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Impact of Clonal Variability in *Vitis vinifera* Cabernet franc on Grape Composition, Wine Quality, Leaf Blade Stilbene Content, and Downy Mildew Resistance

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ABSTRACT: In this study, 10 clones of *Vitis vinifera* Cabernet franc (not yet commercial) have been phenotyped on precocity, grape composition, and assessment of wine quality made by microvinification in 2008−2010. Additionally, two original criteria have been considered: concentration of 3-isobutyl-2-methoxypyrazine (IBMP) in grapes and wines (the green bell pepper flavor) and resistance of grapevines to downy mildew (*Plasmopara viticola*) by stilbene quantification upon infection. Precocity of veraison varied up to four days at veraison. Berry size and yield were highly variable among clones. However, these variables were not correlated. Tanins and anthocyanins varied among clones in grapes and wines. Variations in grape and wine IBMP were not significant. Some clones showed lower susceptibility for downy mildew on leaves. Lower susceptibility was linked to a higher production of stilbenic phytoalexins involved in downy mildew resistance mechanisms.

KEYWORDS: *vine, clones, clonal selection, Vitis vinifera, Cabernet franc, 3-isobutyl-2-methoxypyrazine, IBMP, downy mildew, Plasmopara viticola, stilbene*

INTRODUCTION

Grapevine varieties are not genetically homogeneous.[1] The level of the intravarietal diversity varies among grapevine varieties.[2,3] Grapevines are multiplied by vegetative propagation. A collection of vines propagated from the same mother vine make up a clone.[1] Many studies relate clonal diversity among cultivars from the species *Vitis vinifera* for a broad range of characteristics. Precocity of the phenological cycle varies among clones.[4] Anderson et al.[5] show that clones of *V. vinifera* cv. Pinot noir, originating from California and Champagne (France), express great variability with regard to vigor (assessed by pruning mass measurements), yield, sugar production, and total acidity. Similar results were found by Boso et al.[6] on Albariño clones selected in Galicia, Spain. These authors found that the seed number varied as much as 2-fold among clones. Secondary metabolites are also variable among clones. Belencic and Agosin[7] showed great clonal variability in 3-isobutyl-2-methoxypyrazine (hereafter called IBMP) content in *V. vinifera* cv. Carmenère. Geraniol and linalool are high in *V. vinifera* cv. Chardonnay clone 809 and low in clone 76.[8] Clones vary regarding disease resistance, although underlying mechanisms have rarely been identified. Boso et al. showed interclonal differences in downy mildew (*Plasmopara viticola*) resistance for eight *V. vinifera* cv. Albariño clones.[9] Lower *P. viticola* susceptibility of some of these clones may be due to GLRaV3 virus infection.[10] Downy mildew resistance, even partial, is an important issue, because in maritime climates the number of sprayings against *P. viticola* can be as high as 10 per season.[11] Using partly resistant clones might be a way to reduce the use of pesticides. Clone performances vary with environmental conditions due to clone−environment interactions.[4] Mannini et al.[12] show that *V. vinifera* cv. Nebbiolo clone CVT 142 performs well in a particular environment, while its wines are not appreciated when grown in another environment.

Clonal selection has been carried out in viticulture since the late 1950s. For varieties with great genetic diversity, clonal selection is a major issue in the production of quality wines. In France, the first clones were approved in 1971.[13] The first purpose of clonal selection was the creation of virus-free populations from one healthy mother vine.[14] In a second stage, viticultural criteria were integrated in clonal selection programs. Initially, the basic criteria were yield and grape sugar concentration. Progressively, more sophisticated criteria, such as the concentration of skin phenolic compounds, were integrated in clonal selection programs.[15] Wines were made by means of small-scale vinifications, and sensory attributes of the wine produced were evaluated.[16] Several commercial clones are now available for most varieties. In France, 35 clones are available for *V. vinifera* cv. Cabernet franc.[17]

Production requirements vary in space. High sugar production might be a desired attribute in a cool climate, but is not in a warm climate. Production requirements may also vary over time, depending on modification of the local climatic conditions (global climate change[18]), the evolution of disease pressure, and changes in the desired style of the produced wine. Hence, clonal selection is a never-ending story. Unfortunately,

