The polyphagous plant pathogenic fungus *Botrytis cinerea* encompasses host-specialized and generalist populations

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Abstract

The host plant is often the main variable explaining population structure in fungal plant pathogens, because specialization to hosts contributes to reduce gene flow between populations associated with different hosts. Previous population genetic analysis revealed that French populations of the grey mould pathogen *Botrytis cinerea* were structured by hosts *Solanum* and *Vitis*, suggesting host specialization in this highly polyphagous pathogen. However, these findings raised questions about the magnitude of the specialization in *B. cinerea* and the possibility of specialization to other hosts. Here we report specialization of *B. cinerea* populations to *Solanum* and *Vitis* hosts but not to other tested plants. Population genetic analysis revealed two pathogen clusters associated with *Solanum* and *Vitis* hosts, while the other clusters co-occurred on *Hydrangea*, *Fragaria* and *Rubus*. Measurements of quantitative pathogenicity were consistent with host specialization of populations found on *Solanum*, and to a lesser extent, populations found on *Vitis*. Pathogen populations from *Hydrangea* and *Fragaria* appeared to be generalist, while populations from *Rubus* may be weakly specialized. Our results suggest that the polyphagous *B. cinerea* is more accurately described as a collection of generalist and specialist individuals in populations. This work opens new perspectives for grey mold management, while suggesting spatial optimization of crop organization within agricultural landscapes.

Keywords: Host specialization; grey mould; population structure; molecular epidemiology; cross-pathogenicity; gene flow

Introduction

Fungi are ubiquitous in ecosystems, but most fungal species are not globally distributed and display some kind of population structure (Taylor *et al.,* 2006). In fungal plant pathogens, investigating population structure in relation to host range is particularly relevant, as it is essential for disease management to recognize the different populations of a pathogen, which can have different life history traits, such as resistance to fungicide or phenology (Milgroom & Peever, 2003). Beyond the practical interest in understanding the structure of pathogen populations, studying barriers to gene flow between populations within species may reveal key features of adaptive divergence before they become confounded by other factors. The host plant is often the main variable explaining population subdivision in fungal plant pathogens, because specialization to hosts contributes to reduce gene flow between populations associated with different hosts. Host specialization acts against gene flow due to the reduced viability of migrants and hybrids, and the role of specialization as a barrier to gene flow is reinforced in species that reproduce within their hosts, which is the case for many plant pathogens (Giraud *et al.,* 2010; Giraud *et al.,* 2006; Gladieux *et al.,* 2010). Hence, polyphagous fungal pathogens can form collections of specialist populations, each associated with a limited number of hosts (*i.e.* poly-specialists, such as the rice blast fungus *Pyricularia oryzae*; Gladieux *et al.,* 2018; Valent *et al.,* 2019). Depending on time-based, geographic and genetic factors (Nosil *et al.,* 2009), speciation can then unfold relatively rapidly between pathogen populations (Giraud et al. 2010). The phytopathological literature provides many examples of adaptive radiations (Schluter, 2000) in pathogens, with many sibling pathogen species associated with distinct hosts (Giraud *et al.,* 2006; Tellier *et al.,* 2010). Alternatively, in some pathogens, infection of a novel host is not associated with a specialization process, and merely results in the expansion of the host range of a generalist species. The domestication of plant pathogens, *i.e.* the effective and durable control over pathogen reproduction by humans, remains a major goal in agricultural sciences and knowledge of the intrinsic and extrinsic factors underlying the emergence of new populations specialized onto new hosts –or expansions in host range in generalist species– should help reaching this goal.

Botrytis cinerea (Ascomycota) is a textbook example of a highly polyphagous fungal plant pathogen, causing grey mold on more than 1400 known hosts, in 586 plant genera and 152 botanical families, including high-value crops such as grapevine and tomato (polyphagy index = 54; Elad *et al.,* 2016; Supplementary Information 1). *B. cinerea* is a necrotrophic pathogen that asexually produces conidia that form a sporulating grey layer on the

surface of infected tissues. Conidia are mainly dispersed during spring and summer by wind and human activity. During winter, the fungus develops as a saprobe on plant debris, where it can proceed to undergo sexual reproduction and form melanin-pigmented sclerotia (Carisse, 2016). The analysis of population structure of *B. cinerea* populations infecting wild (e.g. Giraud *et al.*, 1999; Rajaguru & Shaw, 2010) or cultivated (*e.g.* Karchani-Balma *et al.*, 2008; Leyronas *et al.*, 2015) host plants revealed differentiation between sympatric populations from different hosts. However, no correlation was found between population structure and cross-pathogenicity, suggesting that the observed differentiation between populations was not related to host specialization, or that it was instead linked to components of specialization that have not been measured. For example, Leyronas *et al.* (2015) showed lack of genetic differentiation between *B. cinerea* populations found on *Solanum lycopersicum* (tomato) and *Lactuca sativa* (lettuce) grown and patterns of pathogenicity were also consistent with generalism, as strains showed significant differences in quantitative pathogenicity on *S. lycopersicum* hosts, but not on *L. sativa*. Recently, we have found strong genetic differentiation between isolates collected in the greenhouse on *S. lycopersicum* on the one hand and outdoor on *Rubus fruticosus* (wild blackberry) and *Vitis vinifera* (grapevine) on the other hand, suggesting possible specialization of the pathogen to these hosts (Walker *et al.*, 2015). Here, we test the hypothesis of host specialization in *B. cinerea,* by further investigating the genetic structure of *B. cinerea* populations sampled in four French regions on sympatric hosts, including previously tested hosts (e.g. *V. vinifera, S. lycopsersicum* and *R. fruticosus*; Walker *et al.*, 2015) and new hosts (*e.g. Fragaria* x *ananassa* and *Hydrangea macrophylla*), and by measuring quantitative pathogenicity on various hosts using cross-pathogenicity testings. More specifically, we addressed the following questions: (i) What is the population structure of *B. cinerea* on *V. vinifera, S. lycopersicum, R. fruticosus, F.* x *ananassa* and *H. macrophylla* in France? (ii) Are there differences in qualitative or quantitative pathogenicity between identified populations? (iii) Are patterns of population structure and pathogenicity consistent with the specialization of *B. cinerea* to some hosts?

Results

Population subdivision

We characterized 683 isolates from 5 hosts in 15 sites representing 4 areas of France, using 8 microsatellite markers. To examine population structure, we first characterized the partitioning of genetic variation among

87 different factors (host plant and area) using hierarchical analyses of molecular variance (AMOVA) (**Table 2**).
88 With samples grouped by host, variation among hosts was found highly significant (P<0.001; F_{ST}=0.18) and
89 accounted for 18.4% of the molecular variance. Variation due to geography was also found significant but
90 accounted for only 5.5% of molecular variance (P<0.001; F_{ST}=0.06). With samples grouped by host and nested
91 within areas, variation within populations accounted for most of the molecular variance (77,7%; P<0.001;
92 F_{ST}=0.07). Variation among hosts, and among hosts within areas were significant (P<0.001) and accounted for
93 16% and 6% of the molecular variance, respectively. Variation among areas was not significant (P=0.772), but
94 variation among areas within hosts was significant (P<0.001) and accounted for 23.1% of the molecular
95 variation, respectively. These results suggest that hosts (*Solanum, Vitis*, *Fragaria*, *Rubus* and/or *Hydrangea*) and
96 areas (Alsace, Champagne, Loire Valley and Provence) explain a significant proportion of the genetic variance in
97 the dataset, with a stronger effect of the host.
98 We investigated patterns of population subdivision using (i) the Bayesian clustering method implemented in
99 STRUCTURE, assuming a model with admixture and correlated allele frequencies, (ii) the non-parametric
100 multivariate clustering method implemented in the discriminant analysis of principal components (DAPC), and
101 (iii) the maximum likelihood clustering method implemented in SNAPCLUST (**Figure 2**). The three methods
102 converged towards the same clustering solutions at all K value. Models with K=5 captured the most salient
103 features of the structure of the dataset, with two clusters mostly associated with *Vitis* and *Solanum,*
104 respectively, and three clusters found on all hosts but *Solanum*. Increasing K above 5 did not reveal new
105 clusters but mostly introduced heterogeneity in clustering patterns (see also Supplementary Information 4 &
106 5). We calculated the mean pairwise F_{st} between clusters for each K value (Supplementary Information 3) and
107 at K=5 the F_{st} is close to its maximum and displays very close values across all three clustering methods. The ΔK
108 Evanno method (Supplementary Information 6) favors K=4, while goodness of fit statistics (Supplementary
109 Information 7) favors K=5. All subsequent analyses assumed K=5 and isolates were assigned to clusters using
110 the membership proportions estimated with STRUCTURE.
111 Using membership proportions greater than 0.7 to assign multilocus genotypes, clusters tended to be
112 associated with one main host (Figure 3A). Clusters C1 and C2 were almost exclusively associated with *Solanum*
113 and *Vitis,* respectively (96% of isolates from C1 were collected on *Solanum*; 84% of isolates from C2 collected
114 on *Vitis*). The three other clusters were less specific, with the majority of C3 isolates collected on *Vitis* (53%),
115 and the majority of C4 and C5 isolates collected on *Rubus* (50 and 60%, respectively). Isolates from *Fragaria*

