

Evaluation of foliar resistance to downy mildew in different cv. Albariño clones

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Summary

Resistance to downy mildew was studied in different *Vitis vinifera* L. cv. Albariño clones belonging to the collection of the Mision Biológica de Galicia, CSIC (Spain). *V. riparia*, *V. vinifera* cv. Solaris and *V. vinifera* cv. Müller-Thurgau were used as controls. Plants were inoculated with *Plasmopora viticola* in the laboratory using the leaf disc, whole leaf and whole plant techniques. The results were compared with those obtained in the field for the same Albariño clones. The most susceptible group of clones included MBG-2, MBG-14, MBG-12 and MBG-9, while MBG-13, MBG-3 and MBG-6 formed the most resistant group. The remaining clones showed intermediate resistance. These results coincide with observations made in the field. The resistance observed in MBG-12 could have been generated from *in vitro* culture, because this induces changes in the downy mildew resistance.

Key words: Downy mildew resistance, sporulation, leaves, clone, Albariño, artificial inoculation.

Introduction

Downy mildew (*Plasmopora viticola*) is one of the main diseases affecting grapevines. Especially cvs of *Vitis vinifera* L. are highly susceptible (BOUBALS 1959, GALET 1977, 1995, PEARSON and GOHEEN 1996). However, the susceptibility to this pathogen differs between cultivars and even between clones of a single variety (RAVAZ 1914; PÉREZ MARÍN 1992).

The cultivar Albariño (*V. vinifera*) is one of the most important white wine-producing grapevines of north-eastern Spain and northern Portugal. The high rainfall and warm temperatures of these areas favour the development of downy mildew diseases and may cause severe epidemics.

Vitis species from the north-American and east-Asian sphere are an essential source for resistance breeding (ALLEWELDT and POSSINGHAM 1988, ALLEWELDT 1996). To date little attention has been paid to the innate resistance of autochthonic cultivars of *Vitis vinifera*. Especially accessions from ancient vineyards show a broad range of phenotypes. Potentially these cultivars are unknown resources of resistance against downy mildew. This might be the case for cv. Albariño derived from ancient vineyards of north-eastern Spain.

The disease resistance of vines can be tested both in the field and in the laboratory. In the field, vines can either be artificially inoculated or, if the natural conditions are favourable vines can be left untouched and the disease is allowed to develop naturally. Most studies of resistance are, however, performed in the laboratory under controlled conditions. Many studies (BOUBALS 1959, GALET 1977, STAUDT and KASSEMAYER 1995, STAUDT 1997) indicate that different varieties of *Vitis vinifera* vary in their level of resistance to downy mildew. Different inoculation techniques were used in these studies, such as leaf disc inoculation (STEIN *et al.* 1985, DENZER *et al.* 1995, STAUDT and KASSEMAYER 1995), detached leaf inoculation (SONG *et al.* 1998, KIEFER *et al.* 2002), whole plant inoculation (RUMBOLZ *et al.* 2002), *in vitro* dual culture (BARLASS *et al.* 1986), and the leaf single-node technique (LIU *et al.* 2003). Currently, the leaf disc technique is thought to be the most reliable for assessing resistance to downy mildew in the laboratory.

The aim of the present work was to determine whether different Albariño clones show different levels of resistance to downy mildew in the laboratory and to compare the results with field observations.

Material and Methods

Plant material: The resistance to *P. viticola* was tested for 14 clones of *Vitis vinifera* L. cv. Albariño. Of each clone 5 plants derived from the collection of the Misión Biológica de Galicia (MBG; CSIC) were available (Tab. 1). *V. riparia* and *V. vinifera* cv. Solaris (highly resistant) and *V. vinifera* cv. Müller-Thurgau (highly susceptible) were used as controls. All the plants used in the experiments were cultivated in the greenhouse.