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15 years is necessary to select a new clone, making it difficult to market clones that are perfectly adapted to current production requirements. With the need to continue clonal selection, preservation of genetic resources is a critical issue.[19]

The aim of this research was to show how complex criteria can be integrated in clonal selection programs. Among these, cluster morphology, precocity, grape and wine IBMP content (to assess the absence of vegetal character), anthocyanin and tannin contents, sensory assessment of wines obtained by small-scale vinification, and susceptibility to downy mildew are of particular interest. Applying these criteria to clonal selection of Cabernet franc revealed significant genetic diversity for this variety. One clone showed a particularly interesting combination of attributes for the production of red table wines.

MATERIALS AND METHODS

Selection of the Clones. Performance of approximately 600 Cabernet franc mother vines (more than 50 years old and distributed over eight blocks) were assessed over an eight year period (1996−2003). The eight blocks covered approximately 8 ha and were located in the Saint-Emilion region (Bordeaux area), latitude 44°56′, longitude 0°11′. Selection criteria included the cluster morphology (with a preference for loose bunches), berry mass (with a preference for clones producing small berries), berry sugar content (clones with low sugar content at ripeness were eliminated), and yield components (with a preference for moderately yielding clones). All vines were tested for grapevine fanleaf virus (GFLV), *Arabis* mosaic virus (ArMV), and grapevine leafroll virus (GLRV serotype 1, GLRV serotype 3) by means of enzyme-linked immunosorbent assay (ELISA) tests, and only virus-free clones were selected.

Experimental Plot. A total of 31 vines were selected and propagated from cuttings. An experimental vineyard was planted in 2005 with five replicates of ten vines for each clone, grafted on Riparia Gloire de Montpellier rootstock in a gravelly sandy soil in an estate located in Saint-Emilion (Bordeaux, France). Vines were spaced 1.3 m (between rows) × 1.0 m (between vines on the row), resulting in a density of 7692 vines/ha. Vines were trained on a vertical shoot positioned trellising system and cane pruned (one cane of five buds and one spur of two buds). No yield reduction was applied.

Climatic Conditions. Air temperature (at a height of 1.5 m) and rainfall were monitored in an automatic weather station (Météo France, 33700 Mérignac, France) located less than 1 km from the experimental sites.

Yield Components, Precocity, Grape Composition, Wine Composition, and Sensory Analysis. Most of the measurements were concentrated on a set of 10 clones over three years (2008−2010). Previous studies had shown that these were the most promising among the 31 clones initially planted in terms of cluster morphology, berry mass, yield, and grape suger content at ripeness (van Leeuwen, unpublished data). Each measurement was replicated five times. Vine vigor was assessed by means of pruning mass. All clones were harvested on the same day in a given vintage. Yield components (total production per vine, bunch mass, number of bunches per vine, berry mass) were recorded at harvest. Veraison was assessed on two-weekly 150 berry samples, starting at approximately 30% veraison. The date when 50% of the berries reached veraison was recorded. Grape composition (sugar, total acidity, pH, yeast-assimilable nitrogen) was monitored from veraison through harvest by infrared spectroscopy.[20] Phenolic compounds and IBMP[21] were measured on grapes at harvest. Wines were made by small-scale vinifications of 40 kg of grapes in standard conditions. Alcoholic and malolactic fermentations were inoculated. Wines were analyzed after malolactic fermentation by infrared spectroscopy.[20] Wine IBMP content was assessed by GC−MS.[21] Sensory attributes of these wines were judged by a panel of 30 trained professionals (enologists, winemakers). Wines were rated for 21 criteria, including color intensity, color evolution, aroma intensity, intensity of fruity aromas, intensity of vegetal character, mouth feel, amount of tannins, softness of tannins, sweetness, balance, and length.

