GRAPEVINE TRUNK DISEASE

A REVIEW

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EXECUTIVE SUMMARY

Grapevine trunk disease is a high profile condition of the vine which appears to be increasing in incidence and severity globally. Major pathogens include *Botryosphaeria* spp., *Cylindrocarpon* spp., *Eutypa lata*, *Phaeomoniella chlamydosporum*, *Phaeoacremonium aleophilum*, *Phomopsis viticola* and others. Symptoms are variable and inconsistent but generally involve wood & leaf necrosis as well as poor growth and/or establishment, and in very acute cases, vine death. Interaction between these pathogens and their environment appears to be complex and sometimes specific to species and location, with environmental stress thought to play an important role, potentially activating latent infections. Control measures are limited to preventing spread and the severe surgery of infected or entire vines. Vectors for infection have the potential to be varied, but pruning wounds appear to be a principle point of entry. Pruning wound treatments can reduce infection by limiting mycelial growth on open wounds but are subject to variable results and local regulations on the use and availability of fungicides. Further cultural methods as part of viticultural best practice (i.e. site selection) are also important to managing these conditions. Infected mother blocks and pathogen transmission by flawed nursery practices are a significant contributor to trunk disease in young vines.

CONTENTS

INTRODUCTION

This report has been written to review the literature published to date on the issue of grapevine trunk diseases, their symptoms, their epidemiology and their control. Grapevine trunk diseases currently have a high profile globally and the increase in observed symptoms has been associated with recent reductions in the availability of proven treatments, e.g. sodium arsenite, banned in 2001 (Kuntzmann et al. 2010; Lecomte et al. 2003). It is also likely that this increasing awareness is of itself a cause of more frequent observations of symptoms in the field and therefore the increasingly high profile of trunk diseases in general.

Where surveys have been conducted, trunk disease pathogens and symptoms are widespread amongst and within vineyards and nurseries globally, for example 63.6% of Sauvignon blanc vines in California are reported to be infected with *Botryosphaeriaceae* (W. Gubler et al. 2005) and 50% of Tuscan vineyards report Esca symptoms, growing at a rate of 4-5% per year (Fischer & Kassemeyer 2003). In the UK there has been positive molecular identification of *Diplodia seriata* (McNeill 2011, Smart 2011a), *Neofusicoccum parvum* (Smart, 2011) and *Cylindrocarpon destructans* (Smart 2011b) from several vineyards. Unfortunately the lack of a wider data set and commercial sensitivities prevent the publication of further positive identifications of trunk disease pathogens in the UK, however it is strongly suspected that the pathogens responsible for the Esca complex of diseases are also present, i.e. *Phaeomoniella chlamydosporum*, *Phaeoacremonium aleophilum*.

Problems with the management of these diseases begin with the degree of complexity apparent in their interactions with their environment, the host plant and between themselves. The complexity of these reactions extend to the individual species level with species-specific symptoms observed within the same vineyard and between different geographical regions (van Niekerk et al. 2006; van Niekerk, et al. 2011), as well as species-specific responses to environmental stresses (Whiting et al. 2001) and to fungicidal treatments (Alaniz et al. 2011). It is possible that these complex interactions hold the key to understanding disease epidemiology and control, especially in a marginal climate such as the UK's.

MAJOR DISEASES & SYMPTOMS

The simplistic cause and effect visible in other fungal diseases of the grapevine (e.g. *Erisiphe necator* causing powdery mildew) is not always so easily identifiable with trunk diseases. Trunk diseases often present the same way and have several different pathogens present in samples taken from symptomatic vines (Kuntzmann et al. 2010). Nonetheless, there are several diseases recognised globally and these are generally referred to as discreet entities:

BOTRYOSPHAERIA

The taxonomic definitions of the *Botryosphaeriaceae* are still developing as our understanding of the range of species improves, particularly through further methods of DNA analysis (Shiller et al. 2007; Weir & Graham 2008). Anamorphs of *Botryosphaeria* (*Neofusicoccum* and *Diplodia* spp.) are often referred to in the literature (e.g. Halleen & P H Fourie 2000; Amponsah et al. 2009) and there seems to be no consistent naming protocol for published research, although progress towards this is now being made (Crous 2012)

