A New Race of *Venturia inaequalis* Virulent to Apples with Resistance due to the Vf Gene

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ABSTRACT

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The gene Vf from *Malus floribunda* 821 for resistance to scab (*Venturia inaequalis*), has been used successfully for 50 yr in apple breeding programs. Since 1984, scab symptoms have been observed in the field at Ahrensburg, Germany, on seedlings of apple cv. Prima that have been selected as resistant in the greenhouse. In 1988, small scab lesions were found on some Vf selections in the same orchard. The inoculum from Ahrensburg was compared with the inoculum currently used at Angers, France, for selecting apple seedlings for resistance to *V. inaequalis*. All Vf gene cultivars or selections tested were susceptible to the Ahrensburg inoculum, whereas *M. floribunda* 821 itself and the ornamental crabapple Evereste were resistant. The progeny from a cross between a resistant (Vf) and a susceptible cultivar segregate into the five expected classes to Angers inoculum, but were completely susceptible to Ahrensburg inoculum. These results indicate the urgency of diversifying the sources of resistance to *V. inaequalis* in new breeding strategies. The distinction should be made between the resistance of *M. floribunda* 821, which is resistant to Ahrensburg inoculum, and that of the named cultivars and selections, which are susceptible. The new race of *V. inaequalis* is named race 6.

Resistance of apples to *Venturia inaequalis* (Cooke) Wint. coded by the Vf gene, originating from *Malus floribunda* Siebold ex Van Houtte clone 821, has been used in breeding for the last 50 yr. Beginning with the resistant hybrids selected in the United States, breeders in several countries have released 30 scab-resistant cultivars between 1970 and 1989 (11). However, those cultivars have never been grown on a large scale. The genetics of this resistance are not completely understood. The first hypothesis about it was monogenic inheritance, with the dominant allele coding for resistance (5). The progeny originated from a resistant (Vf vf) and a susceptible parent (vf vf) would segregate at 1:1. In fact, such a progeny segregates into five classes (5): the resistant seedlings are distributed through four different classes according to the symptoms, the susceptible seedlings are in the fifth class. Substantial variations from the segregation expected are often observed. The hypothesis of Rousselle et al (13) was for one major gene whose expression could be modified by several minor genes with additive effect. These minor genes could be transmitted either by the resistant or the susceptible parent.

Vf resistance has been considered durable, because Vf cultivars have been free of scab for over 50 yr in the different countries where they have been grown.

Cultivar Prima susceptibility to *V. inaequalis* was first reported from Moldavia in 1981 (3). However, this report was not confirmed and, in contrast, a later article reported continuing resistance of Prima in Moldavia (7).

In 1988, at Ahrensburg, Germany, symptoms of scab were observed in the orchard on the cultivars Prima, Coop 7, 9, and 10, and on some seedlings previously selected as resistant in the greenhouse. However, these observations were not interpreted as a breakdown of the resistance coded by Vf (9).

Under a cooperative research program between France (INRA-Angers) and Germany (Institut für gartenbauliche Pflanzenzüchtung, Ahrensburg), studies on the variability of *V. inaequalis* populations were undertaken in both countries. One of the priorities of this program was to study the pathogenicity of Ahrensburg inoculum under controlled conditions and to determine whether *V. inaequalis* isolates from Ahrensburg could overcome the resistance coded by the Vf gene.

MATERIALS AND METHODS

Laboratory and growth chamber experiments.

Plants. Grafted potted trees and young apple seedlings were used. Nineteen clones were grafted onto seedling rootstocks and grown in pots in the greenhouse (Table 1). The mean number of leaves per shoot was 9–11 when the plants where inoculated. Apple seedlings originated from a cross between the scab-susceptible cv. Chevalier Jaune and the Vf-resistant cv. Baujade, or from a cross between two susceptible cvs. Golden Delicious and Granny Smith. The methods used to obtain the seedlings were described previously (12). The progeny of each cross were randomly divided into two batches, and each subpopulation tested with one type of inoculum; the seedlings had two to three leaves fully expanded when inoculated.

Inocula. In July 1988, *V. inaequalis* infected leaves were collected from seedlings of cv. Prima, in an orchard at the Ahrensburg research station. Some of these hybrids, selected as being scab resistant in the greenhouse, were found to be susceptible in the orchard (10).