Downy Mildew Resistance. Experiments were carried out on nine clones of *V. vinifera* cv. Cabernet franc and one clone of *V. vinifera* cv. Chasselas as a susceptible control. Grafted plants obtained from the grapevine collection mentioned above were maintained in pots in the greenhouse until they developed 10 leaves. Leaves four and five from the top were detached and used for further experiments. It is important to strictly respect the position of the leaves to make sure that leaf age does not interfere with clonal variability in downy mildew susceptibility.[22] *P. viticola* isolates were collected in the experimental vineyard of ACW-Changins-Wädenswil in Nyon (Switzerland) and maintained on rooted grapevine cuttings of Chasselas as described by Gindro et al.[23] Sporangia were collected by vacuum aspiration from sporulating lesions and suspended in water (concentration 2×10^6 sporangia mL^{-1}). Then they were slowly stirred at room temperature, and when the release of zoospores had begun, three different methods of inoculation were applied. In the first treatment, 100 droplets (10 μL each) of the suspension were deposited on the abaxial leaf surface on three leaves per clone in humid chambers on three potted plants. Leaf samples from under the droplets were used for stilbene analyses. In the second treatment, five leaf disks (⌀ = 1 cm) were excised from leaves on three potted plants (insertion level as mentioned before) for each clone, placed in humid chambers at room temperature, and inoculated by spraying 1 mL of sporangial suspension on each leaf disk. These leaf disks were used to determine sporangial density according to Gindro et al.,[24] and the results are expressed as the mean number of sporangia per square millimeter. In the third treatment, abaxial leaf surfaces were inoculated by spraying the aqueous sporangial suspension and maintained in a humid chamber at 18 °C, with alternating light and dark (16 and 8 h, respectively), at 80% relative humidity for seven days before OIV determination.[25]

In 2011, clones were tested for downy mildew susceptibility under field conditions. It was not possible, due to time constraints, to rate all the clones in field conditions. Hence, three clones were chosen for downy mildew resistance ratings in the field: one that had shown high downy mildew susceptibility in laboratory conditions (clone A), one that had shown low downy mildew resistance in laboratory conditions (clone C), and one that showed an intermediate downy mildew resistance in the laboratory (clone B). Four replicates of five adjacent vines were conducted without any spraying. In mid-September, the percentage of leaf area infected with downy mildew was rated on 100 leaves per replicate and averaged.

Stilbene Analysis. At 72 h postinfection (hpi), five regions of leaf corresponding to the area under the droplet surface were cut from each inoculated leaf. Leaf samples were weighed and placed in a tube (1.5 mL), and 100 μL of methanol was added. The tightly closed tubes were first placed in a thermoregulated shaker at 60 °C for 10 min and then placed in an ice bath for 5 min. The methanolic extracts (30 μL) were analyzed for stilbenes as described by Pezet et al.[26] The results are expressed in units of nanograms per milligram fresh weight (FW). Experiments were performed in triplicate (three infection zones on three different leaves from three potted plants at the same insertion level).

Statistical Analysis. Data were analyzed by analysis of variance (ANOVA). Means were separated using the Newman−Keuls test ($p < 0.05$). Sensory assessment data were analyzed by principal component analysis (PCA). The software used was Stat box Pro and Microsoft Excel.

RESULTS AND DISCUSSION

Climatic Conditions. Temperatures were close to average through the growing season in 2008. May was rainy, and rainfall was close to average in the other months. The year 2009 was warm from April through September. Rainfall was above average in April and low in August. Vines faced significant water deficit stress in August 2009 (data not shown). April and July 2010 were warm, and 2010 was one of the driest vintages ever recorded in Bordeaux. April, May, and September 2011 were

Table 1. Performances of 10 Cabernet franc Clones (A–J) (2008–2010)