Botryosphaeria is also one of the most widespread and diverse of the fungal species that contribute to trunk disease, for example of 238 samples taken from symptomatic vines in 43 vineyards in NZ, 88% of the vineyards were infected with *Botryosphaeria* spp. and 68% of the samples, which yielded 336 isolates of *Botryosphaeriaceous* species (Baskarathevan 2011). In other species, *Botryosphaeria* is held to be a weak pathogen that is widespread even in healthy plants (Bonfiglioli 2008), however a survey in California reported no evidence of *Botryosphaeria* spp. in asymptomatic vines in the same vineyard as symptomatic vines with *Botryosphaeria* infections (Harvey 2010), although this is from a third party report of conference proceedings. In a recent study of Texan grapevines, evidence also suggested that *Botryosphaeria* may be more pathogenic than originally suspected (Úrbez-torres et al. 2009) however these conclusions were based on an in vitro assessment of visible lesion length rather than extent of fungal penetration, as with later research that uses a similar methodology, e.g. (Ayres et al. 2011). It is likely that different species of *Botryosphaeria* present different levels of risk, with for example *Diplodia seriata* present in a wide range of plants but not associated with significant vine damage, unlike *Neofusicoccum parvum*.

Botryosphaeria's symptoms are varied and not all vines where *Botryosphaeria* spp. have been found present the same symptoms or present the full range; foliar symptoms are especially variable in their expression (W. Gubler et al. 2005; Sosnowski & Loschiavo 2010):

- Early season leaf chlorosis and wilt
- Visible exterior cankers in advanced cases (Figure 1)
- Slow/stopped shoot growth

Figure 1 – Visible exterior canker (Newsome J, 2011a

- Necrosis of the wood, including light brown discolouration inside the cane, a darkened central pith &/or necrotic wedges in cross-section of severely infected vines
- Bud necrosis
- Bleached bark with fine dark spots (pycnidia)
- Leaf spots
- Bunch rot
- Severe crop loss on vines after 8+ years of infection

(Bonfiglioli & McGregor 2006; Mundy & Manning 2010; W. Gubler et al. 2005; van Niekerk et al. 2006; Wunderlich et al. 2009)

ESCA

Esca is a widely reported disease associated with a number of different fungal species, specifically *Phaeomoniella chlamydosporum* and *Phaeoacremonium aleophilum* but also with the presence of *Botryosphaeria* spp., *Eutypa lata*, *Phomopsis viticola*, *Cylindrocarpon* spp. and others in infected vines (Fischer & Kassemeyer 2003). These pathogens are also found in young vines where Petri Disease, or 'Black Goo' has been diagnosed (Mundy & Manning 2010; Mugnai et al. 1999; Agusti-Brisach et al. 2011; Fleurat-Lessard et al. 2010).

More recent research has created a clearer understanding of the relationship between symptoms and causative pathogens of Esca. Surico (2009) refers to two distinct conditions within the Esca complex, on one hand the distinctive foliar symptoms (Figure 2) and on the other woody necrosis that is familiar from other trunk diseases. Foliar symptoms are shown to be commonly caused by the vascular fungi *Phaeomoniella chlamydosporum* and *Phaeoacremonium aleophilum*; the term 'Grapevine Leaf Stripe Disease' has been used for this condition. Wood necrosis is related to infection by *Fomitiporia mediterranea* (and others) and known as 'true Esca'. Where both symptoms are present then the condition can be called Esca proper (Surico 2009; di Marco et al. 2011).

