Vitis **45** (1), 29–36 (2006)

Assessment of powdery mildew resistance of grape and *Erysiphe necator* pathogenicity using a laboratory assay

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Summary

To develop a quantitative evaluation of grapevine resistance to powdery mildew and of pathogenicity of the causal agent (*Erysiphe necator*), a spot inoculation method was developed using detached leaves of potted plants. The percentage of inoculating spots leading to a colony and the mean diameter of colonies were determined to assess the host-pathogen relationships. Significant differences were found between host cultivars and their ranking was associated with that observed in the vineyard. There was a significant interaction between cultivar and replicate during the whole experiment indicating that the physiological state of detached leaves is important. Aging of tissues was accompanied by a gain in resistance that was considerably more marked in resistant cultivars. Only partly expanded leaves of resistant cultivars that stopped expansion on the agar medium supported the development of the fungus. Significant differences between *E. necator* isolates were also demonstrated, but these variations were less marked than those due to host cultivar and leaf position. Preliminary results obtained with different isolates from the two European genetic groups (A and B) indicate that, on average, group A is less pathogenic.

K e y w o r d s : powdery mildew, *Vitis vinifera*, *Vitis* hybrids, rating method, ontogenic resistance, *Erysiphe necator*, *Uncinula necator,* pathogenicity.

Introduction

Powdery mildew caused by *Erysiphe necator* Schwein. (formally *Uncinula necator* (Schwein.) Burrill (Braun and Takamatsu 2000) is an economically important disease of grapevine (Bulit and Lafon 1978, Pearson 1988). Several asexual cycles of reproduction of the fungus occur during the growing season and may cause losses of yield and quality (Gadoury *et al.* 2001, Calonnec *et al.* 2004, Stummer *et al.* 2005) if there is no or incomplete chemical control.

The most efficient way to minimize powdery mildew is to grow resistant cultivars, however, the elite cultivars originate from *Vitis vinifera* which is the most susceptible host species. Monogenic resistance to *E. necator* has been studied in *Muscadinia* and its hybrids with *Vitis vinifera* (Bouquet 1986, Pauquet *et al.* 2001). In the *Vitis* genus resistance appears to be due to several genes (Boubals 1961, Fisher *et al.* 2004). Methods are needed to identify valuable sources of resistance and to assist breeding programs for cultivars being resistant to powdery mildew. Determination of host resistance was based on field observations (Boubals 1961, Doster and Schnathorst 1985 a, Staudt 1997), tests on leaf disks (Stein *et al.* 1985, Wang *et al.* 1995) or on *in vitro* material (Kitao and Doazan 1990). The results obtained by different methods were found to be correlated (Li 1993). However, data were mostly presented on discrete scales of disease rating. Continuous variables would be more appropriate for quantitative analysis of the relationships between grapevine and *E. necator.*

Moreover, quantitative assessment of the pathogenicity of *E. necator* would enable to choose the most appropriate isolates to rate host genotypes. The diversity of the pathogen with respect to its pathogenicity has been poorly investigated. Kitao and Doazan (1990) reported differences in colony diameter, length of latent period and production of conidia between a few isolates from different European regions. Some evidence for pathogenic specialization was observed when pathogenicity was examined for isolates obtained from a range of *Parthenocissus* and *Vitis* species (Gadoury and Pearson 1991). A quantitative method in a closed-system laboratory environment would thus be particularly useful to address several questions regarding the diversity of the pathogen. For instance, the marked genetic differences between the morphologically similar groups that have been identified in European, Indian and Australian populations of *E. necator* (Délye *et al.* 1997, Stummer *et al.* 2000) suggest that these groups may differ in pathogenicity, but this possibility has not been explored.

The objectives of this study were 1) to develop a method to obtain a simple, quantitative assessment of the interaction between grapevine and *E. necator* and 2) to evaluate the potential of this method using a set of grapevine cultivars with different levels of susceptibility and resistance in the vineyard as well as a set of isolates differing in host origin and genetic group.

Material and Methods

P l a n t m a t e r i a l : Four *Vitis vinifera* cultivars (Cabernet-Sauvignon, Carignan, Cinsault, Grenache) and three resistant interspecific cvs (Villard Blanc, Villard Noir, Jacquez) were used. Villard Blanc and Villard Noir have a complex genealogy involving several *Vitis* species with a majority of *V. rupestris* and *V. vinifera* parentage (Antcliff

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1992). Jacquez probably results from a cross between *V. aestivalis* and *V. vinifera* (GALET 1988). For this study, plants were obtained from one-bud cuttings prepared in spring from one-year old branches collected in the preceding winter and stored at 4 °C. Cuttings were placed at high density in flat boxes filled with vermiculite and maintained for 6-8 weeks in a growth chamber at 25 °C in the light (12 h·d^{-1}, 40 µmol quanta m^{-2} s^{-1}) and 22 °C in the dark. Rooted cuttings were then transferred in 2 liter pots containing a mixture of soil and vermiculite (2:1), fertilized (Osmocote, Scotts Europe B.V., Heerlen, The Netherlands) and grown in the greenhouse. Plants were regularly trimmed to allow the development of new shoots with suitable leaves.