The clones used in this study originated from centuries-old mother plants growing wild in north-eastern Spain, except for MBG-12, MBG-13 and MBG-14 which were generated by micropropagation of the apical meristem (FERRO 1989) of clone MBG-11. After removal from their test tubes, the cultured, ungrafted plantlets were planted in 1986 in a plot at CSIC and subjected to three different types of pruning: Cordon Royat, Sylvoz (intermediate length), and Cordon alto en Cruzeta (long pruning). The ampelographic characteristics of the plants and their fertility were different depending on the pruning system used (MARTÍNEZ and MANTILLA 1993). They were therefore concluded to be 'pruning-dependent clones' and termed MBG-12 (Sylvoz pruning), MBG-13 (Cor-

Table 1

Severity index (%) and resistance ratings of 14 Albariño clones resistant to downy mildew after natural infection and artificial inoculation

Clone	From	Artificial Inoculation					Natural Infection ⁴⁾	
		Leaf Disc	Leaf	Plant ³⁾	Mean ± Deviation	Rating ¹⁾	Plant	Rating ²⁾
MBG1	Specimens centuries old MBG	80	70.00	36.43	62.14 ± 22.82	S	43	S
MBG2	Specimens centuries old MBG	100	96.67	29.13	75.27 ± 39.99	HS	44	S
MBG3	Specimens centuries old MBG	53.30	53.33	37.05	47.79 ± 9.30	R	25	R
MBG4	Specimens centuries old MBG	76.67	75	25.74	59.14 ± 28.93	S	43	S
MBG5	Specimens centuries old MBG	76.67	76.67	32.01	61.78 ± 25.78	S	45	S
MBG6	Specimens centuries old MBG	51.67	50	33.63	45.10 ± 9.97	R	27	S
MBG7	Specimens centuries old MBG	76.67	75	34.28	61.98 ± 24.01	S	48	S
MBG8	Specimens centuries old MBG	76.67	75	24.85	58.84 ± 29.45	S	40	S
MBG9	Specimens centuries old MBG	86.67	78.33	37.10	67.37 ± 26.54	S	38	S
MBG10	Specimens centuries old MBG	76.67	75	32.94	61.54 ± 24.78	S	31	S
MBG11	Specimens centuries old MBG	76.67	63.33	29.97	56.66 ± 24.05	S	44	S
MBG12	<i>In vitro</i> MBG	90	100	43.54	77.85 ± 30.13	HS	50	S
MBG13	<i>In vitro</i> MBG	36.67	36.67	39.23	37.52 ± 1.48	R	10	R
MBG14	<i>In vitro</i> MBG	100	93.33	43.34	78.89 ± 30.97	HS	60	HS

¹⁾ 0-5.0: ER, extremely resistant; 5.0-25.0: HR, highly resistant; 25.0-50: R, resistant; 50-75: S, susceptible; >75: HS, highly susceptible.

²⁾ 0-25: R, resistant; 25-50: S, susceptible; >50: HS, highly susceptible.

³⁾ Pot plants in greenhouse from cuttings.

⁴⁾ Field plants, 2000-2002, Spain (Boso *et al.* 2004)

don Royat), and MBG-14 (Cordon alto en Cruzeta). In 1993, cuttings were taken from each clone and grafted onto 110 R rootstocks. The same was done for the remaining clones from the centuries-old plants belonging to the Misión Biológica collection (each clone was represented by 10 plants and all were Sylvoz-pruned). Cuttings from these clones provided the experimental material used in the evaluation of resistance. These vines were planted in pots and cultivated in the greenhouse.

Inoculum: The method of RUMBOLZ *et al.* (2002) was used to propagate sporangia for the preparation of the inoculum. *Plasmopara viticola* obtained from naturally infected plants in the vineyards of the Staatliches Weinbauinstitut, Freiburg, Germany, were maintained on *V. vinifera* cv. Müller-Thurgau grown in the greenhouse. For the propagation of the inoculum, plants were sprayed with a suspension of sporangia (40,000 sporangia·ml⁻¹ distilled water) on the abaxial leaf side and covered with a wetted polyethene bag overnight. Next day bags were removed and after 5-6 d incubation at 25-26 °C the sporulation was triggered by covering the plant again with a wet plastic bag. On day 6, freshly developed sporangia were collected in centrifuge tubes using a small paintbrush. The suspension for the experimental inoculation was prepared by counting the sporangia with a Fuchs-Rosenthal chamber; a dilution was adjusted to a concentration of 20,000 sporangia·ml⁻¹.

