European Journal of Plant Pathology (2006) 115:195–202
DOI 10.1007/s10658-006-9008-5

A survey of trunk disease pathogens within rootstocks of grapevines in Spain

Angeles Aroca[1], Francesc García-Figueres[2], Lilian Bracamonte[3], Jordi Luque[4], and Rosa Raposo[1,*]
[1]*CIFOR, Instituto Nacional Investigaciones Agrarias (INIA), C. Coruña km. 7.5, 28040, Madrid, Spain;* [2]*Laboratori de Sanitat Vegetal DARP, Via Circulació Nord, Tram VI Carrer 3, Zona Franca, 08040, Barcelona, Spain;* [3]*Dept. Biotecnología, E.T.S.I. Agrónomos, C. Coruña s/n, 28040, Madrid, Spain*
[4]*Dep. Protecció Vegetal, IRTA Centre de Cabrils, Ctra de Cabrils s/n, 08348, Cabrils, Barcelona, Spain;*
**Author for Correspondence (Fax: +0034-91-347-6883; E-mail: raposo@inia.es)*

Accepted 5 April 2006

Key words: Botryosphaeria parva, Grapevine decline, Petri disease, *Phaeoacremonium parasiticum*, *Vitis vinifera*

Abstract

Grapevine trunk disease pathogens, and specifically Petri disease pathogens, can be spread by planting infected plants. Due to the increasing incidence of Petri disease and other young grapevine declines reported lately in Spain, a sampling of plants used before for new vineyards were carried out in 2002 and 2004. A total number of 208 plants (grafted and non grafted) were collected, of which 94 plants (45.2%) were infected with at least one of the following pathogens: *Phaeomoniella chlamydospora*, and species of *Phaeoacremonium*, *Botryosphaeria*, *Cylindrocarpon*, and *Phomopsis*. Species of the genera *Phaeoacremonium* and *Botryosphaeria* isolated in 2004 were identified using morphological and molecular characters. Species of *Phaeoacremonium* identified were *P. aleophilum* and *P. parasiticum*; and those of *Botryosphaeria* were *B. obtusa*, *B. dothidea* and *B. parva*. This is the first report of *P. parasiticum* and *B. parva* occurring on grapevines in Spain. Distribution of pathogens within the plants was studied in 2004. *Phaeomoniella chlamydospora* was not detected in the graft union of any plant; however, species of *Botryosphaeria* and *Phomopsis* were detected along the plant, but mainly in the graft union; *Phaeoacremonium aleophilum* was detected along the grafted plants, but not in rooted rootstocks. The results suggest that infected plants used for new plantings in Spain are an important source of primary inoculum of the pathogens associated with grapevine trunk diseases in the field.

Introduction

Several disease surveys have been carried out in Spanish vineyards in the last few years. These surveys have reported decline symptoms in young grapevines occurring throughout the main production regions (Armengol et al., 2001, 2002). Field symptoms included plants that failed to thrive, with reduced shoot growth and small chlorotic leaves. Severely diseased vines died even in the year they had been planted. In a longitudinal section of stems, frequent internal wood symptoms were dark brown to black streaking. Fungi commonly associated with these symptoms were *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. (causal agents of Petri disease), as well as several species of the genera *Botryosphaeria*, *Phomopsis*, and *Cylindrocarpon* (causal agent of black foot disease) (Armengol et al., 2001).

Young grapevine diseases associated with these pathogens, and Petri disease in particular, are being steadily reported, occurring worldwide where vines are intensively grown (Scheck et al., 1998b; Sparapano et al., 2000; Castillo-Pando et al., 2001). Some of these pathogens are also found affecting old grapevines. For example,

Botryosphaeria obtusa is the causal agent of black dead arm in France (Larignon and Dubos, 2001) and it has been associated with wood symptoms of black to brown streaking and wedge-shaped necrosis (Mugnai et al., 1999). *Botryosphaeria dothidea* is associated with Excoriosis disease in Portugal (Phillips, 2000, 2002) and *Phomopsis viticola* is the causal agent of Phomopsis cane blight and leaf spot (Pearson and Goheen, 1994a). *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp., the causal agents of Petri disease in young plants, are also involved in esca disease in older grapevines (Mugnai et al., 1999; Sparapano et al., 2000). The importance of Petri disease has been emphasized since it was speculated that these fungi act as pioneer organisms in the posterior invasion of the wood decay fungi that cause the typical symptoms of esca inside the trunk and branches (Larignon and Dubos, 1997; Mugnai et al., 1999; Sparapano et al., 2000).

