Genetics of Host-Pathogen Relationships Between *Venturia inaequalis* Races 6 and 7 and *Malus* Species

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ABSTRACT

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Resistance to scab originating from *Malus floribunda* clone 821 is the most widely form of resistance used in apple breeding programs. A dominant gene, named *Vf*, was introgressed from this clone into recent cultivars, although the genetic determinants of the resistance of *M. floribunda* 821 are apparently more complex than a single gene. The appearance of new races overcoming the resistance of cultivars with the *Vf* gene, the parental clone, or both made it possible to undertake a genetic analysis of host-pathogen interactions. The segregation of resistance in progenies of crosses from 'Golden Delicious' $\times$ *M. floribunda* 821 and 'Golden Delicious' $\times$ 'Idared' into five strains of *Venturia inaequalis*—races 1 (strains 104, 1093, and 301), 6 (strain 302), and 7 (strain 1066)—demonstrated the

existence of a second dominant gene in *M. floribunda* 821. This gene, independent of *Vf*, was named *Vfh* because it seemed to induce a hypersensitive reaction. The results obtained with strain 1066, virulent to *M. floribunda* 821, allowed identification of another dominant gene, *Vg*, responsible for the resistance of 'Golden Delicious' to this strain. *Vg* is also carried by 'Florina', which was selected for its *Vf* resistance. The pathogenicity of a progeny originating from a cross between *V. inaequalis* strains 1066 and 301, characterized in vitro on leaf disks of differential genotypes, revealed two independent avirulence genes involved in the pathogenicity toward the *Vg* and *Vf* genes, respectively. These avirulence genes were named *Avr Vg* and *Avr Vf*. The host-pathogen interactions detected are consistent with a gene-for-gene relationship.

Additional keywords: *Malus* $\times$ *domestica*.

Apple scab, caused by *Venturia inaequalis* (Cooke) G. Wint., is the most important disease in apple orchards in most areas where apples are grown (23). It causes damage to leaves and fruits, with negative consequences on yield and fruit quality. Plant breeding programs in numerous countries have attempted to develop resistant varieties by introgressing apple scab resistance of the *Malus floribunda* Siebold ex. Van Houtte 821 clone (an ornamental crabapple) (16) into *Malus* $\times$ *domestica* Borkh. cultivars which have high fruit quality. The initial cross with 'Rome Beauty' was performed in 1914 in the United States by Crandall (6). Among the 66 cultivars resistant to scab obtained by plant breeders between 1970 and 1993, 51 to 53 owed their resistance to the *Vf* gene derived from one of the two hybrids of the original *M. floribunda* 821 $\times$ 'Rome Beauty' cross (7,21). Over the same period, only a few cultivars with resistance under polygenic control were selected, showing that monogenic resistance plays a major role in plant breeding programs for resistance to apple scab. Few cultivars carry genes different from *Vf*, which illustrates the small number of resistance sources used (7,21). The *Vm* gene from *M. micromalus* was introgressed into six selected cultivars, but was overcome quickly by race 5 of *V. inaequalis* (42).

According to the study of Hough et al. (16), the resistance of *M. floribunda* 821 is conferred by a single gene, heterozygous in this clone, with the dominant allele coding for resistance expressed as a hypersensitive reaction. The fact that the progeny of crosses between resistant (*Vf/vf*) and susceptible (*vf/vf*) parents did not provide the expected 1:1 segregation, but a distribution into five classes of symptoms, and a gradual decrease of the initial resistance ob-

served during backcrosses (17,34) suggest that this resistance involves more than one gene. However, its genetic determinants have not yet been specified (12,34).

In comparison to the number of studies on the improvement of apple trees for resistance to scab, few studies have dealt with the variability of pathogenicity in *V. inaequalis*. A good knowledge of the genetic interactions between the host and the pathogen seems essential to establish effective control strategies.

In 1971, Boone (4) reported the identification of 19 avirulence genes established according to interactions between some pathogenic strains and a range of hosts including both ornamental *Malus* species and apple tree cultivars such as McIntosh. However, the genetic determinants of the resistance carried by the different *Malus* clones were not known with certainty. The numerous avirulence genes identified by Boone (4) suggest a high potential number of pathotypes. In this pathosystem, only seven races were defined, according to their ability to overcome resistance sources used by plant breeders (29,32,33,36,42). Race 1 was defined as the population avirulent to all known resistance genes. The appearance of race 6 in Germany in 1993 (29) questions the stability of the resistance given by the *Vf* gene. It further confirms that the resistance of the *M. floribunda* 821 clone, which is resistant to race 6, is more complex than a single major gene (28,29). In 1994, in England, Roberts and Crute (33) found isolates of *V. inaequalis* virulent to *M. floribunda* 821 and to some *Vf* cultivars on an ornamental *M. floribunda*.