Leaf disc test: Leaf discs were prepared according to STAUDT and KASSEMAYER (1995) and RUMBOLZ *et al.* (2002). The leaf surface was sterilised in 75 % ethanol and subse-

quently washed three times in distilled water. After drying with filter paper 12 discs (16 mm diameter) were excised per leaf using a cork borer. Discs were placed bottom side up in Petri dishes containing water agar (0.8 %).

Each disc was inoculated with 50 µl of the sporangia suspension and incubated for 5 d at 25 °C, photoperiod: 16 h light (2.5 W·m⁻²), in a culture chamber. All preparations were repeated in triplicate. Infection symptoms such as frequency of sporulation, necrosis, sporulation severity and density were scored on day 5.

Detached leaf test: The same procedure as in the leaf disc test was used except that whole leaves were placed on the 0.8 % agar (KIEFER *et al.* 2002). Leaves were inoculated with 4 or 5 50 µl drops of the sporangium suspension and incubated for 5 d at 25 °C, photoperiod: 16 h light (2.5 W·m⁻²), in a culture chamber. All preparations were repeated in triplicate. Observations were made on day 5 and the infection symptoms scored as described above.

Plant test: All cv. Albariño clones were planted in pots in the greenhouse (one plant of each clone) and control plants were sprayed with a suspension of sporangia (40,000 sporangia·ml⁻¹ distilled water). The incubation and sporulation was carried out as described above. After 5-6 d the frequency of sporulation and 'oil spots' were documented. All experiments were repeated in duplicate.

The resistance of each clone was scored after the following scheme: 0-5: ER, extremely resistant; 5-25: HR, highly resistant; 25-50: R, resistant; 50-75: S, susceptible; > 75: HS, highly susceptible (STAUDT and KASSEMAYER 1995).

Microscopical analysis: The development of the pathogen was analysed by epifluorescence microscopy (AxioPhot Zeiss, Germany) and a digital imaging system (AxioCam and AxioVision, Zeiss, Germany): Three inoculated discs were removed from each experiment at different times (48, 120 and 144 h) and were stained with anilin blue after clarification with 1M KOH according to KIEFER *et al.* (2002).

Statistical analysis: Each variant was examined by analysis of variance (ANOVA) to determine significant differences among the clones. For each variant, Fisher's protected test (minimum significant difference [MSD] method) (STEEL *et al.* 1997) was used to determine the level of resistance and susceptibility, respectively, for each clone. All calculations were performed using SAS V8.1 software (SAS 2000).

Results and Discussion

The results show that the clones of cv. Albariño have different levels of resistance to downy mildew (Tabs 1 and 2). All variants showed significant differences between clones except for the degree of sporulation.

Similar results were obtained for each of the inoculation techniques (leaf disc, whole leaf and whole plant). All three techniques showed that cv. Albariño is more sensitive to downy mildew infection than cv. Müller-Thurgau, which is actually considered to be one of the most sensitive cultivars. All the Albariño clones showed 100 % sporulation (Tab. 3), and thus were different from the controls. Sporulation in cv. Müller-Thurgau was slightly lower (5.56 % lower, whole leaf method), while *V. riparia* and *V. vinifera* cv. Solaris showed either no (whole leaf and whole plant tests) or very low levels of sporulation (leaf disc test).

