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CURRENT TOPICS - 10TH SPECIAL ISSUE ON GRAPEVINE TRUNK DISEASES

A protocol for the management of grapevine rootstock mother vines to reduce latent infections by grapevine trunk pathogens in cuttings

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Summary. A protocol is offered as a guideline for managers of rootstock mother grapevines, and as a potential research framework for to reduce infections by Grapevine Trunk Disease (GTD) pathogens in rootstock mother vines and cuttings. Latent infections by GTD pathogens in rootstock cuttings are a major source of the pathogens in grafted nursery vines and subsequently in new vineyards. The many pruning cuts made at the crowns of mother vines predispose them to infection which is transmitted to the new shoots via the xylem. Direct penetration by epiphytic inoculum on the bark of the shoots/canes can also occur. Mother vines with unprotected pruning wounds are typically heavily infected, particularly if they are not trellised. Availability of pruning wound treatments is limited in many countries. The spread of GTD pathogen inoculum can be reduced by avoiding sprinkler and flood irrigation, by trellising mother vines so that canopies are off the soil, and by spraying fungicides or painting wounds immediately after canes are harvested. Frequent trunk renewal aids in reducing inoculum. Cuts should be made to retain long internodes on the mother vines, cuttings should not contact the soil and pruning debris should be promptly destroyed. Cutting implements should be disinfested regularly and cuttings should be dipped in a registered fungicide or sterilant. Soaking cuttings increases fungal populations in the basal wounds and softens the bark, favouring penetration by pathogen inoculum. Dormant bench grafting in nurseries produces more GTD-symptomatic vines than field chip budding, so improved management for rootstock mother vines is more important where dormant cuttings are bench grafted. GTD epidemiology in source blocks is summarised, and best practice protocols for mother vine management and pre-grafting stages of propagation are suggested. Similar principles could be applied to scion mother vine management.

Key words: rootstock management, trunk diseases, latent transmission, propagation.

Introduction

The purpose of grapevine propagation is to produce sound, healthy and uniform vines that will be long-lived and productive. However, the outcomes of propagation processes are not always as intended. Vines that appear superficially sound and healthy when they are despatched from nurseries are often found to have defects that have negative impacts on vineyard establishment and productivity (Morton, 2000; Stamp, 2001). Some of these defects are indicative of active infections by grapevine trunk disease

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(GTD) pathogens. Symptoms associated with GTD pathogens include brown streaking and staining in the innermost rings of xylem in the rootstocks, poorly healed graft unions (Wallace *et al*., 2004) with dark staining extending down into the rootstocks and upwards into the scions, and dark staining in the xylem extending upwards from the rootstock bases (Morton, 2012). Some localised internal dark staining around wounds is a normal part of the wound healing process (Hartmann *et al*., 1990), but staining extending more than 5–10 mm from wounded tissues is strongly associated with pathogen infection (Bertsch *et al*., 2013). GTD pathogens, including the causal organisms of Petri disease (Mugnai *et al*., 1999; Gatica *et al*., 2001; Fourie and Halleen, 2004a), Botryosphaeria dieback (Úrbez-Torres, 2011; Whitelaw-Weckert *et al*., 2013) and black-foot (Halleen *et al*., 2006; Agustí-Brisach and Armengol, 2013), are regularly isolated from young vines with these defects, but not from young vines without these defects (Whitelaw-Weckert *et al*., 2013). Although there is a minority view that GTD pathogens are saprobes (Hofstetter *et al*., 2012), there is now a significant body of evidence detailing the pathogenic nature of these organisms (Bertsch *et al*., 2013).

Research and observational evidence indicate that rootstock cuttings are major sources of infections by GTD pathogens in young nursery vines (Halleen *et al*., 2003; Retief *et al*., 2006; Aroca *et al*., 2010; Gramaje and Armengol, 2011; Cardoso *et al*., 2013; Billones-Baaijens *et al*., 2013a). Asymptomatic cuttings taken from infected mother vines are frequent hosts of latent endophytic infections (Fourie and Halleen, 2002). Inoculum is also found lodged on the bark of rootstock cuttings taken from mother vine plantings where GTDs are present (Billones-Baaijens *et al*., 2015a). Once in nurseries, unhygienic processes, particularly the practice of soaking large batches of cuttings in water even for short periods, favour cross contamination of entire batches by inoculum on the rootstock cuttings (Retief *et al*., 2006; Aroca *et al*., 2010; Edwards *et al*., 2007; Gramaje *et al*., 2011; Waite *et al*., 2013).

Although the inoculum sources and epidemiology of GTDs are well documented (Larignon and Dubos, 2000; Eskalen and Gubler, 2001; Rooney-Latham *et al*., 2005a; Moyo *et al*., 2014; Baloyi *et al*., 2016), preventing transmission of trunk diseases in propagation remains particularly challenging. This is partly because the superficially healthy appearance of infected vines and the delay in full disease expression, leads propagators to believe that the internal symptoms of GTDs are normal and are not indicative of disease. Consequently, propagators often believe that no remedial action is required (Jefford, 2018). However, there is now growing acknowledgement in the nursery industry that GTD transmission in planting material is an important issue.