	years measd	A	B	C	D	E	F	G	H	I	J
					Vigor						
pruning mass (g)	2009	656 a	424 b	627 a	599 a	608 a	487 ab	599 a	643 a	639 a	633 a
					Precocity						
veraison date	2009	Aug 2	Aug 5	Aug 4	Aug 5	Aug 5	Aug 4	Aug 4	Aug 3	Aug 4	Aug 5
					Morphology						
cluster compaction rating (1–10)	2008–2010	6.7 a	8.2 b	7.4 a	7.2 a	7.1 a	6.3 a	7.0 a	6.5 a	7.0 a	7.3 a
					Yield Components						
production (kg/vine)	2008–2010	0.984 b	1.230 b	1.154 b	0.995 b	1.188 b	1.478 a	1.033 b	0.969 b	0.989 b	1.111 b
bunch number per vine	2008–2010	7.4 abc	8.0 abc	8.2 abc	6.7 c	8.6 ab	9.2 a	7.7 abc	7.5 abc	6.9 bc	7.4 abc
bunch mass (g)	2008–2010	127 ns	151 ns	141 ns	150 ns	137 ns	155 ns	136 ns	131 ns	146 ns	149 ns
berry mass (g)	2008–2010	1.33 a	1.12 b	1.23 a	1.29 a	1.30 a	1.24 a	1.25 a	1.26 a	1.28 a	1.27 a
					Grape Composition at Harvest						
grape sugar mass (g/L)	2009, 2010	221 ns	225 ns	225 ns	222 ns	225 ns	214 ns	223 ns	223 ns	228 ns	225 ns
total acidity (g of tartrate/L)	2009, 2010	5.0 a	5.3 ab	5.5 b	5.2 a	5.1 a	5.4 ab	5.3 ab	5.0 a	5.2 a	5.3 ab
pH	2009, 2010	3.53 a	3.41 c	3.41 c	3.46 bc	3.45 bc	3.39 c	3.42 c	3.50 ab	3.44 bc	3.42 c
yeast-assimilable nitrogen concn (mg of N/L)	2009, 2010	227 a	163 c	188 bc	193 b	203 ab	185 bc	199 ab	202 ab	185 bc	208 ab
IBMP concn (ng/L)	2009	2.0 ns	3.3 ns	7.3 ns	3.8 ns	3.8 ns	5.0 ns	3.8 ns	3.8 ns	1.8 ns	1.5 ns
					Wine Composition						
alcohol concn (vol %)	2008–2010	13.4 ns	13.4 ns	13.3 ns	13.2 ns	13.2 ns	13.1 ns	13.3 ns	13.4 ns	13.7 ns	13.4 ns
total acidity (g of tartrate/L)	2008–2010	4.2 a	4.5 abc	4.7 bc	4.4 ab	4.5 abc	4.5 abc	4.7 abc	4.3 ab	4.7 abc	4.8 c
pH	2008–2010	3.90 a	3.82 abc	3.71 c	3.85 abc	3.81 abc	3.77 abc	3.75 abc	3.88 ab	3.78 abc	3.73 bc
total phenols (D_{280})	2008–2010	52.7 b	58.0 c	53.7 bc	52.3 b	52.7 b	45.7 a	54.3 bc	50.7 b	55.7 bc	53.7 bc
tanin concn(g/L)	2008–2010	2.5 ab	2.9 a	2.4 abc	2.4 abc	1.9 bc	1.9 c	2.7 a	2.3 abc	2.7 a	2.6 a
anthocyanin concn (mg/L)	2008–2010	540 a	590 a	574 a	557 a	581 a	475 b	581 a	564 a	592 a	601 a
IBMP concn (ng/L)	2008–2010	10.4 ns	8.9 ns	10.5 ns	11.9 ns	9.9 ns	9.9 ns	10.2 ns	9.7 ns	10.3 ns	11.1 ns

warm and dry. In June, July, and August 2011 temperatures and rainfall were close to average.

Precocity. The 50% veraison dates varied in 2009 by three days among the 10 main clones (Table 1) but by as much as eight days in the total population of 31 clones (data not shown). Sugar accumulation was slightly delayed for clones B and F (Figure 1). Sugar accumulation reached a plateau two weeks before harvest for clones A, E, G, H, I, and J. They can be considered as rather early-ripening clones. This variability can be used to adapt Cabernet franc clones to local pedoclimatic conditions. Early-ripening clones are suited to cool locations where full ripeness is difficult to obtain. Late-ripening clones are suited to warm locations where too early ripening might lead to the production of unbalanced wines: high in alcohol content and lacking freshness.