Leaves of potted plants were removed, washed in tap water and disinfected for 1.5 min in a 0.3 % solution of active chloride. After three rinses in sterile distilled water, leaves were dried between several layers of sterile paper. Petiole length was then reduced to 1.5-2 cm. Leaves with their upper surface visible were placed on 8 g·l^{-1} agar medium in 9 cm Petri dishes; the remaining part of the petiole was inserted into the medium.

Fungal isolates: The isolates of *E. necator* used in this study originated from infected material collected in southern France (Ardeche district) in 2000 and 2001. In this region, a population study identified the two genetic groups that exist in Europe (PÉROS *et al*. 2005), and the set of isolates included 5 group A isolates and 4 group B isolates (Tab. 1). Within the two d after collection in the field, infected tissues were brushed onto detached leaves of cv. Cabernet-Sauvignon. The inoculated leaves were then incubated for 10-15 d at 25 °C under 16·h d^{-1} illumination (40 µmol quanta m^{-2} s^{-1}). Either a single or a few conidia from the same conidial chain were then picked up using a glass needle under the binocular microscope. Conidia were spotted on detached leaves from the greenhouse or on leaves of *in vitro* plants, cv. Cinsault, cultured as described by PÉROS *et al.* (1998). The process was repeated once. The isolates were maintained on leaves of *in vitro* plants and subcultured on new leaves every 4-5 weeks under the conditions described above.

Pathogenicity assay: Inoculum was produced on detached leaves of cv. Cabernet-Sauvignon inoculated with infected tissues and incubated for 2-3 weeks under the conditions described above. Detached leaves of potted plants were inoculated using a spot method. About 20-60 conidia were taken from a leaf infected with powdery mildew using a glass needle. Inoculum was deposited on a healthy leaf by gentle contact with the leaf epidermis. On each inoculated leaf, three separate spots were applied approximately in the middle between the petiole insertion point and the lobe extremity, avoiding veins. Inoculated leaves were incubated under the conditions described above. Inoculation was considered successful if the spot resulted in a colony that sporulated. The diameter of the area that sporulated was measured under the binocular microscope 11 d (Experiment 3) or 20 d (other experiments) after inoculation.

Experimental designs and analyses: Five different experiments were performed using complete block designs (Tab. 2). Each experiment was replicated three times and, depending on the experiment, the replicate was studied as a second or a third classifying factor. Isolate FA01 was used in experiments 1 and 2. Cv. Cabernet-Sauvignon was used in experiment 4. Leaves were taken at 5 different positions: the first expanding leaf (position 1), the second expanding leaf (position 2), and the three following leaves (positions 3-5). The different positions were compared in experiment 2 and position 2 and 4 were compared in experiment 4. The leaves used in experiment 1 and 5 were taken at position 2 and those used in experiment 3 were taken at position 1. Statistical analyses were performed using SAS (version 8.1; SAS Institute, Cary, NC, USA). The numbers of colonies that sporulated were compared with χ^2 testing using the FREQ procedure. Analyses of variance for the diameter of the colony were performed using the GLM procedure in SAS and means were compared using paired t-tests or the CONTRAST statement.

Table 1

Origin of *Erysiphe necator* isolates used in this study

Isolate	Sampling date (month/year)	Tissues	Host cultivar	Genetic group[x]
FA01	07/2000	Leaf	Carignan	A
FA03	07/2000	Berry	Jacquez	B
FA09	07/2000	Leaf	nd[y]	A
FA12	07/2000	Leaf	Villard Noir	A
FA16	05/2001	Flagshoot	Carignan	A
FA18	09/2001	Leaf	nd	B
FA19	09/2001	Leaf	nd	B
FA20	09/2001	Leaf	nd	B
PA07	11/2001	Leaf	Gamay	B

[x] Assignment to genetic groups was based on results obtained using Nested allele specific PCR (DÉLYE *et al.* 1999), groups A and B corresponding to groups I and III described in Europe by DÉLYE *et al.* (1997).

[y] Not determined.