The appearance of these two new races that are virulent to the most-used resistance in apple breeding programs provides a good model for studying the genetic interaction between the host and the pathogen. The objective of this study was to better understand the genetic determinants of the resistance given by the *M. floribunda* 821 clone and to identify the *V. inaequalis* genes responsible for the pathogenicity to this clone and *Vf* resistant cultivars.

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MATERIALS AND METHODS

Genetic determinants of host resistance. *Plant material.* The progenies of two crosses were analyzed. The first progeny originated from the open pollination of 'Golden Delicious' by 'Idared' in an isolated orchard planted with these two cultivars. A total of 948 seedlings was tested. The second progeny originated from a cross between 'Golden Delicious' and *M. floribunda* 821 under controlled conditions. A total of 1,655 seedlings was tested. The characteristics of the parents of the two crosses are given in Table 1. For the 'Golden Delicious' × *M. floribunda* 821 cross, unopened flowers on two trees of 'Golden Delicious' were emasculated and pollinated with *M. floribunda* 821 pollen that was previously collected and dried. The two trees were isolated in the orchard under an insect-proof pollination cage. After harvest, the seeds originating from the two crosses were stratified in moist sphagnum moss for 90 days at 2°C. They were then sown in alveolar plates containing a mixture of peat and sand in equal proportions. The seedlings were grown in a greenhouse for 3 to 4 weeks at a temperature ranging from 18 to 20°C. At this stage, each plant had five to seven unfolded leaves and could be inoculated.

The three parental cultivars of the two crosses were grafted on seedling rootstocks. The scions originated from the INRA collection at Angers (Unité d'Amélioration des Plantes). The trees were grown in a greenhouse under the same conditions as the progenies of the crosses and were inoculated when they contained 13 to 25 leaves on actively growing shoots susceptible to the disease (35).

V. inaequalis strains and inoculum preparation. Three strains of race 1, one strain of race 6, and one strain of race 7 were used (Table 2). Strains 301 and 302 originated from the German orchard, where race 6 of the pathogen was identified for the first time (29). Strain 1066 was isolated from sporulating lesions observed on *M. floribunda* 821 after inoculation of the Fl 1 isolate described by Roberts and Crute (33). Previously, strain 1066 was named "English race" (2). However, with this race now being present in several European countries (27), we decided to call it race 7.

For each strain, a conidial suspension was obtained in Roux flasks according to the technique of Keitt and Palmiter (19). This conidial suspension was used to inoculate batches of seedlings obtained from the 'Golden Delicious' × 'Idared' cross as described by Parisi et al. (29). This method made it possible to collect scabbed leaves that were then dried and stored at -20°C. To inoculate the plants to be tested, a new conidial suspension was obtained by rinsing leaves with distilled water. The suspension was adjusted to 250,000 conidia per ml.

Inoculation and grading. The scions and batches of seedlings obtained from the crosses of *Malus* genotypes were inoculated and incubated in a growth chamber under the same conditions as those described by Parisi et al. (29). The incidence and severity of the disease on scions were scored after 14 days of incubation. The incidence corresponded to the percentage of leaves that was scabbed and the severity corresponded to the median of the scores for percentage of the leaf surface infected, rated on the scale of Croxall et al. (8) as modified by Parisi et al. (29). Four to five clonal copies

of each parent and 135 to 374 seedlings originating from the crosses were assessed for each strain studied.

For the progenies of the 'Golden Delicious' × *M. floribunda* 821 and 'Golden Delicious' × 'Idared' crosses, the type of symptoms observed for each seedling after 14 days of incubation was scored using the scale of Chevalier et al. (5). It contains six classes of symptoms: class 0 = no symptoms; class 1 = hypersensitivity (pin-point pits); class 2 = resistance, chlorosis, and necrosis, either together or separate, without sporulation; class 3a = weak resistance, chlorosis, and necrosis, either together or separate, with slight sporulation; class 3b = weak susceptibility, chlorosis, and necrosis, either together or separate, with sporulation; and class 4 = susceptibility, abundant sporulation without chlorosis and necrosis, either together or separate. According to the scale, we considered seedlings in classes 0 to 3a as resistant and seedlings in classes 3b and 4 as susceptible.

Statistical analysis. Chi-squares tests were used to perform pairwise comparisons of the distributions of the progeny from the 'Golden Delicious' × *M. floribunda* 821 cross into symptom classes for the various isolates used. When the values calculated in the contingency tables were smaller than 5, classes were grouped together. The resistant seedling/susceptible seedling ratios observed in the progenies for the two crosses were compared with the theoretical segregation values of one or two dominant genes using the chi-square test for goodness-of-fit. All the chi-squares and their associated probabilities were calculated using the STAT-ITCF software (version 5; Institut Technique des Céréales et du Fourrage, Paris, France). For the contingency tables with one degree of freedom, we did not use the Yates correction factor.

