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Phaeoacremonium species and *Phaeomoniella chlamydospora* in vines showing "hoja de malvón" and young vine decline symptoms in Argentina

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Summary. Vines showing "hoja de malvón" and young vine decline symptoms in Argentina have been examined for the presence in the wood of *Phaeomoniella chlamydospora*, different species of *Phaeoacremonium*, and other fungi. In all the isolations from mature vines with hoja de malvón symptoms *Phellinus* sp. prevailed over *Phaeoacremonium aleophilum*, *Pm. parasiticum,* and *Phaeomoniella chlamydospora*. By contrast in young vines with decline symptoms the most prevalent fungi were species of *Botryodiplodia*. *Pm. aleophilum* was more frequently isolated than *Pm. parasiticum* either in mature or in young vines. This is the first time *Pm. parasiticum* was isolated from diseased grapevines. In mature vines Simple Correspondence Statistical Analysis showed that *Pm. aleophilum* was significantly associated with soft white rot and sectorial brown necrosis, and in terms of the portion affected, with the arms of the vine. *Pa. chlamydospora* was associated with necrosis of the brownish zone bordering the black line, with black spots, and with the base of the vine. *Pm. parasiticum* considered separately or together with *Pm. aleophilum*, was associated with black line necrosis, with the mid-trunk and with the primary vine branches. In young vines, *Pa. chlamydospora* was also associated with the base of the vine, *Pm. aleophilum* with the rootstock stem, and *Pm. aleophilum* and *Pm. parasiticum* with the grafting zone.

Key words: wood diseases, *Vitis vinifera*, decline diseases.

Introduction

Among the different grapevine diseases in Argentina, hoja de malvón is without any doubt, the most important. Its etiology was for long unknown, and no effective control treatments are yet available. As a result the disease has spread, causing economical losses and considerable damage (Gatica *et al.*, 1999).

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Hoja de malvón presents similarities with esca such as wood white rot, but differs from esca in the symptoms on the leaves and in the species of basidiomycetes fungi associated with white rot. Recent studies have shown that a *Phellinus* species, *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingfield & L. Mugnai) Crous & W. Gams, *Phaeoacremonium aleophilum* W. Gams, Crous, M.J. Wingfield & L. Mugnai, *Phaeoacremonium parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M..J. Wingfield and *Botryodiplodia* species 2 are frequently associated with hoja de malvón (Gatica *et al.*, 2000).

Pm. aleophilum and *Pa. chlamydospora* have

been stated to be involved in esca (Larignon, 1991; Mugnai *et al*., 1996; Larignon and Dubos, 1997; Mugnai *et al*., 1999), together with the wood rot agent *Fomitiporia punctata*. Recently, another *Phaeoacremonium* species, *Pm. viticola* J. Dupont, has also been associated with esca of grapevine in France and California (Dupont *et al*., 2000a). Other species such as *Pm. rubrigenum* and *Pm. inflatipes* are rarely found with esca and seem to have a minor incidence in the disease (Dupont *et al*., 2000b). *Pm. parasiticum* has been described from woody plant diseases and, mainly, from human infections (Crous, 1996).

Another disease affecting young grapevines in Argentina is characterized by stunted growth, slow die-back, dark xylem vessels in the rootstock and plant death.

A similar disease, Petri disease[(1)], associated with *Pa. chlamydospora* and *Phaeoacremonium* spp. have been reported from the USA (Morton, 1997; Scheck *et al*., 1998), Italy (Mugnai *et al*., 1999; Sidoti *et al*., 2000), South Africa (Ferreira *et al*., 1994; Theron and Crous, 1998), Portugal (Rego *et al*., 2000), Australia (Pascoe, 1999; 2000) and New Zealand (Clearwater *et al*., 2000).

The present report describes the species of *Phaeoacremonium* and *Pa. chlamydospora* and their frequencies found in different types of necrosis and in different vine portions in both mature and young grapevines with hoja de malvón or decline respectively, in Argentina.

