentially released and disseminated when the lettuces are harvested. This could explain the lower haplotypic diversity of tomato strains compared to lettuce strains in our study, with 52% of tomato strains present in at least two copies compared to only 30% of lettuce strains.

This situation is also compatible with the results of the genetic structure analysis with clone-corrected data. We found a clear genetic structure in two clusters, but this structure did not separate the strains according to their host of origin. However, lettuce strains were distributed quite equitably among the two clusters (55% and 42% - together with 3% that were not assigned) whereas tomato strains were mainly assigned to one cluster (18% and 82%). It may reflect the occurrence of two types of primary inoculum in the greenhouse, from two different origins. Both lettuce and tomato plants are likely to have been exposed to airborne inoculum entering from the outside of the greenhouse. Exchange of air between the inside and the outside of a greenhouse can occur regularly through vents (Leyronas *et al*., 2011) and Bardin *et al.*

(2014) have recently shown that the contribution of external inoculum was non-negligible. In addition to airborne inoculum, the plants may have been confronted to soilborne inoculum, with a greater impact on lettuce whose foliage quickly covers the soil. Further studies will be needed to assess the relative impact of soilborne and airborne primary inoculum in grey mould epidemics on tomato and lettuce crops.

Taken together, our results support the hypothesis of a lack of specialization for tomato or lettuce among the strains of *B. cinerea* examined in our study. Similar observations have been reported for strains collected from grape, kiwifruit, pea and squash in California (Ma & Michailides, 2005) and from strawberry and faba beans in Tunisia (Karnachi-Balma *et al.*, 2008). The polytrophic behaviour of the lettuce and tomato strains of our study was also supported by their systematic ability to produce symptoms on the three plant species tested and by the absence of significant difference in the aggressiveness of the two groups on apple and lettuce. The significant difference observed when strains were inoculated on tomato could be attributed, as hypothesized above, to a progressive selection on tomato of the strains with the best fitness on that plant. This hypothesis could be tested in further experiments by combining genotypic and phenotypic characterization of sequentially sampled strains of *B. cinerea* during a growing season.

In conclusion, our results showed an ability of all examined *B. cinerea* strains to attack both tomato and lettuce and point to a lack of clear host specialization. This implies that the inoculum produced on one host species would be able to trigger a grey mould epidemic on the other species. In rotations implicating tomato and lettuce, caution should be used about possible carry-over of inoculum, as *B. cinerea* can survive on plant debris or in soil as sclerotia (Coley-Smith, 1980). Moreover, possible exchange of airborne inoculum between neighbouring plots (Jarvis, 1962; Harrison & Lowe, 1987), including in greenhouse systems (Leyronas *et al.*, 2011) need to be considered when planning the arrangement of crops on the farm.

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Table 1: Genetic diversity of *B. cinerea* strains collected on lettuce and tomato.

	Sample size	Hnb [a,f]	Allelic richness	No. of distinct MLH [b]	Haplotypic diversity	D* [c]	Evenness	r_d [d,f]
Total	**86**	**0.74 (0.11)**	**9.8**	**43**	**0.49**	**0.947**	**0.835**	**0.15 HS [e]**
Lettuce	44	0.77 (0.12)	9.2	31	0.69	0.969	0.758	0.13 HS
Tomato	42	0.62 (0.12)	4.7	16	0.37	0.912	0.867	0.13 HS

a : unbiased gene diversity (standard deviation between brackets)

b : multilocus haplotype

c : Diversity Simpson's index

d : linkage disequilibrium

e : highly significant at the 5% level

f: computed with clone-corrected data (data set with only one example of each haplotype) to remove bias of overrepresentation of clones.

Table 2: Allelic richness based on minimum sample size (AR) and unbiased gene diversity (Hnb) per microsatellite locus in lettuce and tomato strains of *B. cinerea*

Host of origin	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
Lettuce	9.7	0.84	10.88	0.89	7.6	0.77	2.7	0.50	8.9	0.83	11.4	0.81	8.2	0.79	5.3	0.61	8.3	0.84
Tomato	6	0.71	6	0.74	4	0.65	2	0.51	4	0.42	5	0.51	4	0.69	5	0.51	6	0.75

Table 3: Genetic diversity reported for *B. cinerea* strains collected from crops grown under greenhouse and in open field. In all studies, strains were genotyped using the microsatellites described by Fournier *et al*. (2002)

