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Decline of young grapevines associated with *Phaeoacremonium chlamydosporum* in Sicily (Italy)

Agatino Sidoti[1], Emanuele Buonocore[1], Tullio Serges[1] and Laura Mugnai[2]

[1]Osservatorio per le Malattie delle Piante, Corso Umberto 114, 95024 Acireale, Italy
[2] Dipartimento di Biotecnologie Agrarie – Patologia vegetale, Università,
Piazzale delle Cascine 28, 50144, Firenze, Italy

Summary. In Italy, for the first time, a decline of young grapevines, cultivar Victoria grafted on 775 Paulsen, caused by the fungus *Phaeoacremonium chlamydosporum* has been observed. The decline presented itself with stunted growth, small leaves, chlorosis, leaf dessication and darkening of the xylem vessels in the trunk and roots. Inoculation tests showed that the fungus colonised the wood and roots of young vines.

Key words: *Phaeoacremonim chlamydosporum*, *Vitis vinifera* L., decline, young grapevines, propagation material.

Introduction

In the summer of 1998 we observed a decline of young grapevines (*Vitis vinifera* L.) cultivar Victoria grafted on 775 Paulsen (*V. berlandieri* x *V. rupestris*) in a vineyard in south-eastern Sicily (Italy). The vineyard had been established in 1997 in an open-sided green house on predominantly sandy soil which had earlier been planted to citrus. Rooted cuttings from a national nursery were used, for the purpose of producing green-house table grapes for early harvesting. The vineyard has an area of 2.80 ha and contains about 7,500 plants trained by the "tendone" system and arranged in 2x2 m squares. At the time of inspection the plants showed chlorosis and necrosis of the leaves, stunted growth and darkening of the xylem vessels.

This note reports on the symptoms observed and on the results of the study carried out to establish the causes of the decline.

Corresponding author: A. Sidoti
Fax +39 095 605290
E-mail: osservatorio@mail.gte.it

Materials and methods

Field observations

The observed decline problem affected about 15% of the vines. Distribution of the decline was irregular and infected plants generally grew apart from each other. Affected vines showed a slow but progressive decline with stunted growth, small leaves, shorter internodes, smaller trunks and branches, and discoloration of the leaves and of the wood. From the onset of sprouting, leaves were smaller and had chlorotic spots between the veins and along the edges which in the following months spread over different proportions of the leaf lamina and dried up (Fig. 1). In some cases there was also leaf roll. Wood tissue showed darkening of the xylem vessels of the rootstock, which mostly extended along the entire rooted cutting except for the scion (Fig. 2). The discolorations, which were visible as spots in cross section and as streaks in longitudinal section, were mainly located in the rings adjacent to the pith. Magnification under the light microscope revealed a dark brown to black gummy substance in the vessels.

Fig. 1. Smaller leaves, chlorosis and necrosis at the margin of the leaves in declining vines heavily colonized by *Phaeoacremonium chlamydosporum* (cv. Victoria/775 Paulsen).

Fig. 2. Discoloured xylem in the wood at the base of a 2-year-old grapevine of the cultivar Victoria/775 Paulsen. From the darken tissue *P. chlamydosporum* was frequently isolated.

In some cases the root-vessels also had brown streaking.

Diagnosis procedure

In addition to fungal isolations environmental causes of decline were also considered. It was obvious that declining plants were sparse among very healthy looking vines, which suggested that soil factors were not very likely. It was also noted that cv. Victoria performs very well in the area including in an adjacent vineyard planted with vines from a different source.