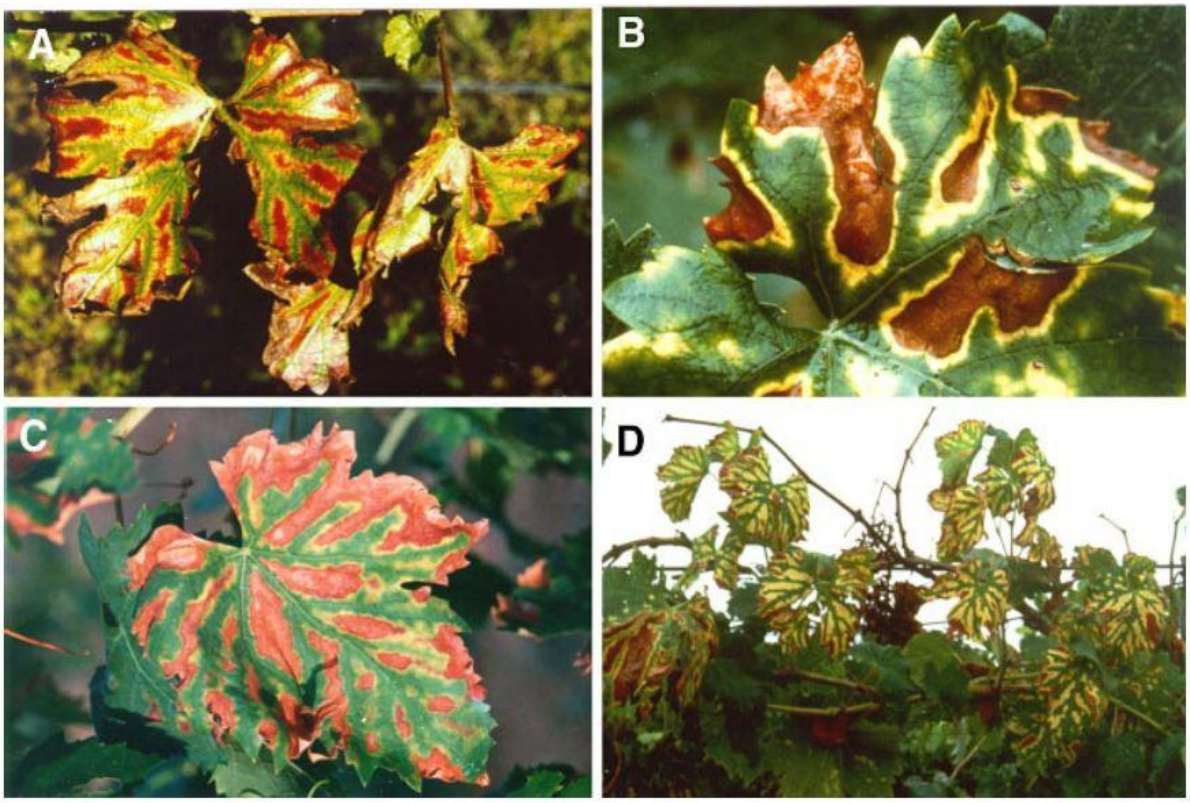

Fig. 4. Foliar symptoms of esca first appear as chlorotic spots that subsequently coalesce, turning dark red in some red cultivars like Cabernet (A), and finally becoming necrotic. Dead tissues appear dark brown to red-brown, depending on the cultivar (B and C). Symptoms often extend to the interveinal areas of the foliar blade, leaving a narrow strip of unaffected tissue along the main veins (D), thus giving the leaves a characteristic tiger-stripe pattern. (*photos courtesy* G. Minervini, [A], and S. Frisullo [D])

Figure 2 - Foliar symptoms of Esca, from Mugnai, 1999

Esca is generally associated with the following symptoms:

- Foliar symptoms – light green spots early season, coalescing to become interveinal chlorosis with red/brown colours, varying according to cultivar; 'tiger-striping', Figure 2
- White rot - rare in young vines but common in older (8 yrs+) vines
- Black/purple spotting on berries, not omnipresent, can lead to cracking
- Black necrosis visible as black dots in latitudinal cross-section
- Apoplexy, i.e. sudden vine death affecting cordons or entire vines – reportedly more common in hot climates (see below, Linking Symptoms & Causes)

(Mugnai et al. 1999; Dula et al. 2007; Letousey et al. 2010; Fleurat-Lessard et al. 2010; Fischer & Kassemeyer 2003)

Foliar symptoms are also reportedly erratic and usually not visible for 3-5 years after planting (D Gramaje et al. 2010; Whiting et al. 2001). It is also reported that environmental stress is a major influence on the expression of Esca symptoms (Fleurat-Lessard et al. 2010) and it could be argued that this argument applies to all trunk diseases, see below Environmental Stress.

