Managing Grapevine Trunk Diseases with respect to etiology and epidemiology: Current Strategies and Future Prospects

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The genus *Vitis* L. (grapevines), with more than 100 species currently described (The Plant List 2013), has been cultivated for over 7,000 years (Mullins et al. 1992). *Vitis vinifera* L. (common grapevine) cultivars are the most widely planted around the globe and have a high commercial value for fresh table-grape, dried fruit and wine production. Cultivation of *V. vinifera* is primarily located in Mediterranean and other temperate climate regions between the latitudes 30° and 50° in both the Northern and Southern Hemispheres. Other *Vitis* species as well as their inter specific hybrids are also important for juice, table or wine production, and in particular for rootstock development. With approximately 7.12 million ha cultivated and 74.5 million t of fruit harvested in 2014, grapevines (*V. vinifera* and *Vitis* spp.) are one of the most extensively grown and economically important woody perennial fruit crops in the

world. The European continent, with 3.55 million ha and 28.9 million t, leads grape production worldwide and is followed by Asia (2.04 million ha), South America (0.53 million ha), North America (0.43 million ha), Africa (0.33 million ha), and Oceania (0.18 million ha) (FAO 2017). In 2014, six countries accounted for approximately 60% of the world's grape production, including The People's Republic of China (12.54 million t), USA (7.12 million t), Italy (6.93 million t), Spain (6.22 million t), France (6.17 million t), and Turkey (4.17 million t) (FAO 2017). Grape-producing countries benefit tremendously from the major economic boost that grape and wine industries provide, no matter what their size. For example, in Canada where only about 12,000 ha of grapevines are cultivated, the industry contributed over CAD$9 billion to the national economy in 2015 (Rimerman 2017). In comparison, the full economic impact of wine and grape products from larger industries such as the American or Australian were estimated to be about USD$161 billion in 2005 (MKF Research 2007) and AUD$40.2 billion in 2015 (Gillespie and Clarke 2015), respectively. In Spain, the economic impact of grape and wine products in 2015 accounted for 1% of the gross domestic product (MAPAMA 2017).

Grapevine cultivation generates substantial production costs, due to the high intial financial investment for vineyard establishement and the costly annual vineyard operations required for production. A significant amount of these costs are associated with intense pest and disease management programs, which include cultural practices and the cost of chemical and/or biological control products and their application (Cooper et al. 2012). This is particularly true for diseases as *V. vinifera* is known to host the widest variety of pathogens of any woody agricultural plant (Martelli 1997). Among them, fungal pathogens are of significant importance since *V. vinifera* is susceptible to 29 fungal diseases (Wilcox et al. 2015), including grapevine trunk diseases (GTDs), which are currently considered one of the most destructive (Bertsch et al. 2013). .

The term GTD is relatively new and was established by Dr. Luigi Chiarappa along with other scientists from around the world in late 1990s to include several symptoms observed in both foliage and vascular tissue of grapevines, which were thought to be caused by a group of fungi that primarily infect grapes through pruning wounds, subsequently colonizing the vascular tissues (Mugnai 2011). However, symptoms of what we today call GTDs as well as fungi associated with them are long known. It has even been suggested that the disease currently known as esca may be as old as vine cultivation (Mugnai et al. 1999). Nonetheless, the first formal record of a GTD dates back to the end of the 19th century in France, where esca foliar symptoms were first described and named 'folletage' and 'apoplexy' and thought to be caused by the the basideomicetous fungi *Stereum hirsutum* and *Phellinus igniarius* (Ravaz 1898, 1909; Viala 1926). Later in 1912, Italian scientist Lionel Petri completed for the first time Koch's postulates and demostrated that *Cephalosporium* and *Acremonium* spp. were responsible for the necrosis observed in the vascular system of young grapevines (Petri 1912). Similarly, studies conducted during the first decade of the 1900s in North America by plant pathologist Donald Reddick at the Cornell University State Agricultural Experiment Station in Geneva New York, showed for the first time that the fungus *Fusicoccum viticolum*, currently known as *Diaporthe ampelina* (syn. *Phomopsis viticola*), was associated with grapevine cankers, dieback and symptoms resembling what we know today as Phomopsis cane and leaf spot, Phomopsis dieback (Úrbez-Torres et al. 2013a) and Eutypa dieback, thus naming the syndrome dead-arm disease of grapevines (Reddick 1914). Accordingly, the term dead-arm disease was commonly used for more than 50 years to describe similar symptoms observed in grapevines around the world (du Plessis 1938; Hewitt 1935; Hiura 1924), including those shown for the first time to be caused by species in the Botryosphaeriaceae family (Chamberlain et al. 1964), now known as Botryopshaeria dieback (Úrbez-Torres 2011). Eutypa dieback, caused by the diatrypaceous fungus *Eutypa lata*, was first reported to

occur in Australia on apricots and grapevines (Carter 1957a, 1957b). In the following decades, *E. lata* wasreported on grapevines in California (English et al. 1962, Moller et al. 1968), Europe and many other countries (Carter 1991). Symptoms of black foot, now recognized as a a GTD, were first described in early 1960s in France under the name of 'gangrene' (Maluta and Larignon 1991) but first associated with a "*Cylindrocarpon*" species in Italy in 1975 (Grasso and Magnano Di San Lio 1975).

To date, up to 133 fungal species belonging to 34 genera have been associated with GTD worldwide, although Koch's postulates have not been completed for all of them. Nonetheless, GTD fungi account for the largest group of pathogens known to infect grapes (Agustí-Brisach and Armengol 2013; Araújo da Silva et al. 2017; Carlucci et al. 2015a, 2017; Cloete et al. 2015; Gramaje and Armengol 2011; Gramaje et al. 2015; Lawrence et al. 2016a; Lombard et al. 2014; Travadon et al. 2015; Trouillas et al. 2010; Úrbez-Torres 2011; Úrbez-Torres et al. 2013a). GTDs are primarily caused by ascomyceteous fungi but some basideomiceteous taxa are also thought to play an important role in this disease complex (Fischer 2002; Cloete et al. 2015). Spores of GTD fungi can infect grapevines through any type of open wound, including those caused by re-training, trimming and de-suckering (Makatini et al. 2014). However, annual pruning wounds are the primary point of entry providing many infection sites each growing season during the life of a vineyard.

Importance and impact of grapevine trunk diseases

Although GTDs have been known since the end of the 19th century, their significance and impact on plant health have only been recognized recently. The recent increase of GTD incidence worldwide is believed to be the consequence of several factors. Firstly, the grapevine planting 'boom' experienced worldwide during the 1990s, which not only increased the movement of potentially contaminated propagated material (Gramaje and Armengol

2011), but has led to the increased area of vineyards around the world reaching an age where symptoms are expressed and therefore becoming more visually prevalent. Secondly, drastic changes in production methods that have greatly favored fungal infection, such as the transformation from traditional low-density head-trained or bush vines to more high density spur-pruned trellis vineyards, often mechanically pruned, the latter presenting a significantly higher number of pruning wounds (Fig. 1). Finally, in some countries, the phasing out of sodium arsenite, benzimidazole fungicides and methyl bromide in the early 2000s, due to environmental and public health concerns (Decoin 2001; EPA 2017), eliminated the most effective chemical products available against GTD fungi. Accordingly, it is well-accepted that GTDs represent one of the major threats to the future economic sustainability of viticulture, causing significant economic losses due to reduced yields, increased crop management costs for cultural and chemical preventive measures, and shortened life span of vineyards (Bertsch et al. 2013; Gramaje et al. 2016; Kaplan et al. 2016). Productivity is reduced over time by death of the spurs, canes, and/or cordons. In severely infected vineyards of North America, yield losses between 30-50% and up to 94% have been reported by Botryosphaeria (Milholland 1991) and Eutypa dieback (Johnson and Lunden 1987), respectiveley. In South Australia, yield losses of 1,500 kg per ha were estimated when 47% of Shiraz vines were affected by Eutypa dieback, leading to losses of AUD$2,800 per ha (Wicks and Davies 1999). The economic impact of Botryosphaeria and Eutypa dieback in California was estimated to be $USD260 million per year (Siebert 2001). In Spain, incidence of GTDs in grape-growing regions of Castilla y León increased from 1.8% in 2001 to 7% in 2006 (Martín and Cobos 2007). In Italy, studies conducted at the end of the 1990s reported about 15% of the young vines in Sicily with symptoms of decline and high mortality within the first year of planting (Sidoti et al. 2000). Esca incidence has reached up to 80% in many mature vineyards of southern Italy (Romanazzi et al. 2009). More recently, GTD incidence and consequent plant

mortality has also been reported to be rising throughout Chinese vineyards (Yan et al. 2013). A survey conducted in the Canadian Province of British Columbia reported 90% of the vineyards with GTD symptoms, and some individual vineyards recorded with up to 54% incidence (Úrbez-Torres et al. 2014a, 2014b). In France, it is estimated that 12% of vineyards are currently economically unviable, due primarily to esca, with an annual estimated loss of €1 billion (Lorch 2014).

Management of GTD is difficult and is influenced by the disease and/or pathogens involved. Information on control measures is limited and varies among geographical regions. Complete eradication is not possible, so control is primarily focused on disease prevention and mitigation (Úrbez-Torres 2011). Since the loss of the most effective preventative chemical products, remedial surgery was the only management strategy left for the grape growing industries to combat GTD (Creaser and Wicks 2004; Sosnowski et al. 2011b). However, this operation can be costly (Epstein et al. 2008). Consequently, the evaluation of novel active ingredients as well as of cultural practices that could effectively reduce infection caused by GTD pathogens, have been the main priority for industry and researchers during the last decade (Úrbez-Torres 2011). In addition, the impact of fungal trunk pathogens transmitted in propagation material on the establishment and longevity of vines is well documented (Gramaje and Armengol, 2011). Nurseries can be a source of infected plant material which results in cross infection of entire batches of cuttings and the nursery vines grown from them. An integrated management program that includes physical, chemical, biological, and/or other control strategies has been suggested as the most effective procedure to reduce infections by fungal trunk pathogens in the nursery (Halleen and Fourie 2016).

In this article, we review the individual diseases and pathogens that cause GTDs, the importance of understanding disease etiology and epidemiology to develop control programs, discuss current strategies and measures available to minimize the economic impact of the

causal pathogens in both young and mature vines, and consider some of the future prospects for effective disease management.

Grapevine trunk diseases: symptoms and fungi involved

Grapevines can be affected by one or more GTDs at the same time since individual vines can be infected with different pathogens due to the multiple infection opportunites throughout a season and over the years. Furthermore some symptomology overlaps among different GTDs making accurate identification in the field difficult. For example, Petri disease and black foot, the two most common GTDs observed in young vineyards (< 5-years-old). Foliar symptoms associated with both diseases (overall stunting, delayed budbreak, shortened internodes, chlorotic foliage with necrotic margins, and witlting of leaves or entire shoots) (Fig. 2A-C) not only overlap but they resemble symptomatology associated with abiotic disorders such as winter damage, spring frost, water stress, and/or nutrient deficiency. However, each disease can be differentiated individually as follows.

Petri disease

Petri disease can be recognized by the presence of dark-colored phenolic compounds in xylem vessels of the trunks, which exude out of the vessels when cut in cross sections and dark streaks in longitudinal section (Fig. 2D and E) (Rooney-Latham et al. 2005). The fungal species associated with Petri disease include: *Phaeomoniella chlamydospora*, 29 species of *Phaeoacremonium*, *Pleurostoma richardsiae* and six species of *Cadophora* (Araújo de Silva et al. 2017; Gramaje and Armengol 2011; Gramaje et al. 2015; Halleen et al. 2007b; Travadon et al. 2015). Among the different *Phaeoacremonium* and *Cadophora* spp. occurring in Petri disease symptomatic vines, *Phaeoacremonium minimum* and *Cadophora luteo-olivacea* are the most prevalent (Mostert et al. 2006; Gramaje et al. 2011).

Black foot

Black foot can be recognized by black, sunken, necrotic lesions on roots and a dark purplish or reddish brown discoloration in the base of the trunk of affected vines. Bark removal reveals black discoloration and necrosis of wood tissue which develops from the base of the rootstock, causing death of young vines (Fig. 2F) (Halleen et al. 2006). Up to 24 species in the genera *Campylocarpon*, *Cylindrocladiella*, *Dactylonectria*, *Ilyonectria*, *Neonectria*, and *Thelonectria* have been reported to cause black foot disease (Agustí-Brisach and Armengol 2013; Carlucci et al. 2017; Lombard et al. 2014).

Eutypa dieback

Foliar symptoms of Eutypa dieback include stunted shoots with chlorotic leaves, that are often cupped and with necrotic margins (Fig. 3A and B). The foliar symptomatology is caused by toxic metabolites produced only by the *E. lata* fungus in the wood (Mahoney et al. 2005; Moller and Kasimatis 1981; Molyneux et al. 2002; Tey-Rulh et al. 1991). Foliar symptoms can appear 3 to 8 years after infection (Carter 1978; Tey-Rulh et al. 1991) and can vary from year to year (Sosnowski et al. 2007a). Bunches on stunted shoots ripen unevenly, are small and, in severe cases, berries shrivel and die (Fig. 3C). Wood symptoms of Eutypa dieback include cordon dieback, with loss of spurs and internal, necrotic, wedge-shaped staining in the cross-section of cordons and trunks (Fig. 3D). External cankers appear as the dieback progresses, characterized by flatten areas of the wood with no bark, leading to eventual vine death (Fig. 3E). Perithecia of the fungus develop in the cankered wood and can be found embedded in the bark (Fig. 3F).

Eutypa dieback is caused by 24 species in the Diatrypaceae (Luque et al. 2012; Pitt et al. 2013a; Rolshausen et al. 2014; Trouillas et al., 2010), the most virulent and common of which is *Eutypa lata* (Carter 1991), and it is the only species known to be responsible for the foliar symptoms (Trouillas and Gubler 2010). Other Diatrypaceous genera include

Anthostoma, *Cryptosphaeria*, *Cryptovalsa*, *Diatrype*, *Diatrypella* and *Eutypella* (Luque et al. 2012; Trouillas et al. 2010).

Botryosphaeria dieback

Botryosphaeria dieback often presents as lack of spring growth from affected spurs (Fig. 3F and I) with shoot dieback, bud and xylem necrosis (Úrbez-Torres 2011). In the case of Botryosphaeria dieback, pycnidia develops from dead/cankered wood (Fig. 3H). The main wood symptom of Botryosphaeria dieback is wedge-shaped perennial cankers, indistinguishable to that of Eutypa dieback (Fig. 3J), or circular to non-uniform central staining of the wood observed in cross-sections of affected wood. However, Botryosphaeria dieback can be distinguished from Eutypa dieback in the field by the lack of foliar symptomatology (Leavitt 1990, Úrbez-Torres et al. 2006, 2008, 2015b).. Botryosphaeria dieback symptoms can appear in the field only one or two years after infections have occurred (Leavitt 1990, Úrbez-Torres et al. 2006) and are mainly observed in mature vineyards (over 8-year-old). However, cankers, dieback and plant death have been recorded in 3- to 5-year-old table-grape vines (Úrbez-Torres et al. 2008).

