Phytopathol. Mediterr. (2006) 45, S43–S54

Botryosphaeria spp. as grapevine trunk disease pathogens

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Summary. Several species of *Botryosphaeria*, including *Botryosphaeria dothidea*, *B. obtusa*, *B. parva* and *B. australis*, were isolated and/or described from declining grapevines in association with a wide range of decline and dieback symptoms. Internal wood symptoms most commonly included black streaking, wedge-shaped necrosis and brown internal necrosis. Several diseases under names such as excoriose, grapevine decline syndrome and black dead arm have been described for decline and dieback symptoms associated with *Botryosphaeria*. This has led to the confusing situation where the same symptoms and causal species are often associated with more than one disease. This review summarises the research on *Botryosphaeria* on grapevine by focusing on the causal species, their associated symptoms, known epidemiology and possible effective control and management strategies.

Key words: etiology, management, epidemiology, grapevine decline.

Introduction

The genus *Botryosphaeria* Ces. & De Not. is currently known to encompass species with wide host ranges, several of which are also known to have an endophytic growth phase, later becoming either saprobic or pathogenic (Smith *et al.*, 1996a, 1996b). Typical disease symptoms include cankers, dieback, wood necrosis, fruit rot, as well as various others. Many of the species associated with these disease symptoms have also been reported from grapevines on which they are associated with a wide variety of disease symptoms (Table 1). *Botryosphaeria* has furthermore gained prominence as a grapevine pathogen due to the fact that it has increasingly been the only pathogen isolated from grapevines exhibiting dieback of shoots, spurs and arms as well as from severe internal wood necrosis symptoms (Fourie and Halleen, 2001; Larignon *et al.*, 2001; Auger *et al.*, 2005). Symptoms are especially severe in cases where the host plant has been subjected to stress (Pusey, 1989a). Confusing overlap of symptoms between diseases is caused by the fact that different disease names are given for certain sets of symptoms in different countries (Hewitt, 1988; Phillips, 1998; Larignon and Dubos, 2001; Leavitt, 2003). Pathogenicity data obtained for the same species in different countries have also been contradictory, which has led to further controversy as to which species of *Botryosphaeria* are the more important pathogens of grapevines (Phillips, 2002; van Niekerk *et al.*, 2004; Taylor *et al.*, 2005).

Diagnosis of *Botryosphaeria* diseases is problematic, since symptoms occurring on grapevines

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in the field closely resemble those of other diseases such as Phomopsis dead arm disease caused by *Phomopsis viticola* (Sacc.) Sacc. and Eutypa dieback caused by *Eutypa lata* (Pers.) Tul. & C. Tul. as was evident from New South Wales, Australia, where *B. obtusa* was regularly isolated from dieback symptoms usually associated with above-mentioned two pathogens (Chamberlain *et al*., 1964; Magarey and Carter, 1986; Castillo-Pando *et al.,* 2001). Accurate identification of the causal species is also difficult since the teleomorphs of *Botryosphaeria* species are very seldom encountered in nature, and rarely form in artificial cultures. The diversity among these teleomorphs is furthermore insufficient to allow clear differentiation at species level. For these reasons taxonomy and identification of *Botryosphaeria* species are based mainly on characters of the anamorph (Denman *et al*., 2000).

The anamorph genera of *Botryosphaeria* have also been the subject of significant revision. The primary reason for this revision was that these genera were not clearly defined when they were initially described, and that up to seven genera have subsequently been introduced. After recent revisions of this complex (Crous and Palm, 1999; Denman *et al.,* 2000; Phillips, 2000; Zhou and Stanosz, 2001; Slippers, 2004a), many of the problems surrounding these genera have been resolved. Currently two main anamorph genera are accepted, namely *Diplodia* Fr. (with dark, thick-walled conidia), and *Fusicoccum* Corda (with hyaline, mostly thin-walled, fusoid conidia). Although Denman *et al*. (2000) treated *Lasiodiplodia* Ellis & Everh. as part of the *Diplodia* complex, the description of *L. gonubiensis* has revealed that *Lasiodiplodia* is probably a separate *Botryosphaeria* lineage in its own right (Pavlic *et al*., 2004).

Species identification based on anamorph morphology alone has, however, become very difficult due to the fact that closely related species are often morphologically indistinguishable (Slippers *et al*., 2004a, 2004c). This fact has led to the increasing use of DNA sequence data in combination with morphological data to distinguish between closely related species. The higher resolution obtained via these techniques, has again resulted in the description of several new *Botryosphaeria* spp., making the task of their identification even more difficult (Slippers *et al*., 2004a, 2004c; van Niekerk *et al*., 2004; Taylor *et al*., 2005). In an attempt to simplify the identification of these species, PCR-based techniques were recently developed (Slippers *et al*., 2004b; Alves *et al*., 2005).