Management of Petri disease and other young grapevine decline diseases relies on the use of pathogen-free plants for new plantings (Surico, 2001). New plants may become infected because of the use of mother plants that harbour fungal infections (Edwards and Pascoe, 2004; Fourie and Halleen, 2004). Infection may also take place in nurseries during the propagation process and storage as it has been reported for *P. chlamydospora* at all stages of the propagation process (Surico, 2001; Whiteman et al., 2003). *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. have been detected in both symptomatic and asymptomatic cuttings (Bertelli et al., 1998).

Intensive studies on the fungi associated with decline symptoms in young grapevines have been carried out in the last few years with regard to species identity. Characterisation of isolated fungi have brought the description of an important number of novel species or the reassignment of former species to novel genus. Thus, the genus *Phaeoacremonium* was proposed for five new species, and for a former species included in the genus *Phialophora* (Crous et al., 1996); in following years, *P. chlamydospora* was redisposed in the *Phaeomoniella* genus (Crous and Gams, 2000), and two other new species were defined (Dupont et al., 2000; Groenewald et al., 2001). There are now seven *Phaeoacremonium* species identified on the basis of morphological characters and DNA phylogeny (*P. aleophilum*, *P. angustius*, *P. inflatipes*, *P. mortoniae*, *P. parasiticum*, *P. rubrigenum*, *P. viticola*) that have been shown to be pathogenic to young grape plants and causing brown wood streaking symptoms. In the same way, *Cylindrocarpon destructans* and *C. obtusisporum* were considered the unique agents of black foot disease of grapevines (Maluta and Larignon, 1991; Scheck et al., 1998a; Rego et al., 2000). However, this genus has been recently reviewed (Halleen et al., 2004) and some new *Cylindrocarpon* species have been defined on *Vitis*, as well as a novel genus named *Campylocarpon*.

Other genera involved in grapevine trunk diseases have been reviewed. Species of *Botryosphaeria* were identified based on molecular and morphological characters (Phillips, 2002; Niekerk et al., 2004; Slippers et al., 2004) , resulting in the definition of new species (Phillips, 2002). Some former species that represented a species complex have been differentiated into several species (Slippers et al., 2004). Nowadays, there are 11 species of *Botryosphaeria* associated with grapevines (Niekerk et al., 2004) which cause a wide range of symptoms including dieback, brown wood streaking and bunch rot. *Phomopsis viticola* is the causal agent of Phomopsis cane blight, and it is the most frequent species of this genus reported from grapevines. However, there are 10 more species reported (Uecker and Ker-Chung, 1992; Merrin et al., 1995; Ker-Chung and Lii-Sin, 1998), of which *P. viticola* and *P. vitimegaspora* have been confirmed as pathogens of grapevines (Niekerk et al., 2005).

Results obtained from vineyard surveys in Spain suggested that plants may have been infected in the nursery. Many pathogens were detected in scattered plants in new vineyards, and in a few of them, plants died in the first year after planting. The objective of this work was to determine the sanitary conditions of vine plants to be used in new plantings. Specifically, the objective was to determine if these plants were infected with any trunk disease pathogen. Sampling of both grafted plants and rooted rootstocks have been done because some growers prefer to plant rooted rootstocks and perform field grafting by themselves. Detection of fungi was done using traditional techniques which involved isolation and growth of fungi on culture media. Identification of fungal taxa was based on morphological and molecular characters. A preliminary report of this study was presented at

the 4th International Workshop on Grapevine Trunk Diseases (Stellenbosch, South Africa, January 2005).