To date, 26 botryosphaeriaceaous taxa in the genera *Botryosphaeria*, *Diplodia*, *Dothiorella*, *Lasiodiplodia*, *Neofusicoccum*, *Neoscytalidium*, *Phaeobotryosphaeria* and *Spencermartinsia* have been associated with Botryosphaeria dieback of grapevines (Pitt et al. 2013b, 2013c, 2013d; Rolshausen et al. 2013; Úrbez-Torres 2011; Yang et al. 2017). Pathogenicty studies have demonstrated that species within the botryosphaeriaceous genera *Lasiodiplodia* and *Neofusicoccum* are among the fastest wood-colonizing fungi and hence the most virulent GTD fungi (Úrbez-Torres et al. 2008; Úrbez-Torres and Gubler 2009a; van Niekerk et al. 2004).

Phomopsis dieback

The most characteristic symptoms attributed to Phomopsis dieback are similar to those resembling Botryosphaeria dieback and include perennial cankers in the framework of the vine and lack of budbreak from infected spurs (Úrbez-Torres et al. 2013a). Symptoms of Phomopsis dieback were shown to be particularly high in vineyards severly affected by Phomopsis cane and leaf spot (Baumgartner et al. 2013; Úrbez-Torres et al. 2013a). Presently, seven species in the genera *Diaporthe* have been shown to be pathogenic on grapevine wood (Baumgartner et al. 2013; Dissanayake et al. 2015; Úrbez-Torres et al. 2013a). Among them, Phomopsis dieback is primarily caused by the most virulent *D. ampelina* (syn *Phomopsis viticola*), which has long been known as the causal agent of the grapevine disease named Phomopsis cane and leaf spot in USA or excoriose in Europe (Phillips 2000; Úrbez-Torres et al. 2013a).

Esca and Grapevine Leaf Stripe diseases

Two forms of the disease, chronic/mild and acute/apoplectic, have been traditionally reported to occur in vineyards. In the chronic or mild form, grapevine leaf stripe disease, leaf symptoms of affected vines are highly variable according to the literature: drying, dropping, reddening and yellowing (Lecomte et al. 2012). The most characteristic foliar symptom of this form corresponds to the 'tiger-stripe' pattern (Fig. 3K) (Surico 2009; Gubler et al. 2015). Leaves display multiple banding discolorations surrounding dry, light or red-brown necrotic tissue on the leaf blade, often bordered by narrow red or yellow blotches. The red color is normally absent in white cultivars. Superficial small redish and dark spots, known as 'black-measles', can also develop on the berry epidermis of white cultivars (Fig. 3L). The acute or apoplectic form, esca, is characterized by a sudden wilting of the entire plant or of one arm or several shoots (Fig. 3M). Leaf symptoms include scorching, dropping and shriveling. The drying of grape clusters is also frequently observed (Mugnai et al. 1999). Foliar symptoms of both forms of esca appear in late spring or summer, and can vary from year to year, similar to

Eutypa dieback foliar symptoms. Cross-sections of esca affected trunks reveal a variety of internal wood symptoms, such as black spots in the xylem eventually surrounded by pink to brown wood discoloration, brown to black vascular streaking, or dry wood with a silver appearance. In older vines, the wood may develop a white to yellow soft rot (Fig. 3N) (Fischer 2002).

The etiology of esca disease has been a matter of discussion among scientists over the last 20 years. A broad range of taxonomically unrelated fungal trunk pathogens and even endophytic bacteria have been isolated from wood tissues of esca diseased vines (Hofstetter et al. 2012; Bruez et al. 2014, 2015, 2016). However, the role of these microorganisms and how they interact with the primary fungi responsible for disease symptoms is still uncertain. The main hypothesis is that young vines infected with the pioneer fungi *P. chlamydospora* and/or species of *Phaeoacremonium*, *P. minimum* being the most prevalent and virulent, can later develop esca symptoms following further colonisation by several basidiomycetous species, although Koch's postulates are to be completed to support the role of the basidiomycete fungi in the symptomatology of the disease. The basidiomycetes belong to the genera *Inocutis*, *Inonotus*, *Fomitiporella*, *Fomitiporia*, *Phellinus* and *Stereum* (Cloete et al. 2015).

Epidemiology of grapevine trunk diseases

Grapevine pathogens responsible for Eutypa dieback, Botryosphaeria dieback, Phomopsis dieback, esca, and grapevine leaf stripe diseases are primarly spread through the dispersion of airborne spores, and for Botryosphaeria dieback and esca pathogens can also be propagated through the use of infected cuttings. Depending on the fungal species, ascospores or conidia are released from perithecia or pycnidia embedded in the bark and/or on the surface of dead grapevine wood (Eskalen and Gubler 2001; Pearson 1980; Rooney-Latham et al. 2005; Trese et al 1980; Úrbez-Torres et al. 2010a; van Niekerk et al. 2010). Additionally,

many of the fungal pathogens known to cause GTD have been also reported to cause cankers and dieback symptoms in a wide range of woody perennial crops (Carter 1991; Gramaje et al. 2016). Accordingly, it is has been demonstrated that some of these hosts can serve as a source of inoculum primarily when near vineyards but can also, in the case of *E. lata*, when located more than 50 km from grapevine-production areas(Ramos et al. 1975a; Petzoldt et al. 1983a). Ascospores and conidia are released under favorable environmental conditions, which are primarily associated with rain events and/or high relative humidity (RH) along with temperatures above freezing, which also favor spore germination (Úrbez-Torres et al. 2010a, 2010b; van Niekerk et al. 2010). Spores are then spread from pycnidia or perithecia by rain-droplets, wind or arthropods until they land on susceptible pruning wounds to germinate and start colonizing new xylem vessels and pith parenchyma cells (Mostert et al. 2006; Moyo et al. 2014). The potential for pruning shears to spread GTD pathogens has been demonstrated recently under greenhouse conditions (Agustí-Brisach et al. 2015). However, successful infection rates were relatively low (between 3.6 and 28.6% depending on the pathogen) and only occurred when pruning shears were pre-inoculated with high inoculum concentrations of 10^4 and 10^6 spores/ml. Carter and Moller (1971) showed that as few as 10 *E. lata* ascospores were likely to land on an apricot branch wound in natural conditions to initiate infection.

It has been shown that spore release and hence, high risk infection periods throughout the growing season vary depending on the fungal pathogen and geographical location but primarily overlap with dormant pruning seasons in both the Northern and Southern Hemispheres (Table 1). Susceptibility of grapevine pruning wounds to GTD fungi primarily depend on the pruning month and the time elapsed between pruning and possible infection events. Studies using artificial inoculations with spores indicate that grapevine pruning wound susceptibility is high when infections occur at the time of pruning but decreases as the interval

between pruning and infection increases over the following weeks and months, with seasonal variation reported between regions, due mainly to climatic differences (Table 2).

There are conflicting reports on the effect of wood age on wound susceptibilty to *E. lata*, with Moller and Kasimatis (1980) reporting significantly less infection on wounds of 1-year-old compared to that of 2 to 4-year-old wood (*Vitis vinifera* cv. Grenache), wheras Trese et al. (1982) reported no difference in *E. lata* infection between 1-2 and 3-year-old wood (*V. labrusca* cv. Concord). More recently, studies conducted in California showed both 1 and 2 year-old pruning wounds to be equally susceptible to infection caused by the botryosphaeriaceous fungi *L. theobromae* and *N. parvum* (Úrbez-Torres and Gubler 2011). It is difficult to make any conlusions from these varying results, as the studies involved different pathogens and grapevine cultivars, and were conducted in different environments. Exposure to rainfall has been linked to susceptibility to *E. lata* infection in apricot trees, based mainly on micro-organism activity in pruning wounds (Carter and Moller 1970, Price 1973) and susceptibility to GTD pathogens has been linked with lignin content and vascular diameter (Hamblin 2015, Pouzoulet et al. 2014, Rolshausen et al. 2008). Further research is necessary on the effect of wood age on GTD susceptibility and should consider the phenolic, vasculature, and micro-organism activity related to wound healing.

The pathogens responsible for black foot are soil-borne. Fungal species known to cause black foot are commonly found in nursery fields and soils and thus, inoculum may already exist in soils before planting (Agustí-Brisach et al. 2011, 2013a; Agustí-Brisach and Armengol 2013; Berlanas et al. 2017). Furthermore, several studies have shown evidence to support an endophytic phase of GTD fungi such as *P. chlamydospora*, *P. minimum* and several Botryosphaeriaceae spp. in grapevines (González and Tello 2011) as they have been isolated from asymptomatic rootstock mother plants (Halleen et al. 2003, 2007a; Edwards and Pascoe 2004; Fourie and Halleen 2004b; Aroca et al. 2010) and mature plants (Hofstetter et

al. 2012). It has been hypothesized that these fungi may become pathogenic to the grapevine following different biotic and/or abiotic stress factors and thus, they have been considered to play a role as latent pathogens in vines (Ferreira et al. 1999). Further investigation is required within the GTD complex to determine what triggers latent pathogens to transition from an endophyte to a pathogen, and cause disease symptoms.

Management of grapevine trunk diseases in nurseries and newly established vineyards

The large number of cuts and wounds made during the different steps of the propagation process in nurseries make the planting material vulnerable to infection by fungal trunk pathogens (Gramaje and Armengol 2011). Presently, no curative measures are known for control of black foot, Petri disease and/or Botryosphaeria dieback in nurseries and young vineyards. These diseases would be best managed by an integrated disease management strategy that combines the use of preventive measures, control options throughout the nursery mother blocks, which are the blocks nurseries used to gather propagation material from, the nursery process, nursery propagation beds, and newly planted nursery vineyards. These strategies are discussed below.

1) Nursery mother blocks

Pruning wound protection. Billones-Baaijens et al. (2015) partially reduced Botryosphaeriaceae infections of current-year shoots by protecting trimming wounds with fungicides. However, control strategies to prevent dormant pruning wound infections by Petri disease pathogens are still scarce. As such, mother vines in the nursery production blocks can accumulate infections by different trunk pathogens over time. Products and strategies to

protect pruning wounds in mother blocks are the same as those applied to mature commercial vineyards; therefore, this issue will be addressed in the next section.

Cultural practices and sanitation. Little attention has been paid to the role of mother vine management in the production of quality propagating material, and there is a paucity of literature on the subject. Several cultivation practices in mother plants can have a direct effect on trunk disease incidence and thus in the quality of graft material. Some nurseries cultivate rootstock mother vines on a trellis (Gramaje and Di Marco 2015), thus providing an increased shoot mass and longer quality shoots relative to rootstock mother vines cultivated along the ground (Waite et al. 2015; Gramaje and Di Marco 2015). Trellising can eliminate potential black foot disease pathogen contamination, but it is more expensive and labor intensive (Hunter et al. 2004). The susceptibility of ground-sprawling shoots to soil borne pathogens will increase as a result of higher temperature and humidity than vertical-positioned shoots, and possible mechanical damage (Whiteman et al. 2007).

Adequate soil moisture and aeration is important since overwatering favors most soilborne pathogenic fungi and reduces aeration in the root zone (Toussoun et al. 1970). Drainage in heavy soil can be accomplished by planting on raised beds and by moving drip irrigation emitters away from the vine (Gubler and Petit 2013). Drip irrigation is often used as the main source of irrigation once the vine root systems are established in both nursery blocks and commercial production vineyards. Overhead watering has been considered a good method of irrigation provided the sprinklers have a uniform distribution pattern and are mounted high enough to clear the foliage (Nicholas et al. 2001); however, this method could enhance foliar disease development (Koike et al. 2007). Recent studies demonstrated that overhead sprinkler irrigation can trigger release of Botryosphaeriaceae conidia and ascospores of the sexual morph of *P. minimum* in some vineyard sites in California (Gubler et al. 2013; Úrbez-Torres et al. 2010a). Burial or removal of dead wood and pruning debris in source blocks is strongly

recommended since numerous fungal fruiting bodies can otherwise be retained in the vineyards and become a potential source of inoculum for new infections (Elena and Luque 2016b).

2) Propagation processes in the nursery

Cultural practices. Viable propagules of black foot and Petri disease pathogens have been detected from washed pruning shears and grafting machines, and from hydration tanks during the propagation process for grafted plants (Retief et al. 2006; Aroca et al. 2010; Gramaje et al. 2011; Agustí-Brisach et al. 2013b; Cardoso et al. 2013; Waite et al. 2013a). Soaking cuttings in water for long periods of time could threaten the phytosanitary status of grapevine planting material, since this process promotes infection by the GTD pathogens (Pollastro et al. 2009; Aroca et al. 2010; Gramaje et al. 2011; Agustí-Brisach et al. 2013b). Grafting and callusing are critical stages in the grapevine propagation process and necessitate making wounds that are inherently vulnerable to contamination with trunk pathogens (Gramaje and Armengol 2011). Contaminated wounds and poorly matched graft unions fail to heal properly, remain open to fungal infection and create structural weaknesses in the finished vines (Stamp 2001). In the callusing stage, high temperatures that are sometimes used in nurseries can create weakened callus unions that may be more susceptible to trunk disease infection (Waite et al. 2015). The dark, humid and warm conditions in callusing rooms are particularly favorable for the growth of some pathogens (Hartmann et al. 2001). Regular treatments of the callusing room with disinfectants are therefore essential elements in trunk disease control. Stress conditions such as dehydration, soaking and anaerobic storage conditions, excessive wounding, exposure to extreme temperatures and toxic fumes from herbicides and fuels should also be avoided during the propagation in the nursery (Waite et al. 2015). Probst et al. (2012) demonstrated that grapevine cuttings and young vines subjected to

the stressful conditions of increasing periods of cold storage before rooting/callusing, also exhibited an increased susceptibility to black foot disease pathogens.

Chemical control. Several important focal points for chemical management have been identified in the nursery process, including time within hydration tanks and callusing boxes, post-grafting, during storage of cuttings and one year-old vines, pre-dispatch, and as wound protectants in both mother blocks and newly established vineyards (Fig. 4). The application of fungicides to control fungal trunk pathogens in the nursery process is difficult. Chemical dips and sprays used for the control of external pathogens do not penetrate grapevine cuttings sufficiently to control fungal pathogens inhabiting the vascular tissues (Waite and May 2005). However, the application of fungicides against trunk disease pathogens during the propogation process is a common practice in grapevine nurseries worldwide, and there are many reports of varying effectiveness (Table 3). In a recent survey performed among 146 European nurseries, only 8% of nurseries reported not using fungicides at any of the stages in the propagation process (Gramaje and Di Marco 2015). Chinosol® (Hydroxyquinoline sulphate) was reported to be the most commonly used fungicide; however, it was reported to be ineffective for the control of *P. chlamydospora* and *P. minimum* in Spanish grapevine nurseries (Gramaje et al. 2009b).