The clones showed differences in the leaf disc and whole leaf tests; they could be differentiated into three groups (Tab. 3). One group was formed by MBG-2, MBG-14, MBG-12 and MBG-9; they were the most susceptible clones showing high disease severity, high sporulation density and a low frequency of necrosis. The second group consisted of MBG-13, MBG-3 and MBG-6; they showed a high frequency of necrosis, low sporulation density and low disease severity. The third group comprised the remaining clones, which showed intermediate characteristics. The control vines *V. vinifera* cv. Solaris and *V. riparia* showed the highest degree of necrosis. The clones MBG-14, MBG-12 and MBG-9 were the most sensitive in all three tests while MBG-3, MBG-13, MBG-6 were consistently the most resistant.

According to some authors (KORTEKAMP *et al.* 1999, STAUDT and KASSEMAYER 1995), cultivars with high prostrate trichoma densities have a certain degree of resistance. When these trichoma were removed, they became more sensitive. These authors suggest that they might therefore be part of a resistance mechanism. However, cv. Albariño has a very high density of trichoma on the abaxial leaf side (LOUREIRO *et al.* 1998, MARTÍNEZ *et al.* 1994) and yet appears to be much more sensitive to downy mildew than cv. Müller-Thurgau, which has a low trichoma density (AMBROSI *et al.* 1998, BOIDRON *et al.* 1995).

Table 2

Variance for frequency of sporulation, necrosis, sporulation severity and density of cv. Albariño clones, evaluated for downy mildew resistance/susceptibility using the leaf disc, leaf and plant method

Source of variation	df	Leaf disc			Means square Plant			Leaf		
		Sporulation	Necrosis	Severity	Sporulation	Severity	Density	Sporulation	Necrosis	Severity
Clone	16	2479.78 ns	2648.87***	2560.35***	2472.42***	4840.60***	3289.75 n.s	1284.68***	2603.06***	2383.57***
Error	34	10.89	132.02	35.29	36.76	918.99	5.44	69.61	54.90	49.01

***: Significant at the 0.001 probability level; n.s: not significant

Table 3

Means values for frequency of sporulation, necrosis, sporulation severity and density of cv. Albariño clones from artificial infection using the leaf disc, leaf and plant method

Clone	Disc Leaf				Leaf				Plant	
	Sporulation	Necrosis	Severity	Density	Sporulation	Necrosis	Severity	Density	Sporulation	Severity
MBG-1	100 a	0.0 d	80 cd	25 d	100 a		70.00 bc	25 e	22.45 cdef	36.43 abc
MBG-2	100 a	0.0 d	100 a	100 a	100 a	0.00 c	96.67 a	100 a	22.64 cdef	29.13 bc
MBG-3	100 a	0.0 d	53.30 d	25 d	100 a	0.00 c	53.33 de	33.33 de	19.26 ef	37.05 abc
MBG-4	100 a	16.66 bcd	76.67 d	50 c	100 a	0.00 c	75 bc	50 c	18.04 ef	25.74 bc
MBG-5	100 a	11.11 cd	76.67 d	50 c	100 a	0.00 c	76.67 b	50 c	18.82 ef	32.01 abc
MBG-6	100 a	22.21 b	51.67 d	33.33 d	100 a	0.00 c	50 ef	41.67 cd	25.22 bcdef	33.63 abc
MBG-7	100 a	34.44 b	76.67 d	50 c	100 a	0.00 c	75 bc	50 c	16.55 f	34.28 abc
MBG-8	100 a	16.66 bcd	76.67 d	50 c	100 a	0.00 c	75 bc	50 c	18.29 ef	24.85 c
MBG-9	100 a	16.66 bcd	86.67 bc	75 b	100 a	0.00 c	78.33 b	75 b	37.28 a	37.10 abc
MBG-10	100 a	0.0 d	76.67 d	50 c	100 a	0.00 c	75 bc	50 c	20.88 def	32.94 abc
MBG-11	100 a	11.11 cd	76.67 d	50 c	100 a	0.00 c	63.33 cd	50 c	30.86 abcd	29.97 abc
MBG-12	100 a	0.0 d	90 b	75 b	100 a	0.00 c	100 a	75 b	32.39 abc	43.54 a
MBG-13	100 a	77.77 a	36.67 e	33.33 d	100 a	3.33 c	36.67 g	33.33 de	29.51 abcde	39.23 ab
MBG-14	100 a	0.0 d	100 a	100 a	100 a	0.00 c	93.33 a	100 a	34.55 ab	43.34 a
Müller-Thurgau	100 a	11.11 cd	36.67 e	33.33 d	94.44 b	22.83 b	40 fg	33.33 de	2.15 g	0.00 d
Riparia	22.21 b	83.33 a	8.33 f	0.00e	0.00c	18.33 b	0.00 h	0.00 f	0.00 g	0.00 d
Solaris	5.55 c	83.33 a	0.00 f	0.00 e	0.00c	83.33 a	0.00 h	0.00 f	0.00 g	0.00 d
L.S.D (0.05)	5.47	19.06	9.85	10.06	3.8733	13.845	12.295	11.618	36.586	36.609