Materials and methods

Sampling and morphological identification of pathogens

A first survey was done in north-eastern Spain in March and April 2002. Seventy-three grapevine plants (56 grafted plants and 17 rooted rootstocks) ready to be planted were collected from nurseries, distributors and growers. One plant was sampled for each rootstock-scion combination originating from different sources. Plants were taken to the laboratory, and 5 cm-long fragments were cut at different parts of each plant (bottom, medium, graft union and shoot). Pieces were washed with tap water, dipped in 96% ethanol and flamed. Once surface-sterilized, pieces were cut longitudinally, and internal wood symptoms were observed. From each piece, five wood chips with black and/or brown streaking were pulled with forceps and placed in Petri dishes with 2% malt extract agar medium (MEA; Conda laboratories, Torrejón de Ardoz, Madrid, Spain) amended with streptomycin sulphate (100 mg l^{-1}, Sigma, Steinheim, Germany).

A second survey was done in central Spain in February and March 2004. This time only agricultural cooperatives were surveyed, which stored the grapevine plants to distribute them to growers for use in new plantings. Plants from nurseries are packed in batches of 50 plants of either a specific combination of rootstock and scion or a cultivar of rooted rootstocks. A sample of 3–5 plants was randomly chosen from each batch. A total number of 135 grapevine plants (110 grafted plants and 25 rooted rootstocks) were collected from 34 different packs of either a rootstock-scion combination or a cultivar of rooted rootstock, that were produced in 13 different nurseries. If plants came from the same nursery, then a different rootstock-scion combination was sampled. Once in the laboratory, 5 cm long pieces at the basal end, internodes (two maximum), 2 cm below graft, and graft union of each plant were obtained. Scion was not analyzed in any plant. Bark of each piece was removed with a scalpel and six thin cross sections (1–2 mm thick) were cut. These disks were immersed in 70% ethanol for 1 min and air-dried under sterile conditions. Disks were plated in streptomycin-amended MEA (3 disks per plate, 6 disks per piece).

All plates were incubated at 25 °C in darkness, and observed daily for mycelial growth. Isolates were transferred to potato dextrose agar (PDA, Conda laboratories) and incubated at 25 °C under black light with 16/8 h light/dark photoperiod. Fungi were morphologically identified after microscopic examination of fruiting structures and conidia (Pearson and Goheen, 1994b; Crous et al., 1996; Crous and Gams, 2000; Dupont et al., 2000; Dubos, 2002; Niekerk et al., 2004). For identification of *Botryosphaeria* species, isolates were grown on 2% water agar (WA; Conda laboratories) with sterilized pine needles at 25 °C under the same light conditions as above to induce sporulation (Slippers et al., 2004). Specifically, identification of *B. lutea* was done on the basis of yellow pigment production on cultures on PDA after 3 days of incubation at 25 °C in darkness (Slippers et al., 2004).

DNA sequencing

Species identification of all isolates identified as *Phaeoacremonium* and of some of *Botryosphaeria* were confirmed by analysis of the sequences of the nuclear 5.8S rDNA and its flanking ITS1 and ITS2 regions. For these isolates, DNA was extracted from freeze-dried mycelia by means of DNeasy Plant Mini kit (Qiagen, GmbH, Germany). The ITS region of DNA was amplified using fungal universal primers ITS1F and ITS4 (Gardes and Bruns, 1993). The PCR reactions were performed in 25 μl volume containing: 1 μl fungal genomic template, 0.2 μM of each primer ITS1F and ITS4 (Sigma-Genosys, Pampisford, United Kingdom), 2 mM $MgCl_2$, 0.2 mM dNTP mix, 2 μl 10× Biotools buffer and 0.75 U of *Taq* polymerase (Biotools, Madrid, Spain). The PCR was performed using the following parameters: an initial denaturation step at 94 °C for 2.5 min, followed by 35 cycles of 15 s denaturation at 94 °C, 30 s annealing at 53 °C and 90 s elongation at 72 °C. The amplification was terminated by an incubation for 7 min at 72 °C. PCR products were analyzed by electrophoresis on 1% agarose gels in TAE buffer and visualized by staining with ethidium bromide. The amplified product was purified using a Mon-

tage PCR kit (Millipore, Bedford, MA, USA) following the manufacturer's protocol.