Table 2

Experiments designed to evaluate the performance of a spot inoculation method of grapevine with *Erysiphe necator*

Exp.	Source of variation (levels)	Blocks	Exp. Unit	Dates of replicates
1	Cultivar (CAB, CAR, CIN, GRE, VB, VN, JAC)[x]	5	4 leaves	21/05, 04/06 and 17/06/02
2	Cultivar (CAB, GRE, VN)			
	Leaf position (n°1 to n°5)[y]	5	2 leaves	21/08, 29/08 and 10/09/02
3	Cultivar (CAB, VN, JAC)			
	Isolate (FA01, FA03, FA12)[z]	3	2 leaves	03/10/02, 17/04 and 01/09/03
4	Isolate (FA01, FA09, FA12, FA16, FA18, FA19, FA20, PA07)			
	Leaf position (n°2 and n°4)	3	2 leaves	04/07, 17/07 and 14/08/03
5	Cultivar (CAB, GRE, VN)			
	Isolate (FA01, FA16, FA18, FA20)	3	2 leaves	10/07, 20/08 and 26/08/03

[x] Cultivars were coded as follows: CAB: Cabernet-Sauvignon, CAR: Carignan, CIN: Cinsault, GRE: Grenache, VB: Villard Blanc, VN: Villard Noir, JAC: Jacquez.
[y] The leaf at position 1 is the first expanding leaf.
[z] Origin of isolates is given in Tab. 1.

Results

Overall performance of the method: During the course of this sudy a total of 1536 detached leaves were used and 4608 inoculating spots were made. The percentage of spots observable 20 d after inoculation (11 d in experiment 3) was 97.7 % indicating a very low rate of missing data. A few leaves, mostly in experiments 1 and 2, showed necrotic lesions or were contaminated by saprophytic fungi. A few inoculating spots were contaminated by other fungi despite the axenic precautions taken during the preparation of inoculum and during inoculation. Leaves at position 2 of the cv. Cabernet-Sauvignon inoculated with isolate FA01 corresponded to the most frequent combination tested (experiment 1, 2, 4 and 5) and colony development was observed in 87 % of the 378 observable spots.

Effect of replicate: The percentage of infection varied in each experiment as a function of the replicate (Tab. 3). In each experiment, χ^2 testing showed significant differences between replicates. Generally one replicate was clearly distinguished from the two other replicates. Analysis of variance for colony diameter also revealed a significant effect of the replicate except in experiment 1 (Tab. 4). Large significant differences for colony diameter between replicates were evidenced in experiment 2, 3 and 5. The effect of replicate interacted with cultivar response in all experiments in which different cultivars were tested and with leaf position in experiment 4, but not in experiment 2. Conversely, the isolate-replicate interaction term was never significant (Tab. 4).

Effect of vine cultivar: In experiment 1, inoculation of Jacquez was not followed by development of the fungus whatever the replicate (Tab. 5). The other cultivars fell into different groups: Cinsault and Carignan with maximal percentage in the three replicates, Cabernet-Sauvignon, Grenache and Villard Blanc with high percentages but with variation between replicates, and Villard Noir with lower percentages. These results were confirmed for the cultivars used in several experiments at leaf position no. 2: the percentage of infection was 92 %, 84 % and 83 % for Cabernet-Sauvignon in experiment 2, 4 and 5, 83 % and 90 % for Grenache in experiment 2 and 5, and 73 % and 51 % for Villard Noir in experiment 2 and 5. In experiment 3, using leaves at position 1, some spots initiated infection in

Table 3

The performance of a spot inoculation method of grapevine with *Erysiphe necator*: differences between replicates in each experiment

	Experiment 1		Experiment 2		Experiment 3		Experiment 4		Experiment 5	
Replicate	% I[x]	D (mm)[y]	% I[x]	D (mm)[y]	% I[x]	D (mm)[y]	% I[x]	D (mm)[y]	% I[x]	D (mm)[y]
1	77a	12.2	49b	7.9a	65a	6.5a	91a	10.6b	71b	10.7a
2	71a	11.3	58a	8.0a	62a	4.3bc	71b	8.1a	82a	12.5a
3	63b	11.4	61a	11.6b	43b	5.1c	71b	10.0b	71b	11.1a

[x] Percentage of infection based on the number of inoculating spots resulting in a colony. Percentage within the same column followed by the same letter are not significantly different at the P = 0.05 level of probability based on paired χ^2 tests.
[y] Mean diameter of sporulating colonies. Only data for inoculating spots resulting in a colony were included. Means within the same column followed by the same letter are not significantly different at the P = 0.05 level of probability based on paired t tests. ANOVA analyses for D are shown in Tab. 4.