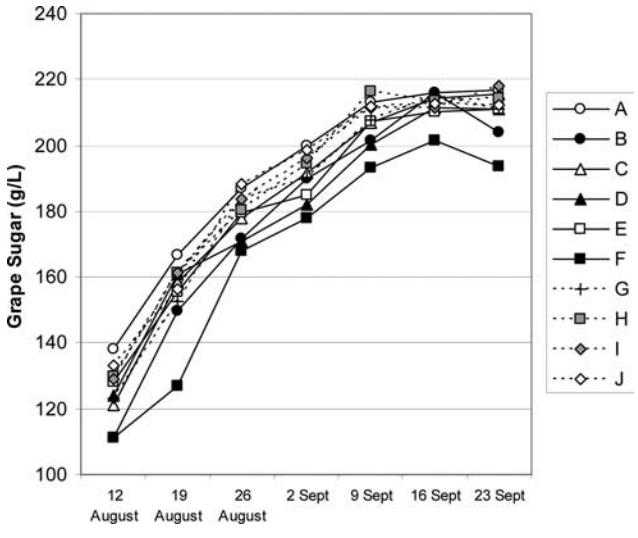

Figure 1. Sugar accumulation from veraison to harvest in 10 Cabernet franc clones in 2009.

Vine Vigor. Pruning mass was measured in 2009 only and varied from 424 to 656 g/vine (Table 1). Pruning mass was significantly lower for clone B compared to the other clones. This great variability in vigor is consistent with the findings of Boso et al.,[6] who found variations in pruning mass ranging from 850 to 2160 g/vine among *V. vinifera* cv. Albariño clones.

Cluster Morphology. Cluster compaction is a major issue in Cabernet franc because compact clusters increase sensitivity toward *Botrytis*. Cluster morphology was assessed from 2008 to 2010 by a visual observation associated with ratings, where highly compact clusters were rated 1 and very loose clusters were rated 10. Among the 10 preselected clones, ratings ranged from 6.3 to 8.2 (Table 1). Clone B obtained consistently high ratings, because its bunches were very loose.

Yield Components. Average production from 2008 to 2010 ranged from 1 to 1.5 kg/vine depending on the clone (Table 1). The average bunch mass ranged from 127 to 155 g, but differences were not statistically significant. The number of bunches per vine varied from 6.7 for clone B to 9.2 for high-yielding clone F. Clone B produced very small berries, but this was not related to low production because it was compensated by a great number of berries per bunch. Differences in berry size might be related to differences in seed number, but this variable was not addressed in this study. Boso et al.[6] showed

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clonal variability in seed number for *V. vinifera* cv. Albariño, but the seed number was not correlated to the berry mass. The production of small berries is an important quality factor for a grapevine variety that is planted to produce red table wines. The skin to juice ratio increases when the berry size decreases. A high skin to juice ratio favors the production of wine with a high concentration in polyphenols, because the latter are mainly located in the skins. Clone B was also the clone with the loosest bunches. This feature is likely to reduce susceptibility to *Botrytis cinerea*. However, no significant differences were recorded at ripeness for these 10 clones with regard to gray mold, because the *B. cinerea* pressure was particularly low in the studied vintages.

Grape Composition at Harvest. The grape sugar content varied among clones by as much as 23 g/L (1.4% in potential alcohol) in a given vintage (data not shown), but average differences were not significant over the 2008−2010 period. Total acidity varied from 4.2 to 4.8 g of tartrate/L, and grape-juice pH values ranged from 3.39 to 3.53 (Table 1). Differences in malic acid were very small and statistically not significant (data not shown). Clone A, which was most early to reach 50% veraison, was low in total acidity and high in pH. The high-yielding clone F was also high in total acidity and low in pH. Yeast-assimilable nitrogen (YAN) was highly variable among clones, which has already been shown on *V. vinifera* cv. Albariño clones.[16] YAN is shown to be a good indicator of vine nitrogen status.[27]

Wine Composition and Sensory Analysis. Early-ripening clones A and H produced wine with a high pH. Wine of clone B, which produced small berries, was high in tannins and anthocyanins, while wine of the high-producing clone F was low in tannins and anthocyanins. IBMP was below the perception threshold (15 ng/L[21]) for all clones and wines. Whereas Belancic and Agosin[7] observed large clonal differences in *V. vinifera* cv. Carmenère, the same variation was not observed for the collection of *V. vinifera* Cabernet franc analyzed here. It is likely that favorable ripening conditions in the three vintages considered in this study leveled potential clonal differences. Clone B showed a tendency to have lower IBMP content in grapes and wine (Table 1), although three-year averages were not significantly different between clones. Wines produced from clone B received consistently good judgment in the three vintages. The wines produced from clone B were darker in color, and the color showed less browning. They were high in tannins, although tannins were soft. Wines from clone C were judged as being typical for Cabernet franc. Clone F was consistently rejected. Its wines showed signs of dilution and marked color browning. An example of the tasting results (vintage 2009) is presented in Figure 2.