Materials and methods

Plant material

Thirty mature (more than 11 years) vines of the cv. Bonarda (1), Cabernet Sauvignon (4), Cereza (1), Chardonnay (3), Chenín (3), Fintendo (2), Gibbi (4), Malbec (5), Nebbiolo (2), Tempranilla (2), Torrontés Riojano (2) and Valency (1) with symptoms of hoja de malvón and 55 young (1–3 years) vines of the cv. Bonarda (1), Cabernet Sauvignon (20), Chardonnay (1), Malbec (14), Merlot (2), Pinot (3), Sangiovese (4), Sauvignon (1) and Syrah (9), with young decline symptoms and, from various vineyards in Mendoza province, were examined and analysed in 1999 and 2000. Young vines were mainly grafted on SO4 (69%); other rootstocks were 110-14 (11%), 1103P (7%), Chenin (2%), Bonarda (1%) and unknown (9%).

Isolation

Tissues from different types of necrosis (soft white rot; black line; brownish zone of hard consistency; sectorial brown zone of hard consistency and black spots) and different vine portions (young vines: root, base of rootstock, rootstock stem, graft union site, scion trunk; mature vines: root, crown, mid-trunk, primary branches, arms) were used for the isolations. Isolations were made in malt extract agar (MEA) using the technique described by Larignon and Dubos (1997).

Observations were recorded on a data-base.

Fungal identification

Phaeoacremonium species and *Pa. chlamydospora* were identified by morphological and molecular methods (Crous *et al*., 1996; Dupont *et al*., 2000b). The other fungi were identified based on morphological and cultural characteristics (Punithalingam and Waller, 1973; Punithalingam, 1976; Gatica *et al*., 2000).

Statistical analysis

The frequencies of *Phaeoacremonium* species and *Pa. chlamydospora* isolations were analysed by multivariate methods: Simple Correspondence Analysis, using the statistical program Portable System to Analyse Data (SPAD.N), 1991, Version 2.52 (France logiciel APP-88 08 006 01, CISIA, Ceresta, 261 rue de Paris, 93556 Montreuil Cedex, France).

Results were expressed as the species of *Phaeoacremonium* and *Pa. chlamydospora* in relation to the type of necrosis in mature vines (necrosis a, soft white rot; b, black line; c, brownish zone of hard consistency; d, sectorial brown zone of hard consistency and p, black spots) and in relation to the portion of the vine they colonised (root, crown or base of the trunk, mid-trunk, primary branches and arms in mature vines; root, base of rootstock, rootstock stem, graft union and scion trunk in young vines).

(1) At the general Assembly of the 2nd ICGTD meeting held in Lisbon 2001 it was unanimously decided that the disease variously known as black goo, young grapevine decline, or Petri vine decline will henceforth be called Petri disease.

Results

Fungal identification

Of the colonies of *Phaeoacremonium* spp. isolated from the stems of young and the trunks of mature grapevines, 21 were selected as pattern cultures. Of these, 12 were identified as *Pm. aleophilum* by their phenotypic characters and their restricted growth at 35°C. This identification was confirmed by PCR-RFLP analyses. Nine cultures were characterized by their greyish-brown colonies and identified by molecular data as *Pm. parasiticum* (Dupont *et al*., in press). The other isolates were identified as *Phaeoacremonium* spp. and *Pa. chlamydospora* by their morphological characteristics.

The other wood pathogenic fungi isolated were identified as *Phellinus* sp, *Botryodiplodia* sp. and *Cylindrocarpon destructans*.

Fungal incidence

Of the total number (7,745) of infected (6,832) and non-infected (913) samples of woody tissues obtained from 30 mature vines with hoja de malvón symptoms, *Phaeoacremonium* spp. occurred in 7% of the samples, *Pa. chlamydospora* in 3%, *Phellinus* sp. in 31%, and *Botryodiplodia* spp. in 8% (Fig. 1). In respect to the total number of colonies isolated *Pa. chlamydospora* and *Pm. aleophilum* were the most frequently isolated species, 30.2% and 28.4% respectively, and *Pm. parasiticum* was isolated with an incidence of 17.1%. *Pm. aleophilum*+ *Pm. parasiticum* before discriminating between species, represented 24.3% of isolations (Fig. 3).