Ten monoconidial isolates were obtained either from tree no. 22 from the cross 81/11 between Prima and A 143/24 (Jonathan × *Malus zumi* o.p.), or tree no. 53 from the cross 81/19 between Prima and Klon 40 (a seedling from open-pollinated cultivar Goldparmäne). These isolates were grown on malt agar medium (1% Cristomalt, Difal, Villefranche-sur-Saône, France). Eight isolates were selected for high sporulation. A conidial suspension was obtained from each isolate by Keitt and Palmiter's technique (8). A conidial suspension of each isolate was applied to young apple seedlings from the cross Golden Delicious × Granny Smith. Leaves with abundant sporulation were rinsed in distilled water to obtain a conidial suspension for each isolate. The inoculum Ahrensburg was a mixture of the eight isolates in equal concentrations. The inoculum Angers was composed of a mixture of aggressive isolates from different French orchards. This inoculum is currently used at INRA-Angers to select scab-resistant apple seedlings and is stored on frozen apple leaves. The inocula were each adjusted to a concentration of $2–3 \times 10^5$ conidia per milliliter.

TABLE 1. Characteristics of 19 apple clones inoculated with *Venturia inaequalis*

Clone	Name or code number	Characteristic
X 972	Golden Delicious	Susceptible to scab
X 4238	Topred	Susceptible to scab
X 2596	Prima	Vf resistant
X 2775	Florina	Vf resistant
X 3183	Baujade	Vf resistant
X 2851	Priscilla	Vf resistant
X 2811	Liberty	Vf resistant
X 4171	NY 61345-2	Vf resistant
X 3191	Idared × Prima	Vf resistant
X 6246	1082-201 × Prima	Vf resistant
X 6247	Britemac × Prima	Vf resistant
X 6517	Coop 28	Vf resistant
X 6518	*Malus floribunda* 821	Progenitor of all the Vf selections
X 2369	*Malus* × *"Perpetu"* Evereste	Ornamental crabapple, scab resistant
X 2225	9-AR2T196 (Vm/vm)	Differential host for race 5 (h5)
X 2250	TSR34T132	Differential host for race 2 (h2)
X 4811	Dolgo	Differential host for race 2 (h2)
X 2249	TSR33T239	Differential host for race 4 (h4)
X 2253	Geneva	Differential host for race 3 (h3)

Inoculation. All experiments were carried out in a growth chamber at INRA-Angers. Conidial suspensions were applied to runoff with a manual sprayer, at a rate of 50 ml per batch of 73 seedlings. Plants were incubated for 48 h in the dark at a temperature of 18 C; constant leaf wetness was maintained using an humidifier for the potted trees and using plastic sheeting covering the young seedlings. After incubation, the seedlings were grown 16–19 C, under a 16-h photoperiod of 60 μE s^{-1} m^{-2} with RH 70–80%. For the potted trees, the light intensity was 70 μE s^{-1} m^{-2} and RH was maintained at 85–98% with an intermittent intermittent humidifier.

Symptom assessment. Symptom assessment of potted trees was performed 14–17 days after inoculation. All leaves were assessed on each shoot, and severity was evaluated using a grading scale derived from Croxall et al (1) (Table 2).

Ten days after inoculation, the young seedlings were classifed according to the grading system of Hough et al (5): class 1: pinpoint pits with no sporulation; class 2: irregular chlorotic or necrotic lesions with no sporulation; class 3: few restricted sporulating lesions; class M: intermediate between class 2 and class 3; class 4: susceptible reaction, extensive and abundantly sporulating lesions.

The susceptible plants of class 4 were eliminated, and a second inoculation was performed to confirm the first one.

Sporulation evaluation. Sporulation was evaluated on young seedlings originating from a cross Golden Delicious × Granny Smith. Five randomly sampled seedlings were collected from a batch of 73; their foliar area was measured (Delta-T Area meter system 22804 R, Cambridge, England). Then, the leaves were shaken for 30 min in a dispersing solution (6 g/L tetra-sodium diphosphate, 1.2 g/L Bacto Peptone, pH 7.0), and the conidia were counted with a hemacytometer.

Symptom assessment. The results on potted trees were first recorded simply on the basis of the presence or absence of symptoms, and then quantified by the percentage of scabbed leaves. Disease severity for each clone was expressed by the median obtained from the scores of all leaves. For each clone, two to six trees have been evaluated (representing 4–22 shoots and 58–280 leaves per clone).

On young seedlings the results were expressed as the percentage of plants in each class. For the susceptible plants, the percentage of leaves with sporulating symptoms was calculated, and the sporulation was evaluated in terms of the number of conidia per square centimeter of leaf area.

