

Occurrence of *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* in grape propagation materials and young grapevines

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Abstract During a 6-year study, grapevine propagation materials and young grapevines were analysed to evaluate the presence of internal wood discolouration and the occurrence of fungal species involved in Petri disease. The intensity of wood discolouration increased with the ageing of the plants. The maximum incidence of dark streaks was observed in the rootstock while necrosis originating from buds or nodes were notably present in the trunk and cordon of older vines. In contrast, the highest levels of brown-red halo symptoms, defined as discoloured areas around the pith, were recorded in the early growth stages. *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* were usually isolated from the rooted-grafts and the 3-year old plants, respectively. The number of infected grapevines increased with age. Most of the *P. chlamydospora* strains were isolated from dark streaks or dots, while *Phaeoacremonium* spp. were detected in brown-red halo symptoms and other symptomatic or asymptomatic wood. The greatest incidence of the two fungal taxa was recorded in the lower parts of the grapevine, including the roots and rootstock.

Keywords Cabernet Sauvignon · Cuttings · Grafts · Petri disease · Prosecco

Introduction

Decline of young grapevine, recently named Petri disease (Surico 2001), has been increasingly reported in developing wine-growing areas worldwide (Bertelli et al. 1998; Halleen et al. 2003; Edwards and Pascoe 2004). Usually foliar symptoms of Petri disease are similar but not typical of esca disease and the presence of soft, spongy, deteriorated wood is not observed. Visual analysis of affected inner woody tissues reveals the presence of colour alterations, mainly due to xylematic vessels turning brown or black and causing the formation of dark streaks. The longitudinal discolourations are visible as small, sparse or converging dots in cross-section.

The study of the microflora isolated from discoloured wood tissues of declining grapevines has led to the identification of two main fungal taxa: *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora*, which are believed to cause brown-red wood necrosis and dark streaks, respectively (Mugnai et al. 1999). Considering that Petri disease could evolve into an esca proper syndrome, it was thought that these fungi acted as precursors of lignicolous basidiomycetes (Larignon 2004).

As reports of such decline involved newly-planted vineyards, the contamination of propagation material

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had been suggested (Scheck et al. 1998; Surico 2001). Usual nursery practices for the production of grafts are based on the selection of graftable rootstock cuttings and scions and on uniting them by means of grafting. After a callusing period of 2 weeks, at a temperature of 30–32°C and 75–90% relative humidity, grafts with some shoots but lacking roots are produced (unrooted grafts). The unrooted grafts are planted in the nursery after a short hardening period outdoors. There they are grown for a season, developing shoots and new roots, until they are uprooted in early winter, pruned, paraffined and packed to be delivered to vinegrowers (rooted grafts). Mother plants and grafts produced in nurseries around the world have been investigated for the presence of such mitosporic fungi and reported positive (Bertelli et al. 1998; Halleen et al. 2003; Edwards and Pascoe 2004).

The aim of this work was to investigate the presence of internal grapevine wood discolouration and the occurrence of *Phaeoacremonium* spp. and *P. chlamydospora* in plant propagation materials and in young grapevines.

Materials and methods

Analysis of nursery propagation materials and of grapevines uprooted from vineyards

The study was carried out over a period of 6 years (1999–2004), on certified virus-free grapevine propagation materials, received from a nursery in north-eastern Italy. In 1999 and 2002, randomly selected scions, including 29 Cabernet Sauvignon (Clone R5), 13 Merlot (Clone R3) and 10 Prosecco (Clone ISV-ESAV 10) as well as 80 Selection Oppenheim 4 (SO4) rootstock cuttings were analysed for internal wood discolouration and fungal contamination (Table 1). Forty-five unrooted grafts of the Prosecco/Kober 5 BB (K5BB) and the same number of the Cabernet Sauvignon/SO4 grafting combinations were analysed in 2002 and 2003.