Symptoms

Although the pathogenicity and virulence of the various *Botryosphaeria* species occurring on grapevines has not yet been fully clarified, various symptoms associated with different *Botryosphaeria* species have been described on grapevines worldwide (Table 1). These symptoms develop slowly and lead to a gradual decline in vigour and yield (Phillips, 1998). This aspect is probably the reason why the most severe losses due to this disease occur in grapevines that are eight years and older (Larignon and Dubos, 2001).

A symptom that is often associated with *Botryosphaeria* species is bud mortality, which leads directly to a reduction in yield. Bud mortality is often the result of young shoots being infected by the fungus early in the season. The infection develops into elongated black lesions on the internodes. These infections might lead to shoot dieback as infected shoots become swollen at the base, with the blackened cortex eventually rupturing. These shoots are consequently very brittle and can easily collapse under their own weight. Some of the shoots, which do not break, may die back. Later in the season, after harvest, the black lesions turn grey or white with black fruiting structures immersed in the host tissue (Phillips, 1998). These shoots have a bleached appearance, similar to Phomopsis cane blight (Phillips, 2002).

Wound infections, especially of pruning wounds, lead to arm and trunk dieback (Fig. 1A), with a dark brown discolouration of the wood that starts at the pruning wounds and spreads down the trunk. In some cases brown streaking of the wood is also encountered with a dark brown, watery discolouration of the trunk (Fig. 1B). In cross-sectioned trunks and arms, the streaking has the appearance of small black spots (Fig. 1C). This symptom is similar to the symptom found in trunks and arms affected by Petri disease, which is caused by *Phaeomoniella chlamydospora* (W.Gams, Crous, M.J. Wingf. & Mugnai) Crous & W. Gams. It differs from Petri disease, however, in that the spots are more diffuse and, in the case of Petri disease, a

Table 1. Symptoms associated with different *Botryosphaeria* species as reported in published literature.

Symptoms	*B.d* [a]	*B.p*	*B.o*	*B.s*	*B.l*	*B.rib*	*B.rho*	*B.a*	*F.vitic*	*F.vitif*	*D.p*	Reference [b]
Bud mortality	X	X	X									4; 10; 11; 15
Shoot dieback	X					X	X					
Elongated black lesions (cankers)	X					X	X					
Bleached canes	X	X	X	X	X		X					
Trunk dieback	X	X	X	X	X		X					1; 9; 11; 12; 13; 14; 15
Wedge-shaped necrotic lesion	X	X	X	X	X	X	X	X				
Arch-shaped lesions								X				
Dark brown wood discolouration	X	X	X	X	X		X			X		
Brown streaking, black spots	X	X	X	X	X		X					
Infected pruning wounds		X	X						X			
Leaf chlorosis		X	X	X		X						4; 5; 6
Fruit rot	X					X	X					2; 3; 7; 8
Graft union failure	X	X	X	X	X		X					1; 9; 11; 12
Pruning debris											X	14
Asymptomatic								X				

[a] *B. d – Botryosphaeria dothidea; B. p – Botryosphaeria parva; B. o – Botryosphaeria obtusa; B. s – Botryosphaeria stevensii; B. l – Botryosphaeria lutea; B. rib – Botryosphaeria ribis; B. rho – Botryosphaeria rhodina; B .a – Botryosphaeria australis; F. vitic – Fusicoccum viticlavatum; F. vitif – Fusicoccum vitifusiforme; D. p – Diplodia porosum.*

[b] 1. Castillo-Pando *et al*., 2001; 2. Kummuang *et al*., 1996a; 3. Kummuang *et al*., 1996b; 4. Larignon and Dubos, 2001; 5. Lehoczky 1974; 6. Lehoczky, 1988; 7. Milholland, 1988; 8. Milholland, 1991; 9. Pascoe, 1998; 10. Phillips, 1998; 11. Phillips, 2000; 12. Phillips 2002; 13. Taylor *et al*., 2005; 14. Van Niekerk *et al*., 2004; 15. Wood and Wood, 2005 .

black tarry substance oozes from the spots, which is not the case with the spots caused by *Botryosphaeria* species (Castillo-Pando *et al*., 2001; Phillips, 2002). Although *Pa. chlamydospora* was mostly isolated from young plants with failed graft unions, *Botryosphaeria* species were also isolated from these failed graft unions (Phillips, 2002).

Another internal wood symptom that can be seen in cross-sectioned arms and trunks is a wedge-shaped necrotic sector (Fig. 1D). This symptom is also found in association with brown wood streaking. This symptom resembles Eutypa dieback, but can be distinguished by the absence of stunted shoot development and small, yellow, malformed leaves (Castillo-Pando *et al*., 2001). A symptom closely resembling the wedge-shaped necrosis, is arch-shaped lesions (Fig. 1E) leading to brown internal necrosis (Fig. 1F), which can be seen in cross-sectioned arms and trunks. These symptoms might be due to earlier infection of wounds such as suckering wounds, severe girdling of trunks and wounds where water shoots were removed.