Downy Mildew Resistance. Resistance to downy mildew was assessed according to counts of sporulation density (7 days after inoculation[23]), OIV referenced downy mildew notations,[25] phytoalexin production (72 h after inoculation[26]), and counts of infected stomata (%). Sporulation was lowest on clone C and highest on clone E (Table 2). According to the OIV ratings (Table 2), clone C showed little damage from downy mildew on leaves while clones A, E, H, I, and J were infected similarly to the *V. vinifera* cv. Chasselas susceptible control. Clone C produced the highest concentrations of one of the most toxic stilbenes for *P. viticola*, δ-viniferin (Figure 3). This may explain why sporulation was lowest on this clone. Clones A, H, and J, as well as the control, which displayed the most severe infection by *P. viticola*, produced the lowest levels of the most toxic

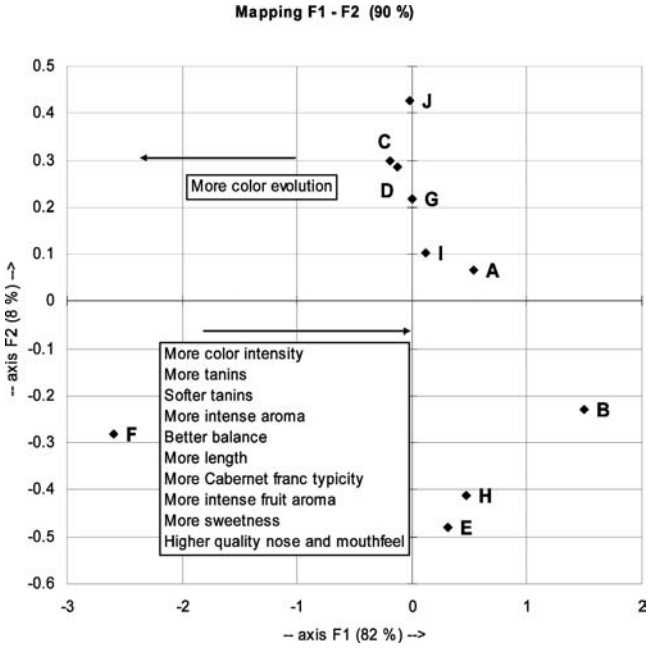

Figure 2. Principal component F_1−F_2 mapping of the results of the tasting of 10 Cabernet franc clones obtained by small-scale vinification in 2009.

stilbenes. Clone B produced a medium amount of toxic stilbenes and showed a medium density of sporulation. These observations confirm that the susceptibility to downy mildew is directly related to the level of sporulation and concentration of toxic stibenes after infection. The various methods of investigation implemented for downy mildew resistance in this study were consistent. OIV ratings were highly correlated to sporulation (R^2 = 0.92, n = 10) and the percentage of infected stomata (R^2 = 0.87, n = 10). Sporulation was correlated to the counts of the infected stomata (%) (R^2 = 0.85, n = 10). Among phytoalexins, δ-viniferin was best correlated to the percentage of infected stomata, sporulation, and OIV ratings (R^2 = 0.48, 0.45, and 0.65, respectively, n = 10). Pterostilbene was correlated to the percentage of infected stomata and OIV ratings (R^2 = 0.34 and 0.34, respectively, n = 10). ε-Viniferin was correlated to OIV ratings (R^2 = 0.35). Previously, Boso et al.[8] have shown clonal differences in downy mildew susceptibility for *V. vinifera* cv. Albariño in field conditions, although underlying mechanisms were not identified. We have shown that variability in downy mildew susceptibility was correlated with differential stilbene production. This observation is in agreement with that of Faria et al.,[28] who showed that a set of 21 *V. vinifera* cv. Touriga Nacional clones could be discriminated in two groups by stilbene synthase−chalcone synthase (StSy−CHS) markers. On the OIV symptoms rating, ranging from 1 (very susceptible) to 9 (very resistant), Cabernet franc clones tested in this study ranged from 1 to 4.5.

