European Journal of Plant Pathology **104:** 785–793, 1998.

Characterization of *Venturia inaequalis* pathogenicity on leaf discs of apple trees

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Accepted 29 July 1998

Key words: apple scab, host-pathogen interactions, *in vitro*, *Malus* × *domestica*, virulence

Abstract

Characterization of pathogenicity on whole plants is required to study host-pathogen interactions between *Malus* × *domestica* and *Venturia inaequalis*. We studied the reliability of an *in vitro* test of pathogenicity on leaf discs. Three strains of *V. inaequalis* (races 1, 6 and an English race) were inoculated *in vitro* onto a range of 16 *Malus* sp. clones including susceptible and resistant clones. The results were compared to those previously obtained *in vivo*. Resistant clones contained the main major known genes, i.e. *Va, Vb, Vbj, Vf, Vg, Vm* and *Vr.* Scab severity and the sporulation of the fungus were assessed 21 days after the inoculation date. The results indicated that it was possible to reproduce incompatible and compatible situations *in vitro*. A null severity corresponded to the avirulence of the strain for the clone considered. The resistance given by the *Vb, Vbj, Vf, Vg, Vm* and *Vr* genes were expressed *in vitro.* Only the clone carrying the *Va* gene and inoculated with the race 6 strain presented a compatible situation which was inconsistent with the observations on the whole plant. Improving this test will facilitate studies on the pathogenicity of *V. inaequalis* populations in relation to resistance genes of the host expressed *in vitro* as well as its genetic determinism.

Introduction

Apple scab is caused by an ascomycete fungus, *Venturia inaequalis* (Cooke) Wint. and is the main disease of apple in most producing countries (MacHardy, 1996). Controlling apple scab requires the application of numerous fungicide treatments during the growing season (Parisi et al., 1995). These treatments must be applied by taking into account ascospore projections and the risks of infection which are assessed as a function of leaf wetness and temperature (Mills and Laplante, 1951). Efficient control requires reliable information about biology and climate, provided for example by agricultural information networks. This is not possible in every production area. Moreover, control strategies based on the massive use of fungicides may face problems of resistance to some active ingredients and might disturb the ecosystem and pollute the environment (Köller, 1994).

Venturia inaequalis and *Malus* × *domestica* interactions seem to follow the gene for gene concept (Flor, 1956). In a differential range of resistant parents, Shay and Williams (1956) defined races 2, 3 and 4 of *V. inaequalis* whose pathogenicity seems to result from an avirulence gene interacting with a resistance gene of the host. But these host genes have not been clearly identified and named. In addition, the genetic determinism of pathogenicity could not be studied for race 5 (Hernàndez Castillo, 1990), which is virulent for the *Vm* gene originating from *Malus micromalus* (Williams and Brown, 1968).

Sierotzki et al. (1994) have confirmed the existence of specific interactions between a range of apple tree varieties considered as susceptible in the orchard and various scab isolates. Certain cultivars carrying unidentified scab resistance genes yet appear susceptible in the orchard because their resistance can easily be overcome by the pathogen (Gessler, 1994). One example is Golden Delicious which is considered

Table 1. Characteristics of 16 apple clones inoculated with strains of *Venturia inaequalis*

Clone	Name or code number	Characteristics	Resistance gene
X 4712	Gala	Scab susceptible	none
X 557	McIntosh	Scab susceptible	none
X 972	Golden Delicious	Scab susceptible	*Vg*
X 3069	Granny Smith	Scab susceptible	none
X 2250	TSR34T132	Differential host for race 2 (h2)	not named
X 2253	Geneva	Differential host for race 3 (h3)	not named
X 2249	TSR33T239	Differental host for race 4 (h4)	not named
X 2225	9-AR2T196	Differential host for race 5 (h5)	*Vm*
X 6518	*Malus floribunda* clone 821	Progenitor of *Vf* selections, scab resistant	*Vf* and *Vfh*
X 2369	Evereste	Ornamental crabapple, scab resistant	*Vf*
X 2775	Florina	Scab resistant	*Vf* and *Vg*
X 4077	Nova Easygro	Scab resistant	*Vf*[1]
X 2241	*Malus pumila* R 127.40.7A	Progenitor of *Vr* selections	probably 3 including *Vr*
X 7189	*Malus baccata*	Scab resistant	*Vb*
X 7218	*Malus baccata jackii*	Scab resistant	*Vbj*
X 7217	PI 172623	Scab resistant	*Va*