Infection by *Botryosphaeria* species can also manifest itself by causing mild chlorosis of the leaves, depending on the extent of wood colonisation. In France, foliar symptoms, which might easily be confused with that of esca disease, have been reported on cv. Cabernet Sauvignon, Cabernet Franc and Merlot that were infected with *B. obtusa* (Larignon *et al*., 2001). These foliar symptoms developed by the end of May (late spring), appearing first on leaves near the base of shoots and then spreading to other leaves on the shoot as the growing season progressed (Larignon *et al*., 2001). These symptoms have not been reproduced by artificial inoculation. Berry rots could be an important *Botryosphaeria* symptom in various parts of the world and could play an important role in the epidemiology of these pathogens and as inoculum sources for wound infections leading to trunk diseases. Fruit rot symptoms associated with *Botryosphaeria* species were reported from France (Larignon and Dubos, 2001) and the USA (Hewitt, 1988; Milholland, 1988, 1991; Kummuang *et al.,* 1996a, 1996b;

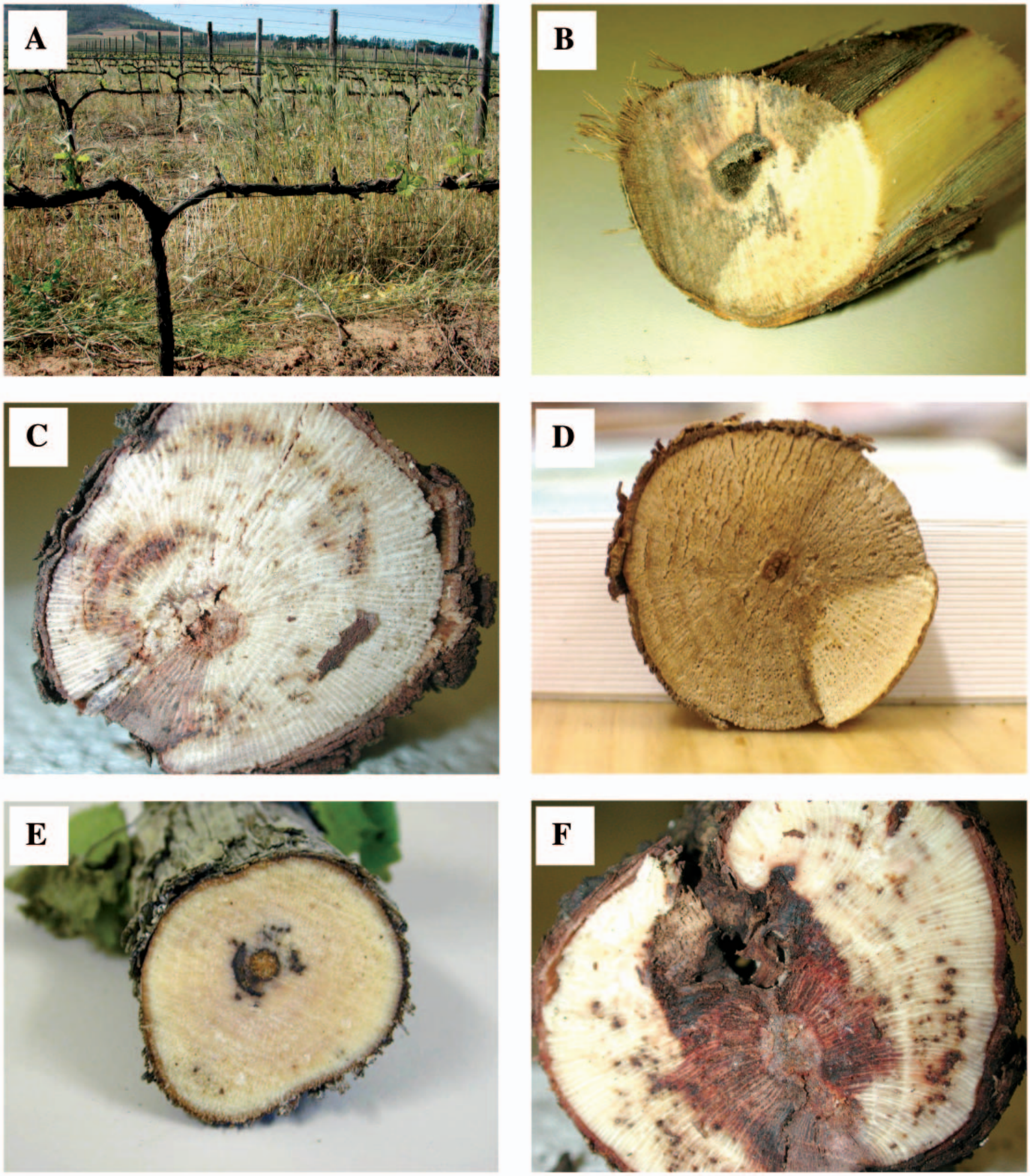

Fig. 1. Profile of symptoms associated with *Botryosphaeria* infection of grapevines: A. spur and arm dieback; B. dark brown, watery discolouration of wood; C. small, brown-black spots, visible in cross-sectioned arms; D. wedge-shaped necrotic sector; E. arch-shaped lesion in vascular tissue; F. brown internal necrosis visible in cross-sectioned arms and trunks.

Leavitt, 2003). When berries become infected, they initially appear water-soaked, and as the rot develops and the skin cracks, the berries become covered in a white mass of mycelium. The berries eventually dry out and become mummified with black pycnidia emerging on the surface. The dried-out berries are seldom seen, due to the fact that they become infected with secondary fungi and yeasts that cause the cluster to turn into a rotten mass known as summer bunch rot (Hewitt, 1988).

Etiology

Eleven *Botryosphaeria* species were reported from grapevine by van Niekerk *et al*. (2004). These species were associated with one or more symptom types and/or occurred saprophytically on grapevine debris (Table 1) and consequently several diseases were described in different grape growing countries. The different *Botryosphaeria* species and the diseases they are associated with are briefly described below.

Botryosphaeria dothidea **(Moug.: Fr.) Ces. & De Not.**