CYLINDROCARPON

Several species of *Ilyonectria spp.* (aka *Cylindrocarpon*) and *Campylocarpon* spp., (Halleen et al. 2006)) are associated with this condition, principally *C. liriodendri*, *C. macrodidymum* and *C. destructans* (Alaniz et al. 2010; Petit & W. Gubler 2005; Jaspers, M. 2011a). Cylindrocarpon presents some differing characteristics from the trunk diseases discussed above in that it primarily attacks the vine roots via the soil, leading to the following symptoms from Halleen et al. (2007), Alaniz et al. (2007), Bleach et al. (2008) and Halleen et al. (2006):

- Secondary root development close to surface
- Reduction in root biomass
- Necrotic lesions under root bark
- Darkened and necrotic pith with spotting &/or darkening of the root xylem
- Poor/delayed budburst
- Weak shoot growth & reduced vigour overall
- Interveinal chlorosis & necrosis
- Poor establishment of young vines

OTHER TRUNK DISEASES

Eutypa (*Eutypa lata*) has a range of symptoms similar to those of *Botryosphaeria* and Esca, generally with reduced shoot growth, inconsistent foliar symptoms (necrotic leaf margins rather than interveinal chlorosis), necrotic wedges within the wood in severe infections and exterior cankers (Sosnowski et al. 2009; W. Gubler et al. 2005; Creaser & T. Wicks 2004).

Phomopsis (*Phomopsis viticola*) is common in UK vineyards and can be identified by brown lesions on bleached shoots, dark spots with chlorotic margins on leaf blades and in severe cases, fruit rot (Úrbez-torres et al. 2009). Generally these symptoms are considered to be mild in the UK, however there is some evidence that *P. viticola* (as with other fungi) plays a part in the expression of other trunk diseases (Úrbez-torres et al. 2009). Urbez-Torres also takes a revisionist stance on the pathogenicity of *P. viticola*, questioning the historical assumption that it is a mild pathogen (Urbez-Torres, 2012).

LINKING SYMPTOMS & CAUSES

The link between the symptoms and the causes of grapevine trunk disease is not a simple and discreet relationship. In Fischer & Kassemeyer (2003) the authors identify multiple pathogens in vines showing symptoms of Esca – these pathogens include *Eutypa lata*, *Diplodia seriata*, *Phomopsis viticola* and *Cylindrocarpon destructans*, all of which are also commonly associated with 'other' trunk diseases. In Texas, detailed and extensive research has also cast doubt on the traditional understanding of cause and effect – i.e. *Eutypa lata* causes the dieback condition known as Eutypa (Úrbez-torres et al. 2009) – where 11 different fungal species were isolated from grapevine cankers associated with Eutypa.

A possible reason for this may lie in the biological processes behind the expression of symptoms common to all trunk diseases, including wood necrosis, reduced shoot/leaf growth and leaf wilting as well as extreme cases of vine death. As early as 1999, Mugnai et al. identify four possible causative processes:

1. Physiochemical changes from the action of air and water, e.g. oxidation
2. Enzyme production by invading fungi leading to cellular breakdown and lignification
3. Vascular occlusion from the xylem-blocking action of tylosis
4. Cell necrosis caused by toxins produced by the fungi and the response of the vine

More recent research in transport systems questions the importance of simple vascular occlusion as a cause of trunk disease symptoms, as multiple redundancies have been identified in xylem transport (Keller M, 2010) and necrotic wedges rarely cover the whole cross-section of the vine (Letousey et al. 2010). Whilst some authors continue to focus on water stress (e.g. Mundy & Manning, 2010) the role of pathogenesis-related (PR) proteins and of the vine's response to infection (e.g. reduced photosynthesis, Letousey et al. 2010) is thought to be increasingly significant, as demonstrated in the detailed analysis of the changes in pre-apoplexy metabolic and genetic expression by Letousey et al. (2010). Recent research has also highlighted the potentially synergistic role of bacteria in the expression of trunk disease symptoms where, for example, symptomatic and asymptomatic vines with the same fungal profiles are observed in the field (Bruez et al, 2012).

If trunk disease symptoms could be definitively linked to PR-proteins and vine pathogenic responses then this would help to explain why multiple fungi have similar symptoms. Equally, infection by one virulent pathogen could lead to a weakened vine that is more vulnerable to infection by other fungi, as hypothesised by Fischer & Kassemeyer (2003). These issues clearly add further complexity and whilst there has been some success in linking symptoms and causative pathogens (e.g. developments in the redefining of the Esca complex of diseases by Surico (2009)) there is still room for debate as to whether trunk diseases in general are discrete diseases at all, but rather a collection of symptoms caused by a range of fungi.

This is an area of interest for further research with the practical goal of exploring new pathways for disease treatment, however as there is currently no curative remedy it is necessary, for now, to focus on epidemiology and control.

