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A review of black foot disease of grapevine

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Summary. Black foot disease of grapevine is a relatively new, and as yet poorly known disease affecting vines in various countries where grapevines are cultivated. The causal organisms, their distribution, associated symptoms, known epidemiology and possible management strategies are discussed. Specific attention is also given to the taxonomy of the fungi involved, and the detection methods being developed to facilitate rapid identification of these pathogens.

Key words: *Campylocarpon fasciculare*, *Campyl. pseudofasciculare, Cylindrocarpon destructans*, *C. obtusisporum*, *C. macrodidymum*, *Vitis* spp.

Introduction

Species of *Cylindrocarpon* Wollenw. are common soil inhabitants, occurring as saprobes or weak pathogens, often associated with roots of herbaceous woody plants (Brayford, 1993). However, two species, *C. destructans* (Zinnsm.) Scholten and *C. obtusisporum* (Cooke & Harkn.) Wollenw., have been reported as the causal agents of black foot disease of grapevines (*Vitis* spp. L.). Scheck *et al.* (1998a) proposed that the common name Cylindrocarpon black foot disease be used with both species, as the disease symptoms were similar. The first record 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 Tasmania (Sweetingham, 1983), Sicily (Grasso, 1984), Portugal (Rego, 1994; Rego *et al.*, 2000, 2001a) and Pennsylvania, USA (Gugino and Travis, 2003). *Cylindrocarpon obtusisporum* has been identified as the causal agent of this disease in Sicily (Grasso and Magnano di San Lio, 1975) and California (Scheck *et al.*, 1998a). Various unidentified species of *Cylindrocarpon* have also been isolated from young vines and from declining vines with basal rot or root necrosis in Chile (Auger *et al.*, 1999), Greece (Rumbos and Rumbou, 2001), Spain (Armengol *et al.*, 2001), South Africa (Fourie *et al.*, 2000; Fourie and Halleen, 2001a) and Australia (Edwards and Pascoe, 2004). In the recent taxonomic study revising the *Cylindrocarpon* spp. associated with black foot disease of grapevines, the primary causal organism was identified as *C. destructans*, while a second species was newly described as *C. macrodidymum* Schroers, Hal-

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leen & Crous (Halleen *et al*., 2004c). Furthermore, two new species were also found to represent an undescribed genus of fungi that was *Cylindrocarpon*-like in morphology, namely *Campylocarpon* Halleen, Schroers & Crous (*Campylocarpon fasciculare* Schroers, Halleen & Crous and *Campyl. pseudofasciculare* Halleen, Schroers & Crous). All four species have been implicated in this disease complex (Halleen *et al*., 2004c).

In this review the causal organisms, their distribution, associated symptoms, known epidemiology and possible management strategies are discussed. Specific attention is also given to the taxonomy of the fungi involved, and the detection methods being developed to facilitate rapid identification of these pathogens.

Symptoms

According to the literature regarding *Cylindrocarpon* spp. associated with grapevine diseases, two scenarios are evident. These scenarios might also be related to the initial source of infection and are therefore treated as 'nursery infections' and 'vineyard infections'.

Nursery infections

This scenario relates to nursery vines or younger vines shortly after transplantation where typical symptoms of vascular streaking are evident. Grasso and Magnano di San Lio (1975) described black foot symptoms from nursery plants with black discolouration and gum inclusions in xylem vessels of affected rootstocks (225 Ruggeri). Scheck *et al*. (1998a) also described dark-brown to black streaking in the vascular tissue of young (2–5-year-old) grapevines investigated in California. Affected vines showed reduced vigour with small-sized trunks, shortened internodes, uneven wood maturity, sparse foliage, and small leaves with interveinal chlorosis and necrosis (Fig. 1). Other symptoms included a reduction in root biomass and root hairs with sunken, necrotic root lesions. The pith of the affected vines was also compacted and discoloured (Scheck *et al*., 1998a) (Fig. 2). Whilst investigating rootstock nurseries in Portugal, Rego *et al*. (2000) also observed black discolouration and brown to dark streaks in wood, mainly at the base of the rootstock (Fig. 3 and 4). Investigation of older vines (2–8-year-old) also revealed the presence of *C. destructans* in the basal end of the rootstocks (Rego *et al*., 2000).