were mostly distributed in C3 and C4, whereas isolates collected from *Hydrangea* were mostly assigned to C4 and C5. Pairwise Jost's *D* between clusters inferred with STRUCTURE were all significantly different from zero and ranged between 0.320 and 0.869 at *K*=5 (Table 3). Highest values were observed for pairs including *Solanum*-associated cluster C1 (range 0.663-0.869) and Rubus-associated cluster C5 (range 0.658-0.869). The smallest differentiation was observed between *Vitis*-associated clusters C2 and C3 (D=0.320).

Patterns of genetic diversity and reproductive mode

We investigated how genetic variability varied across the clusters defined at *K*=5, by estimating the mean number of alleles per locus *Ar,* genic diversity *He,* and Shannon's diversity index *H* (Figure 3B). The *Solanum*-associated cluster C1 was the least variable (*Ar*=2.12; *He*=0.26), while the *Rubus*-associated cluster C4 was the most variable (*Ar* =11.45; *He*=0.77). Intermediate levels of variability were observed for the *Vitis*-associated clusters C2 and C3 (Ar=5.31 and 6.57; *He*=0.51 and 0.69) and the other *Rubus*-associated cluster C5 (*Ar*=4.01; *He*=0.44). Multilocus genotypes were evenly represented in clusters C1, C2, C3 and C5 (1.18<*H*<1.5), but not in C4 (*H*=0.37)

To investigate the reproductive mode of *B. cinerea,* we computed the proportion of genotypes repeated multiple times (clonal fraction 1-G/N) and we estimated multilocus linkage disequilibrium ($\bar{r}_D$; **Figure 3**A). The highest clonal fraction was found in the *Solanum*-associated cluster C1 (CF=0.75), which also displayed a relatively high level of linkage disequilibrium among markers ($\bar{r}_D$=0.18). Clonal fraction was low and in the same order of magnitude (range: 0.11-0.26) across other clusters. $\bar{r}_D$ values were also low for these clusters (range: 0.008-0.20), although significantly different from zero.

Cross-pathogenicity tests

<u>On leaves.</u> We assessed differences in aggressiveness between clusters using cross-pathogenicity tests on *Fragaria, Vitis, Rubus, Solanum* and *Hydrangea* leaves, with *Phaseolus* used as a naïve host (no isolates were sampled on this host). Mean necrosis diameters are reported in **Figure 4** and differences between pairs of hosts of origin are presented in **Table 4**. Our statistical model detected significant effects of the host of origin on the mean necrosis diameters, for several time points after inoculation. Mean necrosis diameters increased over time for all interactions and clusters, and especially for the reference isolate B05.10, indicating that tests

144 were properly carried out. For a given scoring time, aggressiveness of the reference isolate differed among test
145 hosts, *Phaseolus* and *Solanum* showing faster growth of necrosis compared to other hosts. No significant
146 differences (*P*-values > 5%) in aggressiveness were observed among isolates from different clusters inoculated
147 onto the naïve host *Phaseolus*, at any scoring time.
148 On *Fragaria* leaves, the aggressiveness of isolates collected on *Hydrangea* was significantly reduced (range: -
149 0.75 - -4.47mm) compared to isolates collected on *Vitis,* for three out of the four scoring dates. Aggressiveness
150 of other groups of isolates on *Fragaria* were similar to each other. Isolates from *Fragaria* did not exhibit greater
151 growth of necrosis on this host compared to other hosts.
152 On *Hydrangea* leaves, no pairwise differences between isolates collected from different hosts were observed,
153 including for isolates collected on this host.
154 On *Rubus* leaves, isolates collected from *Rubus* did not exhibit the best performance, at any scoring time.
155 Isolates from *Solanum* were more aggressive on *Rubus* than both *Vitis* isolates (range of mean diameter
156 necrosis: 0.36-12.7 mm; significant at all scoring times) and *Rubus* isolates (range of mean diameter necrosis:
157 0.2-11.80; significant for 3 out of 6 scoring times).
158 On *Solanum* leaves, isolates collected from this host exhibited the highest mean aggressiveness, for the three
159 latest scoring dates. Aggressiveness of isolates collected on *Rubus* and *Vitis* was significantly reduced on
160 *Solanum* (range of mean diameter necrosis: -0.25 - -13.20 mm for *Rubus* isolates, -0.33 - -13.60 mm for *Vitis*
161 isolates; statistically significant at all dates but the earliest), compared to isolates collected on *Solanum.*
162 Isolates from *Rubus* and *Vitis* hosts performed similarly on *Solanum* leaves.
163 On *Vitis* leaves, aggressiveness of isolates was highly variable, as indicated by the high proportion of boxplot
164 outliers. No significant difference in aggressiveness was observed among isolates from different hosts on *Vitis*,
165 but the biggest mean necrosis diameters were measured for isolates originating from *Vitis* at the two latest
166 scoring times. In summary, the results of cross-pathogenicity tests support host specialization of *B. cinerea* to
167 *Solanum,* and to a lower extent, to *Vitis;* the differences in aggressiveness between *B. cinerea* populations
168 associated with *Solanum* and *Vitis* were also observed on *Rubus* leaves.
169 <u>On berries.</u> We assessed differences in aggressiveness between clusters using pathogenicity tests on *Solanum*
170 berries after 16 days of inoculation and *Vitis* berries, respectively after 16 and 12 days. Necrosis percentages
171 for each cluster are reported in **Error! Reference source not found.** and differences between pairs of hosts of
172 origin are presented in Table 4. Our statistical model detected a significant effect of the host of origin. On

Solanum berries, isolates collected from this host exhibited the highest mean aggressiveness (46.6% of mean necrosis). Aggressiveness of isolates collected on *Rubus* (28% of mean necrosis) was significantly reduced on *Solanum* compared to isolates collected on *Solanum.* No significant difference was detected with isolated collected on *Vitis* (38.1% of mean necrosis).

On *Vitis* berries, percentages of necrosis were lower than on *Solanum* berries. Isolates collected from this host exhibited higher mean aggressiveness (23.7% of mean necrosis) than isolates collected on *Solanum* (12.2%, *P*-value=0.064) but lower mean aggressiveness than isolates collected on *Rubus* (33.3%, *P*-value=0.052).

Discussion

Host specialization in B. cinerea *populations infecting* Vitis *and* Solanum

Several previous studies investigating the population structure of *B. cinerea* proposed that host plant should be considered as a potent factor structuring populations of the pathogen, based on observed associations between patterns of population subdivision and the host of origin of isolates (see review and examples in Walker, 2016). In a previous (Walker *et al.*, 2015) and the present study, we confirmed that most of the genetic variation segregating in *B. cinerea* populations is explained by hosts, and that the effect of geography is weaker. Two of the clusters identified were associated with *Solanum* and *Vitis,* respectively, while other clusters were observed on all hosts but *Solanum,* although in different frequencies. Cross-pathogenicity experiments were consistent with analyses of population subdivision, with patterns of quantitative pathogenicity consistent with specialization of *Solanum* isolates to *Solanum* host, and specialization of *Vitis* isolates to *Vitis* hosts. This pattern of quantitative pathogenicity was observed after inoculation on leaves, but also in berry tests, which represents the type of symptoms more commonly found in the field.

A pattern of pathogenicity partially consistent with specialization was previously observed in indoor samples of *B. cinerea* collected on lettuce and tomato but differences in pathogenicity were not associated with population subdivision (Leyronas *et al.*, 2015). To the best of our knowledge, this is the first report of host-associated ecological divergence in *B. cinerea,* with patterns of population structure mirroring patterns of quantitative pathogenicity. Indeed, the association between population structure and pathogenicity features suggests that host specialization contributes to reduce gene flow between populations associated with different hosts, due to the reduced viability of migrants and hybrids. The association between adaptation to

201 hosts and barriers to gene flow would be favored by limited migration of gametes (especially non-mobile
202 sclerotia developing on plant debris) between phases of selection by the host and sexual reproduction on (or
203 inside) the host's tissue.

