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1 **Hybridizations between *formae speciales* of *Venturia inaequalis* pave the way for a new**
2 **biocontrol strategy to manage fungal plant pathogens**

3

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13 **Abstract**

14 Hybridization and adaptation to new hosts are important mechanisms of fungal disease
15 emergence. Evaluating the risk of emergence of hybrids with enhanced virulence is then key
16 to develop sustainable crop disease management. We evaluated this risk in *Venturia*
17 *inaequalis*, the fungus responsible for the common and serious scab disease on Rosaceae
18 hosts, including apple, pyracantha and loquat. Field isolates from these three hosts and
19 progenies obtained from five crosses between *formae speciales* isolates collected from
20 pyracantha (f.sp. *pyracantha*) and apple (f.sp. *pomi*) were tested for their pathogenicity on
21 the three hosts. We confirmed a strict host specificity between isolates from apple and
22 pyracantha, and showed that most isolates were able to cause disease on loquat. None of the
23 251 progeny obtained from five crosses between *V. inaequalis* f.sp. *pyracantha* and *V.*
24 *inaequalis* f. sp. *pomi* could infect apple. If confirmed on more crosses, the inability of the
25 hybrids to infect apple could lead to a novel biocontrol strategy based on a sexual hijacking of
26 *V. inaequalis* f.sp. *pomi* by a massive introduction of *V. inaequalis* f.sp. *pyracantha* in apple
27 orchards. This strategy, analogous to the sterile insect approach, could lead to the collapse of
28 the population size of *V. inaequalis* and dramatically reduce the use of chemicals in orchards.

29

30 **Keywords** : Host specificity, Biological control, Crossing experiments, Scab, Apple, Firethorn,
31 *Eriobotrya*, Sexual hijacking

32

33 Plant-pathogenic fungi show variation in the width of their host range, from generalist
34 pathogens that cause disease on multiple host species to specialized pathogens that infect a
35 single host species. Fungal species strictly specialized to different hosts are referred to as
36 *formae speciales*. Reproductive barriers between lineages – including *formae speciales* –

37 specialized to different hosts may be incomplete, which may lead to hybridization (Le Gac and
38 Giraud 2008; Depotter et al. 2016). If hybrids are less fit than their parents on the same host,
39 they should ultimately disappear, but still may act as a “genetic bridge” allowing transfer of
40 pathogenicity traits from one species to another by backcrossing (Brasier 2001; Feurtey and
41 Stukenbrock 2018; Stukenbrock et al. 2012). Hybridization can also result in extreme
42 phenotypes, a phenomenon referred to as transgressive segregation (Rieseberg et al. 1999).
43 Hybrid individuals can display increased virulence, as observed for hybrids between anther
44 smut pathogens *Microbotryum lychnidis-dioicae* and *M. silenes-dioicae* (Gibson et al. 2014),
45 or display an expanded or new host range such as in the triticale pathogen *Blumeria graminis*
46 f.sp. *triticales* that was formed by hybridization between the wheat and rye pathogens *B.*
47 *graminis* f.sp. *tritici* and *B. graminis* f.sp. *secalis* (Menardo et al. 2016). As hybridization of
48 different *formae speciales* can be a driving force in the emergence of new diseases with
49 expanded host ranges we investigated the potential for this phenomenon to occur in the
50 fungal pathogen *Venturia inaequalis*.

51 *V. inaequalis* is responsible for scab disease on apple (*Malus x domestica*), the most cultivated
52 fruit tree in temperate areas worldwide. Apple scab reduces both the quality and quantity of
53 fruit. The control of the disease is mainly based on the use of fungicides. For a sustainable and
54 risk-free environmental control of apple scab, there is a need to develop the use of apple
55 resistant cultivars together with sanitation (Didelot et al. 2016), and to develop new control
56 methods, especially for biological control which lacks efficiency in orchards (Carisse and
57 Rolland 2004; Kohl et al. 2015). *V. inaequalis* also causes scab disease on other Rosaceae
58 hosts, including pyracantha and loquat (Jones and Aldwinckle 1991; Sivanesan 1977). Loquat
59 (*Eriobotrya japonica* Lindl.) is grown for fruit production in regions with subtropical climate,
60 while pyracantha (*Pyracantha* spp) is grown as an ornamental plant sometimes planted in

61 hedges in an area extending from Southwest Europe to Southeast Asia. A strict host specificity
62 has been observed for isolates of *V. inaequalis* from pyracantha, which are able to cause
63 disease on pyracantha but not on apple (*i.e.* *V. inaequalis* f.sp. *pyracantha*), and isolates from
64 apple, which are able to cause disease on apple but not on pyracantha (*i.e.* *V. inaequalis* f.sp.
65 *pomi*) (Le Cam et al. 2002). It was suggested that these two *formae speciales* are in “a process
66 of isolation due to host specialization” (Le Cam et al. 2002). The status of isolates from loquat
67 is less clear. Raabe and Gardner (1972) considered isolates from pyracantha and from loquat
68 as belonging to the same *forma specialis* because they were able to infect both pyracantha
69 and loquat. Sanchez-Torres et al. (2009) showed that four isolates of *V. inaequalis* sampled on
70 apple were not able to cause disease on loquat. Gladioux et al. (2010) used six nuclear loci to
71 show that *V. inaequalis* populations from loquat and those from pyracantha have more
72 recently diverged than *V. inaequalis* populations from apple and those from pyracantha.
73 Recently, using whole genome sequencing data, Le Cam et al. (2019) confirmed that
74 populations of *V. inaequalis* isolated from apple, pyracantha and loquat represent distinct
75 genetic lineages.