The list of fungi associated with GTD symptoms is long and diverse, and continues to expand as research progresses (Gramaje *et al*., 2018). Currently, the identified GTD pathogens include several Botryosphaeriaceae spp., Diatrypaceae spp., *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., *Cadophora* spp., *Campylocarpon* spp., *Cylindrocladiella* spp., *Ilyonectria* spp. *Dactylonectria* spp. and *Neonectria* spp. (Halleen *et al*., 2003; Halleen *et al*., 2004; Fourie and Halleen, 2006; Whiteman *et al*., 2007; Gramaje *et al*., 2011; Petit *et al*., 2011; Billones-Baaijens *et al*., 2013b; Whitelaw-Weckert *et al*., 2013; Lombard *et al*., 2014; Travadon *et al*., 2015). In addition, the biology and epidemiology of GTD pathogens that often occur as mixed infections, is extremely complex (Bertsch *et al*., 2013). GTD pathogens can also remain latent in vines until conditions, including environmental stress and other unknown factors, favour their development (Graniti *et al*., 2000; Giménez-Jaime *et al*., 2006; Zanzotto *et al*., 2007). This makes identification of infected, but asymptomatic vines difficult. Consequently, research investigating potential GTD management strategies is lagging. For this reason, non-specific control measures such as hot water treatment (HWT) of cuttings and the careful use of broad-spectrum biocides, combined with very high levels of nursery sanitation and measures to prevent re-infection in field nurseries, are more likely to be successful compared to control measures that target specific pathogens.

Some fungicide dips have been shown to reduce surface inoculum of some, but not all known GTD pathogens (Rego *et al*., 2009; Billones-Baaijens *et al*., 2015a), thus reducing cross contamination during propagation, but the location of the latent infections within the xylem tissues can make them inaccessible to fungicides that do not completely penetrate the wood. Fungicides that show good efficacy against GTD pathogens include the systemic benzimidazoles, benomyl and carbendazim. Carbendazim has been shown to control GTD pathogen inoculum in the xylem of rootstock cuttings when applied during the initial hydration stage (Gramaje *et al*., 2009b).

Benomyl has been reported by Fourie and Halleen (2004b, 2006) to be an effective control for endophytic *P. chlamydospora* when used in combination with other treatments in a sequence of 1) benomyl drench before cold storage of cuttings followed by HTW before grafting (30 min, 50°C); 2) a 30 min dip in didecyl dimethyl ammonium chloride (Sporekill®); 3) a 1 min dip in Trichoflow® (containing *Trichoderma harzianum*) after grafting, and 4) a further 10 min dip in Trichoflow® prior to planting in field nurseries. Efficacy of Trichoflow® when applied as part of an integrated strategy for trunk diseases has also been reported by Halleen and Fourie (2016). Similarly, applications of Rootshield® (containing *Trichoderma harzianum*) to callused cuttings just prior to field nursery planting showed efficacy against *P. chlamydospora* and improved the quality of cuttings (Di Marco *et al*., 2004; Di Marco and Osti, 2007). In jurisdictions where benomyl is no longer registered for use, Gramaje *et al*. (2009b) and Halleen and Fourie (2016) recommend that carbendazim be used.

Following *in vitro* testing to determine the effects of fungicides on mycelium growth and spore germination of GTD pathogens, Jaspers (2001) indicated that spraying of mother vine source blocks using systemic fungicides, including DMI fungicides, benomyl, carbendazim, pyrimethanil or cyprodinil + fludioxonil, may inhibit internal spread of *P. chlamydospora* and reduce infections through pruning and disbudding wounds. Billones-Baaijens *et al*. (2015a) reported that most Botryosphaeriaceae infections (≈95%) in cuttings of *Vitis vinifera* cv. Sauvignon Blanc and Pinot Noir occurred in the bark and were well controlled by carbendazim. However, the inaccessibility of internal infections in the wood might limit the effectiveness of these fungicides as controls for Botryosphaeriaceae spp. in rootstock cuttings. Furthermore, benomyl and carbendazim are no longer registered for use on grapevines in some jurisdictions, including Australia and Europe. Propagators of grapevines should check fungicide registrations in their jurisdictions before using either of these chemicals.

Hot water treatments of dormant cuttings and young dormant vines has been shown to suppress a range of GTD pathogens (Crous *et al*., 2001), including *P. chlamydospora* and *Phaeoacremonium* spp. (Gramaje *et al*., 2009a), Botryosphaeriaceae spp. (Billones-Baaijens *et al*., 2015a), black foot pathogens (Halleen *et al*., 2007), and *Cadophora luteo-olivacea* (Gramaje *et al*., 2010), but does not completely eradicate all pathogens (Gramaje *et al*., 2009a, 2010; Billones-Baaijens *et al*., 2015b; Elena *et al*., 2015). However, HWT following a soaking treatment with cyproconazole was found to effectively control *P. chlamydospora* in laboratory trials with artificially inoculated cuttings (Serra *et al*., 2011), indicating that HWT in combination with fungicides may be more effective than either HWT or fungicides alone. There is also a risk of damage to the cuttings, particularly if they are sourced from cool climate regions (Graham, 2007; Billones-Baaijens *et al*., 2015a), or held in cold storage for more than 4 weeks after HWT (Gramaje and Armengol, 2012).

The lack of fully effective and reliable controls for latent GTD pathogens in and on rootstock cuttings, and the regular identification of new GTD pathogens, indicate that preventing infections in mother vines, and the cuttings taken from them, is a vital and necessary adjunct to reduce GTDs in grapevine cuttings. Improved general sanitation will also reduce cross contamination of uninfected cuttings during propagation.

Standards for grapevine cuttings and grafted plants exist in several jurisdictions including Australia (Standard for Grapevine Material, AS 5588–2013 (Standards Australia, 2013), New Zealand (New Zealand Winegrowers, 2017), California (California Department of Food and Agriculture, 2016) and Europe (European Plant Protection Organization, 2008). However, the emphasis of these standards is for management of diseases caused by viruses. Adherence to the standards is voluntary and none yet provide comprehensive descriptions of GTDs, their symptoms and their management in propagation.

