## Black-foot disease of grapevine: An update on taxonomy, epidemiology and management strategies

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**REVIEW** 

# Black-foot disease of grapevine: an update on taxonomy, epidemiology and management strategies

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**Summary.** Black-foot is one of the most destructive grapevine trunk diseases in nurseries and young vineyards, causing necrotic root lesions, wood necrosis of the rootstock base, and a gradual decline and death of grapevines. Causal agents of the disease are included into the genera *Campylocarpon*, "*Cylindrocarpon*", *Cylindrocladiella* and *Ilyonectria*. Recent taxonomical studies of *Neonectria* and related genera with "*Cylindrocarpon*"-like anamorphs based on morphological and phylogenetic studies, divided *Neonectria* into five genera. Thus, the current taxonomical position and classification of the causal agents of black-foot disease, mainly "*Cylindrocarpon*" / *Ilyonectria*, comprises one of the main topics of this review. The review also provides an update on geographical distribution, epidemiology and management strategies of the disease.

Key words: Campylocarpon, "Cylindrocarpon", Cylindrocladiella, Ilyonectria, Vitis vinifera.

### Introduction

Black-foot disease of grapevines is a serious disease in most wine and grape-producing regions of the world, particularly in nurseries and young vineyards (Halleen *et al.*, 2006a). The causal agents are included into the genera *Campylocarpon*, "*Cylindrocarpon*", *Cylindrocladiella* and *Ilyonectria* (Crous *et al.*, 1993; Halleen *et al.* 2004; Halleen *et al.*, 2006b; Schroers *et al.*, 2008; Chaverri *et al.*, 2011; Cabral *et al.*, 2012a, c; Lombard *et al.*, 2012). This disease was first described in 1961 (Grasso and Magnano Di San Lio, 1975), and over the last decade, its incidence has increased significantly in most grapevine production areas of the world (Halleen *et al.*, 2006a; Alaniz *et al.*, 2007).

Although these pathogens usually manifest on mature grapevines, they have also been frequently isolated from symptomatic or asymptomatic root-

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stock mother-plants, rooted rootstock cuttings, bench-graft and young grafted vines in different grapevine production areas around the world, being considered the most common pathogenic fungi associated with young nursery vines (Rumbos and Rumbou, 2001; Halleen *et al.*, 2003; Fourie and Halleen, 2004; Oliveira *et al.*, 2004; Aroca *et al.*, 2006; Dubrovsky and Fabritius, 2007; Halleen *et al.*, 2007). Moreover, it is well known that these pathogens are common in the soil causing infection of grafted vines after some months of growth in nursery soils (Halleen *et al.* 2003, 2007; Chaverri *et al.*, 2011).

Characteristic symptoms of black-foot disease include a reduction in root biomass and root hairs with sunken and necrotic root lesions (Rego *et al.*, 2000; Halleen *et al.*, 2006a; Alaniz *et al.*, 2007, 2009; Abreo *et al.*, 2010). In some cases the rootstock diameter of older vines is thinner below the second tier. To compensate for the loss of functional roots, a second crown of horizontally growing roots is sometimes formed close to the soil surface. Removal of rootstock bark reveals black discoloration and necrosis of wood tissue which develops from the base of the rootstock (Figures 1A, 1B). The pith is also compacted and dis-



**Figure 1.** A, black discoloration and necrosis of wood tissue which develops from the base of the rootstock, characteristic of black-foot disease; B, longitudinal section of a rootstock showing dark-brown to black discoloration; C, Un-sprouted grapevine propagation material in a grapevine nursery; D, grapevine plants showing stunded growth, reduced vigour and retarded sprouting in a young plantation.

colored (Scheck *et al.*, 1998b; Larignon, 1999; Fourie and Halleen, 2001; Halleen *et al.*, 2006a).

External symptoms show reduced vigour with small-sized trunks, shortened internodes, uneven wood maturity, sparse foliage, and small leaves with interveinal chlorosis and necrosis (Figures 1C, 1D). Field symptoms of black-foot disease affected vines are frequently indistinguishable from those of caused by Petri disease (Scheck *et al.*, 1998b; Rego *et al.*, 2000; Halleen *et al.*, 2006a; Alaniz *et al.*, 2007, 2009; Abreo *et al.*, 2010). When young vines are infected, death occurs quickly, nevertheless as the vine ages, infection results in a more gradual decline and death might only occur after a year (Gubler *et al.*, 2004). Disease symptoms on mature vines (5 years and older) are

noticed early in the growing season. Affected vines achieve poor new growth, fail to form shoots after winter dormancy, and die by mid-summer. Often shoots also dry and die during the summer. Vines with reduced vegetative growth also die during the subsequent dormant winter period (Halleen *et al.*, 2006a).

## **Causal agents**

## Taxonomy and distribution

The common name black-foot disease was proposed by Scheck *et al.* (1998b), to designate the disease caused by "Cylindrocarpon" destructans (Zinnsm.)

Scholten and "C." obtusisporum (Cooke & Harkn.) Wollenw., which were the two species traditionally reported as the causal agents of basal rot or root necrosis on grapevines. Nevertheless, this disease was already named as "pied noir" in French language since 1969, because of the presence of black necrosis on the base of diseased rootstocks (Badour, 1969).

The first report of "C." destructans on grapevine was made in France in 1961 (Maluta and Larignon, 1991). Since then, it has been isolated from diseased vines in Italy (Grasso, 1984), Portugal (Rego, 1994), California (Scheck et al., 1998b), Argentina (Gatica et al., 2001), Germany (Fischer and Kassemeyer, 2003), Pennsylvania (Gugino and Travis, 2003), New Zealand and South Africa (Halleen et al., 2004), Brazil (Garrido et al., 2004) and Canada (Petit et al., 2011). "Cylindrocarpon" obtusisporum, has also been reported to produce black-foot symptoms on grapevine in Sicily (Grasso and Magnano di San Lio, 1975) and California (Scheck et al., 1998a).

The generic name "Cylindrocarpon" was introduced in 1913 by Wollenweber for anamorphs belonging to Nectria section Willkommiotes Wollenw. This section included species without chlamydospores. Few years later, in 1917, Wollenweber expanded the concept of "Cylindrocarpon" to include species forming mycelial chlamydospores in culture, being "C." destructans the most important member of this group (Brayford, 1993). In 1966, Booth split the genus into four groups based on the presence or absence of microconidia and chlamydospores: (i) "Cylindrocarpon" magnusianum (Sacc.) Wollenw., which was the anamorph of the type species of Neonectria, (ii) "C." cylindroides Wollenw., which was the type species of the genus "Cylindrocarpon", (iii) "C." destructans, which was the anamorph of Neonectria radicicola, and (iv) members of "Cylindrocarpon" species predominantly connected with teleomorphs of the 'Nectria' mammoidea group (Brayford, 1993; Halleen et al., 2006a). "Cylindrocarpon" obtusisporum was originally described from the USA (California) as occurring on Acacia sp., where it was observed to form macroconidia and chlamydospores (Booth, 1966). "Cylindrocarpon" obtusisporum strains identified by Booth (1966) originated from a broad range of host plants in Europe, New Zealand, North America, and, at least partly, formed microconidia.

Traditionally, representatives of all 'Nectria' groups with "Cylindrocarpon" anamorphs were transferred into Neonectria (Rossman et al., 1999;

Mantiri et al., 2001; Brayford et al., 2004). Mantiri et al. (2001) and Brayford et al. (2004) analyzed mitochondrial small subunit (SSU) ribosomal DNA (rDNA) sequence data of some of the species and concluded that the Neonectria / "Cylindrocarpon" species grouped together by this reclassification were monophyletic. However, these authors also found that this overall Neonectria/"Cylindrocarpon" clade included distinct subclades corresponding to at least three of the four groups delineated by Booth (1966). Significant molecular variation among taxa with "Cylindrocarpon"like anamorphs was found by Seifert et al. (2003) in a study on fungi causing root rot of ginseng (Panax quinquefolius L.) and other hosts. The dendrograms in this study, based on partial β-tubulin gene (TUB), and nuclear ribosomal internal transcribed spacer (ITS) region sequences, suggested that subclades including (i) Neon. radicicola, which consisted of numerous phylogenetically distinct units, (ii) Neon. macroconidialis (Samuels & Brayford) Seifert, and (iii) a subclade comprising two distinct isolates, one from V. vinifera in Ontario, Canada and the other from Picea sp. in Quebec, Canada, were monophyletic. Other "Cylindrocarpon" species appeared to be excluded from this monophyletic group (Halleen et al., 2006a).

Significant variation in cultural and morphological characters was observed among "Cylindrocarpon" strain isolates from grapevines in nurseries and vineyards of South Africa, New Zealand, Australia and France, which were morphologically and phylogenetically characterized by Halleen et al., (2004). Thus, these authors described a novel species, "C." macrodidymum Schroers, Halleen & Crous, also associated with black-foot disease of grapevines. Since then, this species has been reported in California (Petit and Gubler, 2005), Portugal (Rego et al., 2005), Chile (Auger et al., 2007), Spain (Alaniz et al., 2007), Uruguay (Abreo et al., 2010), northeastern United States and southeastern Canada (Petit et al., 2011) and Turkey (Özben et al., 2012).

