Assessing the phenotypic and genotypic diversity of Sclerotinia sclerotiorum in France

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Abstract

White mould caused by the ascomycete *Sclerotinia sclerotiorum* affects the production of many economically important crops. The incidence of this disease has recently increased in France, especially in melon crops, which were not affected much in the past. One possible explanation for this situation is the emergence of strains with particular characteristics, including increased aggressiveness to melon. To test this hypothesis, 200 isolates of *S. sclerotiorum* were collected from six host crops (bean, brassica oilseed rape, carrot, lettuce, melon, witloof chicory) in different regions. They were genotyped with 16 microsatellites markers. A subsample of 96 isolates were assessed for their aggressiveness on melon leaves. Overall, the isolates from melon did not show higher aggressiveness on melon leaves than those which originated from other host plants. Moreover, the melon isolates did not present distinctive genetic characteristics in comparison with those from other crops and shared several of the 128 identified multilocus haplotypes with isolates collected from carrot, witloof chicory and oilseed rape. Furthermore the Bayesian analysis of the genetic structure indicated that the host plant is not a structuring factor of the three genetic clusters identified, and it suggested instead the occurrence of an isolation-by-distance process. Possible consequences of these results for the management of white mould and alternative hypotheses to explain the recent changes in disease incidence are presented.

Introduction

Sclerotinia sclerotiorum (Lib) de Bary causes annual crop losses up to several millions of dollars worldwide (Petrofeza and Nasser 2012). This plant pathogenic ascomycete can attack

more than 400 plant species (Purdy 1979; Boland and Hall 1994), including some with economic importance such as oilseed and protein crops and vegetables. In many crops, disease management relies largely on fungicide applications that are guided by risk-prediction models (Clarkson et al. 2014; Lehner et al. 2017), but biological control and preventive cultural methods also play an important role (Barriere et al 2014; Derbyshire and Denton-Giles 2016; Elsheshtawi et al 2017; Kamal et al. 2016; Nicot et al. 2016). Such cultural methods include crop rotation or the spatial arrangement of plots with host crops and non-host crops. However, designing strategies of rotation or landscape use is complicated by the wide host range of the pathogen and the long survival of sclerotia in the soil (Derbyshire and Denton-Giles 2016). Sclerotia can be abundant in the soil and lead either to direct plant infection through myceliogenic germination or to production of ascospores through carpogenic germination (Steadman 1983). Ascospores can be dispersed by wind and thus disseminate the pathogen within and among fields (Kora et al. 2003).

In recent years, French fruit and vegetable growers have been confronted to an increase in white mould incidence, particularly in melon crops which were largely spared in the past. The reasons for this change are not known. As a possible explanation, we hypothesised that isolates with increased aggressiveness on melon may have become prevalent in the production area. Little is known on possible differences in aggressiveness to various host crops among populations of *S. sclerotiorum*, but significant levels of diversity were reported among 120 isolates from Australia examined for their aggressiveness to sunflower (Ekins et al. 2007). In *Botrytis cinerea*, a taxonomically related species also known for its wide host range (Elad et al. 2016), some level of adaptation to the host plant has been suggested, with a clear genetic differentiation according to the host plant of origin among 3546 isolates collected in France from three plant species (Walker et al. 2015a). While no information is available on French populations of *S. sclerotiorum*, some cases of genetic differentiation, and even possibly divergence into separate

76   populations, have been reported in several studies (Aldrich-Wolfe et al. 2015; Clarkson et al.

77   2013; Malvárez et al. 2007).

78   The objectives of the present study were to test the hypothesis that French isolates of *S.*

79   *sclerotiorum* found on melon may be phenotypically and genetically different from those

80   isolated from other crops. For this, we examined a large collection of *S. sclerotiorum* isolates

81   sampled from different host crops, some of which are traditionally cultivated in close vicinity

82   or in rotation with melon.

83

84   **Materials and methods**

85   Isolate collection

86   Two hundred isolates were collected as sclerotia present on diseased plants in crops of carrot

87   (*Daucus carota* L.), melon (*Cucumis melo* L.), bean (*Phaseolus vulgaris* L.), oilseed rape

88   (*Brassica napus* L.), witloof chicory (*Cichorium intybus* var. *foliosum* L.) and lettuce (*Lactuca*

89   *sativa* L.) from different regions of France (Fig. 1). The sclerotia were surface-disinfested with

90   bleach (5%) and rinsed in sterile water. They were then plated on Potato Dextrose Agar (PDA)

91   and incubated for two days at 21°C. A mycelial plug carrying a single piece of hyphal tip was

92   excised from the growing margin of each colony and transferred to fresh PDA medium, as

93   described for the characterization of individual isolates in population genetic studies (Lehner et

94   al. 2016). After four days of incubation, the mycelium was collected from the surface of the

95   agar medium and stored at -20°C before DNA extraction.

96

97   Characterization of the aggressiveness of isolates on melon

98   The aggressiveness of a subsample of 96 isolates of *S. sclerotiorum* was assessed on detached

99   leaves of melon in controlled conditions. These isolates were chosen among the 200 isolates

100   collected on diseased plants, based on their host of isolation (21 isolates from green bean, 11

from oilseed rape, 25 from carrot, 28 from melon and 11 from witloof chicory) and their suitability to represent different haplotypes characterized as described below. The melon cultivar Chubaka (Nunhems Bayer CropScience, France) was chosen because of its susceptibility to *S. sclerotiorum*. Seeds were sowed in compost (Klasmann TS3, Germany). Plants were produced in a heated greenhouse and watered daily with a nutrient solution (Soluveg Essentiel, Angibaud Derome & Spécialités, France). The potted plants were used at the 3–4 leaf stage after 4 weeks of culture. Two leaves per plant were detached and placed in clear polystyrene boxes (250 mm × 178 mm) on moistened filter paper. Mycelial plugs (5 mm in diameter) of each *S. sclerotiorum* isolate were excised from the growing margin of two day-old PDA cultures and deposited onto the melon leaves. Each *S. sclerotiorum* isolate was inoculated on 10 leaves (5 plastic boxes). The plastic boxes were then incubated in a growth chamber at 21°C and a photoperiod of 14 hours for 48 hours. The leaves were then photographed and lesion areas were assessed with image analysis software (Image J, US National Institutes of Health, Bethesda, USA). To compare the aggressiveness of *S. sclerotiorum* isolates, these figures were used to compute an aggressiveness index, expressed as a percentage relative to the size of lesions for a reference isolate (SS44, isolated from melon).