Here we describe the measures that can be taken to reduce inoculum levels in and on grapevine rootstock cuttings, and also prevent cross contamination in the early stages of propagation. We also discuss the potential barriers to adoption of these procedures and suggest ways to mitigate the factors that prevent adoption of best practice.

Rootstock mother vine cultivation

Current practices

With few exceptions, rootstock mother vines are not normally trellised (Gramaje and Di Marco, 2015) in traditional rootstock plantations. Each season, the new canes develop on each mother vine from a single crown without cordons, about 20–40 cm above the ground

and are allowed to grow unchecked along the soil surface. Because rootstock mother vines are vigorous and long canes are preferred, vines are often widely spaced to allow the shoots to sprawl along the ground. Mother vine spacings vary depending on the jurisdiction. Inquiries made by the authors indicate that mother vines in Australia are spaced 2 to 3 m. apart with 3 to 4 m between rows; in Spain, 3 × 3 m spacings are the most common, and in South Africa these are 2.7 × 1.2 m.

As each growing season progresses, the canes, which can be more than 10 m long, eventually form dense mats of leaves and canes, which cover the entire soil surface including the inter-row spaces. For this reason, overhead sprinklers are favoured in Australia, but drip irrigation is more popular in Europe. Overhead sprinklers and keeping the soil surface bare until covered by the canes favouring dispersal of inoculum in wind-blown dust and plant debris, and in rain and irrigation splash (Griffin *et al*., 2001; Úrbez-Torres *et al*., 2010; van Niekerk *et al*., 2010). The multitude of cuts on short spurs made when the canes are harvested, and the proximity of the crowns to the soil surface (generally <500 mm), make rootstock mother vines particularly vulnerable to infection. Persistent wetness in the developing canopies from rain or irrigation may then favour the germination of surface inoculum and penetration of the bark and, potentially, the woody tissues. This scenario is supported by the observations of Úrbez-Torres *et al*. (2010), who reported discharge of spores of Botryosphaeriaceae spp. after rain and irrigation in Californian vineyards. Billones-Baaijens *et al*. (2015b) reported that adjacent *Neofusicoccum* isolates recovered from bark and underlying tissues of rootstock cuttings were sometimes of the same genotype, suggesting that Botryosphaeriaceae pathogens can colonise the bark and potentially reach the underlying woody tissues. Furthermore, the bark of newly developing canes remains green for some time and may present less of a barrier to germinating GTD pathogens than fully lignified bark. Wetting by rain or irrigation might also soften both green and lignified bark and aid the penetration of germinating inoculum.

Because the movement of vehicles is impeded by canes growing in the inter-rows, the traditional method of growing rootstocks also limits the capacity to apply fungicides to reduce surface inoculum of GTD pathogens and other fungi including *Botrytis cinerea* or powdery and downy mildews that can proliferate in nurseries (Hartmann *et al*., 1990). Although rootstocks are generally either tolerant of, or resistant to, powdery and downy mildews (Brewer and Milgroom, 2010; Gessler *et al*., 2011), these pathogens together with *Botrytis*, are potential sources of inoculum that could infect *V. vinifera* bud wood in nurseries, and be carried into new vineyards on the young vines (Daughtrey and Benson, 2005; Jeger *et al*., 2007).

It is important to establish new grapevine rootstock blocks with vines with the lowest possible titres of GTD pathogens, because infected rootstock mother vines are major sources of latent inoculum in the wood and on the surfaces of rootstock cuttings (Halleen *et al*., 2003; Retief *et al*., 2006; Aroca *et al*., 2010; Gramaje and Armengol, 2011; Cardoso *et al*., 2013; Billones-Baaijens *et al*., 2013a). This is not easy to achieve, since it is almost certain that rootstock cuttings taken from extant mother vines will carry latent endophytic and/or epiphytic inoculum. However, some rootstock varieties are likely to be more susceptible than others to the different GTD pathogens (Eskalen *et al*., 2001; Jaspers *et al*., 2007; Alaniz *et al*., 2010; Gramaje *et al*., 2010; Bertsch *et al*., 2013; Billones-Baaijens *et al*., 2014; Gramaje *et al*., 2018). In a study of wood necrosis in rootstock mother vines, Liminana *et al*. (2009) reported that rootstock 1103 Paulsen was least susceptible to GTDs (33% mean percent necrotic area) and 101-14 MGT was the most susceptible (71% mean percent necrotic area). Murolo and Romanazzi (2014) also noted that vines grafted to the drought tolerant 1103 Paulsen rootstock had lower incidence of esca symptoms than those grafted to SO4. The level of inoculum is, therefore, likely to vary with rootstock genotype.

Rootstocks may carry low titres of GTD pathogens even if they have been hot water treated (Whiting *et al*., 2001), and because cuttings carrying latent endophytic GTD pathogens are asymptomatic (Halleen *et al*., 2003; Billones-Baaijens *et al*., 2015a), careful visual examination of external features is insufficient to detect infections. Furthermore, internal symptoms in the wood only become apparent towards the end of the propagation season in nurseries, 6 to 9 months after grafting and callusing (Giménez-Jaime *et al*., 2006). These symptoms are normally only detected by destructive sampling.