Orchard observations.

Symptoms of scab on seedlings of cv. Prima were evaluated twice a year at Ahrensburg (Germany), from 1982 to 1990. Incidence and severity were recorded for all the trees, including no. 22 and no. 53, from which the scab isolates were obtained and were graded as: healthy, slightly infected, moderately infected, or severely infected.

RESULTS

The goal of the first experiment was to study the pathogenicity of the inoculum collected in the Ahrensburg orchard. The results showed that this inoculum was able to induce symptoms on all

TABLE 2. Grading scale used for the evaluation of severity of infection by *Venturia inaequalis*

Score	Percentage of the leaf area with sporulating symptoms (las)
0	No symptoms
1	$0 < las \leq 1$
2	$1 < las \leq 5$
3	$5 < las \leq 10$
4	$10 < las \leq 25$
5	$25 < las \leq 50$
6	$50 < las \leq 75$
7	$75 < las \leq 100$

the cultivars and Vf-resistant selections tested (Table 3). However, disease incidence and severity varied among the cultivars and selections. The cv. Golden Delicious was the most susceptible, but did not differ significantly from Florina and X 3191, which both contain the Vf gene. Prima expressed intermediate susceptibility to Ahrensburg inoculum, whereas the two selections X 6246 and X 6247 were least susceptible based on both incidence and severity.

In a second experiment, involving more cultivars and Vf selections, including the original source of the Vf gene *M. floribunda* 821, and also the differential hosts for races 2, 3, 4, and 5 (Table 4), inoculated cultivars and selections were susceptible to the Ahrensburg inoculum, but they were resistant to the Angers inoculum. In contrast, *M. floribunda* 821 and the ornamental crabapple Evereste were resistant to both inocula. No symptom was ever observed on the differential hosts, whatever the inoculum. Disease incidence and severity on a susceptible host were consistently higher with Angers inoculum than with the Ahrensburg inoculum (Table 5).

The difference in pathogenicity of the two inocula was confirmed by the study of the reaction of two progenies to inoculation (Table 6). All progeny from Golden Delicious × Granny Smith were susceptible to both inocula. The progeny from Chevalier Jaune × Baujade (susceptible × Vf resistant) segregated into the five different classes with the Angers inoculum, but were all susceptible to the Ahrensburg inoculum. Disease incidence on these young susceptible seedlings was identical with both inocula (Table 7). However, sporulation was more abundant on the young leaves inoculated with Angers inoculum than with Ahrensburg inoculum.

In the numerous observations at Ahrensburg from 1982 to 1990, as a whole, the progeny of Prima × A 143/24 was more susceptible than that of Prima × Klon 40 (Fig. 1). For the latter progeny, 20% of the trees remained scab-free even during the years with a high scab pressure (1985–1987). Nevertheless, tree no. 53 showed scab symptoms annually since 1984 and was even severely damaged in 1984, 1986, and 1987 (Fig. 2). All the progeny of Prima × A 143/24 were infected in 1985 and 1987, whereas all the trees were scab free in July 1982. Tree no. 22 showed scab symptoms every year since 1983. During 1988, when the samples were taken from the orchard, no particularly severe scab attack was noted.

TABLE 3. Pathogenicity of Ahrensburg *Venturia inaequalis* inoculum to Vf apple clones

Cultivar	Incidence: Presence of symptoms	Incidence: Percentage of scabbed leaves[y]	Severity: Score[z]
Golden Delicious	+	41.2 a	3
Florina	+	31.3 ab	3
X 3191	+	31.3 ab	3
Prima	+	20.7 b	2
X 6247	+	7.1 c	2
X 6246	+	6.9 c	1

[y] Percentages followed by the same letter do not differ significantly by Student *t* test at $P = 0.05$.
[z] The grading scale is described in Table 2.

TABLE 4. Comparison of the pathogenicity of Angers and Ahrensburg *Venturia inaequalis* inocula to apple clones

Cultivar or clone	Presence of symptoms of susceptibility: Angers inoculum	Ahrensburg inoculum
Golden Delicious	+	+
Topred	+	+
Baujade	−	+
Coop 28	−	+
Florina	−	+
Liberty	−	+
Priscilla	−	+
X 4171	−	+
Evereste	−	−
Malus floribunda 821	−	−
X 2225 (h5)	−	−
X 2250 (h2)	−	−
X 4811 (h2)	−	−
X 2249 (h4)	−	−
X 2253 (h3)	−	−

TABLE 5. Effect of the origin of *Venturia inaequalis* on the incidence and severity of scab symptoms on Golden Delicious apple

Inoculum	Incidence/percentage of leaves with sporulating symptoms[z]	Severity score
Angers	39.3 a	4
Ahrensburg	20.0 b	3

[z] Percentages followed by the same letter do not differ significantly by Student's *t* test at $P = 0.05$.