Vineyard infections

This scenario relates to infections of 2–10-year-old grapevines. Sweetingham (1983) described the death of mature vines (5 years and older) caused by *C. destructans* in Tasmania. Disease symptoms were noticed early in the growing season as affected vines achieved poor new growth, failed to form shoots after winter dormancy, and died by midsummer. Vines with reduced vegetative growth also died during the subsequent dormant winter period. A dark brown discolouration of the wood in the trunk at ground level was observed. This discolouration extended up to 15 cm above ground level, and throughout the below-ground portion of the trunk, and sometimes extended from the trunk into the larger roots for distances up to 10 cm. Sections through symptomatic tissue revealed that the majority of the xylem vessels were plugged with thick-walled tyloses or brown gum, and functional phloem elements were plugged with gum. Further microscopic examination of infected tissue revealed the presence of fungal hyphae in the ray cells of the phloem and younger xylem. Hyphae were not visible in the xylem vessels and rarely in the functional phloem. The presence of hyphae in the ray cells declined towards the centre of the trunk in the discoloured tissue and they were not visible in tissue beyond the zone of discolouration or in tissue of healthy vines. Starch reserves are mainly stored in the ray cells, providing a readily metabolisable carbon source for *C. destructans*, which can produce extracellular amylases (Sweetingham, 1983). Larignon (1999) described black foot disease as a disease affecting mainly young vines between 2 and 8 years of age. Observations in California also support this, and according to Gubler *et al*. (2004) vines up to 10 years old might succumb to the disease. When young vines are infected, death occurs quickly, but as the vine ages, infection results in a more gradual decline and death might only occur after a year (Gubler *et al*., 2004). Larignon (1999) described symptoms similar to Sweetingham (1983) where diseased vines characteristically displayed abnormal, weak vegetation and in some cases did not sprout at all. Often shoots also dried and died during the summer. Furthermore, below-ground symptoms included abnormal root

Fig. 1. Decline symptoms associated with black foot disease including absence of budding, as well as abnormal, weak vegetation.

Fig. 2. Cross-section through a black foot disease infected rootstock revealing severe necrosis extending from the bark to the pith.

Fig. 3. Dark vascular streaking as seen in longitudinal section of a young grapevine infected with *Cylindrocarpon* spp.

Fig. 4. Cross-section of an infected root.

development characterised by shallow growth parallel to the soil surface. A second crown of roots may develop on an upper level of the rootstock to compensate for the loss of functional roots further below (Larignon, 1999; Fourie *et al*., 2000). Roots of the basal crown become necrotic. In some cases the rootstock diameter of older vines is thinner below the second tier (Fourie and Halleen, 2001b). Removal of rootstock bark reveals a brown to black zone beginning at the base of the rootstock extending up along the rootstock. A cross section through the affected area reveals internal necrosis which develops from the bark to the pith (Larignon, 1999; Fourie and Halleen, 2001a).

Taxonomy and phylogeny

Teleomorphs with *Cylindrocarpon* anamorphs were traditionally classified in *Nectria* (Fr.) Fr., but are now considered to belong to *Neonectria* Wollenw. (Rossman *et al*., 1999; Mantiri *et al*., 2001; Brayford *et al*., 2004). Wollenweber based the generic name upon *Neon. ramulariae* Wollenw. (1916). The reintroduction of *Neonectria* resulted from the realisation that *Nectria* was too broadly defined and that its segregation into numerous teleomorphic genera could be corroborated by anamorphic, phylogenetic, and ecological character patterns (Rehner and Samuels 1995; Rossman *et al*., 1999).

Some pre-phylogenetic classification schemes had segregated the teleomorphs of *Cylindrocarpon* species into four infrageneric *Nectria* groups, based on perithecial wall anatomy and ascospore morphology; these groups were centred on '*Nectria*' *radicicola* Gerlach & L. Nilsson, '*Nectria*' *coccinea* (Pers. : Fr.) Fr., '*Nectria*' *mammoidea* Phill. & Plowr., and '*Nectria*' *rugulosa* Pat. & Gaillard (Booth, 1959; Samuels and Brayford, 1990; Samuels and Brayford, 1994). Wollenweber (1917, 1928) created the sections *Chlamydospora* Wollenw. and *Ditissima* Wollenw. for species with and without chlamydospores, respectively.

