*Phytopathologia Mediterranea* (2013) 52, 2, 245–261

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 rootstock 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-

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ISSN (print): 0031-9465
ISSN (online): 1593-2095


**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 μm) (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 MacDonald 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 *Ilyonectria 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)×5–7.5 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 *et al*., 2004), Spain (Alaniz *et al*., 2011b) and Brazil (Correia *et al*., 2012). |
| *Campylocarpon pseudofasciculare* Halleen, Schroers & Crous | South Africa (Halleen *et al*., 2004), Uruguay (Abreo *et al*., 2010), Brazil (Correia *et al*., 2012) and Perú (Álvarez *et al*., 2012) |
| *"Cylindrocarpon" destructans* (Zinssm.) Scholten | France (Maluta and Larignon, 1991), Italy (Grasso, 1984), Argentina (Gatica *et al*., 2001), Germany (Fischer and Kassemeyer, 2003), Pennsylvania (Gugino and Travis, 2003), Brazil (Garrido *et al*., 2004) and Canada (Petit *et al*., 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 *et al*., 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 *et al*., 2008), Uruguay (Abreo *et al*., 2010) Canada (O'Gorman and Haag, 2011), Spain (Martin *et al*., 2011) and Portugal (Cabral *et al*., 2012a) |
| *Cylindrocladiella parva* (P.J. Anderson) Boesew. | South Africa (Van Coller *et al*., 2005), New Zealand (Jones *et al*., 2012) and Spain (Agustí-Brisach *et al*., 2012) |

(*Continued*)

**Table 1.** *Continues.*

| Species | Distribution |
|---|---|
| *Cylindrocladiella peruviana* (Bat., J.L. Bezerra & M.P. Herrera) Boesew. | South Africa (Van Coller *et al*., 2005), Spain (Agustí-Brisach *et al*., 2012) and Perú (Álvarez *et al*., 2012) |
| *Ilyonectria alcacerensis* A. Cabral, Oliveira & Crous | Portugal (Cabral *et al*., 2012c) and Spain (Agustí-Brisach *et al*., 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 *et al*., 2006b), Australia (Whitelaw-Weckert *et al*., 2007), California (Petit and Gubler, 2007), Spain (Alaniz *et al*., 2007), Iran (Mohammadi *et al*., 2009), Switzerland (Casieri *et al*., 2009), Brazil (Russi *et al*., 2010), Uruguay (Abreo *et al*., 2010), northeastern United States and southeastern Canada (Petit *et al*., 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 *et al*., 2004), California (Petit and Gubler, 2005), Chile (Auger *et al*., 2007), Uruguay (Abreo *et al*., 2010), northeastern United States and southeastern Canada (Petit *et al*., 2011), Portugal (Cabral *et al*., 2012c), Turkey (Özben *et al*., 2012) and Spain (Agustí-Brisach *et al*., 2013b). |
| *Ilyonectria novozelandica* A. Cabral, Nascimento & Crous | South Africa, USA and New Zealand (Cabral *et al*., 2012c) and Spain (Agustí-Brisach *et al*., 2013a, b) |
| *Ilyonectria pseudodestructans* A. Cabral, Rego & Crous | Portugal (Cabral *et al*., 2012a) |
| *Ilyonectria robusta* (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 *et al*., 2012c; Agustí-Brisach *et al*., 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 µm wide, which bear several phialides or a penicillus of irregular branches with terminal branches bearing 1 or several phialides. Macroconidia are as in *Ilyonectria*, but typically curved, and with up to 6 septa, [(24–)35–60(–62)×6.5–9 µm], 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,