76 The aim of this study was to investigate the host specificity of *V. inaequalis* isolates from apple,
77 pyracantha and loquat, and to test the hypothesis that crosses between *V. inaequalis formae*
78 *speciales* represent a disease emergence risk by generating hybrids pathogenic on both hosts,
79 or potentially with higher virulence on existing hosts.

80

81 **A strict host specificity was observed on apple and pyracantha, while most *V. inaequalis***
82 **isolates were able to cause disease on loquat.**

83 Thirty-two monoconidial isolates were recovered from scabbed leaves of apple (11 isolates,
84 named *Pomi*), pyracantha (11 isolates, named *Pyr*) and loquat (10 isolates, named *Loq*),

85 sampled from different locations in nine, seven and seven different countries, respectively
86 (Table S1). These isolates were checked as being non-clonal using eight Simple Sequence
87 Repeat markers (SSRs) developed on *V. inaequalis* (Table S2; Guérin et al. 2004; Guérin et al.
88 2007; Tenzer et al. 1999). These isolates were inoculated individually on two apple cultivars
89 (Gala and Top Red), four pyracantha cultivars (Kasan, Orange Charmer, Red Column and Soleil
90 d'or), and two loquat cultivars (Peluche and Alfonso). These cultivars were selected for their
91 susceptibility to scab (Bertrand et al. 1992; Bus et al. 2011; Sanchez-Torres et al. 2009). Plants
92 were grown in a glasshouse in a scab-free environment, and transferred into a growth
93 chamber for the tests of pathogenicity. Actively growing plants with expanding leaves which
94 are highly susceptible to *V. inaequalis* were used in six different experiments, because all
95 isolates could not be inoculated simultaneously across all hosts in the growth chamber. In
96 order to avoid cross-contamination during inoculation, plants were placed in polycarbonate
97 compartments and each compartment was inoculated with a single isolate. Each experiment
98 included isolates of each origin and at least one cultivar of each host, except experiment 1
99 where no pyracantha was present and experiment 6 where no loquat was present. Each
100 isolate was grown on a cellophane sheet placed on malt-agar medium at 17°C to obtain spores
101 (Caffier et al. 2014). Spores were suspended in water by shaking the cellophane sheet. After
102 filtration through medical gauze, the spore suspension was adjusted to a final concentration
103 of 2.5×10^5 spores ml⁻¹ and was inoculated using a manual sprayer on three plants of each
104 cultivar. After inoculation, plants were kept in darkness under a plastic sheet to maintain leaf
105 wetness. Climatic conditions in the growth chamber were set to 95% of relative humidity at
106 17°C for 48 hours. Afterwards, the plastic sheet was removed and the relative humidity was
107 reduced to 80% at day and 90% at night, with 12 hours of light per day. In order to evaluate

108 the ability of each isolate to cause disease, the presence/absence of scab symptoms on each
109 plant was scored at 14, 21 and 28 days post inoculation.

110 Not all *Pomi* and *Pyr* isolates caused symptoms on their respective hosts, while all *Loq* isolates
111 could infect both loquat cultivars (Figure 1). This result suggests the existence of resistance
112 genes in the apple cultivar Top Red and in all four pyracantha cultivars. No *Pyr* isolate was able
113 to cause disease on the apple cultivars, and no *Pomi* isolate was able to cause disease on any
114 of the four pyracantha cultivars (Figure 1), which agrees with previous results and confirms
115 the status of *formae speciales V. inaequalis* f.sp. *pomi* and *V. inaequalis* f.sp. *pyracantha* (Le
116 Cam et al. 2002). All *Pyr* isolates were able to cause disease on loquat, which agrees with data
117 from Raabe and Gardner (1972). However, the reciprocal was not true, as only one of the 10
118 *Loq* isolates was able to cause disease on pyracantha. Nine out of 11 *Pomi* isolates were able
119 to cause disease on the loquat cultivars Peluche and/or Alfonso. No *Loq* isolate was pathogenic
120 on apple except isolate 2305 that was previously described as resulting from a one-off shift
121 from apple to loquat, on the basis of its polymorphism at six nuclear loci (Gladieux et al. 2010).
122 To further investigate the pathogenicity of isolates on loquat and compare their
123 aggressiveness in relation to their host of origin, five isolates from each host were inoculated
124 on both loquat cultivars (three plants per cultivar) in the same experiment. Loquat plants were
125 randomized within three blocks to have in each block one plant replicate for each isolate and
126 each cultivar. We performed a quantitative scoring through visual evaluation of the
127 percentage scabbed leaf area 21 days post inoculation, from no disease to 100% leaf area
128 scabbed. The effect of the host of origin on the percentage scabbed leaf area was tested in R
129 4.0.3 (R Core Team 2020) using a linear mixed effect model with the LMER package
130 (Kuznetsova et al. 2017). The 'isolate' factor was treated as a random factor nested within the
131 'host of origin' factor. The data were Log transformed prior to statistical analysis. This

132 experiment showed that the *Pomi* isolates were significantly less aggressive on the loquat
133 cultivars Peluche and Alfonso than the *Pyr* and *Loq* isolates (Figure 2). There was no significant
134 difference in aggressiveness on loquat between *Pyr* and *Loq* isolates. This result agrees with
135 the scenario proposed by Gladieux et al. (2010), where populations from loquat and
136 pyracantha have diverged more recently than populations from apple and pyracantha.

