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Involvement of *Phaeoacremonium* spp. and *Cylindrocarpon destructans* with grapevine decline in Portugal

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Summary. In an attempt to determine the aetiology of young vine decline in Portugal a study was carried out in rootstock nurseries and in young vineyards during the last few years. Rootstock nurseries located in the most important production areas of Portugal (Ribatejo-Oeste and Beira Litoral) were surveyed. The fungi most frequently isolated from discoloured wood at the base of the stem were *Cylindrocarpon destructans* and *Phaeoacremonium* spp., although *Acremonium* sp., *Phomopsis* sp., *Fusarium* spp., *Fusicoccum* sp., *Gliocladium* sp., *Phoma* sp. and *Sphaeropsis* sp. were also present. Young vines (2- to 8-year-old) showing decline symptoms were collected from various vine-growing regions of Portugal. Disease incidence was variable but decline symptoms were present in several wine and table grapevine cultivars grafted onto different rootstocks (99R, 110R, 1103P, 101-14, 140Ru, 5BB and 161-49). Isolations from symptomatic internal tissues revealed that also *C. destructans* and *P. chlamydosporum* were the predominant fungi isolated from the basal end of the rootstocks and grafting tissues. In the pathogenicity tests carried out with *C. destructans* isolates the pathogen was reisolated from inoculated plants showing black-foot symptoms but never from the controls. Our results point out that both *C. destructans* and *P. chlamydosporum* might be involved with young grapevine decline in Portugal.

Key words: young vine decline, *Cylindrocarpon destructans*, *Phaeoacremonium* spp.

Introduction

Decline symptoms in young grapevines were first seen in Portugal in the early 1990s, mainly in areas where there had been extensive replacement of ancient vineyards with new plantations. Symptoms included reduced vigour, shortened internodes, sparse foliage and chlorotic leaves, frequently leading to death of the plants. In cross-sections of the trunks brown to black discolouration of the wood tissues was evident at the level of the graft. Longitudinal sections revealed that the black discolouration was also present in tissues above and below the graft union. Isolations from discoloured tissues of the trunk consistently yielded the fungus *Cylindrocarpon destructans* (Zins.) Scholten which was subsequently shown to be pathogenic to grapevines (Rego, 1994). This fungus had first been reported as the causal agent of black-foot disease of grapevine in Italy in 1984 (Grasso, 1984) and later it was considered to be responsible for grapevine mortality in some new plantings in France (Maluta and Larignon, 1991). *Cylindrocar-*

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pon obtusisporum (Cook & Harkn.) Wollenweb. has also been reported to cause symptoms of black-foot in Sicilia (Grasso and Magnano di San Lio, 1975) and in California both *Cylindrocarpon* species have been reported to induce dark-brown to black streaking in the vascular elements of trunks (Scheck *et al*., 1998a).

Species of *Phaeoacremonium* have also been associated with young grapevine decline in South Africa (Ferreira *et al*., 1994), United States of America (Morton, 1997; Scheck *et al*., 1998b), in Australia (Pascoe, 1999) and in Italy (Mugnai *et al*., 1999). Symptoms caused by *Phaeoacremonium* spp. on young grapevines are very similar to those incited by species of *Cylindrocarpon* and disease diagnosis cannot be made solely on the basis of visual symptoms of vegetation.

After the first reports of *Cylindrocarpon* black-foot disease in Portugal, other severe outbreaks were observed in young vineyards of certain grape cultivars. These vineyards were located in various different regions of the country but a common feature was that the rootstocks came from a single region, or even from the same nursery (Rego *et al*., 1998). Concerning the origin of the infection in these young vines, it was hypothesised that infected rootstocks are a potential mean by which the pathogen could be spread.

This paper reports the results of surveys of *Cylindrocarpon* black-foot disease in rootstock nurseries and young vineyards in Portugal. The role of species of *Phaeoacremonium* isolated from sampled materials is also discussed.

Materials and methods

During 1998 and 1999, 68 rootstock nurseries located in the most important production areas of Portugal (Ribatejo-Oeste and Beira Litoral) were surveyed. Samples of approximately 100 plants of each rootstock (1103P, S04, 196-17, 5BB, 140Ru, 99R, 41B, 101-14, 110R) were randomly collected from each nursery and a total of 3,340 plants were selected for isolation. Plants were washed with water and their roots cut off. Rootstocks were cut crosswise, and inspected for wood discoloration. Small pieces, approximately 3x2x1□mm in size, of blackened tissues located within 5 cm of the basal end of rootstock were removed. In some cases fragments of affected tissues were also taken from upper levels. Pieces were surface disinfected by immersion in a 1.5% solution of NaOCl for 30 sec, rinsed in sterile distilled water, plated on potato-dextrose agar and then incubated in the dark at 20°C. Plates were examined weekly. Identification of the isolated fungi was based on their microscopical and cultural characters, according to classical manuals. Species of *Cylindrocarpon* and *Phaeoacremonium* were identified following Samuels and Brayford (1990) and Crous *et al*. (1996), respectively. Results are given as a percentage of plants infected with each fungus.

