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The "hoja de malvón" grape disease in Argentina

Marta Gatica[1], Bernadette Dubos [2] and Philippe Larignon[2]

[1]INTA, EEA Mendoza, CC Nº 3, 5507 Luján de Cuyo, Mendoza Argentina
[2]INRA, Centre de Recherches de Bordeaux, BP 81, 33883 Villenave d'Ornon cedex, France

Summary. "Hoja de malvón" is the common name of a disease that has been observed in grapevines of Argentina since the beginning of the century. This paper reports the status of this disease in this country. The disease starts appearing in one of the plant arms. Leaves are smaller than normal, chlorotic and with the edges rolled downwards, the canes and current-year shoots show reduced growth and the clusters are smaller and sparser with berries of non uniform-size. In a cross sectional cut of the trunk two types of necrosis could be observed: a yellowish necrosis of soft consistency, or decay, in a central or sectorial position, surrounded by a black line and a brownish area; and a sectorial light brown necrosis of hard consistency, surrounded by a brown zone. Sometime, black spots are observed at the edge of the damaged areas. Isolations from trunk tissues of different cultivars of grafted and nongrafted plants, 3- to 30-year-old, were done in malt extract agar. *Phellinus* sp., *Botryodiplodia* sp. and *Phaeoacremonium* spp. (*P. aleophilum*, *P. chlamydosporum* and *P. parasiticum*) were isolated. Up to now, *in vitro* pathogenicity tests were conducted only with *Phellinus* sp., *Botryodiplodia* sp. and *P. chlamydosporum*, which were able to cause the decline and death of young plants. Research attempting to quantify the effects, and to study the factors influencing the appearance of the disease, is also being conducted. Only preventive practices are recommended for its control.

Key words: grapevine, trunk disease, wood decay.

Introduction

Argentina has an area of 210,000 ha planted with vines, which represents the 2.7% of the total area of the world's vineyards. It is the sixth world grape producer and the fifth wine producer after France, Italy, Spain and the United States. Vines are cultivated in the west of the country, between 24º and 39º South latitude, in temperate climates with 100 to 400 mm of rainfall, so irrigation is necessary for growth. About 92% of the area under vines is located in the provinces of Mendoza (69%) and San Juan (23%). Vertical shoot position (VSP) (32%) and a local trellis system called "parral" or pergola (67% of the total surface) are the most used training systems.

Powdery mildew, downy mildew, bunch rot and the so called "hoja de malvón" are the most frequent diseases. The "hoja de malvón" has been observed by the grape growers since the beginning of the century. This name refers to the fact that a diseased grapevine leaf resembles a geranium leaf. For many years its presence did not cause serious losses but in the last years its increased spread has begun to cause important damage to vineyards. Since 1996 studies have been carried out in Argentina to identify the causal agent(s), quantify the effects and determine the factors that favour diseases appearance. Two fungi of the *Hymenochae-*

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Corresponding author: M. Gatica
Fax: +54 261 4 963320
E-mail: mgatica@inta.gov.ar

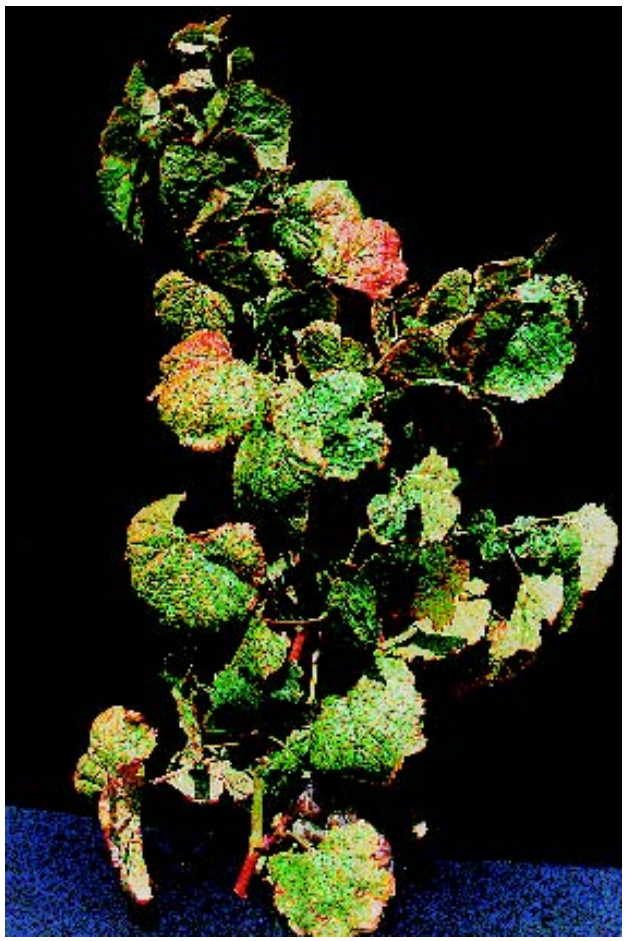

Fig. 1. Shoot and leaves of a grapevine plant of cv. Chenín showing typical symptoms of "hoja de malvón" disease.