Hot-water treatment (HWT). Treating propagation material with hot water at 50°C for 30 minutes is the most effective method to disinfect dormant canes during the propagation process (Crous et al. 2001; Fourie and Halleen 2004a; Waite and May 2005). However, some anecdotal reports of unacceptably high losses when long duration HWT (50°C for 30 or 45 min) is applied to commercial batches of cuttings and rootlings have been published (Ophel et al. 1990; Bazzi et al. 1991; Wample 1993). In Italy, Habib et al. (2009) reported negative side effects on shoot development and growth of graftlings, rootstocks and grafted rootstocks (140 Ruggeri and 1103 Paulsen grafted with the Negroamaro cultivar) treated at 50°C for 45 min

after one growing season. In cooler-climate grapevine regions such as New Zealand it has been shown to cause mortality of cuttings and has led to a recommendation of 48°C treatment for 30 minutes, which also decreases the efficacy against GTD pathogens (Bleach et al. 2013; Billones-Baaijens et al. 2015). On the other hand, studies conducted in Spain have shown that 53°C for 30 minutes significantly improves efficacy against trunk disease pathogens without detrimental effects to cuttings (Gramaje et al. 2010a, 2014). These reports suggest that grapevine cuttings taken from vines grown in warm climates might be superior to cuttings taken from vines grown in cool climates and better able to withstand HWT. In this sense, Crocker et al. (2002) found that in south-eastern Australia cuttings sourced from well-managed vineyards and rootstock plantings in warm climates performed better in propagation than cuttings from vineyards in cool climates, or vineyards that had suffered from water stress in the growing season prior to cutting collection.

Hot-water treatments can be applied to rootstock cuttings pior to grafting (Edwards et al. 2004; Eskalen et al. 2007; Fourie and Hallen, 2004; Halleen and Fourie 2016) or to young grafted vines just prior to dispatch (Fourie and Halleen 2004a; Halleen et al., 2007a; Halleen and Fourie 2016) (Fig. 4). HWT material is susceptible to stresses caused by inappropriate handling practices, such as prolonged cold storage periods after HWT (Gramaje and Armengol 2012) and does not provide 100% control of trunk disease pathogens (Rooney and Gubler 2001; Gramaje et al. 2010a), hence its use remains controversial (Gramaje and Di Marco 2015; Waite et al. 2013b). However, it is well-known that HWT is successful in eliminating pests and other detrimental organisims, such as the bacteria *Agrobacterium vitis* causing crown gall disease and *Xylella fastidiosa* causing Pierce's disease, and the phytoplasma Flavescense dorée (Waite and May 2005; EFSA PLH Panel 2015). Additionally, HWT is required in some countries such as Canada for imported planting material. Other negative effects of HWT include delayed callusing and rooting of cuttings (Waite and May

2005), delayed development or bud death in cuttings and grafted vines (Caudwell et al. 1997; Gramaje et al. 2009a; Laukart et al. 2001), and failed or incomplete healing of graft unions and fermentation in cold storage (Waite and Morton 2007). A summary of the published studies investigating the efficacy of HWT in controlling fungal trunk disease pathogens is provided in Table 4.

Biological control. Most studies on biological control on GTD have examined the application of *Trichoderma atroviride* and *T. harzianum* in nurseries. *Trichoderma* can be found as commercial products in various formulations, including powder, granules/pellets and dowels. Powder can be mixed with water for application by soaking plants during the hydration stage in nurseries. Incidence of *P. chlamydospora* and *Phaeacremonium* spp. in rootstock cuttings was reduced by soaking the planting material in *Trichoderma* formulations (Di Marco et al. 2004; Fourie and Halleen 2004a, 2006; Halleen and Fourie 2016). Dipping young infected plants in *T. atroviride* strain I-1237 resulted in a decreased necrosis caused by *D. seriata* and *P. chlamydospora* in French nurseries (Mounier et al. 2014). More recently, Pertot et al. (2016) demonstrated that the application of *T. atroviride* strain SC1 at the hydration, callusing and pre-planting stages in Italian grapevine nurseries effectively reduced infection by *P. chlamydospora* and *P. minimum*, hydration treatments being the most effective.

Use of resistant rootstocks. In nurseries, research has been focused on determining the susceptibility of grapevine rootstocks to black foot and Petri disease pathogens. In general, the incidence and severity of these diseases has been significantly affected by rootstock genotype. However, none of the rootstocks tested have shown complete resistance to black foot and Petri disease pathogens (Alaniz et al. 2010; Eskalen et al. 2001; Gramaje et al. 2010b; Gubler et al. 2004; Jaspers et al. 2007). More recently, Brown et al. (2013) evaluated the susceptibility of four common grapevine rootstocks to *Cylindrocladiella parva* in pot

experiments in New Zealand and concluded that Riparia Gloire was the most susceptible and Millardet et de Grasset 101-14 the least. Regarding the susceptibility of rootstocks originating from crosses of North American *Vitis* spp. to Petri diseases pathogens, none of the 20 evaluated by Eskalen et al. (2001) were resistant to infection caused by *P. chlamydospora*, *P. inflatipes* or *P. minimum* in controlled conditions. Studies conducted by Gramaje et al. (2010b) showed 161-49 Couderc to be the least susceptible among five grapevine rootstocks vacuum inoculated with *Cadophora luteo-olivacea*, five species of *Phaeoacremonium*, or *P. chlamydospora* under field conditions in Spain (Gramaje et al. 2010b). In the north coast of California, large-scale replanting of grapevine rootstock crosses of *V. berlandieri* × *V. riparia* by new rootstock crosses of *V. riparia* × *V. rupestris* and *V. berlandieri* × *V. rupestris* in the early 1990s resulted in increased signs of plant decline and subsequent death (Gubler et al. 2004). Species of *Phaeoacremonium* and *P. chlamydospora* were later isolated from these affected vines. This information and the results published by Gramaje et al. (2010b) suggest that grapevine rootstock crosses of *V. berlandieri* × *V. riparia* could be the least susceptible to Petri disease pathogens. In contrast, regarding Botryosphaeriaeceae spp., Billones-Baaijens et al. (2014) concluded that 5C and SO4 rootstocks (*V. berlandieri* × *V. riparia)* were the most susceptible to *Neofusicoccum* spp. infection among the six most common genotypes used in New Zealand.

Alternative methods. The use of several ameliorative treatments to reduce disease progress and symptom expression has been reported. The application of electrolyzed acid water to cuttings during the hydration stage was evaluated by Di Marco and Osti (2009) in Italian nurseries, and results showed that this disinfectant was effective in reducing conidial germination of *P. chlamydospora* and *P. minimum* without affecting plant growth and development in the nursery field. The use of ozonation as a novel technique to disinfest grapevine planting material has given inconsistent results. Vigues et al. (2010) concluded that

ozonation was ineffective to control Botryosphaeriaceae spp. and *P. chlamydospora* in French nurseries. More recently, Pierron et al. (2015) evaluated the efficacy of ozonated water *in vitro* and *in planta* and concluded that this method suppressed *P. minimum* spore germination *in vitro* and reduced fungal development by 50% *in planta* at 9 weeks post inoculation of pruning wounds. Further studies are required to determine the effectiveness of this method against internal vascular infections.

3) Nursery propagation beds

Crop rotation in nursery fields. This method may have a limited effect with soil borne pathogens that cause black foot disease, because they produce long-lived spores or can survive as saprophytes for long periods of time. Halleen et al. (2003) concluded that planting grapevine cuttings every second year in a nursery field, followed by a cover crop, may have led to increased black foot disease inoculum. In a nursery field where grapevines had been planted consecutively for two years, followed by three years of rotation with other crops (e.g. potato, cabbage, carrot, garlic, leek and cereals), a high proportion of plants was reported to be infected with "*Cylindrocarpon"* spp. (Rego et al. 2009). However, Jaspers and Billones-Baaijens (2014) recommended rotation of field sites with a mustard crop as the best practice to reduce black foot and Petri disease infections in nursery fields. Black foot disease pathogens were detected in soils during the rotation cycle with crops such as wheat and barley in Portugal (Cardoso et al. 2013) and Spain (Berlanas et al. 2017). Further research is needed to determine the duration of fallow periods for perennial crops and their role in mainainting the fungal inoculum bank in soil.

Alternative methods. The potential of the biofumigant Indian mustard seed meal (*Brassica juncea*) was evaluated in nursery fields and vineyards as an alternative for metham sodium and methyl bromide for the control of black foot pathogens. In Australia and New

Zealand, biofumigation using this treatment significantly improved the growth and yield parameters when buried under diseased grapevines (Whitelaw-Weckert et al. 2014), reduced disease when callused rootstock cuttings were planted into artificially infested soil (Bleach et al. 2010) and significantly reduced black foot inoculum in amended soils (Barbour et al. 2014).

4) Commercial production settings

Site preparation for newly established vineyards. Preplanting care is critical to maintaining the quality of the vines. The vineyard must be ready for planting pre-dispatch with irrigation infrastructure, weed control and cultivation completed (Agustí-Brisach et a. 2011; Waite et al. 2015). Vines should be planted immediately on arrival at the vineyard. Planting in heavy and poorly drained soils should be avoided as it can favor infection by black foot pathogens (Rego et al. 2000; Halleen et al. 2007a). Site preparation should be made based on assessment of inoculum density and distribution in soil. This could be achieved by using a semi-selective culture medium (Berlanas et al. 2017), by fungal isolation from roots of grapevine seedlings used as bait plants (Agusti-Brisach et al. 2013a), or by molecular methods (Agustí-Brisach et al. 2014; Probst et al. 2010; Tewoldemedhin et al. 2011; Úrbez-Torres et al. 2015a). However, there is a need for high throughput, more sensitive and cost-effective molecular methods to be developed and commercialised for industry use.

Pruning wound protection. Products and strategies to protect pruning wounds in young vineyards are the same as those applied to mature commercial vineyards; therefore, this issue will be addressed in the next section.

Biological control. Powder formulations of *Trichoderma* can be mixed with water for application on aerial plant parts as wound protectants with sprayers. It can also be applied to the soil through irrigation emitters or even directly to soil as drenches. Granules or pellets can

be incorporated in compost or directly into soil as soil amendments (Mutawila et al. 2011b). The efficacy of the *Trichoderma* biocontrol products is dependent on the active growth of the fungal active ingredient, which could be compromised by application mixtures containing fungicides and by application of toxic fungicides before and/or after treatment with *Trichoderma* inoculum. In this sense, Mutawila et al. (2015) recently developed benzimidazole resistant mutant *Trichoderma* strains by gamma irradiation, which were effective in protecting pruning wounds against fungal trunk pathogen infections. Incidence of *P. chlamydospora* and *Phaeacremonium* spp. in rootstock cuttings was reduced by applying soil amendments and root drench treatments with *Trichoderma* (Fourie et al. 2001) or

Other biological control agents have recently been evaluated to control fungal trunk pathogens with promising results. The oomycete *Pythium oligandrum* Drechsler was effective in colonizing grapevine roots and reducing the wood necroses caused by *P. chlamydospora* in Cabernet Sauvignon cuttings (Yacoub et al. 2016). Inoculation of roots with the mycorrhizal fungus *Rhizophagus irregularis* (syn. *Glomus intraradices*) reduced both the number of root lesions, as well as disease severity caused by black foot disease pathogens (Petit and Gubler 2006). The effects of beneficial bacteria inhabiting the rhizo- and/or the endosphere of vines in reducing fungal trunk diseases (directly or indirectly) has been reviewed recently by Compant et al. (2013). *In vitro* assays of the heat stable metabolites of *Bacillus subtilis* AG showed promise in reducing the growth of *L. theobromae, P. chlamydospora* and *P. minimum* (Alfonzo et al. 2009). Rezgui et al. (2016) recently identified several *B. subtilis* strains inhabiting the wood tissues of mature grapevines in Tunisia with antagonistic traits against fungal trunk pathogens. The antagonistic activity of 46 bacterial strains isolated from wood tissue and the grape berry surface was evaluated against *N. parvum* (Haidar et al. 2016a) and *P. chlamydospora* (Haidar et al. 2016b) with thirteen strains able to reduce lesion length in inoculated grapevine cuttings.

Cultural practices and sanitation. In some countries, graft unions are usually covered with soil to prevent drying of the callus tissue (Fourie and Halleen 2006); however, this practice could increase the occurrence of black foot disease pathogens in this plant zone. Adequate vine and root development should be allowed to occur prior to placing a heavy fruit load on vines in the early production years in order to avoid stress, thereby reducing the likelihood that endophytic GTD organisms will become pathogenic. However, the factors that lead to symptom expression in establishing vineyards are not well understood and require further investigation.

Management of grapevine trunk diseases in mature vines

Research conducted over the past 50 years has generated a good understanding on the etiology and biology of GTD fungi, identifying high risk infection and susceptibilty periods throughout the year. Knowledge of these infection windows assists in the development of effective management strategies by the use of appropriate cultural practices, remedial control strategies and application of preventative fungicides and/or biological agents to wounds. Currently, it is well-accepted that an integrated pest management (IPM) approach, in which a combination of all of the afformentioned control options is implemented, is probably the most successful strategy to minimize GTD infections in vineyards (Berstch et al. 2013).

Cultural practices

Vineyard sanitation. As fruiting bodies containing the spores of GTD fungi are primarily developed in dead or infected tissues of spurs, cordons and trunks, removing and destroying all diseased wood from the vineyard still remains the best practice to reduce the number of new infections for all GTD pathogens affecting mature plants (Fig. 5A). However, because sourrounding vineyards or orchards can also be a source of inoculum, it would be important that sanitation is implemented across production regions. However, this type of

widescale cooperation would be challenging, and to date there are no known examples of this occurring. Pruning debris has also been shown as a reservoir for GTD inoculum since pycnidia, primarily from Botryosphaeriaceae spp., have been commonly observed the following year on prunings left on the ground of vineyards (Úrbez-Torres et al. 2010a; Elena and Luque 2016b). For this reason, it is also recommended to eliminate the pruning debris from the vineyard. Infected wood and pruning debris can be destroyed by burning, burying, mulching and incorporation into the soil of the vineyard or composting (Fig. 5B and C). Burning has several environmental disadvantages; therefore, this practice is being replaced by other options such as composting or mulching. Lecomte et al. (2006) showed composting of vine material along with sheep manure and garden residues for six months to successfully eliminate inoculum of *D. seriata*, *P. chlamydospora*, *P. minimum* and *E. lata* from grapevine wood tissue. The dormant application of lime sulfur is also recommended in California as a sanitation practice to reduce the inoculum of Botryosphaeria dieback, Eutypa dieback, and esca (Adaskaveg et al. 2015).

Remedial surgery. Remedial surgery, where visibly infected parts of the vine (cordons and/or trunks) are cut and removed, has long been implemented in vineyards to control Eutypa dieback (Carter 1994; Creaser and Wicks 2004; Sosnowski et al. 2011b). The success of remedial surgery is dependent on the removal of all infected wood, including removal of an extra 10-20 cm of apparently healthy tissue beyond any visible staining (Sosnowski et al. 2007b; 2016a; 2016b). Grapegrowers are recommended to identify and flag infected vines and parts of vines in the spring/summer, and remove infected wood in the following winter pruning season, but surgery can be conducted at any time of the year **(**Fig 5D and E). However, the success of remedial surgery is very limited if infection has reached the ground or graft union (Fig. 5F). This is particularly true for esca diseased plants, where internal necrosis is often observed in both scion and rootstock wood of affected plants, thus

completely removing infected wood is difficult (Calzarano et al. 2004). An ancient custom still practiced today in some European countries is believed to delay the recurrence of esca foliar symptoms. This involves opening the trunks of symptomatic vines, in the middle and inserting a stone so as to expose the rotted wood to the air (Fig. 5G) (Surico et al. 2008). However, no scientific evidence has been provided on the effect of this practice.

