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

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Abstract

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

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

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

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

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

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

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

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

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

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

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

Not all *Pomi* and *Pyr* isolates caused symptoms on their respective hosts, while all *Loq* isolates could infect both loquat cultivars (Figure 1). This result suggests the existence of resistance genes in the apple cultivar Top Red and in all four pyracantha cultivars. No *Pyr* isolate was able to cause disease on the apple cultivars, and no *Pomi* isolate was able to cause disease on any of the four pyracantha cultivars (Figure 1), which agrees with previous results and confirms the status of *formae speciales V. inaequalis* f.sp. *pomi* and *V. inaequalis* f.sp. *pyracantha* (Le Cam et al. 2002). All *Pyr* isolates were able to cause disease on loquat, which agrees with data from Raabe and Gardner (1972). However, the reciprocal was not true, as only one of the 10 *Loq* isolates was able to cause disease on pyracantha. Nine out of 11 *Pomi* isolates were able to cause disease on the loquat cultivars Peluche and/or Alfonso. No *Loq* isolate was pathogenic on apple except isolate 2305 that was previously described as resulting from a one-off shift from apple to loquat, on the basis of its polymorphism at six nuclear loci (Gladieux et al. 2010).

To further investigate the pathogenicity of isolates on loquat and compare their aggressiveness in relation to their host of origin, five isolates from each host were inoculated on both loquat cultivars (three plants per cultivar) in the same experiment. Loquat plants were randomized within three blocks to have in each block one plant replicate for each isolate and each cultivar. We performed a quantitative scoring through visual evaluation of the percentage scabbed leaf area 21 days post inoculation, from no disease to 100% leaf area scabbed. The effect of the host of origin on the percentage scabbed leaf area was tested in R 4.0.3 (R Core Team 2020) using a linear mixed effect model with the LMER package (Kuznetsova et al. 2017). The ‘isolate’ factor was treated as a random factor nested within the ‘host of origin’ factor. The data were Log transformed prior to statistical analysis. This

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

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

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

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

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

Spore suspensions from 37 progeny of the cross between 1669 and EU-B04 isolates and from both parents were adjusted to a final concentration of 2.5 x 10^5 spores ml^{-1} and inoculated in a single experiment on three plants of pyracantha cultivar Kasan as described above. Pyracantha plants were randomized within three blocks to have in each block one host replicate for each isolate. At 15, 20, 23 and 27 days post inoculation, disease severity was measured visually as the percentage of sporulating leaf area on the most scabbed leaf on each plant. These 37 progeny produced a large range of disease levels on the pyracantha cultivar Kasan (Figure 3). No progeny was more aggressive than the *Pyr* parent 1669. Only one progeny isolate was unable to cause disease on Kasan, similarly to the parental *Pomi* isolate. All other progenies gave a disease level that was intermediate between the parental *Pomi* isolate EU-B04 (0% leaf area scabbed) and the parental *Pyr* isolate 1669 (90 % leaf area scabbed), suggesting a complex genetic determinism for pathogenicity on this host. This result differed from previous studies in other pathosystems, for which a few genes explained host specificity. For example, two to four genes may be responsible for the pathogenicity of the two sibling species *Ceratocystis manginecan* and *Ceratocystis fimbriata* on *Acacia mangum* and *Ipomoea batatas* (Fourie et al. 2018; Fourie et al. 2019). Similarly, one to two genes have been shown to be responsible for host specificity of *Magnaporthe oryzae* on foxtail millet in a cross between an isolate pathogenic on wheat and an isolate pathogenic on foxtail millet (Murakami et al. 2003), and three genes have been shown to be responsible for host specificity of *M. oryzae* on wheat in a cross between an isolate pathogenic on rice and an isolate pathogenic on wheat (Tosa et al. 2006). The loss of a single locus, AVR-Co39, was involved in the host shift of *M. oryzae* from Setaria millet to rice (Couch et al. 2005). In the same way a single gene was