204 Comparison of pairwise *Jost's D* between clusters suggest that the *Solanum* host induces greater population
205 differentiation, *i.e.* greater reduction of gene flow between populations, compared to other hosts. This may
206 rely on functional variations into the diverse and sophisticated biochemical arsenal of *B. cinerea.* Indeed, this
207 pathogen shows a large repertoire of secreted proteins, secondary metabolites and small interfering RNA
208 (siRNA) that act as effectors in the interactions with its hosts (Amselem *et al.,* 2011; Mbengue *et al.,* 2016;
209 Reino *et al.,* 2004; Siewers *et al.,* 2005; Valero-Jiménez *et al.,* 2019; Weiberg *et al.,* 2013). The possible role of
210 the different fungal effectors in host specialization remain to be investigated, as well as the possible role of the
211 host phytoalexins. Indeed, contrasted pathogenic interactions between fungal pathogens (*e.g. Cladosporium*
212 *fulvum* and *Fusarium oxysporum* f. sp. *lycopersici*) and tomato were explained by variation of tomatine content
213 produced by the host and the production of tomatinase, an enzyme produced by the pathogen and able to
214 detoxify the saponin toxin produced by *Solanum* (Melton *et al.,* 1998; Sarhan & Kiraly, 1981). Then, many
215 molecular mechanisms could be involved in the incomplete host specialization of the grey mold fungus.
216 Actually a recent genome-wide association mapping of *B. cinerea* showed that its virulence against a range of
217 domesticated and wild tomatoes is highly polygenic and involves a diversity of mechanisms (Breeze, 2019;
218 Soltis *et al.,* 2019). Moreover, the previous observation that conidia of *B. cinerea* strains isolated from *Solanum*
219 have a higher germination rate on *Solanum* cutin than conidia of strains isolated from *Vitis* (Cotoras & Silva,
220 2005) suggests that some of the molecular determinants underlying host specialization must be involved from
221 the very early stage of the plant-pathogen interaction. Altogether, this pleads for elucidating the molecular
222 determinants of host specialization in *B. cinerea* and to determine their specificity towards host plants, such as
223 *Solanum* but also within the Solanaceae family. From an evolutionary perspective, disentangling the molecular
224 evolution of these determinants would contribute to unveil speciation in the *Botrytis* genus, in the light of the
225 domestication of solanaceous plants. The biological material characterized in this study, as well as the high
226 quality genomic resources available for *B. cinerea* should allow populations genomic analysis investigating the
227 direction and density of selection exerted by the host, *Solanum* in particular.

228

Lack of host specialization in B. cinerea *populations infecting* Rubus, Fragaria *and* Hydrangea

In this study we extended previous analyses to new hosts, with the aim to better characterize the pattern of host specialization in *B. cinerea.* We found no evidence for host specialization in isolates of *B. cinerea* collected on *Hydrangea* in outdoor nurseries, neither from population subdivision nor from cross-pathogenicity tests. However, our capacity to detect ecological divergence in pathogens from *Hydrangea* may have been limited by the small number of isolates collected on this host, associated with a relatively high proportion of clones. Further prospections may help determining the pathogenicity profile of *Hydrangea*-associated isolates, in particular in environmental conditions favoring *Botrytis* population isolation in *Hydrangea* cropping, such as in cold climatic chambers used to keep plants during vernalization.

The lack of population cluster specifically associated with *Fragaria* was surprising, since Leroch *et al.* (2013) identified using multiple-gene sequencing, a novel clade of *B. cinerea* called group S, associated with strawberry in Germany. Using the detection marker designed by Leroch *et al.* (2013), we did detect a non-negligible proportion of group S isolates in our dataset. However, group S isolates were not assigned into a specific cluster, suggesting that the pattern of population subdivision previously observed, and which led to the description of group S, may result from specific selection pressures such as resistance in fungicide, rather than from host specialization. The lack of specific interactions between *Fragaria* and *Fragaria*-collected isolates in the cross-pathogenicity test supports this hypothesis.

The case of *Rubus*-associated isolates is more puzzling, given that populations sampled from this host were differentiated from other populations in a previous study (Walker *et al.*, 2015) and partially in this one, but tests did not reveal higher aggressiveness of isolates collected from *Rubus,* compared to isolates from other hosts. Surprisingly, the difference in aggressiveness between *Solanum*- and *Vitis*-collected strains was observed when inoculating on *Rubus,* whereas it belongs to a botanical family, the Rosaceae, distant from Solanaceae and Vitaceae. However, plant susceptibility to *Botrytis cinerea* shows very little association to the evolutionary distances between the plant species (Caseys *et al.*, 2018). Henceforth, theses aggressiveness variations could be linked to species-specific interactions. We may assume that the decrease in gene flow is not sufficient to induce host specialization on *Rubus,* compared to *Solanum* and *Vitis.* Considering host specialization as a dynamic process, adaptation to *Rubus* might be only in the early stages.

Concluding remarks

Our study shows that *B. cinerea* is a model of polyphagous fungal plant populations, whose populations are often comprised of generalist individuals, but can also be specialized to particular hosts (*e.g.* on *Solanum*). While revealing key aspects of the process of adaptive divergence, understanding host specialization in *B. cinerea* may also help forecasting disease emergence and implementing sound management strategies. Indeed, host specialization suggests synchrony between host and pathogen development, determining time-shifted epidemics onsets in sympatric hosts within a landscape. Some cultivated hosts may also "filter" some specialized populations, within the collection of generalist and specialist individuals constituting *B. cinerea.* Limited gene flow between populations specialized on different crops may then be accentuated by optimizing spatial crop organization within an agricultural area. Limiting the density of putative high-risk host plants (with low host specialization) in the vicinity of high value crops may help decreasing disease propagation, as well as delaying fungicide resistance evolution.

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Experimental procedures

Sample collection – Population genetics

Botrytis cinerea samples were collected in four areas of France (Alsace, Champagne, Loire Valley and Provence), with one to five collection sites per area (Figure legends

Figure 1). Collection sites were 1 to 173 km apart within areas, and 180 to 703 km apart between areas. Populations from Champagne and Provence were chosen as a subset of a previously published dataset (Walker *et al.*, 2015), keeping the sample size similar to the number of newly characterized strains (n=331) to avoid biases related to unbalanced sample size. Populations from Alsace and the Loire Valley (n=350) were collected specifically for the present study and include hosts that have not been previously characterized. Samples were collected in June in years 2002, 2005, 2006, 2007, 2014 and 2015. Spring is favorable to the sampling of *B. cinerea* populations from various hosts, as spring conditions facilitate primary infections by ascospores and winter-surviving macroconidia. Primary infections can occur on flowers and young ovaries by penetrating through scars left by floral pieces. Samples were collected from five different hosts: (i) strawberry (*Fragaria* x *ananassa,* hereafter referred to as *Fragaria*; diseased berries), (ii) grapevine (*Vitis vinifera,* hereafter referred to as *Vitis;* asymptomatic flower caps), (iii) wild blackberry (*Rubus fruticosus,* hereafter referred to as *Rubus;* asymptomatic berries and floral pieces) from bushes surrounding vineyards and fields, (iv) hydrangea (*Hydrangea macrophylla,* hereafter referred to as *Hydrangea;* symptomatic and asymptomatic flower buds and stems) from outside nurseries and (v) tomato (*Solanum lycopersicum,* hereafter referred to as *Solanum*; symptomatic fruits or petioles;

Area[a]	Location	Host plant[b]	Cultivar	Number of isolates
Alsace	Balbronn	*Fragaria spp.*	Clery	40
		Rubus fruticosus	Wild	27
		Vitis vinifera	Riesling	36
	Sigolsheim	*Fragaria spp.*	Candiss	31
		Vitis vinifera	Pinot auxerrois	51
Champagne	Courceroy	*Solanum lycopersicum*	Moneymaker	21
	Courteron	*Rubus fruticosus*	Wild	15
		Vitis vinifera	Pinot meunier	30
	Foissy-sur-Vanne	*Rubus fruticosus*	Wild	19
		Solanum lycopersicum	Moneymaker	21
	Hautvilliers	*Vitis vinifera*	Pinot noir	31
	Vandières	*Rubus fruticosus*	Wild	9
		Vitis vinifera	Pinot noir	31
Loire Valley	Angers	*Hydrangea macrophylla*	Unknown	33

