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Investigation into the occurrence of esca-associated fungi in cuttings and bench-grafted vines

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Summary. Rootstocks, scions, certified virus-free grafted plants and 1-year old plants were studied to determine the incidence of wood discoloration and the occurrence of fungi associated with esca. On cuttings very little discoloration was observed and fungal infection was negligible. The majority of grafts and vines showed blackening and harboured a variety of fungi, mainly *Phaeoacremonium* spp., with *Phaeomoniella chlamydospora* in second place. The presence of these fungi as endophytes requires further investigation to determine the conditions under which they become pathogenic and to determine when contamination of vine material occurs during nursery operations.

Key words: young esca, grapevine, wood discoloration, fungal colonization.

Introduction

Phaeomoniella chlamydospora has been stated to be the causal agent of black goo or Petri decline(1) (Morton, 1999; Crous and Gams, 2000) and, together with *Phaeoacremonium* species, is frequently reported as a cause of esca. *Phaeomoniella chlamydospora* has been reported in vine propagation material (Bertelli *et al.*, 1998; Porter, 1998; Rego *et al.* 2000) and in rootstock mother-vines (Pascoe and Cottral, 2000).

In Italy 70% of new vines are produced in the nursery by the union of two different cuttings: the scion (the cultivated variety) and the rootstock (the American species), thus having a bi-member plant. The union is done by grafting and ultimately produces a grafted-vine to be sold to growers. The usual grafting procedure is as follows. Cuttings are collected in winter from rootstock and *Vitis vinifera* mother-plants. Rootstocks are immersed in water for 2–4 h and then soaked in a fungicide solution for 2 h. After this treatement they are kept at 2–3°C with 90% humidity, for 2–3 months. Scions are treated in the same way but without the preliminary water bath. After conservation in the refrigerator, the rootstocks are again immersed in water for 12–48 h and then grafted. The rootstocks are 30–38 cm long with 2–3 buds, and are disbudded. The scions are cut much shorter as they have to bear only one bud. Grafting is usually done with a bench "omega-cut" or "lamella-cut" grafting machine. Soon after grafting, plants are put in a paraffin pot at

(1) At the general Assembly of the 2nd ICGTD meeting held in Lisbon 2001 it was unanimously decided that the disease will henceforth be called Petri disease.

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80–82°C, with paraffin containing a cicatrising hormone to facilitate the sealing of the two vine members. The grafted vines are put in forcing boxes, filled with wood shavings, and maintained in a hot house at 30–32°C with 75–90% humidity, for about 2 weeks. After emergence of shoots and roots, the vines are put outdoors to acclimatise, pruned and then paraffined at 78–86°C. Fungicides are applied to protect against *Botrytis cinerea* infections. Plants are then planted in the field where they develop shoots and roots during summer and until the end of winter, when they are uprooted. After uprooting, the grafts are carefully selected, pruned and once more paraffined at 80–82°C to promote their conservation. They are then sold to growers.

The occurrence of esca-associated fungi in vine propagation material was studied at three stages in the nursery: (i) the cutting stage, when 1-bud scions and 2-bud rootstocks were ready to be joined to form the grafted plant; (ii) the grafted-vine stage, when grafted vines were sent to the growers; and (iii) the 1-year old vine stage, when the vines used in the study were uprooted after 1 year of growth.

Materials and methods

Rootstock and scion cuttings

In March 1998, two groups of cuttings were examined before grafting: one in which cuttings were treated with thiophanate-methyl (70 g hl^{-1}), for 48–72 h and another one in which they were left untreated, i.e., brought straight to the laboratory without any water immersion in the nursery. Each of the two groups included 53 scions of Cabernet Sauvignon (R5 clone), 53 of Prosecco (ISV-ESAV 10 clone) and 50 rootstock cuttings of Kober 5 BB (ISV 1 clone).