Genetic determinants of pathogenicity. *Crosses between V. inaequalis strains.* The two strains 1066 and 301 were crossed in vitro under controlled conditions. The method for obtaining perithecia and isolating monoascosporic strains was described by Hernández Castillo et al. (15). To check the viability of ascospores, perithecia were crushed and spread on water agar (15 g/liter; Agar-Agar Merck, Nogent-sur-Marne, France) and observed under a microscope after 24 h at 20°C. The germination percentage of the ascospores was greater than 99%. No aborted ascospores were observed in the asci from the perithecia.

A total of 58 monoascosporic strains was isolated from two perithecia. For each strain, axenic inoculum adjusted to 250,000 conidia per ml was obtained using Keitt and Palmiter's method (19). A supplementary centrifugation step (8,500 × g for 20 min at 4°C) was added to the protocol to remove the residual culture medium.

Testing the pathogenicity of monoascosporic strains. The pathogenicity of the monoascosporic strains was tested in vitro on leaf disks. This method and its correlation with the results obtained in vivo on whole plants were described by Benaouf and Parisi (2). The host range (Table 1) included 'Gala' (susceptible control), 'Golden Delicious' (resistance gene *Vg*), 'Priscilla' (gene *Vf*), 'Florina' (gene *Vf*), and *M. floribunda* clone 821. The grafted trees were grown in a greenhouse as described above. The most susceptible leaves (14) were sampled on growing shoots. Five leaf disks of each differential host were then inoculated in vitro with each monoascosporic strain

TABLE 1. Characteristics of the six apple clones used in analyses of host resistance and genetic determinants of pathogenicity

Clone	Name	Behavior in French orchards	Resistance gene identified
X 922	'Idared'	Susceptible	None
X 972	'Golden Delicious'	Susceptible	<i>Vg</i> ^a
X 4712	'Gala'	Susceptible	None
X 2851	'Priscilla'	Resistant	<i>Vf</i>
X 2775	'Florina'	Resistant	<i>Vf</i>
X 6518	<i>Malus floribunda</i> 821	Resistant	Progenitor of the <i>Vf</i> resistant selections

^a Benaouf and Parisi (1).

TABLE 2. Origins and characteristics of five strains of *Venturia inaequalis* used in the experimentation

Strain	Race	Geographic origin	Host of origin	Date of isolation
104	1	St. Lézin, France	'Golden Delicious'	1978
1093	1	Beaucouzé, France	'Jerseymac'	1994
301	1	Ahrensburg, Germany	81/19-53 ^a	1988
302	6	Ahrensburg, Germany	81/11-22 ^b	1988
1066 ^c	7	Beaucouzé, France	<i>Malus floribunda</i> 821	1993

^a Progeny (*vf/vf*) of a Prima (*Vf/vf*) × K1 40 (*vf/vf*) cross.

^b Progeny (*Vf/vf*) of a Prima (*Vf/vf*) × A143/24 (*vf/vf*) cross.

^c Strain originating from the Fl 1 isolate described by Roberts and Crute (33).

obtained from the cross and the two parent strains, according to the protocol of Benaouf and Parisi (2). After 19 days of incubation (18°C, 70 to 80 µE m⁻² s⁻¹ 16 h per day), the differential host was scored as susceptible to the corresponding strain if more than two disks showed sporulating lesion. The pathogenicity of the different strains was defined following two independent tests.

Statistical analysis. We compared the numbers of avirulent and virulent strains with the theoretical segregation values for one or two avirulence genes using the chi-square test for goodness-of-fit. All the chi-squares and their associated probabilities were calculated with the STAT-ITCF software.

RESULTS

Genetic determinants of host resistance. ‘Idared’ was susceptible to all the strains tested, while ‘Golden Delicious’ was susceptible to strains of races 1 and 6 and resistant to strain 1066 of race 7. The source of *Vf* resistance, *M. floribunda* 821, was resistant to strains of races 1 and 6, but was susceptible to strain 1066 of race 7 (Table 3). These results are in agreement with those of Parisi and Lespinasse (28) and Benaouf and Parisi (1).

The progeny of the ‘Golden Delicious’ × ‘Idared’ cross was susceptible to the four strains of *V. inaequalis* of races 1 and 6 with 97 to 100% of the seedlings with symptoms of classes 3b and 4 (Table 4). The resistant/susceptible ratio to strain 1066 fitted a 1:1 pattern, which corresponds to segregation of a single dominant gene.