Table 4

Analyses of variance for colony diameter after inoculation of detached leaves of grapevine with *Erysiphe necator* in five different experiments designed to study the effects of cultivar, isolate, leaf position and replicate

Experiment[x]	Source of variation[y]	df	Mean square	*F* value	*P*
1	Cultivar	5	0.46	15.5	<0.001
	Cultivar × Replicate	12	0.14	4.6	<0.001
	Replicate	2	0.08	2.8	0.070
2	Replicate	2	2.29	47.2	<0.001
	Leaf position	4	1.99	41.0	<0.001
	Cultivar	2	0.78	16.1	<0.001
	Cultivar × Replicate	4	0.12	2.5	<0.05
	Replicate × Leaf position	8	0.06	1.3	0.25
	Cultivar × Leaf position	8	0.04	0.8	0.57
	Cultivar × Replicate × Leaf position	13	0.03	0.6	0.88
3[z]	Cultivar	1	0.40	25.5	<0.001
	Replicate	2	0.22	14.2	<0.001
	Isolate	2	0.07	4.5	<0.05
	Cultivar × Replicate	2	0.06	4.0	<0.05
	Isolate × Replicate	4	0.03	2.0	0.12
	Cultivar × Isolate	2	0.01	0.9	0.41
	Cultivar × Isolate × Replicate	4	0.01	0.2	0.96
4	Leaf position	1	6.07	116.37	<0.001
	Replicate	2	0.81	15.54	<0.001
	Isolate	7	0.69	13.24	<0.001
	Leaf position × Replicate	2	0.31	5.97	<0.01
	Isolate × Leaf position	7	0.07	1.34	0.24
	Isolate × Replicate	14	0.06	1.22	0.27
	Isolate × Leaf position × Replicate	14	0.05	0.87	0.59
5	Cultivar	2	3.91	68.76	<0.001
	Isolate	3	0.38	6.72	<0.001
	Replicate	2	0.32	5.71	<0.01
	Cultivar × Replicate	4	0.24	4.14	<0.01
	Isolate × Cultivar	6	0.05	0.90	0.50
	Isolate × Cultivar × Replicate	12	0.04	0.77	0.68
	Isolate × Replicate	6	0.03	0.59	0.74

[x] Only data for inoculating spots leading to a powdery mildew colony were included.
[y] In each experiment the sources of variation were ranked in decreasing order of *F* values.
[z] Data for the Jacquez cultivar were not included in the analysis because a many values were lacking.

cv. Jacquez (Fig. 3) and the average percentage of infection was 92 %, 67 % and 9 %, for Cabernet-Sauvignon, Villard Noir and Jacquez.

The diameter of the powdery mildew colony also varied depending on the cultivar (Tab. 4). In experiment 1, the means per cultivar were compared for each replicate (Tab. 5). There were some changes in the ranking of cultivars between replicates as well as in the magnitude of differences between cultivars. In replicate 1 the cultivars were not separated, in replicate 2 only Villard Noir was distinguished from the other cultivars, and in replicate 3 the two hybrids were distinguished from the *V. vinifera* cultivars. Cabernet-Sauvignon and Grenache were not separated in experiment 2 (Fig. 2) and experiment 5 (data not shown) whereas Villard Noir presented colonies with smaller diameters than Cabernet-Sauvignon in experiment 2 (Fig. 2), experiment 3 (Fig. 3) and experiment 5 (data not shown).

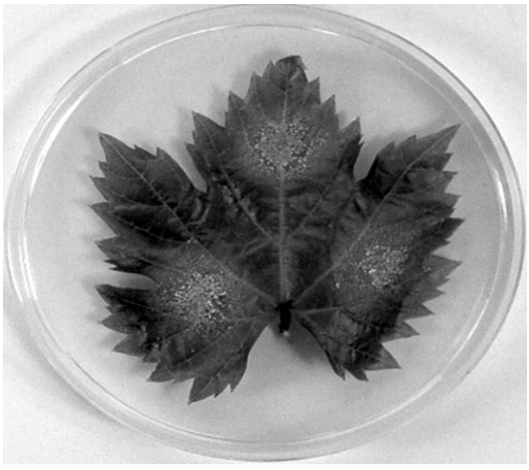

Fig. 1: Detached leaf of grapevine (cv. Carignan) 20 d after inoculation with *Erysiphe necator* using the spot inoculation method.

Table 5

Percentage of infection and mean colony diameter for 7 grapevine cultivars inoculated with *Erysiphe necator* (isolate FA01) on detached leaves at position 2

	Replicate 1		Replicate 2		Replicate 3	
Cultivar	% I[x]	D (mm)[y]	% I	D (mm)	% I	D (mm)
Cinsault	100a	13.4a	100a	12.8a	100a	14.5a
Carignan	100a	13.1a	100a	12.7a	98a	12.1ab
Cabernet-Sauvignon	97ab	11.9a	98a	13.8a	82b	13.7ab
Grenache	93b	12.0a	68c	11.2a	77b	11.6bc
Villard blanc	88b	11.6a	89b	11.5a	42c	6.1d
Villard noir	58c	10.9a	40d	7.4b	28d	9.3c
Jacquez	0	-	0	-	0	-

[x] Percentage of infection based on the number of inoculating spots resulting in a colony. Percentages within the same column followed by the same letter are not significantly different at the P = 0.05 level of probability based on paired χ^2 tests.