Booth (1966) schematically segregated *Cylindrocarpon* species into four groups based on the presence or absence of microconidia and chlamydospores. *Cylindrocarpon magnusianum* (Sacc.) Wollenw., which is the anamorph of the type species of *Neonectria*, *C. cylindroides* Wollenw., which is the type species of the genus *Cylindrocarpon*, *C. destructans*, which is the anamorph of *Neonectria radicicola*, and members of *Cylindrocarpon* species predominantly connected with teleomorphs of the '*Nectria*' *mammoidea* group were core members of the anamorphic groups delineated by Booth (1966). *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.

Currently, representatives of all '*Nectria*' groups with *Cylindrocarpon* anamorphs have been transferred into *Neonectria* (Rossman *et al*., 1999; Mantiri *et al*., 2001; Brayford *et al*., 2004). Mantiri *et al*. (2001) and Brayford *et al*. (2004) analysed 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, 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 *Vitis 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.

Significant variation in cultural and morphological characters was observed among *Cylindrocarpon* strains isolated from grapevines in nurseries and vineyards in South Africa, France, New Zealand, and Australia (Halleen *et al*., 2003; Halleen *et al*., 2004a; Halleen *et al*., unpublished). Halleen *et al*. (2004c) used morphological characters and DNA sequences to characterise these taxa

taxonomically and phylogenetically. Sequences were compared with those of members of the *Neon. radicicola* complex published by Seifert *et al*. (2003) and various other *Neonectria*/*Cylindrocarpon* species deposited at the CBS Fungal Biodiversity Centre (CBS, Utrecht, The Netherlands). Sequences of the partial nuclear large subunit ribosomal DNA (LSU rDNA), internal transcribed spacers 1 and 2 of the rDNA including the 5.8S rDNA gene (ITS), and partial β-tubulin gene introns and exons were used for phylogenetic inference. *Neonectria*/*Cylindrocarpon* species clustered in mainly three groups. One monophyletic group consisted of three subclades comprising (i) members of the *Neonectria radicicola*/*Cylindrocarpon destructans* complex, which contained strains isolated from grapevines in South Africa, New Zealand, and France; (ii) a *Neonectria*/*Cylindrocarpon* species isolated from grapevines in South Africa, Canada (Ontario), Australia (Tasmania), and New Zealand, described as *Cylindrocarpon macrodidymum*; and (iii) an assemblage of species closely related to strains identified as *Cylindrocarpon cylindroides*, the type species of *Cylindrocarpon*. This monophyletic group excluded two other groups, which comprised (i) members of the *Neonectria mammoidea* complex, with anamorphs characterised by curved macroconidia, violet or purple pigments in cultures of most of its members, and the lack of microconidia and chlamydospores; and (ii) two *Campylocarpon* species, *Campylocarpon fasciculare* and *Campylocarpon pseudofasciculare*, isolated from grapevines in South Africa. The latter two clades formed a paraphyletic group in LSU rDNA analysis, but were supported as a monophyletic group in ITS and β-tubulin gene analysis. Analyses of Halleen *et al*. (2004c) therefore excluded *Campylocarpon* and members of the *Neonectria mammoidea* group from *Neonectria*/*Cylindrocarpon*, contradicting the transfer of the mammoidea group to *Neonectria* by Brayford *et al*. (2004). *Campylocarpon* species, though similar in macroconidial morphology to members of the *Neonectria mammoidea* group, can be distinguished by the formation of typical brownish rather than violaceous cultures, as well as by production of brownish hyphae, often in strands, and in *Campyl. pseudofasciculare* by formation of chlamydospores (Halleen *et al*., 2004c).

Strains of the *Neonectria radicicola*/*Cylindrocarpon destructans* complex isolated from grapevines matched those currently placed in *C. destructans* based on morphology and DNA sequences. However, as shown by previous phylogenetic studies (Seifert *et al*., 2003; Halleen *et al*., 2004c), *C. destructans* represents a species complex. Furthermore, it appears that within this complex, different woody hosts have their own unique species, some of which are more host-specific than others. Although Halleen *et al*. (2004a) referred to the primary causal organism of black foot rot of grapevines as *C. destructans*, further work is currently in progress to resolve the taxa accommodated in this species complex on grapevines (Halleen *et al*., unpubished data).