Studies conducted in Australia have shown that making cuts lower down on the trunk (20-30 cm above the ground) improve the likelihood of eradicating the pathogen from the vine (Sosnowski et al. 2011b). Trunks and cordons can be retrained from watershoots, returning vines to full production within a few years (Fig. 5H and I). When infection has reached ground level in trunks of own-rooted vines, layering can be used to self-rejuvenate vines (Ahrens 2010), or healthy canes can be taken from a neighbouring vine to replace a diseased or dead vine (Fig. 5J) (Nicholas et al. 2001). Remedial surgery has also been shown to effectively control Botryosphaeria dieback in California vineyards (Leavitt 1990). More recent studies conducted in Australia on the use of remedial surgery to control Botryosphaeria dieback also revealed the importance of low trunk cuts to ensure all affected wood is removed (Savocchia et al. 2014). Along with providing effective control against some GTDs, remedial surgery offers other benefits, such as retaining a superior clone and maintenance of an established root system, which leads to a rapid return to full production. On the other hand, remedial surgery is a labour intensive practice and highly-skilled workers are needed. Remedial surgery was shown to be an expensive operation with costs of up to USD$4.20/plant or USD$1,960/ha in 2008 (Epstein et al. 2008). Nevertheless, remedial surgery still remains cost-effective if compared against the cost of pulling out and replanting an entire vineyard (Sosnowski and McCarthy 2017). Remedial surgery is more difficult to accomplish in grafted vines, with watershoot production limited in the scion portion when

cuts were made 30-40 cm above the graft union (Savocchia et al 2014). Additionally, grafted vines cannot be retrained if infection has developed beneath the graft union.

Pruning and training. Based on the knowledge gained from the different epidemiological studies conducted in grape-growing regions around the world, reduction of new GTD infections in a vineyard can be effectively achieved by pruning management. No matter which GTD fungi are involved, spore release has generally been shown to correlate with rain events and moderate temperatures. Accordingly, pruning in wet weather should be avoided and conducted during periods when inoculum is less prominent and wound healing is more rapid. Based on the studies conducted by Petzoldt et al. (1981, 1983b), late pruning (mid-February to early March) has since then been recommended to reduce infections caused by *E. lata* in California, with weather conditions then favoring a faster healing of pruning wounds than earlier in the season and also correlating with periods of lower amounts of ascospores in the environment. Similarly, later studies conducted in California also showed late pruning as an effective cultural practice to reduce Botryosphaeria dieback pathogen infections (Úrbez-Torres et al. 2010a; Úrbez-Torres and Gubler 2011). On the other hand, the window to complete this operation is relatively short for the large vineyards of California and so a double-pruning technique was developed. This involves mechanical pre-pruning, leaving long canes (> 40 cm) on existing spurs in early winter coinciding with the highest amount of inoculum present in the environment. The idea behind this technique is that if canes get infected, the pathogen will not have enough time to reach the final two-bud spur left following the final prune in late winter (Weber et al. 2007). In California, inoculum levels of both Botryosphaeria and Eutypa dieback are much lower in late winter reducing the risk of infection in the pruned vines. Double pruning is nowadays a common cultural practice used by grape-growers in California for control of Botryosphaeria dieback, esca and Eutypa dieback (Hersch 2009; Úrbez-Torres and Gubler 2009b; Weber et al. 2007). However, double

pruning is best implemented in specific trellis systems where pre-pruning costs can be minimised by using mechanical pruning systems such as vertical shoot position (VSP), California sprawl, or Geneva double curtain, adding an estimated USD$247 per ha/year into production costs (Hillis et al. 2016). Recent epidemiological studies conducted in Spain showed a much higher level of infection by GTD pathogens in pruning wounds made in late winter (February) than in late Fall (November), suggesting that under those specific geographical and environmental conditions, early pruning can minimize GTD infection rates (Elena and Luque 2016a; Luque et al. 2014). Because enviromental conditions and availability of inoculum throughout the year have been shown to vary among grape-growing regions worldwide, it is imperative to conduct epidemiological studies on a regional basis in order to optimize pruning to minimize GTD development. Although contamination of pruning tools has been reported (Augusti-Brisach et al. 2015), it is not likely to be a major means of spreading trunk disease, but the use of curative fungicide as wound protection will reduce the likelihood of any infection. Removal of watershoots in spring can lead to sporadic infection (Lecomte and Bailey 2011; Makatini et al. 2014), so it is recommended that shoot thinning in wet weather be avoided.

Gu et al. (2005) demonstrated that grapevine trained to a head, rather than to bilateral cordons, showed lower incidences of Eutypa dieback in California. Similarly, a study on esca in France revealed a higher incidence of wood necrosis in the cordons of vines under a ‘Lyre’ training system (cordons of 80 cm in length) versus a ‘Guyot’ training system (cordons of 20 cm in length), probably due to the greater number of pruning wounds and the resulting infection courts with the ‘Lyre’ system (Lecomte et al. 2012). In a 10-year field trial in France, Dumot et al. (2004) reported that foliar symptoms of Eutypa dieback were more prevalent in spur-pruned vines, but after 20 years, greater mortality was reported in cane-pruned vines (Dumot et al. 2012). Therefore, symptoms are expected to be visible earlier on

spur-pruned vines, which have greater numbers and surface areas of pruning wounds than cane-pruned vines. However, large wounds located on the crown of cane-pruned vines can lead to trunk infection, causing vine death in mature vines with fewer visible external symptoms. More recently, Travadon et al. (2016) examined the effects of two pruning systems, minimal or spur-pruning, on the wood mycobiota of Mourvedre and Syrah cultivars. They concluded that minimal pruning system, with fewer pruning wounds per vine, was associated with less wood necrosis and a lower incidence of esca than a standard, spur-pruning system.

Vineyard management. Abiotic factors have been shown to influence the expression of symptoms or the progression of Eutypa dieback, and so knowledge of these factors is important in managing this disease. Foliar symptom expression has been linked to environmental factors with seasonal variation in the incidence of symptoms reported in France (Dumot et al. 2004), USA (Butterworth et al. 2005) and Australia (Sosnowski et al. 2007a). Sosnowski et al. (2007a) associated foliar symptom expression with winter rainfall, suggesting that greater water availability could facilitate the transport of toxins to the foliage in spring. Furthermore, a lower disease incidence was associated with increased temperature in spring, suggesting that more vigorous vine growth and greater plant biomass reduced the concentration of toxic fungal metabolites, and hence the expression of foliar symptoms. Sosnowski et al. (2011a) reported significantly greater foliar symptom expression in potted vines subjected to extreme temperature and moisture conditions (both low and high), although this did not correlate to mycelial growth in the wood tissue of stems.

Regulated deficit irrigation watering and the partial rootzone drying technique control and manage water stress by not providing the full requirement of water to vines, in order to reduce vigour and increase fruit quality whilst also conserving water (McCarthy et al. 2002). Low soil water content (Hardie and Considine 1976; Lovisolo and Schubert 1998; Smart and

Coombe 1983) and high temperature (Kriedemann and Smart 1971) have both been implicated as causes of stress on grapevines. Grapevines under deficit irrigation in a warm, dry environment were reported to be more susceptible to pruning wound infection by *E. lata* (Sosnowski et al. 2011a). However, this practice was later reported to reduce the distance of pathogen colonisation within canes of water stressed field vines (Sosnowski et al. 2016a), leading to the conclusion that water stress was not exacerbating Eutypa dieback wood symptoms. Botryosphaeria dieback has also been associated with water stress. Van Niekerk et al. (2011b) showed that in potted grapevines that were exposed to water stress, lesion length was greater due to *Neofusicoccum australe*, *N. parvum*, *L. theobromae* and *D. seriata* were greater in water-stressed vines that in non-stressed vines. Amponsah et al. (2014) reported that high and low soil moisture levels imposed stress on potted grapevines, making them more susceptible to infection by *N. luteum*. Furthermore, Lawrence et al. (2016b) showed that *N. parvum* caused more severe lesions on potted Cabernet Sauvignon vines subjected to water stress that on control vines not subjected to such stress. However, in a field trial, Sosnowski et al. (2016a) reported no increase in colonisation by *D. seriata* in canes of water stressed vines compared with non-stressed vines. It was also reported here and by Sosnowski et al (2016b) that the distance of *D. seriata* or *E. lata* recovery and lesion length were not correlated, similar to that reported earlier for *E. lata* (Sosnowski et al 2007b). This indicates that lesion length is not a reliable measure of susceptibility to pathogen colonisation.

Esca and Petri disease symptoms are exacerbated in grapevines under water stress, as was shown with one of the main causal pathogens *P. chlamydospora* by Ferreira et al. (1999) and Edwards et al. (2007a, 2007b). Water stress conditions during mid-summer are linked to the occurrence of apoplectic symptoms of esca, while cool and rainy summers favour the development of chronic esca symptoms (Surico et al. 2000). The latter authors reported that in vineyards that gradually slope down to a level area, esca disease symptoms are encountered in

higher frequencies in the level areas where water has accumulated. More recently, Fischer and Kassemeyer (2012) also reported increased wood symptoms in vine cuttings under water stress and inoculated with *P. chlamydospora*. Conflicting reports on the impact of water stress on GTD are most likely due to the different methodologies employed. Evidence, at least for Eutypa and Botryosphaeria dieback, that foliar and wood visual symptoms do not correlate to pathogen colonisation (Sosnowski et al 2007b, 2011, 2016a, 2016b), puts into doubt results based merely on visual foliar and wood symptoms. In addition, the use of deficit irrigation practices are an important strategy for canopy and and fruit quality control, and so it is unlikely that the practice would be eliminated to avoid increasing susceptibility to GTDs, when there is insufficient evidence to do so.

***Wound protection*.** Wound protection is the most effective strategy for controlling GTD when compared with remedial surgery (Sosnowski and McCarthy 2017), and especially if adopted early in the life of the vineyard (Kaplan et al. 2016, Sosnowski and McCarthy 2017). Many products have been evaluated, with the most efficacious listed in Table 5. Pruning wound protection studies on grapevines date back to early 1980s when Moller and Kasimatis (1980) first showed protection against the fungus *E. lata* by applying benomyl and thiabendazole to grapevine pruning wounds. This followed earlier research on pruning wound protection against *E. lata* for apricots (Carter 1971; Carter and Price 1974; Moller and Carter 1969; 1970; Moller et al. 1977a). Ever since, there has been extensive evaluation of the efficacy of grapevine wound treatments for *E. lata*. Similarly, Leavitt (1990) showed for the first time protection of pruning wounds against the Botryosphaeria dieback fungus *L. theobromae* by applying products to grapevine pruning wounds. Grafting mastic, paints and pastes are the most reliable wound protectant, particularly when they are supplemented with fungicides (Fig. 5K) (Moller et al. 1977b; Rolshausen and Gubler 2005; Rolshausen et al. 2010; Sosnowski et al. 2008, 2013; Tulloch 1960). These not only provide a physical barrier

to stop GTD pathogen spores from entering the wounds, but should the physical barrier be compromised by sap flow, rain or cracking when drying, the fungicide can then act on the pathogens. Paint and paste treatments are applied by hand with paint brush or specially designed applicators. This can be very costly, two to four times the cost of application with a tractor mounted sprayer (Sosnowski and McCarthy 2017) and so prohibitive. Hence there is a need for effective liquid formulation fungicides that can be applies with a sprayer.

Of the fungicides evaluated, based on frequency of reports from literature, the methyl benzimidazole carbamate mode of action group (benomyl, carbendazim and thiophanate methyl) are the most effective against both Botryosphaeria and Eutypa dieback pathogens (Table 5). The demethylation inhibitors; tebuconazole and flusilazole, the anilinopyrimidine; pyrimethanil, the quinone outside inhibitor; pyraclostrobin, and the 2,6-dinitro-aniline; fluazinam, are also very effective. Liquid formulation fungicides have been applied with pneumatic sprayers (Carter and Perrin 1985; Munkvold and Marois 1993b) and more efficient strategies of applying with tractor driven sprayers have been developed (Fig. 5L) (Ayres et al. 2017b; Carter and Price 1977; Herche 2009; Lecomte et al. 2003; Ramsdell 1995; Sosnowski et al. 2013; Sosnowski and Mundy 2016) making it more economically viable for annual post-pruning wound protection in large-scale vineyards (Sosnowski and McCarthy 2017).

Regarding the esca disease pathogens, Rolshausen et al. (2010) showed that thiophanate-methyl was very efficient in controlling *P. minimum*, *Phaeoacremonium parasiticum* and *P. richardsiae* but did not perform as well against *P. chlamydospora.* Control of *P. chlamydospora* was better achieved by applying boron to pruning wounds. Díaz and Latorre (2013) evaluated the efficacy of paste and spray fungicide applications in protecting pruning wounds against *D. seriata*, *Inocutis* sp. and *P. chlamydospora* and concluded that mixing of the paste with thiophanate-methyl provided the best control of these pathogens. Fosetyl-Al applications limited the extent of wood necrosis in young vines inoculated with *P.*

chlamydospora (Laukart et al. 2001), and *P. chlamydospora* and *P. minimum* (Di Marco et al. 2011), and these treatments reduced both esca leaf symptom expression and vine mortality under field conditions (Di Marco et al. 2011).

Biological control agents have shown variable results for preventing infection by *E. lata*. *Fusarium lateritium*., a saprophyte associated with apricot wood, showed promise for control of *E. lata* in apricot and grapevine wounds (Carter 1971; Carter and Price 1974; John et al. 2005; Munkvold and Marois 1993a) particularly in combination with benomyl (Carter and Price 1975). The natural epiphyte *Cladosporium herbarum* reduced infection of grapevine wounds by *E. lata* (Munkvold and Marois 1993a; Rolshausen and Gubler 2005), and *Trichoderma* spp. have also provided varying control of *E. lata* in grapevines (Halleen et al. 2010; John et al. 2005; Kotze et al. 2011; Mutawila et al. 2011a, 2015). The bacterial biocontrol agent *Bacillus subtilis* also reduced infection of *E. lata* in pruning wounds (Ferreira et al. 1991; Kotze et al. 2011). Although biological alternatives may offer long-term protection, the 1-2 weeks required for biological control agents to colonise the wound creates a window of susceptibility to infection (Carter and Price 1975; Munkvold and Marois 1993a).