[1] Gianfranceschi et al., 1996.

moderately susceptible to scab in France (Parisi and Trillot, 1993). However, this cultivar carries a resistance gene (Hernàndez Castillo et al., 1994) which has been recently called *Vg* (Bénaouf and Parisi, 1997).

These examples emphasise the need for a better understanding of the host-pathogen interactions in this model. Two new races which are able to overcome the resistance given by the gene *Vf* (Parisi et al., 1993; Roberts and Crute, 1994), which is present in most currently available resistant varieties, have recently appeared. Thus, this study is urgent.

Obtaining apple varieties resistant to scab has long been one of the priorities of plant breeding programmes in several countries. The selection for resistance is made at the seedling stage with a mixed inoculum whose virulence is not always known.

Most studies on pathogenicity of *V. inaequalis* populations consist of *in vivo* tests on grafted potted trees, which limits the number of strains and differential hosts which can be assessed during an experimental season. Different attempts have been made to obtain miniaturized tests on detached leaves (Yepes and Aldwinckle, 1993b) and plants raised *in vitro* or leaves originating from the latter (Guillaumès et al., 1995; Ivanicka et al., 1996). These studies did not make it possible to obtain a simple and reliable test which can be used over a wide range of hosts without *in vitro* culture.

We based our study on tests used in other pathological systems, which make it possible to rapidly assess the virulence of many strains or isolates on host leaf discs, for example *Melampsora larici-populina/Populus* sp. (Pinon, 1992). We evaluated the reliability and the interest of this test in our pathological system using three strains of *V. inaequalis* with known pathogenicity on a range of differential hosts.

Materials and methods

Plant material

The range of hosts consisted of 16 *Malus* × *domestica* and *Malus* sp. clones. These clones were chosen for their susceptibility or resistance to the pathogen (Table 1). The resistant clones carried the main major genes currently known, i.e. *Va, Vb, Vbj, Vf, Vm* and *Vr* (Williams and Kuc, 1969). Two resistance genes have been designated recently, i.e. *Vg* which is present in Golden Delicious (Bénaouf and Parisi, 1997) and a gene responsible for a hypersensitive reaction, *Vfh*, present in *M. floribunda* 821 (Bénaouf et al., 1997). The scions of the different clones originated from the INRA collection at Angers (Station d'Amélioration des Espèces Fruitières et Ornementales) and were grafted on rootstocks of seedling apple trees. Trees were grown in pots in the greenhouse. The most susceptible leaves (the 3 youngest unfolded

Table 2. Origin and characteristics of the three *Venturia inaequalis* strains tested on apple clones

Strain	Origin	Host	Characteristic
104	Saint Lézin, France, 1978	Golden Delicious	Race 1
305	Ahrensburg, Germany, 1988	81/11-22[1]	Race 6
1066	Beaucouzé, France, 1993	*Malus floribunda*, clone 821	Monoconidial strain from Fl 1, English Race[2]

[1] Tree No. 22 of the progeny Prima (*Vf/vf*) × A143/24 (*vf/vf*).
[2] Fl 1 isolate was described by Roberts and Crute in 1994.