[y] Mean diameter of sporulating colonies. Only data for inoculating spots resulting in a colony were included. ANOVA analysis for D is shown in Tab. 4. Means within the same column followed by the same letter are not significantly different at the P = 0.05 level of probability based on t tests.

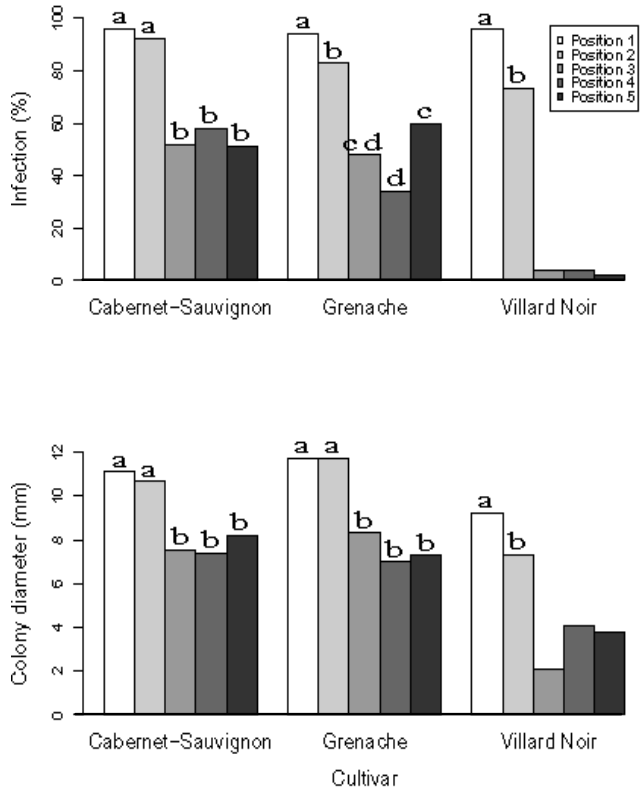

Fig. 2: Effects of cultivar and leaf position on percentage of infection and colony size 20 d after inoculation of detached leaves of grapevine with isolate FA01 of *Erysiphe necator,* (experiment 3). Bars with a different letter for each cultivar were significantly different at P = 0.05.

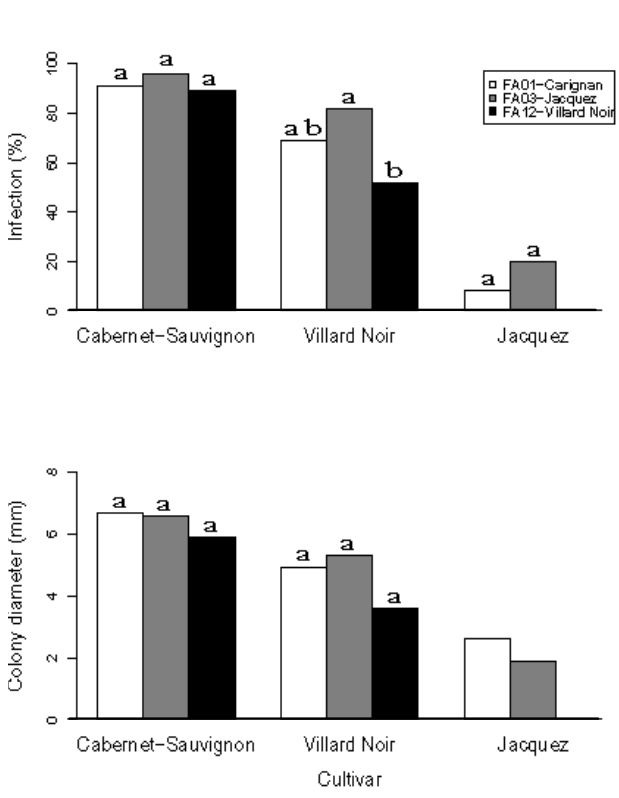

Fig. 3: Effects of cultivar and isolate of *Erysiphe necator* on percentage of infection and colony size 11 d after inoculation of detached leaves of grapevine (experiment 3). In the legend the code of each isolate is followed by the name of the cultivar from which it was collected. For details see Fig. 2.