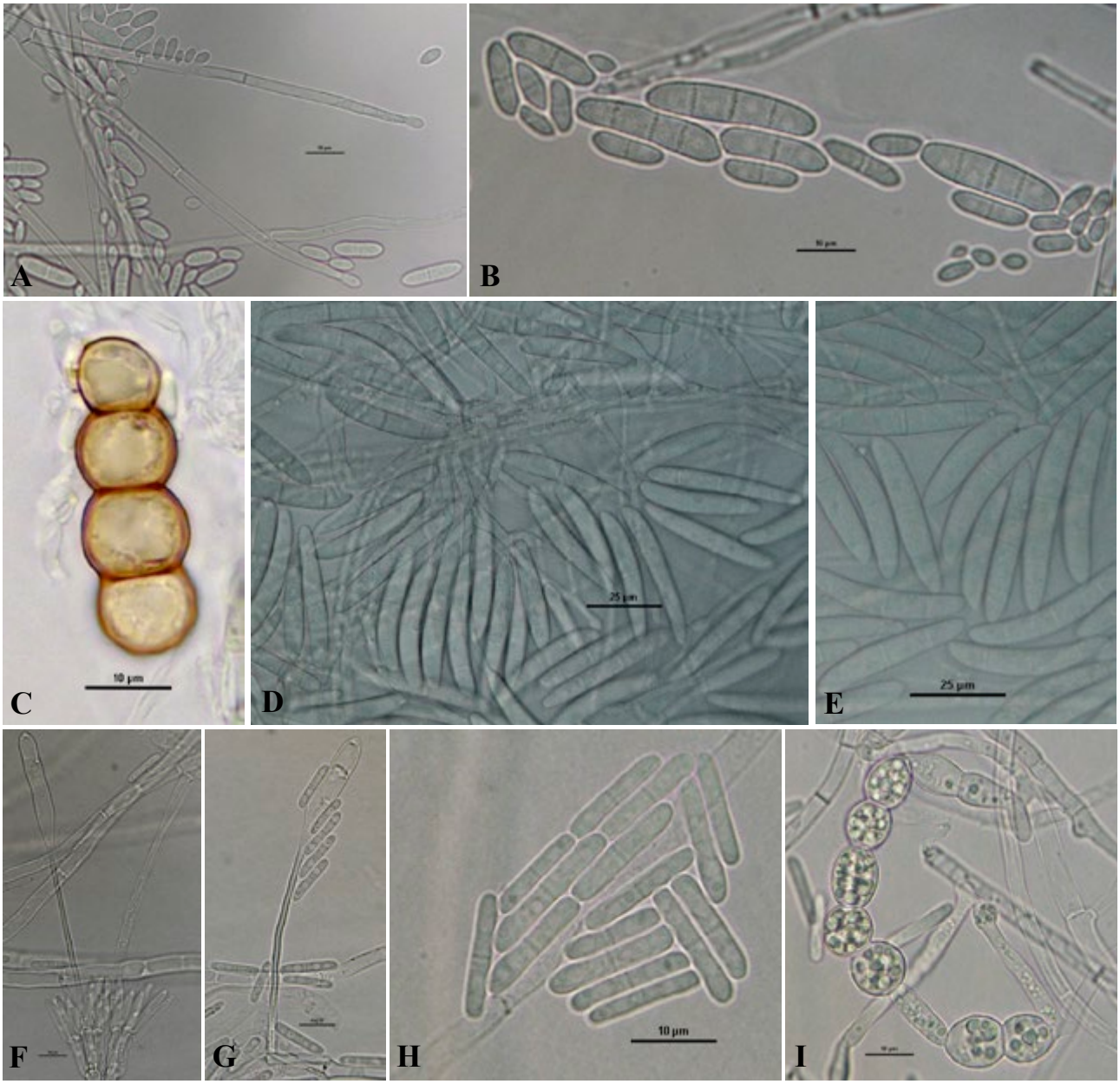


**Figure 2.** A, Conidiophores of *I. liriodendri*; B, Macro- and microconidia of *Ilyonectria liriodendri*; C, Chlamydospores in chains of "*Cylindrocarpon*" *pauciseptatum*; D, Conidiophores of *Campyl. fasciculare*; E, Macroconidia of *Campylocarpon fasciculare*; F, Penicillate conidiophores of *Cylindrocladiella 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 µm; d–e = 25 µm.