Table 3. In vivo pathogenicity of three *Venturia inaequalis* strains on 16 apple clones. Data from Parisi and Lespinasse, 1996 and Bénaouf and Parisi, 1997

	Strain 104 (race 1)			Strain 305 (race 6)			Strain 1066 (English race)		
Clone	I[1]	S[2]		I	S		I	S	
Gala	48	2	(C)[3]	nt[4]	nt		15	2	(C)
McIntosh	31	2	(C)	25	3	(C)	13	3	(C)
Golden Delicious	23	4	(C)	22	3	(C)	0	0	(IC)
Granny Smith	0	0	(IC)	0	0	(IC)	0	0	(IC)
TSR34T132 (h2)	0	0	(IC)	0	0	(IC)	0	0	(IC)
Geneva (h3)	0	0	(IC)	0	0	(IC)	0	0	(IC)
TSR33T239 (h4)	0	0	(IC)	0	0	(IC)	0	0	(IC)
9-AR2T196 (h5, *Vm*)	0	0	(IC)	0	0	(IC)	0	0	(IC)
M. floribunda 821 (*Vfh, Vf*)	0	0	(IC)	0	0	(IC)	8	3	(C)
Evereste (*Vf*)	0	0	(IC)	0	0	(IC)	7	2	(C)
Florina (*Vf, Vg*)	0	0	(IC)	10	1	(C)	0	0	(IC)
Nova Easygro (*Vf*)	0	0	(IC)	19	2	(C)	14	1	(C)
M. pumila R127.40.7A (*Vr*)	0	0	(IC)	0	0	(IC)	0	0	(IC)
M. baccata (*Vb*)	0	0	(IC)	0	0	(IC)	0	0	(IC)
M. baccata jackii (Vbj)	0	0	(IC)	0	0	(IC)	0	0	(IC)
PI 172623 (*Va*)	0	0	(IC)	0	0	(IC)	0	0	(IC)

[1] Incidence, percentage of scabbed leaves.
[2] Severity, score from 0 to 7, grading scale of Parisi et al. (1993).
[3] (C) compatibility or (IC) incompatibility.
[4] Not tested.

leaves) were sampled on growing shoots (Gessler and Stumm, 1984). They were disinfected superficially under sterile conditions in sodium hypochlorite solution (1% active chlorine) for 1 min 30 s, then rinsed three times in sterile deionised water. They were then dried between two sheets of sterile filter paper and cut into discs of 1 cm in diameter. The average number of discs obtained from a leaf was 3. Leaf discs were laid in Petri dishes containing 1% water agar on the basis of 10 discs per clone and per *V. inaequalis* strain in each dish.

Strains of V. inaequalis *and inoculum preparation*

The origin and characteristics of the three monoconidial strains tested are described in Table 2. Strain 1066 was isolated from lesions of the *M. floribunda* 821 clone inoculated with the Fl 1 isolate (Roberts and Crute, 1994). The pathogenicity and the virulence pattern of these three strains were characterized on whole plants of the 16 *Malus* clones according to the technique described by Parisi et al. (1993). Table 3 shows the pathogenicity of the three *V. inaequalis* strains on the 16 *M.* × *domestica* and *M.* sp. clones (Parisi and Lespinasse, 1996; Bénaouf and Parisi, 1997). The Golden Delicious cultivar presents a specific interaction with strain 1066. This variety has a major resistance gene called *Vg*, which is also present in the Florina cultivar (Bénaouf and Parisi, 1997). The Gala cultivar, which was not tested with strain 305 in this study, is susceptible to this strain (L. Parisi, unpubl.).

A sterile conidial suspension was obtained for each strain according to the Keitt and Palmiter tech-

nique (1938). Thirty milliliters of the suspension was centrifuged at 8609 g and 4 °C for 20 min. The supernatant was eliminated and the residue containing the conidia was kept. It was then diluted in 10 ml of sterile water. This step made it possible to eliminate the residual culture medium. The conidial suspensions were adjusted to 2.5×10^5 spores ml^{-1} and kept at − 18 °C.