Effect of leaf position: In experiment 2, there was a dramatic decrease in the percentage of infection between leaf position 2 and leaf position 3 (Fig. 2). This effect was considerably more noticeable in Villard Noir than in the two *V. vinifera* cultivars. In Cabernet-Sauvignon inoculated in experiment 4, there was a significant difference between the leaves taken at positions 2 and 4 ($\chi^2 = 16.6$, $P < 0.001$) but the magnitude of the difference (84 % vs 72 %) was less marked than in experiment 2 (92 % *vs.* 58 %).

The diameter of the powdery mildew colony varied with leaf position (Tab. 4, Fig. 2). The decrease was not linear: leaves at positions 1 and 2 showed similarly high susceptibility, except for Villard noir, and then the size of the colony size decreased sharply and was almost the same at the three

other positions. For Villard Noir, means for leaves at positions 3 to 5 were not subjected to t-tests because of limited data. In Cabernet-Sauvignon inoculated in experiment 4, there was a significant difference between leaves at position 2 and 4 (11.6 mm vs 7.4 mm), *i.e.* a bigger difference than that observed in experiment 2 (10.7 *vs.* 7.4 mm).

Effect of the isolate: In the experiment investigating the pathogenicity of three isolates with respect to their host origin (experiment 3), we inoculated leaves at position 1 and some infections were observed on cultivar Jacquez (Fig. 3). No differences between isolates were evidenced in Cabernet-Sauvignon, in contrast to the hybrids for which isolate FA12 originating from Villard Noir displayed lower percentages of infection. Data on colony diameter for Jacquez were not included in the variance analysis (Tab. 4). The cultivar-interaction term was not significant indicating that the ranking of Cabernet-Sauvignon and Villard Noir was not influenced by the isolate inoculated. Despite an overall significant effect of the isolate (Tab. 4), the means per isolate did not show significant differences either for Cabernet-Sauvignon or for Villard Noir (Fig. 3).

In experiment 4, designed to compare the pathogenicity of 8 isolates from two genetic groups on leaves of cv. Cabernet-Sauvignon, the percentage of infection varied and isolate FA19 resulted in the lowest percentage of infection (Fig. 4). On average, groups A and B did not differ significantly (79 % *vs.* 76 %, P = 0.32). The analysis of variance performed for the diameter of powdery mildew colonies revealed an effect of the isolate (Tab. 4) and paired t identified isolate FA09 as the less pathogenic isolate (Fig. 4). We then compared the means of groups using the statement CONTRAST in SAS. There was a significant difference between groups A and B (8.6 *vs.* 10.5 mm, P < 0.001). Thus, on average, group A showed 18 % reduction in pathogenicity compared to group B. However, the data obtained with isolate FA09 from group A might have made a major contribution to this result.

In experiment 5, we selected the two isolates from each group that had produced the largest diameters in experiment 4 and compared their pathogenicity using three cultivars. There were significant differences for the percentage of infection (Fig. 5) and, on average, groups A and B differed significantly (69 % *vs.* 81 %, P < 0.001). The analysis of variance performed for the diameter of powdery mildew colonies revealed an effect of the isolate (Tab. 4), however paired t-tests did not distinguish isolates (Fig. 5). Using CONTRAST, a significant difference was observed between groups A and B (10.6 *vs.* 12.3 mm, P < 0.001), and, on average, group A showed 14 % less pathogenicity compared to group B. The isolate-cultivar interaction term was not significant (Tab. 4).

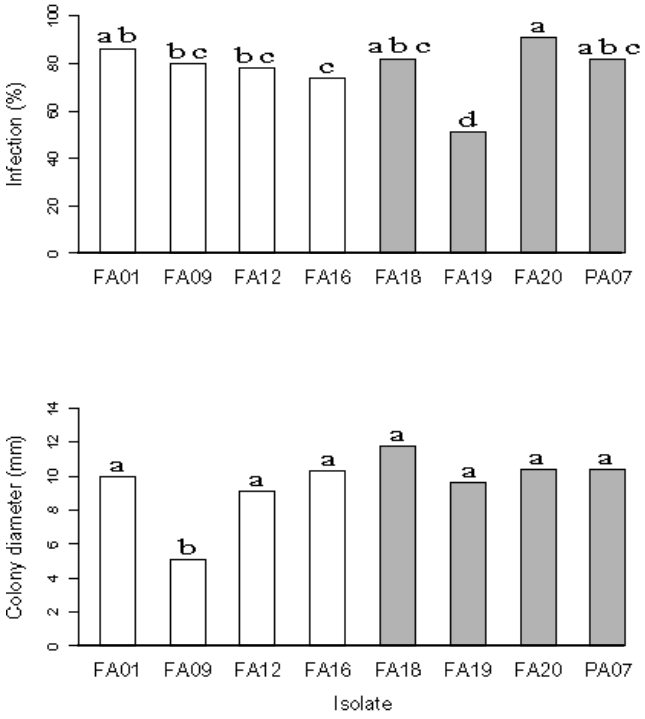

Fig. 4: Percentage of infection and colony size measured 20 d after inoculation of detached leaves of Cabernet-Sauvignon taken at two leaf positions and inoculated with isolates of *Erysiphe necator* from two genetic groups (experiment 4). Data for the two leaf positions were combined. White and grey bars represent group A and group B, respectively. For details see Fig. 2.