Analyzing the distribution of the progeny of the ‘Golden Delicious’ × *M. floribunda* 821 cross into the different classes of symptoms showed that the distributions obtained with two strains, 104 and 1093, of race 1 (Fig. 1) were similar, which was confirmed by statistical analysis (Table 5). We observed a majority of seedlings with a hypersensitive reaction (class 1). The ratios between resistant and susceptible individuals corresponded to the segregation of two independent, dominant genes (Table 6). A more detailed analysis of the distribution into the different classes showed a distribution into the three groups of classes, (0, 1), (2, 3a), and (3b, 4), close to 2:1:1 (Fig. 1). This was confirmed by statistical analysis. However, the fit was better for strain 104 (Table 7).

TABLE 3. In vivo incidence and severity of the disease after inoculation of the parents of two apple crosses with five strains of races 1, 6, and 7 of *Venturia inaequalis*

Strain	Race	<i>Malus × domestica</i> cultivars				<i>Malus floribunda</i>	
		‘Idared’		‘Golden Delicious’		Clone 821	
		Incidence ^a	Severity ^b	Incidence	Severity	Incidence	Severity
104	1	34	4	30	2	0	0
1093	1	42	3	27	3	0	0
301	1	40	3	36	4	0	0
302	6	31	4	26	2	0	0
1066	7	33	3	0	0	21	3

^a Percentage of scabbed leaves.
^b On a scale from 0 (no visible symptoms) to 7 (scabbed leaf surface > 75%) (29).

TABLE 4. Segregations in the progeny of the ‘Golden Delicious’ × ‘Idared’ cross inoculated with five strains of races 1, 6, and 7 of *Venturia inaequalis*

Strain	Race	No. of seedlings tested	No. of seedlings		Expected ratio (R:S)	χ^2	<i>P</i> ^a
			Resistant (R)	Susceptible (S)			
104	1	219	0	219	0:1		
1093	1	135	4	131	0:1		
301	1	207	1	206	0:1		
302	6	128	1	127	0:1		
1066	7	259	131	128	1:1	0.035	0.852

^a Probability associated from a χ^2 test for goodness-of-fit to the theoretical ratios indicated.

Strain 301 gave a segregation pattern markedly different from those observed with the other two strains of race 1. In the progeny of the ‘Golden Delicious’ × *M. floribunda* 821 cross, the resistant/susceptible ratio observed was close to 1:1, and not 3:1 as expected (Table 6). This ratio could correspond to the segregation of a single dominant gene. However, the probability associated with the chi-square value was 0.034 (Table 6). The distribution into the different classes of symptoms was also different at *P* = 0.05 (Fig. 1; Table 5), with fewer individuals in class 1 and a majority in class 4. A similar type of distribution and segregation, i.e., that of a dominant resistance gene (*P* = 0.913) (Table 6), was observed with strain 302 of race 6 (Fig. 1; Table 5).

With strain 1066, we obtained a distribution significantly different (*P* = 0.05) from those obtained with the other four strains (Fig. 1; Table 5), with two peaks of individuals in classes 2 and 4. The resistant/susceptible ratio observed was of the 1:1 type (Table 6), i.e., the segregation of a single dominant gene.

Genetic determinants of pathogenicity. The pathogenicity of the two parental strains 301 and 1066 on the host range chosen to characterize the progeny is given in Table 8. The two strains induced susceptibility symptoms in ‘Gala’. Strain 301 was virulent to ‘Golden Delicious’ and avirulent to the *Vf* cultivars Priscilla and Florina and to *M. floribunda* clone 821. Strain 1066 was virulent to ‘Priscilla’ and *M. floribunda* 821 and avirulent to ‘Golden Delicious’. ‘Florina’ remained resistant to strain 1066, although this strain overcame the *Vf* gene. This cultivar appeared to have another resistance factor to strain 1066. These results were confirmed in pathogenicity tests of these two strains to leaf disks in vitro (data not shown).

All the monoascosporic strains from the 301 × 1066 cross that were tested in vitro on leaf disks were virulent to ‘Gala’ (Table 9).