	Beaulieu-sur-Layon	*Vitis vinifera*	Chenin	39
	Cheverny	*Fragaria spp.*	Darselect	29
		Rubus fruticosus	Wild	10
		Vitis vinifera	Pinot noir	2
	Cour-Cheverny	*Fragaria spp.*	Gariguette	27
		Rubus fruticosus	Wild	10
		Vitis vinifera	Pinot noir	15
Provence	Lançon-Provence	*Rubus fruticosus*	Wild	5
		Solanum lycopersicum	Grenache	7
		Vitis vinifera	Alison	43
	La Farlède	*Vitis vinifera*	Rolle	21
	Pierrelatte	*Solanum lycopersicum*	Hipop	6
	Sarrians	*Rubus fruticosus*	Wild	20
		Solanum lycopersicum	Emotion/Alison	18
		Vitis vinifera	Grenache	34
Total				681

Table 1). Strains from asymptomatic hosts were collected after incubating plant material in a moist chamber at room temperature until sporulation. Single spores were isolated for each sample and cultured on malt-yeast-agar (MYA; 20 g.L^{-1} malt extract, 5 g.L^{-1} yeast extract, 15 g.L^{-1} agar) at 23°C, under continuous light until sporulation. Seven hundred and one single-spored isolates were produced and kept as conidial suspensions in glycerol 20% at -80°C until use.

Microsatellite genotyping

DNA was extracted after 7 days of cultivation on MYA (malt extract 20 g.L^{-1}; yeast extract 5 g.L^{-1}; agar 15 g.L^{-1}) medium at 21 °C in the dark. DNA was extracted using the "DNeasy® 96 Plant Kit" (QIAGEN®) following manufacturer protocol. Strains were genotyped at eight microsatellite markers (Bc1, Bc2, Bc3, Bc4, Bc5, Bc6, Bc7 and Bc10; Fournier *et al.*, 2002) in multiplex polymerase chain reactions by Eurofins Scientific (Nantes, France). Markers with overlapping allelic size ranges bore distinct fluorochromes, as detailed in Supplementary Information 2. Allele size was scored automatically after binning analysis.

Population subdivision

We used hierarchical AMOVA in Arlequin v3.5 (Excoffier & Lischer, 2010) to estimate population differentiation within and between samples grouped according to their area or host plant of origin. To investigate population subdivision, we used three different methods. First, we used the model-based Bayesian clustering implemented in the STRUCTURE v2.3.4 software (Pritchard *et al.*, 2000). STRUCTURE was run with admixture and correlated allele frequencies options, with 500,000 Markov Chain Monte Carlo iterations, following 100,000 burn-in steps, and results were processed with STRUCTURE HARVESTER (Earl & vonHoldt, 2012). Second, we used

the Discriminant Analysis of Principal Components DAPC, which is a multivariate analysis of population subdivision implemented in the ADEGENET 2.1.1 (Jombart & Ahmed, 2011) package in R 3.4.3. Third, we inferred the population structure using the maximum likelihood method SNAPCLUST implemented in the ADEGENET 2.1.1 package in R 3.4.3. Genetic differentiation between the clusters identified with STRUCTURE was measured using Weir & Cockerham's F_{st} in GENEPOP 4.7.0 (Rousset, 2008) to choose an appropriate number of clusters (K) and *Jost's D* in GENALEX 6.5 (Peakall & Smouse, 2012).

Genetic diversity and mode of reproduction

All calculations were performed on clusters identified with STRUCTURE, considering only genotypes with a membership coefficient greater than or equal to 0.7 in one cluster. We used POPPR 2.8.2 (Kamvar *et al.*, 2015; Kamvar *et al.*, 2014) to calculate the number of unique genotypes *G* and the clonal fraction $1 - G/N$ with *N* the number of individuals in the population, and the $\bar{r}_D$ estimator of multilocus linkage disequilibrium. Allelic richness *Ar,* the mean number of alleles per locus, was estimated using a rarefaction method to standardize sample size in ADZE v1.0 (Szpiech *et al.*, 2008). Gene diversity (*He*, called expected heterozygosity, in diploids) was estimated using GENETIX (Belkhir *et al.*, 1996-2004). Shannon's diversity index, which captures the richness and evenness of allelic profiles, was computed using GENALEX 6.5 (Peakall & Smouse, 2012).

Sample collection – Pathogenicity tests

Cross-pathogenicity tests were carried out using single-spored isolates previously collected from sympatric *Vitis, Rubus* and *Solanum* hosts (Walker *et al.*, 2015), and single-spored isolates newly collected from *Fragaria, Hydrangea* and *Vitis* hosts (this study). Seventy-three isolates (7 to 33 isolates per host plant) were used in total (Supplementary Information 8). Only isolates with membership coefficient greater than or equal to 0.7 in the cluster they represent were used, and the laboratory reference strain B05.10 (Buttner *et al.*, 1994), whose genome is sequenced, was used as a control. Strains were cultivated and kept as described in previous sampling procedure.

Cross-pathogenicity tests

Isolates were cultivated on MYA at 23°C in the dark to prevent sporulation. Inoculations were performed using 1mm-diameter young mycelial plugs collected on the margin of the colonies, deposited on detached leaves (the mycelium facing the plant tissue), between leaf veins, and without wounding the leaf. Isolates were inoculated onto the following plants: *Solanum* (cultivar Moneymaker; 2-3 week-old plantlets cultivated at 20°C with 8h dark), *Vitis* (cultivar Marselan; adult plants cultivated in greenhouse), *Rubus* (wild plants collected in the surroundings of our laboratory), *Fragaria* (cultivar Daroyal; one-year adult plants cultivated in greenhouse) and *Hydrangea* (cultivar Leuchtfeuer; adult plants cultivated in greenhouse). *Phaseolus vulgaris* (green bean, hereafter referred to as *Phaseolus*; cultivar Caruso; 2 week-old plantlets cultivated at 20°C with 8h dark) was used as a naive test host, differing from the previous collection hosts. Two sets of tests were conducted (Table 1): isolates from Champagne were inoculated onto *Vitis, Rubus, Solanum* and *Phaseolus*; isolates from Alsace and Pays de Loire were compared together on *Vitis, Fragaria* and *Hydrangea*.

Inoculations were performed between May and July, when *Vitis, Fragaria* and *Hydrangea* have young leaves. Leaves were detached with sterilized scissors on the day of inoculation, and they were chosen to be as similar as possible in terms of size, age, and stalk position. Each plant species was challenged with all isolates within a week. Experiments were repeated twice on *Phaseolus, Rubus, Fragaria* and *Hydrangea,* and four repeats were carried out on *Solanum* and *Vitis*. For each test, ten plugs per isolate were randomly distributed across leaves; the reference isolate B05.10 was also deposited as a control on each leaf. The number of plugs (from different isolates) on each leaf differed across hosts, as they differ in leaf surface: 5 per *Solanum* leaf, 3 per *Phaseolus* leaflet, 3 per *Vitis* and *Fragaria* leaf, and 4 per *Hydrangea* leaf. Inoculated leaves were incubated in 30 x 30 x 2 cm transparent plastic boxes, over paper moisturized with 40 ml sterile water and these moist chambers were incubated in climate chambers at 23°c with 8h of daily obscurity. Two diameters of each lesion surrounding plug inoculation were measured daily, during between 2 to 9 days depending on the plant species and the speed of necrosis maturation. Lesions were scored until they coalesced with neighboring lesions. For each test and each lesion, plug, leaf and box numbers were recorded in order to include this information in statistical tests. Altogether, the dataset on leaves was comprised of 25,863 measurements of lesion diameter.

For berry tests, isolates were cultivated on MYA at 23°C under continuous light to promote sporulation. Only isolated collected from *Solanum, Vitis* and *Rubus* were used in berry tests (a subset of isolates described in Supplementary Information 8). Ten-day old cultures were used to produce spores suspension in sterile water at

5.10^5 spores/ml, the day of the test. 10 µl droplets of spore suspension were deposited on the unwounded apical side of *Vitis* (white cultivar Mélissa) and *Solanum* (unknown cultivar of cherry tomato) ripe berries. Berries were previously cleaned twice for 5 mn in sterile water with 10% Tween80, rinsed two times for 5 mn in pure water and dried before use. Ten inoculated berries per isolate were stabilized using individual gaskets in a 24 x 18 x 10 cm transparent plastic box over paper moisturized with 20 ml sterile water. A set of ten non-inoculated berries was added in each test to detect natural contamination of the berries. These moist chambers were incubated as for leaf tests. The test was repeated two times for each isolate. The percentage of necrotic area was scored for each berry after 16 and 12 days for *Solanum* and *Vitis,* respectively. Altogether, the dataset on berries was comprised of 1600 measurements of necrosis surface.