Isolate genotyping

Genomic DNA was extracted in 96-well plates from aliquots of 100 mg (fresh weight) of frozen fungal material, following the Dneasy Plant extraction Kit protocol (Qiagen). Sixteen microsatellite markers designed for *S. sclerotiorum* by Sirjusingh and Kohn (2001) were amplified with forward primers conjugated with the following fluorescent dyes: FAM for loci 5-3, 7-2, 7-3, 8-3, 12-2, 13-2, 17-3, 36-4, 42-4, 55-4, 92-4, 106-4 and HEX for loci 5-2, 9-2, 110-4 and 114-4 (MWG). Reverse primers did not carry any fluorescent dye. All markers were amplified at a hybridization temperature of 55°C, except 8-3, 42-4, 55-4 and 92-4 which were

126 amplified at 60°C. To determine the size of the microsatellites, the PCR products were diluted

127 and multiplexed prior to scanning with the help of an ABI 3730 sequencer (Applied

128 Biosystems). The multiplexing consisted of mixing in a same well the PCR products of several

129 markers. The markers were separated in three mixes: one for markers 7-2, 12-2, 13-2, 36-4,

130 106-4 and 114-4, a second one for markers 7-3, 8-3, 9-2, 17-3 and 42-4 and a third one for 5-2,

131 5-3, 55-4 92-4, and 110-4. In each well, 600LIZ was used as a standard size marker.

132 GeneMapper software version 4.1 (Applied Biosystems) was then used for the microsatellite

133 size analysis. Complete microsatellite size profiles (referred to as "haplotypes" hereafter) were

134 obtained for all isolates.

135

136 Genetic diversity of *S. sclerotiorum* isolates

137 Unbiased gene diversity (Hnb) and unbiased allelic richness were computed separately for the

138 isolates collected on every plant species with the Genetix software (Belkhir 1996-2004). The

139 number of different multilocus haplotypes (MLH) was computed with GenClone 1.0 software

140 (Arnaud-Haond and Belkhir 2007). We used the index of haplotypic diversity (based on the

141 number of individuals and the number of distinct MLH), which estimates the proportion of

142 haplotypes present in a population and takes a value of 1 when a population is composed

143 exclusively of unique haplotypes (Arnaud-Haond et al. 2007).

144 To assess the possible exchange of inoculum between crops often cultivated in rotation or in

145 close vicinity, a separate analysis was carried out on a subsample of 124 isolates (40 from melon

146 and 84 from carrot) collected between October 2014 and January 2016 in two adjacent districts

147 (Gironde and Charente-Maritime) in southwestern France (Fig. 1). The software FSTAT

148 version 2.9.3 (Goudet 1995) was used to compute allelic richness per locus corrected for a

149 minimum sample size of 40 isolates.

150

Genetic structure

To investigate the genetic structure of *S. sclerotiorum* isolates we used the discriminant analysis of principal components (DAPC) implemented in the R package adegenet (Jombart 2008; Jombart et al. 2010). DAPC requires prior genetic groups that can be either determined by users or defined without any a priori on sample assignments using a k-means clustering approach. To avoid possible bias, only one representative was kept for isolates which presented an identical haplotype and were collected from a same plot, resulting in a group of 141 isolates included in the analysis. First, we tested for a potential host specialization of *S. sclerotiorum* by assigning the isolates to six distinct clusters corresponding to the host species from which they have been sampled. As *S. sclerotiorum* may be structured by other factors than the host plant, such as geography, we also performed a DAPC without any assumption 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 implemented in adegenet. The best clustering solution was determined, using Bayesian Information Criterion (BIC). We used the optimisation procedure implemented in adegenet to select the optimal number of principal components to be retained in the DAPC analyses (Jombart et al., 2010). The distribution of isolates among clusters and their probability of assignation were analysed. An isolate 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 FST values using the software Arlequin version 3.5 (Excoffier et al. 2005). To further investigate the genetic structure resulting from the assumption-free DAPC analysis, a multivariate analysis was conducted using the dudi.hillsmith function from the R package "ade4". The analysis was performed by considering for each isolate: the cluster number as a factor and the year of sampling as well as the geographic coordinates as continuous variables to consider both temporal and spatial genetic structure. Finally, a test for isolation-by-distance was conducted

176 using the R package "genepop" (Rousset 2008). The analysis was performed using the a-like

177 statistics as pairwise genetic distance, the logarithm of the geographic distance, a minimal

178 distance of 10 meters between isolates to exclude pairs sampled within the same field and 10000

179 permutations for the Mantel test between genetic and geographic distance matrix. As the

180 sampling size varied among the plots with quite an over representation of south-western France,

181 due to two highly sampled localities, we also performed the clustering, multivariate and

182 isolation-by-distance analyses on 10 subsampled datasets. These 10 datasets included only one

183 isolate from each plot. Isolates from the most represented plots were different among the 10

184 sub-samplings.