Inoculation and disease evaluation

The 16 apple clones were inoculated simultaneously with a conidial suspension (2.5×10^5 ml^{-1}) with a sprayer used for chromatography (Sigma, Saint-Louis, USA). The Petri dishes containing the leaf discs were incubated in the dark at 17 °C for 48 h for the germination of conidia and the formation of appressoria. Leaf discs were then dried between two sheets of sterile filter paper and distributed into 10 Petri dishes containing 1% water agar; each Petri dish contained 16 leaf discs, one disc of each clone of *Malus* sp. These dishes were placed in an illuminated growth chamber (Sanyo MLR 350, Japan) under the following experimental conditions: photoperiod of 16 h (light intensity: 50 to 80 μmol $s^{-1}m^{-2}$) – 8 h of darkness, constant temperature 18 ± 1 °C.

Symptoms were assessed 21 days after inoculation by observing leaf discs with a binocular microscope (Leica Wild M3Z, OSI, Elancourt, France) at a magnification × 100 with incident light (Schoti KL500 electronic, OSI, France). The scabbed surface was scored using the grading scale described by Parisi et al. (1993): score 0 = no visible symptom; score 1 = 0% < percentage of scabbed leaf surface (sls) ⩽ 1%; score 2 = 1% < sls ⩽ 5%; score 3 = 5% < sls ⩽ 10%; score 4 = 10% < sls ⩽ 25%; score 5 = 25% < sls ⩽ 50%; score 6 = 50% < sls ⩽ 75%; score 7 = sls > 75%. Two tests were carried out in 1995 and 1996. For each test, disease severity was determined by the median obtained from the scores given to the 10 replications for each clone and strain.

In 1996, the sporulation per cm^2 of leaf surface was assessed for each disc by centrifuging the latter at a low rate (1500 g) in 1 ml of dispersing solution (tetra-sodium diphosphate 6.0 g l^{-1}, bactopeptone 1.2 g l^{-1}, normal chlorhydric acid 15 ml l^{-1}, pH 7). The concentration in conidia of each suspension was estimated using a hematocytometer (Mallassez cell). The surface area of the leaf disc was 0.78 cm^2 and we calculated the number of conidia per cm^2.

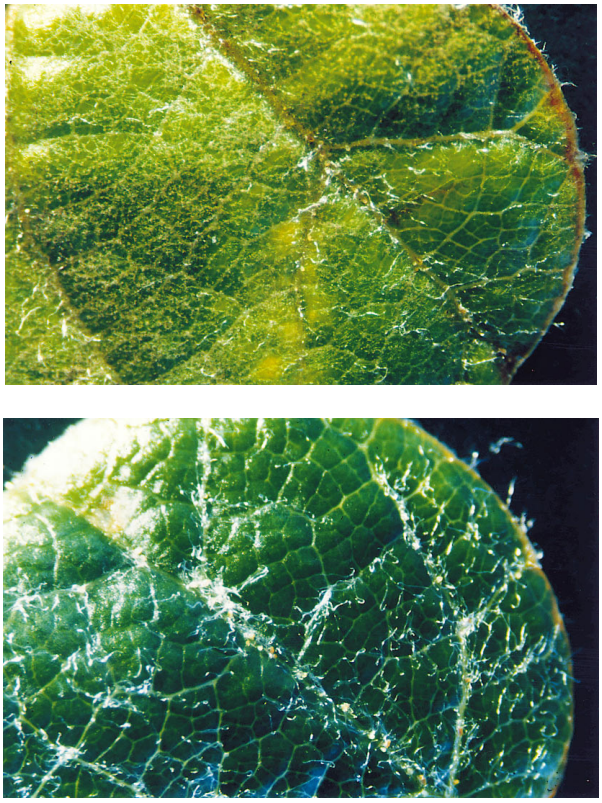

Figure 1. Apple leaf discs reaction 21 days after inoculation with the strain 104 of *Venturia inaequalis*: (top) Golden Delicious with sporulation of the pathogen; (bottom) Florina with no sporulation and no resistance symptom.