Development of non-destructive tests for GTD pathogens

Serological tests have been recently developed to detect low amounts of proteins secreted by *P. chla-*

mydospora, although their implementation has been limited to detection in woody tissues (Cardoso *et al*., 2014; Fleurat-Lessard *et al*., 2010). Czemmel *et al*. (2015) reported four candidate genes from grapevine leaves, that were expressed when latent infections of *Neofusicoccum parvum* were present in host plants. Although these results are promising developments in the search for non-destructive options to detect GTD-causing fungi, further research is required to determine if these responses are consistent for all or most grapevine cultivars and GTD pathogens.

Development of PCR methods for detecting GTD pathogens

Polymerase Chain Reaction (PCR) technology is useful for detecting known GTD pathogens in water and callusing media used in nurseries (Edwards *et al*., 2007; Aroca *et al*., 2010; Agustí-Brisach *et al*., 2013, Billones-Baaijens *et al*., 2013a), in nursery soil samples (Agustí-Brisach *et al*., 2014) and in samples of woody tissues (Aroca and Raposo, 2007; Pouzoulet *et al*., 2013). PCR could also be used to test small pieces of host tissue from the tips of developing shoots without destroying entire vines. This would assume that the pathogens concerned, and thus their DNA, was present in the shoot tips. However, it is rare to find only one causative pathogen in GTD affected cuttings and vines. Most GTDs are caused by complexes of fungal pathogens representing several taxa, rather than single infections. Furthermore, unless multiplex PCR is used, multiple taxa are not able to be detected in a single test. The low levels and uneven distribution of latent GTD pathogen inoculum in cuttings, that tends to be concentrated in the basal parts of shoots (Billones-Baaijens *et al*., 2013b), also means that sampling for PCR from shoot tips is unlikely to be a reliable and cost-effective means of detecting GTD pathogen infections. In an effort to overcome these limitations, Úrbez-Torres *et al*. (2015) have developed promising macro-array technology that enables simultaneous detection of DNA from many different taxa. However, DNA based methods are unable to distinguish between viable or dead organisms. An RNA based analysis would reveal metabolically active fungal taxa in field samples (Eichmeier *et al*., 2018). Therefore, the use of DNA and RNA analyses would give an overview of all fungal taxa present in field samples, but enable discrimination between active and dead or inactive taxa in each sample. However, these methods have yet to be developed as rapid and inexpensive tests for industry use. Furthermore, the need for destructive sampling has not been resolved. Until a cheap, rapid and non-destructive method for detecting GTD pathogens is developed, tissue culture remains the most practical means of propagating rootstock mother vines.

Tissue culture

Although fungal infections are normally eliminated during tissue culture, there remains a small, but real, chance that some GTD pathogens are latent in the explants until they have become established in nurseries (Leifert and Cassells, 2001). Bacteria and viruses are more likely than fungal pathogens to be carried into nurseries as latent infections in tissue-cultured explants (Leifert and Cassells, 2001). However, elimination of serious viruses from grapevine rootstocks has been achieved using a combination of thermotherapy and tissue culture (Alley and Golino, 2000). Providing tissues are taken from plants shown to be free of viruses, the likelihood of these pathogens being transmitted in tissue-cultured rootstocks is very low. However, very little is known about the roles of bacteria that are often isolated from wood infected by GTD pathogens. Some of these bacteria may be beneficial, neutral or antagonistic, but there is some evidence that others may be opportunistic pathogens when accidentally introduced into the woody tissues of cuttings and young vines (Cole and Waite, 2006). Control of potentially pathogenic bacteria *in vitro* is, therefore, an important consideration when propagating "clean" mother vines.

The use of tissue culture to produce "clean" rootstock plants risks the elimination of potentially beneficial endophytes. Endophyte communities in grapevines are large, complex and variable (West *et al*., 2010). Apart from *Trichoderma* spp., which are sometimes used in nurseries to suppress GTD pathogens including *P. chlamydospora* (Fourie *et al*., 2001), the effects of endophyte communities on grapevines are not yet well fully understood (Pancher *et al*., 2012).

In the event that tissue-cultured vines cannot be used, new rootstock mother vines should be propagated from cuttings taken from the distal portions of canes to reduce the probability that the new vines carry latent GTD pathogen infections (Billones-Baaijens *et al*., 2015b). It is important that such cuttings be hot water treated and treated with non-specific disinfect-

ants to destroy internal and surface inoculum at the beginning of propagation cycles. To date, testing of fungicides against GTD pathogens in propagation has been limited, and not all fungicides tested have been effective against all pathogens included in trials (Fourie and Halleen 2006; Halleen *et al*., 2007; Nascimento *et al*., 2007; Gramaje *et al*., 2009b; Rego *et al*., 2009; Halleen and Fourie, 2016). Treatments with one fungicide are unlikely to be uniformly effective against the entire suite of GTD pathogens that might be carried by a large batch of cuttings or grafted vines. Soaking of cuttings also favours cross contamination (Retief *et al*., 2006; Aroca *et al*., 2010; Edwards *et al*., 2007; Gramaje *et al*., 2011; Billones-Baaijens *et al*., 2013a; Waite *et al*., 2013), and causes oxygen starvation in plant tissues (Perata and Alpi, 1993). Therefore, treatments to destroy bark inhabiting inoculum should be applied as short dips rather than as long soaking treatments. The precise required duration of the dipping time depends on the chemical being used, and propagators should follow respective label directions for particular products.