185

186 Statistical analyses

187 Statistical analyses were performed with Statistica (version 12, Statsoft). Non-parametric tests

188 were used to determine significant differences between gene diversity and allelic richness, of

189 carrot and melon isolates (Mann and Whitney). As normality test indicates that aggressiveness

190 indices of *S. sclerotiorum* isolates are not normally distributed (Lilliefors normality test, $P <$

191 0.0001), we carried out a non-parametric analysis of variance (Kruskal-Wallis test) to evaluate

192 an isolate effect. In order to test the significance of the association between the level of

193 aggressiveness of isolates and their plant of origin, a non-parametric analysis of variance

194 (Kruskal-Wallis test) was realized. Data are reported in the text as mean values ± standard error

195 of the mean. Statistical inferences were made at the 5 % level of significance, unless indicated

196 otherwise.

197

198

199 **Results**

200 Aggressiveness on melon

201 All tested isolates of *S. sclerotiorum* were able to cause symptoms on detached leaves of melon,

202 regardless of their host of origin. However, they displayed a wide range of aggressiveness (Fig

203 2), with significant differences among isolates ($P_{Kruskal-Wallis} < 0.05$). When grouped according

204 to their host of origin, the relative aggressiveness of isolates from melon was not higher than

205 that of others (Fig 3) and no significant differences were found between groups ($P_{Kruskal-Wallis} =$

206 0.19).

207

208 Overall genetic diversity of *S. sclerotiorum* isolates

209 Among the 16 markers used to characterize the 200 isolates, one (locus 36-4) was

210 monomorphic, showing a single 411 pb allele, while all others were polymorphic with a number

211 of alleles ranging from 3 (for locus 12-2) to 24 (for locus 106-4) (Table 1). A total of 128

212 different multilocus haplotypes (MLH) were identified (Table 2), among which 35 were

213 represented by at least 2 isolates. The most frequent MLH was represented by 8 isolates (all

214 from carrot) but 10 of the MLH in multicopies were represented by isolates collected from

215 different host plants, including melon (Fig. 4). Isolates from melon shared haplotypes with

216 isolates from carrots (4 haplotypes), oilseed rape (3 haplotypes) and witloof chicory (2

217 haplotypes) but not with isolates from bean or lettuce. The geographic distance between the

218 collection sites of two isolates sharing the same haplotype could be up to 700 km, as for example

219 melon isolates collected in the South West and witloof chicory isolates collected in the North

220 of France (MLH 2 and 34 in Fig. 4).

221 The values of gene diversity were in the same range (0.52 to 0.55) for the isolates collected

222 from the 6 different host crops (Table 2). In contrast, haplotypic diversity values showed greater

223 variation. The carrot isolates had the lowest haplotypic diversity (0.57) and oilseed rape isolates

224 had the highest (1.0) as they all had a different haplotype.

225

Regional focus on melon and carrot isolates

The group of melon isolates and that of carrot isolates from the two adjacent southwestern districts of France were not markedly differentiated on the basis of genetic diversity (Table 3). There was no significant difference of allelic richness and gene diversity between carrot isolates and melon isolates ($P_{Mann\&Whitney} = 0.67$ and P= 0.86 respectively). The two groups of isolates shared three haplotypes. One haplotype (MLH 2 in Fig. 4) was shared by 2 melon isolates collected in spring 2014 and 3 carrot isolates collected in autumn 2014, one (MLH 4) was shared by melon isolates collected in spring 2014 and carrot isolates collected in autumn 2014 and 2015 and the third haplotype (MLH 32) was shared by a melon isolate collected in spring 2014 and a carrot isolate collected in spring 2015. The group of melon isolates and that of carrot isolates were significantly (P<0.05) but very slightly genetically differentiated, with F_{ST} values respectively of 0.029 and 0.019 for the complete data and for the clone-corrected data.

Genetic structure

When the DAPC was performed on six a priori groups corresponding to host plants, only 22 isolates, out of the 141 included in the analysis, were assigned to one of the six clusters with a posterior probability greater than 0.8. Among those 22 isolates, 19 were assigned to the cluster corresponding to the host species from which they have been sampled. To run the DAPC without assumption about the factors shaping the genetic structure of *S. sclerotiorum,* we first determined the optimal number of groups in our data using a k-means clustering analysis with a maximum number of clusters fixed to 10. The BIC associated to each clustering solution showed a rapid decrease and a sharp elbow at K=3, which gives very low support to higher clustering solutions. For K=3, all isolates were assigned to one of the three clusters with a posterior probability equal to 1 (Fig. 5). These three clusters were significantly differentiated (P<0.001) with F_{ST} values reaching 0.25-0.28. Results of the multivariate analysis suggested

that *S. sclerotiorum* isolates were somewhat geographically structured with a rough gradient-like pattern from Northwest, where more isolates from cluster 1 were found, to Southeast where isolates from cluster 3 were more frequent (Online ressource 1). Isolates from cluster 2 were more evenly distributed across space. This pattern suggests that the three genetic clusters retrieved by the DAPC analysis reflect an isolation-by-distance process as supported by the result of the Mantel test conducted on genetic and geographic distances (Mantel test p-value = 0.0145; slope estimate = 0.030 - $CI_{95\%[}$0.019-0.046]; Online resource 2).

When analysing the 10 subsampled datasets, we still found that the optimal clustering solution was K=3 in all cases. Although the decrease in the sampling size decreased the power of the multivariate analysis, results from the 10 subsampled datasets still suggested a trend for a longitudinal gradient with isolates from cluster 1 being mostly located West while cluster 3 appeared most represented in the west and cluster 2 more evenly distributed across space (Online resource 1). As for the complete dataset, Mantel tests between genetic and geographic distances were significant for nine of the subsampled datasets (p-values ranged from 0.001 to 0.01). Although not significant, the Mantel test for the 10th subsampled dataset also showed a clear trend for an isolation by distance pattern (p-value = 0.097).