EPIDEMIOLOGY

Generally, the infection vectors and disease cycle for the pathogenic species of fungi identified above are all similar. The fungi overwinter in diseased wood and develop fruiting bodies during high humidity periods. Spores are released by rainfall and spread by rain splashes and wind (Ayres et al. 2011; Mundy & Manning 2010). It has also been shown that trunk disease fungi can survive for extended periods in soils across a range of temperature and humidity (Tello, Gonzalez & Andres, 2012). During extended rainfall events, spores will be produced for up to 36 hours before fruiting bodies are exhausted and take 12 days to recharge (Sosnowski et al. 2009). Lab-based research has shown that the fungi will produce conidia in the temperature range 6 – 30 °C (Copes & Hendrix 2004) and in the same paper, Copes & Hendrix hypothesise that conidial production may be initiated at less than 6 °C with the release of mature conidia delayed until the temperature rises. However this would need to be demonstrated before it is of practical use as information to influence pruning conditions.

Initial infection occurs on any exposed, unlignified wood such as found on pruning wounds; given the favourable climatic conditions during winter pruning (i.e. high humidity/rainfall) entry via pruning wounds is considered the primary infection vector for all the fungi above except *Cylindrocarpon* spp. (Halleen et al. 2010; M. R. Sosnowski et al. 2008; Philippe E Rolshausen et al. 2010; Lecomte et al. 2003 and many more). This can be demonstrated by dissection of the vine where necrotic areas of the wood are clearly traceable back to pruning wounds, see Figure 3.

Figure 3 - Cross section of vine showing fungal infection entry point in pruning wound (Newsome J, 2011b)

As well as pruning wounds, there is some evidence that *Botryosphaeria* conidia are found in vineyard standing water (although not aerial spores) all year round (Amponsah et al. 2009) and therefore present a potential infection threat for summer trimming as well as winter pruning. Similarly, Epstein et al. (2008) state they have evidence of springtime infection through wounds left after bud-rubbing, although this is from a limited sample of 14 vines that had were known to have had severe *Botryosphaeria* infection before cutting back. Alarmingly, Amponsah (2012) has demonstrated that all vine tissue types have the potential to be infected, even dormant buds with no physical injuries. Epstein et al. also tested the blades of secateurs after pruning infected vines and found evidence of *Botryosphaeria* infections on 24% of sampled blades. Whilst their experiments were not in depth, they do indicate the potential for pruning equipment to be a vector for infection when moving between infected and uninfected vines. Finally, Whitelaw-Weckert et al. (2006) present their findings that forced inoculation of soil with *Botryosphaeria* can lead to infection of the root structure in potted vines, indicating that soil may also be a potential infection vector for fungi other than Cylindrocarpon. The authors are the first to admit that their research is not conclusive but they do state that this has "*serious implications*".

Nonetheless, pruning wounds remain the most common and most likely vector for infection of established vines by the fungi that cause trunk disease, whether through airborne spores or possibly (as observed in South Africa), the physical transferral of spores from the bodies and feet of vineyard insects (Moyo et al, 2012). Variables such as wound size, age, weather conditions during pruning, etc., are all relevant to the risk of infection (Halleen et al. 2010). Pruning wounds are susceptible for up to 7 weeks from the initial cut (W. Gubler et al. 2005), although this varies with the temperature. That is, Sosnowski et al. (2009) report that wound healing (i.e. lignification) is significantly accelerated in warmer spring temperatures (10-14 days in spring vs. 4-6 weeks in winter).

Warmer temperatures also promote the growth of other microorganisms that compete with invading fungi and the rising sap is reportedly able to physically obstruct and remove fungal spores (Munkvold & Marois 1995; Sosnowski et al. 2009; W. Gubler et al. 2005) although there is no specific research on the latter point. This is contrary to the position taken by Mundy & Manning (2010) who report that rising sap can increases the risk of infection by creating damp pruning wounds – in this case the presence of sap on pruning wounds might also serve to activate latent infections within the wood itself (Morton, 2012). Research has demonstrated the presence of pathogens in the healthy vine tissue vine even before pruning (van Niekerk et al. 2011, Billones-Baajiens et al 2012) and so the role of rising sap in trunk disease epidemiology is as yet unclear.