DNA regions were sequenced using an ABI PRISM Dye Terminator Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). All samples were sequenced in both directions using the same primers as in the PCR amplifications. Nucleotide sequence data of the ITS1, ITS2 regions, and the 5.8S rDNA gene were compared with the FASTA programme using the EMBL nucleotide sequence database (http://www.ebi.ac.uk/embl/).

Data analysis

Frequency of infected plants was calculated for each pathogen. Distribution of each pathogen within the plant was analyzed by calculating the percentage of fragments in which the pathogen was detected. Correspondence analysis on the data from each plant fragment sampled in 2004 was performed with BMDP software release 7.1. The following variables were considered in the analysis: location of plant fragment (basal, medium, below graft and graft union), nursery from which plant was produced (13 sampled), rootstock cultivar (110 R, 140 Ru, 161–49, SO_4 and 41 B), internal wood symptoms (black and/or brown vascular discolouration, brown parenchymatic tissue in total/sector cross-section, presence of wounds, internal necrosis and no symptoms), wood pathogens detected (*B. parva*, *B. obtusa*, *B. dothidea*, *P. aleophilum*, *P. parasiticum*, *P. chlamydospora*, *Cylindrocarpon* spp. *Phomopsis* spp.), and number of disks from which a specific pathogen was isolated (represented on a 1–3 numeric scale with 1 = 1–2 disks out of 6; 2 = 3–4 disks; 3 = 5–6 disks).

Results

Total number of plants sampled in 2002 and 2004 was 208, out of which 166 plants (79.8%) were grafted grapevines and 42 plants (20.2%) were rooted rootstocks (Table 1). The percentage of grafted plants that were infected with at least one grapevine trunk pathogen was 48.8% and that of infected rooted rootstocks was 31%. Percentage of plants that were infected in each sampling was 34.2% (25 plants) in 2002, and 53.3% (72 plants) in 2004 (Table 1). Plants were infected with at least one of the following pathogens: *P. chlamydospora*, and species of *Phaeoacremonium*, *Botryosphaeria*, *Cylindrocarpon*, and *Phomopsis*. In 68 plants out of the total number of infected plants (97 plants) only one pathogen was detected, while in 22, 6, and 1 plants, respectively, two, three and four pathogens were present. The three fungi most frequently isolated from rootstocks in both samplings were *P. aleophilum*, *P. chlamydospora* and *B. parva* (Table 2). *Phaeoacremonium parasiticum* was isolated from only one plant in 2004 in a fragment just below the graft union; this is the first report of the occurrence of *P. parasiticum* in Spain. Identification of this species and all other *Phaeoacremonium* species isolated in 2004 were corroborated by analysis of the sequence of the ITS region in the nuclear ribosomal DNA.

Table 1. Proportion of infected grapevine plants in grafted and non-grafted rootstocks sampled before planting in Spain in 2002 and 2004

	Sampled plants (No.)	Infected plants (%)
Grafted	166	48.8
Non-grafted	42	31.0
Total	208	45.2
Total 2002	73	34.2
Total 2004	135	53.3

Botryosphaeria species detected in vine plants were *B. obtusa* (12 and 14% of infected plants in 2002 and 2004, respectively), *B. parva* (23.6% of infected plants in 2004) and *B. dothidea* (8.3% of infected plants in 2004) (Table 2). No attempt was made to distinguish between *B. dothidea* and *B. parva* in 2002 analysis, so they were grouped as *Botryosphaeria* spp. (Table 2), but differentiated from *B. obtusa*. In 2004, *B. dothidea* and *B. parva* were identified on the basis of their conidial morphology and ITS sequence data, according to Pennycook and Samuels (1985) and Slippers et al. (2004).