Discussion

The present study was initiated to test the hypothesis that *S. sclerotiorum* isolates collected from melon may be differentiated from isolates collected from other host crops. To our knowledge, this was the first investigation of the genetic characteristics of this pathogen in France and the first to provide data on isolates collected from melon and from witloof chicory.

Overall, our results did not provide any support for the original hypothesis. The isolates from melon did not show higher aggressiveness on melon leaves than those which originated from other host plants, and they did not present distinctive genetic characteristics. The genetic

structure of the isolates was clearly not influenced by their host plant of origin, as more than 84% of them failed to be assigned to host-based a priori clusters in the discriminant analysis of principal components.

In general, the genetic properties of French isolates of *S. sclerotiorum* were comparable to those previously reported in studies where microsatellite markers were used (Table 4). In the present study, only one microsatellite locus (36-4) among the 15 used for isolate genotyping showed a single allele for all isolates and 17.5% of multilocus haplotypes were shared by at least two isolates. Among those shared haplotypes, nearly one third concerned isolates collected on different host crops, including melon. This is consistent with previous reports that MLH of *S. sclerotiorum* were shared between isolates from various crops in Iran (Hemmati et al. 2009), the UK (Clarkson et al. 2013; 2017) and the USA (Aldrich-Wolfe et al. 2015). In these studies, the distance between sampling sites could reach several hundred kilometres. This was also the case in the present study, with haplotypes shared by isolates collected up to 700 km apart.

The specific comparison of melon and carrot isolates from adjacent districts of southwestern France has cast further doubts on the original hypothesis of the present study. It showed that the two crops, often cultivated in rotation or in close vicinity in that region, could be infected by isolates with identical MLH at periods separated by several months, suggesting the exchange of inoculum in time and space at a scale of 100 km. When considering isolates at a national scale, however, both the discriminant analysis of principal components and the Mantel test revealed some level of geographic structure, likely to result from an isolation-by-distance process.

All these findings have implications for the management of white mould. Rotation strategies for the management of white mould could be complicated by possible long distance transport of inoculum. Although specific information is lacking for airborne inoculum *S. sclerotiorum*, viable fungal spores have been recovered at large distances from their sources (Brown and

301 Hovmøller 2002). Our results suggest that the elaboration of rotation schemes should be

302 adapted according to the geographic scale of inoculum transport. As ascospores of *S.*

303 *sclerotiorum* are anemophilic and may be largely responsible for inoculum dissemination

304 (Abawi and Grogan 1979), further work should focus on the trajectories of air masses between

305 production areas and their possible role in inoculum transport. Our results may also be relevant

306 for chemical control of white mould. Fungicide resistance of *S. sclerotiorum* is increasingly

307 reported in oilseed rape production in various countries, including France (Derbyshire and

308 Denton-Giles 2016; Kaczmar et al. 2000; Lehner et al. 2015; Walker et al. 2015b; Zhu et al.

309 2016) and its management is likely to be complicated by inoculum dissemination both in oilseed

310 rape and in a variety of other host crops. All the more so as the active substances are often

311 registered for white mould control in several crops (including melon) in addition to oilseed rape.

312 Clearly, melon could not be used as a low-risk alternative crop in rotation strategies to manage

313 white mould and the recent increase in disease incidence in French melon production may not

314 be explained by the emergence of a distinct "melon" isolate. Other hypotheses need to be

315 considered, including a possible role of the substantial increase in the frequency of fungicide-

316 resistant isolates documented in France in recent years (Walker et al. 2015b), which results in

317 a reduction of control efficacy in the field. An increase in the acreage of susceptible crops (eg

318 oilseed rape) in the South- and Center-West of France, together with decreased rotation time

319 between susceptible crops, may also have played a role. Melon crops themselves may be

320 substantial contributors of inoculum, as moulded fruits are not picked at harvest. If left to rot

321 in the field, these fruits are likely to produce large numbers of sclerotia which could then

322 contribute to the soil inoculum load for the following crops over several years and possibly for

323 crops in neighbouring plots. In this context, it could be particularly valuable to implement

324 measures that reduce the amount of inoculum released on the soil by diseased plants, through

325 the removal of rotten melons after harvest or through the application of biocontrol agents

326 capable of destroying freshly produced sclerotia.

327

328 **Compliance with ethical standards**

332 The authors declare that this research was performed according to the ethical standards.

333 The authors declare to have no conflict of interest.

334 The research did not involve human participants and/or animals.

335 All the authors agreed to submit this manuscript in this current version.

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Table 1 Polymorphism of the 16 microsatellites markers designed by Sirjusingh and Kohn (2001) used in the present study to characterize the genetic diversity of the 200 *Sclerotinia sclerotiorum* isolates

Microsatellite locus	Number of alleles	Size range (bp)
5-2	4	310-318
5-3	12	293-335
7-2	4	159-171
7-3	4	206-214
9-2	10	354-384
12-2	3	212-218
13-2	13	274-354
17-3	7	339-360
106-4	24	402-598
110-4	6	366-386
114-4	19	335-411
8-3	8	230-264
36-4	1	411
42-4	4	406-420
55-4	9	146-222
92-4	4	372-382

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518   **Table 2** Genetic diversity and linkage disequilibrium of *Sclerotinia sclerotiorum* isolates

519   collected from six different crops in France

	Sample size	Hnb [a]	Allelic richness[b]	No. of distinct MLH [c]	R[d]
Total	**200**	**0.58 (0.24)**	**8.25**	**128**	**0.64**
carrot	88	0.54 (0.23)	4.2	51	0.57
melon	55	0.55 (0.26)	4.6	40	0.72
bean	20	0.52 (0.20)	3.8	18	0.89
witloof chicory	15	0.53 (0.24)	3.2	11	0.71
lettuce	12	0.54 (0.26)	4.2	11	0.91
oilseed rape	10	0.55 (0.27)	3.7	10	1

520   a : unbiased gene diversity (standard deviation between brackets)

521   b : unbiased allelic richness corrected for a minimum sample size of 10 isolates.