Each year with the exception of 2004, rooted grafts were analysed after an entire season of cultivation in the nursery (Table 2). From the year 2000 to 2002, samples were taken out from the graft batches received and planted outdoors in small experimental vineyard plots. Prosecco/K5BB grafts were planted in

Table 1 Analysed nursery propagation materials

Type	Cultivar	Number of pieces in the year			
		1999	2002	2003	Total
Scions	Cabernet Sauvignon	19	10	0	29
	Merlot	13	0	0	13
	Prosecco	0	10	0	10
Graftable rootstock cuttings	SO4	80	0	0	80
Unrooted grafts	Prosecco/K5BB	0	25	20	45
	Cabernet Sauvignon / SO4	0	25	20	45

two different areas, one on a plain (Spresiano, Treviso) and one on a hillside (Vittorio Veneto, Treviso) while the Cabernet Sauvignon/SO4 grafts were planted only on the plain. While Cabernet Sauvignon is a worldwide cultivated variety, Prosecco is a local white berry cultivar, used to produce a still, semi-sparkling or fully sparkling wine, the Prosecco DOC of Conegliano-Valdobbiadene (www.Prosecco.it).

Plants were grown following the usual agronomic practices of fertilisation and pest management with soil ploughing along the rows. In the summer months, vineyards were visually inspected for the presence or the absence of foliar symptoms of esca disease or other symptoms. Beginning in 2001, at the end of every winter, samples of the planted grapevines were uprooted from the vineyard and inspected in the laboratory for internal symptoms and fungal detection. Young grapevines were collected up to an age of 4 years (Table 2).

Morphological features and internal wood discolouration

In the laboratory, main morphological features of the grafts and uprooted grapevines, including dimension, weight and root development, were recorded. Since 2002, the morphology of the grafts has been scored according to the procedure described by Morton (2000). The rootstock diameter was measured and the quality of grafting unions assessed by observing the

Table 2 Analysed rooted grafts and young grapevines

		Number of pieces in the year						Total
		1999	2000	2001	2002	2003	2004	
Prosecco/K5BB								
Rooted grafts	Analysed	100	50	50	25	15	0	240
	Planted		50	50	50			
Young grapevines ^a	1-Year old			10	10	10	0	30
	2-Year old				10	6	6	22
	3-Year old					6	6	12
	4-Year old						6	6
Cabernet Sauvignon/SO4								
Rooted grafts	Analysed	100	50	50	25	25	0	250
	Planted		50	50	50			
Young grapevines ^a	1-Year old			10	10	10	0	30
	2-Year old				10	6	6	22
	3-Year old					6	6	12
	4-Year old						6	6

^a Resulting after the planting of rooted grafts in the vineyard

percentage of callus formation. Structural malformations were recorded, if present. The growth of the scion was evaluated and a general quality value expressed, where the lowest class (rating = 1) was assigned to the technically unsuitable materials.

Scions and rootstock cuttings were transversely cut at every node while grafts were transversely sectioned at six positions (A = basal end, B = first basal internode, C = first node, D = 4 cm below the grafting point, I = grafting point, II = scion). The 1 or 2 year-old uprooted plants were cut at two more positions upward along the cane (III and IV); all the positions were observed. Older grapevines were cut and inspected at each node along the trunk. In the last 2 years the roots were also carefully observed.

Wood discolourations were recorded in the following symptom classes: dark dots (DD), brown-red halo symptoms surrounding the pith and later evolving into a brown necrosis (BHP) and necrosis originating from bud or node (NBN). In addition, different wood alterations were observed and clustered (Other). Sections without any visible colour or tissue alteration were also recorded (Asymptomatic). Since 2002, plants have also been cut longitudinally and the presence of dark streaks (DS) investigated. The two classes (DD and DS) were combined and named DS/D (dark streaks or dots).

Fungal isolation

Sections, <1 mm thick, were cut from flame-sterilised wood samples, visually inspected and placed under sterile conditions on Petri dishes (9 cm diam.) containing malt extract agar (MEA) (malt extract 15 g; agar 12.5 g; 1 l distilled water) amended with 50 mg l⁻¹ tetracycline hydrochloride (Fluka, CH). The dishes were incubated at 25°C for 30–45 days, to permit the development of fungal colonies. Four to twenty wood sections per scion and two per rootstock cuttings were sampled. Wood sections of the grafts were sampled at six positions (A, B, C, D and I + II) while wood sections from 1 or 2 year-old plants were cut in eight positions, as previously reported. Older plants were sampled in 14 positions: two in the roots and three each in the rootstock, trunk, cordon and canes, respectively.