Frequency of pathogens detected in plants sampled in 2004 was further analyzed. Distribution of pathogens along the plants was studied by the fragment from which the pathogen was detected in basal, internodes one and two, below graft and graft (Figure 1). *Phaeomoniella chlamidospora* was detected most in the basal end (74%) and internode 1 (25%) of the plant, and not detected at all in the

Table 2. Frequency of grapevine trunk disease pathogens in the total infected plants sampled before planting in Spain in 2002 and 2004

	2002				2004			
	No. plants	%	Grafted	Non-grafted	No. plants	%	Grafted	Non-grafted
Phaeomoniella chlamydospora	7	28.0	6	1	27	37.5	24	3
Phaeoacremonium aleophilum	9	36.0	9	0	21	29.2	21	0
Phaeoacremonium parasiticum	nd[a]	nd	nd	nd	1	1.4	1	0
Botryosphaeria spp.	4	16.0	3	1				
Botryosphaeria parva	nd[a]	nd	nd	nd	17	23.6	17	0
Botryosphaeria obtusa	3	12.0	2	1	10	13.9	9	1
Botryosphaeria dothidea	nd	nd	nd	nd	6	8.3	6	0
Phomopsis spp.	3	12.0	2	1	12	16.7	11	1
Cylindrocarpon spp.	4	16.0	4	0	11	15.3	7	4

[a]nd: Not determined.

graft union. *Phaeoacremonium aleophilum* was detected below the graft union (63.6%) and in the graft union (36.4%), and it was found alike in the basal end and in internode 1 (13.6%). *Cylindrocarpon* spp. was detected in the lower part of the rootstock (72.7% in the basal end and 9% in the internode 1) and also in the upper part of the plants (18 and 9% in the graft union and below graft, respectively). *Botryosphaeria dothidea*, *B. obtusa* and *Phomopsis* spp. were most frequently isolated from the graft union, and *B. parva* was found in all fragments of the rootstocks and graft union. *Phomopsis* spp. was not detected in any fragment analyzed at the basal end of the rootstock, whereas *Phaeoacremonium* spp., *Cylindrocarpon* spp., *B. dothidea*, and *B. obtusa* were not detected in internode 2.

Frequency of infected and healthy plants was compared in grafted and non-grafted plants (Table 1). A χ^2 test of independent groups showed no differences in the infection rate between grafted and non-grafted plants (χ^2 value = 3.643; degrees of freedom = 1; $p > 0.05$). However, it is striking that *P. aleophilum* was not detected in rooted rootstocks in samples of either year (Table 2).

Symptoms observed in the internal wood of plant fragments were not grapevine trunk pathogen-specific. Results of the correspondence analysis showed no association among any of the studied variables (data not shown). That is, none

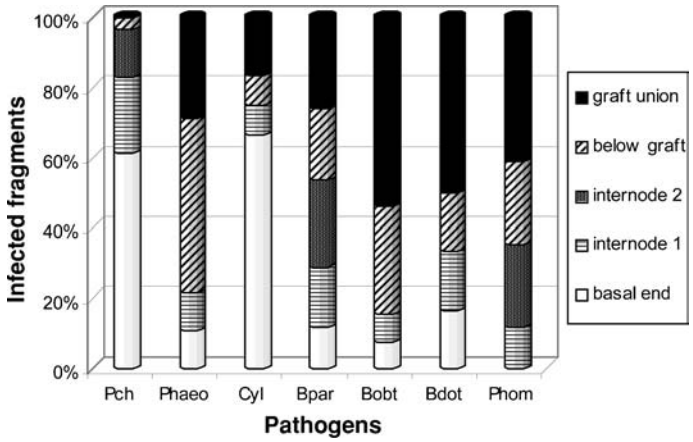

Figure 1. Distribution of each pathogen within the infected plants sampled in 2004. Values are given as the percentage of fragments where the pathogen was detected (a pathogen may be detected in more than one fragment within the plant). Pch: *Phaeomoniella chlamydospora*; Phaeo: *Phaeoacremonium* spp.; Cyl: *Cylindrocarpon* spp.; Bpar: *Botryosphaeria parva*; Bobt: *B. obtusa*; Bdot: *B. dothidea*; Phom: *Phomopsis* spp.

of the described symptoms was correlated with any pathogen or fragment from which the pathogen was isolated. Nursery or rootstock cultivars were not associated with any particular pathogen. Most importantly, *P. aleophilum* and *Phomopsis* spp. were detected respectively in two and one fragments below the graft union, with no internal wood symptoms.