Kotze et al. (2011) found benomyl to be less effective in pruning wound protection as compared with *Trichoderma* spp. treatments when wounds were inoculated with *D. ampelina*, *D. seriata*, *E. lata, N. australe*, *N. parvum*, *L. theobromae* and *P. chlamydospora* 7 days after application. Di Marco et al. (2004) reported the potential of *T. harzianum* to protect pruning wounds against artificial infection by *P. chlamydospora* under field conditions. Mutawila et al (2016) recently reported that pruning early in the season in combination with the application of *T. atroviride* approximately 6 h after pruning could significantly reduce wound infection by GTD pathogens. More recently, several natural compounds, including garlic extract, lactoferrin, tea tree oil, chitosan, oligosaccharide, lichen extract, lemon peel extract, and vanillin have also shown promise for control of GTD, but further research is required before

recommendations can be made for widescale application (Ayres et al. 2017b; Cobos et al. 2015; Nacimento et al 2007; Sosnowski et al. 2013).

It is important to note that for most of the research reported, an artificially large number of pathogen spores were applied (i.e., >500 spores per wound) in order to ensure a substantial incidence of infection in untreated controls for statistical analysis. This can be seen in the high recovery rates reported in inoculated versus non-inoculated controls, which reflect natural infection. Carter and Moller (1971) showed that as few as 10 *E. lata* ascospores were expected to land on a wound on a stone fruit tree in natural conditions, leading to 13-45% recovery of the fungus. More recently, Elena et al. (2015b) showed that dose ranges between 100 and 2000 conidia of *D. seriata* and *P. chlamydospora* and between 100 and 500 ascospores of *E. lata*, were required to obtain robust recovery percentages of 50 to 70%. Therefore, the large number of ascospores/conidia used in most field trials represent significantly greater "disease pressure" than that which may be expected to occur naturally, and therefore the efficacy of wound treatments evaluated would likely be even greater than results indicate. Ayres et al (2017b) reported increased efficacy of fungicide wound treatments when inoculum doses of *E. lata* were reduced from 1000 to 200 spores/wound.

Disease resistance

There have been reports of varying susceptibilty of *Vitis vinifera* cultivars to GTD. Reports on the resistance or susceptibility to Eutypa dieback of cultivars grown in France, based on foliar symptoms in the vineyard, showed that of 32 cultivars assessed, five were categorised as resistant (cvs. Aligote, Grolleau, Merlot, Semillon and Sylvaner) and all others listed as moderately to highly susceptible (Carter 1991). Based on three surveys conducted in South Australia over the past 40 years (Wicks 1975; Highet and Wicks 1998; Loschiavo et al. 2007), the cvs. Grenache, Cabernet Sauvignon and Shiraz were recorded with the highest incidence of Eutypa dieback foliar symptoms and cvs. Merlot, Riesling, Pinot noir, Sauvignon

blanc, Chardonnay and Semillon with the least. The growth of *E. lata* in grapevine wood varied and cvs. Merlot, Gamay, Grenache and Semillon were recorded with half of the rate of dieback compared with cvs Cabernet Sauvignon and Shiraz (Sosnowski et al. 2007b). For Botryosphaeria dieback, studies on lesion length in canes of several cultivars of *V. vinifera* and other *Vitis* spp. indicated variation in susceptibility (Billones-Baaijens et al. 2014; Guan et al. 2016; Pitt et al. 2013a; Savocchia et al. 2007; Travadon et al. 2013; Úrbez-Torres and Gubler 2009a). Sosnowski et al. (2016b) reported vast variation in GTD symptoms on mature vines in a *V. vinifera* germplasm repository and found significant differences in rate of pathogen colonization of grapevine canes by *E. lata* and *D. seriata* between cultivars and rootstocks suggesting possible tolerance or resistance.

Esca symptoms in the vineyard have been reported with varying incidence between cultivars, rootstocks and clones of grapevine (Fussler et al. 2008; Marchi 2001; Murolo and Romanazzi 2014). Furthermore, inoculations with *P. minimum* and *P. chlamydospora* have indicated differential susceptibility of grapevine cultivars (Feliciano et al. 2004; Landi et al. 2012). No evidence of qualitative resistance to the causal agents of Botryosphaeria dieback, esca, Eutypa dieback and Phomopsis dieback was found among several commercial and wild *Vitis* spp. in California under greenhouse conditions (Travadon et al. 2013).

Little is known about the mechanisms of resistance to GTD. Relatively high lignin levels has been associated with wood and cane tissue of grapevine cultivars having more tolerance to *E. lata* infection (Hamblin 2015; Rolshausen *et al.* 2008). Furthermore, tolerance has also been correlated to xylem vessel diameter for both esca pathogens (Pouzelet et al. 2014) and *E. lata* (Hamblin 2015). Recently, Pierron et al. (2016) reported gene expression by grapevine woody tissue cells when inoculated with *P. minimum* and *P. chlamydospora*, suggesting the activation of defence mechanisms. These results warrant further investigation as such traits may be useful markers when selecting for tolerant cultivars or new genotypes.

Furthermore, in the shorter term, rootstocks and clones found to be more tolerant to GTD can be recommended for future plantings, which will contribute to vineyard longevity.

Future prospects for effective management of grapevine trunk diseases

The global increase in incidence of GTD, along with the difficulty of effectively managing these diseases, has positioned GTD as a top research priority for the grape and wine industry worldwide. Although reduction in the availability of efficient chemical controls since 2000 has played a role in the impact that GTD has on grapevine health today, it is also a consecuence of changes experienced in viticulture in the past 30 years. Increase of plant density in vineyards, more common use of double cordon, spur-pruned vines and mechanization of vineyard practices, in particular pruning, have favoured the increased infection with GTD pathogens on grapes. Furthermore, the overall rise in production costs, particularly labour, reduces the ability for growers to increase inputs, such as protection of pruning wounds. Adding further challenges, the etiology of grapevine trunk diseases has become more complex in recent years with the emergence and description of many more fungal pathogens. Therefore, there is a need to build on the advances over the past few decades in our understanding of how these factors favour the development of GTD, as well as further improving the efficiency of new strategies for disease management. Here, we discuss the future direction of GTD research that is required to fill the existing gaps in our knowledge.

Minimizing infection in planting material. It is imperative to start with the healthiest planting material possible. An example of successful production of clean plant material to minimize the impact of disease can be found with viruses. Many clean grapevine plant programs currently exist around the world, which provide grape material that test negative for known viruses and virus-like organisms to nurseries and/or growers for propagation (Rowhani

et al. 2005). Considering the known existence of GTD fungi in propagated grapevine material and their impact on the health of newly-established vineyards, there would be significant value to industry in similar clean plant programs for GTD. However, there are several challenges associated with the biology of GTD fungi for developing such a program. For instance, compared to grapevine viruses, which are generally phloem-limited and thus, can be reliably detected by serological or PCR-based methods from green tissues such as leaves and/or petioles, GTD fungi primarily colonize the xylem tissues of the plant and are well-known to be unevenly distributed throughout the vine. For example, their absence at the base of the rootstock does not guarantee their absence in the graft-union or scion. Additionally, the process of cutting canes from mother vines predisposes them to infection by trunk disease pathogens. Furthermore, infected cuttings may initially have no visible internal or external symptoms, but they may become apparent after a certain period of time. These factors make detection of these fungi challenging, as it requires destructive sampling from different parts of the plant. Accordingly, it is currently not possible to assure that propagation material is free of GTD fungi by non-destructive sampling. Recently, serological tests have been developed to detect low amounts of proteins secreted by *P. chlamydospora*; however, implementation has been limited to woody tissues (Cardoso et al. 2014; Fleurat-Lessard et al. 2010). Another study reported four candidate genes from leaves, which express with latent infections of *N. parvum* in the plant (Czemmel et al. 2015). Although these results are promising for non-destructive options to detect GTD fungi, further research is needed to determine if these responses are consistent among grapevine cultivars and GTD fungi, and to develop alternative non-destructive detection tools with the ultimate goal of including GTD fungi within the current clean grapevine certification programs.

In order to produce plant material with minimal levels of fungal infection, cost-effective, quantitative detection protocols need to be developed that can be used for routine

diagnostics on propagation material. A DNA-macroarray tool for the simultaneous detection and identification of all fungi associated with black foot and petri disease directly from plants or soil has been recently developed (Úrbez-Torres et al. 2015a) and with further development and validation has potential to provide a cost-effective, high throughput method of diagnosis. The link between presence of GTD pathogens and disease expression is still largely unknown. For instance, some species may occur in grapevine wood as latent pathogens, without any disease symptoms ever becoming evident, until in some cases, the grapevines are subjected to stress, such as waterlogging. The future direction of research needs to investigate the thresholds of infection in planting material by determining the minimum quantity of each GTD pathogen, or combinations of pathogens, that will be likely to manifest into diseased plants. Furthermore, understanding the effect of different growing conditions and stress factors (water stress, waterlogging, overcropping, winter-kill, nutrition or J-rooting) on vine establishment will assist in future site selection and other management decisions.

Minimizing infections in nursery soils. Nursery soils are one of the main sources of inoculum for soilborne pathogens (black foot) where vines are infected prior to distributing them to growers. There is an urgent need to develop novel management strategies, including the evaluation of fumigation and solarisation, to eliminate GTD fungal inoculum from soil and to protect grapevine roots from pathogen infections. In addition, the biology and ecology of soilborne pathogens associated with GTD is still poorly understood. The low success of crop rotation for managing black foot disease in grapevine nurseries could be explained by the broad host ranges and long-lived inoculum of these fungi in soil. Future research should be focused on improving soil structure through addition of composts and mulches which would improve water drainage and aeration of soil and hence reduce anaerobic conditions that lead to black foot and other soil pathogen invasion. Asymptomatic secondary hosts, specifically rotational crops and weeds, can maintain populations of black foot and Petri disease

pathogens in grapevine nurseries and young vineyards. Some species are actually able to colonize weeds, even though these hosts do not show symptoms of decline (Agustí-Brisach et al. 2011). Pathogen diversity maintained by asymptomatic hosts may have a detrimental long-term consequence for disease management. A better understanding of this phenomenon and its likelihood would be useful in managing GTD in the nursery.

Wound protection. There are many strategies available to control wound infection by GTD pathogens. This includes the use of a number of chemicals with different modes of activity to reduce the development of resistant strains that may be associated with the long term use of a single compound for disease control, and to provide alternative non-chemical or biological control strategies that will enable growers to minimise chemical inputs. However, limited products are registered for use on grapevines, and only in some countries, with many species of the taxonomically variable pathogens yet to be evaluated. Future research should be focussed on expanding the range of chemical and alternative options available to industries worldwide. Application of wound treatments by hand is labour intensive and costly. Recent research in California, Australia and New Zealand has clearly demonstrated the efficient application of fungicide wound protectants with tractor driven sprayers (Ayres et al 2017b; Hersche 2009; Sosnowski and Mundy 2016), and future research should adapt this strategy for application of alternative compounds and biociontrol products, and expand for use in industries worldwide. Further optimization of the critical timing for application of wound protection treatments is being addressed by determining curative and preventative properties of fungicides (Ayres et al. 2017a), and together with localised regional data on wound susceptibility and spore dispersal at different pruning times (Ayres et al. 2016, Billones-Baaijens et al. 2017), will ultimately provide decision support and recommendations for industry to ensure protection of wounds for the duration of wound susceptibility. Future research is required to provide similar information for the many environmentally diverse

grape growing regions around the world and expand critical timing application to other active ingredients, alternative compounds and biological controls.

Breeding for disease resistance. The use of tolerant cultivars, clones and rootstocks would be the least expensive, easiest, safest, and one of the most effective means of controlling GTD. Cultivation of tolerant cultivars or rootstocks would not only reduce losses from the disease, but also would markedly decrease the need for spray treatments and curatve control strategies, and reduce the level of toxic chemicals in the vineyard environment. Previous studies have shown that grapevine cultivars and rootstocks have different levels of susceptibility to GTD pathogens (Eskalen et al. 2001; Gramaje et al. 2010b; Sosnowski et al 2016b; Travadon et al. 2013).

Development of grapevine cultivars and rootstocks of commercial interest with improved tolerance against GTD is of utmost importance for management of these diseases. To date, there is no single gene/gene product that has been identified as putatively providing significant control of GTD. Furthermore, it seems very possible that a single gene product (or pyramid thereof) effective against one pathogen or pathogen group within the GTD complex would not be effective against others. Although the technical capacity for the development of transgenic grapevines is well established (Pretorius and Høj 2005), there is currently no prospect for developing such resistant genotypes. In addition, there is significant public resistance to genetic modification of grapevines (Janardhan 2007). Pedneault and Provost (2016) recently listed several additional limitations once resistant cultivars and rootstocks are obtained: agronomic practices need to be adapted for different growing requirements, a lack of enological experience with new cultivars, and legal issues with growing resistant cultivars for wine production in many countries. Conventional grapevine breeding for resistance to trunk pathogens combined with agronomic yield and quality traits face the difficulties of the lack of knowledge on sources of genetic resistance for these diseases as well as the time

required for classical breeding approaches. Modern techniques, such as gene mapping, marker assisted selection, *in vitro*-culture, genetic engineering and pyramiding of resistance, are useful for understanding the nature, level and durability of resistance and can help reducing those difficulties (Töpfer et al. 2011). In future, continued efforts to identify sources of tolerance or resistance to GTD pathogens are required, followed by use of these modern techniques to qualify traits and develop germplasm with decreased susceptibility.

Genetics and genomics. The study of genetic variation and the population biology of GTD pathogens with appropriate markers are still scarce in the literature. The examination of the population genetic structure of fungi associated with GTD at a global scale would allow us to i) assess the relative importance of sexual vs asexual reproduction, ii) identify putative founder populations or reconstruct routes of introduction, iii) examine the genetic relatedness of GTD populations from all grape producing regions of the world, iv) identify highly virulent strains within a population of a specific fungal species, v) breed for resistance: which pathogen to screen against, and vi) develop robust and precise diagnostic tools. All of this knowledge is important for development of targeted disease management strategies and disease-resistant cultivars (Grünwald and Goss 2011). This intercontinental approach to study the population genetic structure of a GTD pathogen has been accomplished for *E. lata* by Travadon et al. (2012). Genotyping by sequencing (GBS) is a relatively novel approach based on next generation sequencing that could be very suitable for population studies of GTD pathogens (Elshire et al. 2011).

The genomes of the GTD fungi *Botryosphaeria dothidea* (Joint Genomics Institute (JGI), http://1000.fungalgenomes.org), *Dactylonectria macrodidyma* (Malapi-Wight et al. 2015), *Diplodia seriata* (Morales-Cruz et al. 2015), *E. lata* (Blanco-Ulate et al. 2013a), *Neofusicoccum parvum* (Blanco-Ulate et al. 2013b), *P. minimum* (Blanco-Ulate et al. 2013c) and *P. chlamydospora* (Antonielli et al. 2014; Morales-Cruz et al. 2015) have been sequenced

in their entirety. This improves our ability to locate, identify, compare, isolate, and manipulate the genes associated with the mechanisms of pathogenesis and virulence in the pathogens (Morales-Cruz et al. 2015), and of resistance in their host plants, as well as manipulate the introduction of them into specific locations of the plant genome where they would be more effective. For instance, Morales-Cruz et al. (2017) recently benefitted from the availability of annoted genomes of the most releveant GTD fungi to develop and optimize a community-level transcriptomics approach that can monitor simultaneously the virulence activities of multiple GTD pathogens *in planta*.