"Cylindrocarpon" obtusisporum and "C." macrodidymum had been considered as two different species associated with black-foot disease of grapevines. Nevertheless, Halleen et al. (2004) suggested the possibility that Grasso and Magnano di San Lio (1975) and Scheck et al. (1998a) misidentified "C." obtusisporum and that it was in fact "C." macrodidymum. In this sense, Halleen et al. (2004) indicated that macroconidia of "C." macrodidymum measure [(26–)34–36–38(–45)×(4–)5.5–6–6.5(–8) µm], whereas those of

the type of "C." obtusisporum measure (30-35×4-5 um) (Cooke, 1884). However, the shape of the macroconidia distinguishes "C." macrodidymum from the type of "C." obtusisporum, which Cooke (1884) described as having conidia with obtuse ends. Booth (1966) described macroconidia of similar shape in "C." obtusisporum. According to Booth, however, 2–3-septate macroconidia of "C." obtusisporum measure (34–50×6–7.5 μm). "Cylindrocarpon" obtusisporum isolates obtained from California formed perithecia when cross-inoculated with "C." macrodidymum, giving further evidence to support the misidentification theory. This was also confirmed by sequence comparisons (Halleen et al., 2006a). In 2005, Petit and Gubler confirmed the presence of "C." macrodidymum in the USA, and concluded that black-foot disease in California is caused by "C." macrodidymum and "C." destructans (Petit and Gubler, 2005).

Moreover, Halleen et al., (2004) established a new genus, Campylocarpon Halleen, Schroers & Crous, which is "Cylindrocarpon"-like in morphology, associated with black-foot disease of grapevines. Species of this genus and members of the former "Nectria" mammoidea group, are excluded from Neonectria/"Cylindrocarpon", because phylogenetic analyses revealed that these species are phylogenetically not closely related to Neonectria/"Cylindrocarpon" genera (Halleen et al., 2004; Schroers et al., 2008). From this genus, two species were included as the causal agents of black-foot disease: Campylocarpon fasciculare Schroers, Halleen & Crous, which has been reported in South Africa (Halleen et al., 2004), Brazil (Correia et al., 2012), and Spain (Alaniz et al., 2011b) and Campyl. pseudofasciculare Halleen, Schroers & Crous, which has been reported in South Africa (Halleen et al., 2004), Uruguay (Abreo et al., 2010), Brazil (Correia et al., 2012) and Perú (Álvarez et al., 2012).

As highlighted before, "C." destructans was originally identified as the causal agent of black-foot disease (Maluta and Larignon, 1991), but the status of "C." destructans as the causal agent of the disease was since then questioned. In fact, Halleen et al. (2006b), compared "C." destructans strains isolated from diseased grapevines in France, New Zealand, Portugal and South Africa with "C." destructans-like anamorphs obtained from various herbaceous or woody hosts. DNA analyses of their ITS and TUB showed that these isolates were genetically identical with "C." liriodendri J.D. MacDon. & E.E. Butler,

which was first associated with root rot of tulip poplar (Liriodendron tulipifera L.) in California by Mac-Donald and Butler (1981). Thus, because these species had identical sequences, "C." destructans isolates collected from asymptomatic or diseased grapevines affected by black-foot disease were renamed as "C." liriodendri, associating "C." destructans only with root rot on other herbaceous or woody hosts (Halleen et al., 2006b). In addition, in order to clarify the taxonomy of "C." destructans causing black-foot in California, Petit and Gubler (2007) also compared "C." destructans isolates obtained from grapevines in California with "C." liriodendri isolates from South Africa. All of them were identical, and consequently "C." destructans isolates were also renamed as "C." liriodendri. This species has been later reported as a black-foot pathogen of grapevine in Australia (Whitelaw-Weckert et al., 2007), Spain (Alaniz et al., 2007), Brazil (Russi et al., 2010), Iran (Mohammadi et al., 2009), Switzerland (Casieri et al., 2009), Uruguay (Abreo et al., 2010) and northeastern United States and southeastern Canada (Petit et al., 2011). The teleomorphs of "C." liriodendri and "C." macrodidymum were described as Neonectria liriodendri Halleen, Rego & Crous and N. macrodidyma Halleen, Schroers & Crous (Halleen et al., 2004, 2006b).

In 2008, a new species associated with black-foot disease of grapevines, "C." pauciseptatum Schroers & Crous, was described in New Zealand and Slovenia (Schroers et al., 2008). To date, this species has been isolated from affected grapevines in Uruguay (Abreo et al., 2010), Canada (O'Gorman and Haag, 2011), Spain (Martin et al., 2011) and Portugal (Cabral et al., 2012a). Phylogenetic studies carried out in New Zealand and Slovenia by Schroers et al. (2008), indicated that "C." pauciseptatum is the closest phylogenetic sister-taxon of "C." macrodidymum and both species are closely related to the "C." destructans-complex, which also includes "C." liriodendri.

Thus, at this moment, "C." destructans, "C." liriodendri, "C." macrodidymum, "C." obtusisporum, "C." pauciseptatum, Campyl. fasciculare and Campyl. pseudofasciculare were considered as the main species associated with young vines showing symptoms of black-foot disease in most of grapevine producing areas worldwide. In addition, other "Cylindrocarpon" species have been associated occasionally with black-foot disease of grapevine: "Cylindrocarpon" didymum (Harting) Wollenw. in Canada (Petit et al., 2011), "C." olidum (Wollenw.) Wollenw. in Spain (De Francisco

et al., 2009) and "C." olidum var. crassum Gerlach in Uruguay (Abreo et al., 2010).

Chaverri et al. (2011) performed a phylogenetic study of Neonectria, "Cylindrocarpon" and related genera with "Cylindrocarpon"-like anamorphs. Morphological and molecular phylogenetic analyses data accumulated over several years have indicated that Neonectria sensu stricto and "Cylindrocarpon" sensu stricto are phylogenetically congeneric, while Neonectria sensu lato and "Cylindrocarpon" sensu lato do not form a monophyletic group, suggesting that Neonectria/"Cylindrocarpon" represents more than one genus. Thus, based on results of the phylogenetic study, these authors divided Neonectria into five genera based on a combination of characters linked to perithecial anatomy and conidial septation: Neonectria/"Cylindrocarpon" sensu stricto (Booth's groups 1 and 4), Rugonectria, Thelonectria (group 2), Ilyonectria (group 3) and anamorph genus Campylocarpon. According to this, only Neonectria has "Cylindrocarpon" anamorphs, while the remaining genera have "Cylindrocarpon"-like anamorphs, and since then are referred to as "Cylindrocarpon". Consequently, "C." liriodendri and "C." macrodidymum were included into Ilyonectria genus, with I. radicicola as the type species, and re-identified as *Ilyonec*tria liriodendri (Halleen, Rego & Crous) Chaverri & Salgado and I. macrodidyma (Halleen, Schroers & Crous) P. Chaverri & Salgado, respectively (Chaverri et al., 2011).

Moreover, Cabral et al. (2012a) were able to delineate 12 new taxa in the *I. radicicola*-complex, previously known as the "C." destructans-complex, by using a multi-gene DNA analysis supported by morphological characters. Other *Ilyonectria* species within *I.* radicicola-complex have been also found associated with black-foot disease of grapevine: Ilyonectria europaea A. Cabral, Rego & Crous, I. lusitanica A. Cabral, Rego & Crous, I. pseudodestructans A. Cabral, Rego & Crous and I. robusta (A.A. Hildebr.) A. Cabral, Rego & Crous, reported in Portugal (Cabral et al., 2012a, 2012c). Another *Ilyonectria* spp., *I. vitis* has also been described in Portugal (Cabral et al., 2012a), and isolates belonging to Neonectria mammoidea group have also been associated with the disease in Canada (Petit et al., 2011). Soon thereafter, following this study, Cabral et al. (2012c), demonstrated the existence of polymorphism into *I. macrodidyma-*complex. This hypothesis was in agreement with the results obtained by Alaniz et al. (2009), who already detected

relevant genetic diversity in "C." macrodidymum by using inter-simple sequence repeat (ISSR) technique. However, previous phylogenetic analysis showed low variation in the large subunit (LSU) ribosomal DNA (rDNA), TUB and ITS sequences of "C." macrodidymum isolates obtained from grapevine in different countries (Halleen et al, 2004; Petit and Gubler, 2005; Alaniz et al., 2007). Thus, in order to clarify this hypothesis, Cabral et al. (2012c) performed a phylogenetic study of I. macrodidyma-complex by using ITS, TUB, histone H3 gene (HIS) and translation elongation factor 1- $\alpha$  (TEF) sequence analysis. Consequently, six new species of Ilyonectria (I. alcacerensis A. Cabral, Oliveira & Crous, I. estremocensis A. Cabral, Nascimento & Crous, I. novozelandica A. Cabral & Crous, I. torresensis A. Cabral, Rego & Crous, and Ilyonectria sp. 1, I. sp. 2,) and I. macrodidyma, which are morphologically rather similar, were recognised into the *I. macrodidyma*-complex. All these species have been reported in Portugal, with the exception of I. novozelandica which has been reported in South Africa, USA, New Zealand (Cabral et al., 2012a, 2012c). Recently, I. alcacerensis, I. macrodidyma, I. novozelandica, and I. torresensis have also been found on grapevines in Spain (Agustí-Brisach et al., 2013a, 2013b).

Regarding, "C." pauciseptatum, it is not clear in which genera it has to be included, although it is very similar in morphology to *I. anthuriicola* A. Cabral & Crous (Cabral *et al.*, 2012a).