One of the diseases that *B. dothidea* is associated with is excoriose. This disease is especially well known in Portugal and France (Phillips, 1998; Larignon and Dubos, 2001). For many years, *Phomopsis viticola* was regarded as the causal organism of excoriose in the USA (Phillips, 2000). In Europe, the causal organism had been regarded as *Macrophoma flaccida* (Viala & Ravaz) Cavara. After *P. viticola* was reported from Germany, this fungus was considered as the sole cause of excoriose and dead arm in Europe and the USA. However, in 1997 Phillips and Lucas showed that *M. flaccida* is synonymous with *Fusicoccum aesculi* Corda, which is the anamorph of *Botryosphaeria dothidea*. In a subsequent study (Phillips *et al*., 2002), the identity of the isolates used by Phillips (1998, 2000) was clarified and *B. dothidea* was found to be relatively uncommon on grapevines (Phillips, 2002).

Botryosphaeria dothidea (as *Macrophoma flaccida*) was also found to be widely distributed in association with excoriose in Portugal (Tomaz and Rego, 1990). This led to the conclusion that the cause of excoriose might be *B. dothidea*. However, doubt still remained as to the pathogenicity of *B. dothidea* on grapevines, which led to the study of Phillips (1998, 2000) where the pathogenicity of *B. dothidea* and *P. viticola* was evaluated in Portugal. The results showed that the symptoms caused by the two fungi are very similar and that these symptoms corresponded with the symptoms commonly associated with excoriose. This confirmed that *B. dothidea* was indeed a causal organism of excoriose (Phillips, 1998, 2000). However, as mentioned above, when Phillips *et al*. (2002) re-examined the isolates referred to as *B. dothidea*, they confirmed that the cause of excoriose is in fact *B. parva*. In France *B. dothidea* was identified as one of the causal organisms of black dead arm disease (Larignon *et al*., 2001).

In Portugal, *B. dothidea* was also reported as one of the *Botryosphaeria* species isolated from failed graft unions of young plants (Phillips, 2002). In the USA, *F. aesculi*, the anamorph of *B. dothidea*, was reported as the causal organism of Macrophoma rot of Muscadine grapes (Milholland, 1988, 1991; Kummuang *et al*., 1996a, 1996b).

Botryosphaeria parva **Pennycook & Samuels**

In Portugal, *B. parva* is associated with grapevine decline syndrome (Phillips, 2002). It was isolated from more than one type of symptom: trunk dieback, with dark brown discoloration of the wood, and small black spots (characteristic of Petri disease) that can be seen when looking at a cross section of the trunk and arms of affected grapevines. In some cases it was isolated from dead tissue surrounding necrotic buds (Phillips, 2000; Castillo-Pando *et al*., 2001; Phillips, 2002).

In South Africa, *B. parva* was isolated from asymptomatic rootstock mother vines and nursery plants. Furthermore, it was also isolated from wedge-shaped necrotic sectors or half-moon shaped lesions that can be seen in cross sections of arms and trunks. *B. parva* was also isolated from fruiting bodies occurring on pruning debris picked up from the vineyard floor (van Niekerk *et al*., 2003).