A second species described from grapevines, *C. macrodidymum,* formed micro- and macroconidia, but rarely formed chlamydospores. Its predominantly 3-septate macroconidia were more or less straight, minutely widening towards the tip, and had an apical cell slightly bent to one side. Its teleomorph, *Neonectria macrodidyma*, was obtained in mating experiments, and was characterised by smooth to finely warted ascospores, smooth to finely warted perithecia, and moderately sized angular to subglobose cells in the outer region of the perithecial wall. *Campylocarpon* spp. were characterised by mostly 3–5-septate, curved macroconidia, and by the lack of microconidia (Halleen *et al*., 2004c). Cultural and morphological differences between, and geographical distribution of *Cylindrocarpon* and *Campylocarpon* species associated with black foot disease of grapevine are summarised in Table 1.

What happened to *C. obtusisporum*? 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* was raised by Halleen *et al*. (2004c). Macroconidia of *C. macrodidymum* measure (26–)34–36–38(–45)×(4–)5.5–6–6.5(–8) μm (Halleen *et al*., 2004c), 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. *C. obtusisporum* isolates obtained from California formed

Table 1. Summary of morphological and cultural differences between, and geographical distribution of *Cylindrocarpon* and *Campylocarpon* species associated with black foot disease of grapevine.

Characteristics	*Cylindrocarpon*		*Campylocarpon*	
	C. destructans	*C. macrodidymum*	*Campyl. fasciculare*	*Campyl. pseudofasciculare*
Microconidia	✓	✓	×	×
Macroconidia	✓ Straight or curved 3 septate (1-5)	✓ More or less straight Mostly 3 septate (1-3)	✓ Curved 3–4 septate (1–5)	✓ Curved 3–5 septate (2–6)
Chlamydospores	✓	✓ rare	×	✓ sparse
Cardinal temperatures for growth	Min: Not determined, but <4°C Opt: 20–25°C Max: ≤30°C	Min: Not determined, but <4°C Opt: 20–25°C Max: ≤30°C	Min: 10°C Opt: 30°C Max: Not determined, but ≥35°C	Min: 10°C Opt: 30°C Max: Not determined, but ≥35°C
Distribution	South Africa New Zealand Australia France Italy Portugal USA	South Africa New Zealand Australia Canada USA	South Africa	South Africa
Habitat	Roots and rootstocks	Roots and rootstocks	Roots and rootstocks	Roots

✓, Present.
×, Absent.

perithecia when cross-inoculated with *C. macrodidymum,* giving further evidence to support the misidentification theory (Halleen *et al*., unpubl. data). This was also confirmed by sequence comparisons (Ulrike Damm, University of Stellenbosch, personal communication). Petit and Gubler (2005) recently 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*.

Molecular detection

Hamelin *et al*. (1996) designed species-specific primers (Dest1 and Dest4) to detect *C. destructans* from conifer seedlings. Using these primers in direct PCR assays on DNA extracted from *C. destructans* cultures obtained from grapevines in Portugal, Nascimento *et al*. (2001) obtained a DNA fragment of 400 bp. However, Nascimento *et al*. (2001) were unable to distinguish between *C. destructans* and *C. obtusisporum* when using these primers, because an amplification of the same size was obtained for isolates of *C. obtusisporum*. Furthermore, these primers could also not detect *C. destructans* from artificially inoculated potted grapevines. The nested PCR assay developed by Hamelin *et al*. (1996) was therefore modified by Nascimento *et al*. (2001). The universal primer ITS4 and the fungus-specific primer ITS1F were used in a first-stage fungus-specific amplification, followed by a second-stage amplification with the primers Dest1 and Dest4 using the PCR product from stage one. This is a simple and reliable method for detection of *Cylindrocarpon* spp. directly from infected grapevines (Nascimento *et al*., 2001).

Damm *et al*. (2005) developed a method for the extraction of fungal DNA from soil to study the epidemiology of grapevine trunk disease pathogens in South African grapevine nurseries and

vineyards. The extracted DNA was tested for *Cylindrocarpon* spp. by using the primers Dest1 and Dest4. *Cylindrocarpon* spp. were detected in 66% of the samples investigated (Damm *et al*., 2005). Species-specific primers are currently being developed for detection of all the species involved in black foot disease in South Africa (Halleen *et al*., in prep.).