Finally, another genus, Cylindrocladiella Boesew., which is also *Cylindrocarpon*-like in morphology, has recently been associated with black-foot disease of grapevines (Van Coller et al., 2005; Agustí-Brisach et al., 2012; Jones et al., 2012). This genus was established by Boesewinkel (1982) to accommodate five Cylindrocladium-like species producing small and cylindrical conidia. This decision was based on the fact that species of Cylindrocladiella had different conidiophores branching patterns, conidial shapes, dimensions, cultural characteristics and teleomorphs from those of Cylindrocladium (Van Coller et al., 2005; Lombard et al. 2012). Since then, several taxonomic studies of these fungi have relied on morphologically and to lesser extent on DNA sequence comparisons of the ITS and TUB gene regions, recognizing nine species of Cylindrocladiella (Crous and Wingfield, 1993; Victor et al., 1998; Van Coller et al., 2005). Lombard et al. (2012), have just described 18 new Cylindrocladiella species based on morphological and phylogenetic studies employing ITS, TUB, HIS and TEF gene regions. Nevertheless, only two species into this genus have been found associated with black-foot disease on grapevines: *Cylindrocladiella parva* (P.J. Anderson) Boesew., which has been reported in South Africa (Van Coller *et al.*, 2005), New Zealand (Jones *et al.*, 2012) and Spain (Agustí-Brisach *et al.*, 2012) and *Cyl. peruviana* (Bat., J.L. Bezerra & M.P. Herrera) Boesew., which has been reported in South Africa (Van Coller *et al.*, 2005), Perú (Álvarez *et al.*, 2012) and Spain (Agustí-Brisach *et al.*, 2012).

A list of all "Cylindrocarpon" / Ilyonectria, Campylocarpon and Cylindrocladiella species, which have been reported associated with black-foot disease of grapevine and their geographical distribution, is presented in Table 1.

## Morphological and cultural characterization

"Cylindrocarpon" / Ilyonectria, Campylocarpon and Cylindrocladiella species have characteristic distinctive morphological and cultural patterns (Figures 2

and 3; Table 2). The anamorphs of "Cylindrocarpon" / Ilyonectria produce abundant microconidia and chlamydospores. Macro- and microconidia apparently are produced from the same conidiophores which are 40–160 µm long, generally simple, unbranched or sparsely branched, irregularly or verticillately branched, rarely densely branched, and with cylindrical phialides. Macroconidia are straight or curved, hyaline, 1–3-septate, rarely > 3-septate [25–  $50(-55)\times5-7.5$  µm], generally with a prominent basal or lateral abscission scar or hilum. Microconidia are ellipsoidal to ovoid, hyaline, 0-1-septate, with a lateral or basal hilum [3–15×2.5–5(–6) μm] (Figures 2A, 2B). Chlamydospores are abundant, generally intercalary, globose, single or in chains, becoming brownish. In addition, colony morphology on PDA is very heterogeneous (Figure 2C). Aerial mycelium is floccose to felted, and the colour varies from white to yellow or light to dark brown. The margin of the colony can be entire, slightly lobulated, or lobulated (Figures 3A-3H) (Booth, 1966; Samuels and Brayford, 1990; Chaverri et al., 2011).

**Table 1.** Fungal species which have been reported associated with black-foot disease of grapevines and their geographical distribution.

Species	Distribution	
Campylocarpon fasciculare Schroers, Halleen & Crous	South Africa (Halleen <i>et al.</i> , 2004), Spain (Alaniz <i>et al.</i> , 2011b) and Brazil (Correia <i>et al.</i> , 2012).	
Campylocarpon pseudofasciculare Halleen, Schroers & Crous	South Africa (Halleen <i>et al.</i> , 2004), Uruguay (Abreo <i>et al.</i> , 2010), Brazil (Correia <i>et al.</i> , 2012) and Perú (Álvarez <i>et al.</i> , 2012)	
"Cylindrocarpon" destructans (Zinssm.) Scholten	France (Maluta and Larignon, 1991), Italy (Grasso, 1984), Argentina (Gatica <i>et al.</i> , 2001), Germany (Fischer and Kassemeyer, 2003), Pennsylvania (Gugino and Travis, 2003), Brazil (Garrido <i>et al.</i> , 2004) and Canada (Petit <i>et al.</i> , 2011)	
"Cylindrocarpon" didymum (Harting) Wollenw.	Canada (Petit et al., 2011)	
"Cylindrocarpon" obtusisporum (Cooke & Harkn.) Wollenw.	Sicily (Grasso and Magnano di San Lio, 1975) and California (Scheck <i>et al.</i> , 1998a)	
"Cylindrocarpon" olidum (Wollenw.) Wollenw.	Spain (De Francisco et al., 2009)	
"Cylindrocarpon" olidum var. crassum Gerlach	Uruguay (Abreo et al., 2010)	
"Cylindrocarpon" pauciseptatum Schroers & Crous	New Zealand and Slovenia (Schroers <i>et al.</i> , 2008), Uruguay (Abreo <i>et al.</i> , 2010) Canada (O'Gorman and Haag, 2011), Spain (Martin <i>et al.</i> , 2011) and Portugal (Cabral <i>et al.</i> , 2012a)	
Cylindrocladiella parva (P.J. Anderson) Boesew.	South Africa (Van Coller <i>et al.</i> , 2005), New Zealand (Jones <i>et al.</i> , 2012) and Spain (Agustí-Brisach <i>et al.</i> , 2012)	

(Continued)

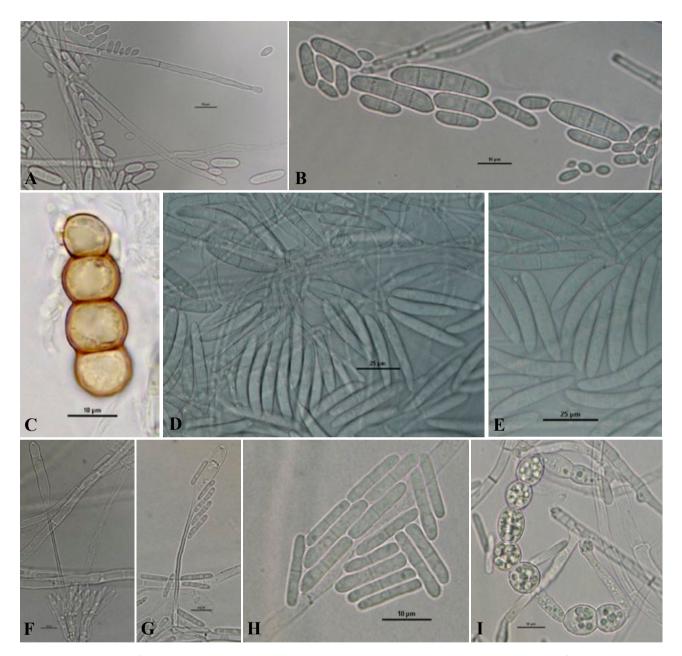
Table 1. Continues.

Species	Distribution	
Cylindrocladiella peruviana (Bat., J.L. Bezerra & M.P. Herrera) Boesew.	South Africa (Van Coller <i>et al.</i> , 2005), Spain (Agustí-Brisach <i>et al.</i> , 2012) and Perú (Álvarez <i>et al.</i> , 2012)	
Ilyonectria alcacerensis A. Cabral, Oliveira & Crous	Portugal (Cabral <i>et al.</i> , 2012c) and Spain (Agustí-Brisach <i>et al.</i> , 2013b)	
Ilyonectria estremocensis A. Cabral & Crous	Portugal (Cabral et al., 2012c)	
Ilyonectria europaea A. Cabral, Rego & Crous	Portugal (Cabral et al., 2012a)	
Ilyonectria liriodendri (Halleen, Rego & Crous) Chaverri & Salgado	France, New Zealand, Portugal and South Africa (Halleen <i>et al.</i> , 2006b), Australia (Whitelaw-Weckert <i>et al.</i> , 2007), California (Petit and Gubler, 2007), Spain (Alaniz <i>et al.</i> , 2007), Iran (Mohammadi <i>et al.</i> , 2009), Switzerland (Casieri <i>et al.</i> , 2009), Brazil (Russi <i>et al.</i> , 2010), Uruguay (Abreo <i>et al.</i> , 2010), northeastern United States and southeastern Canada (Petit <i>et al.</i> , 2011),	
Ilyonectria lusitanica A. Cabral, Rego & Crous	Portugal (Cabral et al., 2012a)	
Ilyonectria macrodidyma (Halleen, Schroers & Crous) P. Chaverri & C. Salgado	Australia, France, New Zealand and South Africa (Halleen <i>et al.</i> , 2004), California (Petit and Gubler, 2005), Chile (Auger <i>et al.</i> , 2007), Uruguay (Abreo <i>et al.</i> , 2010), northeastern United States and southeastern Canada (Petit <i>et al.</i> , 2011), Portugal (Cabral <i>et al.</i> , 2012c), Turkey (Özben <i>et al.</i> , 2012) and Spain (Agustí-Brisach <i>et al.</i> , 2013b).	
Ilyonectria novozelandica A. Cabral, Nascimento & Crous	South Africa, USA and New Zealand (Cabral <i>et al.</i> , 2012c) and Spain (Agustí-Brisach <i>et al.</i> , 2013a, b)	
Ilyonectria pseudodestructans A. Cabral, Rego & Crous	Portugal (Cabral et al., 2012a)	
<i>Ilyonectria robusta</i> (A.A. Hildebr.) A. Cabral, Rego & Crous	Portugal (Cabral et al., 2012a)	
Ilyonectria torresensis A. Cabral, Rego & Crous	Australia, Canada, New Zealand, Portugal, South Africa, Spain and USA (Cabral <i>et al.</i> , 2012c; Agustí-Brisach <i>et al.</i> , 2013a, b)	
Ilyonectria vitis A. Cabral, Rego & Crous	Portugal (Cabral et al., 2012a)	
Ilyonectria sp. 2 (Cabral et al., 2012c)	Portugal (Cabral et al., 2012c)	
Isolates belonging to Neonectria mammoidea group	Canada (Petit et al., 2011)	

Campylocarpon is similar to "Cylindrocarpon" / Ilyonectria, although Campylocarpon spp. produce macroconidia mostly curved, while microconidia are absent and chlamydospores are rare or also absent. Conidiophores appear arising laterally from single or fasciculate aerial hyphae or from creeping substrate hyphae, singly or in loose or dense aggregates (Figure 2D). Conidial heads form pionnotes-like aggregates. Conidiophore show a stipe base to 16  $\mu$ m wide, which bear several phialides or a penicillus of irregular branches with terminal branches bearing 1 or several phialides. Macroconidia are as in Ilyonec-

tria, but typically curved, and with up to 6 septa,  $[(24-)35-60(-62)\times6.5-9 \mu m]$ , apical cell obtuse, basal cell obtuse or with inconspicuous hilum (Figure 2E). Regarding colony morphology on PDA, aerial mycelium is abundant, covering the whole or sectors of the colony, white to off-white or slightly brownish, thickly cottony to felty, intermingled with or giving rise to erect white or brown hyphal strands. This strands sometimes are partly covered by off-white slime (Figures 3I, 3J) (Halleen *et al.*, 2004; Chaverri *et al.*, 2011).