522   c: multilocus haplotype

523   d: haplotypic diversity

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Table 3 Allelic richness (AR; based on a minimum sample size of 40) and unbiased gene diversity (Hnb) per microsatellite locus in 84 carrot and 40 melon isolates of *Sclerotinia sclerotiorum* collected from two adjacent districts in southwestern France

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Host crop	7-2[a]		12-2		13-2		106-4		114-4	
	AR	Hnb	AR	Hnb	AR	Hnb	AR	Hnb	AR	Hnb
carrot	2.8	0.50	3.0	0.59	6.6	0.70	16.8	0.85	10.5	0.85
melon	3.0	0.60	3.0	0.55	11	0.81	20.0	0.94	10.0	0.84

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Host crop	7-3		8-3		17-3		42-4		9-2	
	AR	Hnb	AR	Hnb	AR	Hnb	AR	Hnb	AR	Hnb
carrot	3.0	0.59	5.8	0.55	4.0	0.59	2.0	0.23	5.8	0.34
melon	4.0	0.57	6.0	0.76	6.0	0.45	2.0	0.03	7.0	0.43

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Host crop	55-4		5-3		92-4		5-2		110-4	
	AR	Hnb	AR	Hnb	AR	Hnb	AR	Hnb	AR	Hnb
carrot	8.7	0.82	8.6	0.67	3.0	0.63	2.8	0.24	4.9	0.60
melon	5.0	0.76	10.0	0.64	3.0	0.59	2.0	0.25	5.0	0.67

[a] Microsatellite locus 36-4 is not shown in this table as it was monomorphic in this study

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Table 4 Characteristics of the loci, sample size and origin of isolates in published studies on

Sclerotinia sclerotiorum using microsatellites markers

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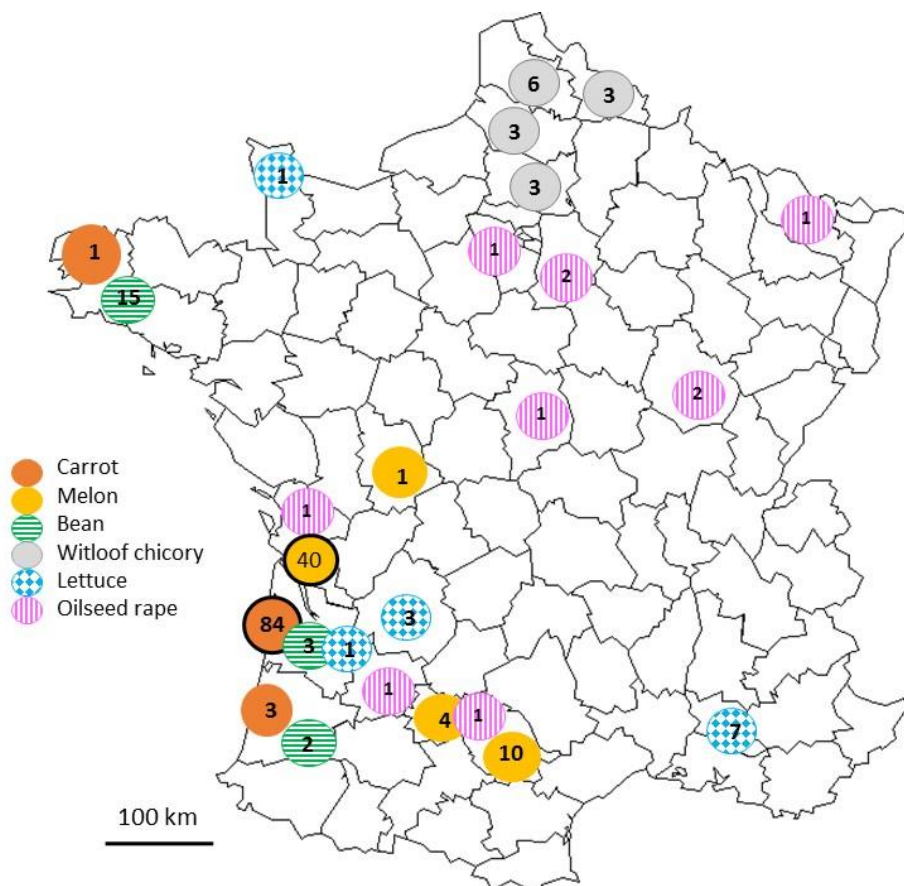| Nb polymorphic loci	Nb monomorphic loci	Nb isolates	Host plants	Country	R[a]	Reference
5	0	156	basil, bean, cabbage, lettuce, potato	USA	0.07	Winton et al. 2006
5	0	65	bean, oilseed rape, cucumber, lettuce, tomato, wild sinapsis	Iran	0.67	Barari et al. 2010
6	1	276	broad bean, canola, tobacco	Iran	0.28	Hemmati et al. 2009
6	0	60	eggplant	Turkey	nd	Tok et al. 2016
7	1	105	canola	Australia	0.53	Sexton et al. 2006
7	1	36	oilseed rape	Turkey	0.63	Met-Turk et al. 2007
8	0	30/29	canola	China/USA	0.96-0.64	Attanayake et al. 2013
8	0	384	oilseed rape, carrot, celery, lettuce, meadow buttercup, pea	UK	0.59	Clarkson et al. 2013
8	0	54	Chinese cabbage, clover, Jerusalem artichoke, lettuce, potato, pumpkin	Norway	0.92	Warmington 2014
8	0	200	beans (snap, dry, baby lima), soybean	USA	0.13	Dunn et al. (2017)
8	0	800	carrot, camelina, oilseed rape, celery, Chinese cabbage, lettuce, lupin, meadow buttercup, pea,	UK, Norway, Australia	from 0.57 to 0.94	Clarkson et al. (2017)