Several studies have reported associations between GTD pathogen spore discharge and rain events (Larignon and Dubos, 2000; Rooney-Latham *et al*., 2005b; Van Niekerk *et al*., 2010; Úrbez-Torres *et al*., 2010). Spore release has also been observed in the absence of rain events where overhead irrigation is applied (Rooney-Latham *et al*., 2005b). It is possible that the practice of soaking cuttings promotes the discharge and germination of bark inhabiting GTD pathogen inoculum. This could then either penetrate directly through the bark, or infect cut ends and disbudding wounds on cuttings during soaking.

Regardless of the initial propagation method, nursery sanitation must be very stringent to prevent re-infection occurring during propagation (Waite *et al*., 2014). Contact with untreated cuttings, dirty work benches, dust and untreated water in nurseries should be avoided to prevent infection of cut ends and disbudding wounds. Pruning wounds made on 1-year-old wood of *V. vinifera* have been shown to be susceptible to infection by *P. chlamydospora*, *Pm. minimum*, *Diaporthe ampelina* and Botryosphaeriaceae spp. for periods up to 4 months after pruning, although disease incidence decreased with increasing time (Eskalen *et al*., 2007; Serra *et al*., 2008; Úrbez-Torres and Gubler, 2011; Van Niekerk *et al*., 2011). Young vines have also been shown to acquire GTD pathogen infections through wounds made during propagation in addition to infections acquired from the mother vines (Fourie and Halleen, 2006; Gramaje and Armengol, 2011). Fresh wounds made on rootstock cuttings during propagation are probably susceptible to infection for the duration of callusing and cutting establishment. Infection events during cutting establishment show as dark staining in xylem rings of the wood extending for several centimetres away from the graft unions, and disbudding wounds and upwards from the base of vines. Although not yet confirmed by research, uniform staining along the whole length of inner (oldest) rings of rootstock xylem is indicative of cuttings that have acquired infections directly from the mother vines. Both types of symptoms are often seen in the same vine, indicating multiple infection events before and during the propagation processes (Whitelaw-Weckert *et al*., 2013).

Establishment of new plantings of rootstock mother vines

In order to reduce the chances of rootstock mother vines becoming contaminated by inoculum from water splash and raised dust from the soil, it is recommended that the vines be trellised with crowns 1.2–1.4 m above soil level. Canes can then be kept off the ground by training emerging shoots horizontally, vertically, or at an angle (often 45°) (Figure 1). Horizontal trellising normally consists of strips of wire mesh laid parallel to, but above the soil surface. However, reports from nurseries indicate that it is difficult to detach the canes from the mesh when the cuttings are being harvested. Vertical and angled trellis systems are reported to be more satisfactory if shoots are thinned early in the season and canes are positioned on the wires as they develop. It is also reported that this system yields larger quantities of good quality straight cuttings than from vines allowed to spread along the ground (Gramaje and Di Marco, 2015).

Soil preparation to remove all weeds and correct pH and any nutrient deficiencies is also recommended before trellis is constructed and irrigation systems are installed. Planting of biofumigant crops such as mustard in the season prior to planting the new rootstock vines may also be advantageous to assist with the control of nematodes (Rahman *et al*., 2011) and soil borne pathogens, including those causing black foot (Bleach *et al*., 2008; Mundy, 2015).

Bare soils increase raised dust and dispersal of soil borne pathogens in dry windy weather (Griffin *et al*.,