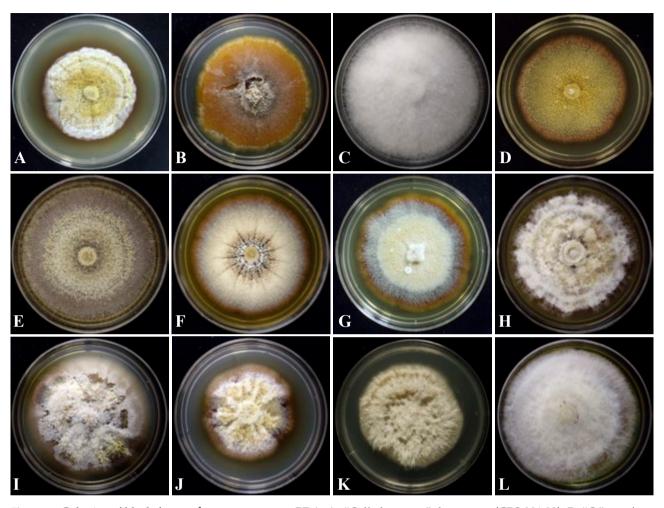
Cylindrocladiella species produce hyaline, single, subverticillate, as well as penicilliate conidiophores,



**Figure 2.** A, Conidiophores of *I. liriodendri*; B, Macro- and microconidia of *Ilyonectria liriodendri*; C, Chlamydospores in chains of "Cylindrocarpon" pauciseptatum; D, Conidiophores of Campyl. fasciculare; E, Macroconidia of Campylocarpon fasciculare; F, Penicillate conidiophores of Cylindrocladiella parva; G, Terminal vesicles of Cyl. parva; H, Conidia of Cyl. parva; H, Chlamydospores in chains of Cyl. parva. Scale bars: a-c,  $f-i=10~\mu m$ ;  $d-e=25~\mu m$ .

with primary and secondary branches. The phialides are terminal, hyaline, in whorls of 2–4, with or without obvious collarets. In general, stipe is centrally arranged on conidiophores, with a single basal

septum, terminating in a thin-walled, hyaline vesicle of characteristic shape (Figures 2F, 2G). Conidia are cylindrical, rounded at both ends, straight, hyaline, (0)–1-septate, [(9–)11–13(–15)×2–4 µm], sometimes



**Figure 3.** Colonies of black-foot pathogens grown on PDA. A, "Cylindrocarpon" destructans (CBS 301.93); B, "C." pauciseptatum; C, "C." obtusisporum; D, Ilyonectria alcacerensis; E, I. liriodendri; F, I. novozelandica; G, I. macrodidyma; H, I. torresensis; I, Campylocarpon fasciculare; J, Campyl. pseudofasciculare; K, Cylindrocladiella parva; L, Cyl. peruviana.

becoming swollen at one end with age (Figure 2H). Chlamydospores are abundant or moderate, more frequently arranged in chains than clusters (Figure 2I). Aerial mycelium ranges from dark to light brown (Figures 3K, 3L) (Crous and Wingfield, 1993; Lombard *et al.*, 2012).

## **Epidemiology**

Campylocarpon, "Cylindrocarpon", Cylindrocladiella and Ilyonectria species are generally regarded as pathogens and/or saprobes of a wide range of angiosperm and gymnosperm hosts and substrates in temperate, sub-tropical and tropical regions worldwide

(Victor et al., 1998; Chaverri et al., 2011; Lombard et al., 2012). In addition to grapevine, they have also been associated with root rot diseases of other economically important hosts (Chaverri et al., 2011; Lombard et al., 2012), such as: Actinidia chinensis Planch. (Erper et al., 2011), Liriodendron tulipifera L. (MacDonald and Butler, 1981), Olea europaea L. (Úrbez-Torres et al., 2012), Panax quinquefolius L. (Rahman and Punja, 2005), Persea americana Mill. (Vitale et al., 2012), Pinus radiata D. Don (Agustí-Brisach et al., 2011b) or Pinus sylvestris L. (Menkis and Burokiene, 2012). Lombard et al. (2013) have just reported black foot rot disease associated with the cultivation of Proteaceae cut flowers in South Africa, and described four new Ilyonec-

**Table 2.** Summary of distinctive morphological and cultural features of "Cylindrocarpon" / Ilyonectria, Campylocarpon and Cylindrocladiella genera associated with black-foot disease of grapevines.

Characteristics	"Cylindrocarpon"/llyonectria	Campylocarpon	Cylindrocladiella
Conidiophores			
	40–160 µm long, generally simple, unbranched or sparsely branched, irregularly or verticillately branched, rarely densely branched, and with cylindrical phialides	Appear arising laterally from single or fasciculate aerial hyphae or from creeping substrate hyphae, singly or in loose or dense aggregates	Hyaline, single, subverticillate, as well as penicillate, with primary and secondary branches
Conidia			
Microconidia	Abundant, ellipsoidal to ovoid, hyaline, 0–1-septate, with a lateral or basal hilum	Absent	Cylindrical, rounded at both ends, straight, hyaline, (0) –1-septate, sometimes becoming swollen at one end with age
Macroconidia	Straight or curved, hyaline, 1–3-septate, rarely > 3-septate, generally with a prominent basal or lateral abscission scar or hilum	Mostly curved, hyaline, with up to 6- septate, apical cell obtuse, basal cell obtuse or with inconspicuous hilum	Absent
Chlamydospores	Abundant, generally intercalary, globose, single or in chains, becoming brownish	Rare or also absent	Abundant or moderate, more frequently arranged in chains than clusters
Colony color	White to yellow or light to dark brown	White to off-white or slightly brownish	Dark to light brown

*tria* spp. included into the *I. radicicola*-complex: *I. capensis* L. Lombard & Crous, *I. leucospermi* L. Lombard & Crous, *I. protearum* L. Lombard & Crous and *I. vredehoekensis* L. Lombard & Crous.

In grapevine, black-foot pathogens are frequently isolated from rootstock mother-plants, rooted rootstock cuttings, bench-grafts and young grafted vines (Rego et al., 2001a; Fourie and Halleen, 2002, 2004; Halleen et al., 2004; Oliveira et al., 2004; Petit and Gubler, 2005). During the last decade, several surveys of young vineyards have been carried out in different grapevine growing areas worldwide in which black-foot pathogens were isolated from plants used in new plantings (Armengol et al., 2001; Fourie and Halleen, 2001; Rego et al., 2001b; Rumbos and Rumbou, 2001; Petit and Gubler, 2005; Aroca et al., 2006; Giménez-Jaime et al. 2006; Alaniz et al., 2007; Mohammadi et al., 2009; Abreo et al., 2010). Recently, studies carried out in commercial grapevine nurseries in Spain by Agustí-Brisach et al. (2013a), have demonstrated that inoculum of *Ilyonectria* spp. is also present at the different stages of the grapevine nursery propagation process and suggest that infections caused by these pathogens can also occur during this process. Moreover, these authors confirmed that during the rooting phase in nursery fields the number of plants infected with black-foot pathogens increases markedly (Agustí-Brisach et al., 2013a). These results are in agreement with those obtained by Cardoso et al. (2012), who detected inoculum sources of black-foot pathogens in a commercial grapevine nursery in Portugal. All together these results suggest that new plants are infected during the propagation process in nurseries and that even the planting material used in the propagation process might be infected with these pathogens (Rego et al., 2000; Halleen et al., 2003; Aroca et al., 2010; Cardoso et al., 2012; Agustí-Brisach et al., 2013a).

Regarding the role of soil from nursery fields and vineyards as another important source of inoculum, Halleen *et al.* (2003), concluded that black-foot pathogens from soils infected grafted grapevines

once planted in open-rooted nurseries, whereas these pathogens rarely occurred in rootstock propagation material prior to planting. During the grapevine propagation process, at the time of planting, the susceptible basal ends (especially the pith area) of most of the nursery cuttings are partly or even fully exposed, and the young callus roots also break during the planting process, resulting in small wounds susceptible to infection by soilborne pathogens. The occurrence of black-foot pathogens in the graft union might be explained by the nursery practice of covering graft unions with soil for a period of approximately 5 weeks to prevent drying of the callus tissue. Recently, Agustí-Brisach et al. (2011a, 2013b, 2013c) have confirmed the presence of black-foot pathogens in soils from rootstock mother fields, nursery fields and vineyards by fungal isolation from roots of grapevine seedlings used as bait plants, multiplexnested PCR and qPCR, and fungal isolation from roots of weeds collected from the three types of soil.