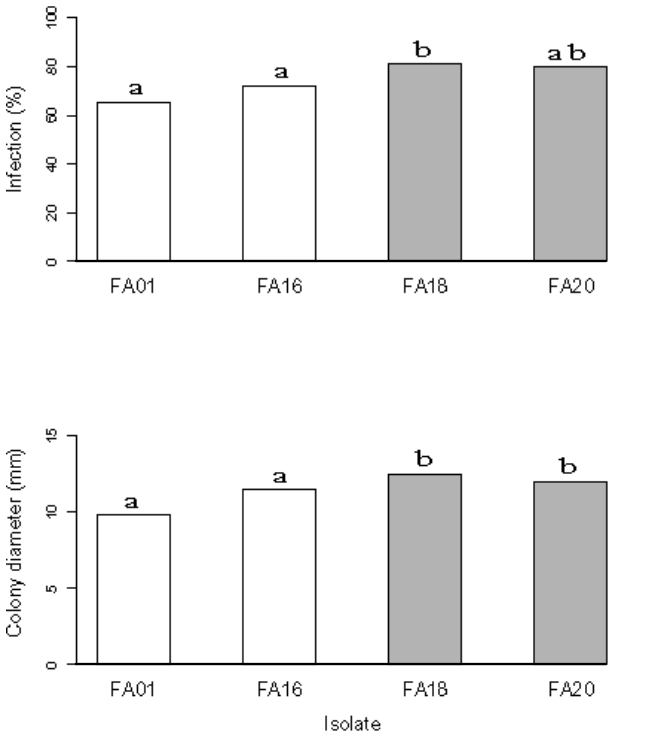

Fig. 5: Percentage of infection and colony size measured 20 d after inoculation of detached leaves of three cultivars of grapevine inoculated with isolates of *Erysiphe necator* from two genetic groups (experiment 5). Data for the three cultivars were combined. White and grey bars represent group A and group B, respectively. For details see Fig. 2.

Discussion

The method developed here allows the interaction between grapevine and *E. necator* to be studied under controlled conditions. After the collection of leaves in the greenhouse all operations are performed in a controlled labora-

tory setting that ensures reproducible conditions. The spot method allows dry conidia to be deposited on specific areas of detached leaves. In very susceptible cultivars and in the most susceptible tissues, the percentage of spots that led to sporulation was very high in different replicated experiments. The diameter of the resulting colony provides another character that can easily be measured under the binocular microscope. Although the latency period of *E. necator* from inoculation to occurrence of conidiophores with mature conidia is 5-6 d, we performed observations only 20 d after inoculation. This decision was made because: (1) preliminary data indicated that measurements at 10 d gave similar results but with more variability and therefore less precision in the statistical analysis, (2) under the binocular microscope measurement of larger colonies is easier, and (3) a few colonies in resistant genotypes took longer to develop and infection percentage can consequently be underestimated if observations are made earlier. However, when the smallest leaves were inoculated it was easier to perform the measurements earlier and in experiment 3, we consequently measured the colonies 11 d after inoculation. In contrast to the two characters we measured for this study, more time and resources would be needed to estimate the length of the incubation period after successive observations or to determine the number of conidia produced per colony. Furthermore, previous studies on grapevine powdery mildew (Boubals 1961, Coutinho and Martins 1985, Kitao and Doazan 1990) demonstrated that characters like colony size, incubation time and spore production are correlated.

Without fungicide treatments, cultivars of *Vitis vinifera* are affected by destructive epidemics. Some differences have been found among *V. vinifera* cultivars and Grenache was previously considered to be less susceptible than Cabernet-Sauvignon, Cinsault and Carignan (Boubals 1961, Li 1993). This result was not corroborated in our study since Grenache was not always distinguished from very susceptible cultivars. In contrast to *V. vinifera* cultivars, the three hybrids are not subject to epidemics even in the absence of fungicide treatments. In experiment 1, the fungus did not develop on leaves of Jacquez but the two other resistant hybrids showed colonies that were not always significantly smaller than those observed on *V. vinifera* cultivars. Moreover, there was a significant interaction between cultivars and replicates with variations in both, the rank of cultivars and the magnitude of the differences between them. The three replicates of experiment 1 were completed in a two-month period and a likely explanation was the difference in the growing conditions of the plants that produced the leaves. Chellimi and Marois (1991) also explained the variability in their results by differences in the physiological state of host tissues.