Epidemiology

Investigation of diseased vines in Tasmania showed that wood discolouration did not originate from the base of the trunk (Sweetingham, 1983). In fact, the discolouration and fungal hyphae first became evident in the buried portion of the trunk, 2–12 cm below ground surface (Sweetingham, 1983), suggesting that infection occurred at a later stage in the vineyard. Gubler *et al*. (2004) was also of the opinion that the presence of the pathogens in vineyards probably plays a larger role in disease development than infected nursery material.

Rego *et al*. (1998) speculated that rootstock nurseries might be the origin of these infections in Portugal, since severe outbreaks only occurred in vineyards where the rootstocks were sourced from the same region or even the same nursery. Surveys of rootstock nurseries located in Ribatejo-Oeste and Beira Litoral confirmed that infected rootstocks were the most likely way in which the pathogens are disseminated, although the initial source of infection was still unknown (Rego *et al*., 2000). Investigation on the occurrence of decline pathogens in canes of rootstock mother vines in Portugal and South Africa revealed extremely low levels of *Cylindrocarpon* spp. (Rego *et al*., 2001b; Fourie and Halleen, 2002). A survey of 34 certified rootstock mother blocks in six production areas, where isolations were made from the basal and pruning wound ends of 2-year-old pruning stubs, again revealed the low incidence (av. 0.17%) of *Cylindrocarpon* spp. inside rootstock mother vines (Fourie and Halleen, 2004c). An investigation of fungi occurring in asymptomatic nursery vines supported these findings in that *Cylindrocarpon* spp. were hardly ever isolated from callused grafted cuttings prior to planting in nurseries (Halleen *et al*., 2003). However, once planted in the nurseries, *Cylindrocarpon* spp. were isolated from the roots, rootstocks and graft unions. Infection of the roots occurred first, followed by infection of the rootstocks. At the time of planting, the basal ends (especially the pith area) of most of the cuttings are partly or even fully exposed for infection by soilborne pathogens. Callus roots often break during the planting process, resulting in small wounds susceptible to infection. The presence of *Cylindrocarpon* spp. in graft unions might be explained by the nursery practice where graft unions are covered with soil for a 5-week-period to prevent drying of the callus tissue (Halleen *et al*., 2003). *Cylindrocarpon* spp. occurred in graft unions of 15% of nursery grapevines investigated by Aroca and Raposo (2005). This suggests that the recommendation of Stamp (2001), namely that the graft union should be fully healed when a vine is removed from the callusing chamber 2–4 weeks after grafting, is not always followed in practice.

The production of chlamydospores would also allow *Cylindrocarpon* spp. to survive for extended periods in soil (Booth, 1966; Halleen *et al*., 2004c). However, very little information is currently available regarding the survival of these pathogens, and the role of chlamydospores during subsequent infections. In a related hypocrealean genus, *Cylindrocladium* Morgan, chlamydospores were shown to remain viable up to 15 years (Crous, 2002), which suggests that this could indeed be a very important aspect to consider in further epidemiological studies of *Cylindrocarpon*.

Rumbos and Rumbou (2001) argued that fungal infection alone could not be the sole reason of young grapevine decline in Greece, since the incidence of decline pathogens [*Cylindrocarpon* spp., *Phaeomoniella* (*Pa*.) *chlamydospora* (W. Gams, Crous & M.J. Wingf. & L. Mugnai) Crous & W. Gams, *Phaeoacremonium* spp. and *Botryosphaeria* spp.] were too low, and were present in too low a percentage of young vines. *Cylindrocarpon* spp. were isolated from only 1–4% of young vines. It was therefore speculated that abiotic factors such as lesions from improperly healed rootstock disbudding sites, and graft unions made in the nursery, as well as improper storage and transportation conditions of propagated material, could also play a role in enhancing grapevine decline (Rumbos and Rumbou, 2001).