taceae family and a *Botryodiplodia* sp. were found to be associated with the disease (Gatica *et al.*, 1998). In 1998 the INTA-INRA Agreement between Argentina and France allowed researchers from these two institutions to increase their knowledge and experience about diseases of vinewood and their appearance in both countries.

This is a report about the situation of knowledge of the disease in Argentina.

Description of symptoms

The symptoms appear first in one of the plant arms, affecting canes, leaves, clusters and trunks. Leaves are smaller than normal, chlorotic and with the edges rolled downwards (Fig. 1). The canes and shoots of the year show reduced growth and the clusters are smaller and sparser with berries of non uniform-size. In a cross sectional cut of the trunk two types of necrosis are observed: a yellowish necrosis, of soft consistency, in a central or sectorial position, surrounded by a black line and a brownish area, and a sectorial light brown necrosis of hard consistency, surrounded by a brown zone (Fig. 2). These two types of necrosis can also be observed in the same cut. Sometimes black spots are observed at the border of the damaged areas. Wood weakness develops from the top to the bottom of the plant. During its development, the disease spreads to other arms until the plant becomes unproductive and finally dies. Upon the trunks of some affected vines, the development of a light brownish fructification from a *Hymenochaetaceae* fungus has also been observed.

Estimation of damage

Research to quantify the effects of the disease, and to study the factors favouring its appearance, is also being conducted. In a survey done in the north and central part of Mendoza province, the relationships: a) between different crop features such as variety, plant age, trellis systems, pruning and disease incidence and, b) between yield and disease severity, were studied. About 46% of the vineyards had less than 10% of their vines infect-

Fig. 2. Wood necrosis symptoms in a cross sectional cut of cv. Chenín.

Fig. 3. Plantlets of cv. C. Sauvignon inoculated with *Phellinus* sp. showing reduced growth and reddish leaves (two flasks at the left). Control: flask at the right.

ed, while 30% had an incidence (I) between 11 and 20%. A positive correlation between I≥10% and vines grown in the "parral" system (P=0.067), and also between I≥10% and vines more than 20 years old (P=0.01) was found by chi squared test. There was also a positive correlation between I≥20% and heavy pruning (P=0.053). The data of yield and severity measured in vines of the cultivars Criolla Grande and Cereza grown with a "parral" trellis system, were adjusted to an exponential model (P=0.0001; r^2=0.69) (Gatica *et al.*, 1997).

Etiological studies

Isolation and identification of the pathogens

Since November 1998, isolations from 1,491 trunk tissue pieces of eleven plants were done in malt extract agar (Larignon, 1991; Larignon and Dubos, 1997). Plants corresponded to cultivars Cabernet Sauvignon, Merlot, Cereza, Sultanina and Chenín. Non-grafted plants, 15- to 30-year-old, and 3-year-old grafted plants, were examined.

Phellinus sp., *Botryodiplodia* sp., *Phaeoacre-*

Table 1. Frequency distribution of the organisms isolated from different zones of decayed wood.

Organisms isolated	Frequency (%)[a]					
	Zone a[b]	Zone b[c]	Zone c[d]	Zone d[e]	Zone e[f]	Zone p[g]
Phellinus sp.	36.8	15.8	15.8	5.3	26.3	0.0
Botryodiplodia sp.	21.4	0.0	14.2	7.1	35.7	21.4
Phaeoacremonium spp.	11.1	44.4	22.2	11.1	0.0	11.1

[a] Percent values were calculated over the total number of isolates of each fungus.
[b] Soft white decay.
[c] Black line bordering the decay.
[d] Brownish zone of hard consistency.
[e] Sectorial brown zone of hard consistency.
[f] Margin of sectorial brown zone.
[g] Black spots.

Table 2. Plantlets showing symptoms in *in vitro* pathogenicity tests.