DISCUSSION

For the first time, these results clearly demonstrate that the Vf-resistance present in recently released cvs. and selections is overcome by *V. inaequalis*. However, the resistance carried by *M. floribunda* 821 and that of Evereste has remained effective.

The Ahrensburg inoculum, which induces symptoms on all the Vf-resistant selections tested, has a specific pathogenicity compared to the Angers inoculum; the latter does not induce symptoms on any of the same hybrids. The differential virulence of the Ahrensburg inoculum is confirmed by the study of the progeny of Chevalier Jaune × Baujade.

These results show that the Ahrensburg inoculum is less aggressive as yet, than the Angers inoculum on a host not having the Vf gene. However, these results need to be extended by studying the aggressiveness of each strain individually.

Low aggressiveness of these virulent strains could explain the nature of disease expression at the Ahrensburg orchard. Although the observations made from 1982 to 1990 clearly demonstrated that the disease occurred on most of the seedlings derived from Prima × A 143/24 and Prima × Klon 40, few symptoms were recorded on other Vf-resistant cultivars or selections. In 1988, for the first time, some symptoms appeared on Prima, Coop 7, 9, and 10. In 1990, most of the Vf selections were not infected; this includes cvs. Florina, Prima, Liberty, and Priscilla, which were susceptible in the growth chamber experiments with the

TABLE 6. Effect of the origin of *Venturia inaequalis* inoculum on the percentage of plants in the different classes of symptoms in two apple progenies

Cross	Percentage of plants in each class and total population[z]: Angers inoculum 1	2	M	3	4	T	Ahrensburg inoculum 1	2	M	3	4	T
Chevalier Jaune × Baujade	1	2.9	33.2	7.8	55.1	488	0	0	0	0	100	435
Golden Delicious × Granny Smith	0	0	0	0	100	144	0	0	0	0	100	141

[z] Class designations 1—pinpoint pits with no sporulation; 2—irregular chlorotic or necrotic lesions with no sporulation; M—intermediate class between 2nd and 3rd; 3—few restricted sporulating lesions; 4—susceptible reaction, extensive, and abundantly sporulating lesions.

TABLE 7. Effect of the origin of *Venturia inaequalis* inoculum on the incidence of symptoms and sporulation on susceptible apple seedlings from a progeny of Golden Delicious × Granny Smith

Inoculum	Percentage of leaves with sporulating symptoms	Sporulation/number of conidia/cm² of leaf area
Angers	93.7	$5.3\ 10^6$
Ahrensburg	93.5	$3.6\ 10^6$

Ahrensburg inoculum. In 1990, symptoms in the orchard were only seen on one Vf selection: 5002 (Laxton's Superb × Priam), but in 1991 several Vf selections were attacked, although often only to a low degree.

The differences between the symptoms seen in the growth chamber and in the orchard could be explained by the different environmental conditions, which are optimum for the disease in the growth chamber. Moreover, in the growth chamber the dose of the inoculum is higher than in the orchard. In addition, the isolates carrying the new virulence determinant could have a low fitness and could therefore be infrequent in the *V. inaequalis* orchard population. As the capacity for sexual reproduction is a prerequisite for the survival of the fungus during winter, its