Environmental factors considered were:

– water: table grape vineyards in Sicily are all abundantly irrigated;
– site characters: no difference could be detected in the position and history of the site compared with those of adjacent unaffected green-house vineyards;
– herbicide applications: same as adjacent green-houses and no apparent herbicide symptoms;

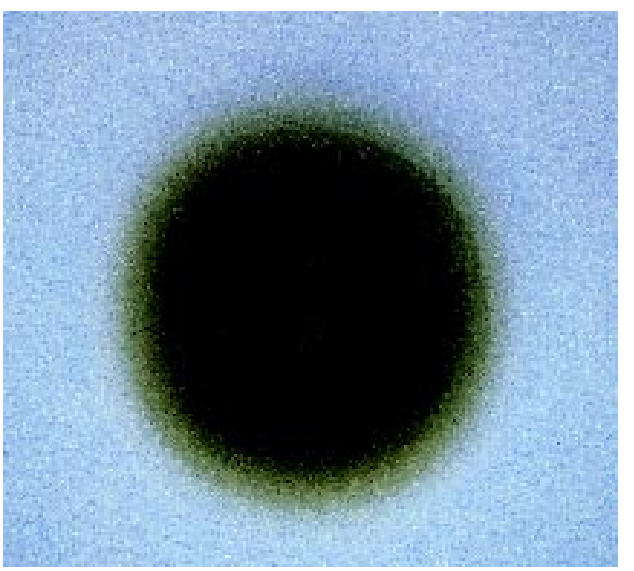

Fig. 3. A three week old colony of *Phaeoacremonium chlamydosporum* growing on malt agar, isolated from a declining vine, cv. Victoria/775 Paulsen.

– soil type: the same as the adjacent, unaffected vineyard;
– nutrients: some aspects of the foliar symptoms suggested a lack of K or Mg, but a soil analysis did not reveal any lack of nutrients. Nevertheless a foliar fertilization containing the main microelements and nitrogen was applied from the very first signs of decline.
– Virus testing was not performed as foliar symptoms were not typical of common viruses.

Fungal isolation

Fungal isolation was carried out to detect the presence of pathogenic species. Declining vines showing all typical symptoms were selected in July and September. Isolations on malt agar and potato dextrose agar were carried out analysing about 100 wood fragments sampled from roots and from darkened wood tissue of various parts of the trunk (1-year cane, graft union, mid-trunk and base of the rootstock) for each of 10 rooted cuttings.

Pathogenicity test

The pathogenicity of the fungus most commonly isolated was determined by inoculating IT in 20 one-year-old rooted cuttings of cv. Matilde grafted on Kober 5BB *(V. Berlandieri* x *V. riparia),* and of cv. Regina grafted on S04 *(V. Berlandieri* x *V. riparia)*. The inoculation was done in March 1999. Each of 10 rooted cuttings had their roots pruned to approx. 10-15 cm long and was then dipped for 30 min into a suspension of conidia and mycelium at a concentration of $1x10^6$ spores/ml. The remaining rooted cuttings were inoculated by inserting a 3-4-mm diameter agar plug colonised by the mycelium into an artificial wound at the graft union, which was then protected by moist cotton and a plastic film. Control vines were dipped in sterile water or received sterile agar plugs only. All plants were transferred to 15 litre capacity plastic pots containing steam-sterilised soil, and maintained outdoors. Six months after inoculation the ability of the fungus to colonise the wood and roots of the artificially inoculated vines was examined.

Results

Phaeoacremonium chlamydosporum W. Gams, Crous, M.J. Wingf. & L. Mugnai was consistently isolated from diseased wood tissues. Other fungi occurred with such low frequency that they are considered to be opportunistic infections or contaminants. Table I reports the relative frequencies of the main microorganisms isolated from each site in the trunk and roots of 10 young grapevines showing decline. The frequency of isolation of *P. chlamydosporum* (calculated over the total number of non sterile wood fragments) from the various samples ranged from 16 to 85%. The fungus was identified from all sites where isolations were done except for 1-year-old canes. Generally it occurred with high frequencies that reached as much as 100% of the fertile fragments analysed from some sites. For example, 70% of the fragments from the root isolations tested yielded only *P. chlamydosporum*. The main morphocultural char-

Table I. Isolation of *Phaeoacremonium chlamydosporum* carried out on various sites of 10 plants from decline of young grapevines (Victoria/775 Paulsen) observed in Sicily.