137

138 **Most progenies from a cross between f.sp. *pomi* and f.sp. *pyracantha* can infect pyracantha,**
139 **but none can infect apple.**

140 As *V. inaequalis* f.sp. *pyracantha* and *V. inaequalis* f.sp. *pomi* isolates can be crossed *in vitro*
141 (Le Cam et al. 2002), we tested the hypothesis that hybrids may have increased virulence on
142 one of the hosts (apple or pyracantha) or that they may have an extended host range (able to
143 cause disease on both apple and pyracantha).

144 We performed five different crosses, three crosses between one *Pyr* isolate (1669, 1381 and
145 1383) and one *Pomi* isolate (EU-B04 and 2556) and two crosses between one *Pyr* isolate (1387
146 and 2299) and a mixture of five *Pomi* isolates (104 + 2416 + 2429 + 2444 + 2557, Table S1,
147 Table 1), on sterile foliar disks of apple cultivar Gala, as described in Le Cam et al. (2002). The
148 use of a mixture of isolates enabled us to diversify the origin of the *Pomi* parent. Mature
149 pseudothecia were recovered from the leaf disks and crushed individually to obtain a
150 suspension of ascospores that was spread on malt (10g per liter) agar. After 24 to 48 hours of
151 incubation at 17°C, germinated ascospores were picked up individually with a needle under a
152 stereomicroscope and transferred to new medium. To ensure the hybrid status of each
153 progeny, we performed a multilocus genotyping using eight SSRs (Table S2; Guérin et al. 2004;
154 Guérin et al. 2007; Tenzer et al. 1999; D. Sargent, *unpublished data*). Two hundred and fifty-

155 one progeny, checked as being hybrids by SSRs, and the parental isolates were grown on
156 cellophane sheets.

157 Spore suspensions from 37 progeny of the cross between 1669 and EU-B04 isolates and from
158 both parents were adjusted to a final concentration of 2.5×10^5 spores ml⁻¹ and inoculated in
159 a single experiment on three plants of pyracantha cultivar Kasan as described above.

160 Pyracantha plants were randomized within three blocks to have in each block one host
161 replicate for each isolate. At 15, 20, 23 and 27 days post inoculation, disease severity was
162 measured visually as the percentage of sporulating leaf area on the most scabbed leaf on each
163 plant. These 37 progeny produced a large range of disease levels on the pyracantha cultivar
164 Kasan (Figure 3). No progeny was more aggressive than the *Pyr* parent 1669. Only one progeny
165 isolate was unable to cause disease on Kasan, similarly to the parental *Pomi* isolate. All other
166 progenies gave a disease level that was intermediate between the parental *Pomi* isolate EU-
167 B04 (0% leaf area scabbed) and the parental *Pyr* isolate 1669 (90 % leaf area scabbed),
168 suggesting a complex genetic determinism for pathogenicity on this host. This result differed
169 from previous studies in other pathosystems, for which a few genes explained host specificity.
170 For example, two to four genes may be responsible for the pathogenicity of the two sibling
171 species *Ceratocystis manginecan* and *Ceratocystis fimbriata* on *Acacia mangum* and *Ipomoea*
172 *batatas* (Fourie et al. 2018; Fourie et al. 2019). Similarly, one to two genes have been shown
173 to be responsible for host specificity of *Magnaporthe oryzae* on foxtail millet in a cross
174 between an isolate pathogenic on wheat and an isolate pathogenic on foxtail millet (Murakami
175 et al. 2003), and three genes have been shown to be responsible for host specificity of *M.*
176 *oryzae* on wheat in a cross between an isolate pathogenic on rice and an isolate pathogenic
177 on wheat (Tosa et al. 2006). The loss of a single locus, AVR-Co39, was involved in the host shift
178 of *M. oryzae* from *Setaria* millet to rice (Couch et al. 2005). In the same way a single gene was

179 shown to be involved in the host specificity of *Blumeria graminis* f.sp. *secalis* on wheat (Tosa
180 1994).

181 The 251 progeny obtained from the five different crosses were inoculated on apple, either
182 four seedlings obtained from seeds collected from the apple cultivar Gala or six apple plants
183 grafted on the rootstock MM106 (three Gala and three Golden Delicious) at a concentration
184 of 2 to 4 x 10⁵ spores ml⁻¹. The viability of each inoculum was checked by counting germinated
185 and non-germinated spores over a total of 100 spores for each isolate 24 hours after the
186 deposition of the suspension on malt agar incubated in darkness at 17°C. Only progeny with a
187 concentration higher than 8 x 10⁴ viable spores ml⁻¹ were kept for analysis. Nine experiments
188 were needed to test all progeny. In each experiment, two parental isolates (a *Pomi* isolate and
189 a *Pyr* isolate) were used as control to check that the conditions were suitable for scab
190 infection. None of the 251 progeny were pathogenic on apple, *i.e.* all progeny behaved as the
191 *Pyr* parental isolates 1669, 1381, 1383, 1387 and 2299 on apple, whereas the *Pomi* isolate
192 used as a control gave at least 45% and 80% leaf area scabbed (mean of the most infected leaf
193 of three plants for each cultivar), on Gala and Golden Delicious plants, respectively. This result
194 is consistent with the study of Tosa et al. (2006) on *M. oryzae*, which showed that none of the
195 94 progeny obtained from a cross between an isolate pathogenic on rice and an isolate
196 pathogenic on wheat was virulent on rice. Comparative genomic analysis of these progeny
197 and parental isolates are needed to understand pathogenicity mechanisms and to identify
198 putative genes involved in specificity of *V. inaequalis* on apple and pyracantha.