CYLINDROCARPON

Cylindrocarpon infections can be traced back to the soil in which the vine is planted, as the infection extends from the bark towards the rootstock pith, commonly at the base of the rootstock (Bleach et al. 2008; Halleen et al. 2006). Open wounds therefore may not be a significant infection vector for Cylindrocarpon unless the wound is somehow exposed to infected soil, as is sometimes the case in nurseries where a new graft union can be covered with soil to prevent drying out (Halleen et al. 2006). There is some evidence that *Cylindrocarpon* spp. can persist in weed cover (Agusti-Brisach et al. 2011) although this data was geographically specific to Spain. It has also been demonstrated that *Cylindrocarpon* spp. can infect a vine via planting in infected soil in the space of just one growing season, with the proportion of infected rootstock on one block in a South African nursery increasing from 1% to 50% over the course of one summer (Halleen et al. 2003).

NURSERIES & PROPAGATION

Of major concern in the spread of Cylindrocarpon and other trunk diseases is the role of nurseries and infected rootstock play. Global studies have shown that there can no longer be any doubt that nurseries are a source of infected plant material, e.g. France (Larignon, 2012), Spain (Agusti-Brisach et al, 2012), Australia (Whitelaw-Weckert M (2012). Nursery practices are considered to contribute in a number of ways, for example Waite (2010) reports that (unspecified) trunk disease pathogens were isolated from a range of nursery equipment and material including soaking water and grafting benches. These findings are supported by other authors, e.g. Aroca et al. (2010)(D Gramaje & J Armengol 2011) and by evidence presented of infected planting material, e.g. Halleen et al. (2003). Practices such as growing all rootstock in the same mother blocks and the

burying of newly grafted vines can only contribute to the risk of soil-borne infections. These conditions can then be exacerbated by poor planting practices and/or site selection that unduly stress new vines leading to poor establishment and/or vine failure.

Since the banning of sodium arsenite, nurseries are limited in the actions they can take to minimise infection. Hot water treatment has received a great deal of attention and shows some positive results in reducing pathogen population densities (Casieri et al. 2009). However the precise methodology and efficacy is still debated alongside the long term impact on the vine. It also appears that the climate in which the cuttings are grown has an impact on their response to hot water treatment with cool climate cutting more sensitive to heat (D Gramaje et al. 2009; Waite & Morton 2007). Therefore it is apparent that time and temperature should be varied to minimise harm to the vine whilst maximising protection – exact parameters still need to be established conclusively. Other researchers have found that fungicidal treatments (e.g. soaking) can help reduce infection prior to grafting (Rego et al. 2009) however it is not known if these practices are being taken up by nurseries. Soaking in untreated water has the potential to be a significant vector for infection (Waite et al, 2012) and it is questionable whether this practice is of any benefit to the propagation process.

CONTROL METHODS

There are no curative treatments for grapevine trunk diseases. Some research has been carried out on trunk injections of fungicide however these are labour-intensive, costly and either shown to be ineffective (Darrieutort & Lecomte 2007) or only of very limited impact (Dula et al. 2007). Therefore, treatment of these conditions must focus on management and the minimisation of spread. There are three principle areas on which current practices focus.

PRUNING WOUND TREATMENTS

Generally, the goal for pruning wound treatments is to inhibit mycelial growth on the wound itself and/or physically seal the wood to prevent infection. A variety of fungicides and paints have been deployed and tested globally with benzimidazoles (e.g. carbendazim and benlate) found to be the most effective (Sosnowski et al. 2008; W. Gubler et al. 2005; Halleen et al. 2010; Sosnowski et al. 2009) although there are questions over the longevity of benlate treatments (Halleen et al. 2010; W. Gubler et al. 2005). Some biological agents have also shown potential as pruning wound treatments if applied to open wounds to prevent infection. For example, both *Trichoderma harzianum* (John et al. 2008) and *Bacillus subtilis* (Ferreira et al. 1991) were shown to be effective against *Eutypa lata* in preliminary trials, however results of field and lab trials since have been inconsistent (Sosnowski et al. 2009; Halleen et al. 2010). One possible cause of this is the time it takes for bio-control agents to become established on the wound, during which a window of opportunity is open for infection (Sosnowski et al. 2009). If biological agents can become established though, they provide effective long term protection when compared against chemical controls which can be short-lived (Mugnai, 2012). Unfortunately, regulation and commercial decisions on availability restrict the options available in the UK, see Appendix 1 – Potential UK Pruning Wound Treatments.