Fungal species	Strain No.	'C. Sauvignon'		'Tempranilla'		'Malbec'	
		No. of plantlets tested	Plantlets showing symptoms (%)	No. of plantlets tested	Plantlets showing symptoms (%)	No. of plantlets tested	Plantlets showing symptoms (%)
Phellinus sp.	LCP 00 4369	18	100	16	100	16	100
P. chlamydosporum	LCP 99 4302	28	63	22	45	30	34
Botryodiplodia sp.	LCP 00 4370	24	100	25	100	29	100

monium aleophilum W. Gams, Crous, M.J. Wingf. & L. Mugnai, *P. chlamydosporum* W. Gams, Crous, M.J. Wingf. & L. Mugnai, and *P. parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf. were isolated from non-grafted plants. In young grafted plants (Merlot/101-14 MG and C. Sauvignon/1103 P), only species of *Phaeoacremonium* were isolated.

Phellinus sp. was most frequently isolated from the soft decayed areas and from the margin of sectorial brown zones, *Phaeoacremonium* sp. from the black line bordering the decay and *Botryodiplodia* sp. from the margin of sectorial brown zones (Table 1).

Pathogenicity test

Plantlets from the cv. C. Sauvignon, Malbec and Tempranilla were multiplied *in vitro* in glass flasks containing 50 ml of Galzy medium (Mauro *et al.,* 1988; Gregory and Tizio, 1997) and grown under controlled conditions: 25ºC, 16-h day 90 μE m^{-2} s^{-1}. After 30 days, when the plants had 3-4 nodes, a 5 mm disc of inoculum was placed in the culture medium where the plants grew.

a) *Inoculation with* Phellinus *sp*. Thirty days after the inoculation, symptoms in all the plants which grew in the inoculated medium were observed, while no symptoms appeared in the control plants (Table 2). The symptoms were: plantlets of reduced growth, darkened and reddish stems, chlorotic leaves, with reddish edges (some of them rolled downwards) and death of plants. The cv. C. Sauvignon showed leaves with reddish edges 23 days after the inoculation (Fig. 3).

b) *Inoculation with* Phaeoacremonium chlamydosporum**.** The three cultivars started showing symptoms 26 days after the inoculation. After 40 days, some of the plants showed the same symptoms as described above. The frequency of plants with symptoms depended on the cultivar (Table 2). Control plants remained healthy.

c) *Inoculation with* Botryodiplodia *sp*. Six days after the inoculation, all the inoculated plants of the cv. Tempranilla and Malbec became desiccated and died, while the controls were healthy; only 71% of 'C. Sauvignon' plants died first, the rest showed reddish leaves and died afterwards (Table 2).

Reisolations from the stems of affected plants with visible mycelium, were positive in all cases. In the case of *Phellinus*-inoculated flasks however, only 10% of reisolations from the roots of plants without visible mycelium of the fungus were positive.

Management of the disease

In Argentina, all practices to reduce the incidence of the disease are preventive. No chemical treatments are applied except for the protection of the large pruning wounds with fungicide (e.g.: benomyl, carboxin) mixed with latex paint. Prophylactic measures recommended are: to identify and mark plants with symptoms, to prune diseased plants separately, to disinfect pruning tools, to burn pruning material, to eradicate and burn affected plants, and to use healthy plants or cut new plants from healthy shoots to replace diseased ones.

Conclusions

The isolated fungi are those mentioned in the international literature as causal agents of wood diseases (Mugnai *et al.*, 1996; Chiarappa, 1997; Larignon and Dubos, 1997; among many other).

The results from the pathogenicity tests indicate that all the organisms tested were able to produce decline and death of very young grapevines grown *in vitro*.

In *Phellinus* sp. pathogenicity tests, there were two factors suggesting that fungal phytotoxic metabolites could be involved: 1) the occurrence of plants with symptoms but without evident mycelium development and, 2) the fact that some symptoms on these plants were the same as those found in flasks inoculated with *P. chlamydosporum*.

It is also interesting to compare esca and "hoja de malvón". Both diseases present similarities like wood necroses, and the presence of some species of *Phaeacremonium* (*P. aleophilum* and *P. chlamydosporum*), mainly in the areas surrounding the soft necrosis. However there are also differences, such as the symptoms on the leaves and the species of *Phellinus* identified until now.

It is necessary to continue studies with great number of isolates in order to detect the presence and frequency of the microorganisms involved, as well as to perform more pathogenicity tests so as to ascertain the role of each fungus in the development of the disease.

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