TABLE 5. Pairwise comparisons of the distributions observed in classes of symptoms of the ‘Golden Delicious’ × *Malus floribunda* 821 progeny inoculated with strains of races 1, 6, and 7 of *Venturia inaequalis*

Strain 1	Strain 2	df	χ^2	<i>P</i> ^a
104 (race 1)	1093 (race 1)	5	5.0	0.416
104 (race 1)	301 (race 1) ^b	3	13.7	0.003
104 (race 1)	302 (race 6)	5	39.6	0.000
104 (race 1)	1066 (race 1) ^c	3	90.9	0.000
1093 (race 1)	301 (race 1) ^b	3	37.0	0.000
1093 (race 1)	302 (race 6)	5	53.0	0.000
1093 (race 1)	1066 (race 7) ^c	3	112.2	0.000
301 (race 1) ^b	302 (race 6)	3	8.2	0.042
301 (race 1)	1066 (race 7) ^d	2	38.4	0.000
302 (race 6)	1066 (race 7) ^c	3	80.3	0.000

^a Probability from a χ^2 test of independence.
^b When the calculated theoretical numbers were lower than 5, classes of the scale of Chevalier et al. (5) were grouped together: (0, 1) and (3b, 4).
^c When the calculated theoretical numbers were lower than 5, classes of the scale of Chevalier et al. (5) were grouped together: (0, 1) and (2, 3a).
^d When the calculated theoretical numbers were lower than 5, classes of the scale of Chevalier et al. (5) were grouped together: (0, 1), (2, 3a), and (3b, 4).

TABLE 6. Resistant/susceptible segregations obtained in the progeny of a ‘Golden Delicious’ × *Malus floribunda* 821 cross inoculated with five strains of races 1, 6, and 7 of *Venturia inaequalis*

Strain	Race	No. of seedlings tested	No. of seedlings		Expected ratio ^a (R:S)	χ^2	<i>P</i> ^b
			Resistant (R)	Susceptible (S)			
104	1	374	267	107	3:1	2.599	0.107
1093	1	307	230	77	3:1	0.001	0.975
301	1	303	170	133	1:1	4.520	0.034
302	6	322	162	160	1:1	0.012	0.913
1066	7	349	183	166	1:1	0.828	0.363

^a Based upon the model that resistance is conditioned by a single dominant gene.
^b Probability from a χ^2 test for goodness-of-fit to the theoretical ratios indicated.

An avirulence gene to the *Vg* gene of 'Golden Delicious' segregated in a 1:1 ratio in the progeny of the pathogen (Table 9). A 1:1 ratio was also observed to 'Priscilla' (gene *Vf*), indicating that an avirulence gene also segregated to this cultivar. The virulent and avirulent strains to 'Priscilla' were also virulent and avirulent, respectively, to the progenitor of *Vf* resistance, *M. floribunda* 821. The distribution between strains avirulent and virulent to 'Florina' fitted a 3:1 ratio (Table 9), which corresponds to the segregation of two avirulence genes. As this cultivar contains the *Vf* gene, one of the two avirulence genes was the one leading to the resistance of 'Priscilla'. The phenotypic distribution of the progeny indicates that all the strains that were virulent to 'Florina' were also virulent to 'Golden Delicious' (Table 10). The second avirulence gene segregating to 'Florina', therefore, seemed to be the same as that identified with 'Golden Delicious'.

The progeny of the 301 × 1066 cross showed equivalent numbers between four types of strains: two parental strains and two recombinant strains (Table 10). The two avirulence genes that segregated in this progeny were independent.

DISCUSSION

Genetic determinants of host resistance. The results regarding the parents of the crosses and the segregation analysis of the progenies of 'Golden Delicious' crossed with 'Idared' and *M. floribunda* 821 showed that the resistance of 'Golden Delicious' to strain 1066 of race 7 is provided by a single gene that is present in this cultivar in the heterozygous state. BÉnaouf and Parisi (1) named this gene *Vg*. Analyzing the distribution into the different classes of symptoms in the progeny of the 'Golden Delicious' × *M. floribunda* 821 cross showed that *Vg* codes for a resistance reaction, which is not a hypersensitive reaction but is expressed by chlorosis and leaf necrosis (class 2). The segregation of virulence in the progeny of the cross between *V. inaequalis* strains 301 and 1066 showed that 'Florina' also carries the *Vg* gene.

Analyzing the segregation of the progeny of the cross 'Golden Delicious' × *M. floribunda* 821 inoculated with strains 104 and 1093 showed that the resistance of *M. floribunda* 821 to race 1 is conditioned by two independent major genes. The 2:1:1 distribution

Fig. 1. Distribution of the progeny originating from the 'Golden Delicious' × *Malus floribunda* 821 cross into the different classes of symptoms after inoculation with five strains of *Venturia inaequalis*. The grading scale was that described by Chevalier et al. (5): class 0 = no symptoms; class 1 = hypersensitivity (pinpoint pits); class 2 = resistance, chlorosis, and necrosis, either together or separate, without sporulation; class 3a = weak resistance, chlorosis, and necrosis, either together or separate, with slight sporulation; class 3b = weak susceptibility, chlorosis, and necrosis, either together or separate, with sporulation; and class 4 = susceptibility, abundant sporulation without chlorosis and necrosis, either together or separate.