Other countries have faced similar issues with the availability of potential treatments, e.g. South Africa (Halleen et al. 2010) and a three phase approach to finding an appropriate solution has generally been taken:

1. **Survey** – Given the potential for species-specific responses to treatments (Alaniz et al. 2011) and the potential complexity of interactions between the pathogens themselves and their environment, a comprehensive survey of indigenous trunk disease fungi is acknowledged as an important first step to establishing a treatment protocol, e.g. (Halleen et al. 2010; Mundy & Manning 2007; W. Pitt et al. 2010; Fischer & Kassemeyer 2003; W. Pitt et al. 2008). In these surveys, morphological identification of pathogens is problematic given their similar physical characteristics (van Niekerk et al. 2006; Weir & Graham 2008; Alaniz et al. 2007) and more expensive molecular analysis considered far more accurate.

2. **In-vitro Trials** – A long list of potential treatments is developed based on locally-available/regulated treatments and tested in the lab against indigenous species. The majority of in-vitro tests follow an established methodology to plate, treat and assess the degree of mycelial growth, (e.g. Alaniz et al.

2011; Halleen et al. 2010 also Sosnowski et al. 2008). However this can be time-consuming and there is sometimes a disconnect between lab results and field results (Bleach et al. 2008; Ayres et al. 2011; Halleen et al. 2010). Recently, a new technique using single-node plantlets has been trialled as a more time-efficient method to simulate field conditions (Ayres et al. 2011; Mundy & Robertson 2010). This method, particularly with the improvements put in place by Ayres et al., shows some promise, however it remains unproven against complex environmental interactions in the field.

3. **In-vivo Trials** – Given this complexity, field trials are the definitive method for assessing pruning wound treatments. Experiments such as those in Rolshausen et al. (2010), Alaniz et al. (2011) and Sosnowski et al. (2008) have produced actionable, location and species-specific results. This may be particularly relevant in the UK, see below Environmental Stress.

Results of trials published to date are summarised below:

Active Ingredient	**Botryosphaeria**		**Cylindrocarpon (field trials)**	**Eutypa lata**		
	In vitro	Field trials		In vitro	Field trials	
Carbendazim	(5)		(1)	(3)		(3)
Benomyl	(6)			(2,3)		(2,3)
Copper oxychloride			(1)			
Copper ammonium acetate	(6)					
Kresoxim-methyl	(6)					
Boscalid	(6)					
Captan			(1)			
Iprodione	(5)	(6)				
Thiram			(1)			
Prochloraz	(6)		(1)			
Flusilazole	(5,6)		(1)	(2)	(2)	(3)
Imazalil			(1)			(3)
Tebuconazole	(5, 6)			(2)		
Didecyldimethylammonium			(1)			
Hydroxyquinoline sulphate			(1)			
Fenarimol	(5)			(2)		
Myclobutanil	(5)			(2)		
Cyprodinil + fludioxonil	(5)					(3)
Pyraclostrobin	(5)					(3)
Pyrimethanil (Scala)	(5,6)					(3)
Fluazinam	(5)					(3)
Spiroxamine	(5)					

Penconazole	(5)					
Fenhexamide	(5)					
Quinoxyfen	(5)					
cyproconazole + iodocarb (paste)						(3)
Boron (n.b. potentially phytotoxic)				(4)		(3,4)
Acrylic paint						(3)
Acrylic paint with fungicide						(3)
Trichoderma harzianum						(2)
Bacillus subtilis						(2)

Table 1 - Collated results of pruning wound trials

Key:
Ineffective (reference)
Partial, limited or reduced effectiveness (reference)
Effective (reference)

References
1 - Alaniz, S. et al., 2011
2 - Halleen, F et al. 2010
3 - Sosnowski, M. et al 2008
4 - Rolshausen & Gubler 2005
5 - Pitt, W et al 2008
6 - Bester et al. 2007

CULTURAL CONTROLS

Further vineyard management protocols that can reduce the spread of grapevine trunk diseases are well covered in a number of papers, particularly Sosnowski et al. (2008), and cover practices such as vineyard hygiene (removing and burning cuttings), modifying pruning practice where applicable to minimise spread (do not prune when raining) and pruning as late in the season as possible. Further studies have suggested double-pruning (Weber et al. 2007) in climates with a dry early spring for the second pass, removing infected dead wood from nearby trees (W. Gubler et al. 2005) and mycorrhizal additions to new plantings (Bleach et al. 2008), however the latter has been shown to be inconsistent and other unpublished research suggests it has no value beyond improving vine vigour in general (Jaspers, M 2011a).