Botryosphaeria obtusa **(Schwein.) Shoemaker**

Botryosphaeria obtusa occurs on a wide variety of woody hosts and can live as saprobe on dead wood and bark (Castillo-Pando *et al*., 2001). It has been recognised as a wound pathogen, and can cause dieback symptoms as well as cankers. In France (Larignon *et al*., 2001) and Italy (Cristinzio, 1978; Rovesti and Montermini, 1987), *B. obtusa* was reported to be one of the causal organisms of black dead arm of grapevine. In Portugal, *B. obtusa* was found associated with reduced growth, reduced bud burst and death of arms (Phillips, 2002).

This species is also associated with grapevine decline syndrome as it was isolated from more than one type of symptom (Phillips, 2002). Similar to *B. parva*, *B. obtusa* was also isolated from spots resembling those associated with Petri disease (Castillo-Pando *et al.*, 2001; Phillips, 2002). It is also associated with decline or dieback in young plants where it was isolated from failed graft unions, similarly to *B. dothidea* and *B. parva* (Phillips, 2002).

Another symptom type found in association with *B. obtusa*, is wedge-shaped necrotic sector seen in the trunk and arms of the grapevines in cross section. This symptom is also found in association with brown wood streaking (Larignon *et al.*, 2001; Phillips, 2002). In Australia, *B. obtusa* was found to be the causal organism of dieback of 'Semillon' grapevines in New South Wales and in Western Australia as it was isolated from declining grapevines, which exhibited internal wedge-shaped and arch-shaped lesions similar to those described in California, USA (Castillo-Pando *et al.*, 2001; Taylor *et al.*, 2005; Urbez *et al.*, 2005). In South Africa, this species was frequently isolated from asymptomatic nursery plants, pruning wound stubs as well as from arch-shaped lesions occurring internally in the arms and trunk, similar to *B. parva* (van Niekerk *et* al., 2003).

Botryosphaeria stevensii **Shoemaker**

Botryosphaeria stevensii is another of several *Botryosphaeria* spp. that are associated with grapevine decline syndrome (Phillips, 2002). Similar to *B. parva* and *B. obtusa*, it was in some cases isolated from the small black spots that resemble the spots commonly associated with Petri disease (Castillo-Pando *et al.,* 2001; Phillips, 2002). Isolations made from dead tissue surrounding dead buds often yielded *B. stevensii,* with *B. parva* also encountered in some cases (Phillips, 2002).

Botryosphaeria stevensii is also associated with another disease, black dead arm, which was first described in Hungary in 1974, and is a well-known disease of grapevines in France (Lehoczky, 1974, 1988; Larignon and Dubos, 2001; Larignon *et al.*, 2001). Symptoms described for black dead arm are leaf chlorosis, wilting and in some cases fruit rot. In the xylem of infected spurs, arms and trunks black streaks develops which expand to form large necrotic sectors in the wood. In Australia, *B. stevensii* was also isolated from declining grapevines, which internally also exhibited wedge-shaped and arch-shaped lesions (Pascoe, 1998; Taylor *et al.*, 2005).

Botryosphaeria lutea **A.J.L. Phillips**

This species, like others discussed by Phillips (1998, 2000, 2002), is also associated with grapevine decline syndrome. It was also isolated from disease symptoms resembling that of Petri disease, with spots being visible in the cross section of the trunk or arms (Castillo-Pando *et al.,* 2001; Phillips, 2002). Similar to *B. parva* and *B. obtusa*, it was also isolated from bleached, dormant canes that resemble the bleached canes normally associated with Phomopsis cane and leaf spot (Phillips, 2002).

Botryosphaeria ribis **Grossenb. & Duggar**

In Australia, this species was mostly associated with fruit rot symptoms in vineyards and it was concluded that it might not be a pathogen of shoots or canes in that country (Pascoe, 1998). However, the possibility exists that it can be involved with symptoms such as shoot dieback, cankers on shoots, trunk dieback, brown streaking and wedge-shaped lesions (Table 1).

Botryosphaeria rhodina **(Berk. & M.A. Curtis) Arx**

In Australia and California, *Botryosphaeria rhodina* was isolated from the necrotic wedge-shaped symptom that was described for grapevine decline syndrome in Portugal (Pascoe, 1998; Phillips, 2002; Taylor *et al.*, 2005; Urbez *et al.*, 2005; Wood and Wood, 2005). In South Africa, it was isolated from the above-mentioned symptom as well as from arch-shaped lesions, and asymptomatically from nursery plants (van Niekerk *et al.*, 2003).