In young vines, out of the total number (2,979) of infected (2,724) and non-infected (255) samples of woody tissues, *Phaeoacremonium* spp. occurred in 5% of samples, *Pa. chlamydospora* in only 1%, *Phellinus* sp. in 0.03%, and *Botryodiplodia* spp. in 16% of samples (Fig. 2). The frequency of *Pm. aleophilum* (30.7%) over the total number of obtained colonies was similar to that observed in mature vines. The incidence of *Pm. parasiticum* (21.1%) and *Pm. aleophilum*+*Pm. parasiticum* (32.3%) was slightly greater in young than in mature vines, while that of *Pa. chlamydospora* (15.9%) was significantly lower (Fig. 3).

The isolation data of each *Phaeoacremonium* species and *Pa. chlamydospora* were divided by

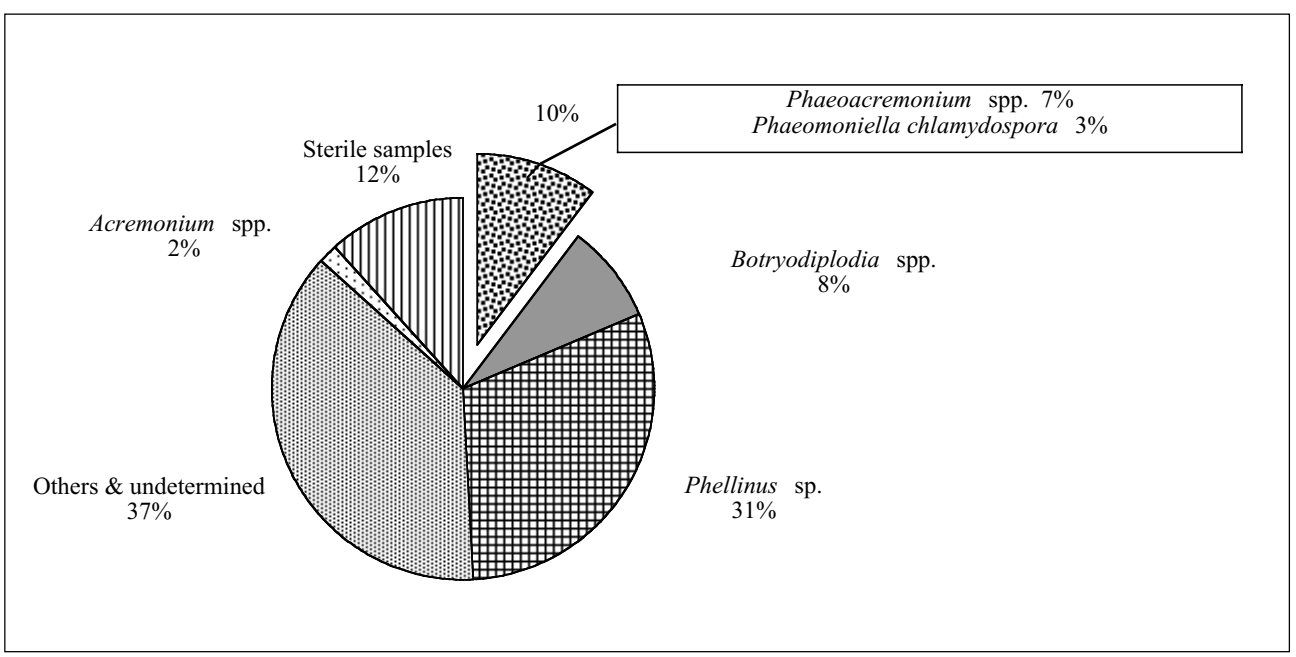

Fig. 1. Percentage of *Phaeoacremonium* species and *Phaeomoniella chlamydospora* out of the total microflora isolated from mature vines showing hoja de malvón symptoms.