199

200 **Towards a new strategy of biocontrol against apple scab.**

201 While hybridization between divergent populations or phytopathogenic species is often
202 depicted as a threat for agriculture because it can increase the host range of the pathogen or

203 enhance its virulence, we showed that hybridization between *formae speciales* of *V. inaequalis*
204 can be detrimental to fungal pathogens. If confirmed on more crosses, our finding that hybrids
205 are non-pathogenic on apple paves the way for a new biocontrol method to collapse pathogen
206 population size in apple orchards. Indeed, we propose to exploit this finding for biological
207 control based on a sexual hijacking strategy, similar to the sterile insect technique proposed
208 by Vanderplank (1944) to control the tsetse fly, which is largely developed now on many insect
209 species (Klassen and Curtis 2005).

210 As sexual reproduction is the main way for *V. inaequalis* f.sp. *pomi* to survive in winter in
211 temperate areas and as heterothallic mating is induced on dead leaves after leaf fall
212 (MacHardy 1996), a massive introduction in autumn of *Pyr* isolates representing the two
213 mating types by spraying of spores on the senescent leaves before or just after leaf fall should
214 lead to the production of hybrid ascospores unable to cause disease on apple in spring. This
215 strategy is expected to be efficient to reduce primary inoculum throughout most regions of
216 apple production, except maybe in regions with mild winter where *V. inaequalis* can survive
217 as asexual spores within buds (Holb et al. 2005; Passey et al. 2017). To maximize impact on
218 apple scab, this strategy may be associated with other methods, like sanitary measures and
219 use of resistant cultivars (Didelot et al. 2016).

220 Because the proposed biocontrol strategy is based on an organism which is widely distributed,
221 that has not undergone genetic modification, and whose effect is highly specific, we do not
222 expect any toxicity to humans or the environment, with the possible exception of pyracantha
223 shrubs located in the immediate vicinity of orchards. The efficiency of this sexual hijacking
224 strategy remains to be evaluated experimentally in orchards, and so does the risk of
225 emergence of hybrids pathogenic on apple trees or other cultivated Rosaceae hosts. Since this

226 strategy could be implemented with other fungi having *formae speciales* and a sexual stage,
227 our work opens a new area of research in the biocontrol of plant pathogenic fungi.

228 Patent for this novel strategy of biocontrol

229 Patent application PCT/FR2020/052580 « Méthode de bio-contrôle pour lutter contre la
230 propagation des champignons et oomycètes phytopathogènes », jointly filed on 21/12/2020
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244

245 **References**

- 246 Bertrand, H., Cadic, A., and Belin, J. 1992. *Pyracantha*: origine, description et clé de
247 détermination des principaux taxons. SEDA/PHM-Revue Horticole, Paris, France.
- 248 Brasier, C. M. 2001. Rapid evolution of introduced plant pathogens via interspecific
249 hybridization. *Bioscience* 51:123-133.
- 250 Bus, V. G. M., Rikkerink, E. H. A., Caffier, V., Durel, C. E., and Plummer, K. M. 2011. Revision
251 of the nomenclature of the differential host-pathogen interactions of *Venturia*
252 *inaequalis* and *Malus*. *Annu. Rev. Phytopathol.* 49:391-413.
- 253 Caffier, V., Lasserre-Zuber, P., Giraud, M., Lascostes, M., Stievenard, R., Lemarquand, A., van
254 de Weg, E., Expert, P., Denancé, C., Didelot, F., Le Cam, B., and Durel, C. 2014. Erosion
255 of quantitative host resistance in the apple - *Venturia inaequalis* pathosystem. *Infect.*
256 *Genet. Evol.* 27:481-489.
- 257 Carisse, O., and Rolland, D. 2004. Effect of timing of application of the biological control
258 agent *Microsphaeropsis ochracea* on the production and ejection pattern of
259 ascospores by *Venturia inaequalis*. *Phytopathology* 94:1305-1314.
- 260 Couch, B. C., Fudal, I., Lebrun, M. H., Tharreau, D., Valent, B., van Kim, P., Notteghem, J. L.,
261 and Kohn, L. M. 2005. Origins of host-specific populations of the blast pathogen
262 *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic
263 clones on rice and weeds of rice. *Genetics* 170:613-630.
- 264 Depotter, J. R. L., Seidl, M. F., Wood, T. A., and Thomma, B. 2016. Interspecific hybridization
265 impacts host range and pathogenicity of filamentous microbes. *Curr. Opin. Microbiol.*
266 32:7-13.
- 267 Didelot, F., Caffier, V., Orain, G., Lemarquand, A., and Parisi, L. 2016. Sustainable
268 management of scab control through the integration of apple resistant cultivars in a
269 low-fungicide input system. *Agric. Ecosyst. Environ.* 217:41-48.
- 270 Feurtey, A., and Stukenbrock, E. H. 2018. Interspecific gene exchange as a driver of adaptive
271 evolution in Fungi. *Annu. Rev. Microbiol.* 72:377-398.
- 272 Fourie, A., Wingfield, M. J., Wingfield, B. D., van der Nest, M. A., Loots, M. T., and Barnes, I.
273 2018. Inheritance of phenotypic traits in the progeny of a *Ceratocystis* interspecific
274 cross. *Fungal Biol.* 122:717-729.
- 275 Fourie, A., van der Nest, M. A., de Vos, L., Wingfield, M. J., Wingfield, B. D., and Barnes, I.
276 2019. QTL mapping of mycelial growth and aggressiveness to distinct hosts in
277 *Ceratocystis* pathogens. *Fungal Genet. Biol.* 131: 103242.
- 278 Gibson, A. K., Refregier, G., Hood, M. E., and Giraud, T. 2014. Performance of a hybrid fungal
279 pathogen on pure-species and hybrid host plants. *Int. J. Plant Sci.* 175:724-730.
- 280 Gladieux, P., Caffier, V., Devaux, M., and Le Cam, B. 2010. Host-specific differentiation
281 among populations of *Venturia inaequalis* causing scab on apple, pyracantha and
282 loquat. *Fungal Genet. Biol.* 47:511-521.
- 283 Guérin, F., Gladieux, P., and Le Cam, B. 2007. Origin and colonization history of newly
284 virulent isolates of the phytopathogenic fungus *Venturia inaequalis*. *Fungal Genet. Biol.*
285 44:284-292.
- 286 Guérin, F., Franck, P., Loiseau, A., Devaux, M., and Le Cam, B. 2004. Isolation of 21 new
287 polymorphic microsatellite loci in the phytopathogenic fungus *Venturia inaequalis*.
288 *Mol. Ecol. Notes* 4:268-270.