Discussion

Results presented here show that vine plants used for new plantings in Spain were infected with trunk disease pathogens, and specifically with fungi causing Petri disease. Grapevine trunk diseases are described as a general decline of young plants, stunted growth, chlorosis of leaves and brown wood-streaking (Mugnai et al., 1999). Plants with these symptoms have been previously described in field surveys carried out in Spanish new vineyards (Armengol et al., 2001, 2002). Our results indicate that the poor sanitary condition of the plants used for new vineyards may contribute to the incidence of grapevine trunk diseases reported in the last few years in Spain (Armengol et al., 2001).

Fungal pathogens detected in the sampled vine plants were *P. chlamydospora*, several species of *Phaeoacremonium* and species of *Botryosphaeria*, *Phomopsis* and *Cylindrocarpon*. Species of *Phaeoacremonium* isolated here were *P. aleophilum* and *P. parasiticum*. This is the first report of *P. parasiticum* occurring on vines in Spain, and was only detected in one plant out of 135 sampled in 2004. *Phaeoacremonium parasiticum* may have occurred in Spanish vines before, but the fact that *Phaeoacremonium* isolates were identified only on a morphological basis may have hampered the identification of species less frequent than *P. aleophilum*. In this work, nucleotide sequences of the ITS region for all *Phaeoacremonium* isolates were used in the identification to the species level. *Phaeoacremonium aleophilum* and *P. parasiticum* can be accurately identified from their sequence of the ITS region (Mostert et al., 2005); therefore, we concluded that only these two species occurred in the sampled plants. Other studies have also detected *Phaeoacremonium* species other than *P. aleophilum* (Auger et al., 2005; Eskalen et al., 2005; Overton et al., 2005). Even so, *P. chlamydospora* and *P. aleophilum* are the most frequently isolated species from young vines showing decline (Mugnai et al., 1999) as was found in this work.

Three species of *Botryosphaeria* were isolated in this study, namely *B. obtusa*, *B. parva*, and *B. dothidea*. *Botryosphaeria obtusa* and *B. dothidea* were known to occur in grapevines in Spain (Armengol et al., 2001), but not *B. parva*. A survey in Spanish grapevines during 1999 to 2001 showed only *B. obtusa* and *B. dothidea*, in 61 and 6.4% of the sampled vineyards, respectively (Armengol et al., 2001). However, *B. dothidea* identified by Armengol et al. (2001) may have included any of the species in which the *B. dothidea* complex was later differentiated (*B. ribis*, *B. parva*, *B. lutea* and *B. dothidea*) (Phillips, 2002; Niekerk et al., 2004; Slippers et al., 2004). *Botryosphaeria obtusa* and *B. dothidea* complexes are generally associated with black dead arm in France (Larignon and Dubos, 2001) and decline of older grapevines, but they have also been reported to occur in nurseries in South Africa, detected in apparently healthy mother plants (Halleen et al., 2003; Fourie and Halleen, 2004).

The percentage of infected plants was lower in 2002 than in 2004 (25% of 73 sampled plants and 69% of 135, respectively) (Table 1). There are two possible reasons for this. One is that nurseries where sampled plants had been produced were different in both years. In 2002, nurseries were located in north-eastern Spain while in 2004 they were located in eastern Spain. The other reason is that the method for isolating fungi in the laboratory was slightly modified in 2004: thin disks of the whole cross-section (either with symptoms or symptomless) instead of chips (from brown streaking wood) taken from longitudinal sections were placed on the growth media.