Fungal identification

During the incubation period, Petri dishes were observed daily for the appearance of fungal colonies. Individual strains were subcultured in new Petri dishes (6 cm diam.) containing MEA and incubated at 25°C in the dark. The macroscopic features of the colonies (i.e. growth, colour, exudates, etc.) and

details of reproductive structures were observed using optical microscopes. Identification was carried out by using the available literature (Barnett and Hunter 1972; Crous et al. 1996; Crous and Gams 2000) and confirmed by comparison with strains previously identified by CBS (Utrecht, The Netherlands), for the two most relevant taxa to this study.

Since no differences were seen between varieties, except for the morphological features, data were pooled and then analysed by ANOVA using the CoStat Statistical Software, Rel. 4.2 (CoHort Software, Berkeley, CA, USA). Percentage data were arcsin-square root transformed prior to analysis and treatment means were separated with Student-Newman-Keuls test at $P \leq 0.05$.

Results

Morphological features

The mean length of unrooted grafts (rootstock and scion), after callusing, was 38.5 cm with a mean fresh weight of 22 g. The mean length of rooted grafts, after one season of cultivation in the nursery and ready to be sent to the growers, was 41.5 cm with a mean fresh weight of 54 g. During 5 years of observation, 90% of Prosecco/K5BB grafts and 93% of Cabernet Sauvignon/SO4 had normally developed roots (mean fresh weight = 10 g). Other data recorded on the propagation materials are shown in Table 3. In the unrooted grafts, the mean diameter of K5BB rootstock (9.0 cm) was significantly higher than in SO4 (7.5 cm), while the grafting combination of

Cabernet Sauvignon/SO4 showed a significantly reduced callus formation of 46.7% from the Prosecco/K5BB combination. Differences were also observed between the unrooted and rooted grafts in the same grafting combination. The general quality rating of the rooted grafts was similar in the two cultivars and sufficient to consider all the analysed grafts as plantable (Morton 2000).

Mean diameters of the uprooted grapevines are shown in Table 4. A constant increase in diameter was observed as the grapevines were ageing at all positions except for canes, in which the development was different from year to year. Generally, dimensions were similar in the two cultivars: higher levels were only found in the cordon of Cabernet Sauvignon 4-year old grapevines.

Internal wood discolouration

Wood discolouration was not observed in scions, nearly absent in rootstock cuttings and generally low in the further growth stages (Fig. 1). In grafts and 1 year-old grapevines greater discolouration scores were recorded at the basal end of the rootstock (position A) and at the graft union (position I). The intensity of wood discolouration increased with the ageing of the plants. Higher levels than in the trunk and cordon were constantly recorded in the rootstock; minimum levels were observed in the canes. Wood discolouration was more diffuse in the grafting combination Cabernet Sauvignon/SO4 than Prosecco/K5BB (data not shown).

In the unrooted and in the rooted grafts, the most widespread symptoms were the dark streaks or dots

Table 3 Morphological features of the analysed propagation materials

		Rootstock diameter (mm)	Graft point callusing (%)	Rating ¹ (1–5)		
				Structure	Scion growth	Quality
Unrooted grafts	Prosecco/K5BB	9.0 b ²	81.9 b	3.9 ns	3.2 ns	3.8 a
	Cabernet Sauvignon/SO4	7.5 c	46.7 c	4.0 ns	3.2 ns	3.0 b
Rooted grafts	Prosecco/K5BB	10.1 a	93.2 a	3.5 ns	3.5 ns	3.9 a
	Cabernet Sauvignon/SO4	10.2 a	96.2 a	3.5 ns	3.3 ns	3.8 a

¹ After Morton (2000), modified. Structure was evaluated with respect to the presence of malformations such as bending, distortion, flatness. Quality is expressed as a comprehensive evaluation on the plantability of the graft. 1 = Insufficient, 2 = Poor, 3 = Fair, 4 = Good, 5 = Excellent