^{a)} Mean separation by Fisher's protected test (least significant difference (LSD) method), at $p \leq 0.05$. Means with the same letter are not significantly different.

Epifluorescence microscopy was used to monitor *P. viticola* infection in cv. Albariño whole leaves during the infection cycle. The different stages of development were examined from the penetration of the pathogen into the host tissue and the colonization of the mesophyll until the production of sporangiophores and sporangia. By the second

day after inoculation, differences became visible between some clones with respect to the developmental stage of *P. viticola* (Figure). For example, in MBG-5, the development of the pathogen appeared to be delayed when compared to the other clones. In MBG-1 and MBG-13, few haustoria were apparent and the hyphae were small. In contrast,

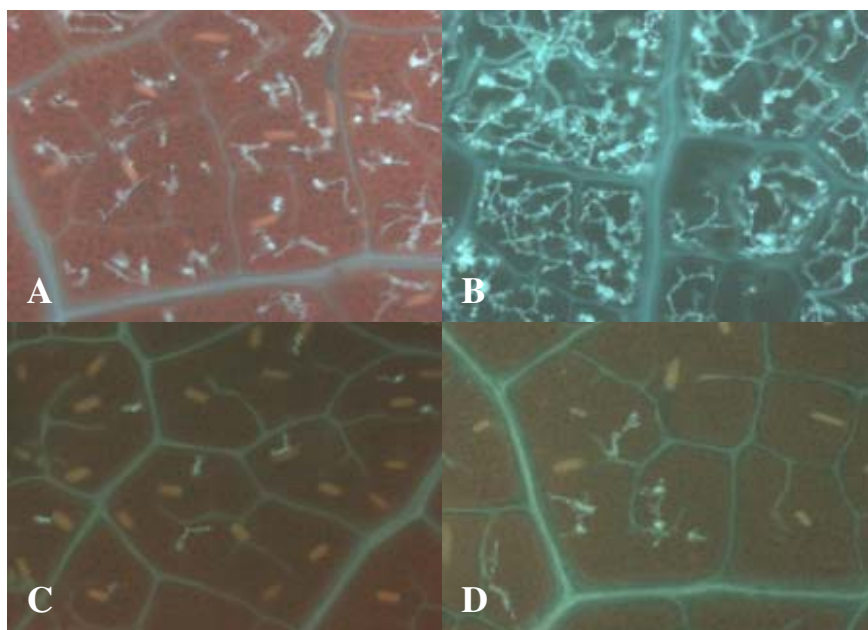


Figure: Mycelium development over 2 d, (Anilin blue staining, epifluorescence, obj. 40x). **A:** the most resistant clones (MBG-3 and MBG-13): few infections and only small hyphae; **B:** the most susceptible clones (MBG-2 and MBG-14): many infections and different sized hyphae; **C** and **D:** control plants (*V. rupestris* and *V. vinifera* cv. Solaris).

MBG-9, MBG-2, MBG-14 and MBG-12 showed many well developed haustoria and different sized hyphae. By the 5th or 6th day, however, no differences were visible between the clones; the intercellular spaces of the meso-phyll were completely colonized by hyphae.