Statistical analysis

A statistical comparison of the distributions of the scabbed surface scores was made to determine whether there was a significant difference between the two experiments carried out in 1995 and 1996. Both tests gave similar results. So, the median severity score was calculated on the basis of the two test results (20 discs for each clone and strain) considered as two independent replications.

The statistical analysis dealt with the scabbed surface scores and was performed using the SAS software (SAS Institute, Inc., Cary, NC). The subpopulation chosen for this study corresponded to the set of situations for which at least one disc presented a symptom of the disease. The non parametric Krushkal Wallis test, which was carried out on all data, indicated a significant difference at the threshold of $P = 0.05$. This enabled us to compare the rank means by pair for each strain-close association (Sprent, 1992).

The sporulation variable was transformed with the base 10 logarithmic function (Schwarz, 1993). After

Table 4. Reaction of leaf discs of 16 apple clones to three strains of *Venturia inaequalis*

Clone	Strain 104 (race 1)					Strain 305 (race 6)					Strain 1066 (English race)				
	S[1]	N[2]		So[3]	N	S	N		So	N	S	N		So	N
Gala	3.5	20	(C)[4]	232	10	6	20	(C)	518	10	3	20	(C)	186	10
McIntosh	2.5	20	(C)	188	10	5	20	(C)	443	10	2	20	(C)	174	10
Golden Delicious (*Vg*)	5	20	(C)	264	10	7	20	(C)	563	10	0	0	(IC)	0	0
Granny Smith	0	5	(IC)	3	2	0	1	(IC)	0	0	0	7	(IC)	18	3
TSR34T132 (h2)	0	0	(IC)	0	0	0	0	(IC)	0	0	0	0	(IC)	0	0
Geneva (h3)	0	0	(IC)	0	0	0	0	(IC)	0	0	0	0	(IC)	0	0
TSR33T239 (h4)	0	0	(IC)	0	0	0	0	(IC)	0	0	0	0	(IC)	0	0
9-AR2T196 (h5, *Vm*)	0	5	(IC)	4	2	0	9	(IC)	3	3	0	5	(IC)	6	3
M. floribunda 821 *(Vfh, Vf)*	0	0	(IC)	0	0	0	0	(IC)	0	0	2	18	(C)	125	9
Evereste (*Vf*)	0	0	(IC)	0	0	0	0	(IC)	0	0	1.5	18	(C)	102	9
Florina (*Vf, Vg*)	0	0	(IC)	0	0	3	19	(C)	110	10	0	0	(IC)	0	0
Nova Easygro (*Vf*)	0	0	(IC)	0	0	3	19	(C)	174	10	1	15	(C)	89	8
M. pumila R127.40.7A (*Vr*)	0	0	(IC)	0	0	0	0	(IC)	0	0	0	0	(IC)	0	0
M. Baccata (*Vb*)	0	0	(IC)	0	0	0	0	(IC)	0	0	0	O	(IC)	0	0
M. baccata jackii (*Vbj*)	0	0	(IC)	0	0	0	0	(IC)	0	0	0	0	(IC)	0	0
PI 172623 (*Va*)	0	0	(IC)	0	0	3	18	(IC)	131	9	0	8	(IC)	31	4

[1] Severity, score from 0 to 7 (median of the scores of the 20 discs from two experiments).
[2] Number of scabbed discs.
[3] Mean of sporulation, number of conidia per $cm^2 \times 10^3$ (results from one experiment).
[4] (C) *In vivo* compatibility or (IC) *in vivo* incompatibility.

checking the criteria of normality in the distribution and homogeneity of residual variances (Barklett test), an analysis of variance was then made on the size of the subpopulation considered as being compatible during the experiment. The analysis of variance and the Student Newman and Keuls test of comparison between means were performed using the ANOVA procedure of the SAS software at $P = 0.05$.