Plant host	Growing system	Location	Gene diversity	Mean number of allele/locus	Haplotypic diversity[a]	Reference
lettuce	***greenhouse***	***France (South West)***	***0.77***	***9.20***	***0.69***	***present study***
tomato	***greenhouse***	***France (South West)***	***0.62***	***4.70***	***0.37***	***present study***
tomato	greenhouse	Tunisia (Cap Bon+North)	0.62	3.89	0.48	Karnachi-Balma *et al.*, 2008
strawberry	greenhouse	Tunisia (Cap Bon)	0.70	7.11	0.87	Karnachi-Balma *et al.*, 2008
grapevine	greenhouse	Tunisia (Grand Tunis)	0.70	5.89	0.90	Karnachi-Balma *et al.*, 2008
grapevine	open field	France (Bourgogne)	0.51	3.62	1	Fournier & Giraud, 2008
chickpea	open field	Bangladesh	0.54	na	0.47	Isenegger *et al*., 2008
grapevine	open field	France (Anjou)	0.59	4.87	0.97	Fournier & Giraud, 2008
grapevine	open field	Tunisia (North)	0.60	3.78	0.75	Karnachi-Balma *et al.*, 2008
grapevine	open field	France (Champagne)	0.64	4.75	1	Fournier & Giraud, 2008
grapevine	open field	France (Alsace)	0.64	5	1	Fournier & Giraud, 2008
rooibos	open field	South Africa	0.67	5.6	0.48	Wessels *et al*., 2013

grapevine	open field	France (Bordelais)	0.69	5.75	1	Fournier & Giraud, 2008
faba bean	open field	Tunisia	0.75	6.33	1	Karnachi-Balma *et al.* 2008
strawberry	open field	Tunisia (Cap Bon)	0.80	8.33	0.95	Karnachi-Balma *et al.* 2008
raspberry and strawberry	open field	Hungary	0.82	na	0.37	Asadollahi *et al.,* 2013
strawberry	open field	England	0.86	17.10	na	Rajaguru & Shaw, 2010
primrose	open field	England	0.87	9.9	na	Rajaguru & Shaw, 2010
blackberry	open field	England	0.88	20.80	na	Rajaguru & Shaw, 2010
dandelion	open field	England	0.88	13.8	na	Rajaguru & Shaw, 2010

a: haplotypic diversity index directly available or computed (on the basis of reported information) as the ratio of (number of distinct MLG - 1) over (sample size - 1)

na : data not available

Figure 1: Experimental set up combining a lettuce crop (on each side of a greenhouse) grown together with a tomato crop (in the middle).

Figure 2: Scatter plots from Discriminant Analysis in Principal Components. A: K value *a priori* fixed to 2; B: no *a priori* on the value of K.