Statistical analysis

We tested effects of hosts of origin on leaf necrosis. For each tested host and for each day after inoculation, we used a linear mixed model with diameter of necrosis measured on leaf as the response variable, the host of origin as fixed effect and the isolate, leaf and test box as random effects (using the R packages lmer and lmerTest; Bates *et al.*, 2015; Kuznetsova *et al.*, 2017). We compared the mean of diameter of necrosis by hosts of origin of isolates using a *post-hoc* Tukey test (using the R package emmeans; Lenth *et al.*, 2019).

We further tested effects of hosts of origin on percentage of necrosis on fruit. For each tested host and for each day after inoculation, we used a generalized linear mixed model with percentage of necrosis as the response variable, the host of origin and the test as fixed effects and isolate as a random effect. We compared the necrosis percentage by hosts of origin of isolates using a *post-hoc* Tukey test.

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Tables

Area[a]	Location	Host plant[b]	Cultivar	Number of isolates
Alsace	Balbronn	*Fragaria spp.*	Clery	40
		Rubus fruticosus	Wild	27
		Vitis vinifera	Riesling	36
	Sigolsheim	*Fragaria spp.*	Candiss	31
		Vitis vinifera	Pinot auxerrois	51
Champagne	Courceroy	*Solanum lycopersicum*	Moneymaker	21
	Courteron	*Rubus fruticosus*	Wild	15
		Vitis vinifera	Pinot meunier	30
	Foissy-sur-Vanne	*Rubus fruticosus*	Wild	19
		Solanum lycopersicum	Moneymaker	21
	Hautvilliers	*Vitis vinifera*	Pinot noir	31
	Vandières	*Rubus fruticosus*	Wild	9
		Vitis vinifera	Pinot noir	31
Loire Valley	Angers	*Hydrangea macrophylla*	Unknown	33
	Beaulieu-sur-Layon	*Vitis vinifera*	Chenin	39
	Cheverny	*Fragaria spp.*	Darselect	29
		Rubus fruticosus	Wild	10
		Vitis vinifera	Pinot noir	2
	Cour-Cheverny	*Fragaria spp.*	Gariguette	27
		Rubus fruticosus	Wild	10
		Vitis vinifera	Pinot noir	15
Provence	Lançon-Provence	*Rubus fruticosus*	Wild	5
		Solanum lycopersicum	Grenache	7
		Vitis vinifera	Alison	43
	La Farlède	*Vitis vinifera*	Rolle	21
	Pierrelatte	*Solanum lycopersicum*	Hipop	6
	Sarrians	*Rubus fruticosus*	Wild	20
		Solanum lycopersicum	Emotion/Alison	18
		Vitis vinifera	Grenache	34
Total				681

Table 1. Populations of *B. cinerea* collected in four French areas, on four host plants between 2002 and 2015.

[a] Isolates were sampled at multiple dates between 2002 and 2015 in Alsace (June 2015), Champagne and Provence (September 2005 to June 2007) and Loire Valley (June 2002 to June 2015).

[b] Isolates collected on *Rubus, Vitis* and *Hydrangea* were retrieved from asymptomatic flower parts or young fruits. Isolates from *Solanum* and *Fragaria* were isolated from symptomatic fruits.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	*P*-value	Fixation indices
Hosts						
Among hosts	4	277	0.56	18.4	**< 0.0001**	0.184
Within hosts	696	1740	2.50	81.6		
Total	700	2017	3.06			
Geography						
Among areas	5	104	0.16	5.5	**< 0.0001**	0.055
Within areas	695	1913	2.75	94.5		
Total	700	2017	2.91			
Host > Geography						
Among hosts	4	277	0.49	16.2	**< 0.0001**	0.162
Among hosts within areas	11	112	0.19	6.1	**< 0.0001**	0.223
Within populations	685	1628	2.38	77.7	**< 0.0001**	0.073
Total	700	2017	3.06			

Table 2. Hierarchical analysis of molecular variance (AMOVA) with host of collection or sampling location as grouping factor (upper tables) and host of collection nested within geographic origin as grouping factor (lower table).

P-values in bold are significant at the 5% confidence level. d.f., degrees of freedom.

Cluster	C1	C2	C3	C4
C2	0,754*			
C3	0,633*	0,320*		
C4	0,782*	0,623*	0,579*	
C5	0,869*	0,666*	0,701*	0,658*

Table 3. Pairwise *Jost's D* between clusters inferred with STRUCTURE at *K*=5.

Solanum, *Vitis*, *Vitis*, *Rubus* and *Rubus* were found as the main hosts for C1, C2, C3, C4 and C5, respectively (**Error! Reference source not found.**). Isolates were assigned into clusters when their membership coefficient was greater than 0.7 for a given cluster. Non-assigned isolates weren't used in this analysis.

*indicates that the pairwise *D* is significant at the 5% confidence levels.

Test host	Days after inoculation	Effect of the host of collection[a]	Test effect[b]	Control *vs.*					*Fragaria vs.*				*Hydrangea vs.*			*Rubus vs.*		*Solanum vs.*
				Fragaria	*Hydrangea*	*Rubus*	*Solanum*	*Vitis*	*Hydrangea*	*Rubus*	*Solanum*	*Vitis*	*Rubus*	*Solanum*	*Vitis*	*Solanum*	*Vitis*	*Vitis*
Leaf tests : Pairwise difference in mean necrosis diameter for individuals from various hosts of collection (mm)[c]																		
Fragaria	2	0.005**	***	0.49	1.00	-	-	0.25	0.51	-	-	-0.25	-	-	-0.75**	-	-	-
	3	0.057.		0.27	1.32	-	-	-0.27	1.05	-	-	-0.53	-	-	-1.58*	-	-	-
	4	0.3		-0.07	1.48	-	-	-1.34	1.55	-	-	-1.27	-	-	-2.82	-	-	-
	5	0.035*		-1.20	2.33	-	-	-2.14	3.53	-	-	-0.94	-	-	-4.47*	-	-	-
Hydrangea	2	0.46	.	0.43	0.36	-	-	0.44	-0.07	-	-	0.017	-	-	0.08	-	-	-
	3	0.31	**	1.74	0.61	-	-	1.81	-1.13	-	-	0.08	-	-	1.20	-	-	-
	4	0.17	***	2.82	-0.22	-	-	2.77	-3.04	-	-	-0.05	-	-	2.99	-	-	-
	5	0.17	***	4.09	-0.61	-	-	3.81	-4.70	-	-	-0.28	-	-	4.42	-	-	-
Phaseolus	2	0.3	***	-	-	2.41	1.65	1.46	-	-	-	-	-	-	-	-0.75	-0.95	-0.19
	3	0.3	***	-	-	5.57	3.89	3.81	-	-	-	-	-	-	-	-1.67	-1.76	-0.08
	4	0.28	***	-	-	8.30	4.86	5.93	-	-	-	-	-	-	-	-3.44	-2.36	1.08
	5	0.099.		-	-	5.93	-1,00	7.54	-	-	-	-	-	-	-	-6.93	1.60	8.54·
Rubus	2	0.049*		-	-	0.27	0.05	0.41	-	-	-	-	-	-	-	-0.2	0.14	0.36*
	3	0.029*	***	-	-	0.76	0.11	0.79	-	-	-	-	-	-	-	-0.65	0.03	0.68*
	4	0.0031**	***	-	-	2.58	0.29	2.39	-	-	-	-	-	-	-	-2.29*	-0.20	2.10**
	5	0.0084**	***	-	-	3.84	0.57	3.78	-	-	-	-	-	-	-	-3.28*	-0.06	3.21*
	8	0.005**		-	-	9.04	-2.78	9.96	-	-	-	-	-	-	-	-11.80*	0.92	12.70**
	9	0.044*	***	-	-	8.1	0.49	9.15	-	-	-	-	-	-	-	-7.60	1.05	8.65*
Solanum	2	0.091.	***	-	-	0.33	0.08	0.41	-	-	-	-	-	-	-	-0.25	0.08	0.33 ·
	3	1.1e-06***	***	-	-	5.54*	1.24	5.40*	-	-	-	-	-	-	-	-4.31***	-0.15	4.16***
	4	0.00024***	***	-	-	9.37	1.10	9.67	-	-	-	-	-	-	-	-8.27**	0.31	8.57***
	5	0.0006***	***	-	-	15.70	2.44	16,00	-	-	-	-	-	-	-	-13.20**	0.34	13.60***
Vitis	2	0.4	***	0.20	0.08	0.42	0.21	0.35	-0.12	0.22	0.02	0.15	0.336	0.132	0.27	-0.20	-0.07	0.13
	3	0.26	***	1.33	1.11	1.37	0.65	0.67	-0.22	0.04	-0.68	-0.66	0.26	-0.456	-0.43	-0.72	-0.69	0.02
	4	0.12	***	2.36	2.25	3.15	1.45	1.14	-0.11	0.79	-0.90	-1.22	0.895	-0.798	-1.11	-1.69	-2.01	-0.31
	5	0.037*	***	3.82	4.09	4.03	1.93	0.73	0.27	0.22	-1.89	-3.09	-0.0541	-2.16	-3.36	-2.11	-3.31	-1.20
	9	0.15	***	-	-	14.2	8.74	4.76	-	-	-	-	-	-	-	-5.50	-9.47	-3.98
Berry tests : Pairwise log odds ratio from various hosts of collection																		
Solanum	16	0.02401 *	-	-	-	0.52	-0.77	-0.24	-	-	-	-	-	-	-	-1.30*	-0.77	0.52
Vitis	12	0.000663 ***	***	-	-	-0.57	0.74	0.097	-	-	-	-	-	-	-	1.31***	0.66·	-0.64·