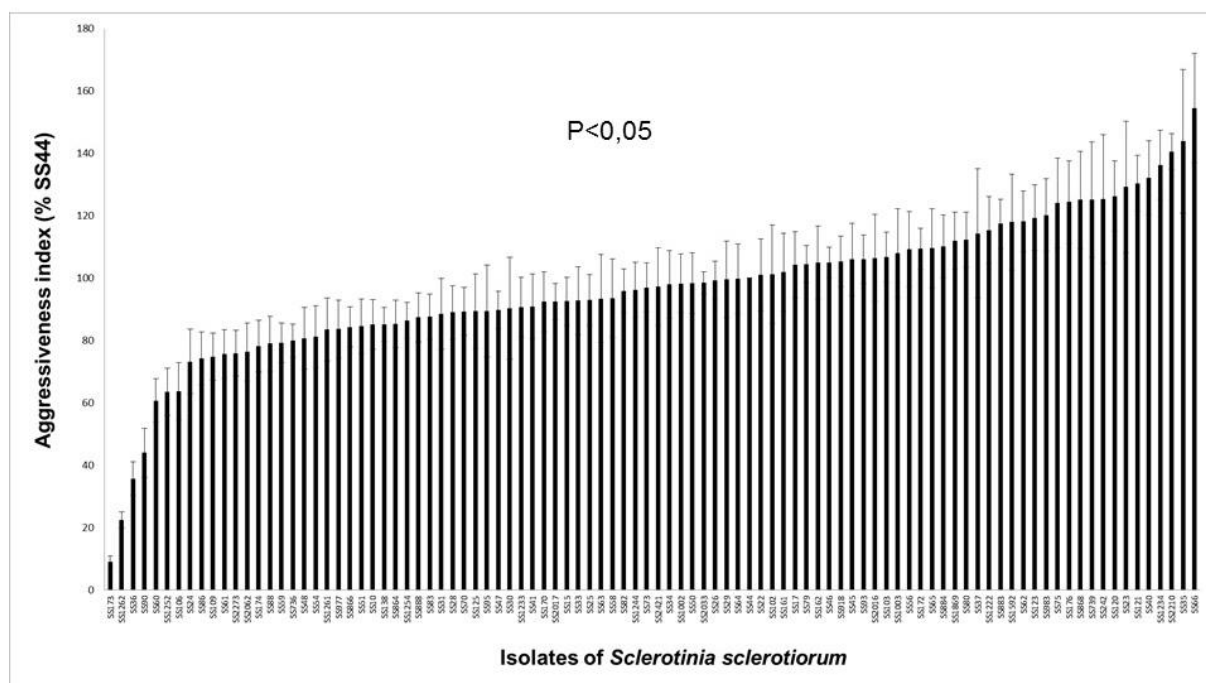|				potato, pumpkin, turnip rape			
10	0	79	dry bean	Brazil	0.64-0.71	Gomes et al. (2011)
10	0	118	common bean	Brazil	0.58	Lehner et al. 2015
11	14	167	potato	USA	0.86	Atallah et al. 2004
12	1	145	canola, carrot, dry bean, houndstongue, potato, tobacco, safflower, soybean, sunflower	USA	0.53	Aldrich-Wolfe et al. 2015
15	1	200	bean, oilseed rape, carrot, lettuce, melon, witloof chicory	France	0.64	**Present study**

546   [a] : haplotypic diversity as indicated in the cited publications or calculated (according to Arnaud-

547   Haond *et al.* 2007) in the present study from published data.

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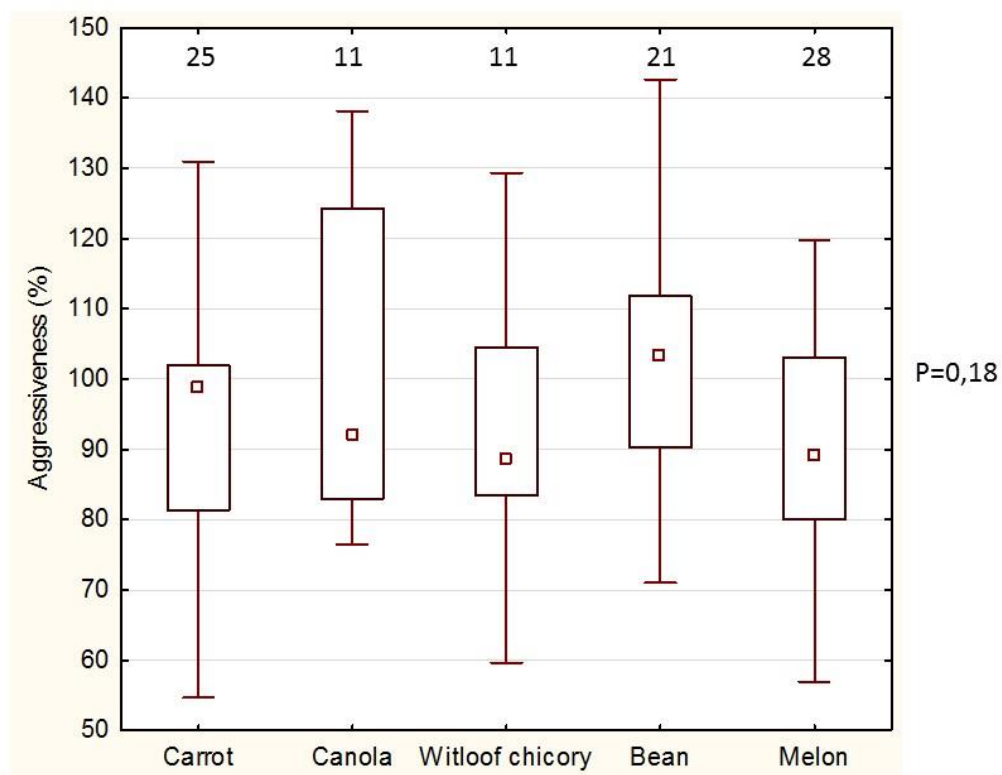550