In April 1999 only treated scions (76 Cabernet Sauvignon, R5 clone; 52 Merlot, R3 clone) and rootstock cuttings (80 SO4, ISV-VCR 6 clone) were collected from the same nursery.

In both years, cuttings and scions were cut and analysed for internal wood discoloration. Wood sections less than 1 mm thick, 2 per rootstock and 1 per scion, covering the entire cross-sectional area of the wood, were cut out under sterile conditions and incubated at 25°C for 30–40 days on malt extract agar amended with 50 mg l^{-1} tetracycline hydrochloride for isolation of fungi (Serra *et al*., 2000).

Bench-grafted rooted vines

Certified virus-free bench-grafted vines of two cultivars, Prosecco ISV-ESAV 10 grafted on Kober 5 BB, and Cabernet Sauvignon-R5 grafted on SO4, were studied in the winter of 2000 and 2001. One hundred vines per cultivar were collected each year from one nursery: half of these were used for the laboratory investigations, and half were planted in the vineyard for further study.

In each year grafted rooted plants were weighed, nodes counted and the occurrence of abnormal roots recorded. As roots usually grow from the bottom of the rootstock, all roots growing from different positions along the axis were considered abnormal due to stress. The 50 grafted vines per cultivar selected were transversely sectioned at five positions along the axis (Fig. 1): bottom of rootstock (A), first internode (B), first node (C), 4 cm below graft union (D), graft union (I). In both years, grafted vines were analysed for internal wood discoloration. Then wood sections, one at each sampling position along the rootstock and two at the graft union (Ia and Ib), were cut, and incubated as described above. About 300 wood sections per cultivar were cut out and incubated every year.

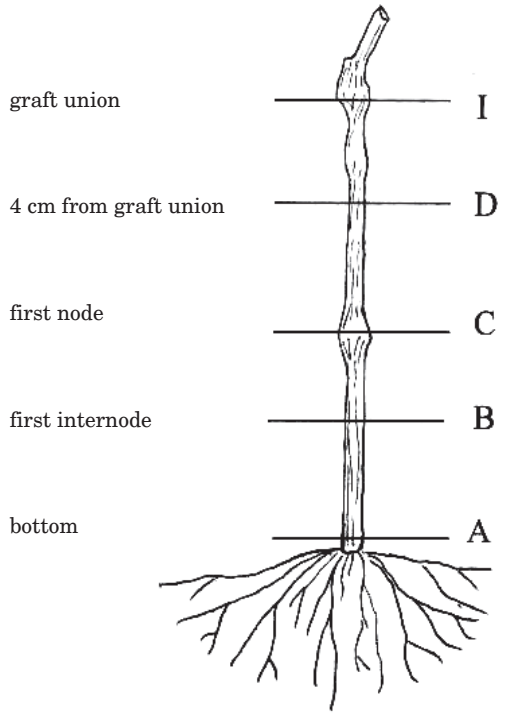

Fig. 1. Positions along the axis of a grafted-rooted vine where isolations were made.

1-year-old vines

In April 2001, 10 of the 50 vines planted in the vineyard in 2000 were uprooted and analysed. The vines were cut along the rootstock and at the graft union, as described for grafted vines, and also at three positions (II, III and IV) along the 1-year-old shoot. At each position a section of wood was cut out as described above. A total of 80 wood sections were cut from each cultivar. At each sampling position, the occurrence and position of any wood discoloration was recorded.

Results

Rootstock and scion cuttings

A small percentage of rootstocks and scions showed wood blackening, with little difference between treated and untreated material (Table 1). Only Cabernet Sauvignon scions showed any substantial blackening in 1998, mainly due to superficial lesions. Colonies on incubated wood segments were very few, particularly on untreated segments. Most fungi isolated were common saprophytes (e.g., *Alternaria* spp., *Aureobasidium* spp., *Cladosporium* spp. and *Penicillium* spp.) or they failed to sporulate and so could not be identified. Immersion in water before disinfecting the cuttings seemed to favour development of a broader saprophytic mycoflora. However, this did not include any of the fungi known to be involved in esca. For these reasons, only normally treated cuttings were analysed in 1999. More tests are needed, to ascertain the real effect of water immersion and fungicide practices.