Campylocarpon, "Cylindrocarpon", Cylindrocladiella and *Ilyonectria* species are known to be saprobes in soil, which can occur on dead plant substrata, or act as weak pathogens of plants infecting wounds of roots and stems of various hosts through wounds and/or natural openings (Fourie and Halleen, 2006; Halleen et al., 2006a, 2007; Schroers et al., 2008; Probst et al., 2012). Furthermore, the production of chlamydospores in most species of these genera may allow them to survive for extended periods in soil (Halleen et al., 2004). However, very little information is currently available regarding the survival of these pathogens, and the role of chlamydospores during subsequent infections (Halleen et al., 2006a). The effect of temperature, pH and water potential  $(\Psi_s)$  on mycelial growth, sporulation and chlamydospore production of I. liriodendri, I. macrodidymacomplex and "C". pauciseptatum isolated from grapevines was studied by Agustí-Brisach and Armengol (2012). Three isolates per species were incubated on potato dextrose agar (PDA) under different temperature, pH, and  $\Psi_s$  conditions. All isolates were able to grow over a range of temperatures from 5 to 30°C, with an optimum temperature between 20 to 25°C, but they did not grow at 35°C. Active mycelial growth was observed in a broad range of pHs, from 4 to 8. Regarding the effect of  $\Psi_s$ , in general, mycelial growth was higher on amended media at -0.5, -1.0 or/and -2.0 MPa compared with that obtained on nonamended PDA (-0.3 MPa), and it was reduced at

 $\Psi_s$  values lower than -2.0 MPa. Regarding the sporulation, most of the isolates were able to sporulate at all temperatures, pH, and  $\Psi_s$ , showing a broad range of variation. In all studied conditions, *I. liriodendri* was found as the species with the highest capacity of sporulation. In general, chlamydospore production was not much affected by temperature, pH and  $\Psi_s$  values tested. Chlamydospores were observed in PDA cultures of all isolates at all pH values studied, while just some isolates did not produced them at 5 and  $10^{\circ}\text{C}$  or -4.0 and / or -5.0 MPa.

Disease risk may be increased by the stresses imposed on young grapevines in nurseries and vineyards. Environmental factors and vineyard management practices, including poor drainage, soil compaction and inadequate planting holes, which cause poor root development, as well as poor nutrition, heavy cropping of young vines and effects of pests and pathogens could be considered as stress factors (Probst et al., 2012). High temperatures during summer also play an important role in symptom expression, since the compromised root and vascular system of diseased plants would not be able to supply enough water to compensate for the high transpiration rate during periods of high temperatures (Larignon, 1999). The processes of nursery propagation and vineyard establishment include many practices that cause stress on young vines. During the grapevine propagation process, wounds produced during cutting and bench-grafting, the early development of roots and shoots in the nursery field, uprooting and trimming, extended cold storage and excessive time in containers prior to establishment in the vineyard are all traumatic to the young plants. In addition, after planting out in the field, these vines are again stressed by the need to develop roots and shoots in an environment that is often selected to limit shoot growth (Probst et al., 2012). Research studies carried out in New Zealand by Brown et al. (2012), confirmed that stress factors such as defoliation can contribute to black-foot disease severity in voung vines.

There are few reports about virulence diversity of "Cylindrocarpon" / Ilyonectria spp. to grapevine. Alaniz et al. (2009) detected virulence diversity in I. macrodidyma-complex showing that the isolates belonging to ISSR groups G6 and G7 were significantly more virulent than other isolates of I. macrodidyma-complex (ISSR groups G3, G4 and G5) and I. liriodendri (ISSR groups G1 and G2). Recently, research stud-

ies carried out by Cabral *et al.* (2012b), in which they compared the virulence of *Ilyonectria* spp. isolates, revealed that described species such as *I. lusitanica*, *I. estremocensis* and *I. europaea* are more virulent to grapevine than the species previously accepted as the main causal agents of black-foot, such as *I. liriodendri* and *I. macrodidyma*.

Black-foot pathogens are often part of disease complexes with other fungi or nematodes (Brayford, 1993). In the case of declining grapevines, they are often isolated in association with other fungi such as Petri disease pathogens, Botryosphaeriaceae, *Phomopsis* spp., *Pythium* spp. or *Phytophthora* spp. (Halleen *et al.*, 2007).

## **Management strategies**

Presently, no curative control measures are available to eradicate black-foot pathogens in nurseries as well as in vineyards (Oliveira *et al.*, 2004; Halleen *et al.*, 2007). During the last years, research has been specially focused in the development of procedures and chemical products to prevent or reduce black-foot disease infection of grapevine woody tissues during the propagation process with promising results including, the use of hot-water treatments, biological control, applications of chitosan, use of arbuscular mycorrhizal (AM) fungi or fungicides (Alaniz *et al.*, 2011a).

In vineyards, management strategies recommended for prevention and disease management mainly involve the prevention and/or correction of predisposing stress situations (Halleen *et al.*, 2007). In nurseries, where there are many opportunities for infection by black-foot pathogens during the propagation process, there have recently been advances in the development of procedures and products to prevent or reduce the infection of woody tissue by these pathogens. Thus, good hygiene and wound protection are of the utmost importance in order to obtain a healthy vine, which is fundamental to the successful beginning and sustainability of all grape vineyards (Gramaje and Armengol, 2011).

In this context, a sanitation program is required to improve the quality of grapevine planting material. Chemical, physical, and biological control, and other management strategies have to be used to decrease the incidence and severity of black-foot pathogens during the nursery propagation process as well as during the growing season in vineyards.

#### Chemical control

Studies carried out in Portugal by Rego et al. (2006) indicated that the fungicides benomyl, prochloraz and the mixtures of carbendazim with flusilazole and cyprodinil with fludioxonil inhibited mycelial growth of "C." destructans in vitro, whereas tebuconazole and difenoconazole were less effective. In vivo studies on potted grapevines proved that benomyl, tebuconazole, and the mixtures of carbendazim with flusilazole and cyprodinil with fludioxonil significantly improved plant growth and decreased disease incidence compared with non-treated vines. In a later study carried in a commercial nursery, these authors found that fludioxonil and the mixtures of cyprodinil with fludioxonil and pyraclostrobin with metiram reduced the incidence and severity of black-foot pathogens on grapevine plants grown in a commercial field with grapevine cultivation history (Rego et al., 2009)

In studies performed in semi-commercial nursery trials in South Africa, grapevine rootstock and scion cuttings were soaked in some chemical products prior to cold storage, prior to grafting and prior to planting in field nurseries. Natural infection levels in basal stem and graft unions of uprooted nursery grapevines were evaluated eight months after planting. Among the different products tested, benomyl didecyldimethylammonium chloride and captan were consistently the best treatments as growth parameters were not negatively influenced and pathogen incidences in basal ends and graft unions of uprooted plants were reduced (Fourie and Halleen, 2006).

In a later study carried out also in South Africa, Halleen *et al.* (2007) evaluated various chemical preplanting treatments for prevention of infection by black-foot and Petri disease pathogens. A total of 13 fungicides were evaluated *in vitro* against "C." liriodendri, "C." macrodidymum, Campyl. fasciculare and Campyl. pseudofasciculare. Results indicated that benomyl, flusilazole, imazalil and prochloraz were effective in reducing mycelial growth of black-foot pathogens. Nevertheless, only benomyl and imazalil showed some effect to control these pathogens in semi-commercial field trials. However, the results were inconsistent, perhaps because of generally low and varying infection levels in the roots and rootstocks, respectively

Recently, Alaniz *et al.* (2011a) conducted a pot assay with several fungicides in order to determine their potential to prevent infections caused by "C." *liriodendri* and "C." *macrodidymum* during the rooting

phase in the grapevine propagation process. Results showed that captan, carbendazim, copper oxychloride, didecyldimethylammonium chloride, hydroxyquinoline sulfate, and prochloraz decreased the root disease severity values in both species compared with the control treatment; but only captan, carbendazim, and didecyldimethylammonium chloride presented a percentage of reisolation values significantly different from the control treatment in the case of the cuttings inoculated with "C." liriodendri, and prochloraz in the case of those inoculated with "C." macrodidymum.

#### Hot-water treatment

The use of hot water treatment (HWT) has been reported as a promising method for the control of black-foot disease pathogens in grapevine propagation material. HWT of rootstock cuttings prior to grafting or HWT of dormant nursery plants after uprooting has been strongly recommended for their effectiveness in reducing infection levels in nursery plants (Gramaje and Armengol, 2011).

Halleen et al. (2007), evaluated the effect of HWT at 50°C for 30 min on dormant nursery grapevines after uprooting. In this study, no black-foot pathogens were isolated from rootstock and roots of plants which were subjected to HWT, whereas these pathogens were isolated from 16.8% of rootstocks and from 4.1% of roots from control plants. Gramaje *et al*. (2010) evaluated the effect of HWT in vitro on conidial and mycelial growth of "C." liriodendri and "C." macrodidymum at a range of temperature from 41 to 49°C for 30, 45 or 60 min. Conidial germination was inhibited by treatments above 45°C for 45 min, while treatments above 48°C for 45 min were necessary to inhibit the mycelial growth. These results suggest that standard HWT protocols at 50°C for 30 min may be sufficient to control black-foot pathogens in grapevine propagation material.