In the USA, the anamorph of *B. rhodina, Lasiodiplodia theobromae* (Pat.) Griffiths & Maubl.*,* which was previously known as *Diplodia natalensis* Pole-Evans*,* is regarded as the causal organism of cane dieback and bunch rot symptoms known as Diplodia cane dieback and bunch rot. *L. theobromae* is also associated with other symptoms of this disease, which includes lesion development on the shoots that could spread to the arms, causing dead arm symptoms (Hewitt, 1988). *B. rhodina* was recently reported to be the cause of a similar cane dieback disease of cv. Dawn Seedless Table grapevines from Western Australia (Wood and Wood, 2005).

Botryosphaeria australis **Slippers, Crous & M.J. Wingf.**

Slippers *et al*. (2004c) recently described this species from *Acacia* spp. and *Sequoiadendron* spp. in Australia. It was for the first time reported to occur on grapevines by van Niekerk *et al*. (2004) who isolated it from asymptomatic mother plants as well as pruning wound stubs. Taylor *et al*. (2005) also reported it occurring on grapevines in Western Australia and obtained isolates from declining grapevines exhibiting wedge-shaped and arch-shaped lesions internally in the arms. In both these studies artificial inoculations indicated that *B. australis* is more virulent than the other species tested, and possibly represents an important grapevine pathogen.

Fusicoccum viticlavatum **Niekerk & Crous**

Van Niekerk *et al*. (2004) described this asexual species from grapevines in the Western Cape province of South Africa where it was isolated from pruning wound stubs. Artificial inoculations made onto mature grapevine wood indicated that this species could also prove to be an important pathogen of grapevines.

Fusicoccum vitifusiforme **Niekerk & Crous**

This species was also described by van Niekerk *et al*. (2004) from grapevines in the Western Cape province of South Africa, where it was isolated from brown internal necrosis in the arms of declining vines. Similarly to *F. viticlavatum,* this species could also be an important pathogen of grapevines as indicated by the lesions caused by artificial inoculations onto mature grapevine wood.

Diplodia porosum **Niekerk & Crous**

This species is currently known to occur only in South Africa, where it was collected on pruning debris from vineyard floors. Artificial inoculations again indicated this species' potential importance as grapevine pathogen (van Niekerk *et al*., 2004).

Fusicoccum viticlavatum, *F. vitifusiforme* and *D. porosum*, as well as *B. australis*, are newly described species and very little is known about their epidemiology and pathogenicity. Currently their status as potential pathogens are based on pathogenicity tests (van Niekerk *et al*., 2004) and the association of especially *B. australis* and *F. vitifusiforme* with symptomatic tissue (unpublished results).

Epidemiology

Very little is currently known about the epidemiology of *Botryosphaeria* diseases of grapevines. The large, and ever increasing number of *Botryosphaeria* species occurring on grapevines, is also complicating epidemiological studies of this pathogen. It has been shown that different species need different climatic conditions to form fruiting structures. A wide range of climatic conditions would therefore be conducive to *Botryosphaeria* infection (Copes and Hendrix, 2004).

Phillips (1998) described excoriose as a disease occurring wherever grapevines are grown, with infection of young shoots occurring early in the season. This infection is more severe when wet weather is encountered. This observation is supported by Lehoczky (1974, 1988) who reported that *B. stevensii*, the causal organism of Black Dead Arm in Hungary, overwintered in diseased wood of grapevines, and that pycnidial development and spore release coincided with rainy weather. This same phenomenon was observed in apple and peach orchards by Sutton (1981) and Pusey (1989b) who found that the ascospores of *B. dothidea*, *B. obtusa* and *B. rhodina* were released during or soon after periods of rain and high relative humidity, leading to prolonged periods of wetness.

The increased infection severity of grapevine shoots during wet weather, as described by Phillips (1998), could therefore be attributed to higher inoculum availability as it is known that during periods of wetness the pycnidia and pseudothecia of *Botryosphaeria* hydrate and start to produce and release conidia and ascospores. Furthermore, the longer the period of wetness and high relative humidity extends, the more spores are produced and released (Michailides and Morgan, 1993). Michailides and Morgan (1992) and Pusey and Bertrand (1993) found that an increase in wetness duration combined with the high inoculum levels present led to an increase in severe infections of pistachio and peach trees. Lehoczky (1974, 1988) also found that pruning wounds caused by mechanical injury served as points of infection with the optimal temperature for infection ranging from 23 to 26°C. This is supported by findings of Halleen and Fourie (2005), who isolated several *Botryosphaeria* species from 1-year-old pruning wound stubs.