In general, the laboratory results agreed with those of natural infections in the field (Boso *et al.* 2004), although MBG-2 was more susceptible in the field than in the laboratory. MBG-6 and MBG-13 were the most resistant in both experimental situations. MBG-13 was produced by micropropagation methods, which may be the reason for its resistance. Note that its mother clone, MBG-11, and its sister clones MBG-12 and 14, showed behaviours similar to the rest of the clones. The divergent behaviour might be due to the different pruning methods.

As described by MARTÍNEZ and MANTILLA (1994), micropropagated, low-pruned, non-grafted Albariño plants tend to maintain juvenile characteristics (leaves with a deep sinus, strong anthocyanin pigmentation of the veins, high density of erect trichoma, few or no prostrate trichoma, and zero fertility). The same authors showed that grafting of these plants onto rootstocks led to the disappearance of these juvenile characteristics, *i.e.*, they formed the characteristics of typical Albariño adults (MARTÍNEZ and MANTILLA 1993). Our observations over recent years (unpubl.) show that MBG-12, MBG-13 and MBG-14, when grafted and planted in plots at the Misión Biológica de Galicia, show agronomic and ampelographic behaviours similar to the rest of the clones. The only explanation we can offer for the improved resistance seen in MBG-12 is that *in vitro* culture induces changes in the downy mildew resistance mechanisms which do not revert to those of typical adults after grafting.

Of the resistant clones, MBG-13 was the only clone to show necrosis. This may be a hypersensitive response.

Obtaining clones with resistance to downy mildew under laboratory and field conditions is of interest not just to viticulturists and nurseries, but also to researchers working on genetic markers of downy mildew resistance or breeders being who are involved in plant improvement programs.