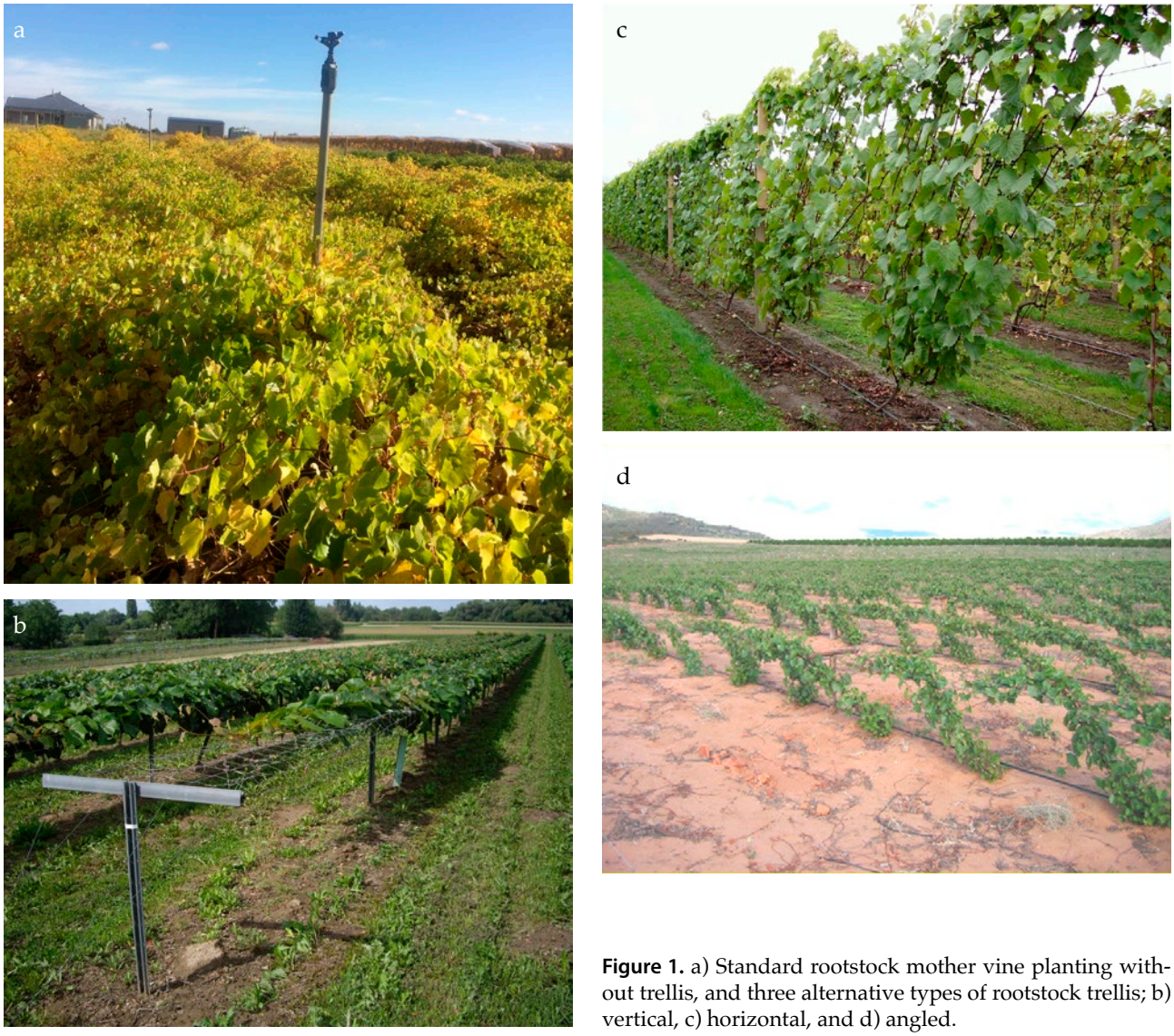

Figure 1. a) Standard rootstock mother vine planting without trellis, and three alternative types of rootstock trellis; b) vertical, c) horizontal, and d) angled.

2001). Rain, wind and sprinkler splash dispersal of inoculum from the soil can be reduced by using drip irrigation, planting the inter-row areas with permanent swards, and by applying organic mulch to the under-vine rows. Organic mulches in the under-vine rows also favour beneficial microorganisms and help to moderate soil temperatures in the root zones (Pinamonti, 1998). Mulches of pruned grapevine canes should not be used since GTD pathogen inoculum is known to be present in, and on, canes and other vine parts normally discarded during pruning (Elena and Luque, 2016a; Billones-Baaijens *et al*., 2015b).

Although untested in the context of GTD epidemiology, wind breaks of non-host species may reduce the risks of GTD from wind borne inoculum, particularly in environments where windy weather coincides with rain events, or where raised dust commonly occurs. However, GTD pathogens, particularly Botryosphaeriaceae spp., Diatrypaceae spp., and *Phaeoacremonium* spp., are known to infect a wide range of woody plants (Gramaje *et al*., 2015; Spies *et al*., 2018), so it is preferable to construct windbreaks from inorganic materials rather than potential host plants.

Management of rootstock mother vines

Tissue-cultured rootstock mother vines may be free of GTD and other pathogens when first planted, but they are unlikely to remain so. As mentioned above, pruning wounds on 1-year-old wood of *V. vinifera* have been shown to be susceptible to GTD pathogen infections for periods up to 4 months after pruning (Eskalen *et al*., 2007; Serra *et al*., 2008; Úrbez-Torres and Gubler, 2011; Van Niekerk *et al*., 2011). Therefore, the multitude of cuts that are made to the crowns of mother vines when the canes are harvested are susceptible to infection by rain and wind-borne inoculum. These cuts on the crowns should be protected as soon as they are made, using suitable wound treatments such as thiophanate-methyl, benomyl, pyraclostrobin or tebuconazole, depending on the pathogens present (Rolshausen *et al*., 2010; Díaz and Latorre, 2013, Gramaje *et al*., 2018). It is especially important that pruning wound protection begins from the first year after planting. It is also suggested that leaving long internodes on spurs on mother vine crowns when cuttings are harvested to reduce the chances of trunk disease organisms penetrating the crowns (Amponsah *et al*., 2012).

The types and availability of pruning wound treatments vary between jurisdictions, and their efficacy against the known GTD pathogens is variable (Rolshausen *et al*., 2010). Products that have not been tested, or are of doubtful efficacy, should be avoided. Fungicidal wound treatments applied as pastes block the grapevine xylem vessels, thus providing better protection than the same products applied as sprays (Díaz and Latorre, 2013; Gramaje *et al*., 2018). A comprehensive list of pruning wound treatments was presented by Gramaje *et al*. (2018). However, it is likely that the trunks of mother vines will become infected with time. Short-trunk and trellised vines will benefit from frequent trunk renewal to reduce the levels of inoculum in mother vine crowns (Calzarano *et al*., 2004). This is most easily achieved by layering ("marcottage"), since it is likely that simply cutting the trunks, which are very short and close to the ground, will not remove all the infected tissues. When done over a few years the trunks may be replaced with no loss of productivity. However, "marcottage" may not be permissible in certification schemes in some European jurisdictions. Propagators contemplating the use of "marcottage" should first check with the vine certification authorities in their respective jurisdictions.