Pathogenesis

As is the case with many other *Cylindrocarpon* species causing disease on other crops, environmental factors and host stress may also play an important part in disease development (Brayford, 1993). Stress conditions that favour development of black foot disease include malnutrition, poor water drainage, soil compaction, heavy crop loads on young plants, planting of vines in poorly prepared soil and improper plant holes (Larignon, 1999; Fourie *et al*., 2000; Fourie and Halleen, 2001a; Halleen *et al*., 2004c). Soil compaction and/or poor soil preparation will most likely contribute to poor root development (J-rooting and pothole effect) (Fourie *et al*., 2000; Halleen *et al*., 2004c). High temperatures during summer also play an important role in symptom expression. The deficient root system and altered vascular system of infected vines would not be able to supply enough water to compensate for the high transpiration rate during high temperatures (Larignon, 1999). *Cylindrocarpon* species are often part of disease complexes with other fungi or nematodes (Brayford, 1993). The example of apple replant disease is well-documented. In the case of declining vineyards, *Cylindrocarpon* spp. are often isolated together with other pathogens from the same diseased vines. These pathogens include *Pa. chlamydospora*, *Phaeoacremonium* spp. (Petri disease pathogens), *Botryosphaeria* spp., *Phomopsis* spp., *Pythium* spp. and *Phytophthora* spp. (Fourie *et al*., 2000; Fourie and Halleen, 2001c; Edwards and Pascoe, 2004; Oliveira *et al*., 2004). Disease symptoms associated with these pathogens overlap in many respects, thereby making correct diagnosis based on visual symptoms nearly impossible.

Grasso and Magnano di San Lio (1975) induced black discolouration of wood in the basal area of rooted cuttings (225 Ruggeri) similar to the symptoms observed in diseased nursery vines 60 days after artificial inoculation with *C. obtusisporum*. Scheck *et al*. (1998a) completed Koch's postulates by dipping the roots of cv. Carignane seedlings in a spore suspension of *C. obtusisporum*. Typical black foot symptoms appeared on 92% of the plants after 8 weeks. In the same experiment 67% of the plants developed symptoms after inoculation with *Phaeomoniella chlamydospora,* and 71% with *Phaeoacremonium inflatipes* W. Gams, Crous & M.J. Wingf. (recently re-identified as *Pm. aleophilum* W. Gams, Crous, M.J. Wingf. & L. Mugnai), demonstrating its virulence despite the fact that *Cylindrocarpon* spp. are generally recognised as relatively weak pathogens (Scheck *et al*., 1998b).

The first pathogenicity study with *C. radicicola* (= *C. destructans*) on grapevines was actually conducted on berries of grape variety Gordo Blanco when the fungus was consistently isolated from small, black necrotic spots on pedicels and blossom ends of Ohanez berries (Taylor, 1956). However, the inoculated fungus could invade green berries only when the skin was first ruptured and was therefore considered to be a secondary invader of already damaged tissue. Sweetingham (1983) failed to initiate infection of the basal trunk region and roots of potted 'Cabernet Sauvignon' vines when potting media were amended with *C. destructans*, despite the presence of *C. destructans* on the surface of below-ground parts. Mycelium plugs inserted into scalpel wounds in the vascular tissue of the buried portion of the trunk also resulted in no infection beyond the inoculation site. However, when 6-month-old own-rooted 'Cabernet Sauvignon' vines were inoculated with a spore suspension applied to the potting mixture directly adjacent to the trunk and the plants were then subjected to waterlogged treatments, symptoms appeared within 90 days. Leaves became chlorotic and some abscised, and vascular discolouration extending upward from the base of the cuttings was also observed in some plants. Rego *et al*. (2000) conducted pathogenicity studies with rooted cuttings of '99R' rootstock by dipping the roots in a conidial spore suspension of *C. destructans*. Typical black foot symptoms including root lesions, vascular discolouration and necrosis developed within two months. Similar results were obtained in studies conducted with rooted cuttings of cv. Seara Nova (Oliveira *et al*., 1998) and cv. Periquita (Rego *et al*., 2001a). However, in the latter study 13 *C. destructans* isolates, collected over a period of seven years, were used. Although all the isolates proved to be pathogenic, variation in virulence was observed and it was not correlated with the age of the cultures. All the isolates significantly reduced plant height and most significantly reduced the number of roots. In most cases the stunting could be explained by the shortened internodes, although it appeared as if the most virulent strains reduced the number of internodes.