² Values followed by the same letter along the column do not differ statistically (Student-Newman-Keuls test, $P \leq 0.05$)

ns = not significant

Table 4 Mean diameter (mm) of uprooted grapevines

Cultivar/Rootstock	Age	Position			
		Rootstock	Trunk	Cordon	Canes
Prosecco/K5BB	1-year old	12.7 d ¹	—	—	6.6 d
	2-year old	22.4 c	—	—	10.5 bc
	3-year old	29.9 b	26.3 b	17.3 b	11.6 abc
	4-year old	38.0 a	36.8 a	18.3 b	9.3 cd
Cabernet Sauvignon/SO4	1-year old	12.9 d	—	—	8.0 d
	2-year old	23.1 c	—	—	13.6 a
	3-year old	28.2 b	28.7 b	20.9 b	13.0 ab
	4-year old	34.8 a	34.6 a	25.8 a	9.8 bcd

¹ See Table 3

and the brown-red halo around the pith, respectively (Fig. 2). In the older grapevines, there was an increase of necrosis originating from buds or nodes, particularly along the trunk and the cordon. A higher incidence of dark streaks and dots was usually recorded in the rootstocks, except in the rooted grafts where brown-red halo symptoms prevailed. Cane and root samples were mostly asymptomatic.

Fungal isolation and identification

Phaeoacremonium spp. occurred in 7.4% and *P. chlamydospora* in 1.6%, of the total number of colonies isolated (5603), respectively. About 230 weakly growing colonies were lost due to contamination during the isolation procedure. In

total, about one third of the wood sampled tissues did not produce any fungal colonies (data not shown). No significant differences were noticed in the results obtained in the different grafting combinations (data not shown).

Concerning the presence of *Phaeoacremonium* spp. and *P. chlamydospora*, a slow but constant increase in the percentage of affected grapevines was ascertained (Fig. 3). Colonies of both the taxa were usually absent from scions and rootstock cuttings and showed a very low occurrence in the unrooted grafts (Fig. 4). The incidence of *Phaeoacremonium* spp. was much higher in the fungal population isolated from the rooted grafts but it decreased in the 4 year-old grapevines while *P. chlamydospora* slowly increased in the 1 and 3 year-old plant stages.

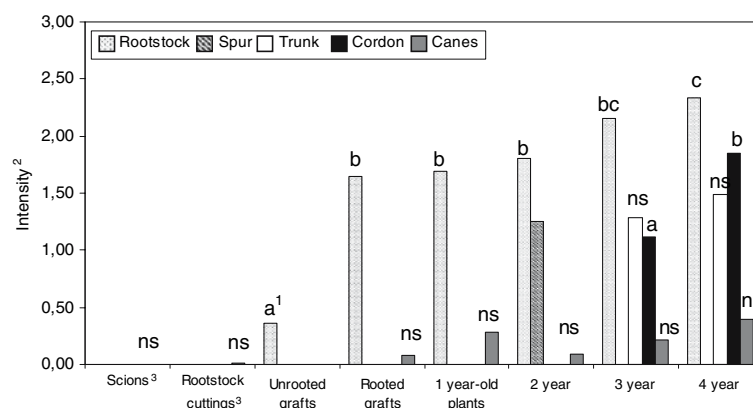


Fig. 1 Intensity of wood discolouration at different growth stages and positions in the analysed grapevine materials (combined data). ¹ Values of each histogram series with the same letter do not differ statistically (Student-Newman-Keuls

test, $P \leq 0.05$), ns = not significant, ² Scale: 0 = absent, 1 = light, 2 = medium, 3 = strong, ³ Scions and rootstock cuttings were classified as ‘Canes’

Fig. 2 Distribution (%) of the various symptoms at different growth stages and positions in the analysed grapevine materials (combined data)

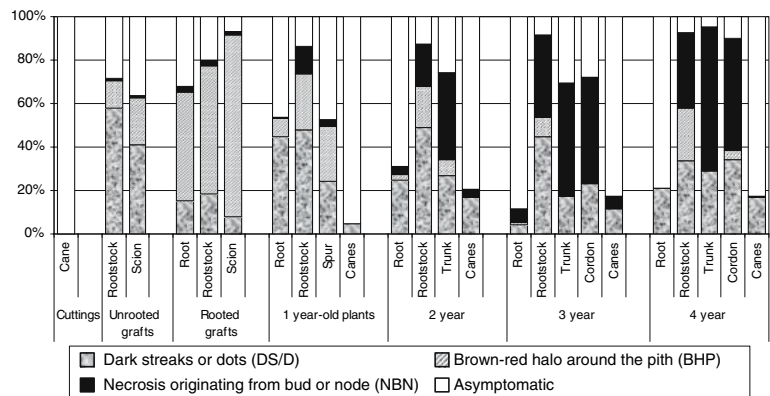


Fig. 3 Percentage of grapevines affected by *Phaeoacremonium* spp. (Pm) and *Phaeomoniella chlamydospora* (Pch) at different growth stages in the analysed grapevine materials (combined data).