- 289 Holb, I. J., Heijne, B., and Jeger, M. J. 2005. The widespread occurrence of overwintered
290 conidial inoculum of *Venturia inaequalis* on shoots and buds in organic and integrated
291 apple orchards across the Netherlands. *Eur. J. Plant Pathol.* 111:157-168.
- 292 Jones, A. L., and Aldwinckle, H. S. 1991. Compendium of apple and pear diseases. St. Paul,
293 Minn.
- 294 Klassen, W., and Curtis, C. F. 2005. History of the Sterile Insect Technique. Pages 3-36 in:
295 Sterile Insect Technique. V. A. Dyck, J. Hendrichs and A. S. Robinson, eds. Springer,
296 Dordrecht, The Netherlands.
- 297 Kohl, J., Scheer, C., Holb, I. J., Masny, S., and Molhoek, W. 2015. Toward an integrated use of
298 biological control by *Cladosporium cladosporioides* H39 in apple scab (*Venturia*
299 *inaequalis*) management. *Plant Dis.* 99:535-543.
- 300 Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. 2017. LmerTest Package: Tests in
301 Linear Mixed Effects Models. *J. Stat. Softw.* 82:1-26.
- 302 Le Cam, B., Parisi, L., and Arène, L. 2002. Evidence of two *formae speciales* in *Venturia*
303 *inaequalis*, responsible for apple and pyracantha scab. *Phytopathology* 92:314-320.
- 304 Le Cam, B., Sargent, D., Gouzy, J., Amselem, J., Bellanger, M. N., Bouchez, O., Brown, S.,
305 Caffier, V., De Gracia, M., Debuchy, R., Duvaux, L., Payen, T., Sannier, M., Shiller, J.,
306 Collemare, J., and Lemaire, C. 2019. Population genome sequencing of the scab
307 fungal species *Venturia inaequalis*, *Venturia pirina*, *Venturia aucupariae* and *Venturia*
308 *asperata*. *G3.* 9:2405-2414.
- 309 Le Gac, M., and Giraud T. 2008. Existence of a pattern of reproductive character
310 displacement in Homobasidiomycota but not in Ascomycota. *J. Evol. Biol.* 21 :761-72.
- 311 MacHardy, W. E. 1996. Apple scab: biology, epidemiology, and management. APS press, St.
312 Paul, Minnesota, USA.
- 313 Menardo, F., Praz, C. R., Wyder, S., Ben-David, R., Bourras, S., Matsumae, H., McNally, K. E.,
314 Parlange, F., Riba, A., Roffler, S., Schaefer, L. K., Shimizu, K. K., Valenti, L., Zbinden, H.,
315 Wicker, T., and Keller, B. 2016. Hybridization of powdery mildew isolates gives rise to
316 pathogens on novel agricultural crop species. *Nat. Genet.* 48:201-205.
- 317 Murakami, J., Tomita, R., Kataoka, T., Nakayashiki, H., Tosa, Y., and Mayama, S. 2003.
318 Analysis of host species specificity of *Magnaporthe grisea* toward foxtail millet using
319 a genetic cross between isolates from wheat and foxtail millet. *Phytopathology*
320 93:42-45.
- 321 Passey, T. A. J., Robinson, J. D., Shaw, M. W., and Xu, X. M. 2017. The relative importance of
322 conidia and ascospores as primary inoculum of *Venturia inaequalis* in a southeast
323 England orchard. *Plant Pathol.* 66:1445-1451. R Core Team. 2020. R: A language and
324 environment for statistical computing. R Foundation for Statistical Computing
325 [<http://www.R-project.org>], Vienna, Austria.
- 326 Raabe, R. D., and Gardner, M. W. 1972. Scab of Pyracantha, Loquat, Toyon, and Kageneckia
327 *Phytopathology* 62:914-916.
- 328 Rieseberg, L. H., Archer, M. A., and Wayne, R. K. 1999. Transgressive segregation, adaptation
329 and speciation. *Heredity* 83:363-372.
- 330 Sanchez-Torres, P., Hinarejos, R., and Tuset, J. J. 2009. Characterization and pathogenicity of
331 *Fusicladium eriobotryae*, the fungal pathogen responsible for loquat scab. *Plant Dis.*
332 93:1151-1157.
- 333 Sivanesan, A. 1977. The taxonomy and pathology of *Venturia* species. *Bibliotheca*
334 *Mycologica*, Vaduz.