Biological Control Agents (BCA). Investigation of BCA able to prevent or at least reduce the development of GTD should be considered a research priority based on the restrictions and difficulties that chemicals are facing in most countries around the world. Successful biological control of GTDs with antagonistic microorganisms is practiced to a rather limited extent. Experimentally, biological control can be obtained against trunk disease pathogens, but most of the studies so far have been applied in one year-old grafted vines under greenhouse conditions and field applications are still mostly ineffective. Research in this field should focus on i) the development of effective treatments with microbial agents, ii) searching for existing or new BCA strains with the potential to degrade phytotoxic disease factors of trunk disease pathogens through the use of in-depth microbial ecology studies, an approach which has been recently initiated by targeting microbial DNA (Bruez et al. 2014, 2015, 2016). In this regard, the shotgun sequencing of the community mRNAs (metatransciptomics), presents an even greater improvement for microbial ecology studies because, unlike other methods targeting DNA, this approach can differentiate between viable and dead microorganisms since it targets the metabolically active fraction of the microbiome (Moralez-Cruz et al. 2017). iii) Investigating the action mechanisms of BCA and the role of

plant defense activation following colonization, and iv) studying the effects of mycorrhization on roostock response to trunk disease infections.

Cultural practices (training systems and pruning techniques). It has been shown that training systems and pruning techniques can influence the level of Eutypa dieback (Dumot et al. 2004; 2012; Gu et al. 2005) and esca disease (Lecomte et al. 2012; Travadon et al. 2016) in vineyards. Recently, there has been greater emphasis placed on the importance of pruning systems for managing GTD (Smart 2014; Lee 2016) so there is a need to scientifically evaluate the variables of different pruning systems, such as proximity of wounds to the trunk, wound surface area and blocking the flow of sap in vascular tissue, by wrapping too tighly on the wire or from natural dessication extending from wounds, in order to corroborate the visual observations being reported.

Epidemiology: alternate hosts. Recent reports indicate that the prevalence of GTDs on tree fruit crops are significantly greater than previously recognized in California (Inderbitzin et al. 2010; Úrbez-Torres et al. 2013b, 2016), Chile (Espinoza et al. 2009), Iran (Mohammadi et al. 2015), Italy (Carlucci et al. 2015b), South Africa (Cloete et al. 2011) and Spain (Gramaje et al. 2012a; Olmo et al. 2016). Fruit orchards should be definitely considered as potential inoculum sources of GTD pathogens. Pathogenic or saprobic survival of these GTD pathogens in fruit orchards could have serious implications for disease management practices employed on farms where vineyards are planted adjacent to woody perennial crops, such as almond, olives and *Prunus* spp. Future research should focus on understanding the epidemiological relevance of these findings and on developing management strategies for trunk diseases in these other hosts.

Responses of the plant to stress and impact on longevity. Water stress has been reported to increase the expression and progression of disease symptoms for Eutypa dieback (Butterworth et al. 2005; Dumot et al. 2004; Sosnowski et al. 2007a; 2011), Botryosphaeria

dieback (Amponsah et al. 2014; Lawrence et al. 2016b; van Niekerk et al. 2011b) and esca (Edwards et al. 2007a; 2007b; Ferreira et al. 1999; Fischer and Kassemeyer 2012; Surico et al. 2000). However, recent results by Sosnowski et al (2016a) provide evidence to the contrary, showing distance of recovery of *E. lata* and *D. seriata* from the wound site to be less in stressed vines compared with well watered vines. The assessment of pathogen reisolation from canes may account for this difference, as lesion length was reported to have little correlation with recovery distance of the pathogen from any given inoculation site. Therefore future research should focus on pathogen colonisation as well as symptom expression to more fully understand the effect of stress factors on pathogen activity and disease development. Nutritional stress may also be a factor with reports of reliance on nitrogen (Dumot et al. 2012) and carbon (Amorabe et al. 2005) by *E. lata*. The physiological and biochemical responses of grapevine tissue to stress when infected by GTD pathogens should be the focus of future research, as it may assist in better understanding of the role of stress on vineyard longevity, and hence assist in developing more effective management strategies.

Conclusion

Fungal trunk diseases are some of the most destructive diseases of grapevine in all grape growing areas of the world. Management of GTDs has been intensively studied for decades with some great advances made in our understanding of the causal pathogens, their epidemiology, impact and control. However, due to the breadth and complexity of the problem, no single effective control measure has been developed. Management of GTD must be holistic and integrated, with an interdisciplinary approach conducted in both nurseries and vineyards that integrates plant pathology, agronomy, viticulture, microbiology, epidemiology, biochemistry, physiology and genetics. In this review, we identify a number of areas of future

prospect for effective management of GTDs worldwide, which, if addressed, will provide a positive outlook on the longevity of vineyards in the future.

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Figure legends

Figure 1. Total number of pruning wounds made in a traditional low-density head trained (bush vines) vineyard (**A**) are significantly lower than in a high density spur-pruned trellis vineyards (**B**).

Figure 2. Petri disease and black foot foliar and vascular symptoms. **A,** poor vigour vines showing chlorotic leaves affected by Petri disease. **B,** vine affected by black foot showing overall stunting with short shoot internodes. **C,** sudden wilting of leaves and shoots is a characteristic symptom of severe Petri disease or black foot infected vines. Rootstock cross- (**D**) and longitudinal-section (**E**) showing dark xylem vessels and necrotic streaks infected by Petri disease fungi. **F,** wood necrosis at the basal end of the rootstcok in black foot infected vines.

Figure 3. Symptoms of grapevine trunk diseases in mature plants. **A** and **B,** foliar symptoms of Eutypa dieback include stunted shoots with chlorotic leaves often cupped and with necrotic margins. **C,** bunches on stunted shoots affected by Eutypa dieback ripen unevenly, are small and, in severe cases, shrivel and die. **D,** cankers and internal, necrotic, wedge-shaped staining in the cross-section of a cordon characteristic of Eutypa dieback. **E,** spurs, cordons, and/or trunks infected by both Eutypa and Botryosphaeria dieback develop external cankers that can be characterized by flattened areas of the wood. *Eutypa lata* perithecia (**F**) and Botryosphaeriaceae species pycnidia (**G**) can be found embedded in the bark in cankered areas. Cordon (**H**) and spur (**I**) dieback along with lack of spring growth can be observed in vines affected by Botryosphaeria dieback. **J,** wedge-shape canker in a Botryosphaeria dieback infected cordon similar to those observed in Eutypa and Phomopsis dieback affected vines. **K,** 'tiger-stripes' symptoms on leaves of a red cultivar characteristic of grapevine leaf stripe disease. **L,** small, round and dark spots symptoms on berries known as black measles. **M,** esca acute or apoplectic form is characterized by a sudden wilting of the entire plant or of one arm

or several shoots. **N,** cross-section showing a central white rot surrounded by black spots and sectorial necrosis of an esca infected vine.

Figure 4. Control measures available throughout the different steps of propagation in grapevine nurseries.

Figure 5. Grapevine trunk disease management. **A,** removal of all diseased wood from the vineyard is critical to reduce inoculum**. B** and **C,** composting of mulched wood in large piles. **D** and **E,** cutting trunks during remedial surgery. If infection extends to the ground or graft-union (**F**) then remedial surgery will be ineffective. **G,** an ancient custom of inserting a stone to expose the rotten wood to the air (stones indicated by arrows). **H** and **I,** low cuts made near the ground to improve the likelihood of eradicating grapevine trunk disease infection from the vines, so that symptoms will not recur. **J,** Layering from a neighboring vine in order to replace a vine missing due to trunk disease. **K,** pruning wound protection by using a mastic or paste. **L,** applying protective pruning wound treatment using a vineyard sprayer.

Table 1. Grapevine trunk disease spore trapping studies, showing spore dispersal throughout the year in grape-growing regions of both Northern and Southern Hemispheres

				Relative spore availability (ascospores or conidia) [d]											
				Fall			Winter			Spring			Summer		
Reference	**Location**	**Disease[a] / Pathogen[b]**	**Years[c]**	**Early**	**Mid**	**Late**	**Early**	**Mid**	**Late**	**Early**	**Mid**	**Late**	**Early**	**Mid**	**Late**
						-------	---------	---------	---------						
Moller and Carter (1965)*	Australia	ED / *E. l.*		M	H	H	M	L	L	L	H	M	H	M	L
Ramos et al. (1975a)*	California	ED / *E. l.*	2	L	H	H	L	H	H	L	H	H			
Pearson (1980)	New York	ED / *E. l.*	2	L	L	L	M	H	H	H	H	M	L	L	L
Trese et al. (1980)	Michigan	ED / *E. l.*	2	H	H	M	L	L	L	H	H	M	L	L	L
Petzoldt et al. (1983b)*	California	ED / *E. l.*	2	H	H	H	M	M	M	H	H	H	L	L	L
Eskalen and Gubler (2001)	California	Esca / *P. c.*	1						L	L	L	L	L	L	
		Esca / *P .i.*							ND	L	L	M	L	ND	
		Esca / *P .m.*							H	H	M	L	L	L	
Amponsah et al. (2009)	New Zealand	BD / *Bot.* spp.	1	L	M	H	H	L	M	M	M	L	H	H	H
Kuntzman et al. (2009)	France	BD / *D. m.*	2	H	H	L	L	ND	ND	L	H	L	L	L	H
		BD / *D. s.*		M	L	L	L	L	L	L	L	M	M	L	L
Trouillas (2009)	California	ED / *E. l.*	2	H	H	H	H	M	L	H	M	L	L	L	L
Úrbez-Torres et al. (2010a)	California	BD / *Bot.* spp.	2	L	L	M	H	H	H	M	L	L	ND	ND	ND
van Niekerk et al. (2010)	South Africa	BD / *Bot.* spp.	2				H	H	H	H					
		ED / *E. l.*					H	H	M	L					
		PD / *D. a.*					H	M	M	L					
Cloete (2015)	South Africa	Esca / Basidio.	2				H	H	L	H	M	L			
Valencia et al. (2015)	Chile	BD / *Bot.* spp.	1	H	H	H	H	H	H	L	L	L	ND	ND	ND

* Studies conducted on apricot

[a] ED. Eutypa dieback, BD, Botryosphaeria dieback

[b] *E. l. Eutypa lata, P. c. Phaeomoniella chlamydospora, P. i. Phaeoacremonium inflatipes, P. m. Phaeoacremonium minimum, D. s. Diplodia seriata, D. m. Diplodia mutila, Bot.* spp. Botryosphaeriaceae species, Basidio. Basidiomycetes species

[c] Number of years the study was conducted.

[d] Dashed line represents pruning season in both Northern and Southern Hemispheres. H: high, M: medium, and L: low number of spores trapped; ND: no spores detected; Blank: no spore trapping conducted.

Table 2. Seasonal effects on grapevine pruning wound susceptibility to trunk disease pathogens

				Pruning wound susceptibility[d]											
				Fall			Winter			Spring			Summer		
Reference	Location	Disease[a] / Pathogen[b]	Years[c]	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late	Early	Mid	Late
				---	---	---	---	---	---	---	---	---	---	---	---
Carter and Moller (1967)*	Australia	ED / *E. l.*	1		H		H		L						
Carter and Moller (1970)*	Australia	ED / *E. l.*	2			H (4)	H (4)	H (4)	M						
Ramos et al. (1975b)*	California	ED / *E. l.*	1	H (6)			H (6)			H (6)					
Moller and Kasimatis (1980)	California	ED / *E. l.*	1						H (3)						
Petzoldt et al. (1981)	California	ED / *E. l.*	1				H (3)		M (2)	L					
Trese et al. (1982)	Michigan	ED / *E. l.*	2			L	L		H	M	M	M			
Munkvold and Marois (1995)	California	ED / *E. l.*	2			H (4)	H (4)	H (3)		L (2)					
Chapuis et al. (1998)	France	ED / *E. l.*	3				H (6)	H (7)	L (2)						
Eskalen et al. (2007)	California	Esca / *P. c.* & *P. m.*	1						H (8)						
Serra et al. (2008)	Italy	Esca / *P. c.*	3					H (4)	M (2)	L (2)					
		Esca / *P. m.*						M (4)	M (6)	H (2)					
		BD / *D. s.*						H (16)	H (12)	H (8)					
Úrbez-Torres and Gubler (2011)	California	BD / *L. t.* & *N. p.*	2			H (10)	H (10)	H (12)	H (2)	L (2)					
van Niekerk et al. (2011a)	South Africa	ED / *E. l.*	2					M (3)	M (3)						
		BD / *N. a.*						M (3)	H (3)						
		Esca / *P. c.*						L (3)	M (3)						
Ayres et al. (2016)	Australia	ED / *E. l.*	1				H (2)	H (2)	H (2)						
		BD / *D. s.*	1				H (16)	H (8)	H (16)						
		BD / *N. l*	1				H (8)	H (4)	H (2)						
Elena and Luque (2016a)	Spain	BD / *D. s.*	2			H (2)			H (4)						
		Esca / *P. c.*				H (2)			H (4)						

* Studies conducted on apricot

[a] ED. Eutypa dieback, BD, Botryosphaeria dieback

[b] *E. l. Eutypa lata, P. c. Phaeomoiella chlamydospora, P. m. Phaeoacremonium minimum, D. s. Diplodia seriata, L. t. Lasiodiplodia theobromae, N. p. Neofusicoccum parvum, N. a. Neofusicoccum australe, N.l. Neofusicoccum luteum.*

[c] Number of years the study was conducted.

[d] Dashed line represents pruning season in both Northern and Southern Hemispheres. Grey background indicates pruning and artificial inoculation months, evaluation was not conducted in other months; H: high, M: medium, and L: low susceptibility of pruning wounds; Number between parenthesis represent duration of pruning wound susceptibility (weeks).