A survey of young grapevines (2- to 8-year-old) showing decline symptoms was carried out from 1996 to 1999. A total of 154 diseased plants of different cultivar/rootstock combinations were obtained from distinct vine growing regions of Portugal. Cross and longitudinal sections of the woody stem of each plant were examined in order to follow the development of wood browning. Isolations were made from different zones of necrotic tissues, following the method described above.

Rooted cuttings of 99R rootstock were used for pathogenicity experiments of *C. destructans* isolates. Plants were uprooted and their roosts washed. For each fungal isolate, twelve plants were inoculated by dipping roots in a conidial suspension containing ca. 10^8 spores per ml for 30 min. Control plants were similarly treated but sterile distilled water was used instead of inoculum. Rooted cuttings were planted in 500 ml plastic pots containing an artificial mixture of soil, peat and sand (2:1:1 v/v) and maintained in a growth-chamber at 24°C with a 12 h fluorescent light/12 h dark cycle. Disease symptoms were recorded two months later and reisolations were made by the method described above for isolations.

Results and discussion

Concerning the survey of rootstock nurseries a high number of plants exhibited, in longitudinal section, black discolouration and brown to dark streaks in the wood, mainly at the base of the rootstock. In cross section the streaks appeared as dark spots, sparsely distributed or in groups. The fungi most frequently isolated from discoloured wood at the base of rootstock were *C. destructans* (37.0%), *Phaeoacremonium* spp. (29.8%), *Acremonium* sp. (9.0%), *Phomopsis* sp. (7.8%), *Fusarium* spp. (4.9%),

Fusicoccum sp. (4.1%), *Gliocladium* sp. (2.9%), *Phoma* sp. (1.1%) and *Sphaeropsis* sp. (0.6%). These fungi were also isolated, but less frequently, from the upper parts of the rootstocks. As for *Phaeoacremonium* species, *P. chlamydosporum* was predominant, although *P. aleophilum* was also isolated. Considering the most frequently isolated fungi, namely *C. destructans* and *Phaeoacremonium* spp., both fungi were present in all tested rootstocks (Table 1).

In what concerns the decline in young vines (2- to 8-years-old), the incidence was generally low, but some severe outbreaks occurred. Wine cultivars (Fernão Pires, Malvasia Fina, Tinta Roriz, Jaen, Alfrocheiro, Trincadeira, Seara Nova, Boal Branco, Tinta Barroca, Touriga, Touriga Francesa, Perrum, and Moscatel de Setúbal) and table grape cultivars (Cardinal, D. Maria) grafted onto various rootstocks (99R, 110R, 1103P, 101-14,140Ru, 5BB, 161-49) were affected. Isolations made from symptomatic internal tissues yielded several different genera of fungi, namely, *Cylindrocarpon*, *Phaeoacremonium*, *Fusicoccum*, *Phomopsis*, *Natrassia*, *Sphaeropsis*, *Truncatella*, *Fusarium*, and *Gliocladium*. Among these, *C. destructans* and *P. chlamydosporum* were the fungi most frequently isolated from the basal end of the rootstocks and from the graft region of young plants.

In addition to the typical symptoms of decline, a canker was observed in some young grapevines. In most cases the canker was located at the base of the current season's shoot, but occasionally they were found on branches of the main stem. The bark was blackened and deeply cracked and the canes had an oval cross-section compared with the cylindrical aspect of healthy canes. *P. chlamydosporum* was frequently isolated from the rootstocks and cankers of the affected plants. Further studies are needed to determine if *P. chlamydosporum* is involved with the production of cankers.

Typical black foot symptoms developed in rooted cuttings inoculated with isolates of *C. destructans*. These included root lesions, vascular discolouration, and necrosis, all of which developed within two months after inoculation. Depending on the isolate tested, between 16 and 66% of the plants developed root symptoms and 33-67% died. The pathogen was reisolated from inoculated plants but never from the control treatments.