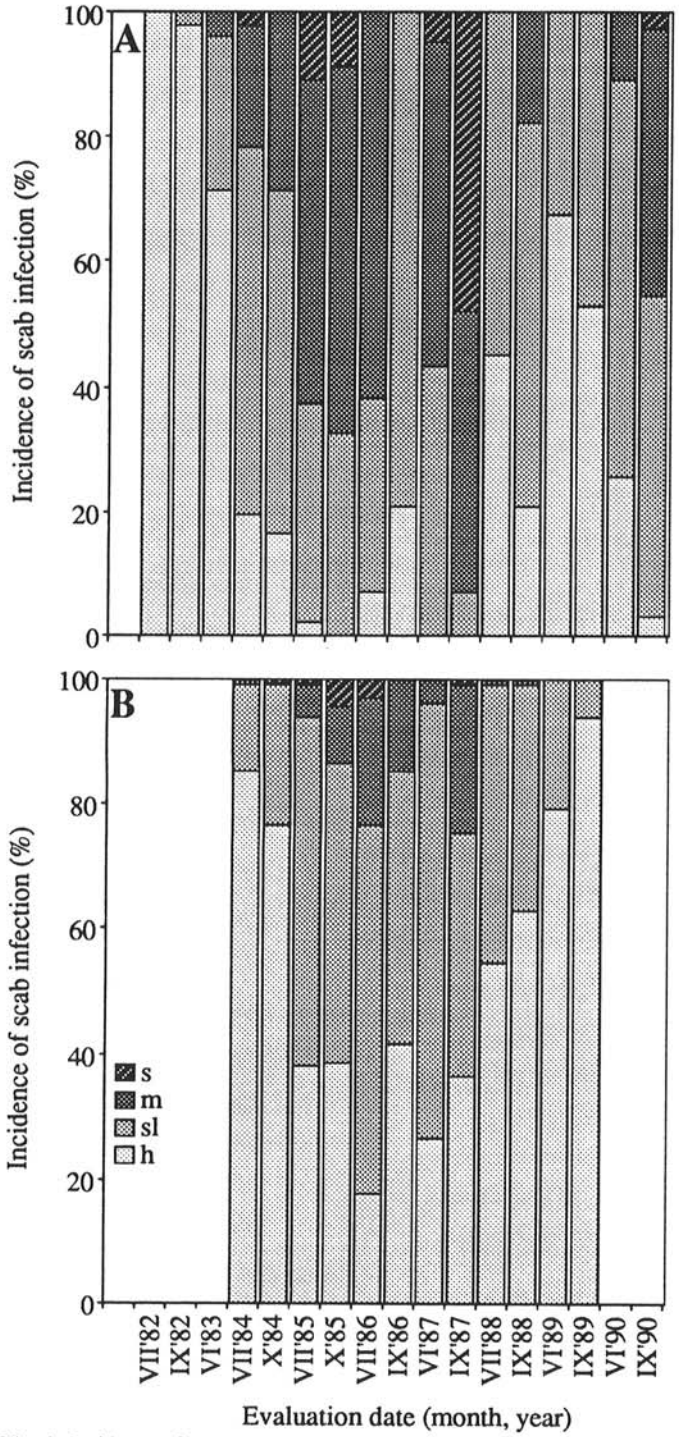

Fig. 1. Incidence of scab on apple seedlings in two progenies: **A,** Prima × A143/24 and, **B,** Prima × Klon 40, in the years 1982 to 1990. Grading scale: healthy (h), slightly infected (sl), moderately infected (m), and severely infected (s).