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References

- ALLEWELDT, G.; 1996: Genetics of grapevine breeding. *Prog. Bot.* **58**, 441-454.
- ALLEWELDT, G.; POSSINGHAM, J. V.; 1988: Progress in grapevine breeding. *Theor. Appl. Genet.* **75**, 669-673.
- AMBROSI, H.; RÜHL, E. H.; MÜNCH-DETTWEILLER, E.; SCHMID, J.; SCHUMANN, F.; 1998: Farbatlas Rebsorten. 330 Sorten und ihre Weine. Ulmer, Stuttgart.
- BARLASS, M.; MILLER, R. M.; ANTCLIFF, A. J.; 1986: Development of methods for screening grapevines for resistance to infection by downy mildew. I. Dual culture *in vitro*. *Am. J. Enol. Vitic.* **37**, 61-66.
- BOIDRON, R.; BOURSQUOT, J. M.; DOAZAN, J. P.; LECLAIR, P.; LEGUAY, M.; WALTER, B.; 1995: Catalogue des variétés et clones de vigne cultivés en France. Ministère de l'Agriculture, de la Pêche et de l'alimentation, CTPS.
- BOSO, S.; SANTIAGO, J. L.; MARTÍNEZ, M. C.; 2004: Resistance of eight different clones of the grape cultivar Albariño to *Plasmopara viticola*. *Plant Dis.* **88**, 741-744.
- BOUBALS, D.; 1959: Contribution à l'Étude des Causes de la Résistance de Vitacées au Mildiou de la Vigne (*Plasmopara viticola* (B. et C) Ber. et de T.)) et leur Mode de Transmission Héritaire. Thèse Doct. Sci. Ann. Amélior. Plantes.
- DENZER, H.; STAUDT, G.; SCHLÖSSER, E.; 1995: The behaviour of *Plasmopara viticola* on resistant and susceptible grapevine varieties. *Vitis* **34**, 113-117.
- FERRO, E.; 1989: Aplicación de las Técnicas de Cultivo *in vitro* de Ápices Caulinares en el Saneamiento de Clones Seleccionados de Vid, cv. Albariño. Tesis Doct., Univ. Santiago de Compostela.
- GALET, P.; 1977: Les Maladies et les Parasites de la Vigne. Tome I: Les Maladies dues à des Végétaux. Imprimerie Le Paysan du Midi., Montpellier.
- GALET, P.; 1995: Précis de Pathologie Viticole. 2^{ème} ed. Imprimerie JF Impression, Montpellier.
- KIEFER, B.; RIEMANN, M.; BÜCHE, C.; KASSEMAYER, H.-H.; NICK, P.; 2002: The host guides morphogenesis and stomatal targeting in the grapevine pathogen *Plasmopara viticola*. *Planta* **215**, 387-393.
- KORTEKAMP, A.; WIND, R.; ZYPRIAN, E.; 1999: The role of hairs on the wettability of grapevine (*Vitis spp.*) leaves. *Vitis* **38**, 101-105.
- LIU, S. M.; SYKES, S. R.; CLINGELEFFER, P. R.; 2003: A method using leafed single-node cuttings to evaluate downy mildew resistance in grapevine. *Vitis* **42**, 173-180.
- LOUREIRO, M. D.; MARTÍNEZ, M. C.; BOURSQUOT, J. M.; THIS, P.; 1998: Molecular marker analysis of *Vitis vinifera* "Albariño" and some similar grapevine cultivars. *J. Am. Soc. Hort. Sci.* **123**, 842-848.
- MARTÍNEZ M. C., MANTILLA J. L. G.; 1993: Elimination des caractères juvéniles typiques de *Vitis vinifera* L., cv. Albariño, provenant de culture *in vitro*, par utilisation du greffage. *Bull. O I V (Off. Int. Vigne Vin)* **66**, 749-750.
- MARTÍNEZ, M. C.; LOUREIRO, M. D.; MANTILLA, J. L. G.; 1994: Importancia y validez de distintos parámetros ampelométricos de hoja adulta utilizados en la distinción de cultivares de *Vitis vinifera* L., *Inv. Agr. Prod. Prot. Vege.* **9**, 377-189.
- PEARSON, R. C.; GOHEEN, A. C.; 1996: Plagas y enfermedades de la vid. Ed. Mundi-Prensa, Madrid.
- PÉREZ MARIN, J. L.; 1992: Los Parásitos de la Vid. Estrategias de Protección Razonada. Ministerio de Agricultura, Pesca y Alimentación. Ediciones Mundi-Prensa, Madrid.
- RAVAZ, L.; 1914: Le Mildiou. Masson éd, Paris.
- RUMBOLZ, J.; WIRTZ, S.; KASSEMAYER, H.-H.; GUGGENHEIM, R.; SCHAFFER, E.; BÜCHE, C.; 2002: Sporulation of *Plasmopara viticola*: Differentiation and light regulation. *Plant Biol.* **4**, 413-422.
- SAS, 2000: SAS/STAT User's Guide, Versión 6, 4th ed. SAS Institute Inc., Cary, North Carolina, USA.
- SONG, R. G.; LU, W. P.; LI, C. Y.; WANG, J.; SHEN, Y. J.; 1998: Inheritance of resistance to *Plasmopara viticola* in intraspecific cross of *Vitis amurensis* Rupr. *Acta Hort. Sin.* **25**, 117-122.
- STAUDT, G.; KASSEMAYER, H.-H.; 1995: Evaluation of downy mildew (*Plasmopara viticola*) resistance in various accessions of wild *Vitis* species. *Vitis* **34**, 225-228.
- STAUDT, G.; 1997: Evaluation of resistance to grapevine downy mildew (*Uncinula necator*) in accessions of *Vitis* species. *Vitis*, **36**, 151-154.
- STEIN, U.; HEINTZ, C.; BLAICH, R.; 1985: Die *in vitro*-Prüfung von Rebsorten auf Oidium- und Plasmopara-Resistenz. *Z. Pflanzenkr. Pflanzenschutz* **94**, 355-369.
- STEEL, R. G. D.; TORRIE, J. H.; DICKEY, D. A.; 1997: Principles and procedures of statistics. A biometrical approach. 3rd ed. MacGraw-Hill, New York.

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