obtained in the three groups of classes, (0, 1), (2, 3a), and (3b, 4), suggests that one of these two genes induces a resistance reaction of class 1 (very few seedlings were observed in class 0). The resistance allele of this gene would have an epistatic effect on the second resistance gene, which would induce a reaction of class 2 or 3a. The segregation of resistance to races 1 and 6 in crosses between an inbred line of 'Florina' and to dihaploids from 'Golden Delicious' showed that *Vf* was expressed as a reaction of class 2 or 3a against avirulent strains (3). This was confirmed by the observations of plant breeders, who detected very few seedlings expressing pinpoint symptoms (class 1) in the progeny of recent *Vf/vf* selections crossed with a susceptible parent (20,34). We can hypothesize that the gene of *M. floribunda* 821 inducing a reaction of class 2 to 3a is the *Vf* gene, which was transmitted to recent selections. We propose that the other gene carried by this clone be named *Vfh*, with *h* indicating that the resistance induced by this gene is expressed as a hypersensitive reaction.

With strain 301, we obtained in the progeny of the 'Golden Delicious' × *M. floribunda* 821 cross a different segregation (close to 1:1) than that obtained with the other two strains of race 1 (3:1). This could indicate that a single resistance gene segregates in this progeny inoculated with this strain. As strain 301 was not virulent to cultivars carrying the *Vf* gene, the most probable hypothesis is that this strain is virulent to the *Vfh* gene. Demonstrating this pathogenic characteristic was impossible without analyzing the segregation of this cross, since our differential host range did not include a cultivar bearing *Vfh* only.

With strain 302 of race 6, we demonstrated that a single resistance gene segregated in the progeny of the 'Golden Delicious' × *M. floribunda* 821 cross. As this strain was characterized by its virulence to the *Vf* gene (28), our results suggest that it is avirulent to the gene *Vfh* carried by *M. floribunda* 821.

One of the first studies on the genetic determinants of the scab resistance of *M. floribunda* 821 was carried out with an inoculum composed of a mixture of *V. inaequalis* isolates (16), which could have introduced a bias in the assessment of the interaction between the host and the pathogen. In our study, all the tests for characterizing the progenies were carried out with monosporic strains of the pathogen, including those of races 6 and 7.

In 1989, Gessler (12) hypothesized the existence of two resistance genes in *M. floribunda* 821. He suggested that one of these genes induced a reaction of class 1 and was lost as early as the

TABLE 7. Test of a genetic hypothesis to explain the observed segregation of the progeny of a 'Golden Delicious' × *Malus floribunda* 821 cross after inoculation with two race 1 strains of *Venturia inaequalis*

Strain	No. of seedlings tested	Observed phenotype			Expected ratio R ₁ :R ₂ :S	χ^2	<i>P</i> ^a
		Classes 0, 1 (R ₁)	Classes 2, 3a (R ₂)	Classes 3b, 4 (S)			
104	374	164	103	107	2:1:1	5.743	0.057
1093	307	155	75	77	2:1:1	0.055	0.973

^a Probability from a χ^2 test for goodness-of-fit to the theoretical ratios indicated.

TABLE 8. In vivo incidence and severity of the disease in five apple clones after inoculation with the two parental strains used in the *Venturia inaequalis* cross

Apple clones	<i>Venturia inaequalis</i> strains			
	301 (race 1)		1066 (race 7)	
	Incidence ^a	Severity ^b	Incidence	Severity
'Gala'	23	3	22	3
'Golden Delicious'	36	4	0	0
'Priscilla'	0	0	21	3
'Florina'	0	0	0	0
<i>Malus floribunda</i> 821	0	0	21	3

^a Percentage of scabbed leaves.

^b Score varying from 0 (no visible symptoms) to 7 (scabbed leaf surface >75%) according to the grading scale described by Parisi et al. (29).

first crosses in plant breeding programs or rapidly overcome by the pathogen. The second gene seemed to induce reactions of classes 2 and 3 and to have been transmitted to apple selections. More recently, Parisi and Lespinasse (28) tested the susceptibility of different apple clones to race 6 by studying the pedigrees of different selections with resistance due to the *Vf* gene. They showed that one (F₂ 26829-2-2) of the two *Vf* resistant F₂ hybrids originating from the 'Rome Beauty' × *M. floribunda* 821 cross remained resistant to race 6; it was the most used in scab-resistant apple breeding programs. The second (F₂ 26830-2) was susceptible. The results obtained with the pedigrees having the F₂ 26829-2-2 hybrid as the initial resistant parent indicate that resistance to race 6 seems to be lost as early as the second cross in scab-resistant apple breeding programs. Our results confirm the existence of a second dominant gene in *M. floribunda* 821, which explains the resistance of this clone to race 6. Our results and those of Parisi and Lespinasse (28) also support the hypothesis of Gessler (12), that the *Vfh* gene must have been lost as early as the first crosses in breeding programs or overcome by the pathogen more rapidly than had the *Vf* gene. The fruits of *M. floribunda* 821 are very small and of poor quality (16). The *Vfh* gene may be closely linked to one of these undesirable characteristics; this linkage could explain the loss of the *Vfh* gene during the breeding programs.