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Fig.1 Geographic localization of the 200 isolates collected on 6 different crops in France (numbers of isolates are indicated in the circles). The isolates taken into account in the regional focus are indicated by circles surrounded by black line

Carrot
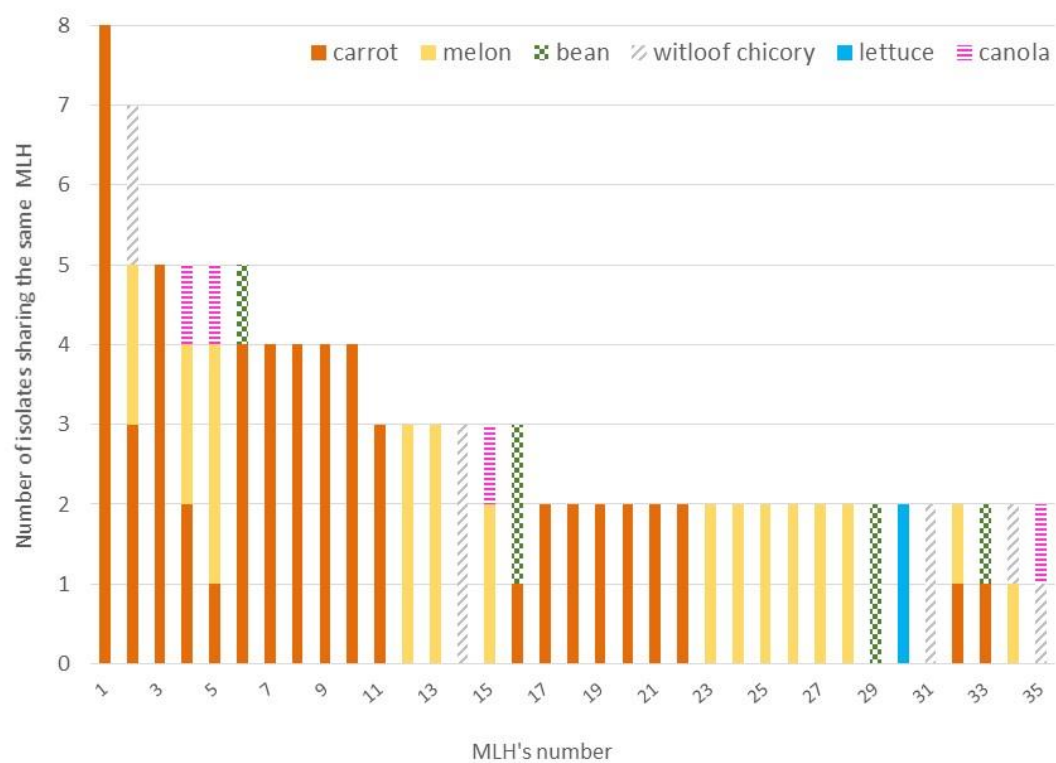Melon
Bean
Witloof chicory
Lettuce
Oilseed rape

100 km

Fig. 2 Distribution of the aggressiveness (in % relative to the reference isolate SS44) of the 96 isolates of *S. sclerotiorum* on melon leaves. Each histogram represents the mean of ten values. Verticals bars indicate the standard error of the mean.
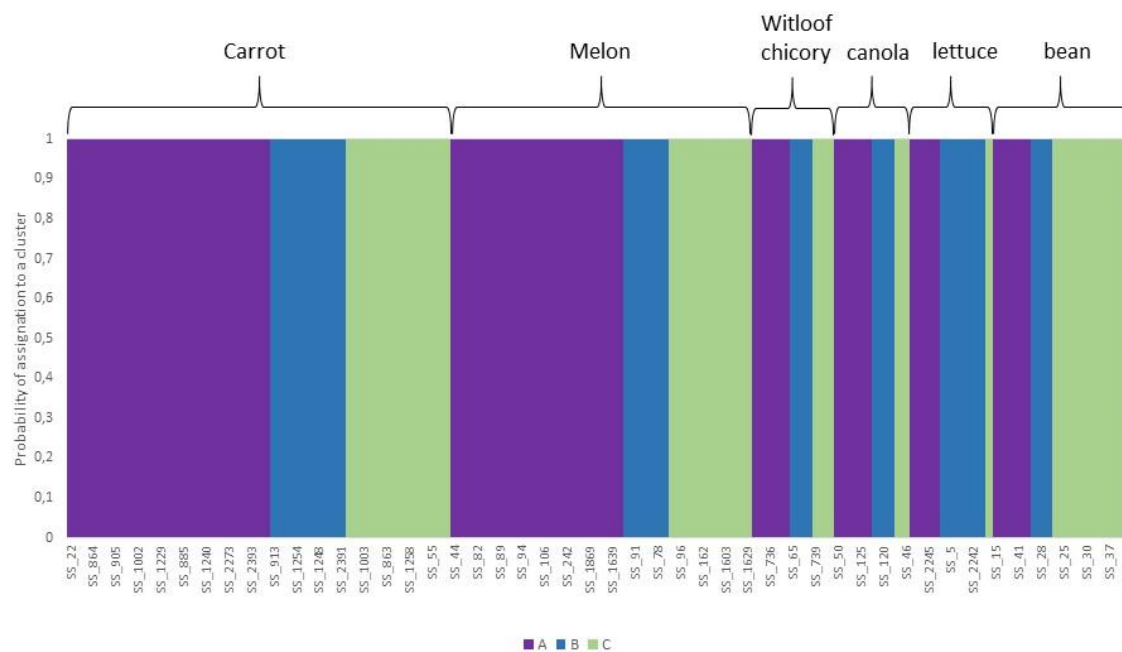
Fig. 3 Box plot representing the extent of aggressiveness (% relative to the reference isolate SS44) on melon of the isolates of *S. sclerotiorum* collected on 5 crops. The numbers above each boxplot corresponds to the number of isolates collected on each of the 5 plant species. For each boxplot, median (□), 25-75% values (▯) and maximum and minimum values (I) are represented