## **Biological control**

The potential use of biocontrol agents as wound protectants and growth stimulants in grapevine nurseries has also been reported. Research studies conducted in a semi-commercial nursery trial in South Africa, showed the growth stimulating attributes of commercial products of *Trichoderma*, as well as the positive effect on natural infection by grapevine trunk pathogens. Although *Trichoderma* treatments

notably reduced the incidence of these pathogens in roots of nursery grapevines, low levels of them were recorded (Fourie et al., 2001). Fourie and Halleen (2006), performed soak-treatments of propagation material by using products containing T. harzianum Rifai obtaining inconsistent results. Then, Halleen et al. (2007) evaluated the effect of products containing T. harzianum in soil as a potential biological control agent of grapevine trunk diseases, showing that the incidence of black-foot pathogens in nursery grapevines was not reduced by the effect of T. harzianum. These authors pointed out that, in general, the growth stimulating effect due to Trichoderma, which significantly improved root development, would possibly make plants more tolerant to black-foot disease when subjected to stress. However, the potential use of Trichoderma as biocontrol agent should be studied further to develop application methods that may ensure a more consistent efficacy (Fourie and Halleen, 2006; Halleen et al., 2007).

### Other management strategies

Given the difficulty of controlling grapevine trunk pathogens using the measures previously described, other management strategies such as host resistance, biofumigation or mycorrhizal colonization have been studied as alternatives to control black-foot disease on grapevines

In a research study carried out in California, it was noted that the rootstocks Vitis riparia 039-16 and Freedom had a good degree of resistance to this disease (Gubler et al., 2004). However, in a later study, Jaspers et al. (2007) evaluated the susceptibility of the more commonly planted grapevine rootstocks in New Zealand such as Riparia Glorie, Schwarzman, K5BB, 140-R, 3309C and 420A, under greenhouse conditions showing that all rootstock varieties included in the study were susceptible to black-foot pathogens to some degree. These findings were in agreement with those obtained by Alaniz et al. (2010), who evaluated the susceptibility of the grapevine rootstocks most commonly used in Spain (110-R, 1103-P, 140-R, 161-49C, 196-17C, Fercal and SO4) to "C." liriodendri and "C." macrodidymum and found that all rootstocks inoculated were affected by the disease, being the rootstock 110-R the most susceptible to both pathogens.

Green crops of *Brassica* species such as mustard (*B. juncea* [L.]) Coss.) and rape (*B. napus* L.) incorpo-

rated into the soil release volatile isothiocvanates, which are known to suppress pathogenic fungal species. Thus, the potential of the biofumigation using these crops have been evaluated in nursery fields and vineyards as a possible alternative for methyl bromide and metham sodium for the control of black-foot pathogens (Stephens et al., 1999; Bleach et al., 2010). Studies conducted by Stephens et al. (1999) showed that this biofumigant did not reduce the percentage of root or stem tissue containing this pathogen at harvest. However, in a New Zealand experiment which crops of mustard, rape and oats (Avena sativa L.) were grown in a vineyard previously infested by black-foot pathogens, showed that biofumigation using mustard was the most effective, reducing disease incidence in rootstocks (Bleach et al., 2010). It appeared that mustard meal incorporated into infested soil was as good as growing the plants and incorporating the plant into the soil. These findings indicated that biofumigation using mustard may be highly effective for reducing soilborne black-foot pathogens inoculum and the incidence of the disease (Bleach et al., 2010). Consequently, this may give a valuable control tool for growers who replant into a pathogen-contaminated site after the removal of infected plants in an established vineyard.

Compost also is known to suppress pathogenic fungal species. In fact, Gugino and Travis (2003) evaluated the efficacy of several types of compost on the suppression of "C." destructans. In this study, the population of "C." destructans was monitored over time in soilless mixes amended with 0, 10, 25 and 50% compost using serial soil dilution plating. The preliminary results indicated an increasing reduction in the "C." destructans population as the amount of compost increased from 0 to 50%. Moreover, several microorganisms were isolated from these composts also demonstrating antagonism toward "C." destructans in vitro.

Regarding the use of the endomycorrhizal symbiosis as alternative control measure, Petit and Gubler (2006) indicated that grapevines inoculated with an arbuscular-mycorrhizal (AM) fungus, *Glomus intraradices* N.C. Schenck & G.S. Sm., were less susceptible to black-foot disease than nonmycorrhizal plants. Even though "C." macrodidymum was consistently recovered from both mycorrhizal and nonmycorrhizal plants, disease severity was significantly lower when vines were preinoculated with *G. intraradices*. These findings were in agreement with those obtained by

Bleach *et al.* (2008), who evaluated the impact of *G. mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe and *Acaulospora laevis* Gerd. & Trappe on grapevine establishment in soils infested with black-foot pathogens. In this study, the AM associations also improve health and growth of young grapevine plants. Although the mechanisms by which AM fungi protect plants against soilborne pathogens is poorly understood, it is often hypothesized that they include improving nutrition of the host, competition for infection sites and changes to root ultrastructure. Results from this study suggest that preplant applications of AM fungi may help prevent black-foot disease in the nursery and in the vineyard (Petit and Gubler, 2006; Bleach *et al.*, 2008).

The use of chitosan which is a high molecular-weight polymer that is non-toxic and biodegradable has also been evaluated as another control measure for grapevine trunk pathogens. In a research study carried out in Portugal, Nascimento *et al.* (2007) explored the *in vitro* and *in vivo* fungicidal effect of chitosan on some of the most important grapevine wood fungi. The results showed that chitosan was effective in reducing mycelial growth of all fungi and significantly improved plant growth and decrease diseased incidence compared with untreated plants. Moreover, the effect of chitosan against "C." liriodendri was similar to that achieved with some selected fungicides such as tebuconazole and mixtures of carbendazim with flusilazole, and cyprodinil with fludioxonil.

## Literature cited

Abreo E., S. Martínez, L. Betucci and S. Lupo, 2010. Morphological and molecular characterization of *Campylocarpon* and *Cylindrocarpon* spp. associated with black foot disease of grapevines in Uruguay. *Australasian Plant Pathology* 39, 446–452.

Agustí-Brisach C., D. Gramaje, M. León, J. García-Jiménez and J. Armengol, 2011a. Evaluation of vineyard weeds as potential hosts of black-foot and Petri disease pathogens. *Plant Disease* 95, 803–810.

Agustí-Brisach C., A. Pérez-Sierra, F, García-Figueres, C. Montón and J. Armengol, 2011b. First report of damping-off caused by *Cylindrocarpon pauciseptatum* on *Pinus radiata* in Spain. *Plant Disease* 95, 874.

Agustí-Brisach C., S. Alaniz, D. Gramaje, A. Pérez-Sierra, J. Armengol, E. Landeras and P.M. Izquierdo, 2012. First report of *Cylindrocladiella parva* and *C. peruviana* associated with black-foot disease of grapevine in Spain. *Plant Disease* 96, 1381.

Agustí-Brisach C. and J. Armengol, 2012. Effects of temperature, pH and water potential on mycelial growth, sporu-

- lation and chlamydospore production in culture of *Cylindrocarpon* spp. associated with black foot of grapevines. *Phytopathologia Mediterranea* 51, 37–50
- Agustí-Brisach C., D. Gramaje, J. García-Jiménez and J. Armengol, 2013a. Detection of *b*lack-foot disease pathogens in the grapevine nursery propagation process in Spain. *European Journal of Plant Pathology* 137: 103-112.
- Agustí-Brisach C., D. Gramaje, J. García-Jiménez and J. Armengol, 2013b. Detection of black-foot and Petri disease pathogens in natural soils of grapevine nurseries and vineyards using bait plants. *Plant and Soil* 364, 5–13.
- Agustí-Brisach C., L. Mostert and J. Armengol, 2013c. Detection and quantification of *Ilyonectria* spp. associated with black-foot disease of grapevine in nursery soils using multiplex, nested PCR and real-time PCR. *Plant Pathology*, DOI 10.1111/ppa.12093.
- Alaniz S., M. Leon, J. García-Jiménez, P. Abad and J. Armengol, 2007. Characterization of *Cylindrocarpon* species associated with black-foot disease of grapevine in Spain. *Plant Disease* 91, 1187–1193.
- Alaniz S., P. Abad-Campos, J. García-Jiménez and J. Armengol, 2011a. Evaluation of fungicides to control Cylindrocarpon liriodendri and Cylindrocarpon macrodidymum in vitro, and their effect during the rooting phase in the grapevine propagation process. Crop Protection 30, 489–484.
- Alaniz S., C. Agustí-Brisach, D. Gramaje, M.I. Aguilar, A. Pérez-Sierra and J. Armengol, 2011b. First report of *Campylocarpon fasciculare* causing black foot disease of grapevine in Spain. *Plant Disease* 95, 1028–1029.
- Alaniz, S., J. Armengol, M. León, J. García-Jiménez and P. Abad-Campos, 2009. Analysis of genetic and virulence diversity of Cylindrocarpon liriodendri and C. macrodidymum associated with black foot disease of grapevine. Mycological Research 113, 16–23.
- Alaniz S., J. García-Jiménez, P. Abad-Campos and J. Armengol, 2010. Susceptibility of grapevine rootstocks to Cylindrocarpon liriodendri and C. macrodidymum. Scientia Horticulturae 125, 305–308.
- Álvarez L.A., D. Tamayo, C. Castilla, C. Munive, C. Agustí-Brisach, D. Gramaje and J. Armengol, 2012. Occurrence of grapevine trunk pathogens in nurseries and vineyards in the northern and southern coast of Peru. *Phytopathologia Mediterranea* 51, 425 (abstract).
- Armengol J., A. Vicent, L. Torné, F. García-Figueres and J. García-Jiménez, 2001. Fungi associated with esca and grapevine declines in Spain: a three-year survey. *Phytopathologia Mediterranea* 40, 325–329.
- Aroca A., F. García-Figueres, L. Bracamonte, J. Luque and R. Raposo, 2006. A survey of trunk pathogens within rootstocks of grapevines in Spain. *European Journal of Plant Pathology* 115, 195–202.
- Aroca A., D. Gramaje, J. Armengol, J. García-Jiménez and R. Raposo, 2010. Evaluation of the grapevine nursery propagation process as a source of *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* and occurrence of trunk disease pathogens in rootstock mother vines in Spain. *European Journal of Plant Pathology* 126, 165–174.
- Auger J., M. Esterio and I. Pérez, 2007. First report of black foot disease of grapevine caused by Cylindrocarpon macro-