Diplodia cane dieback and bunch rot is also favoured by high relative humidity and rain (Hewitt, 1988). It was stated that the causal organism *L. theobromae*, anamorph of *B. rhodina*, overwinters inside diseased wood and on pruning debris on the vineyard floor from where propagules are released during wet periods. These propagules are then wind or splash dispersed by rain or sprinkler irrigation to infect wounds (Hewitt, 1988). Debris as a source of inoculum also plays a role in the development of Macrophoma rot, where it is believed that the causal organism, *B. dothidea*, overwinters in dried-out berries on the vineyard floor (Milholland, 1988, 1991; Kummuang *et al*., 1996a, 1996b). Van Niekerk *et al.* (2003) isolated several *Botryosphaeria* species from pruning debris, further demonstrating the importance of debris on the vineyard floor as a source of inoculum. In pistachio orchards, pruning debris that was left on the orchard floor after winter pruning were also reported as a major source of inoculum for infection during the next season (Michailides, 1991; Ahimera *et al*., 2004).

Apart from infection of grapevines in the field, infection can already take place during the propagation stages. Fourie and Halleen (2002) isolated *Botryosphaeria* spp. from apparently healthy canes of rootstock mother vines and concluded that these infections most likely originated from infections that were found inside the mother vines (Fourie and Halleen, 2004). Propagation material might therefore already be infected prior to grafting. Halleen *et al*. (2003) isolated *Botryosphaeria* from a number of young, apparently healthy, nursery plants, while Phillips (2002) isolated *Botryosphaeria* from failed graft unions of young plants. These early infections could remain latent until the young grapevines are exposed to stress or favourable conditions for disease development, which might then lead to decline and sometimes death of grapevines at a young age. It was found that early infections of pistachio shoots and buds remained latent until favourable environmental conditions for symptom development occurred (Ahimera *et al*., 2003).

Recent studies have found regional differences in the occurrence of *Botryosphaeria* species, possibly due to climate differences between the regions studied. In a survey of *Botryosphaeria* spp. in Western Australian vineyards, Taylor *et al*. (2005) observed differences in the regional occurrence of four identified species, *B. obtusa*, *B. australis*, *B. rhodina* and *B. stevensii*. *Botryosphaeria rhodina* occurred in the Swan District of Western Australia only. This species is regarded as a tropical and subtropical pathogen around the world (Burgess *et al*., 2003), and the cooler climate of the south-west of Western Australia might inhibit the establishment of this species into this region. *Botryosphaeria rhodina* was also the predominant species isolated by Leavitt (2003) in the warmer states of California, Arizona and in Mexico. *Botryosphaeria australis* and *B. stevensii* was isolated only in the Margaret and Pemberton/Manjimup regions and never in the Swan Districts region, while *B. obtusa* was the most cosmopolitan species, occurring in all the regions surveyed (Taylor *et al*., 2005). During a survey of *Botryosphaeria* spp. occurring on grapevines in California, Gubler *et al*. (2005) similarly observed that *B. obtusa* occurred in all the counties surveyed except for Riverside, where only *L. theobromae* (*B. rhodina*) was isolated. *Botryosphaeria dothidea* occurred more frequently in the northern counties.

Management

The management of *Botryosphaeria* diseases is extremely difficult since the information on effective disease control measures is very limited. In many instances recommended control measures vary from one country to another, and even from one region to another (Milholland, 1991). Fungicides have been registered for disease control in only a few countries. In France, sodium arsenite was previously registered for the control of black dead arm, associated with *Botryosphaeria*, and the recommended treatment was the same as for the treatment of esca (Lehoczky, 1974, 1988; Larignon and Dubos, 2001). However, this fungicide has been banned due to the environmental pollution it caused and the build-up of arsenite in sprayed crops that could possibly be dangerous to user and consumer health (Decoin, 2001; Lyubun *et al*., 2002).

In the USA, Macrophoma rot (*B. dothidea*) is controlled by the application of protective fungicides such as maneb at bloom, continuing throughout the ripening period (Milholland, 1988).

Since many of the *Botryosphaeria* species that occur on grapevines are regarded as wound pathogens, it is important to prevent unnecessary wounding of the plants (Lehoczky, 1988; Milholland, 1988, 1991; Larignon and Dubos, 2001). Some research has therefore gone into the protection of pruning wounds with fungicides and/or biological control agents (Larignon and Dubos, 2001).

In vitro fungicide tests done in South Africa indicated that benomyl, tebuconazole, prochloraz manganese chloride, flusilazole and fenarimol were the most effective in inhibiting mycelial growth of *B. australis*, *B. obtusa*, *B. parva* and *B. rhodina* (Bester and Fourie, 2005). These fungicides, except for prochloraz manganese chloride, are registered for use on grapevines in South Africa and in previous studies were shown to be effective against *Pa. chlamydospora*, *P. viticola* and *E. lata*. In Australia, tebuconazole, fenarimol, spiroxamine and fluazinam were shown to inhibit *in vitro* mycelial growth of *B. obtusa* and *B. lutea* (Savocchia *et al*., 2005). In trials done by Leavitt (2003), it was shown that iprodione, benomyl, captan and penconazole were all effective in reducing the infection of pruning wounds by *L. theobromae* (*B. rhodina*) when it was applied prior to inoculation in a glasshouse.