Bench-grafted rooted vines

The length of grafted plants was 30–35 cm for the rootstock and 4–7 cm for the scions; the weight was 60–70 g per plant, with an average of 2.5 nodes. In the two years of trials 79% of cv. Prosecco vines and 92% of cv. Cabernet Sauvignon had normally developed roots. Vines appeared healthy and thriving. Nevertheless, wood blackening was observed at the graft union (I), and at all bottom sampling positions (A) of the grafts. At position A, wood blackening appeared as a thick ring around the pith. The incidence of blackening decreased higher up the vine (99% at position B, 96.5% at position C and 90.0% at position D) while it here appeared as single spots (black streaks in longitudinal sections), sometimes linked together in a thin ring.

In 2000, 227 fungal colonies developed from wood segments of cv. Prosecco and 259 from wood segments of cv. Cabernet Sauvignon. In 2001 the corresponding figures were 291 and 267. The fungal population consisted of different groups, including *Trichoderma* spp., *Gliocladium* spp., *Penicillium* spp., *Fusarium* spp., *Alternaria* spp., and several unidentified non-sporulating fungi. Isolation percentages of *Phaeoacremonium* spp. and *P. chlamydospora,* are shown in Table 2. The former species occurred in many of the grafted plants and were frequently isolated from both the rootstocks

Table 1. Percentage of rootstock (K5BB, SO4) and scion (Cabernet Sauvignon, Prosecco, Merlot) cuttings with wood blackening and percentage of wood sections colonized by fungi in a two years trial (1998, 1999).

Data surveyed	1998						1999		
	Untreated			Treated[a]			Treated[a]		
	Cabernet Sauvignon	Prosecco	K5BB	Cabernet Sauvignon	Prosecco	K5BB	Cabernet Sauvignon	Merlot	SO4
Blackened cuttings	22.6	0	0	7.6	0	0	1.3	1.9	3.7
Colonized wood segments	1.9	0	2	7.5	9.8	15	9.2	21.1	16.9

[a] Soaking in thiophanate-methyl, 70 g hl^{-1} for 48–72 h.

Table 2. Percentage of esca-associated (*Phaeoacremonium* spp., *Pm.* spp.; *Phaeomoniella chlamydospora, Pch)* and other fungi from wood segments taken from different positions on the rootstock and from the graft area of bench grafted rooted vines of two rootstock and scion combinations, and incidence of colonized vines in 2000 and 2001.

Rootstock/scion combination	Year	Fungus	Wood section[a]						Incidence on grafted vines No. (%)
			A	B	C	D	Ia + Ib	Total	
Prosecco/Kober 5BB	2000	*Pm.* spp.	4.4	0	9.1	9.1	8.1	6.6	26
		Pch	0	0	0	3	1.2	0.9	4
		Others	95.6	100.0	90.9	87.9	90.7	92.5	n.d.
	2001	*Pm.* spp.	1.7	13.2	13.3	21.3	14	12.4	46
		Pch	1.7	1.9	0	2.1	0	1v	4
		Others	96.6	84.9	86.7	76.6	86	86.6	n.d.
Cabernet Sauvignon/SO4	2000	*Pm.* spp.	3.5	22.9	18.0	20	7.9	12.4	44
		Pch	1.8	0	5.1	2.5	3.4	2.7	14
		Others	94.7	77.1	76.9	77.5	88.7	84.9	n.d.
	2001	*Pm.* spp.	5.6	20	6.5	4.6	11.1	9.8	42
		Pch	0	2.5	6.4	0	0	1.1	2
		Others	94.4	77.5	87.1	95.4	88.9	89.1	n.d.