Regular monitoring programmes are advised to check for the presence of external symptoms of GTDs, including foliar symptoms, cankers and dead wood, and the presence of insect pests and foliar pathogens. A non-destructive technique to biopsy mature vines to aid identifying infected vines was also been developed by Mundy and Vanga (2017). This takes small core samples from vine trunks under sterile conditions and uses PCR to identify fungal and bacterial DNA in the core samples. A standard spray programme, such as those used to control powdery or downy mildews and other fungal pathogens, may also be useful for reducing the levels of bark inhabiting GTD pathogens (Jaspers, 2001). The products and strategies for protecting pruning wounds in mature commercial vineyards are also applicable to rootstock mother blocks (Gramaje *et al*., 2018). Some of the fungicides that might be useful for reducing inoculum on canes in mother vine blocks include benomyl, prochloraz, triforine (Jaspers, 2001), cyprodinil formulated with fludioxonil (Rego *et al*., 2009) or azoxystrobin (Gramaje *et al*., 2009b). These fungicides, particularly benomyl, are not registered in all jurisdictions and growers are advised to check respective registrations before applying any of these fungicides.

Harvesting and early stage processing of rootstock cuttings

Recent studies by Elena and Luque (2016b) indicated that the length of the stem internodes remaining after pruning can affect cane colonization by GTD pathogens. Therefore, cutting each cane to retain the longest possible internodes at the crown as well as wound protection can help avoid infection. Further, retaining a long internode at both ends of the rootstock cutting can reduce infection; if necessary the cuttings can be recut in the nursery and dipped in fungicide.

Harvesting and early stage processing of cuttings is critical to preventing accidental contamination from soil, water and tools. Harvesting cuttings in wet weather should be avoided, and harvested canes should not contact the soil. Instead, they should be placed in clean, disinfected containers, and directly transported to the nursery. When canes are severed from the mother vine, each cut should be made just below a bud to retain a long internode on the mother vine, and pruning debris should be removed promptly and destroyed by burning or composting. Pruning wounds should be treated immediately with an ap-

propriate fungicide wound paint, paste or spray, and cutting implements should be disinfested between each vine with a broad-spectrum biocide such as ethanol. Every effort should be made to prevent dehydration of the cuttings, heat stress or exposure to toxic chemicals including fuel and herbicides during transit to the nursery. Further details of best handling and transport practices were described by Waite *et al*. (2014).

On arrival at the nursery, the cuttings should be processed immediately to avoid stress and cross contamination. Treating the cuttings is recommended to control epiphytic inoculum of GTD and other potential pathogens by brief dipping (less than 30 min) in non-specific biocides or registered fungicides is recommended. However, this option may not be available in some jurisdictions (including Europe), where there are currently no pesticides registered for this purpose. Soaking of cuttings in water, treated or otherwise, can cause oxidative stress in cutting tissues (Pfister-Sieber and Brändle, 1994), and favours the spread of GTDs that might not be controlled by the treatments. Alternatively, the biocontrol agent *Trichoderma* can be used as a surface treatment for the control of GTDs in place of chemical treatments (Pertot *et al*., 2016). However, it is important to use the correct strain of *Trichoderma* in the appropriate conditions to maximise efficacy.

Cuttings should be stored at 2–3°C in clear plastic bags that are perforated to allow air to penetrate. Dehydration of the cuttings can be prevented by including a small amount of new and clean vermiculite or paper towel that has been moistened with treated water and then squeezed dry. Cuttings must be surface dry when placed in storage. The growth of superficial moulds is favoured by placing wet cuttings in storage.

The common practice of dormant bench grafting in nurseries (with omega or whip and tongue grafts) is reported to produce more GTD-symptomatic vines than field chip budding or cleft grafting (Mary *et al*., 2017). It is hypothesised that the high rate of graft union contamination during bench grafting is caused by the cutting action of the knife mechanisms pushing fragments of bark and/or water containing hyphae from the bark of cuttings into the graft unions, as opposed to the less invasive cuts made during chip budding in the field where the cutting action of the knives and the dry surface of the rootstocks makes graft union contamination less of a risk. In addition, the bark of cuttings that have been soaked becomes soft, blackened and crumbly, increasing the risk of bark fragments being pushed into the graft unions during bench grafting.

The warm (26–29°C), moist and dark conditions of callusing rooms also favour the growth of the microorganisms introduced into graft unions during bench grafting. In contrast, use of transparent budding tape, diurnal temperature fluctuations and the high light intensity in the field may inhibit the germination and establishment of GTD pathogen inoculum in the graft unions of field budded vines.

During the establishment of bench grafted vines, the development of the contaminating pathogens interferes with graft healing and causes dark staining and streaking in xylem tissues. This can be seen extending downwards into the rootstocks (Wallace *et al*., 2004) and upwards into the scions as the growing season progresses. It follows that improved management of rootstock mother vines to reduce the titres of GTD and other potential pathogen inocula is particularly important where dormant cuttings are bench grafted (Halleen *et al*., 2003).

Harvesting vines from field nurseries

Conditions may be wet when 1-year-old vines are dug from field nurseries, and if the vine roots and shoots are trimmed in the field there is further opportunity for infection of pruning wounds on the scion stubs before the vines are waxed. It has been observed by the senior author, in Australia, England and the USA, that when vines are delivered to customers, staining extending from the cut surfaces on the scions down to the graft unions occurred beneath the wax of some vines. Therefore, it is recommended that light trimming is only done in the field, and final trimming before waxing be done in clean areas under cover in nurseries.