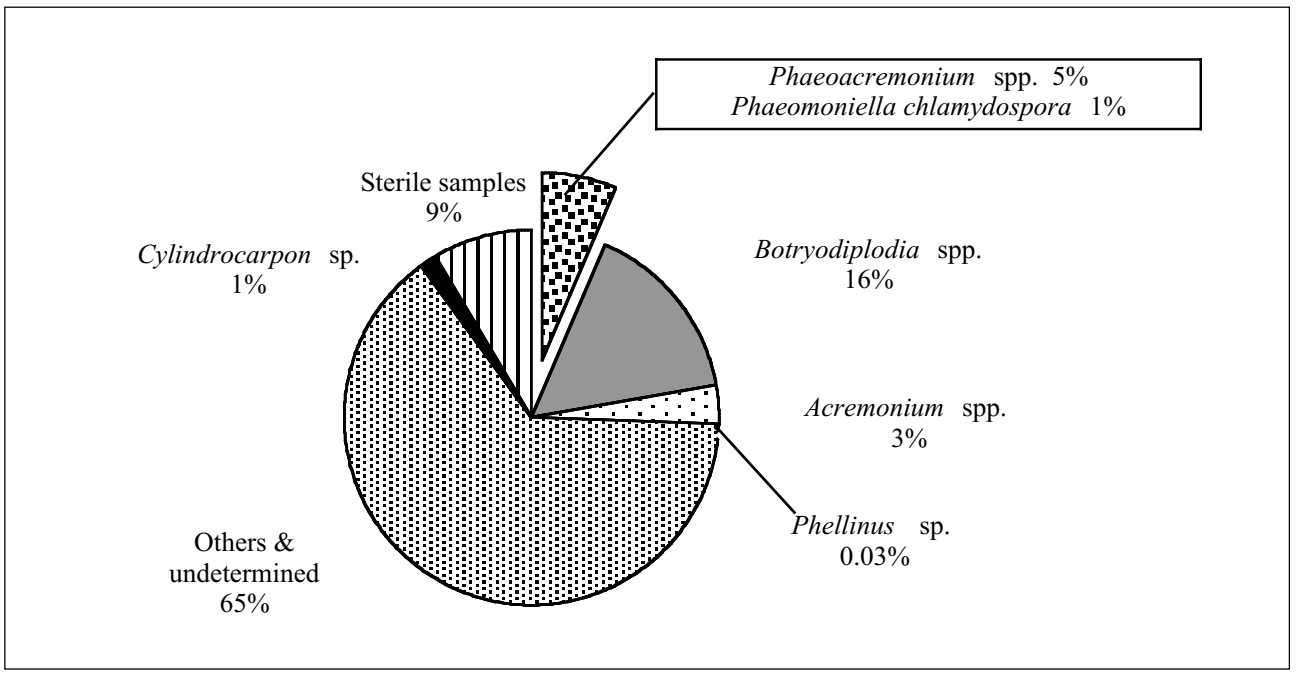

Fig. 2. Percentage of *Phaeoacremonium* species and *Phaeomoniella chlamydospora* out of the total microflora isolated from young vines showing decline symptoms.