[a] A, bottom of the rootstock; B, first internode; C, first node; D, 4 cm below graft union; Ia+Ib, graft union.
n.d., not determined.

and the graft union, whereas *P. chlamydospora* isolation rates from grafted plants were much lower.

1-year old vines

None of the uprooted 1-year-old vines showed external symptoms of stunting or stress. Wood blackening occurred with 100% frequency at the lowermost section point (position A); incidence decreased higher up along the vine (90% at position B, 85% at position C, 90% at position D) but rose again 100% at the graft union (shoot section I). Along the shoot, blackening decreased rapidly (on 60% of vines at shoot section II, 10% at section III, 0% at section IV). Incubation of wood segments produced 91 colonies in cv. Prosecco and 76 in cv. Cabernet Sauvignon. Most of the isolated fungi were *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp. and some non-sporulating, unidentified species. The percent isolations for *Phaeoacremonium* spp. and *P. chlamydospora* were lower than those recorded for the corresponding rooted grafted plants (Table 3) with *P. chlamydospora* being absent from cv. Prosecco and only isolated in low numbers from Cabernet Sauvignon.

Discussion

The low incidence of internal wood blackening and fungal colonization in vine cuttings and scions suggest that, though possible (Ferreira, 1999; Pascoe and Cottral, 2000; Rego et al., 2000), the involvement of propagating material in the transmission of the fungi causing the esca complex, under normal conditions is not very relevant.

Moreover, in a trial carried out using scions and rootstocks from esca-diseased vines it was found that the newly produced grafts either failed to survive, or could not be used because of their stunted or declining condition (unpublished data).

Thus much care must be taken when choosing propagating material. Furthermore, the lack of efficacy of the disinfecting treatment applied here to reduce fungal colonisation of propagation materials, underlines the importance of selecting efficient procedures.

The occurrence of the blackish streaks in the grafted vines, as well as the isolation of *Phaeoacremonium* spp. and *P. chlamydospora,* suggest that colonisation of wood occurred soon after graft-

Table 3. Percentage of esca-associated (*Phaeoacremonium* spp., *Pm.* spp.; *Phaeomoniella chlamydospora, Pch)* and other fungi isolated from wood segments of 1-year-old vines of two rootstock and scion combinations, uprooted in 2001, and mean incidence of colonized vines.

Rootstock/scion combination	Fungus	Wood section[a]									
		Rootstock				Graft union	Shoot			Total	Incidence on vines No. (%)
		A	B	C	D	I	II	III	IV		
Prosecco/Kober 5BB											
	Pm. spp.	0	0	0	18.2	0	0	0	0	2.2	20
	Pch	0	0	0	0	0	0	0	0	0	0
	Others	100	100	100	81.8	100	100	100	100	97.8	n.d.
Cabernet Sauvignon/SO4											
	Pm. spp.	0	9.1	0	9.1	9.1	28.6	33.3	0	9.2	60
	Pch	0	0	0	0	9.1	0	0	0	1.3	10
	Others	100	90.9	100	90.9	81.8	71.4	66.7	100	89.5	n.d.

[b] A, bottom of the rootstock; B, first internode; C, first node; D, 4 cm below graft union; I, graft union; bottom of the shoot (II), middle (III) and, edge of the shoot (IV).
n.d., not determined.

ing, when the grafted vines were forced, or somewhat later, when the rooted grafted vines were developing in the nursery field. After one year in the vineyard, the isolation percentages for *Phaeoacremonium* spp. and *P. chlamydospora* (2001 data) were substantially lower than in the original stock of grafted vines (2000 data). This suggests that, in the first year of cultivation in the vineyard, the percentage of fungi involved in esca could be masked, or actually reduced, by competition with other micro-organisms in the complex natural environment. Observation will be continued to monitor the fungal population in the remaining planted vines, relating those fungi to the evolution of external symptoms.