¹ See Fig. 1

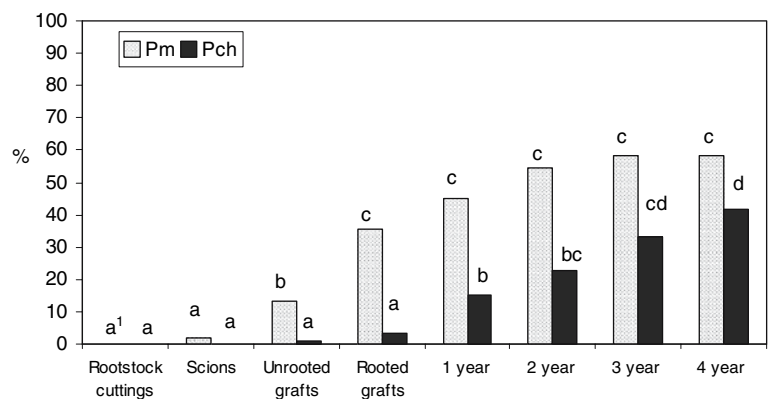
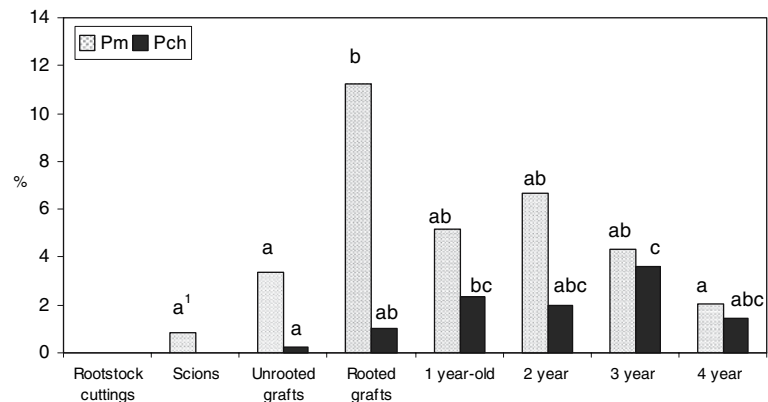


Fig. 4 Occurrence (%) of *Phaeoacremonium* spp. (Pm) and *Phaeomoniella chlamydospora* (Pch) on the total fungal population isolated at different growth stages in the analysed grapevine materials (combined data).

¹ See Fig. 1

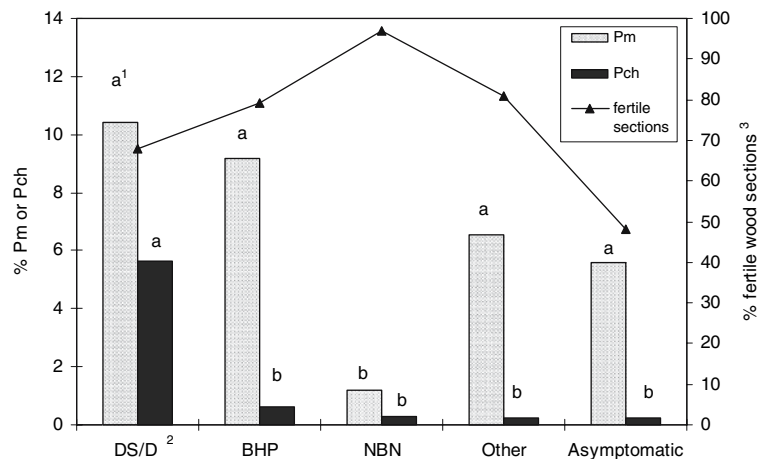


The higher isolation percentages in *Phaeoacremonium* spp. were recorded on dark wood streaks or dots (DS/D) and brown-red halo symptoms (BHP), but this species was also isolated from apparently healthy tissues (asymptomatic) (Fig. 5). Statistical analysis showed significant differences only between the incidence in necrosis (NBN) and other symptoms. *P. chlamydospora* was mainly isolated from wood

streaks and dots (DS/D) with significant differences between all other classes, where it was almost absent. Nevertheless, about 30% of the wood sections with dark streaks and dots did not produce any fungal colonies. The isolation values of these taxa from necrotic tissues (NBN) were very low even if this symptom had a high incidence of wood sections producing fungal colonies (fertile sections). The