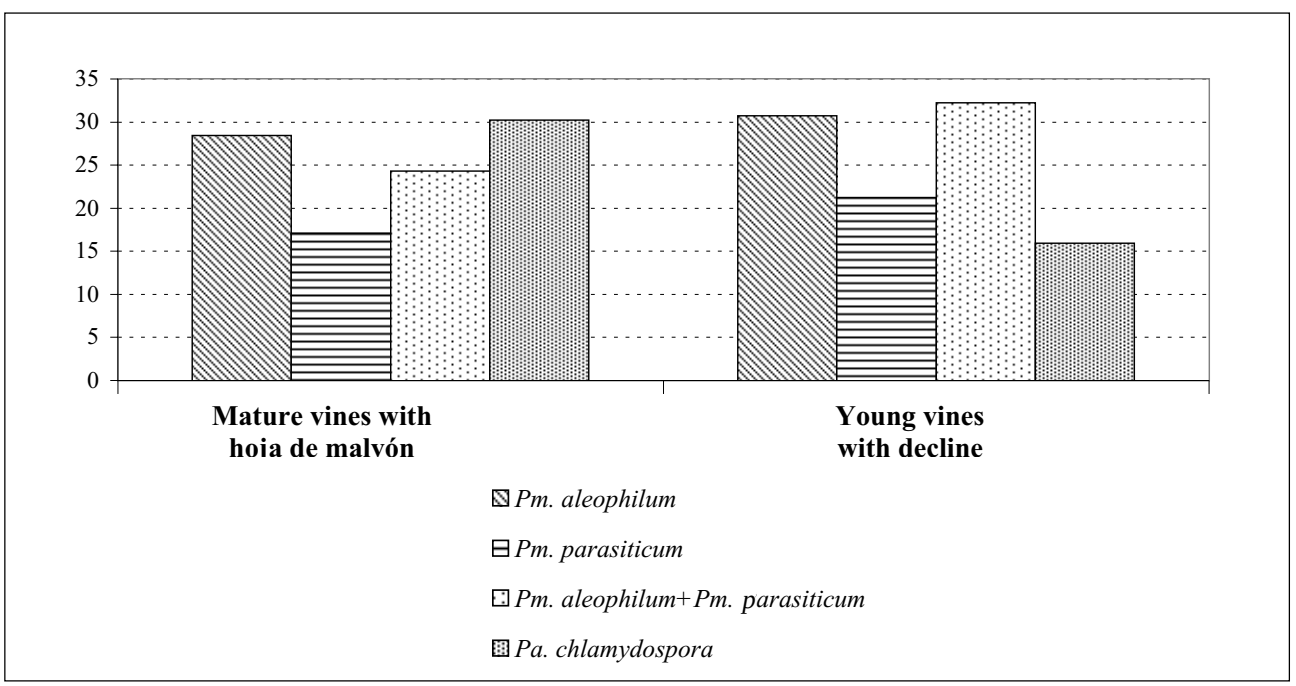

Fig. 3. Percent incidence of *Phaeoacremonium* species and *Phaeomoniella chlamydospora* isolated from mature and young vines.

cluster analysis into 5 classes according to the type of necrosis from which they were isolated (Table 1). Data significance was given by the high test value obtained by the statistical analysis in the characterisation of the classes: 19.55 (Critical Value: 2, $P \leq 0.05$).

With regard to the type of necrosis observed in mature vines affected by hoja de malvón the percentage over all colonies of *Phaeoacremonium* spp. and *Pa. chlamydospora* was 4.93% in necrosis a (soft white rot), 43.36% in b (black line surrounding white rot), 20.61% in c (brownish zone of hard consistency), 13.27% in d (sectorial brown zone of hard consistency), and 17.83% in p (black spots).

Pm. aleophilum was considered to represent necrosis a (test value=5.27; $P \leq 0.0000$) because 69.23% of all colonies from this type of necrosis belonged to this specie and of all isolated *Pm. aleophilum* colonies (28.45%), 12% belonged to necrosis a.

Necrosis b was characterized by *Pm. aleophilum+Pm. parasiticum* (test value=9.98; $P \leq 0.0000$) and *Pm. parasiticum* (test value=3.03; $P \leq 0.0012$). Of all colonies from necrosis b, *Pm. aleophilum+Pm. parasiticum* represented 41.69%, while 74.48% of the total colonies of *Pm. aleophilum+Pm. parasiticum* (24.27%) came from necrosis b. In particular, *Pm. parasiticum* represented 21.87% of all colonies from necrosis b, and 55.56% of all colonies of *Pm. parasiticum* belonged to necrosis b.

Necrosis c was characterised by *Pa. chlamydospora* (test value=3.43; $P \leq 0.0003$); it represented 41.72% of the colonies in necrosis c, and 28.45% of all colonies of *Pa. chlamydospora* belonged to this necrosis.

Pm. aleophilum was considered to represent necrosis d (test value=5.69; $P \leq 0.0000$). Of all colonies isolated from this necrosis, *Pm. aleophilum* represented 53.33%, while 24.89% of the total colonies of *Pm. aleophilum* came from necrosis d.

Necrosis p was characterised by *Pa. chlamydospora* (test value=16.64; $P \leq 0.0000$), 90.07% of the colonies in necrosis d; 53.14% of all colonies of *Pa. chlamydospora* belonged to necrosis p.