The use of fungicides as wound treatments or applied as sprays on hosts such as proteas and apples have been shown to reduce the levels and severity of infection by various *Botryosphaeria* species (Von Broembsen and Van der Merwe, 1990; Brown-Rytlewski and McManus, 2000; Denman *et al*., 2004). When fungicides were applied as topical wound treatments to prevent infection of pruning wounds by *B. dothidea*, external canker formation was reduced but the pathogen was still isolated from internal tissues (Brown-Rytlewski and McManus, 2000). Good protection of pruning wounds against *E. lata* infection was also achieved with winter, post pruning spray applications of benomyl (Ramsdell, 1995). However, weak persistence of fungicides and the possible risk of phytotoxicity when applying high dosages are factors that will influence the long-term protection of pruning wounds with only one fungicide application (Brown-Rytlewski and McManus, 2000; Larignon and Molot, 2004). It is furthermore also important that more than one fungicide is needed to allow the alternate use of the fungicides, and thus prevent resistance build-up in *Botryosphaeria* spp., as was seen with resistance development to iprodione (Ma *et al*., 2000).

A number of biological control agents such as *Fusarium lateritium* Nees, *Cladosporium herbarum* (Pers.) Link, *Trichoderma harzianum* Rifai and *Bacillus subtilis* have been shown to be effective in reducing mycelial growth of *E. lata* in culture as well as reducing the colonisation of living grapevine cuttings (Carter and Price, 1974; Ferreira *et al*., 1991; Munkvold and Marois, 1993; John *et al*., 2004). Similarly to *E. lata*, a number of biological control agents were shown to be effective in inhibiting *Botryosphaeria* spp. *in vitro*. Several *Trichoderma* spp. have been tested and shown to be mycoparasites of several *Botryosphaeria* species (KeXiang *et* al., 2002; Chen *et al*., 2003). Furthermore, several *Trichoderma* strains were able to colonise and survive in grapevine pruning wounds (Halleen and Fourie, 2005). *Paenibacillus lentimorbus* was shown to be an antagonist of *B. dothidea*. When applied to pistachio pruning wounds before inoculation with *B. dothidea* conidia, it greatly reduced wound infection compared to the untreated, inoculated controls (KeXiang *et al*., 2002; Chen *et al*., 2003).

A management strategy that is also widely recommended to prevent or reduce infection of pruning wounds by *Botryosphaeria* is the removal of pruning debris from the vineyard after pruning (van Niekerk *et al*., 2004).

Remedial surgery, where the infected parts of the grapevine are cut away and the vine retrained, is generally recommended for the management of grapevines infected with *E. lata*. However, it can also be used to prolong the lifespan of a grapevine infected with *Botryosphaeria*. For this measure to be effective, it is very important that all infected parts of a vine should be removed (Savocchia *et al*., 2005; Sosnowski *et al*., 2005).

In the management of these infected vines it is furthermore important to prevent any stress condition that could predispose the vines to more severe infection. It has been shown that *Botryosphaeria* infection is more serious when the host plant is subjected to stress (Boyer, 1995; Ma *et al*., 2001). The same phenomenon is considered to occur with grapevines, but it is currently based on anecdotal evidence and needs to be scientifically proven.

In an effort to produce disease free plant material, Fourie and Halleen (2005) recommended the integration of a number of management strategies that were effective in reducing the infection levels of nursery plants with other grapevine trunk pathogens such as *Pa. chlamydospora*, *Phaeoacremonium* spp., *Cylindrocarpon* and *Campylocarpon* spp. This entails the protection of pruning wounds in rootstock mother blocks, chemical and/or biological amendments in hydration water, hot water treatment of rootstock cuttings and/or dormant nursery plants, hygienic grafting, wound protection during nursery stages and soil amendments with *Trichoderma* formulations. These measures might also be effective against *Botryosphaeria* and need to be evaluated.

Conclusion

Botryosphaeria species seem to be ubiquitous wherever grapevines are grown and their role as important trunk disease pathogens is increasingly being recognised. However, the *Botryosphaeria* spp. isolated from decline or dieback symptoms are rarely identified to species level. Moreover, indistinguishable morphological differences between closely related species, apparent absence of the teleomorph states and the multiplicity of anamorph states complicate species-identification. Researchers from different grape growing countries gave different disease names, such as excoriose, grapevine decline syndrome and black dead arm, to the decline and dieback diseases caused by *Botryosphaeria*, which led to the confusing situation where the same symptoms and causal species was often associated with more than one disease. Accurate species identification, aided by molecular techniques, should promote research on etiology, epidemiology and management of these important diseases.

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Accepted for publication: July 8, 2005