Table 4. Results of cross-pathogenicity leaf tests observed over post-inoculation time for *B. cinerea* isolates representative of their host of collection. Isolates collected on *Fragaria*, *Hydrangea*, *Vitis*, *Rubus* and *Solanum* (hosts of origins, in columns) and a reference isolate used as a control were inoculated on *Fragaria*, *Hydrangea*, *Phaseolus*, *Rubus*, *Solanum* and/or *Vitis* leaves (test hosts, in row). Necrosis were scored between 2 and 16 days after inoculation, depending on the test host.

527 [a] *P*-value indicating if lesion size according to host of origin was significantly different, on a given test host.

528 [b] Significance of the *P*-value, indicating if test conditions (test, leaf and box repeat) significantly interacted with the lesion size on a given test host.

529 [c] Values indicate the mean difference of necrosis diameter (in mm) between isolates collected on a host (2nd row) compared to isolates collected on a second one (3rd row),

530 on given test host. Either positive or negative, these values indicate on which test host a given group of isolates had better growth.

531 ·,*, ** and *** indicates that the *P*-value is significant at the 10%, 5%, 1% and 1‰ confidence levels, respectively. – indicates that this interaction was not tested.

532

Figure legends

Figure 1. Map of *B. cinerea* populations collected from four French areas on various host plants between 2002 and 2015.

Each color represents an area with Alsace in red, Champagne in blue, Loire Valley in green and Provence in yellow.

Figure 2. Barplots of the STRUCTURE, DAPC and SNAPCLUST analysis showing genetic subdivision of the 681 *B. cinerea* isolates collected between 2002 and 2015 into a varying number of genetic clusters (K=2 to K=10).

Individuals are sorted out by their area of origin (black vertical bars) and then by their sampling host (grey vertical bars). The areas names are indicated at the top of the chart and the host of origin at the bottom in abbreviated form (*Vv*: *V. vinifera*; *Rf*: *R. fruticosus*; *Fxa*: *F. x ananassa*; *Sl*: *S. lycopersicum* and *Hm; H. macrophylla*). Each cluster is colored according to the most represented host of its members (red for the *Solanum*-associated cluster, shades of green for *Vitis*-associated clusters, shades of purple for *Rubus*-associated clusters and shades of pink for *Fragaria*-associated clusters).

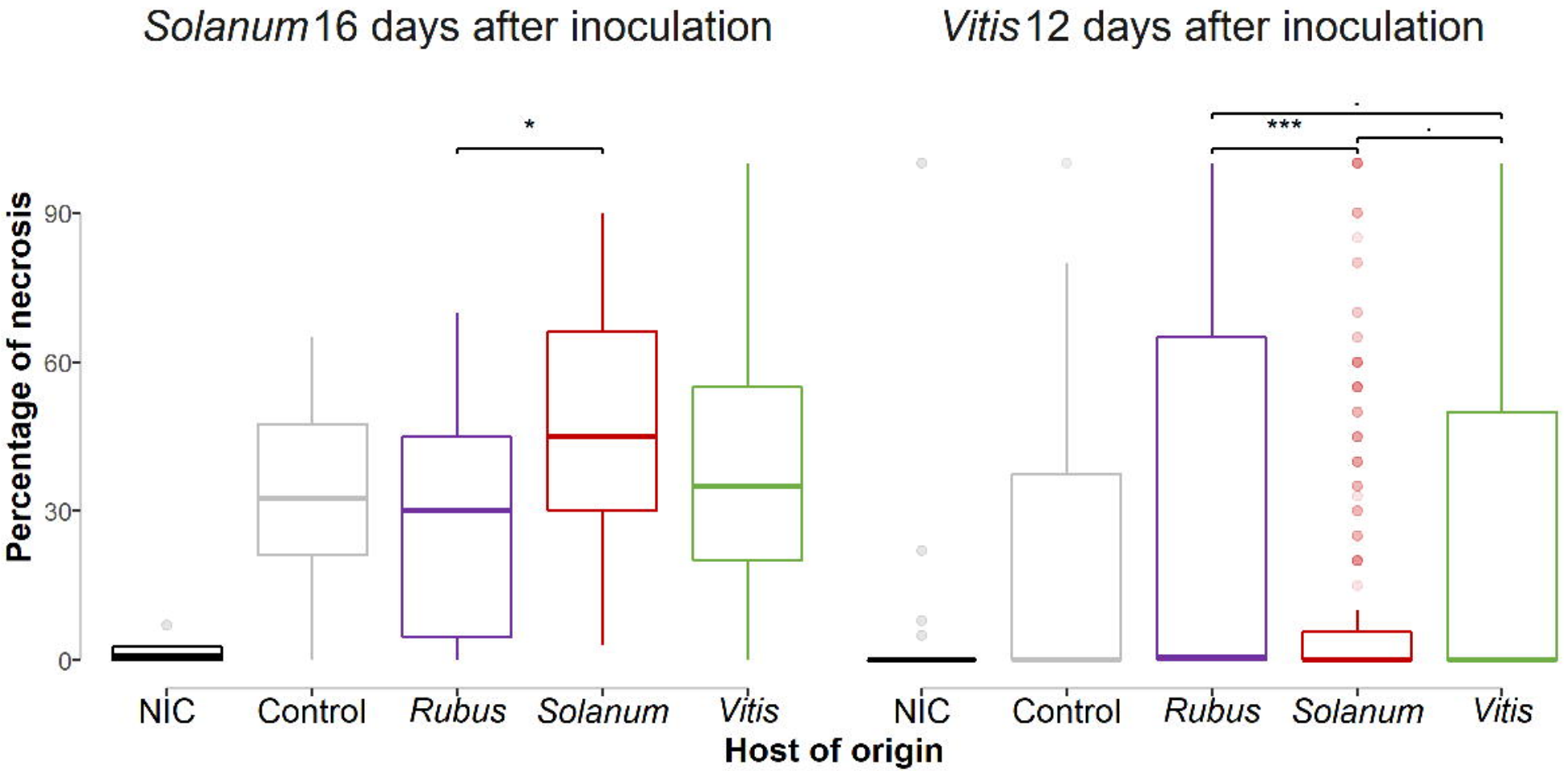
Figure 3. Patterns of diversity and reproduction mode measured in the five clusters delimited by the STRUCTURE partitioning analysis at K=5.