Following the same procedure as above, isolation data of each *Phaeoacremonium* species and *Pa. chlamydospora* were divided into 5 classes according to the vine portion of mature vines from which they were isolated (Table 2). Test value: 13.67. The percentage of colonies of *Phaeoacremonium* spp. and *Pa. chlamydospora* classed by mature vine portion was 0.13% in the roots, 36.24% in the crowns, 16.38% in the mid-trunks, 8.19% in the primary branches and 39.06% in the arms. In the roots no *Phaeoacremonium* spp. had a frequency that characterised this vine portion as a class. *Pa. chlamydospora* characterized the crowns (test value=6.2; $P \leq 0.0000$), *Pm. parasiticum* the mid-trunks (test value=6.02; $P \leq 0.0000$), *Pm. aleophilum+Pm. parasiticum* the primary branches (test value=9.06; $P \leq 0.0000$) and *Pm. aleophilum* the arms (test value=9.55; $P \leq 0.0000$).

The incidence of *Phaeoacremonium* spp. and *Pa. chlamydospora* classed on vine portion in young vines with symptoms of decline is shown in Table 3 (test value=10.87). Of 189 *Phaeoacremonium+ Pa. chlamydospora* colonies, 3.17% were in the roots, 14.81% in the base of the rootstocks, 45.56% in the rootstock stems, 32.80% in the graft unions and 2.65% in the scion trunks. In the roots and scion trunks no *Phaeoacremonium* sp. was frequent enough to characterise these vine portions, but *Pa. chlamydospora* characterized the base of the rootstocks (test value=9.3; $P \leq 0.0000$), *Pm. aleophilum* the rootstock stems (test value=4.63; $P \leq 0.0000$), and *Pm. aleophilum+Pm. parasiticum* the graft unions (test value=4.09; $P \leq 0.0000$).

Discussion

Of the different *Phaeoacremonium* species, *Pm. aleophilum* was more frequently isolated than *Pm. parasiticum* from both mature and young vines. *Pm. parasiticum* was the only species that had never been reported before from grapevine. When genera were compared, *Phaeomoniella* was much less frequent than *Phaeoacremonium* in both mature and young vines and it was less common in young vines than in mature vines.

Simple Correspondence Statistical Analysis was used to associate the different species of *Phaeoacremonium* with the different types of necrosis in crosswise cuts, and also with the vine portions infected. In mature vines, *Pm. aleophilum* was significantly associated with soft white rot and sectorial brown necrosis, and, as regards the vine portion infected, with the arms of the vine. *Pa. chlamydospora* was associated with necrosis of the brownish zone bordering the black line, with black spots,

Table 2. Number of colonies and percent incidence of *Phaeoacremonium* species and *Phaeomoniella chlamydospora* in mature vineyards, classed by the vine portion from which they were isolated.

Fungal species	Root			Crown			Mid-trunk			Primary branches			Arms			Total colonies		
	No.[a]	%[b]	%[c]	No.[a]	%[b]	%[c]	No.[a]	%[b]	%[c]	No.[a]	%[b]	%[c]	No.[a]	%[b]	%[c]	%[b]	No.	%
Pm. aleophilum	0	0	0	36	19.25	13.33	15	8.02	12.3	7	3.74	11.48	129	68.98	44.33	100	187	25.1
Pm. parasiticum	0	0	0	41	31.3	15.19	47	35.88	38.52	6	4.58	9.84	37	28.24	12.71	100	131	17.58
Pm. aleophilum+ *Pm. parasiticum*	1	0.53	100	68	35.98	25.19	18	9.52	14.75	48	25.4	78.69	54	28.57	18.56	100	189	25.37
Pa. chlamydospora	0	0	0	125	52.52	46.3	42	17.65	34.43	0	0	0	71	29.83	24.4	100	238	31.95
Total	1	0.13	100	270	36.24	100	122	16.38	100	61	8.19	100	291	39.06	100	100	745	100

[a],[b],[c] See Table 1.

Table 1. Number of colonies and percent incidence of *Phaeoacremonium* species and *Phaeomoniella chlamydospora* classed by type of necrosis in mature vines.