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

The 251 progeny obtained from the five different crosses were inoculated on apple, either four seedlings obtained from seeds collected from the apple cultivar Gala or six apple plants grafted on the rootstock MM106 (three Gala and three Golden Delicious) at a concentration of 2 to 4 x 10^5 spores ml^{-1}. The viability of each inoculum was checked by counting germinated and non-germinated spores over a total of 100 spores for each isolate 24 hours after the deposition of the suspension on malt agar incubated in darkness at 17°C. Only progeny with a concentration higher than 8 x 10^4 viable spores ml^{-1} were kept for analysis. Nine experiments were needed to test all progeny. In each experiment, two parental isolates (a *Pomi* isolate and a *Pyr* isolate) were used as control to check that the conditions were suitable for scab infection. None of the 251 progeny were pathogenic on apple, *i.e.* all progeny behaved as the *Pyr* parental isolates 1669, 1381, 1383, 1387 and 2299 on apple, whereas the *Pomi* isolate used as a control gave at least 45% and 80% leaf area scabbed (mean of the most infected leaf of three plants for each cultivar), on Gala and Golden Delicious plants, respectively. This result is consistent with the study of Tosa et al. (2006) on *M. oryzae*, which showed that none of the 94 progeny obtained from a cross between an isolate pathogenic on rice and an isolate pathogenic on wheat was virulent on rice. Comparative genomic analysis of these progeny and parental isolates are needed to understand pathogenicity mechanisms and to identify putative genes involved in specificity of *V. inaequalis* on apple and pyracantha.

Towards a new strategy of biocontrol against apple scab.

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

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

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

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

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

Patent for this novel strategy of biocontrol

Patent application PCT/FR2020/052580 « Méthode de bio-contrôle pour lutter contre la propagation des champignons et oomycètes phytopathogènes », jointly filed on 21/12/2020 by INRAE, Université d'Angers, Institut National Supérieur des Sciences Agronomiques, Agroalimentaires, Horticoles et Paysage.

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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**

Pyr isolate	*Pomi* 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

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Legends of the figures

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

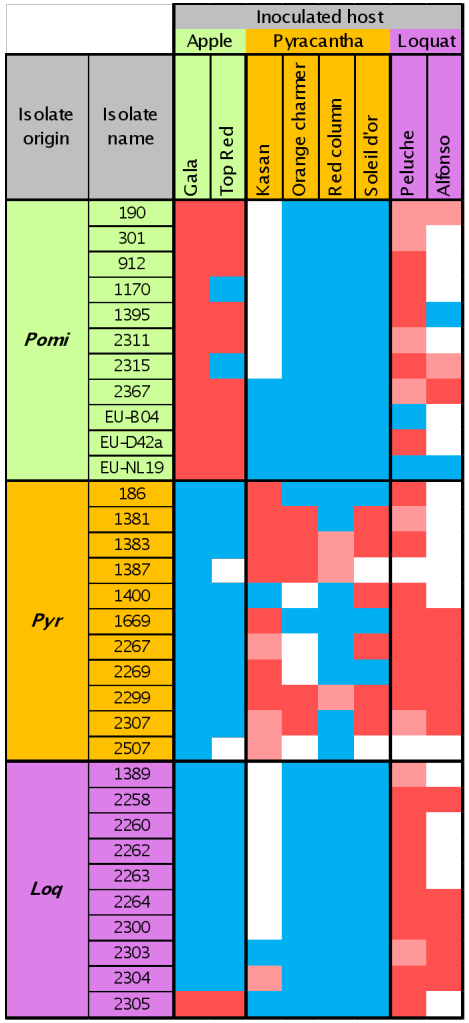
Figure 2. Boxplots of the percentage leaf area scabbed on two cultivars of loquat Alfonso and Peluche 21 days after inoculation with isolates of *Venturia inaequalis* isolated from three hosts: apple (*Pomi* in dark grey), pyracantha (*Pyr* in middle grey) and loquat (*Loq* in light grey). For each origin,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

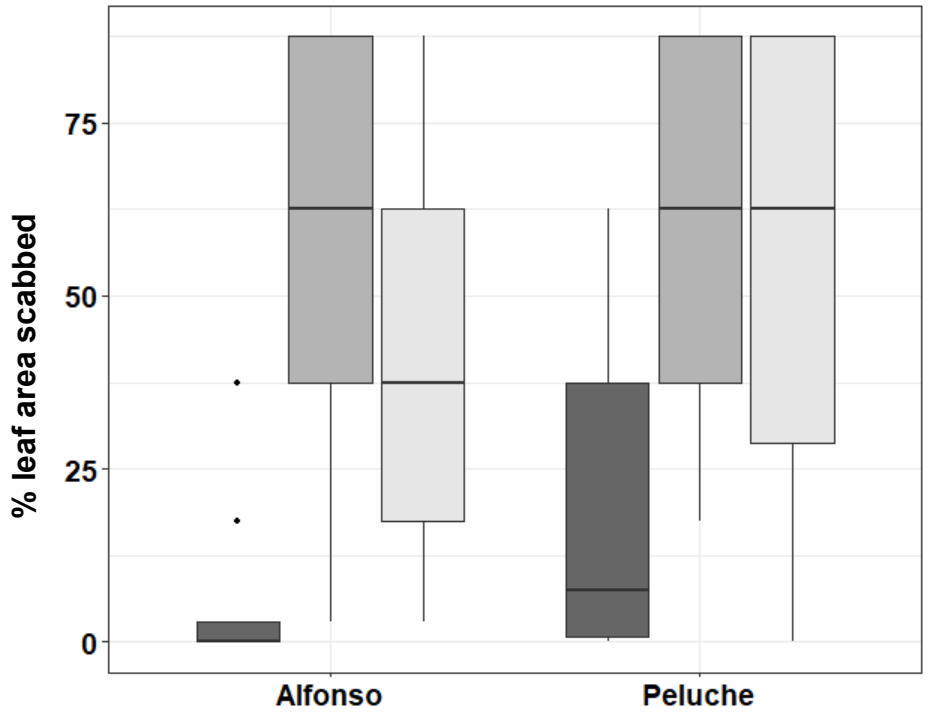
Figure 3. Percentage leaf area scabbed on the pyracantha cultivar Kasan after inoculation with 37 progeny of the cross between the *Venturia inaequalis* f.sp. *pomi* isolate EU-B04 (*Pomi* isolate) and the *V. inaequalis* f.sp. *pyracantha* isolate 1669 (*Pyr* isolate). The pathogenicity of the parents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