Fig. 5 Occurrence (%) of *Phaeoacremonium* spp. (Pm) and *Phaeomoniella chlamydospora* (Pch) on the total fungal population isolated in each symptom class and percentage of fertile wood sections (combined data).

¹ See Fig. 1, ² see Fig. 2, ³ expressed as the ratio between the number of wood sections producing fungal colonies (fertiles) and the total number of wood sections $\times 100$.



smallest numbers of fertile sections were collected from asymptomatic tissues.

A significantly lower occurrence of *Phaeoacremonium* spp. was observed in the cordons and canes than in other grapevine positions (Fig. 6). *P. chlamydospora* was more frequently isolated from rootstocks than from cordons while in roots and trunks its occurrence was lower but without statistical differences from the other positions. The presence of *P. chlamydospora* in the cordon was observed in a 4 year-old Cabernet Sauvignon grapevine only.

Experimental vineyard inspections

Plants of both cultivars developed a horizontal cordon and an early canopy in the third year after planting. In 2003, very high temperatures were recorded in summer, but artificial irrigation supported the devel-

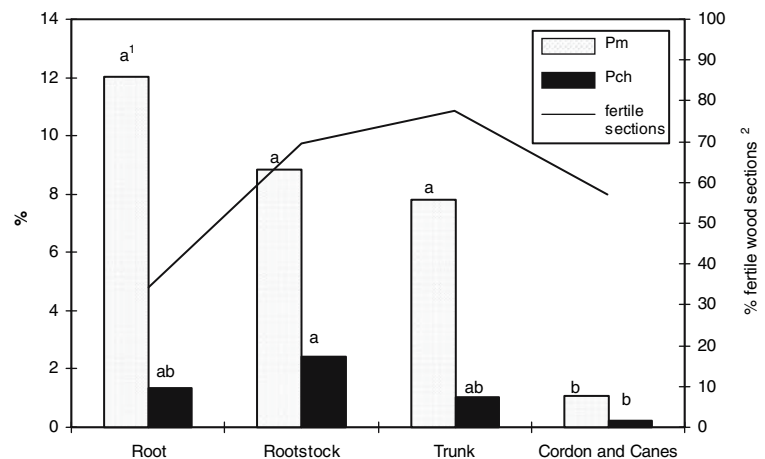
opment of the grapevines. During 5 years of observation, no foliar symptoms of Petri disease or esca were observed. Leaf chlorosis and magnesium deficiency symptoms, as described by Dalmaso and Eynard (1990), were visible each year on some Prosecco grapevines (around 5% of the total plants).

Discussion

The morphological data showed an overall general healthy condition of the analysed propagation materials. The reduction in callus formation in the Cabernet Sauvignon/SO4 unrooted grafts was observed also in other circumstances and could be due to a varietal effect (Borgo, CRA-ISV, personal communication). The growth of the young grapevines in the early years of cultivation was well managed

Fig. 6 Occurrence (%) of *Phaeoacremonium* spp. (Pm) and *Phaeomoniella chlamydospora* (Pch) on the total fungal population isolated at different positions and percentage of fertile wood sections (combined data).

¹ See Fig. 1, ² see Fig. 5



and the reduced development of the canes in the 4 year-old plants was probably due to the high temperatures recorded in the summer of 2003. Neither stunting nor withering were noticed in the observed young grapevines.