Fungal species	Soft white rot			Black line			Brownish zone			Sectorial brown zone of hard consistency			Black spots			Total colonies		
	No.[a]	%[b]	%[c]	No.[a]	%[b]	%[c]	No.[a]	%[b]	%[c]	No.[a]	%[b]	%[c]	No.[a]	%[b]	%[c]	%[b]	No.	%
Pm. aleophilum	27	12	69.23	102	45.33	29.74	39	17.33	23.93	56	24.89	53.33	1	0.44	0.71	100	225	28.45
Pm. parasiticum	6	4.44	15.38	75	55.56	21.87	32	23.7	19.63	17	12.59	16.19	5	3.7	3.55	100	135	17.07
Pm. aleophilum+ *Pm. parasiticum*	4	2.08	10.26	143	74.48	41.69	24	12.5	14.72	13	6.77	12.38	8	4.17	5.67	100	192	24.27
Pa. chlamydospora	2	0.84	5.13	23	9.62	6.71	68	28.45	41.72	19	7.95	18.1	127	53.14	90.07	100	239	30.21
Total	39	4.93	100	343	43.36	100	163	20.61	100	105	13.27	100	141	17.83	100	100	791	100

[a] No. of colonies.
[b] Percent over total No. of each isolated fungus.
[c] Percent over total No. of isolates from each necrosis.

Table 3. Number of colonies and percent incidence of *Phaeoacremonium* species and *Phaeomoniella chlamydospora* in young vines classed by the vine portion from which they were isolated

Fungal species	Root			Base of the rootstock			Rootstock stem			Graft union site			Scion trunk			Total colonies		
	No.[a]	%[b]	%[c]	No.[a]	%[b]	%[c]	No.[a]	%[b]	%[c]	No.[a]	%[b]	%[c]	No.[a]	%[b]	%[c]	%[b]	No.	%
Pm. aleophilum	2	3.45	33.33	1	1.72	3.57	42	72.41	47.73	12	20.69	19.35	1	1.72	20	100	58	30.69
Pm. parasiticum	4	10	66.67	0	0	0	20	50	22.73	16	40	25.81	0	0	0	100	40	21.16
Pm. aleophilum+ Pm. parasiticum	0	0	0	3	4.92	10.71	21	34.43	23.86	33	54.1	53.23	4	6.56	80	100	61	32.28
Pa. chlamydospora	0	0	0	24	80	85.71	5	16.67	5.68	1	3.33	1.61	0	0	0	100	30	15.87
Total	6	3.17	100	28	14.81	100	88	45.56	100	62	32.8	100	5	2.65	100	100	189	100

[a], [b], [c] See Table 1.

and with the base of the vine. *Pm. parasiticum,* by itself and together with *Pm. aleophilum,* was associated with black line necrosis, and with the mid-trunk and the primary branches. In young vines, *Pa. chlamydospora* was associated with the base of the vine; *Pm. aleophilum* with the rootstock stem, and *Pm. aleophilum+Pm. parasiticum* with the grafting zone. Even though in this study *Pm. aleophilum+Pm. parasiticum* were isolated in significant numbers from the graft-union area, other studies that examined the total microflora (Césari and Gatica, 2001) have shown that *Botryodiplodia* is the most important fungus isolated in the graft-union, and that the occurrence of *Pm. aleophilum* in soft white rot is somewhat less significant.

In the scion trunks of young vines, and in the roots of both mature and young vines, the frequencies of *Phaeoacremonium* species and *Pa. chlamydospora* were not significant.

Attempts to associate the frequencies of the different *Phaeoacremonium* species and *Pa. chlamydospora* with necrosis types and vine portions have been reported from other countries. In France, *Pa. chlamydospora* was found in black line and brownish zone of hard consistency (Larignon and Dubos, 1997); this, and findings in Italy and Portugal associating this fungus with black spots and brownish zone of hard consistency (Mugnai *et al*., 1996; Chicau *et al*., 2000) are consistent with the results reported in this paper.

The occurrence of *Pm. aleophilum* in sectorial brown necrosis also agrees with Mugnai *et al*., 1996.

In young vines, *Pa. chlamydospora* was less common than *Phaeoacremonium* spp. or genera of other fungi. This was not consistent with the literature data, which report it as the most frequent fungus in young vines (Sidoti *et al*., 2000; Pascoe and Cottral, 2000; Rego *et al*., 2000).

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