Auger *et al*. (1999) also observed dark streaking of vascular elements in roots of 'Flame Seedless' vines inoculated with *Cylindrocarpon* sp. Inoculation of 6-month-old potted grapevine rootstocks ('Ramsey') with *C. destructans*, *C. macrodidymum*, *Campyl. fasciculare* and *Campyl. pseudofasciculare* resulted in death, as well as reduced root and shoot mass of inoculated plants (Halleen *et al*., 2004c).

Disease management

Curative control

No fungicides are registered for the control of black foot disease in vineyards. Recommendations to farmers have thus far been based on the prevention and/or correction of predisposing stress factors.

Plant material

Plant material should be sourced from reputable nurseries that are subjected to standards as certified by the plant improvement associations in the different countries. Good quality planting material would ensure that nursery defects such as small and incomplete root systems, rootstock lesions, incomplete graft unions, etc., which are all detrimental to field performance, be limited (Stamp, 2001).

Very little information is currently available regarding rootstock susceptibility. Gubler *et al*. (2004) reported that the rootstocks *Vitis riparia* 'O39-16' and 'Freedom' appear to show some resistance towards *C. destructans*.

Soil preparation and vineyard activities

Soil compaction might be natural in some soils or may be the consequence of certain cultural practices. Compacted layers should be broken up during the soil preparation stages for new establishments to make the subsoil accessible to roots (Larignon, 1999). Plant holes should be deep and big enough to facilitate proper root development (Fourie *et al*., 2000). Excessive movement of farm vehicles result in soil compaction, especially when the soil is wet or poorly drained, and this should therefore be avoided (Larignon, 1999; Halleen *et al*., 2004c). New vineyards should not be established on heavy, poorly drained soils (Larignon, 1999; Gubler *et al*., 2004). Drainage in heavy soils can be achieved by planting on berms and moving drip irrigation emitters away from the vine (Gubler *et al*., 2004). Waterlogged situations can also be the consequence of drip irrigation systems where the drippers are positioned in such a way that the trunk is maintained in a waterlogged environment for most of the year, especially in excessive irrigation regimes (Sweetingham, 1983). Planting of certified vines according to best practice procedures and thereafter carefully managed in such a way that roots can develop properly to such an extent that it can carry a decent crop, should go a long way in ensuring successful establishment of a new vineyard.

Soil health is another important aspect to take into consideration. Preliminary results regarding the suppression of *C. destructans* by means of composted soil amendments have recently been published. Several microorganisms isolated from the compost have demonstrated antagonism towards *C. destructans* (Gugino and Travis, 2003).

Fluctuations in soil organic matter may result in changes to the populations of bacteria and actinomycetes able to produce antibiotics (Whitelaw-Weckert, 2004). Whitelaw-Weckert (2004) investigated the effect of mulch and organic matter from herbicide treated weeds on the populations of vineyard soil bacteria and actinomycetes and their effect on *C. destructans*. *In vitro* evaluations revealed that 70% of the bacteria and actinomycetes from a herbicide inter-row treatment inhibited *C. destructans*. Populations of these microorganisms were also seven times higher in soil from this treatment compared to the herbicide under-vine only and no herbicide treatments.

Nursery practices

As mentioned previously, research has shown that black foot disease fungi infect grapevine cuttings when planted in infested nursery soils (Halleen *et al*., 2003). Control methods should therefore focus on preventing or eradicating infection in the basal ends of these cuttings. *In vitro* studies conducted in South Africa revealed that benomyl, flusilazole and prochloraz manganese chloride were the most effective fungicides (Halleen *et al*., 2005). Nursery trials were conducted to evaluate the effectiveness of various physical, chemical and biological treatments aimed at protecting the basal ends of rootstocks against infection. After callusing, the basal ends of grafted cuttings were dipped