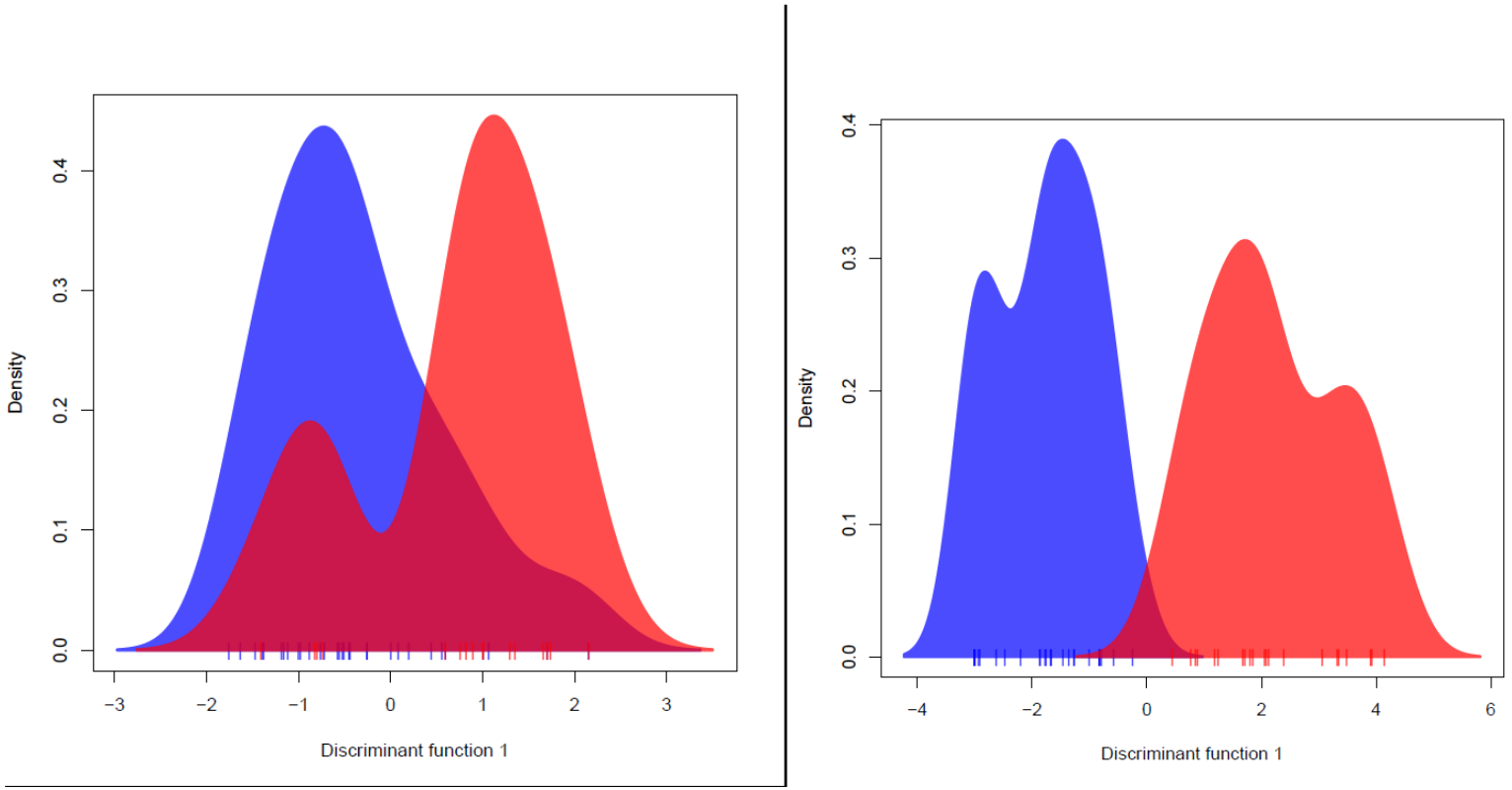

Figure 3: Neighbor-joining tree (based on the distance of Cavalli-Sforza & Edwards (1967)) showing the genetic relationships among 31 strains of *Botrytis cinerea* collected from lettuce and 16 strains collected from tomato. The analysis was done on the set of data corrected for clonality. Support for the nodes was assessed by bootstrapping loci 1500 times. Red boxes identify tomato strains. Circles identify haplotypes common to lettuce strains and tomato strains.

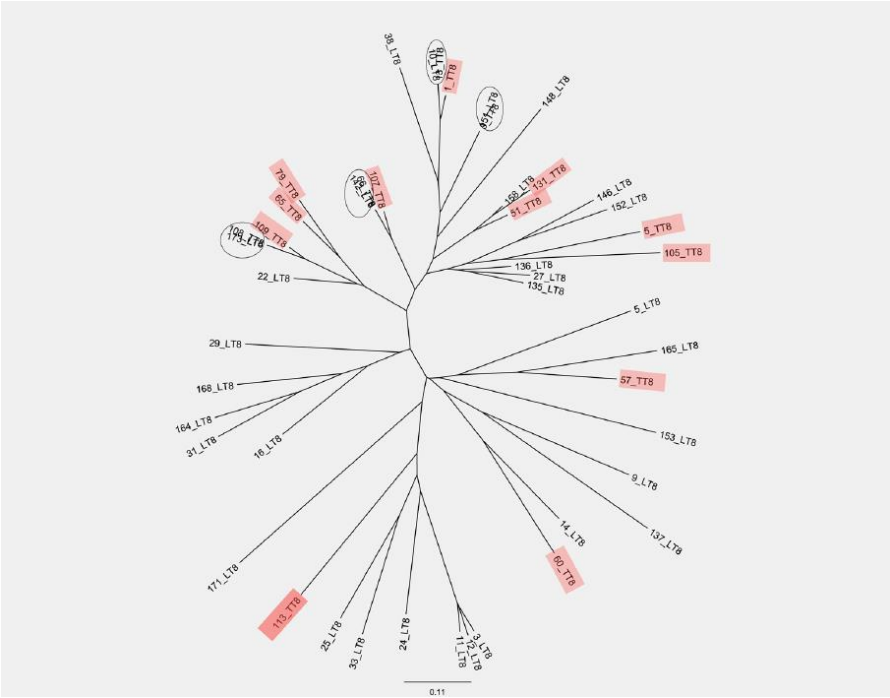

Figure 4: A: Aggressiveness on lettuce of lettuce strains (green bars) and tomato strains (red bars) of *B. cinerea*. B: Aggressiveness on tomato of lettuce strains and tomato strains of *B. cinerea*.

Error bars indicate the standard deviation of the mean.