Rousselle et al. (34) observed differences in segregation of the progenies of several crosses in comparison to the segregation expected from monogenic inheritance. They hypothesized that the *Vf* gene itself induces a resistance reaction that is expressed by a symptom of class 3. The symptoms of classes 1 and 2 could be explained by the presence of an increasing number of modifying genes. The distribution into the different classes of symptoms of the progeny of the 'Golden Delicious' × *M. floribunda* 821 cross that was inoculated with strains 301 and 302 did not show the disappearance of the class of symptoms corresponding to the gene overcome by the virulent strain. The hypersensitive or classes 2 and 3a symptoms observed with these two strains could correspond to a residual resistance due to the *Vfh* and *Vf* genes when overcome. These residual resistances have already been studied in other host-pathogen systems (25,30). This type of resistance seems to have an additive effect and shows the major role played by the genetic background of resistant cultivars, which is sometimes difficult to

TABLE 9. Pathogenicity of the progeny originating from the cross of strains 1066 and 301 of *Venturia inaequalis*, as expressed on in vitro leaf disks

Clones	No. of strains		Ratio (Avr:Vr)	χ^2	<i>P</i> ^a
	Avirulent (Avr)	Virulent (Vr)			
'Gala'	0	58	0:1		
'Golden Delicious'	30	28	1:1	0.069	0.793
'Priscilla'	27	31	1:1	0.276	0.599
<i>Malus floribunda</i> 821	27	31	1:1	0.276	0.599
'Florina'	42	16	3:1	0.207	0.649

^a Probability associated to the value of the χ^2 test for goodness-of-fit to the theoretical ratios indicated.

TABLE 10. Phenotypic distribution of the progeny originating from the cross of strains 1066 and 301 of *Venturia inaequalis*

Type of strain	No. of monosporic isolates ^a	<i>Malus</i> sp. clones			
		'Priscilla'	'Golden Delicious'	'Florina'	<i>M. floribunda</i> 821
301	12	— ^b	+ ^c	—	—
1066	15	+	—	—	+
Recombinant 1	15	—	—	—	—
Recombinant 2	16	+	+	+	+

^a There was no significant difference between the proportions observed and the theoretical proportions at *P* = 0.05 according to the χ^2 test for goodness-of-fit (df = 3, χ^2 = 0.621, *P* = 0.892).

^b Avirulence.

^c Virulence.

recognize (31). Another hypothesis is that the *M. floribunda* 821 clone also carries quantitative resistance. This resistance, which is masked by that of two major genes in the interaction with race 1, could reinforce the expression of the resistance of one of them in the interaction with strains 301 and 302. This hypothesis would be consistent with the gradual decrease of the initial resistance during the backcrosses observed by Rousselle et al. (34). The nature of the interaction between the two resistance genes, *Vf* and *Vfh*, as well as their behavior when resistance is overcome, needs to be investigated. Tracking of the qualitative or quantitative resistance by molecular markers in the resistant host could prove valuable in this respect.

Genetic determinants of pathogenicity. The segregation in the progeny of the cross between *V. inaequalis* strains 301 and 1066 revealed two independent avirulence genes conditioning virulence to the *Vf* and *Vg* resistance genes. These two genes were, therefore, named *Avr Vf* and *Avr Vg*. The 1:1 ratio of virulent and avirulent strains toward the *M. floribunda* 821 clone confirms the hypothesis of a single avirulence gene, *Avr Vf*, segregating in the cross. This cross did not allow us to show the genetic determinants of pathogenicity toward the *Vfh* resistance gene, as the two parent strains were virulent to this gene.

By demonstrating the two avirulence genes corresponding to the *Vg* and *Vf* resistance genes in the heterozygous host, we can propose genotypes for the *V. inaequalis* strains studied. Thus, strain 1066 and the progenies avirulent to 'Golden Delicious' expressed the *Avr Vg*⁺ allele, while strain 301 and the virulent progenies expressed the corresponding *Avr Vg*⁻ allele. Strain 301 and the progenies avirulent to 'Priscilla' expressed the *Avr Vf*⁺ allele, while strain 1066 and the virulent progenies expressed the corresponding *Avr Vf*⁻ allele.