In summary, results presented here demonstrate that plants used for new plantings are infected with pathogens associated with grapevine trunk diseases. The sanitary condition of these plants may contribute to the high incidence of decline of young plants observed in recent years and threaten the sustainability of these vineyards in the long term. Further studies are required to investigate the sources of infection during the propagation process in nurseries and to confirm that mother plants with no infection symptoms are in fact healthy plants. Cultural and molecular techniques

are needed to speed up the detection process as well as the identification of pathogens involved.

Acknowledgements

The authors thank A. Bernao and Viticulture Cooperatives for help in sampling plants. This research was supported by Project RTA03-058-C2 (Programa Nacional de Recursos y Tecnologías Agrarias, Ministerio de Educación y Ciencia, Spain). Angeles Aroca was supported by a grant from INIA.

References

Armengol J, Vicent A, Torné L, García-Figueres F and García-Jiménez J (2001) Fungi associated with esca and grapevine declines in Spain: A three-year survey. Phytopathologia Mediterranea 40: 325–329.

Armengol J, Vicent A and García-Jiménez J (2002) El decaimiento y muerte de vides jóvenes (Enfermedad de Petri) en España. Phytoma España 138: 91–93.

Auger J, Pérez I, Esterio M, Navia V, Gubler WD and Eskalen A (2005) Fungi associated with grapevine wood decay and young vine decline in Chile. In: 4th International Workshop on grapevine trunk disease (pp. 25). Stellenbosch, South Africa.

Bertelli E, Mugnai L and Surico G (1998) Presence of *Phaeoacremonium chlamydosporum* in apparently healthy rooted grapevine cuttings. Phytopathotogia Mediterranea 37: 79–82.

Castillo-Pando M, Somers A, Green CD, Priest M and Sriskathades M (2001) Fungi associated with dieback of Semillon grapevines in the Hunter Valley of New South Wales. Australasian Plant Pathology 30: 59–63.

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

Crous PW, Gams W, Wingfield MJ and VanWyk PS (1996) *Phaeoacremonium* gen. nov. associated with wilt and decline diseases of woody host and human infections. Mycologia 88: 786–796.

Dubos B (2002) Maladies cryptogamiques de la vigne, Éditions Féret, Bourdeaux, Francia.

Dupont J, Laloui W, Magnin S, Larignon P and Roquebert MF (2000) *Phaeoacremonium viticola*, a new species associated with Esca disease of grapevine in France. Mycologia 92(3): 499–504.

Edwards J and Pascoe IG (2004) Occurrence of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* associated with Petri disease and esca in Australian grapevines. Australasian Plant Pathology 33: 273–279.

Eskalen A, Tooney-Latham S and Gubler WD (2005) Occurrence of *Togninia fraxinopennsylvanica* perithecia and *Phaeoacremonium* species in California vineyards. In: 4th International Workshop on Grapevine Disease (pp.13). Stellenbosch, South Africa.

Fourie PH and Halleen F (2004) Occurrence of grapevine trunk disease pathogens in rootstock mother plants in South Africa. Australasian Plant Pathology 33: 313–315.

Gardes M and Bruns TD (1993) ITS primers with enhanced specificity for Basidiomycetes: Application to the identification of mycorrhizae and rusts. Molecular Ecology 2(2): 113–118.

Groenewald M, Kang J-C and Crous PW (2001) ITS and β tubulin phylogeny of *Phaeoacremonium* and *Phaeomoniella* species. Mycological Research 105(6): 651–657.

Halleen F, Crous PW and Petrini O (2003) Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. Australasian Plant Pathology 32: 47–52.

Halleen F, Schroers H-J, Groenewald JZ and Crous PW (2004) Novel species of *Cylindrocarpon* (*Neonectria*) and *Campylocarpon* gen. nov. associated with black foot disease of grapevines (*Vitis* spp.). Studies in Mycology 50: 431–455.

Ker-Chung K and Lii-Sin L (1998) *Phomopsis vitimegaspora*: A new pathogenic *Phomopsis* from vines. Mycotaxon 65: 497–499.