The absence of wood discolouration in scions and the extremely rare presence in rootstock cuttings is consistent with the low frequency of such symptoms reported in a previous work (Zanzotto et al. 2001). Early discolourations were visible in the unrooted grafts but became clear and widespread only in the rooted grafts, after one season of cultivation in the nursery. After planting the grapevines in the vineyard, the intensity of colour alterations increased in the cordon, probably as a result of the accumulation of additional pruning wounds each year. The scarcity of colour alterations on the canes could be due to the fact that these organs are regenerated each year after pruning. Dark streaks or dots were frequently noticed in the rootstock while they were less present in the trunk and the cordon. Eskalen et al. (2001) demonstrated the ability of *P. chlamydospora* and *Phaeoacremonium* spp. to produce dark streaks on rootstocks and also the presence of shorter streaks (<5 cm length) in non-inoculated samples. Frequent colour alterations in rooted grafts were also observed by other authors and explained as the consequence of abiotic causes (Frisullo et al. 1992; Triolo et al. 1993; Rumbos and Rumbou 2001; Stamp 2001). The length of streaks or their presence in unwounded plants, together with the isolation of pathogenic species, could provide support for a biotic origin of these symptoms. In our study, brown-red halo symptoms were observed around the pith of the rootstocks and trunks in very young grapevines. The higher incidence of necrosis close to buds or nodes in older plants could be due to the increasing number of wounds. Most of the asymptomatic wood samples were collected from roots and canes.

Concerning the occurrence of *Phaeoacremonium* spp. and *P. chlamydospora*, the first taxon was much more frequent than the second in all the analysed grapevine materials. Infections by *Phaeoacremonium* spp. and *P. chlamydospora* were never observed in the scions and rootstock cuttings, with the exception of a *Phaeoacremonium* spp. colony isolated on Prosecco. Nevertheless, other authors (Ridgway et al. 2002; Whiteman et al. 2004) reported the isolation of these fungi from various propagation

materials. This may be explained by the different origins of the analysed nursery materials. In our study the presence of these fungi on the canes of young plants occurred very seldom. This is consistent with the extremely rare isolation, or absence, of *P. chlamydospora* from the canes of mother plants reported by other authors (Larignon et al. 2004; Oliveira et al. 2004). The increasing incidence of plants affected by *P. chlamydospora* in the older sampled grapevines would suggest a progressive contamination of healthy plants in the vineyard or a delayed development of latent fungal infections already present in the rooted grafts. Further studies are needed to clarify these aspects.

The observation that the occurrence of *Phaeoacremonium* spp. had greatly increased in the rooted grafts suggests a wider exposure of such materials to fungal infections and is consistent with the hypothesis of Larignon et al. (2004) about a possible contamination of rooted grafts during their cultivation in the nursery. The reduction of *Phaeoacremonium* spp. incidence in further growth stages could be due to competition with other fungal species or to conditions less favourable for its development. In contrast, *P. chlamydospora* exhibited a gradually increasing incidence in the fungal population during the first years of cultivation, as also reported by Gatica et al. (2001), who observed an increase of *P. chlamydospora* occurrence in older grapevines.

P. chlamydospora strains were significantly isolated from wood with dark streaks and dots, as previously reported by other authors (Mugnai et al. 1999; Larignon 2004). *Phaeoacremonium* spp. colonised wood showing brown-red halo symptoms. Other fungi colonised the necrotic tissues close to buds or wounds, where the incidence of the two investigated species was quite low. Additionally, about one third of the wood sections with the dark streaks or dots were not infected with any fungus. Based on these results, the simple appearance of such symptoms in the grapevine wood should not be taken as evidence of the presence of either *P. chlamydospora* or *Phaeoacremonium* spp.

Phaeoacremonium spp. and *P. chlamydospora* colonies were isolated mostly from the lower part of the grapevine, confirming the observations of other authors (Bertelli et al. 1998; Halleen et al. 2003). A very low occurrence of these species was recorded in canes and cordons, suggesting that contamination in

young grapevines is more likely to occur in other parts of the grapevine.

Though a considerable amount of the uprooted grapevines bore at least one *Phaeoacremonium* spp. or *P. chlamydospora* colony in their tissues, no classic foliar symptoms of either esca or Petri disease were observed in the vineyards during the 4 years after planting. The lack of symptoms could have been favoured by the absence of stress factors, the importance of which in the development of esca syndrome has recently been evaluated (Ferreira et al. 1999; Corino et al. 2004; Corti et al. 2004).

In conclusion, Petri disease has not yet been seen as a problem in newly-planted vineyards in this viticultural region of Italy, when propagation materials of satisfactory technical quality are used and good agronomic practices are followed.

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