Fig. 4 Multilocus haplotypes represented by more than one isolate

Fig. 5 Assignment of *S. sclerotiorum* isolates collected from 6 host plants to 3 genetic clusters (clonally corrected data set).
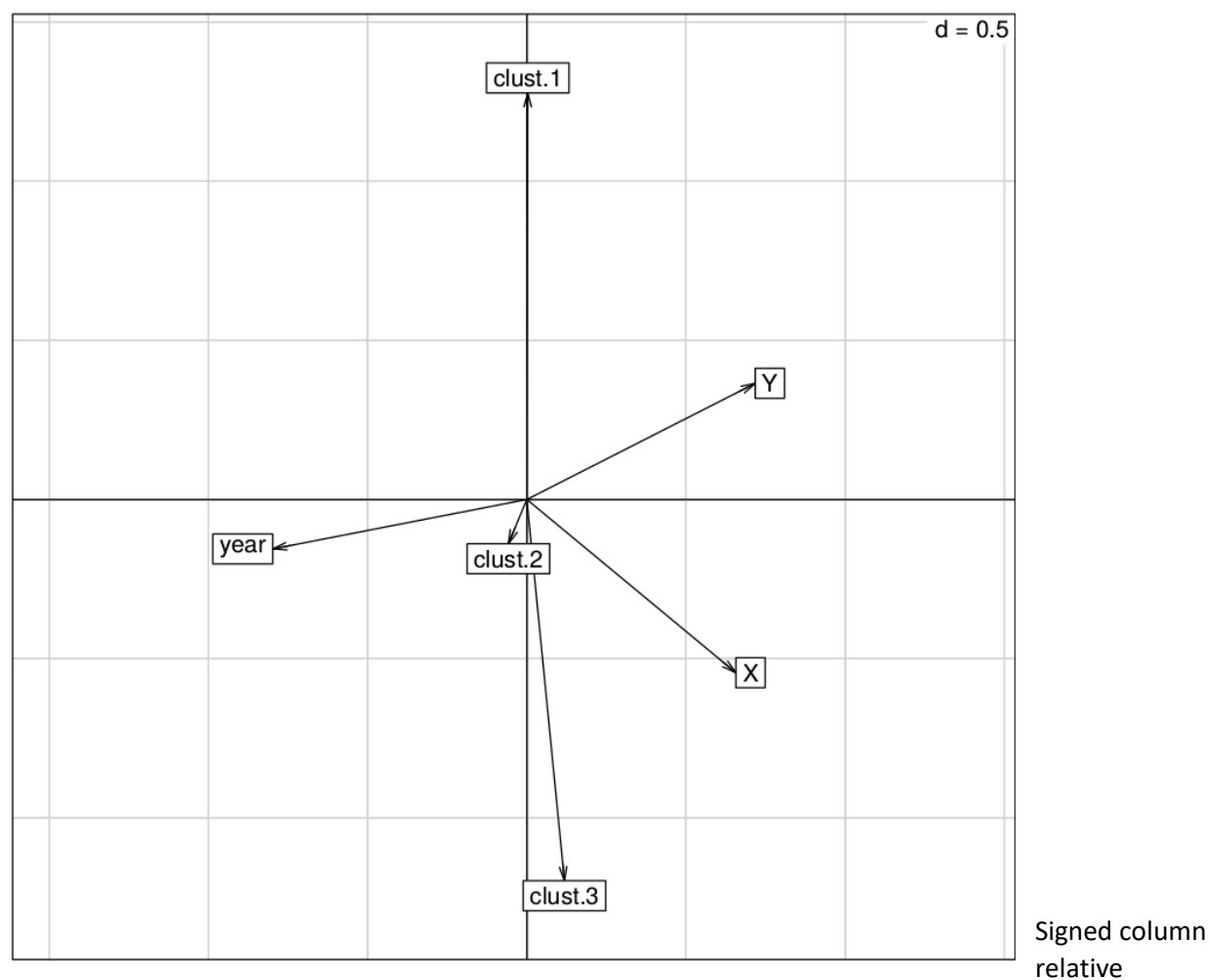
ESM. 1 Results of the multivariate analysis performed by considering for each isolate the cluster number as a factor and the year of sampling as well as the geographic coordinates as continuous variables.

Result of the Hill and Smith multivariate analysis

Projection of the variables on the first and second axis

Signed column relative

contributions of variable on the first and second axis

	Axis1	Axis2
clust.1	3.082e-04	61.401
clust.2	-3.248e-01	-1.878
clust.3	4.278e-01	-43.928
year	-6.370e+01	-2.416
X	4.312e+01	-29.645
Y	5.146e+01	13.309

ESM. 2 Results of the test for isolation-by-distance