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SHORT NOTES

Occurrence of *Cylindrocarpon* spp. in nursery grapevines in California

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Summary. *Cylindrocarpon liriodendri* and *C. macrodidymum* are causal agents of black foot disease of grapevines in California. Together with *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora, Cylindrocarpon* spp. are also known to be associated with decline of young vines. Infected vineyard soils are one source of the *Cylindrocarpon* infections, but nurseries have also been shown to contribute to the origin of the inoculum. To study incidence of *Cylindrocarpon* spp. in nursery grapevines in California, randomly selected, non-symptomatic grapevines from various nurseries were tested in a PCR assay for the presence of *Cylindrocarpon* spp. Results of this study suggested that *Cylindrocarpon* spp. are the most common pathogens associated with young nursery vines in California, being detected in 26% of the samples. *Phaeoacremonium aleophilum* was found in 19% and *P. chlamydospora* in 4% of the nursery samples.

Key words: *Cylindrocarpon destructans*, *Cylindrocarpon macrodidymum*, black foot disease, grapevine decline, PCR detection.

Introduction

Black foot disease, caused by *Cylindrocarpon liriodendri* MacDon. & Butler, *C. destructans* (Zinsm.) Scholten and *C. macrodidymum* Schroers, Halleen & Crous, affects grapevines throughout the main viticultural regions of the world (Halleen *et al.,* 2006a,b). Symptoms of the disease appear as necrotic root crowns, sunken root lesions, xylem necrosis and streaking (Fig 1). Infected vines often show stunted growth with shortened internodes and scarce, sometimes chlorotic foliage. Eventually, the whole vine dies. Young vines, up to 10 years old, are most susceptible to the disease. In California, the disease is fairly recent but causes significant losses to growers every year due to replanting (Petit and Gubler, 2005).

Infected vineyard soils are one source of *Cylindrocarpon* inoculum, but nurseries have also been shown to contribute to the disease incidence. Studies done in South Africa have shown that new infections are frequently established when cuttings come in contact with infected nursery soil during nursery practices such as covering of grafted cuttings with soil to prevent drying of the callused tissue and, more commonly, after planting of callused cuttings (Halleen *et al.,* 2003, 2006a).

In California, vine decline pathogens such as *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora,* are common in young nursery vines (e.g. Stamp, 2001). However, less information is availa-

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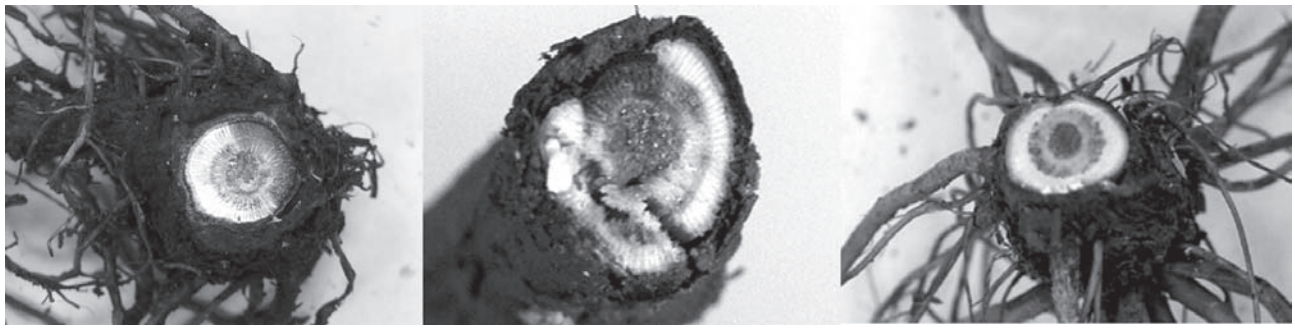

Fig. 1. Symptoms of vascular streaking and xylem necrosis are commonly observed in vines infected with *Cylindrocarpon* spp.

ble about the occurrence of *Cylindrocarpon* sp. in nursery material. The purpose of this study was to investigate the incidence of *Cylindrocarpon* spp. in young, rooted cuttings in California nurseries. Since at the time of the study no sequence information was available about *Cylindrocarpon* species in California, genus specific PCR primers were designed based on sequence information in databases to simultaneously detect *C. liriodendri* and *C. macrodidymum*.