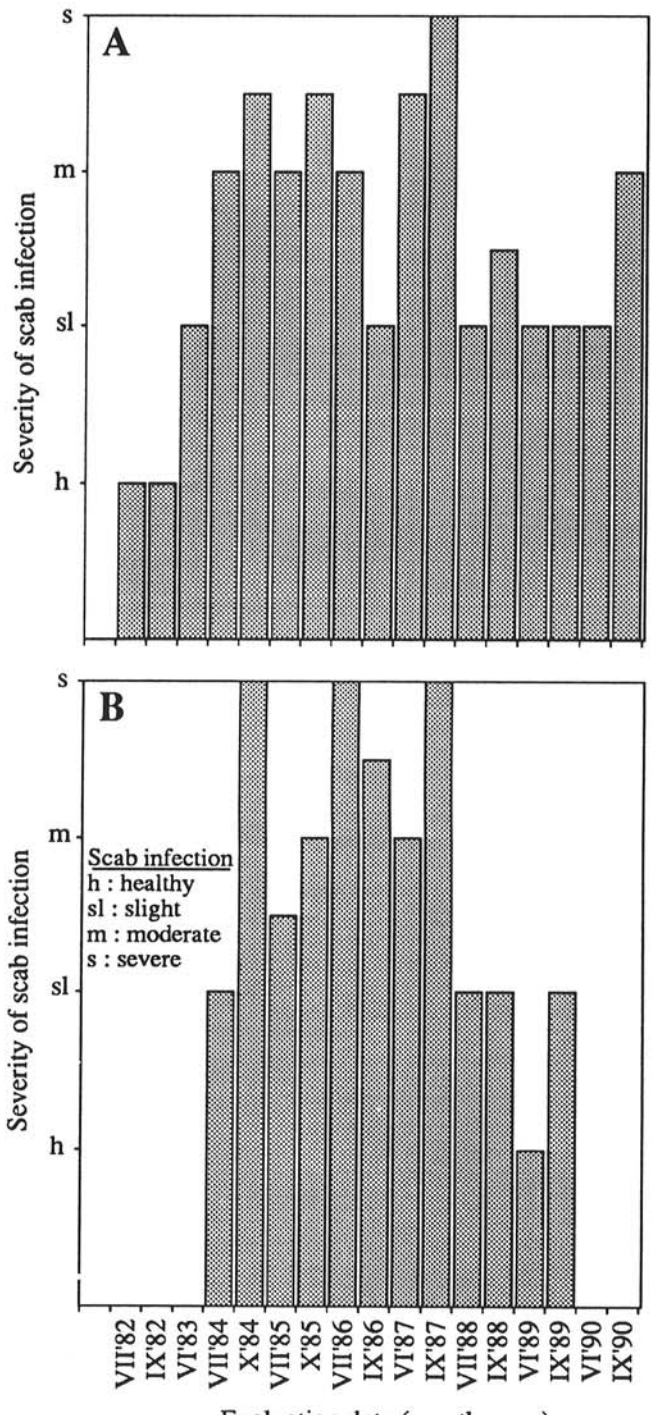

Fig. 2. Severity of scab on the single trees 81/11-22 (tree no. 22 of the progeny **A,** Prima × A143/24) and **B,** 81/19-53 (tree no. 53 of the progeny Prima × Klon 40) in the years 1982-1990.

study could help to explain their behavior; such research is now in progress.

It is significant that *M. floribunda* 821, the progenitor of all the current Vf selections, still appears to be resistant. This might be related to the long breeding process, from *M. floribunda* 821 to the release of new cultivars. In fact, this clone's resistance was originally expressed as a class 1 reaction (18). The two F_2, 26829-2-2 and 26830-2, gave a class 2 reaction (14). In the backcrosses with both of these F_2, type 2, 3, and M reactions were observed. Moreover, all the current Vf selections available originate from these two F_2. These results confirm the hypothesis that the resistance of *M. floribunda* 821 was not solely due to a single gene. Williams and Kúc (18) suggested that the initial level of resistance in *M. floribunda* 821 was controlled either by a block of closely linked genes, or by a single qualitative gene (class 3 reaction) closely linked to one or several modifier genes. Gessler (4) postulated that the resistance of *M. floribunda* 821 was controlled by two genes, one having been lost early in the breeding program, the other coding for type 2 or 3 reactions.

At present, we cannot decide in favor of one or the other of these hypotheses, but it seems clear that *M. floribunda* 821 resistance was rapidly eroded or partly lost.

In the case of Prima in particular, it is known that this cultivar has a relatively low level of resistance (class 3) (6). In its progeny, most of the seedlings have class M reaction (13); this could explain why the first observations of scab symptoms on previously resistant seedlings were made on Prima progenies.

The resistance of the ornamental crabapple Evereste to the Ahrensburg inoculum must also be underlined. Nevertheless, we do not know if the resistance of Evereste is due solely to the Vf gene; Evereste is a seedling from an open-pollinated Vf hybrid (2).

These results emphasize the urgency of defining new breeding strategies. Currently, out of 34 scab-resistant cultivars, 30 possess the Vf gene, and two the Vm gene which is overcome by *V. inaequalis* race 5. It is very important to diversify the sources of resistance and to combine at least two independent genes in the same cultivar; this strategy or others have to be developed as soon as possible even as the epidemiology and rate of spread of new virulent isolates remains to be determined.

This study emphasizes the difference between the genetic background of *M. floribunda* 821 (resistance not overcome) and that of new selections deriving from it (resistance overcome by race 6); it also stresses the meaning of the Vf symbol. Originally, Williams et al (17) used this symbol to name the resistance carried by six *Malus* species after having shown by allelism tests that it was controlled by the same gene. The letter V refers to *Venturia* and the letter f to *M. floribunda,* one of the first species used as a parent for breeding resistance. Using this same symbol for both *M. floribunda* 821 and the selections derived from it now seems wrong. The symbol Vf could be kept for naming the resistance of the recent selections.

Therefore, we propose that the new race of *V. inaequalis* that overcomes the Vf gene be named race 6; its differential virulence distinguishes it from the races 2, 3, 4, and 5 previously identified (15,16).

The still stable resistance of *M. floribunda* 821 is more complex than the resistance coded by the Vf gene.

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