Results

Disease symptoms began to appear 14 days after inoculation in compatible reactions. They were recognizable after 21 days of incubation and corresponded to a typical development of the disease accompanied by sporulation of the pathogen (classes 3b and 4 symptoms following the scale of Chevalier et al., 1991). No atypical symptoms, as the saprophyte colonization of the leaves by the fungus observed on shoots-tips (Chevalier and Parisi, 1991; Yepes and Aldwinckle, 1993a) were observed on this leaf discs test. In the case of incompatibility, no symptoms of resistance reactions were identified by macroscopic observations or using a binocular microscope. No sporulation was observed in this case (Figure 1).

Table 4 shows the results obtained in the two experiments. The 13 compatible situations were shown with the test on leaf discs. For these compatible reactions, the number of scabbed discs varied from 15 to 20 over the 20 replications, severity varied from 1 to 7; the sporulation mean, evaluated in the 1996 experiment, varied from 89 000 to 563 000 conidia per cm^2.

An incompatible situation is defined as the absence of symptoms on all inoculated discs. The test detected only 28 of the 35 incompatible reactions showed *in vivo* (Table 3). Six of the seven remaining situations presented a null median severity score and the number of scabbed discs varied from 1 to 9 with a sporulation mean varying from 3 000 and 4 000 conidia per cm^2. The PI 172623 clone, from which the resistance gene *Va* originates and which was inoculated with strain 305, although compatible *in vitro*, cannot be considered as a compatible situation *in vivo*. A severity score of 3, 18 scabbed discs out of 20 and a sporulation mean of 131 000 conidia per cm^2 were observed.

By comparing pairwise the rank means of the leaf surface scores, it was possible to distinguish between incompatible situations which are not recognised by the test on leaf discs and compatible situations at the threshold of $P = 0.05$ (Table 5). The single exception

Table 5. Paired comparison of rank means (leaf disc scabbed surface score) of three strains of *Venturia inaequalis* on 10 apple clones

Strain	Apple clone	Rank	mean	Severity[1]	
305	Granny Smith	60	a[2]	0	(IC[3])
1066	9-AR2T196 (h5, *Vm*)	83	ab	0	(IC)
104	Granny Smith	89	ab	0	(IC)
104	9-AR2T196 (h5, *Vm*)	89	ab	0	(IC)
305	9-AR2T196 (h5, *Vm*)	105	b	0	(IC)
1066	PI 172723 (*Va*)	107	b	0	(IC)
1066	Granny Smith	107	b	0	(IC)
1066	Nova Easygro (*Vf*)	154	c	1	(C)
1066	Evereste (*Vf*)	182	cd	1.5	(C)
1066	*Malus floribunda* 821 (*Vf, Vfh*)	203	de	2	(C)
1066	McIntosh	226	de	2	(C)
104	McIntosh	230	ef	2.5	(C)
305	PI 172623 (*Va*)	251	fg	3	(IC)
305	Florina (*Vf, Vg*)	267	fg	3	(C)
305	Nova Easygro (*Vf*)	269	fg	3	(C)
1066	Gala	269	fg	3	(C)
104	Gala	294	gh	3.5	(C)
104	Golden Delicious (*Vg*)	328	hi	5	(C)
305	McIntosh	345	i	5	(C)
305	Gala	356	i	6	(C)
305	Golden Delicious (*Vg*)	368	i	7	(C)

[1] Severity based on the median score of the 20 or 19 leaf discs tested from two experiments.
[2] Values with the same letters do not differ significantly according to the comparison test based on rank mean at $P = 0.05$.
[3] (C) *In vivo* compatibility or (IC) *in vivo* incompatibility.

was the interaction between the *Va* gene and strain 305.

Similarly, it was possible to distinguish between highly susceptible clones (severity score from 5 to 7) and slightly susceptible clones (severity score 1 to 2). There was a large number of intermediate situations between these two extremes which were present in two susceptibility groups, f and g (Table 5).