Materials and methods

A sample of 165 non-symptomatic rooted grapevine cuttings were randomly selected from various nurseries in California to be tested by PCR for *Cylindrocarpon* spp., *Phaeoacremonium aleophilum* and *P. chlamydospora*. DNA was extracted from 0.1 to 0.2 g samples taken from the xylem of graft union and rootstock by macerating the tissue in 5 ml of extraction buffer (Osman and Rowhani, 2006). DNA from the fungal cultures was extracted using the same method. Two μl of the DNA extract was used as template in a 25 μl reaction for PCR amplification, combined with 1× PCR buffer, 1× sucrose red dye solution (2% sucrose, 0.1 mM cresol red), 0.5 μM of both forward and reverse primers, 5 mM DTT, 1.24 mM $MgCl_2$, 0.2 mM dNTPs, and 0.5 U Taq polymerase. Primers used for the detection of *P. aleophilum* and *P. chlamydospora* were published by Tegli *et al.*, (2000). Genus-specific primers for *Cylindrocarpon* sp. (Cyl-F: 5'-CCAAACCCCTGTGAACATAC-3', Cyl-R:5'-TGTGCTACTACGCAGAGGAA-3') were designed in this study for the simultaneous detection of *C. liriodendri* and *C. macrodidymum*. PCR amplification of samples included a 94°C initial denaturation step for 4 min, 35 cycles of 30 s denaturation at 94°C, 45 s primer annealing at 56°C and 1 min primer extension at 72°C, followed by 5 min of final extension at 72°C. PCR products were separated by electrophoresis in 1.5% agarose gel and 1× TBE buffer, and visualized by ethidium bromide staining under UV light.

Results and discussion

Development of PCR assay for detection of *Cylindrocarpon* spp.

Genus-specific PCR primers designed in this study to detect *Cylindrocarpon* spp. amplified a 395 bp product from both *C. liriodendri* and *C. macrodidymum* (Fig. 2). Primers were specific to *Cylindrocarpon* sp. since no amplification was observed

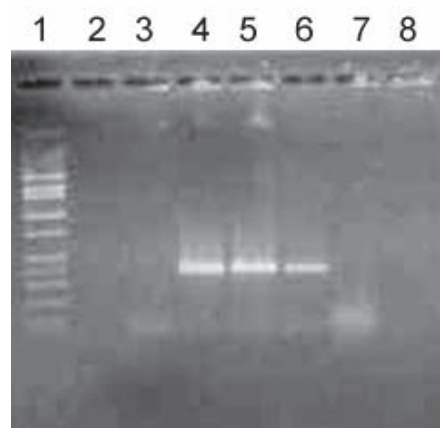

Fig. 2. PCR detection of *Cylindrocarpon* spp. using genus-specific primers. Lane 1, 100 bp DNA ladder; 2, no DNA; 3, healthy grapevine; 4, *Cylindrocarpon macrodidymum* DNA; 5, *C. liriodendri* DNA; 6, *C. macrodidymum*-infected grapevine plant; 7, *Phaeomoniella chlamydospora* DNA; 8, *Phaeoacremonium aleophilum* DNA.

when DNA from healthy grapevines, or from other fungi, such as *P. chlamydospora* or *P. aleophilum,* was used as template (Fig. 2). Sequence comparisons of California strains of *C. liriodendri* and *C. macrodidymum* also showed later on that the primer sequences are conserved between *C. liriodendri* and *C. macrodidymum* isolates (data not shown). Indeed, the genus-specific primers may prove to be more useful in detecting California strains of *C. liriodendri,* since the existing primers (Hamelin *et. al.,* 1996) may fail to detect some of the California strains due to one base-pair mismatch in the 3' end of the primer sequence. The genus-specific primers may also become useful in detecting other *Cylindrocarpon* spp. such as *C. destructans*. However, in order to validate the use of the primers for detecting *Cylindrocarpon* sp., more *Cylindrocarpon* isolates and its relative species need to be tested.

Incidence of *Cylindrocarpon* spp. in California nurseries

The results of this study showed that *Cylindrocarpon* spp. are the most common pathogenic fungi associated with young, asymptomatic nursery vines in California. *Cylindrocarpon* spp. were detected throughout the year, in an average of 26% of nursery samples. *P. aleophilum* and *P. chlamydospora* were found in 19% and 4% of the samples respectively (Table 1). *Cylindrocarpon* spp. were also common in symptomatic, older grapevines obtained from vineyards, with 50% of the samples testing positive for *Cylindrocarpon* sp.

Table 1. Percent incidence of infected vines in randomly selected, non-symptomatic grapevines in California nurseries. A total of 165 rooted grapevine cuttings from various nurseries was tested by PCR for *P. aleophilum* (Pal), *P. chlamydospora* (Pch) and *Cylindrocarpon* spp. (Cyl).

Testing period	Pal (%)	Pch (%)	Cyl (%)
January–June 2005	28	4	29
July–December 2005	6	2	17
Whole year 2005	19	4	26

Since these results were based on random samples of various genetic backgrounds and sources rather than proper sampling methods, the results can only be considered suggestive. However, the data obtained in this study clearly suggest that to avoid introducing infected vines to their vineyards, growers should test the nursery material prior to planting.

Acknowledgements

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