- 335 Stukenbrock, E. H., Christiansen, F. B., Hansen, T. T., Dutheil, J. Y., and Schierup, M. H. 2012.
336 Fusion of two divergent fungal individuals led to the recent emergence of a unique
337 widespread pathogen species. *Proc. Natl. Acad. Sci. U. S. A.* 109:10954-10959.
- 338 Tenzer, I., Degli Ivanissevich, S., Morgante, M., and Gessler, C. 1999. Identification of
339 microsatellite markers and their application to population genetics of *Venturia*
340 *inaequalis*. *Phytopathology* 89:748-753.
- 341 Tosa, Y. 1994. Gene-for-gene interactions between the rye mildew fungus and wheat
342 cultivars. *Genome* 37:758-762.
- 343 Tosa, Y., H Tamba, H., K Tanaka, K., and S Mayama, S. 2006. Genetic analysis of host species
344 specificity of *Magnaporthe Oryzae* isolates from rice and wheat. *Phytopathology*
345 96:480-484.
- 346 Vanderplank, F. 1944. Hybridization between *Glossina* species suggested new method for
347 control of certain species of *Tsetse*. *Nature* 154:607-608.
- 348

349 **Table 1. Description of the crosses between *Venturia inaequalis* f.sp. *pomi* (*Pomi*) and *V. inaequalis* f.sp.**
 350 ***pyracantha* (*Pyr*) and pathogenicity of 251 progeny on apple**

| <i>Pyr</i> isolate | <i>Pomi</i> isolate | Number of progeny non pathogenic on apple / tested on apple |
|--------------------|--|--|
| 1669 | EU-B04 | 76 / 76 |
| 1381 | 2556 | 44 / 44 |
| 1383 | 2556 | 44 / 44 |
| 1387 | mixture of isolates (104, 2416, 2429, 2444, 2557) | 49 / 49 |
| 2299 | mixture of isolates (104, 2416, 2429, 2444, 2557) | 38 / 38 |

351

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353 Legends of the figures

354 **Figure 1.** Pathogenicity of 32 isolates of *Venturia inaequalis* isolated from apple (*Pomi*), pyracantha (*Pyr*) or loquat
355 (*Loq*) on two cultivars of apple, four cultivars of pyracantha and two cultivars of loquat. Each isolate was
356 inoculated under controlled conditions on three plants of each cultivar. In red: presence of scab symptoms (with
357 light red indicating less than 5% of the leaf area with scab symptoms), in blue: absence of scab symptoms, in
358 white: not tested.

359 **Figure 2.** Boxplots of the percentage leaf area scabbed on two cultivars of loquat Alfonso and Peluche 21 days
360 after inoculation with isolates of *Venturia inaequalis* isolated from three hosts: apple (*Pomi* in dark grey),
361 pyracantha (*Pyr* in middle grey) and loquat (*Loq* in light grey). For each origin, five isolates were tested (*Pomi*:
362 190, 1395, 2315, 2367, EU-NL19; *Pyr*: 1669, 2267, 2269, 2299, 2309; *Loq*: 2258, 2264, 2300, 2303, 2304). Each
363 isolate was inoculated under controlled conditions on three plants of each cultivar. The box represents the lower
364 and upper quartiles. The thick horizontal line represents the median. The whiskers represent the highest and
365 lowest values falling within 1.5 times the interquartile range. The black dots represent outliers. *Pomi* isolates are
366 significantly less aggressive than *Pyr* and *Loq* isolates ($P = 0.0095$ and $P = 0.0164$, respectively, linear mixed effect
367 model).

368 **Figure 3.** Percentage leaf area scabbed on the pyracantha cultivar Kasan after inoculation with 37 progeny of
369 the cross between the *Venturia inaequalis* f.sp. *pomi* isolate EU-B04 (*Pomi* isolate) and the *V. inaequalis* f.sp.
370 *pyracantha* isolate 1669 (*Pyr* isolate). The pathogenicity of the parents EU-B04 and 1669 are presented on the
371 left and right sides, respectively. Each isolate was inoculated under controlled conditions on three plants of
372 cultivar Kasan, and scoring was performed at four dates: 15, 20, 23 and 27 days post inoculation.