Table 3. Chemical treatments evaluated during the propagation process in nurseries for control of grapevine trunk disease pathogens

Active ingredient	Formulation[a]	Disease[b]	Pathogens[c]	Procedure	Effectiveness[d]	References
Azoxystrobin	SC	BF	*"C."d.*	Soaking rooted cuttings prior to planting	1	Rego et al. 2006
Benomyl	WP	PD	*P.c., P.*spp.	Soaking cuttings prior to grafting	3	Fourie and Halleen 2004
	WP	BD	Bot.spp.	Soaking cuttings prior to cold storage and prior to grafting, and soaking grafted plants pior to planting	3	Fourie and Halleen 2006
		BF	*"C."*spp., *Ca.*spp.			
		PD	*P.c., P.* spp.			
	WP	BF	BFP	Soaking grafted plants prior to planting	1	Halleen et al. 2007a
		PD	*P.c, P.*spp..		2	
	WP	BD	Bot.spp.	Soaking cuttings prior to cold storage, prior and after to grafting, and before planting	3	Halleen and Fourie 2016
		BF	*Ca.*sp., *D.*sp., *I.*sp.,		1	
		PD	*P.c., P.*spp*., Pl.r.*		3	
	WP	BF	*"C."d.*	Soaking rooted cuttings prior to planting	3	Rego et al. 2006
Captan	SC	BD	Bot.spp.	Soaking cuttings prior to cold storage and prior to grafting, and soaking grafted plants pior to planting	3	Fourie and Halleen 2006
		BF	*"C."*spp., *Ca.*.spp			
		PD	*P.c.*			
	WP	BF	*I.l., D.m.*	Soaking cutting prior to callusing and rooting	2	Alaniz et al. 2011
Carbendazim	SC	PD	*P.c., P.m*	Soaking cuttings in hydration tanks	3	Gramaje et al. 2009b
	SC	BD	Bot.spp.	Soaking cuttings prior to cold storage, prior and after to grafting, and before planting	3	Halleen and Fourie 2016
		BF	*Ca.*sp., *D.*sp., *I.*sp.		1	
		PD	*P.c., P.*spp*., Pl.r.*		3	
	SC	BF	*I.l., D.m.*	Soaking cutting prior to callusing and rooting	2	Alaniz et al. 2011
	SC	BD	*N.l.*	Soaking cuttings after cold storage	3*	Billones-Baaijens et al. 2015
			Bot.spp.	Soaking cuttings prior to rooting and planting	3	
Carbendazim + flusilazol	SC	BF	*"C.d."*	Soaking rooted cuttings prior to planting	3	Rego et al. 2006
	SC	BF	*I.l.*	Soaking rooted cuttings prior to planting	2	Nascimento et al. 2007
		PD	*P.c.*			
Carpropamid	-	BF	*"C".d.*	Soaking rooted cuttings prior to planting	1	Rego et al. 2006
Copper oxychoride	SL	BF	*I.l., D.m.*	Soaking cutting prior to callusing and rooting	2	Alaniz et al. 2011
Cubiet	SL	PD	*P.c., P.m.*	Soaking cuttings in hydration tanks	1	Gramaje et al. 2009b
Cyprodinil	WG	BD	Bot.spp.	Soaking cuttings prior to grafting	1	Rego et al. 2009
		BF	"*C.*"spp.			
	WG	BF	*"C.d."*	Soaking rooted cuttings prior to planting	1	Rego et al. 2006
Cyprodinil +fludioxonil	WG	BD	Bot.spp.	Soaking cuttings prior to grafting	3	Rego et al. 2009
		BF	"*C.*"spp.			
	WG	BF	*"C".d.*	Soaking rooted cuttings prior to planting	3	Rego et al. 2006
	WG	BF	*I.l.*	Soaking rooted cuttings prior to planting	3	Nascimento et al. 2007
		PD	*P.c.*			
Didecyldimethylammonium chloride	EW	BD	Bot.spp.	Soaking cuttings prior to cold storage and prior to grafting, and soaking grafted plants pior to planting in field nurseries	3	Fourie and Halleen 2006
		BF	*"C."*spp., *Ca.*spp.			
		PD	*P.c.*	Soaking cuttings in hydration tanks		
	EW	PD	*P.c., P.m.*	Soaking cuttings prior to cold storage, prior and after to grafting, and before planting	3	Gramaje et al. 2009b
	EW	BD	Bot.spp.		2	Halleen and Fourie 2016
		BF	*Ca.*sp., *D.*sp., *I.*sp.		1	
		PD	*P.c., P.*spp*., Pl.r.*	Soaking cutting prior to callusing and rooting	2	
	EW	BF	*I.l., D.m.*		2	Alaniz et al. 2011

Difenoconazole	EC	BF	*"C."d.*	Soaking rooted cuttings prior to planting	1	Rego et al. 2006
Fludioxinil	WG	BD	Bot.spp.	Soaking cuttings prior to grafting	1	Rego et al. 2009
		BF	"*C.*"spp.			
Flusilazol	EW	BF	BFP	Soaking grafted plants prior to planting	1	Halleen et al. 2007
		PD	*P.c., P.*spp.		1	
	EC	BD	*N.l.*	Soaking cutting after cold storage	1*	Billones-Baaijens et al. 2015
Fosetyl-Al	WG	BF	*"C."d.*	Soaking rooted cuttings prior to planting	1	Rego et al. 2006
Hydroxyquinoline sulphate	SC	BD	Bot.spp.	Soaking cuttings prior to cold storage and prior to grafting, and soaking grafted plants pior to planting in field nurseries	2	Fourie and Halleen 2006
		BF	*"C."*spp. "*Ca.*"spp.			
		PD	*P.c.*	Soaking cuttings in hydration tanks		
	SL	PD	*P.c., P.m.*	Soaking cutting prior to callusing and rooting	1	Gramaje et al. 2009b
	SL	BF	*I. l., D. m.*		2	Alaniz et al. 2011
Imazalil	EC	BF	BFP	Soaking grafted plants prior to planting	1	Halleen et al. 2007
		PD	*P. c., P.* spp		2	
	SC	BF	*I. l., D.m.*	Soaking cutting prior to callusing and rooting	2	Alaniz et al. 2011
Phosphorous acid	SL	PD	*P.c., P.* spp.	Soaking cuttings prior to grafting	1	Fourie and Halleen 2004
Prochloraz	WP	BF	BFP	Soaking grafted plants prior to planting	1	Halleen et al. 2007
		PD	*P.c., P.* spp.		2	
	WG	BF	*"C".d.*	Soaking rooted cuttings prior to planting	1	Rego et al. 2006
	WP	BF	*I. l., D.m.*	Soaking cutting prior to callusing and rooting	2	Alaniz et al. 2011
Pyraclostrobin + metiram	WG	BD	Bot.spp.	Soaking cuttings prior to grafting	2	Rego et al. 2009
		BF	"*C.*" spp.			
Pyrimethanil	SC	BF	*"C."d.*	Soaking rooted cuttings prior to planting	1	Rego et al. 2006
Tebuconazole	WG	BF	*"C." d.*	Soaking rooted cuttings prior to planting	3	Rego et al. 2006
	WG	BF	*I.l.*	Soaking rooted cuttings prior to planting	2	Nacimento et al. 2007
		PD	*P.c.*			
	SC	BD	*N.l.*	Soaking cutting after cold storage + 0.5 mL/L adjuvant	3*	Billones-Baaijens et al. 2015
Thiabendazole	SC	BF	*"C."d.*	Soaking rooted cuttings prior to planting	1	Rego et al. 2006
Thiophanate-methyl	-	PD	*P.c., P.m.*	Soaking cutting prior to cold storage, prior to grafting, during stratification and prior to planting	3	Kun and Kocsis 2014
	WG	BD	*N.l.*	Soaking cuttings after cold storage	1*	Billones-Baaijens et al. 2015
Thiram	-	PD	*P.c., P.m.*	Soaking cutting prior to cold storage, prior to grafting, during stratification and prior to planting	3	Kun and Kocsis 2014
Tolylfluanid	-	BF	*"C."d.*	Soaking rooted cuttings prior to planting	1	Rego et al. 2006
Trifloxystrobin	WG	BF	*"C."d.*	Soaking rooted cuttings prior to planting	1	Rego et al. 2006

[a] WP, wettable powder; WG, water dispersible granule; EC, emulsifiable concentrate; SC, suspension concentrate; EW, emulsion oil in water; SL, soluble concentrate.

[b] BD: 'Botryosphaeria dieback'; BF: 'black-foot' disease; PD: 'Petri' disease

[c] Bot. spp.; Botryosphaeriaceae spp.; BFP: 'black-foot' pathogens; *C.l.*: *Cadophora luteo-olivacea*; *Ca*.sp.: *Campylocarpon* sp.; *Ca*.spp.: *Campylocarpon* spp.; "*C".d.*: "*Cylindrocarpon" destructans*; "*C*".spp.: "*Cylindrocarpon*" spp.; *D.m.*: *Dactylonectria macrodidyma*; *Da*.sp.: *Dactylonectria* sp.; *I.l.*: *Ilyonectria liriodendri; I.*sp.*: Ilyonectria* sp.*; N.l.: Neofusicoccum luteum; Pl.r.: Pleurostoma richardsiae; P.m.: Phaeoacremonium minimum; P.*spp.*: Phaeoacremonium* spp.*; P.c.: Phaeomoniella chlamydospora;*

[d] 1: ineffective; 2: limited or reduced effectiveness; 3: effective (eliminating or significantly reducing fungal infection)

*Superficial fungal infection

Table 4. Summary of the published studies examining the efficacy of hot-water treatment (HWT) in controlling grapevine trunk disease pathogens

Treatment	Disease[a]	Pathogens[b]	Country	Results and effectiveness[c]	References
50 ºC / 30 min	BD	*Bot.* spp.	South Africa	Completely eliminated fungi stems of treated cuttings (3)	Crous et al. 2001
	BF	*"C."* sp			
	PD	*P.c.*			
50 ºC / 30min	PD	*P.c.*	Australia	Not very effective as a curative treatment (1)	Laukart et al. 2001
51 ºC / 30min	PD	*P.c., P.in.*	USA	*In vitro*, slight reduction in growth rate of *P. c.* but no effect on *P. in.* (2)	Withing et al. 2001
51 ºC / 30min	PD	*P.c., P.in.*	USA	Ineffective in eliminating pathogens from dormant wood (1)	Rooney and Gubler 2001
50 ºC / 30min	PD	*P.c., P.m.*	Australia	Reduced the infection level of *P.c.*, but it was not effective against *P. m.* (2)	Edwards et al. 2004
50 ºC / 30 min	PD	*P.c., P.* spp.	South Africa	Effective in reducing the infection caused by *P.c.* and *P.* spp. (3)	Fourie and Hallen 2004
50 ºC / 30 min	PD	*P.c.*	New Zealand	Reduced the incidence of the pathogen (3)	Graham 2007
50 ºC / 30 min	BF	BFP	South Africa	Effective in eradicating fungal infection from uprooted dormant plants (3)	Halleen et al. 2007a
	PD	*P.c., P.* spp.			
49, 50, 51, 52, 52, 54, 55 ºC / 30, 45 or 60 min	PD	*C.l., P.ci., P.h., P.in., P.ir., P.f., P.m., P.p., P.sc., P.si., P.v.*	Spain	*In vitro*, up to 53ºC for 30 min required to reduce growth and germination (3)	Gramaje et al. 2008, 2010a
50, 51, 52, 53, 54 ºC / 30, 45 or 60 min	PD	*P.c., P.m.*	Spain	53ºC for 30 min significantly reduced the incidence pathogens (3)	Gramaje et al. 2009a
50 ºC / 45 min	PD	*P.c.*	Italy	Reduced the frequency of isolation of the pathogen (3)	Habib et al. 2009
41, 42, 43, 44, 45, 46, 47, 48, 49 ºC / 30, 45 or 60 min	BF	*"D. m.* complex", *I.l.*	Spain	*In vitro,* 48ºC for 30 min inhibited growth and germination (3)	Gramaje et al. 2010a
50 ºC / 45 min	BD	Bot. spp.	France	Reduced pathogen infections (3)	Vigues et al. 2010
	PD	*P.c.*			
53 ºC / 30 min	BD	*L.t.*.	Peru	Highly effective against *L.t.*, and reduced *P.p.* in dormant cuttings (3)	Munive et al. 2012
	PD	*P.p.*			
50°C / 30 min	BD	*N.l., N.p.*	New Zealand	Reduced the incidence of *N.l.* but not *N.p.* (2)	Billones-Baaijens et al. 2015
50, 51, 53 ºC / 30 min	BD	*D.s., N.l., N.p., S.v., L.t., N.v.*	Spain	Reduced survival in artificially inoculated canes after 30 min at 51ºC (3)	Elena et al. 2015a
50°C / 30 min	BD	Bot. spp.	South Africa	Eradicated black-foot pathogens and reduced the incidence of *P.c.*, *P.* spp and Bot. spp in dormant grafted vines (3)	Halleen and Fourie 2016
	BF	BFP			
	PD	*P.c., P.*spp.			

[a] BD: 'Botryosphaeria dieback'; BF: 'black-foot' disease; PD: 'Petri' Disease

[b] *Bot*. spp.; Botryosphaeria sp.; Bot. spp.; Botryosphaeriaceae spp.; BFP: 'black-foot' pathogens; *C.l.*: *Cadophora luteo-olivacea*; "*C*".sp.: "*Cylindrocarpon*" sp.; "*D.m.* complex": *Dactylonectria macrodidyma* complex; *D.s.: Diplodia seriata; I.l.: Ilyonectria liriodendri; L.t.: Lasiodiplodia theobromae; N.l.: Neofusicoccum luteum; N.p.: Neofusicoccum parvum; N.v.:Neofusicoccum vitifusiforme; P.ci.: Phaeoacremonium cinereum; P.f.: Phaeoacremonium fraxinopennsylvanicum; P.h.: Phaeoacremonium hispanicum; P.in.: Phaeoacremonium inflatipes; P.ir.: Phaeoacremonium iranianum; P.m.: Phaeoacremonium minimum; P.p.: Phaeoacremonium parasiticum; P.sc.: Phaeoacremonium scolyti; P.si.: Phaeoacremonium sicilianum; P.*spp.*: Phaeoacremonium* spp.*; P.v.: Phaeoacremonium viticola; P.c.: Phaeomoniella chlamydospora; S.v.: Spencermartinsia viticola*

[c] 1: ineffective; 2: limited or reduced effectiveness; 3: effective (eliminating or significantly reducing fungal infection)

Table 5. Natural and chemical treatments evaluated in the laboratory, greenhouse and vineyard for control of grapevine trunk disease pathogens with respect to pruning wound protection. Only treatments providing at least 50% reduction in recovery from wounds or mycelial growth on agar compared with the appropriate control treatment are included. Treatments were applied at a range of active ingredient concentrations and inoculum doses.