Larignon P and Dubos B (1997) Fungi associated with esca disease in grapevine. European Journal of Plant Pathology 103: 147–157.

Larignon P and Dubos B (2001) Le Black Dead Arm: Maladie nouvelle à ne pas confondre avec lEsca. Phytoma 5: 30–31.

Maluta D and Larignon P (1991) Pied-noir: Mieux vaut prevenir. Viticulture 11: 71–72.

Merrin S, Nair NG and Tarran J (1995) Variation in *Phomopsis* recorded on grapevine in Australia and its taxonomic and biological implications. Australasian Plant Pathology 24: 44–56.

Mostert L, Groenewald JZ, Gams W, Summerbell RC, Robert V and Crous PW (2005) Delimitation of new species in *Pheoacremonium* and the development of an identification system. In: 4th International Workshop on Grapevine Trunk Diseases (IWGTD) (pp. 12). Stellenbosh, Stellenbosch, South Africa.

Mugnai L, Graniti A and Surico G (1999) Esca (Black Measles) and brown wood-streaking: Two old and elusive diseases of grapevines. Plant Disease 83: 404–418.

Niekerk van J, Crous PW, Groenewald JZ, Fourie PH and Halleen F (2004) DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. Mycologia 96(4): 781–798.

Niekerk van J, Groenewald JZ, Farr DF, Fourie PH, Halleen F and Crous PW (2005) *Phomopsis* spp. on grapevines: Characterisation and pathogenicity. In: 4th International Workshop on Grapevine Trunk Diseases (IWGTD) (pp. 30). Stellenbosh, Stellenbosch, South Africa.

Overton B, Stewart EL and Wenner NG (2005) Molecular phylogenetics of grapevine decline fungi from Pennsylvania and New York. In: 4th International Workshop on Grapevine Trunk Disease (pp. 27). Stellenbosch, South Africa.

Pearson RC and Goheen AC (1994a) Phomopsis cane blight and leaf spot. In: Pearson RC and Goheen AC (eds.) Compendium of Grape Diseases (pp. 17–18) APS Press, St. Paul (MN), USA.

Pearson RC and Goheen AC (1994b) Compendium of Grape Diseases, APS Press, St. Paul (MN), USA.

Pennycook S and Samuels GJ (1985) *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (kiwifruit) in New Zealand. Mycotaxon 24: 445–458.

Phillips AJL (2000) Excoriose, cane blight and related diseases of grapevines: A taxonomic review of the pathogens. Phytopathologia Mediterranea 39(3): 341–346.

Phillips AJL (2002) *Botryosphaeria* species associated with diseases of grapevines in Portugal. Phytopathologia Mediterranea 41: 3–18.

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

Scheck H, Vasquez SJ and Gubler WD (1998a) First report of Black-Foot Disease, caused by *Cylindrocarpon obtusisporum*, of grapevine in California. Plant Disease 82: 448.

Scheck H, Vasquez SJ, Fogle D and Gubler WD (1998b) Grape growers report losses to black-foot and grapevine decline. California Agriculture 52(4): 19–23.

Slippers B, Crous PW, Benman S, Coutinho TA, Wingfield BD and Wingfield MJ (2004) Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. Mycologia 96(1): 83–101.

Sparapano L, Bruno G, Ciccarone C and Graniti A (2000) Infection of grapevines by some fungi associated with esca. II. Interaction among *Phaeoacremonium chlamydosporum*, *Phaeomoniella aleophilum* and *Fomitiporia punctata*. Phytopathologia Mediterranea 39: 125–133.

Surico G (2001) Towards commonly agreed answers to some basic questions on esca. Phytopathologia Mediterranea 40: S487–S490.

Uecker F and Ker-Chung K (1992) A new *Phomopsis* with long paraphyses. Mycotaxon 64: 425–433.

Whiteman S, Jaspers MV, Stewart A and Ridgway HJ (2003) Identification of potenctial sources of *Phaeomoniella chlamydospora* in the grapevine propagation process. Phytophathologia Mediterranea 43: 152–153.