Microorganism isolated	Frequency of isolation per isolation site (%)[a]				
	1- year cane	Graft union	Mid trunk	Base of rootstock	Roots
P. chlamydosporum	0	66	71	53	70
Bacteria	32	4.4	7	24	30
Other fungi[b]	78	29.6	22	23	0

[a] The percentage isolation of each microorganism was obtained by dividing the number of fragments infected with that organism by the total number of fertile fragments from that site and multiplying by 100. Each value represents the mean of 10 plants.
[b] *Alternaria* sp., *Fusarium* sp., *Gliocladium* sp., *Trichoderma* sp., Mycelia sterilia.

acteristics of the isolates obtained (Fig. 3) were in all correspondent to the ones reported in Crous *et al.*, 1996 for *P. chlamydosporum*.

In the first six months following both artificial inoculation tests no changes were noted on the aerial organs of the infected grapevines, neither formation of chlorosis nor reduction in growth and size. In the roots (1st inoculation method), and at the graft union (2nd inoculation method) dark streaks were found extending from the inoculum site all along the trunk. *P. chlamydosporum* was readily reisolated from the wood of inoculated plants but not from any of the control plants.

Discussion

Although after six months from artificial inoculation tests no external symptoms were produced, it was induced the formation of a dark wood streaking and the accumulation of a dark gummy substance in xylem tissue i.e. inoculation tests confirmed that the fungus had colonised the wood and roots of young vines and had produced the same internal symptoms as the declining vines observed in the field.

P. chlamydosporum is one of the fungi most commonly associated with esca, a disease which has spread significantly in Sicily in recent years, especially in young plantings (Schilirò *et al.,* 1996). This same fungus is also associated with slow decline of young grapevines, the visible symptoms of which are wood darkening and gummy deposits in the wood vessels. To date declines have been reported from the South Africa, USA and Australia (Ferreira, 1994; Morton, 1995, 1997; Pascoe, 1998; Scheck *et al.,* 1998). In 1912, Petri had already reported a decline of young grapevines in Sicily associated with two isolates of *Cephalosporium* and one of *Acremonium*. More recently, another mitosporic fungus, *Phialophora parasitica* Ajello, Georg & Wang, has been isolated in South Africa from young vines affected by "slow dieback" and showing wood darkening (Ferreira, 1994). Following Crous *et al.,* (1996), these fungi were assigned to the new genus *Phaeoacremonium* and the fungus studied by Ferreira (1994) to *P. chlamydosporum*. The taxonomic position of *Phaeoacremonium* have recently been re-examined, leading to the suggestion that *P. chlamydosporum* should be transferred to another genus (Dupont *et al.,* 1998).

The high frequency of *P. chlamydosporum* isolations from the graft union and the lower part of the rootstock, and the fact that a different crop was grown before the vineyard was established, make it probable that the rooted cuttings were infected before planting, and hence that the fungus in the young plants was introduced by infected propagation material (Bertelli *et al.,* 1998; Surico *et al.,* 1998; Mugnai, unpublished data). However the presence of the fungus in a high proportion of root pieces, suggests that another way of entry for the fungus may have been through the roots.

In conclusion *Phaeoacremonium chlamydosporum* is reported for the first time in Italy as the causal agent of a decline of very young grapevines. The fact that this fungus occurs even on rooted cuttings of Cabernet Sauvignon grafted on 1103 Paulsen *(V. Berlandieri* x *V. rupestris*) and Matilde grafted on Kober 5BB (*V. Berlandieri* x *V. Riparia*) and ready for outplanting (Sidoti, unpublished data) shows that the health of vine propagation material should be controlled more closely, with greater attention being paid also to wood-attacking fungi. The Regional Phytosanitary Services can play an important role in this area.

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