Analysis of variance of sporulation per cm^2 of leaf surface indicated a significant difference at the threshold of 0.001 for the strain and clone variables (Table 6). It showed that the two variables, strain and clone, are independent ($P = 0.50$).

For the compatible reactions, comparison tests of means (Student, Newman and Keuls) made it possible to distinguish between the three strains of *V. inaequalis*, strain 305 sporulating more than strain 104 and strain 1066 sporulating the least (Table 7). Comparing the results by clone (Table 7) showed that susceptible varieties in the orchard (Golden Delicious and Gala) had higher sporulation than the varieties carrying the genes *Vf* and *Vfh*.

Table 6. Variance analysis of sporulation per cm^2 (Log_{10}) of leaf disc, for the compatible interactions, as a function of *Venturia inaequalis* strains and apple clone variables

Source of variation	df	Mean square	*F* value	*P*
Apple clone	6	0.8	8.1	< 0.001
Strain	2	1.5	14.7	< 0.001
Interaction apple clone × strain	4	0.1	0.8	0.500

Discussion

This study shows that it is possible to reproduce the host-pathogen interactions between *Venturia inaequalis* and *Malus* sp. on leaf discs *in vitro.*

A typical development of the disease was observed in all the compatible situations studied, such as Gala cultivar inoculated with strain 104 and *Malus floribunda* 821 clone inoculated with strain 1066. The severity scores obtained following the *in vitro* tests were comparable with those obtained for the *in vivo* characterization of *V. inaequalis* strains.

It is possible to distribute the clones studied into two groups of susceptibility as a function of the scabbed surface scores. The first group would correspond to a slight susceptibility and to the *Malus* sp. clones presenting a resistance gene, e.g. *M. floribunda* 821, Florina, overcome by the race 6 and English race strains of *V. inaequalis*. The second group would correspond to a higher susceptibility with the Gala, McIntosh and Golden Delicious varieties.

The host-pathogen interactions between strains 104, 305, 1066 and the resistance given by the genes *Vg* and *Vf* are expressed with the method chosen in our study, in the case of compatible as well as incompatible situations. We also checked the reaction of clones carrying other major genes (*Vb, Vbj* and *Vr*). In all the cases mentioned previously, the response of the *in vitro* test was of the all or nothing type: the presence or absence of scabbed discs characterized the virulence or avirulence of *V. inaequalis* strains.

The test indicated a limited number of slightly scabbed discs for the incompatible interaction regarding the resistance gene *Vm*, which is responsible for a hypersensitive reaction *in vivo.* The interaction

Table 7. Comparison of means (Log_{10}) for sporulation per cm^2 of leaf disc, for the compatible interactions, as a function of *Venturia inaequalis* strains and apple clone variables

Classification	Number of leaf discs tested	Mean[1] of sporulation		Student, Newman and Keuls test[2]
		Per cm^2	Log_{10}	
by strain				
305	50	221 500	5.19 ± 0.42	a
104	30	138 300	5.02 ± 0.32	b
1066	46	90 000	4.87 ± 0.36	c
by apple clone				
Golden Delicious	20	253 000	5.30 ± 0.30	a
Gala	30	191 000	5.16 ± 0.38	a
McIntosh	30	163 300	5.08 ± 0.36	ab
Malus floribunda 821	9	85 000	4.84 ± 0.33	b
Nova Easygro	18	90 000	4.80 ± 0.42	b
Evereste	9	69 300	4.76 ± 0.32	b
Florina	10	67 000	4.75 ± 0.30	b

[1] Mean of sporulation per cm^2 and mean of Log_{10} (sporulation) $\pm$ standard deviation.
[2] Values with the same letters do not differ significantly according to the t test of Student, Newman and Keuls ($P = 0.05$).