Active ingredient	Formulation	Disease[a]	Pathogens[b]	References
-	Paint	ED	*E.l.*	Moller et al. 1977*; Sosnowski et al. 2008
Benomyl	Liquid	ED	*E.l.*	Carter 1971*; Carter and Price 1974*; Glendloff et al. 1983; Halleen et al. 2010; Moller and Carter 1970*; Moller et al. 1977*; Moller and Kasimatis 1980; Munkvold and Marois 1993b; Pearson 1982; Sosnowski et al. 2008
		BD	*D.s., L.t., N.a, N.p.*	Bester et al. 2007
		BD	*Bot.* spp.	Halleen et al. 2010
	Liquid & Paste	BD	*D.s.*	Díaz and Latorre 2013
		esca	*P.c.*	Díaz and Latorre 2013
	Paint	ED	*E.l.*	Sosnowski et al. 2008
		BD	*L.t.*	Leavitt 1990
Benomyl + *Fusarium lateritium*	Liquid	ED	*E.l.*	Carter and Price 1975*
Boron	Liquid	ED	*E.l.*	Rolshausen and Gubler 2005; Sosnowski et al. 2008
	Paste & Paint	ED	*E.l.*	Rolshausen et al. 2010; Rolshausen and Gubler 2005; Sosnowski et al. 2008
	Paste	BD	*B.d., D.s., L.t., S.v.*	Rolshausen et al. 2010
Boron + *Cladosporium herbarum*	Liquid	ED	*E.l.*	Rolshausen and Gubler 2005
Captan	Paste	BD	*L.t.*	Leavitt 1990
	Liquid	BD	*Bot.* spp.	Foiurie and Halleen 2006
Carbendazim	Liquid	ED	*E.l.*	Bourbos and Barbopoulou 2005; Gramaje et al. 2012; Sosnowski et al. 2008, 2013
		ED	*C.a., D.v., E.le., E.c., E.m.*	Gramaje et al. 2012b
		esca	*P.c.*	Mutawila et al. 2015
		BD	*N.l.*	Amponsah et al. 2012
Carbendazim + Flusilozole	Liquid	ED	*E.l.*	Lecomte et al. 2003
Cyproconazole + Iodocarb	Paste	ED	*E.l.*	Rolshausen et al. 2010; Sosnowski et al. 2008;
		BD	*B.d., D.s., L.t., S.v.*	Rolshausen et al. 2010
Didecyldimethyl-amonium chloride	Liquid	BD	*Bot.* spp.	Halleen et al. 2010
Fenbuconazole	Liquid	BD	*L.t.*	Leavitt 1990
Fenarimol	Liquid	ED	*E.l.*	Munkvold and Marois 1993b
		BD	*D.s., L.t., N.a., N.p.*	Bester et al. 2007
Fluazinam	Liquid	ED	*E.l.*	Ayres et al. 2017b; Bourbos and Barbopoulou 2005; Gramaje et al. 2012b; Sosnowski et al. 2008, 2013
		ED	*C.a., D.v., E.le., E.c., E.m.*	Gramaje et al. 2012b
	n.a.	BD	*D.s., N.l.*	Savocchia et al. 2005
Flusilazole	Liquid	ED	*E.l.*	Halleen et al. 2010; Munkvold and Marois 1993b; Sosnowski et al. 2008
		BD	*D.s., L.t., N.a., N.p.*	Bester et al. 2007
		BD	*Bot.* spp.	Halleen et al. 2010
	n.a.	BD	*D.s., N.l.*	Savocchia et al. 2005

	Liquid	BD	*N.l.*	Amponsah et al. 2012
-	Grafting mastic	ED	*E.l.*	Tulloch 1960
Hydrogen peroxide	Liquid	BD	*Bot.* spp.	Halleen et al. 2010
Halogenated alcohols + water	Liquid	BD	*Bot.* spp.	Fourie and Halleen 2006
Hydroxyquinoline sulphate	Liquid	BD	*Bot.* spp.	Fourie and Halleen 2006
Imazalil	Liquid	ED	*E.l.*	Sosnowski et al. 2008
Mancozeb	Liquid	BD	*N.l.*	Amponsah et al. 2012
Myclobutanil	Liquid	ED	*E.l.*	Munkvold and Marois 1993b
		BD	*L.t.*	Herche 2009
Penconazole	Liquid	ED	*E.l.*	Sosnowski et al. 2008
	Paste	BD	*L.t.*	Leavitt 1990
Prochloraz manganese chloride	Liquid	BD	*D.s., L.t., N.a., N.p.*	Bester et al. 2007
Prothioconazole + tebuconazole	Liquid	ED	*E.l.*	Ayres et al. 2011; Gramaje et al. 2012b
		ED	*C.a., D.v., E.le., E.c., E.m.*	Gramaje et al. 2012b
Pyraclostrobin	Liquid	ED	*E.l.*	Ayres et al. 2017b; Gramaje et al. 2012b; Rolshausen et al. 2010; Sosnowski et al. 2008
		ED	*C.a.., D.v., E.le.*	Gramaje et al. 2012
		BD	*B.d., D.s., L.t., S.v.*	Rolshausen et al. 2010
	Liquid & Paste	BD	*D.s.*	Díaz and Latorre 2013
		esca	*P.c.*	Díaz and Latorre 2013
Pyrimethanil	Liquid	ED	*E.l.*	Sosnowski et al. 2008; Sosnowski et al. 2013; Ayres et al. 2016
Spiroxamine	n.a.	BD	*D.s., N.l.*	Savocchia et al. 2005
Tebuconazole	Liquid	ED	*E.l.*	Ayres et al. 2017b; Gramaje et al. 2012b; Halleen et al. 2010; Sosnowski et al. 2013
		ED	*C.a., D.v., E.le., E.c., E.m.*	Gramaje et al. 2012b
	Paint & Gel	ED	*E.l.*	Sosnowski et al. 2013
	Liquid	BD	*D.m.*	Pitt et al. 2012
	Liquid & Paste	BD	*D.s*	Díaz and Latorre 2013
	Liquid	BD	*N.l.*	Amponsah et al. 2012
Thiophanate-methyl	Liquid	ED	*E.l.*	Moller et al. 1977*; Rolshausen et al. 2010
		BD	*B.d., D.s., L.t., S.v.*	Herche 2009; Rolshausen et al. 2010
		esca	*P.c.*	Mutawila et al. 2015
	Liquid & Paste	BD	*D.s.*	Díaz and Latorre 2013
		esca	*P.c.*	Díaz and Latorre 2013
	Liquid	BD	*N.l.*	Amponsah et al. 2012
Triadimefon	Liquid	ED	*E.l.*	Munkvold and Marois 1993b

[a] ED: 'Eutypa dieback', BD: 'Botryosphaeria dieback'

[b] *E.l.: Eutypa lata; D.s.:Diplodia seriata, L.t.: Lasiodiplodia theobromae; N.a.: Neofusicoccum australe; N.p.: N. parvum; Bot.* spp.: Botryosphaeriaceae spp.; *P.c.*: *Phaeomoniella chlamydospora; B.d.:Botryosphaeria dothidea; S.v.: Spencermartinsia viticola; C.a. : Cryptovalsa ampelina; D.v. : Diatrypella vulgaris; E.le. : Eutypa leptoplaca; E.c.: Eutypella citricola; E.m.: Eutypella microtheca; N.l.: Neofusicoccum luteum;*

*Research conducted on apricot

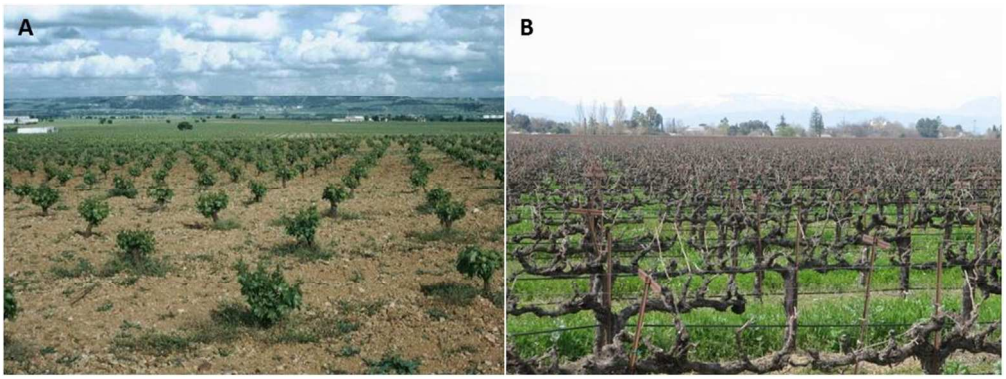

Figure 1. Total number of pruning wounds made in a traditional low-density head trained (bush vines) vineyard (A) are significantly lower than in a high density spur-pruned trellis vineyards (B).

188x70mm (150 x 150 DPI)

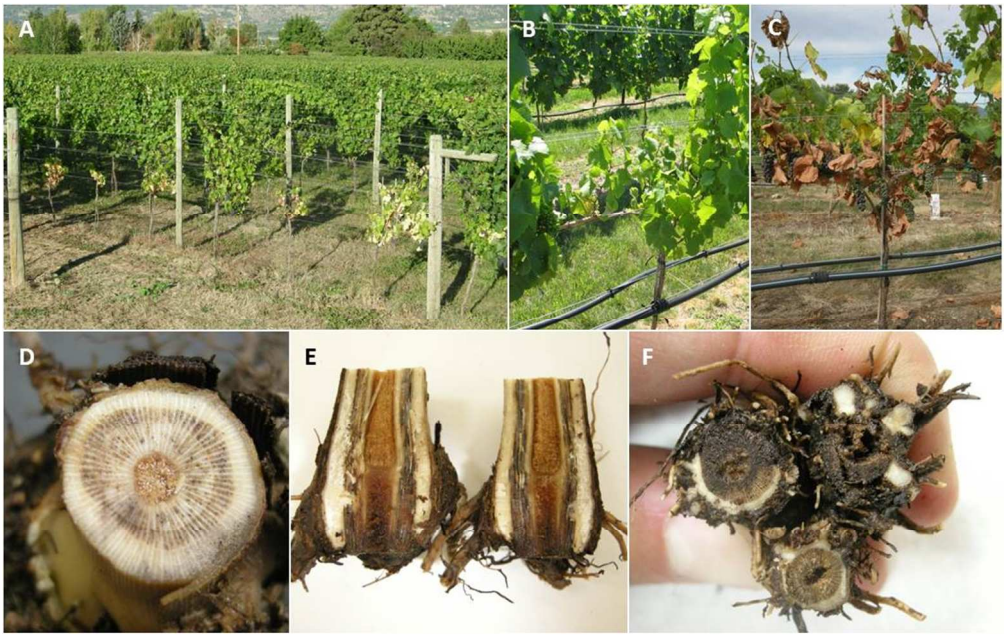

Figure 2. Petri disease and black foot foliar and vascular symptoms. A, poor vigour vines showing chlorotic leaves affected by Petri disease. B, vine affected by black foot showing overall stunting with short shoot internodes. C, sudden wilting of leaves and shoots is a characteristic symptom of severe Petri disease or black foot infected vines. Rootstock cross- (D) and longitudinal-section (E) showing dark xylem vessels and necrotic streaks infected by Petri disease fungi. F, wood necrosis at the basal end of the rootstcok in black foot infected vines.

189x119mm (150 x 150 DPI)

Figure 3. Symptoms of grapevine trunk diseases in mature plants. A and B, foliar symptoms of Eutypa dieback include stunted shoots with chlorotic leaves often cupped and with necrotic margins. C, bunches on stunted shoots affected by Eutypa dieback ripen unevenly, are small and, in severe cases, shrivel and die. D, cankers and internal, necrotic, wedge-shaped staining in the cross-section of a cordon characteristic of Eutypa dieback. E, spurs, cordons, and/or trunks infected by both Eutypa and Botryosphaeria dieback develop external cankers that can be characterized by flattened areas of the wood. Eutypa lata perithecia (F) and Botryosphaeriaceae species pycnidia (G) can be found embedded in the bark in cankered areas. Cordon (H) and spur (I) dieback along with lack of spring growth can be observed in vines affected by Botryosphaeria dieback. J, wedge-shape canker in a Botryosphaeria dieback infected cordon similar to those observed in Eutypa and Phomopsis dieback affected vines. K, 'tiger-stripes' symptoms on leaves of a red cultivar characteristic of grapevine leaf stripe disease. L, small, round and dark spots symptoms on berries known as black measles. M, esca acute or apoplectic form is characterized by a sudden wilting of the entire plant or of one arm or several shoots. N, cross-section showing a central white rot surrounded by black

spots and sectorial necrosis of an esca infected vine.

188x255mm (150 x 150 DPI)

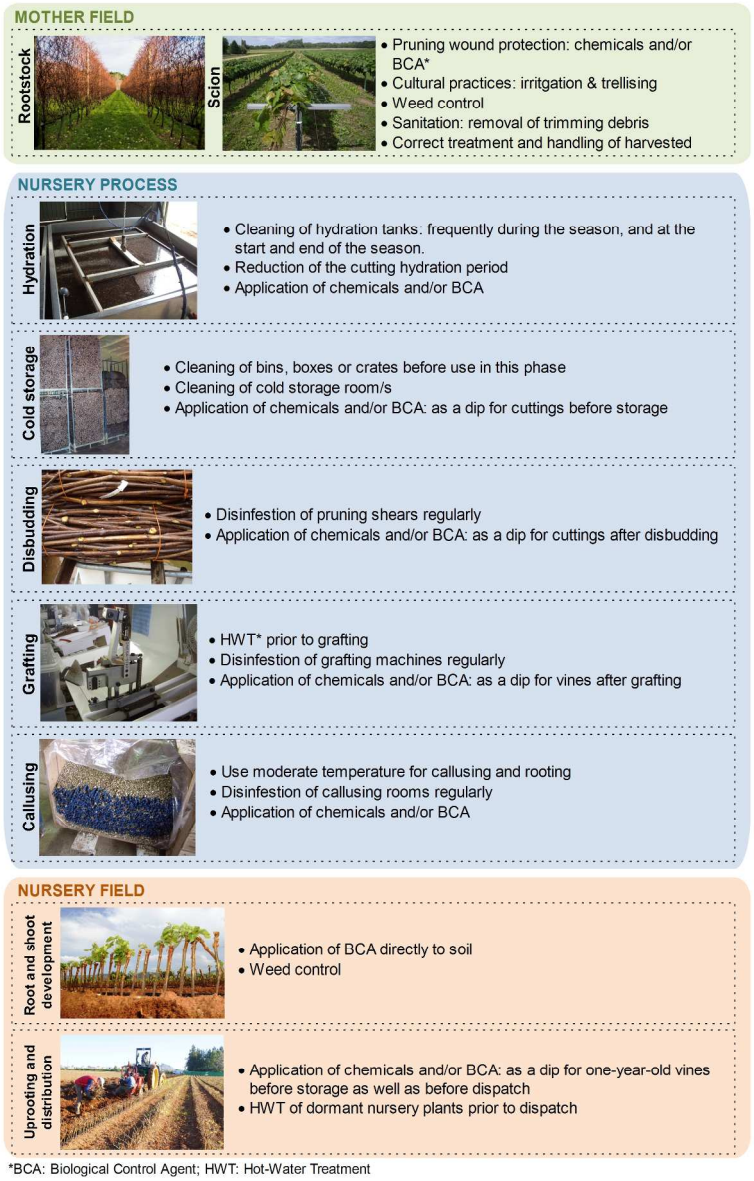

Figure 4. Control measures available throughout the different steps of propagation in grapevine nurseries.

205x302mm (300 x 300 DPI)

Figure 5. Grapevine trunk disease management. A, removal of all diseased wood from the vineyard is critical to reduce inoculum. B and C, composting of mulched wood in large piles. D and E, cutting trunks during remedial surgery. If infection extends to the ground or graft-union (F) then remedial surgery will be ineffective. G, an ancient custom of inserting a stone to expose the rotten wood to the air (stones indicated by arrows). H and I, low cuts made near the ground to improve the likelihood of eradicating grapevine trunk disease infection from the vines, so that symptoms will not recur. J, Layering from a neighboring vine in order to replace a vine missing due to trunk disease. K, pruning wound protection by using a mastic or paste. L, applying protective pruning wound treatment using a vineyard sprayer.

190x207mm (150 x 150 DPI)