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

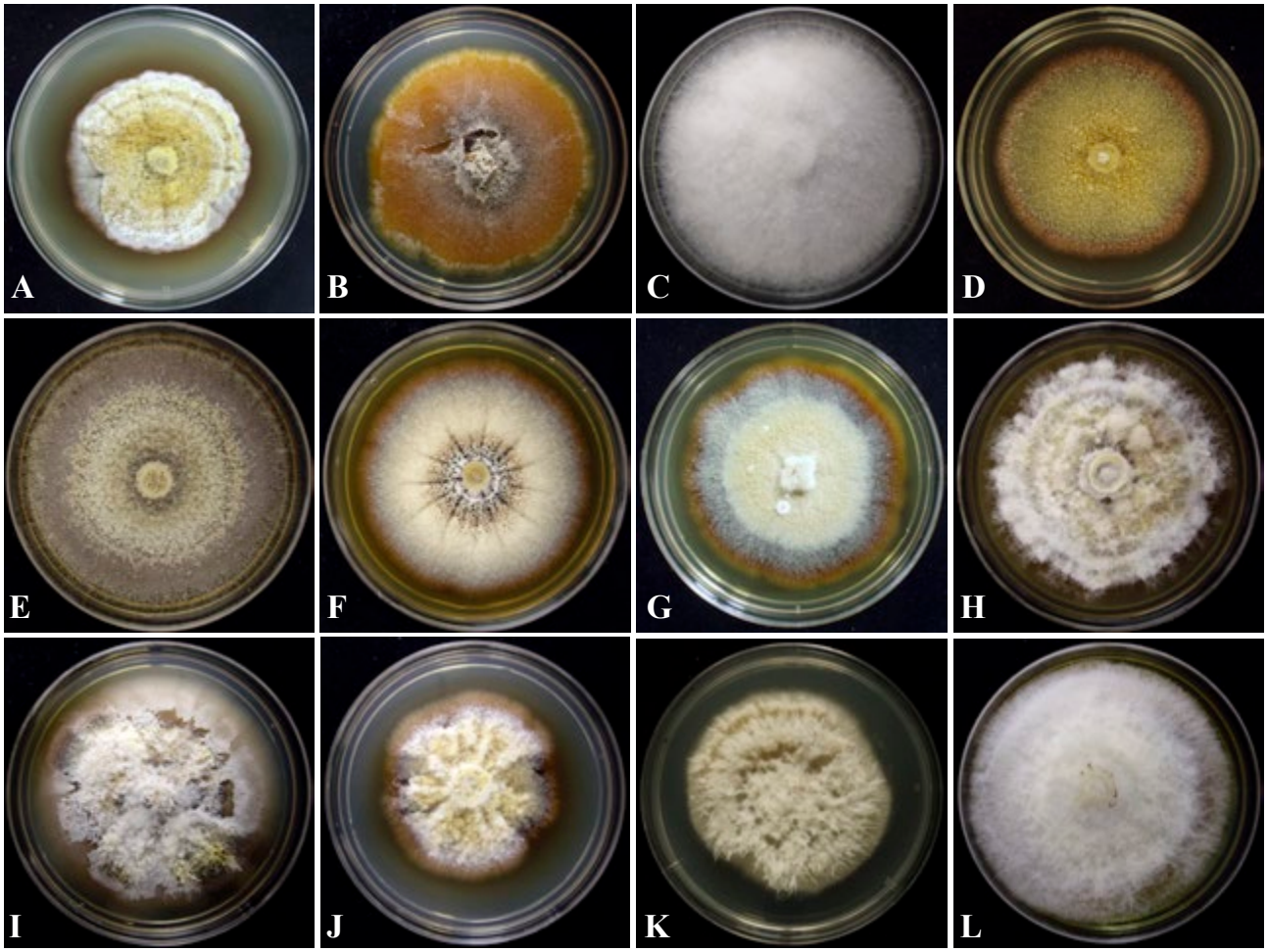


**Figure 3.** Colonies of black-foot pathogens grown on PDA. A, "*Cylindrocarpon*" *destructans* (CBS 301.93); B, "*C.*" *pauciseptatum*; C, "*C.*" *obtusisporum*; D, *Ilyonectria alcacerensis*; E, *I. liriodendri*; F, *I. novozelandica*; G, *I. macrodidyma*; H, *I. torresensis*; I, *Campylocarpon fasciculare*; 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"/Ilyonectria* | *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, multiplex-nested 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. macrodidyma*-complex 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 30ºC, with an optimum temperature between 20 to 25ºC, but they did not grow at 35ºC. 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 10ºC 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 young 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 50ºC 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 49ºC for 30, 45 or 60 min. Conidial germination was inhibited by treatments above 45ºC for 45 min, while treatments above 48ºC for 45 min were necessary to inhibit the mycelial growth. These results suggest that standard HWT protocols at 50ºC 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 isothiocyanates, 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.

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*Accepted for publication: June 17, 2013*