The health of the surveyed material, as shown by the morphological records, and the apparent health of the one-year-old uprooted vines suggested that the colonising fungi were behaving as endophytes (Ferreira *et al*. 1999; Whiting *et al*., 2001). The high incidence of black streaking could have been due to other, biotic or abiotic causes. Nursery forcing operations can expose vines to stress agents (Frisullo *et al*., 1992; Triolo *et al*., 1993) and grafting can induce damaging changes in plant physiology (Bavaresco and Lovisolo, 2000). Rootstock disbudding and accidental wounds provide access to colonization by a wide range of ubiquitous, fast growing fungi. However differences in the symptoms induced by those different agents are not well understood at present.

In conclusion, the occurrence of *Phaeoacremonium* spp. and *P. chlamydospora* as endophytes in vines requires further investigation to determine when contamination occurs during nursery operations and what are its causes. This is necessary in order to apply effective control measures and to prevent grapevine decline.

Literature cited

Bavaresco L. and C. Lovisolo, 2000. Effect of grafting on grapevine chlorosis and hydraulic conductivity. *Vitis* 39, 89–92.

Bertelli E., L. Mugnai and G. Surico, 1998. Presence of *Phaeoacremonium chlamydosporum* in apparently healthy rooted grapevine cuttings. *Phytopathologia Mediterranea* 37, 179–82.

Crous P.W. and W. Gams, 2000. *Phaeomoniella chlamydospora* gen. et comb. nov., a causal organism of Petri grapevine decline and esca. *Phytopathologia Mediterranea* 39, 112–118.

Ferreira J.H.S., 1999. Researching *Phaeoacremonium* in root-stocks: summary, highlights and excerpts. In: *Black goo – Occurrence and Symptoms of Grapevine Declines. IAS/ICGTD Proceedings 1998.* (L. Morton ed.) International Ampelography Society, Fort Valley, VA, USA, 94–97.

Ferreira J.H.S., P.S. van Wyk and F.J. Calitz, 1999. Slow dieback of grapevine in South Africa: stress-related predisposition of young vines for infection by *Phaeoacremonium chlamydosporum*. *South African Journal of Enology and Viticulture* 20(2), 43–46.

Frisullo S., A. Caponero and M. Cirulli, 1992. Ricerche sulle cause dell' "imbrunimento del legno" delle barbatelle di vite. *Petria* 2, 171–182.

Morton L., 1999. On the trail of black goo. In: *Black goo – Occurrence and Symptoms of Grapevine Declines*. *IAS/ICGTD Proceedings 1998*. (L. Morton ed.) International Ampelography Society, Fort Valley, VA, USA, 11–16.

Pascoe I. and E. Cottral, 2000. Developments in grapevine trunk diseases research in Australia. *Phytopathologia Mediterranea* 39, 68–75.

Porter M., 1998. Guerrilla research in the North Coast region of California. In: *Black goo – Occurrence and Symptoms of Grapevine Declines*. *IAS/ICGTD Proceedings 1998*. (L. Morton ed.) International Ampelography Society, Fort Valley, VA, USA, 11–16.

Rego C., H. Oliveira, A. Carvalho and A. Phillips, 2000. Involvement of *Phaeoacremonium* spp. and *Cylindrocarpon destructans* with grapevine decline in Portugal. *Phytopathologia Mediterranea* 39, 76–79.

Serra S., M. Borgo and A. Zanzotto, 2000. Investigation into the presence of fungi associated with esca of young vines. *Phytopathologia Mediterranea* 39, 21–25.

Triolo E., A. Panattoni and S. Cerri, 1993. Alterazioni cromatiche del legno in barbatelle di vite: osservazioni anatomo-patologiche e analisi numerica della dinamica di sviluppo. In: *Atti della Giornata di Studio: "La ricerca sperimentale in corso per la Viticoltura in Toscana"*, Maggio 1993, S. Felice, Siena, Italy, 243–245.

Whiting E.C., A. Khan and W.D. Gubler, 2001. Effect of temperature and water potential on survival and mycelial growth of *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. *Plant Disease* 85, 195–201.

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