In 2011 clones A, B, and C were tested in field conditions without any spraying. By mid-September, 15.7% of the leaf area of clone A was infested with *P. viticola*, while this percentage was only 10.5% for clone C (Table 2). Mildew sensibility was intermediate for clone B (13.4% of leaf area infested with *P. viticola*). No symptoms of downy mildew were observed on flowers or young berries, probably because the spring of 2011 was particularly dry in the Bordeaux area. These observations

Table 2. Characteristics Related to Downy Mildew Resistance of 10 Cabernet franc Clones (A–J)

	year measd	A	B	C	D	E	F	G	H	I	J
ε-viniferin content (ng/mg FW)	2009	2.3 de	2.6 de	11.8 b	2.9 de	6.9 c		19.8 a	1.9 e	6.6 c	3.1 d
δ-viniferin content (ng/mg FW)	2009	2.8 c	5.3 b	13.4 a	1.3 e	5.3 b		5.9 b	1.7 de	5.3 b	2.4 cd
pterostilbene content (ng/mg FW)	2009	3.8 g	8.2 e	8.7 d	10.8 a	9.7 c		6.1 f	1.8 h	10.3 b	2.1 h
no. of sporangia/mm^2	2009	130 b	67 e	36 f	88 d	144 a		70 e	122 b	60 e	113 c
OIV rating (1–9)	2009	1 c	3 b	4.5 a	2 b	1 c		3 b	1 c	1 c	1 c
downy mildew on leaves (% of area)	2011	15.7 a	13.4 ab	10.5 b							

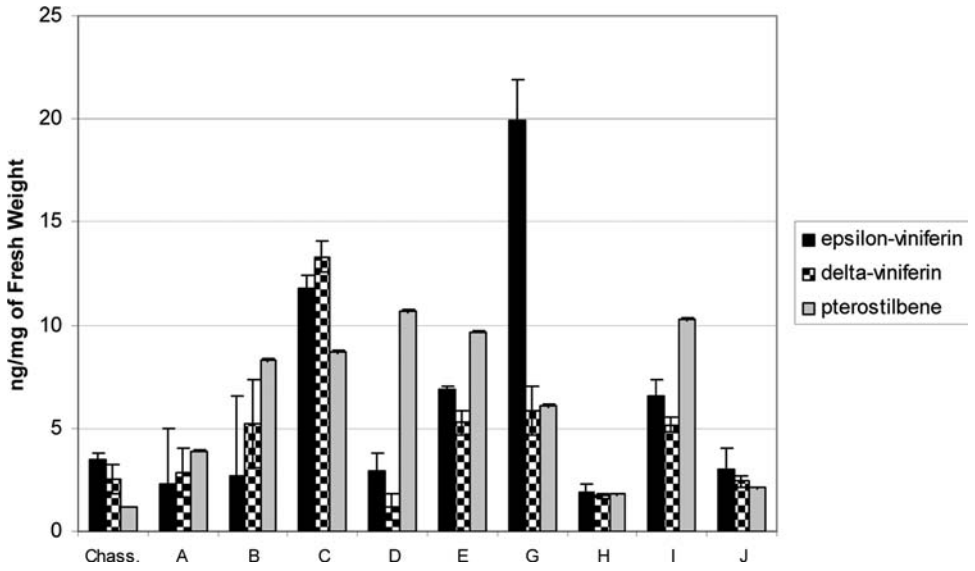

Figure 3. Leaf blade concentrations of ε-viniferin, δ-viniferin, and pterostilbene in nine clones of Cabernet franc. Comparison with the highly sensible reference *V. vinifera* cv. Chasselas. Error bars indicate the standard error.

confirmed slightly reduced downy mildew resistance for clone C in field conditions.

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Notes

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