Ultimately though, infected vines that are no longer economically viable must be addressed either by grubbing up or cutting back. Sosnowski et al. (2009) recommend cutting back to clean wood with no necrosis + 10 cm. The authors also make recommendations as to methods for training up water shoots to minimise loss of production and the risk of new shoots being infected.

On the purchasing, preparation and planting of new material there is little specific research on reducing infection risk at planting although there is much debate on the role of nurseries, see above. It is reported in unpublished data that fungicidal dips of Switch (cyprodinil + fludioxinil) can be effective in reducing the risk of infection when planting in soil which is known to harbour soil-borne fungi such as *Cylindrocarpon* spp. (Jaspers,

M. 2011a). Unfortunately, regulatory approval for the use of Switch as a dip has not been given in the UK (Cooper, C. 2011). Bio-fumigation of soil with a mustard crop has also been reported to have some impact on infection levels of Cylindrocarpon (Jaspers M., 2011a; Harvey 2010)

ENVIRONMENTAL STRESS

Given the fungal preference for humidity and the spread of spores through rain and wind, it is clear that environmental stresses play a significant role in the epidemiology of grapevine trunk diseases. As ever with these complex diseases, behaviour can vary by species:

"Variation in response of isolates within two of the Phaeoacremonium species in response to water potential was not expected. Usually, isolates of soil fungi would be expected to respond similarly, but physiological variation of isolates within species is not unusual" (Whiting et al. 2001)

It is also hypothesised by several authors that environmental conditions act as triggers to infections that would not otherwise have meaningful impact on vine yield and health (Bonfiglioli & McGregor 2006; van Niekerk et al. 2006) and the extent of this impact is of particular relevance in the UK where environmental stress is common. Bonfiglioli & McGregor (2006) refer explicitly to vineyards in "*sub-optimal*" areas in New Zealand with heavy, poorly drained soil that are showing the most damage from Botryosphaeria infections. Therefore, a logical further control method in the UK is to follow best practice in site selection to maximise drainage, exposure and other positive variables as well as utilise viticultural techniques such as canopy management to minimise negative environmental stresses such as shading.

FURTHER RESEARCH

Several research avenues present themselves as particularly relevant to the UK:

- A detailed survey of UK pathogens to establish the severity, spread and indigenous species using molecular identification techniques. Several methods exist and could be followed, e.g. the postal sampling system used in New Zealand (Jaspers, M 2011b) which used molecular analysis, or the longer-term method used by the extensive National Grapevine Trunk Diseases Survey in France which gathered and rigorously analysed data from vineyards but did not use molecular analysis to determine species (Fussler et al. 2008).
- Temperature and epidemiology. An investigation of the impact of temperatures below 6 °C on germination and sporulation
- Sap and epidemiology. Establishing whether rising sap reduces or increases infection would assist with the timing of pruning.
- Further research and, critically, an education program aimed at nurseries with the goal of minimising the infection of vineyard planting material
- Further investigation into the role of PR proteins in vine symptoms

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APPENDIX 1 – POTENTIAL UK PRUNING WOUND TREATMENTS

The treatment list below was developed by Chris Cooper, the UKVA's pesticides expert after an extensive literature review, (Cooper & Smart 2011).

TREATMENT LIST FOR TRUNK DIEBACK TRIAL

1. Control +pathogen(untreated/inoculated)
2. Control (untreated/inoculated)
3. A branded acrylic paint, Dulux water based gloss (choice of colours is up to you) e.g. Dulux Weathershield Quick Dry Exterior Gloss.
4. Alcohol((Propanol as Propeller a 50-70% solution) sprayed onto the cut
5. Alcohol sprayed onto the cane/cut followed by Acrylic paint
6. Nativo as a spray alone 180g/50lt.
7. Nativo as in treatment 6 followed by Acrylic Paint as in treatment 3
8. Bezel (Tebuconazole) used as the trial standard, (if we can get hold of some, no longer commercially available))
9. 5% Brushing Boron (Ultra Gel 12) applied only to cut
10. Borax stirred into acrylic paint to give a 5% solution.
11. Arbrex (a latex wood sealant).
12. Vinevax (via FAST) as Duncan wishes it applied
13. Cuprokylt Liquid (Copper Oxychloride) applied by brush
14. Serenade ASO 1lt in 10lt