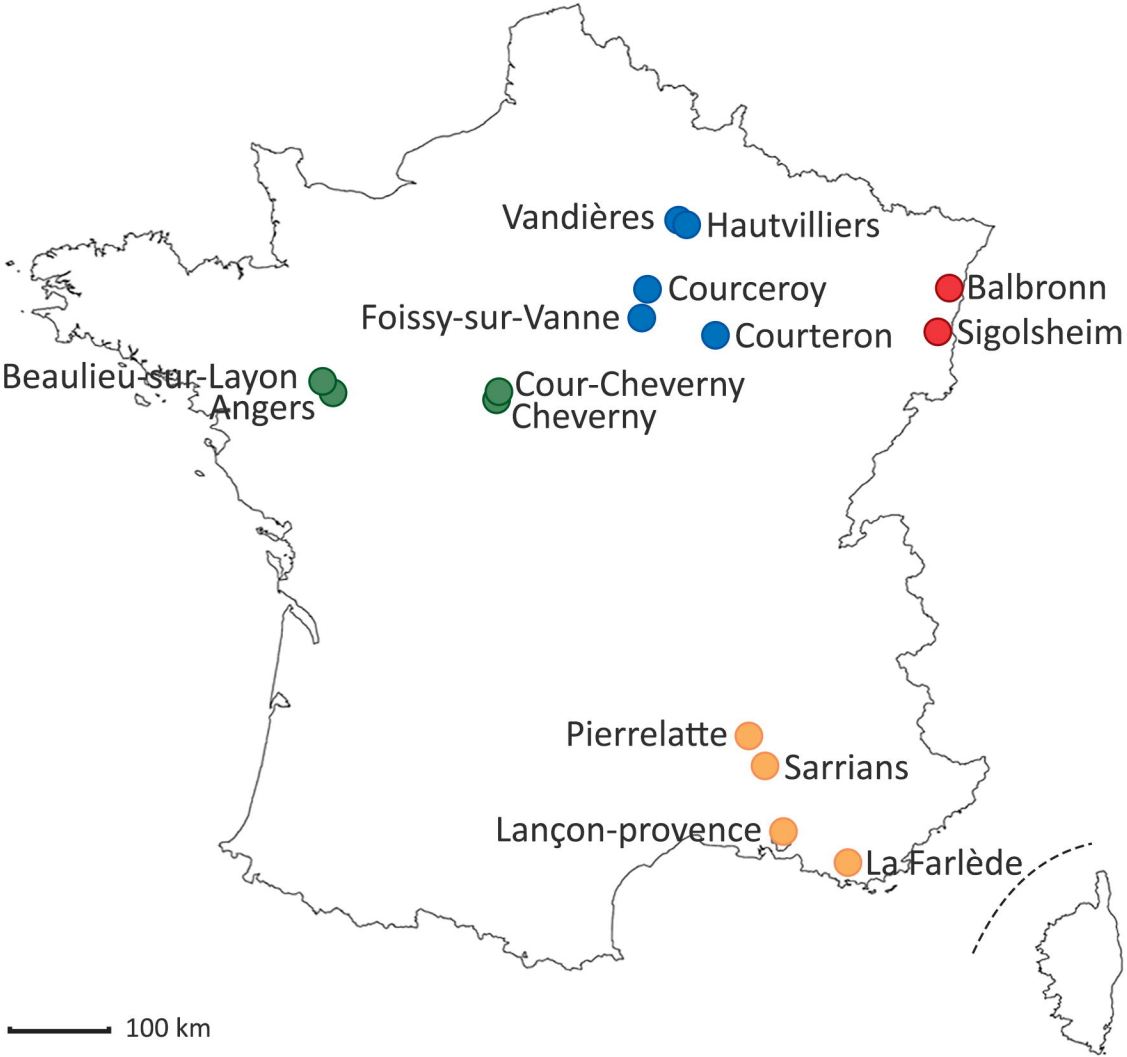
(A) Proportions of sampling hosts represented in inferred clusters (left), clonal fraction (middle) and $\bar{r}_D$ (estimation of multilocus linkage disequilibrium ; right). Isolates from *Solanum* are colored in red, *Vitis* in green, *Rubus* in purple, *Hydrangea* in blue and *Fragaria* in pink. (B) Diversity is estimated *via* allele richness A_r (mean number of alleles per locus; left), the expected heterozygosity *He* (mean expected heterozygosity over the eight loci; middle) and the Shannon's diversity index *H* (mean over the eight loci; right).

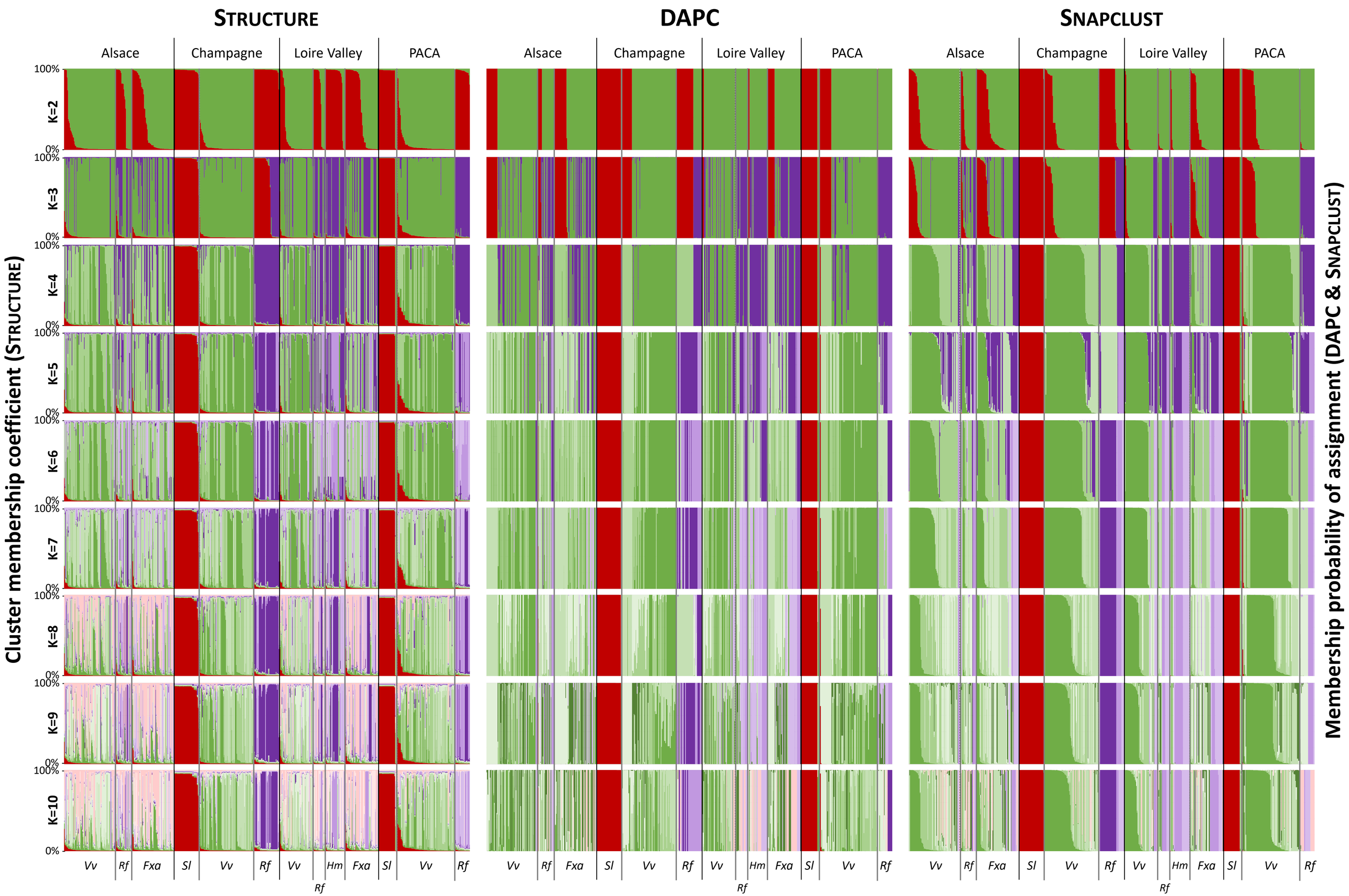
Figure 4. Evolution of mean necrosis diameter over time, for *B. cinerea* isolates representative of their host of origin and inoculated on a range of test hosts.