- didymum in Chile. Plant Disease 91, 470.
- Badour C., 1969. Gangrène ou Pied noir. Le Vigneron Champenois 5, 197–201.
- Bleach C.M., R.J. Cope, E.E. Jones, H.J. Ridgway and M.V. Jaspers, 2008. Impact of mycorrhizal colonisation on grapevine establishment in *Cylindrocarpon* infested soil. *New Zealand Plant Protection* 61, 311–316.
- Bleach C.M., E.E. Jones and M.V. Jaspers, 2010. Biofumigation using brassicaceous plant products to control *Cylindrocarpon* black foot disease in New Zealand soils. *Phytopathologia Mediterranea* 49, 128.
- Boesewinkel H.J., 1982. Cylindrocladiella, a new genus to accommodate Cylindrocladium parvum and other small-spored species of Cylindrocladium. Canadian Journal of Botany 60, 2288–2294.
- Booth C., 1966. The genus *Cylindrocarpon*. *Mycological Papers* 104, 1–56.
- Brayford D., 1993. Cylindrocarpon. In: Methods for Research on Soilborne Phytopathogenic Fungi. (Singleton L.L., Mihail J.D., Rush C.M., ed.). APS Press, St. Paul, MN, USA, 265 pp.
- Brayford D., B.M. Honda, F.R. Mantiri and G.J. Samuels, 2004. Neonectria and Cylindrocarpon: the Nectria mammoidea group and species lacking microconidia. Mycologia 96, 572–597.
- Brown D., E.E. Jones, H.J. Ridgway and M.V. Jaspers, 2012. Effect of partial defoliation on *Cylindrocarpon destructans* infection of grapevines. *New Zealand Plant Protection* 65, 256–261.
- Cabral A., J.Z. Groenewald, C. Rego, H. Oliveira and P.W. Crous, 2012a. Cylindrocarpon root rot: multi-gene analysis reveals novel species within the Ilyonectria radicicola species -complex. Mycological Progress 11, 655–688.
- Cabral A., C. Rego, P.W. Crous and H. Oliveira, 2012b. Virulence and cross-infection potential of *Ilyonectria* spp. to grapevine. *Phytopathologia Mediterranea* 51, 340–354.
- Cabral A., C. Rego, T. Nascimento, H. Oliveira, J.Z. Groenewald and P.W. Crous, 2012c. Multi-gene analysis and morphology reveal novel *Ilyonectria* species associated with black foot disease of grapevines. *Fungal Biology* 116, 62–80.
- Cardoso M., I. Diniz, A. Cabral, C. Rego and H. Oliveira, 2012. Unveiling inoculum sources of Black foot pathogens in a commercial grapevine nursery. *Phytopatologia Mediterranea* 51, 442–443.
- Casieri L., V. Hofstetter, O. Viret and K. Gindro, 2009. Fungal communities living in the wood of different cultivars of young *Vitis vinifera* plants. *Phytopathologia Mediterranea* 48, 73–83.
- Chaverri P., C. Salgado, Y. Hirooka, A.Y. Rossman and G.J. Samuels, 2011. Delimitation of Neonectria and Cylindrocarpon (Nectriaceae, Hypocreales, Ascomycota) and related genera with Cylindrocarpon-like anamorphs. Studies in Mycology 68, 57–78.
- Cooke M.C., 1884. Synopsis pyrenomycetum. Grevillea 12, 97.
  Correia K.C., M.P.S. Câmara, M.A.G. Barbosa, R. Sales Jr., C. Agustí-Brisach, D. Gramaje, J. García-Jiménez, P. Abad-Campos, J. Armengol and S.J. Michereff, 2012. Fungal species associated with trunk diseases of table grapes in Northeastern Brazil. Phytopathologia Mediterranea 51, 427.
- Crous P.W. and M.J. Wingfield, 1993. A re-evaluation of Cylin-

- drocladiella, and a comparison with morphologically similar genera. Mycological Research 97, 433–448.
- Crous P.W., A.J.L. Phillips and M.J. Wingfield, 1993. New records of *Cylindrocladium* and *Cylindrocladiella* spp. in South Africa. *Mycological Research* 42, 302–305.
- De Francisco M.T., L. Martin, R. Cobos, P. García-Benavides and M.T. Martin, 2009. Identification of *Cylindrocarpon* species associated with grapevine decline in Castilla y León (Spain). *Phytopathologia Mediterranea* 48, 167 (abstract).
- Dubrowski S. and A.L. Fabritius, 2007. Occurrence of *Cylindrocarpon* spp. in nursery grapevines in California. *Phytopathologia Mediterranea* 46, 84–86.
- Erper I., B. Tunali, C. Agustí-Brisach and J. Armengol, 2011. First report of *Cylindrocarpon liriodendri* on kiwifruit in Turkey. *Plant Disease* 95, 76.
- Fisher M. and H.H. Kassemeyer, 2003. Fungi associated with Esca disease of grapevine in Germany. *Vitis* 42, 109–116.
- Fourie P.H. and F. Halleen, 2001. Diagnosis of fungal diseases and their involvement in 13 dieback disease of young vines. *Wynboer* 149, 19–23.
- Fourie P.H. and F. Halleen, 2002. Investigation on the occurrence of *Phaeomoniella chlamydospora* in canes of rootstock mother vines. *Australasian Plant Pathology* 31, 425–426.
- Fourie P.H. and F. Halleen, 2004. Occurrence of grapevine trunk disease pathogens in rootstock mother plants in South Africa. *Australasian Plant Pathology* 33, 313–315.
- Fourie P.H. and F. Halleen, 2006. Chemical and biological protection of grapevine propagation material from trunk disease pathogens. *European Journal of Plant Pathology* 116, 255–265.
- Fourie P.H., F. Halleen, J. van der Vyver and W. Schrueder, 2001. Effect of *Trichoderma* treatments on the occurrence of decline pathogens on the roots and rootstocks of nursery plants. *Phytopathologia Mediterranea* 40, S473–S478.
- Garrido L.R., O.R. Sônego and A.F. Urben, 2004. *Cylindrocar*pon destructans causador do "Pie-Preto" da videira no Rio Grande do Sul. *Fitopatologia Brazileira* 29, 548–550.
- Gatica M., C. Césari, S. Magnin and J. Dupont, 2001. *Phaeoacremonium* species and *Phaeomoniella chlamydospora* in vines showing "hoja de malvón" and young vine decline symptoms in Argentina. *Phytopathologia Mediterranea* 40, S317–S324.
- Giménez-Jaime A., A. Aroca, R. Raposo, J. García-Jiménez and J. Armengol, 2006. Occurrence of fungal pathogens associated with grapevine nurseries and the decline of young vines in Spain. *Journal of Phytopathology* 15, 598–602.
- Gramaje D., S. Alaniz, P. Abad-Campos, J, García-Jiménez and J. Armengol, 2010. Effect of hot-water treatments *in vitro* on conidial germination and mycelial growth of grapevine trunk pathogens. *Annals of Applied Biology* 156, 231–241.
- Gramaje D. and J. Armengol, 2011. Fungal trunk pathogens in the grapevine propagation process: Potential inoculum sources, detection, identification, and management strategies. *Plant Disease* 95, 1040–1055.
- Grasso S. and G. Magnano Di San Lio, 1975. Infezioni di *Cylindrocarpon obtusisporum* su piante di vite in Sicilia. *Vitis* 14, 38–39.
- 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.
- Gubler W.D., K. Baumgartner, G.T. Browne, A. Eskalen, S.