In Portugal, *C. destructans* and *P. chlamydosporum* are consistently isolated from young grapevines showing decline symptoms. Disease incidence and severity are increasing in the major grape-growing regions forcing growers to replant young vineyards. Both fungi incite similar field symptoms which Scheck *et al.* (1998a) collectively referred to as "decline symptoms". Also, our results point out that *C. destructans* and *Phaeoacremonium* spp. were isolated both from rootstocks and from grapevine plants with decline symptoms. However, some symptomatic tissues were apparently sterile, suggesting that wood discolouration can also be due to abiotic causes.

Our study has revealed that infected rootstocks are the most likely way in which these two pathogens are disseminated, but the initial source of infection is not known. Propagation material may already be infected when it is taken from the moth-

Table 1. Frequency of isolation of *Cylindrocarpon destructans* and *Phaeoacremonium* spp. from nine different rootstocks.

Rootstock	No. of plants examined	% plants with *Cylindrocarpon destructans*	% plants with *Phaeoacremonium* spp.
1103P	570	25.0	38.4
S04	515	33.4	19.6
196-17	827	30.2	18.0
5BB	220	47.0	40.5
140Ru	163	28.0	25.1
99R	740	26.5	16.2
41B	100	30.0	21.0
101-14	40	47.0	7.5
110R	165	28.5	17.0

er plants, or infection may take place in the nursery (Bertelli *et al*., 1998), or in the soil through the roots and wounds. At present we do not know which of these alternatives occurs in Portugal. Therefore, further research must be aimed at determining the source of infection. The role played by each or both of these fungi in young grapevine decline should also be studied.

Literature cited

Bertelli E., L. Mugnai and G. Surico, 1998. Presence of *Phaeoacremonium chlamydosporum* in apparently healthy rooted grapevine cuttings. *Phytophathologia Mediterranea*, 37, 79-82.

Crous P.W., W. Gams, M.J. Wingfield and P.S. van Wyk, 1996. *Phaeoacremonium* gen.nov. associated with wilt and decline diseases of woody hosts and human infections. *Mycologia*, 88, 786-796.

Ferreira J.H.S., P.S. van Wyk and E. Venter, 1994. Slow dieback of grapevine: association of *Phialophora parasitica* with slow dieback of grapevines. *South African Journal of Enology and Viticulture*, 15, 9-11.

Grasso S., 1984. Infezioni di *Fusarium oxysporum* e di *Cylindrocarpon destructans* associate a una moria di giovani piante di vite in Sicilia. *Informatore Fitopatologico*, 1, 59-63.

Grasso S. and G. Magnano di San Lio, 1975. Infezioni di *Cylindrocarpon obtusisporum* su piante di vite in Sicilia. *Vitis*, 14, 36-39.

Maluta D.R. and P. Larignon, 1991. Pied-noir: mieux vaut prévenir. *Viti,* 11, 71-72.

Morton L., 1997. Update on black goo. *Wines and Vines*, 78, 62-64.

Mugnai L., A. Graniti and G. Surico, 1999. Esca (black measles) and brown wood-streaking: two old and elusive diseases of grapevines. *Plant Disease*, 83, 404-418.

Pascoe I., 1999. Grapevine trunk diseases in Austalia: diagnostics and taxonomy. *In*: Black Goo - Occurrence and Symptoms of Grapevine Declines. IAS/ICGTD Proceedings 1998 (L. Morton, ed.) International Ampelography Society, Fort Valley, Virginia, USA, 56-77.

Rego M.C., 1994. Nova e grave micose da videira em Portugal. Agente responsável: *Cylindrocarpon destructans* (Zins.) Scholten. *Publicação do Laboratório de Patologia Vegetal "Veríssimo de Almeida"*, 67, 1-4.

Rego M.C., M. Pádua, A. Carvalho and H. Oliveira, 1998. Contributo para o estudo da espécie *Cylindrocarpon destructans* (Zin.) Scholten em material vitícola nacional. *In*: Actas 4º Simpósio de Vitivinicultura do Alentejo, 20-22 Maio, 1998, Évora, Portugal, Vol. 1, 129-135.

Samuels G.J. and D. Brayford, 1990. Variation in *Nectria radicicola* and its anamorph, *Cylindrocarpon destructans*. *Mycological Research*, 94, 433-442.

Scheck H.J., S.J. Vasquez, D. Fogle and W.D. Gubler, 1998a. Grape growers report losses to black-foot and grapevine decline. *California Agriculture*, 52(7-8), 19-23.

Scheck H.J., S.J. Vasquez, W.D. Gubler, 1998b. First report of three *Phaeoacremonium* spp. causing young grapevine decline in California. *Plant Disease*, 82, 590.