Host-pathogen interactions. The resistance provided by the *Vf* gene has long been considered as durable by plant breeders, because this resistance was not overcome despite its intensive use for more than 50 years in apple breeding programs. However, the new cultivars carrying this resistance have not yet been cultivated in large areas.

Our study showed a host-pathogen interaction of Flor's (10) classic gene-for-gene type. The *Avr Vf* avirulence gene corresponded to the *Vf* resistance gene. For some pathosystems, studying the interactions from a molecular point of view, it has been possible to specify the mutations leading to the appearance of new pathotypes (18,39,40). For the *Cladosporium fulvum*-*Lycopersicon esculentum* pathosystem, it has been shown in the laboratory that the mutation of a single base pair in an avirulence gene could make the strain virulent toward a cultivar having the corresponding resistance gene (9,18). *V. inaequalis* has an obligatory sexual reproduction stage that involves annual genetic recombinations and a high variability of the pathogen toward a perennial host population. In such a context, the selection pressure exerted by a major resistance gene present in the host population should enhance the appearance of corresponding pathotypes. This might have occurred when the *Vm* resistance gene to apple scab was introduced and immediately overcome in orchards (42). The relatively late appearance of pathotypes virulent to the *Vf* resistant cultivars could be explained by a genetic mechanism presumably more complex than a single major gene and by the low selection pressure exerted by the host due to the limited areas where *Vf* cultivars were grown. Currently, race 6 remains in its initial orchard, while race 7, which appeared later in England, has been detected in other European countries (27).

This study allowed us to show the presence of a major resistance gene (*Vg*) in the genome of 'Florina', which has been selected for its *Vf* resistance (22). This gene was unwittingly introduced by plant breeders from 'Golden Delicious', susceptible to scab in the orchard, and often used in breeding programs. Breeders probably introduced other unidentified sources of resistance (major or polygenic genes) originating from resistant parents as well as from susceptible parents into the genome of resistant *Vf* selections.

Sierotzki et al. (37) confirmed the existence of specific interactions between a range of apple cultivars considered as susceptible in the orchard and different isolates of *V. inaequalis*. Some susceptible cultivars would thus carry resistance genes to *V. inaequalis* that are not identified because they can easily be overcome by the pathogen (13). The durability of *Vf* could be due to the combination of resistance genes other than the *Vf* gene in the genome of the selected cultivars, because the fungus must simultaneously develop pathogenic factors for virulence and aggressiveness.

Based on our results, it should be possible to define a new pathotype of *V. inaequalis* (strain 301) avirulent to the *Vf* gene and virulent to the *Vfh* gene. It has been difficult up to now to identify such a pathotype, because we did not have an apple clone with only the *Vfh* gene in the differential host range.

A better knowledge of the genetic variability of the host and the pathogen is necessary to establish effective disease control strategies: breeding for a durable resistance and strategies for resistance genes deployment. Genetic analysis is essential before assessing the nature of the host-pathogen interactions. Even if the gene-for-gene relationship is often suspected in different pathosystems, it has not always been proven (9). The genetic analyses carried out in this study encourage us to develop this approach to look for other avirulence genes and the corresponding resistance genes.

The identification of two avirulence genes in *V. inaequalis* constitutes a major step in the knowledge of the pathogenicity toward the *Vf* resistant cultivars. In the host, different research programs made it possible to localize genomic regions of practical interest, in particular the resistance gene *Vf*, on genetic maps (11,24,41). More recently, a dominant gene conferring resistance to strain 1066 of race 7 was localized on a linkage group of the map generated in the European Apple Genome Mapping project (24). This linkage group is different from that carrying the *Vf* gene. This gene would apparently correspond to the *Vg* gene due to the presence of 'Golden Delicious' in the genealogy of one of the two mapped parents and the great similarity of the resistance symptoms expressed (C. E. Durel, INRA, Centre d'Angers, Unité d'Amélioration des Plantes, France, *personal communication*).

In such a pathosystem in which interactions are clearly defined, the localization of molecular markers linked to avirulence genes can then be undertaken because *V. inaequalis* shows a high rate of polymorphism, as revealed by random amplified polymorphic DNA analysis (38). We are currently using the bulked segregant analysis (26) to determine whether this type of marker is reliable as a tool for characterizing pathotypes in a pathogen with an obligatory sexual phase and, eventually, to establish a genetic map of *V. inaequalis* to determine a cloning strategy for avirulence genes. All these studies should make it possible to investigate the host-pathogen relationships at the molecular level in the *V. inaequalis*/*Malus* pathosystem.

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