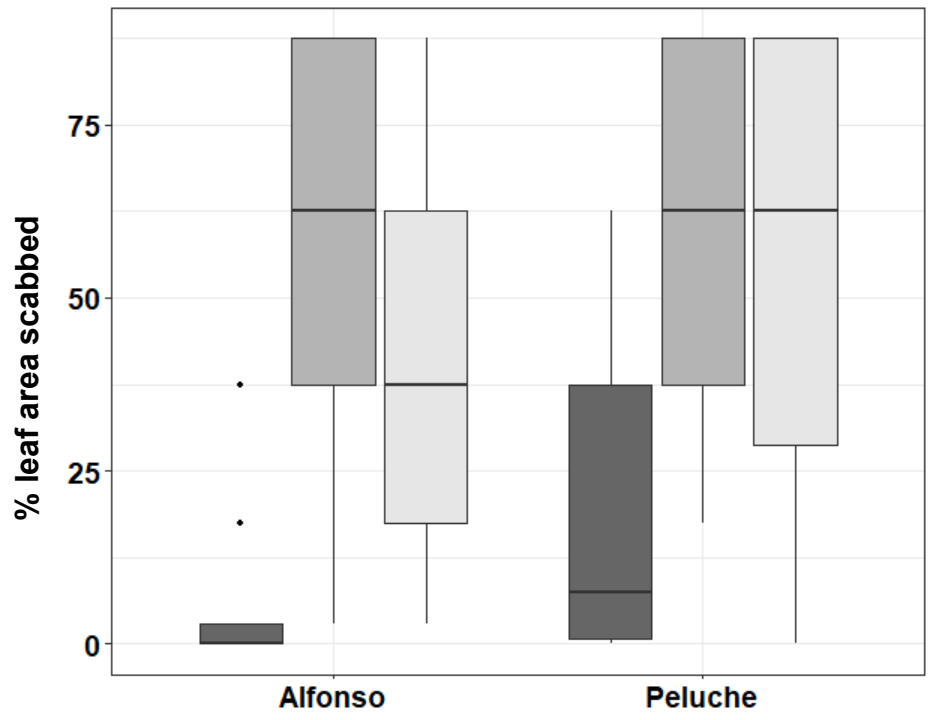
373

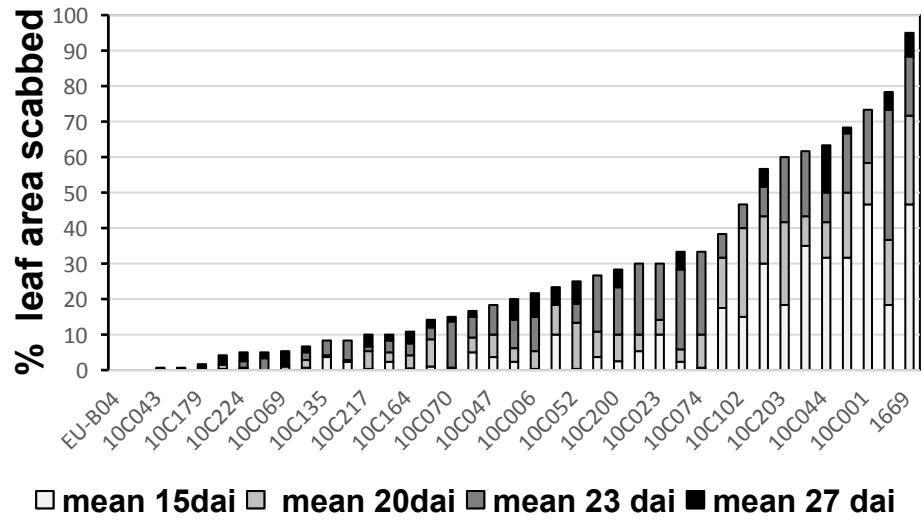
374 Titles of the e-Xtras

375 Table S1. Description of the origin of the *Venturia inaequalis* isolates used in the present study and indication of
376 their mating type

377 Table S2. Simple Sequence Repeats used to check the clone status of each sampled isolate of *Venturia inaequalis*
378 and the hybrid status of each progeny isolate

| | | Inoculated host | | | | | | | |
|----------------|--------------|-----------------|---------|------------|----------------|------------|-------------|-----------|-----------|
| | | Apple | | Pyracantha | | | Loquat | | |
| Isolate origin | Isolate name | Gala | Top Red | Kasan | Orange charmer | Red column | Soleil d'or | Peluche | Alfonso |
| <i>Pomi</i> | 190 | Red | | | Blue | Blue | Blue | Light Red | Light Red |
| | 301 | Red | | | Blue | Blue | Blue | Light Red | Light Red |
| | 912 | Red | | | Blue | Blue | Blue | Light Red | Light Red |
| | 1170 | Red | Blue | | Blue | Blue | Blue | Light Red | Light Red |
| | 1395 | Red | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2311 | Red | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2315 | Red | Blue | | Blue | Blue | Blue | Light Red | Light Red |
| | 2367 | Red | | | Blue | Blue | Blue | Light Red | Light Red |
| | EU-B04 | Red | | | Blue | Blue | Blue | Light Red | Light Red |
| | EU-D42a | Red | | | Blue | Blue | Blue | Light Red | Light Red |
| | EU-NL19 | Red | | | Blue | Blue | Blue | Light Red | Light Red |
| <i>Pyr</i> | 186 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 1381 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 1383 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 1387 | Blue | White | | Blue | Blue | Blue | Light Red | Light Red |
| | 1400 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 1669 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2267 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2269 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2299 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2307 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2507 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| <i>Loq</i> | 1389 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2258 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2260 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2262 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2263 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2264 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2300 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2303 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2304 | Blue | | | Blue | Blue | Blue | Light Red | Light Red |
| | 2305 | Blue | Red | | Blue | Blue | Blue | Light Red | Light Red |