model between this gene and *V. inaequalis* was chosen by Guillaumès et al. (1995) for studying the host-pathogen relations on plants raised *in vitro*. In this case, the hypersensitive reaction was observed only on rooted plants. In 1991, Chevalier and Parisi reported that atypical symptoms of the disease (slightly sporulating superficial mycelium) could appear on non-rooted micropropagated Golden Delicious plantlets raised *in vitro* and inoculated with the 104 strain of *V. inaequalis*. These reactions (hypersensitivity or atypical symptoms) were not observed for the 9-AR2T196 clone (gene *Vm*) under our experimental conditions. With our test, a null severity made it possible to distinguish the incompatible interaction between the *Vm* gene and the three *V. inaequalis* strains from compatible situations. If the number of replications is large enough, a null severity makes it possible to conclude that the pathogen strains are avirulent. It could be the same for the Granny Smith variety which presented an *in vivo* resistance to the three *V. inaequalis* strains tested and a very small number of scabbed discs *in vitro.* Parisi and Lespinasse (1996) assume that the resistance of Granny Smith is polygenic. Studying this type of interaction was not the aim of our test on leaf discs. This test is probably better adapted for studying the expression of major genes of resistance than that of polygenically-determined resistance.

The limits of the test for characterizing pathogenicity on *in vitro* leaf discs are better shown in the case of interaction between the clone providing resistance *Va* (X 7217) and the *V. inaequalis* strains tested. For this clone, we noted a compatible situation with the race 6 strain which was not consistent with *in vivo* observations. The resistance given by the *Va* gene to this strain is not expressed *in vitro* and cannot be characterized with this test, whereas the resistance to strain 104 is expressed. The fact that the resistance of certain major genes, such as *Vf* and *Vg*, is expressed on *in vitro* leaf discs in all the incompatible situations detected *in vivo*, while that coded by the *Vm* and *Va* genes is partially expressed could indicate that the resistance mechanisms induced by these genes are different. Other interaction steps, such as the elicitation of defense reactions, could be disturbed *in vitro* in the case of *Vm* and *Va*.

In this study we did not choose the model involving *in vitro* detached leaves originating from *in vitro* growing explants because we would have been limited in the choice of the host range. Previous studies on the *in vitro* characterization of *V. inaequalis* pathogenicity were carried out on susceptible cultivars, such as Golden Delicious, or cultivars resistant through the *Vf* or *Vm* genes (Yepes and Aldwinckle, 1993a, 1993b; Ivanicka et al., 1996; Guillaumès et al., 1995).

By increasing the number of host-pathogen interactions (three strains of *V. inaequalis* $\times$ 16 clones of *Malus* sp.) we showed the reproducibility of compatible and incompatible situations compared to the behaviour of the *in vivo* range. This test made it possible to considerably reduce the surface area required

for the plant material for characterizing pathogenicity. We thus can envisage testing a larger number of *V. inaequalis* strains within one season. In particular, it will be easy to study a progeny originating from a cross between two pathogen strains whose virulence or avirulence for a resistance gene present in the host would be expressed *in vitro*. This test will make it possible to study the genetic determinism of *V. inaequalis* pathogenicity on major resistance genes, *Vg* and *Vf*.

Miniaturizing the test makes it possible to study the variability of *V. inaequalis* populations originating from different orchards with a broader host range. It will be essential to check the behaviour of *in vitro* detached leaves of each *Malus* sp. clone introduced into this host range. These studies will allow us to better understand the variability and the host-pathogen interactions between *Malus* × *domestica* and *Venturia inaequalis*. This knowledge is necessary for elaborating selection strategies for durable resistance.

Acknowledgements

The authors thank Dr. Roberts for providing the Fl 1 isolate of *Venturia inaequalis*. Plant material was kindly provided by the Station d'Amélioration des Espèces Fruitières et Ornementales, I.N.R.A. Angers. We thank also Dr. Hartman for helpful suggestions. This work was partly supported by the 'Région Pays de Loire' (grant number BTH00262).

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