Barriers to adoption of best practice

Since it came to prominence in the 1990s (Morton, 1995; Mugnai *et al*., 1999), the issue of GTD transmission in propagating and planting material has been a challenging concept for propagation industries and grape growers. The slow decline of young vines infected with GTD pathogens and the resulting time between infection events and the decline of apparently healthy young vines (Mugnai *et al*., 1999; Rego *et al*.,

2000; Rumbos and Rumbou, 2001; Halleen *et al*., 2003; Edwards and Pascoe, 2004; Giménez-Jaime *et al*., 2006; Whitelaw-Weckert *et al*., 2013), has been a significant barrier to recognition of the problems associated with pathogen transmission in propagation. The problems caused by pathogens transmitted in planting material were, and continue to be, attributed to other factors such as poor planting practices and environmental stresses (Waite, 2013). This slow, but insidious progression of GTD after infection is in contrast to most other grapevine diseases. Acceptance of GTDs as the primary cause of young vine decline has been slow, and the uptake of measures to prevent GTD transmission in propagation has languished in nurseries around the world. Furthermore, because clean planting material can become infected by external inoculum within a few years after planting in a vineyard, there is a perception that there is little point in preventing infections in nurseries (Yobregat *et al*., 2018). However, propagation-acquired infections are known to result in defective vines with poorly healed graft unions (Wallace *et al*., 2004) and weak root systems (Halleen *et al*., 2007). Young vines are often inspected without reference to any of the published standards, prior to despatch from nurseries, so defective vines with infections by GTD pathogens are planted by vineyard owners and managers who assume the purchased plants are healthy. There is also good evidence that young vines with propagation-acquired infections often succumb very soon after planting, or are slow to establish and do not become fully productive (Whitelaw-Weckert *et al*., 2013). Post-planting infection events are thus likely to further increase the disease burden for young vines and increase the risks of vine failures.

The low prices that propagators receive for rootstock cuttings and grafted vines (Table 1) is a significant disincentive for nurseries to adopt practices designed to prevent infection and transmission in rootstock cuttings. Propagators fear that the added costs associated with obtaining clean mother vines, installing trellis systems and training the vines to grow on trellis will not be recovered through increased productivity, and will threaten the viability of their businesses. Furthermore, the low value placed on planting material by grape growers, and the quest for the cheapest plants available, means that propagators may not be able to recoup the additional costs of production by charging premia for high quality vines (Waite, 2013). However, improved propagation practices that reduce the number of cuttings and vines that are discarded before sale will reduce the 40-60% loss rates currently reported by nurseries, thus compensating for any additional costs of production.

Table 1. Prices (Euros, as at June 2017) of grapevine rootstock cuttings and bare rooted grafted vines in different countries. Prices may vary depending on variety and grade.

Country	Vine price
Australia	2.72–3.00
Austria	1.30–1.50
France	1.40–1.65
Germany	1.55
Hungary	0.90
Italy	1.50–1.60
France	1.40–1.65
South Africa	1.00–1.50
New Zealand	3.00–3.10
Spain	1.40–1.65
Switzerland	3.16–4.36
USA	3.20–5.00

Summary and discussion

The health and quality of grapevine planting material are fundamental to the longevity and productivity of vineyards. However, the diversity of GTD pathogens transmitted during propagation and the difficulties associated with their control, means that preventing transmission in propagation is the most effective means of controlling the spread of GTDs in planting material. Furthermore, because rootstock cuttings can be major sources of pathogen inoculum, it is vital that rootstock mother vines are proactively managed to ensure that infections in and on cuttings are minimised.

To achieve these aims and ensure that the high health status is maintained in cuttings from well-managed mother vines throughout propagation processes, it is recommended that the best practice outlined in this review is followed, for mother vine and nursery management. The best practice protocol is summarised below:

Mother vine management

1. Propagate new mother vines under very high sanitary conditions, preferably by tissue culture, to ensure the lowest possible titre of GTD pathogens. If tissue culture is not available, rootstock cuttings should be treated with a combination of hot water treatment and (depending on the jurisdiction) appropriate registered fungicide and or biocontrol agent prior to propagation.
2. Hot water treat rooted vines before planting out in the mother vine block, unless the vines are propagated by tissue culture and grown in pots rather than a field nursery.
3. Preferably trellis mother vines, and maintain crowns 1-1.4 m above the soil surface.
4. Treat pruning wounds with an effective fungicide, and/or biocontrol agent.
5. Make all pruning cuts to retain long internodes.
6. Remove and destroy pruning debris by burning or composting.
7. Institute soil management practices, such as mulches and inter row swards, to reduce rain and irrigation splash and raised dust.
8. Use Brassica biofumigants as inter-row cover crops.
9. Renew mother vine trunks at 5-10 year intervals, to reduce likelihood of systemic infections and delay the need to replace mother vine plantings.
10. Remove mother vines at the appropriate time/age (e.g. approx. 15 years, or before, if disease is present) to ensure that propagation material is harvested from healthy mother vines and reduce build-up of GTD pathogen inoculum.

Nursery practices

1. Keep rootstock cuttings off the ground and away from contact with soil and plant debris.
2. Dip freshly cut rootstock cuttings in a general-purpose biocide.
3. Avoid soaking of cuttings, to reduce cross contamination and oxidative stress. Soaking poses serious risks to cutting and vine health. Nurseries that choose to soak cuttings should do this for short periods (< 1 h) and change the water after every batch.
4. Maintain the highest levels of general nursery sanitation.

There are barriers to the adoption of these practices, including the costs of implementation, the low prices received for cuttings and vines, and the low value placed on planting material by grape growers. This situation has arisen because the effects of pathogen infections on cuttings and young vines are frequently mistaken for other causes. The costs of GTDs to nurseries and grape growers is largely unknown. A detailed study to determine the costs associated with the transmission of GTDs in cuttings and young vines would significantly contribute to the understanding of the financial impacts of GTDs and the economic value of quality vines.

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