Titles of the e-Xtras

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

Table S2. Simple Sequence Repeats used to check the clone status of each sampled isolate of *Venturia inaequalis* 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
Pomi
190
301
912
1170
1395
2311
2315
2367
EU-B04
EU-D42a
EU-NL19
Pyr
186
1381
1383
1387
1400
1669
2267
2269
2299
2307
2507
Loq
1389
2258
2260
2262
2263
2264
2300
2303
2304
2305


% leaf area scabbed
75
50
25
0
Alfonso
Peluche

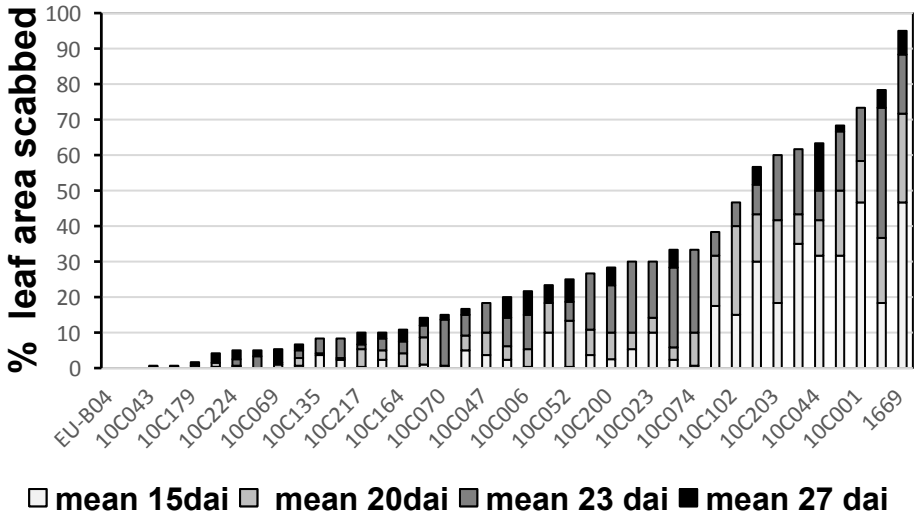

% leaf area scabbed
100
90
80
70
60
50
40
30
20
10
0
EU-B04
10C043
10C179
10C224
10C069
10C135
10C217
10C164
10C070
10C047
10C006
10C052
10C200
10C023
10C074
10C102
10C203
10C044
10C001
1669
mean 15dai
mean 20dai
mean 23 dai
mean 27 dai

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

Host of origin	Isolate	Sampling year	Sampling country	Sampling cultivar	Mating type
Apple (*Pomi*)	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 (*Pyr*)	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 (*Loq*)	1389	1999	France	unknown	a[1]
	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]

[1] Mating types defined with primer sequences F: CCCCTCTGACTCTGAACAGC and R: TGTCGAAATCGTCACTCTGC for Mat a, and primer sequences F: CACCTCTTTCCAGCAGAAGG and R: CGATCTGCAGGAACTTGTCA for Mat α

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

Table S2. Simple Sequence Repeats used to check the clone status of each sampled isolate of *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]TCGAGATCCTCAAACTTCCTT R: TTTTAACTGTGCGGCCTG	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: AAGGTTCAGGCACTGGAG	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: GTACTCTCTCGGCCTAAACTCG		Sargent, unpublished		x