Senescing leaves became more resistant to *E. necator* and this effect was evidenced for both the success of infection and the diameter of the colony. An effect of tissue age on powdery mildew development has already been described by Delp (1954) and Doster and Schnathorst (1985 b). However, in contrast to the latter authors, we did not observe that resistance increased linearly with an increase in leaf maturity but that a threshold separated very susceptible tissues from more resistant ones. In addition, we showed that the effect of leaf position was considerably more pronounced in the resistant hybrids than in the *V. vinifera* cultivars. This rapid decrease in susceptibility with tissue age probably explains the lack of powdery mildew in vineyards of resistant hybrids. Their youngest tissues can be infected but the development of the fungus is generally arrested during aging of tissues before sporulation occurs. In contrast, the youngest leaves placed in Petri dishes stop expanding and are therefore artificially maintained in a susceptible status. This also provides an explanation for the results in experiment 1, *i.e.*, the resistant hybrids were not clearly distinguished from susceptible cultivars because the inoculation was performed in the expanding leaf at position 2. With leaves at position 3-5, the magnitude of differences between susceptible and resistant cultivars increased. Data presented here thus suggest that leaves at position 3 have to be inoculated to clearly distinguish resistant from susceptible cultivars. On the other hand, the youngest leaves have to be used to compare resistant cultivars since very resistant hybrids like Jacquez only developed infection if the first expanding leaf was inoculated.

Our results confirm that age-related resistance, often called 'ontogenic resistance', is expressed in grapevine leaves inoculated with *E. necator*. Doster and Schnathorst (1985 b) hypothesized that a resistance factor inhibits penetration of *E. necator* in old-aged leaves and consequently slows colony development. The fungus indeed needs to repeatedly penetrate and form secondary haustoria to colonize the tissue surface. Ontogenic resistance to powdery mildew has been investigated more precisely in grape berries (Ficke *et al.* 2002, 2003, 2004; Gadoury *et al.* 2003). Only berries inoculated with *E. necator* within 3 weeks after bloom developed severe disease symptoms (Ficke *et al.* 2002). In resistant berries, the pathogen is stopped within 24 h after arrival of the inoculum, *i.e.* after the formation of the appressorium but before it is able to form haustoria and secondary hyphae. Investigations have shown that cuticle thickness, formation of papilla, accumulation of phenolics or pathogenesis-related proteins may only in part explain ontogenic resistance (Ficke *et al.* 2002, 2004). The most likely but still unknown mechanism may involve biochemical or ultrastructural modifications of the cuticle or cell wall (Ficke *et al.* 2004). As observed for leaves, resistant hybrids expressed ontogenic resistance in berries earlier than cultivars of *V. vinifera* (Ficke *et al.* 2003).

The method described in this paper is also suitable for analyzing the variation in pathogenicity within *E. necator.* However, the differences between isolates of *E. necator* were less marked than the differences in tissue susceptibility due to the cultivar or the leaf position. It would probably be necessary to increase the number of replicates to measure the differences between isolates more precisely. This method could also be used to check a range of host cultivars against a range of isolates from different hosts to analyze specialization in the fungus. The limited data presented here suggest that isolates collected on resistant hybrids did not appear to have developed a higher degree of pathogenicity. It is very likely that inoculum infecting resistant hybrids originated

from neighboring cultivars of *V. vinifera* where the epidemic started. Two experiments indicated that, on average, group A displayed lower pathogenicity than group B. However, to corroborate this result, further experiments in the laboratory with different sets of isolates from both groups are needed. If confirmed, differences in pathogenicity between genetic groups could contribute to changes in the global population with respect to their relative frequency. Therefore, lower pathogenicity in group A than in group B could be another explanation for the shift that was observed during the growing season from a population consisting of group A isolates to a population consisting of group B isolates in vineyards (DÉLYE *et al.* 1999).

In conclusion, the method presented here has several potential applications such as cultivar assessment, analysis of host-specialization and studies on the variation in pathogenicity in the fungus. Although the detached leaf assay may not fully reproduce the host-pathogen relationships in the vineyard, we would expect the significant differences evidenced between cultivars or isolates for only one infectious cycle in a Petri plate to be amplified in the vineyard due to the polycyclic development of powdery mildew during the growing season.

Acknowledgements

This work was partially supported by the "Comité Technique Permanent de la Sélection" (grant No. C0047). We thank Dr. A. BOUQUET et Dr. A. DOLIGEZ for critical reading of the manuscript.

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Received June 23, 2005