1 **Table S1. Description of the origin of the *Venturia inaequalis* isolates used in the present**
2 **study and indication of their mating type**

| Host of origin | Isolate | Sampling year | Sampling country | Sampling cultivar | Mating type |
|------------------------------|--------------------------|---------------|------------------|----------------------------|----------------|
| Apple (<i>Pomi</i>) | 104 | 1978 | France | Golden Delicious | a ² |
| | 190 | 1987 | France | Golden Delicious | a ¹ |
| | 301 | 1988 | Germany | 81/19-53 | α ² |
| | 912 | 1992 | Spain | unknown | α ¹ |
| | 1170 | 1995 | The Netherlands | Discovery | a ¹ |
| | 1395 | 1995 | Colombia | Golden Dorsett | α ¹ |
| | 2311 | 2006 | Algeria | Gala | α ¹ |
| | 2315 | 2006 | USA | Gala | α ¹ |
| | 2367 | 2005 | China | Fuji | a ¹ |
| | 2416 | 2006 | France | J25 | a ¹ |
| | 2429 | 2006 | France | J25 | a ¹ |
| | 2444 | 2007 | France | Gala | a ¹ |
| | 2556 | 2006 | France | J108 | α ¹ |
| | 2557 | 2009 | France | E063 | a ¹ |
| | EU-B04 | 1998 | Belgium | Golden Delicious Reinhardt | α ² |
| | EU-D42a | 1998 | Germany | Prima | a ¹ |
| | EU-NL19 | 1998 | The Netherlands | Golden Delicious | α ¹ |
| Pyracantha (<i>Pyr</i>) | 186 | 1987 | Ireland | Red Column | a ² |
| | 1381 | 1998 | France | unknown | a ¹ |
| | 1383 | 1998 | France | unknown | a ¹ |
| | 1387 | 1998 | France | unknown | α ¹ |
| | 1400 | 1995 | Germany | unknown | a ¹ |
| | 1669 | 2001 | USA | unknown | a ² |
| | 2267 | 2003 | United Kingdom | Thornless | a ¹ |
| | 2269 | 2003 | Sweden | unknown | a ² |
| | 2299 | 2004 | France | unknown | α ¹ |
| | 2307 | 2004 | Chile | unknown | a ¹ |
| | 2507 | 2004 | Chile | unknown | α ² |
| | Loquat (<i>Loq</i>) | 1389 | 1999 | France | unknown |
| 2258 | | 2006 | Marocco | unknown | a ¹ |
| 2260 | | 2006 | Portugal | unknown | α ¹ |
| 2262 | | 2004 | France | unknown | a ¹ |
| 2263 | | 2006 | Spain | Algerie | α ² |
| 2264 | | 2006 | Spain | unknown | a ¹ |
| 2300 | | 2006 | Iran | unknown | a ¹ |
| 2303 | | 2004 | New Zealand | unknown | a ¹ |
| 2304 | | 2004 | New Zealand | unknown | a ¹ |
| 2305 | | 2003 | Chile | unknown | a ¹ |

3

4 ¹ Mating types defined with primer sequences F: CCCTCTGACTCTGAACAGC and R: TGTCGAAATCGTCACTCTGC for Mat a,
5 and primer sequences F: CACCTTTTCCAGCAGAAGG and R: CGATCTGCAGGAAGTGTCA for Mat α

6 ² Mating types defined by genome sequencing (Le Cam et al., 2019)

7

8 **Table S2. Simple Sequence Repeats used to check the clone status of each sampled isolate of**
 9 ***Venturia inaequalis* and the hybrid status of each progeny isolate**

| Primer name | Primer sequences (5' - 3') | Primer locus | Reference | Clone status | Hybrid status |
|-------------|--|--------------|----------------------|--------------|---------------|
| M1 | F: [HEX]TCGAGATCCTCAAACCTTCCTT R: TTTAACTGTGCGGCCTG | 1tc1a | Tenzer et al, 1999 | x | x |
| M2 | F: [FAM]CGATTGGGGATATGAAGACTT R: TTAGTAATCAAATCGCACCCA | 1tc1b | Tenzer et al, 1999 | x | x |
| M4 | F: [FAM]AGCGCTAGGTCGTGAAATC R: TTTCTGAAGTGTGTGGGACAT | 1aac3b | Tenzer et al, 1999 | x | |
| M15 | F: [TAMRA]GCACCTGCTCTGTCTATCTC R: AAGGTTCAAGGCACTGGAG | Vica9/152 | Guerin et al., 2004 | x | |
| M20 | F: [TAMRA]TGTCAGCCACGCTAGAAG R: CACCGGACGAATCATGC | Vicacg8/42 | Guerin et al., 2004 | x | x |
| M42 | F: [TAMRA]CCAGACCTCCTTATTCAC R: TAACTCCTGAAGACGGCATG | | Guerin et al., 2007 | x | x |
| M43 | F: [HEX]GCCTGGTTGTGGATCTGTC R: ATCCTGCTACATCGACCTTC | Viga7/116 | Guerin et al., 2004 | x | x |
| M51 | F: [HEX]TCGCGCATCACTATCTACAC R: AGACAGGAATGTGGTGGAAG | Vica9/X | Guerin et al., 2004 | x | |
| 283036 | F: [6- FAM]CAAGCTGAAAGGGCAAAGAG R: ATATGGGGCATTGGGAAACT | | Sargent, unpublished | | x |
| 276191 | F: [6- FAM]TGAGGGGAGAGATTTTGGTG R: ATGGTGGGGCTTGACTAATG | | Sargent, unpublished | | x |
| 139795541 | F: [HEX]GTGGTTATGTTGTGGGAGTGG R: GTA CTCTCTCGGCCTAAACTCG | | Sargent, unpublished | | x |

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