in various treatments prior to planting. Additional treatments involved soil amendments with *Trichoderma* formulations and hot water treatment (50°C for 30 min) of dormant nursery grapevines. Nursery plants were uprooted after eight months (Halleen *et al*., 2004b). The incidence of black foot disease pathogens in the basal ends was not significantly and/or consistently reduced by the majority of chemical and biological treatments investigated. However, no black foot disease fungi were isolated from the plants that were subjected to hot water treatment (Halleen *et al*., 2005). Halleen *et al*. (2005) therefore recommended that hot water treatment of dormant nursery grapevines be included in an integrated strategy for the proactive management of black foot disease in grapevine nurseries. Previously this treatment was also recommended for the eradication of several pests and diseases from dormant propagation material and/or nursery grapevines, including *Meloidogyne javanica* (Treub) Chitwood (Barbercheck, 1986), *Phytophthora cinnamomi* Rands (Von Broembsen and Marais, 1978), phytoplasmas (Caudwell *et al*., 1997), and the causal organism of Pierce's disease (Goheen *et al*., 1973). It was also found to be effective in reducing crown gall (Ophel *et al*., 1990), as well as *Pa. chlamydospora* and *Phaeoacremonium* spp. that cause Petri disease of grapevines (Fourie and Halleen, 2004a).

The following fungicides inhibited mycelial growth of *C. destructans in vitro*: prochloraz, benomyl, cyprodinil + fludioxonil and carbendazim + flusilazole, whilst tebuconazole and difenoconazole were less effective (Rego *et al*., 2005). Cyprodinil + fludioxonil, azoxystrobin, trifloxystrobin and tolyfluanide effectively reduced spore germination. *In vivo* studies on potted grapevines proved that benomyl, tebuconazole, carbendazim + flusilazole and cyprodinil + fludioxonil significantly improved plant growth and decreased disease incidence compared with non-treated vines (Rego *et al*., 2005).

In South Africa, the same soil in grapevine nurseries has been used for decades. Standard nursery practice of a two-year rotation system, whereby cuttings are planted every second year, alternated with a cover crop, might have led to a build-up of soilborne pathogens such as species of *Cylindrocarpon* (Halleen *et al*., 2003). In earlier studies these species appeared insignificant (Marais 1979, 1980). The duration of this rotation period and the type of cover crop should therefore be investigated to establish its effect on pathogen populations.

Biological control

Gubler *et al*. (2004) reported that the mycorrhizal fungus *Glomus intraradices* Schenck & Smith provided excellent control against black foot disease if applied to grapevines in advance of *Cylindrocarpon* inoculation.

The growth stimulating attributes of *Trichoderma* Pers. treatments (dips, soil amendments and drenches with products containing propagules of selected strains of *Trichoderma harzianum* Rifai, [Agrimm Technologies Ltd., Christchurch, New Zealand]), and the effect thereof on the occurrence of decline pathogens including *Cylindrocarpon* spp. were investigated in South African nurseries (Fourie *et al*., 2001). The treatments consisted of rootstock drenches with Trichoflow-T™ before and directly after grafting, planting of grafted vines in planting furrows pre-inoculated with Trichopel™, and monthly root drenches with Trichogrow™. The treatments reduced the incidence of *Cylindrocarpon* spp. in nursery grapevines and significantly improved root development, which would undoubtedly make plants more tolerant when subjected to stress (Fourie *et al*., 2001).

Conclusions

Black foot disease of grapevine is a relatively new, and as yet poorly known disease affecting vines in various countries where grapevines are cultivated. The diversity of species associated with the disease has been confirmed by recent studies. The fact that these species have the ability to infect grapevines as early as the nursery stage, has clearly placed the emphasis on the importance of suitable control methods to prevent or eradicate these infections. Chemical treatments evaluated under field situations thus far were not very successful, although some promising results were recently obtained with potted grapevines. Soil amendments with *Trichoderma* spp., mycorrhizae and compost appear to be an effective measure to boost plant resistance, especially when these plants are subjected to stress situations. The reduction of black foot pathogens in uprooted, dormant nursery grapevines caused by hot water treatment, clearly demonstrated the potential of this control

measure to be included in an integrated strategy for the pro-active control of grapevine trunk disease pathogens in grapevine nurseries. However, apart from these measures, no cure is known for declining grapevines in vineyards. Recommendations to farmers are therefore aimed at the prevention and reduction of predisposing stress situations, such as soil compaction and poor drainage. Considering all these factors, it is clear that soil preparation and establishment of new vineyards should be done according to best practice procedures. Planting of certified vines, followed by efficient vineyard management should ensure successful establishment of new vineyards.

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