- Rooney-Latham, E. Petit and L.A. Bayramian, 2004. Root diseases of grapevines in California and their control. *Australasian Plant Pathology* 33, 157–165.
- Gugino B.K. and J.W. Travis, 2003. Suppression of *Cylindro-carpon destructans* utilizing composted soil amendments. *Phytopathology* 93, S31.
- Halleen F., P.W. Crous and O. Petrini, 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., P.H. Fourie and P.W. Crous, 2006a. A review of black foot disease of grapevine. *Phytopathologia Mediter*ranea 45, S55–S67.
- Halleen F., P.H. Fourie and P.W. Crous, 2007. Control of black foot disease in grapevine nurseries. *Plant Pathology* 56, 637–645.
- Halleen F., H.J. Schroers, J.Z. Groenewald and P.W. Crous, 2004. Novels species of Cylindrocarpon (Neonectria) and Campylocarpon gen. nov. associated with black-foot disease of grapevines (Vitis spp). Studies in Mycology 50, 431–455.
- Halleen F., H.J. Schroers, J.Z. Groenewald, C. Rego, H. Oliveira and P.W. Crous, 2006b. Neonectria liriodendri sp. nov., the main causal agent of black foot disease of grapevine. Studies in Mycology 55, 227–234.
- Jaspers M.V., C.M. Bleach and I.C. Harvey, 2007. Susceptibility of grapevine rootstocks to Cylindrocarpon disease. Phytopathologia Mediterranea 46, 114.
- Jones E.E., D.S. Brown, C.M. Bleach, B. Pathrose, C. Barclay, M.V. Jaspers and H.J. Ridgway, 2012. First report of *Cylindrocladiella parva* as a grapevine pathogen in New Zealand. *Plant Disease* 96, 144.
- Larignon P., 1999. Black foot disease in France. In: Black Goo Occurrence and Symptoms of Grapevine Declines - IAS/ ICGTD Proceedings 1998 (L. Morton, ed.), 8 International Ampelography Society, Fort Valley, VA, USA, 89–90.
- Lombard L., C.M. Bezuidenhout and P. Crous, 2013. Ilyonectria black foot rot associated with *Proteaceae*. Australasian Plant Pathology 42, 337–349.
- Lombard L., R.G. Shivas, C. To-Anun and P.W. Crous, 2012. Phylogeny and taxonomy of the genus *Cylindrocladiella*. *Mycological Progress* 11, 835–868.
- MacDonald J.D. and E.E. Butler, 1981. Cylindrocarpon root rot of tulip poplar. *Plant Disease* 65, 154–157.
- Maluta D.R. and P. Larignon, 1991. Pied-noir: mieux vaut prévenir. *Viticulture* 11, 71–72.
- Mantiri F.R., G.J. Samuels, J.E. Rahe and B.M. Honda, 2001. Phylogenetic relationships in *Neonectria* species having *Cylindrocarpon* anamorphs inferred from mitochondrical ribosomal DNA sequences. *Canadian Journal of Botany* 79, 334–340.
- Martin M.T., L. Martin, M.J. Cuesta and P. García-Benavides, 2011. First report of *Cylindrocarpon pauciseptatum* associated with grapevine decline from Castilla y León, Spain. *Plant Disease* 95, 361.
- Menkis A. and D. Burokiene, 2012. Distribution and genetic diversity of the root-rot pathogen *Neonectria macrodidyma* in a forest nursery. *Forest Pathology* 42, 79–83.
- Mohammadi H., S. Alaniz, Z. Banihashemi and J. Armengol, 2009. Characterization of Cylindrocarpon liriodendri associated with black foot disease of grapevine in Iran. Journal of Phytopathology 157, 642–645.

- Nascimento T., C. Rego and H. Oliveira, 2007. Potential use of chitosan in the control of grapevine trunk diseases. *Phyto-pathologia Mediterranea* 46, 218–224.
- O'Gorman D.T. and P. Haag, 2011. Trunk diseases of wine grapes in Okanagan Valley. In: *Canadian Plant Disease Survey* 91, 152–154.
- Oliveira H., M.C. Rego and T. Nascimento, 2004. Decline of young grapevines caused by fungi. *Acta Horticulturae* 652, 295–304.
- Özben S., F. Demirci, K. Degirmenci and S. Uzunok, 2012. First report of *Cylindrocarpon macrodidymum* associated with black foot disease of grapevine in Turkey. *Plant Disease* 96, 762.
- Petit E. and W.D. Gubler, 2005. Characterization of *Cylindrocarpon* species, the cause of black foot disease of grapevine in California. *Plant Disease* 89, 1051–1059.
- Petit E. and W.D. Gubler, 2006. Influence of *Glomus intraradices* on black foot disease caused by *Cylindrocarpon macrodidymum* on *Vitis rupestris* under controlled conditions. *Plant Disease* 90, 1481–1484.
- Petit E. and W.D. Gubler, 2007. First report of *Cylindrocarpon liriodendri* causing black foot disease of grapevine in California. *Plant Disease* 91, 1060.
- Petit E., E. Barriault, K. Baumgartner, W.F. Wilcox and P.E. Rolshausen, 2011. *Cylindrocarpon* species associated with black-foot of grapevine in northeastern United States and southeastern Canada. *American Journal of Enology and Viticulture* 62, 177–183.
- Probst C., E.E. Jones, H.J. Ridgway and M.V. Jaspers, 2012 *Cylindrocarpon* black foot in nurseries-two factors that can increase infection. *Australasian Plant Pathology* 41, 157–163.
- Rahman M. and Z.K. Punja, 2005. Factors influencing development of root rot on ginseng caused by Cylindrocarpon destructans. Phytopathology 95, 1381–1389.
- Rego M.C.F., 1994. Nova e grave doença da videira em Portugal. Agente responsável: *Cylindrocarpon destructans* (Zins.) Scholten. Publicação do Laboratório de Patologia Vegetal "Veríssimo de Almeida" 67, 1–4.
- Rego C., A. Carvalho, T. Nascimento and H. Oliveira, 2001a. First approach on the understanding of inoculum sources of Cylindrocarpon destructans and Phaeomoniella chlamydospora concerning grapevine rootstocks in Portugal. Bulletin IOBC/WPRS 24, 67–72.
- Rego C., L. Farropas, T. Nascimento, A. Cabral and H. Oliveira, 2006. Black foot of grapevine: sensitivity of *Cylindrocarpon destructans* to fungicides. *Phytopathologia Mediterranea* 45, S93–S100.
- Rego C., T. Nascimento, A. Cabral, M.J. Silva and H. Oliveira, 2009. Control of grapevine wood fungi in commercial nurseries. *Phytopathologia Mediterranea* 48, 128–135.
- Rego C., T. Nascimento, A. Cabral and H. Oliveira, 2005. Fungi associated with young vine decline in Portugal: results of nine years surveys. OILB WPRS Bulletin 29, 123–126.
- Rego C., T. Nascimento and H. Oliveira, 2001b. Characterisation of *Cylindrocarpon destructans* isolates from grapevine in Portugal. *Phytopathologia Mediterranea* 40, S343–S350.
- 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.
- Rossman A.Y., G.J. Samuels, C.T. Rogerson and R. Lowen, 1999. Genera of Bionectriaceae, Hypocreaceae and Nectriaceae (Hypocreales, Ascomycetes). Studies in Mycology 42, 1–260.
- Rumbos I. and R. Rumbou, 2001. Fungi associated with esca and young grapevine decline in Greece. *Phytopathologia Mediterranea* 40, S330–S335.
- Russi A., R. Nalin, G. Dequigiovanni, R. Gava, V. Quecini, L.R. Garrido and P. Ritschel, 2010. Study of the genetic variability of Brazilian populations of *Cylindrocarpon* spp., causal agent of grapevine black foot. *Phytopathologia Mediterranea* 49, 111–112.
- 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 and W.D. Gubler, 1998a. First report of black-foot disease, caused by *Cylindrocarpon obtusisporum*, of grapevine in California. *Plant Disease* 82, 448.
- Scheck H.J., S.J. Vasquez, D. Fogle and W.D. Gubler, 1998b. Grape growers report losses to black-foot and grapevine decline. *California Agriculture* 52, 19–23.
- Schroers H.J., M. Zerjav, A. Munda, F. Halleen and P.W. Crous, 2008. *Cylindrocarpon pauciseptatum* sp. nov., with notes on *Cylindrocarpon* species with wide, predominantly 3-septate macroconidia. *Mycological Research* 112, 82–92.
- Seifert K.A., C.R. McMullen, D. Yee, R.D. Reeleder and K.F. Dobinson, 2003. Molecular differentiation and detection of ginseng-adapted isolates of the root rot fungus *Cylindrocarpon destructans*. *Phytopathology* 93, 1533–1542.
- Stephens P.M., C.W. Davoren and T. Wicks, 1999. Effect of methyl bromide, metham sodium and the biofumigants Indian mustard and canola on the incidence of soilborne fungal pathogens and growth of grapevine nursery stock. *Australasian Plant Pathology* 28, 187–196.
- Úrbez-Torres J.R., F. Peduto and W.D. Gubler, 2012 First report of *Ilyonectria macrodidyma* causing root rot of olive trees (*Olea europaea*) in California. *Plant Disease* 96, 1378.
- Van Coller G.J., S. Denman, J.Z. Groenewald, S.C. Lamprecht and P.W. Crous, 2005. Characterisation and pathogenicity of *Cylindrocladiella* spp. associated with root and cutting rot symptoms of grapevines in nurseries. *Australasian Plant Pathology* 34, 489–498.
- Victor D., P.W. Crous, B.J.H. Janse, W.H., Van Zyl, M.J. Wing-field and A. Alfenas, 1998. Systematic appraisal of species complexes within *Cylindrocladiella*. *Mycological Research* 102, 273–279.
- Vitale A., D. Aiello, V. Guarnaccia, G. Perrone, G. Stea and G. Polizzi, 2012. First Report of Root Rot Caused by *Ilyonectria* (=Neonectria) macrodidyma on Avocado (Persea americana) in Italy. *Journal of Phytopathology* 160, 156–159.
- Whitelaw-Weckerd M.A., N.G. Nair, R. Lamont, M. Alonso, M.J. Priest and R. Huang, 2007. Root infection of Vitis vinifera by Cylindrocarpon liriodendri in Australia. Australasian Plant Pathology 36, 403–406.
- Wollenweber H.W., 1913. Ramularia, Mycosphaerella, Nectria, Calonectria. Phytopathology 3, 197–242.

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