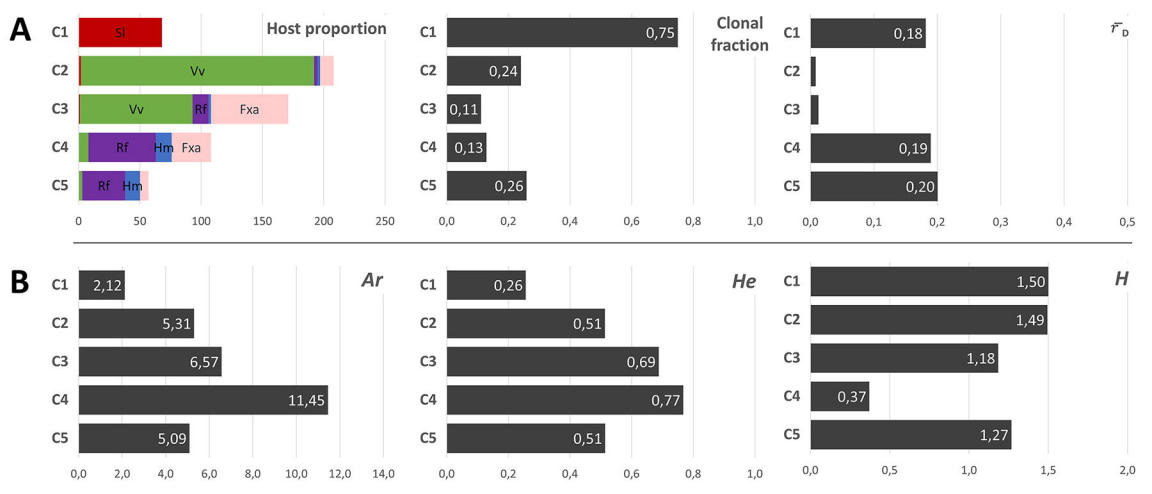
Each boxplot represents a group of 7-33 isolates collected on *Fragaria* (pink), *Hydrangea* (blue), *Rubus* (purple), *Solanum* (red), *Vitis* (green) and a reference isolate used as a control (grey). These isolate subgroups were

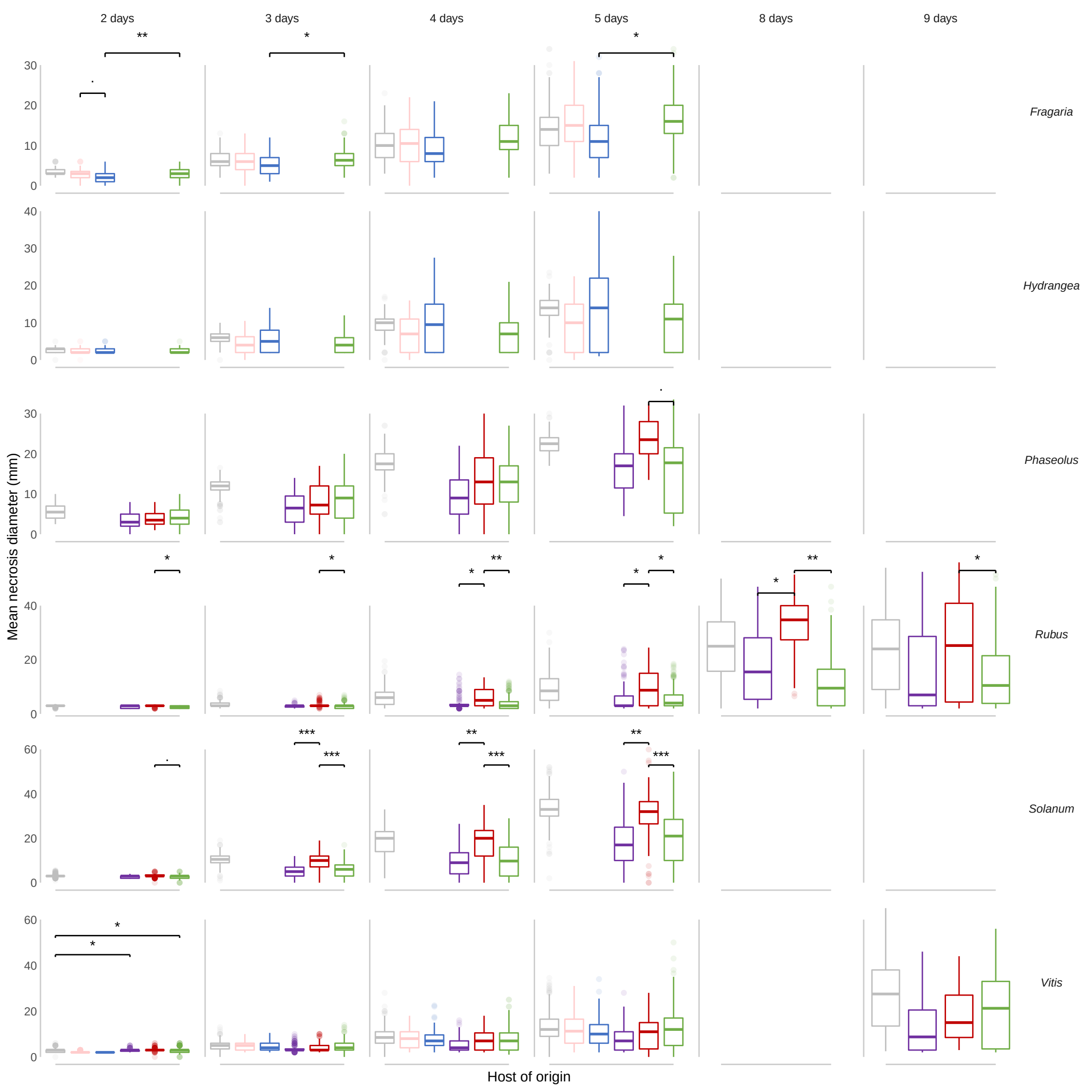
560 tested on leaves from *Fragaria, Hydrangea, Phaseolus, Rubus, Solanum* and *Vitis* (test hosts). Lesions were
561 scored between 2 and 9 days after inoculation, depending on the test host.
562 ˙, *, ** and *** indicates tests of difference of mean necrosis diameter between two groups of isolates with *P*-
563 value lower than the 10%, 5%, 1% and 1‰, respectively.
564
565 **Figure 5**. Percentage of necrosis for *B. cinerea* isolates representative of their host of origin and inoculated on
566 *Vitis* and *Solanum* hosts.
567 Each boxplot represents a group of 16-34 isolates collected on *Rubus* (purple), *Solanum* (red), *Vitis* (green), a
568 reference isolate used as a control (grey) and a not inoculated control (NIC, black). These isolate subgroups
569 were tested on *Solanum* and *Vitis* berries (test hosts) and percentage of necrosis were measured 16 days and
570 12 days, respectively, after inoculation.
571 ˙, *, ** and *** indicates tests of difference necrosis percentage between two groups of isolates with *P*-value
572 lower than 10%, 5%, 1% and 1‰, respectively.


Solanum 16 days after inoculation
Vitis 12 days after inoculation
Percentage of necrosis
90
60
30
0
*
***
.
.
NIC
Control
Rubus
Solanum
Vitis
NIC
Control
Rubus
Solanum
Vitis
Host of origin


Vandières
Hautvilliers
Courceroy
Foissy-sur-Vanne
Courteron
Balbronn
Sigolsheim
Beaulieu-sur-Layon
Angers
Cour-Cheverny
Cheverny
Pierrelatte
Sarrians
Lançon-provence
La Farlède
100 km


STRUCTURE
DAPC
SNAPCLUST
Alsace
Champagne
Loire Valley
PACA
Cluster membership coefficient (STRUCTURE)
Membership probability of assignment (DAPC & SNAPCLUST)
K=2
K=3
K=4
K=5
K=6
K=7
K=8
K=9
K=10
100%
0%
Vv
Rf
Fxa
Sl
Hm


A
Host proportion
C1
C2
C3
C4
C5
Sl
Vv
Vv
Rf
Fxa
Rf
Hm
Fxa
Rf
Hm
0
50
100
150
200
250
Clonal fraction
0,75
0,24
0,11
0,13
0,26
0,0
0,2
0,4
0,6
0,8
1,0
$\bar{r}_D$
0,18
0,19
0,20
0,0
0,1
0,2
0,3
0,4
0,5
B
Ar
2,12
5,31
6,57
11,45
5,09
0,0
2,0
4,0
6,0
8,0
10,0
12,0
14,0
He
0,26
0,51
0,69
0,77
0,51
0,0
0,2
0,4
0,6
0,8
1,0
H
1,50
1,49
1,18
0,37
1,27
0,0
0,5
1,0
1,5
2,0


2 days
3 days
4 days
5 days
8 days
9 days
Fragaria
Hydrangea
Phaseolus
Rubus
Solanum
Vitis
Mean necrosis diameter (mm)
Host of origin