Grapevine trunk diseases: complex and still poorly understood

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This review presents an overview of eutypa dieback, esca and botryosphaeria dieback, the predominant grapevine trunk diseases worldwide. It covers their symptomatologies in the trunk, leaves and berries; the characteristics of the different fungal species associated with them; and host–pathogen interactions. Here, the host–pathogen relationship is defined at the cytological, physiological and molecular levels. Currently available experimental tools for studying these diseases, both in vitro and in the field, are discussed. Finally, a progress report on their control, which, since the ban of sodium arsenite, comprises chemical, biological and/or sanitation methods, is presented.

Keywords: Botryosphaeriaceae, esca, Phaeoacremonium, Vitis vinifera

Introduction

Eutypa dieback, esca and botryosphaeria dieback are three significant grapevine trunk diseases that involve one or several xylem-inhabiting fungi (Larignon & Dubos, 1997; Mugnai et al., 1999; Larignon et al., 2009). Phaeo- monielia (Pa.) chlamydospora (Crous & Gams, 2000), Phaeoacremonium (Pm.) aleophilum (Crous et al., 1996), Eutypa lata (Rappaz, 1984), Fomitiporia mediter- ranea (Fischer, 2002) and several members of the Botryosphaeriaceae are the main species that have been associated with these diseases worldwide (Moller & Kasimatis, 1978; Larignon & Dubos, 1997; Graniti et al., 2000; Fischer, 2006; Larignon et al., 2009; Ú rbez-Torres, 2011).

These three diseases, described as early as the end of the 19th century, mainly attack the perennial organs of the grapevine (Vitis vinifera), leading to leaf and berry symptoms and death. As a result, grapevine trunk diseases are detrimental to the resilience of the wine-growing heritage (Larignon et al., 2009). Moreover, no grapevine taxa, either cultivated or wild, are known to be resistant to trunk diseases (Surico et al., 2006; Wagschal et al., 2008; Larignon et al., 2009). Over the past few decades, the frequency of symptoms of these diseases has increased considerably worldwide. For example, disease incidence values that were estimated over 4 years in approximately 700 French vineyards, including affected trunk disease and dead plants, showed that approximately 10% of pro- ductive plants were affected (Grosman, 2008; Grosman & Doublet, 2012). Sodium arsenite was the sole treatment that had a potential effect against these diseases, especially esca (Fussler et al., 2008; Larignon et al., 2008), but it has been prohibited, beginning in 2000, because of its toxicity both to the environment and to humans (Bisson et al., 2006; Spinosi & Fe ´votte, 2008). The lack of strategies for fighting the diseases, new pruning practices and the necessary protection of the environment could exacerbate the situation (Chiarappa, 2000; Graniti et al., 2000).

Because these pathogens have never been isolated from the leaves of infected plants, it was hypothesized that the leaf and berry symptoms are actually caused by extracel- lular compounds produced by fungi in the discoloured woody tissues of the trunk and which are then translocat- ed to the leaves through the transpiration stream (Mugnai et al., 1999). A variety of metabolites biosynthesized by these fungi have been already identified in eutypa dieback
(Renaud et al., 1989; Tey-Rulh et al., 1991; Andolfi et al., 2011), esca (Evidente et al., 2000; Tabacchi et al., 2000; Abou-Mansour et al., 2004; Bruno et al., 2007) and botryosphaeria dieback (Martos et al., 2008; Djoukeng et al., 2009, Evidente et al., 2010). The esca disease name derives from the Latin for ‘tinder’. In early 1900, the term ‘esca’ was used by grape growers in southern Italy for referring to apoplexy (Surico, 2009), probably because of the presence of rotted trunk wood noted mainly in apoplectic plants, which was in fact used as tinder. The association of apoplexy and/or rotted trunk wood with particular foliar discolorations led, with time, to the use of ‘esca’ for the latter, even in absence of apoplexy and/or rotted trunk wood. Although results of many research studies have led to esca being defined as a complex of diseases (esca disease complex), the term ‘esca’ is still commonly used to refer to most of the diseases forming the complex. The characterization of grapevine trunk diseases is crucial, not only for studying their phytotoxic properties, but also because their detection in grapevines represents a useful tool for an early diagnosis of trunk diseases (Fleurat-Lessard et al., 2010). Numerous studies have dealt with various aspects of these diseases and the fungi associated with them (i.e. epidemiology, pathogenicity and host–pathogen interactions), but the causes of symptom development remain elusive (Larignon et al., 2009; Surico, 2009; Camps et al., 2010).

Eutypa dieback, esca and botryosphaeria dieback are slow perennial diseases, the symptoms of which usually appear on mature grapevines (i.e. 7 years and older). Year to year, an unpredictable discontinuity in the expression of symptoms is a characteristic trait of these diseases (Mugnai et al., 1999; Surico et al., 2000; Wagschal et al., 2008), which can occur alone or together on the same plant.

This review presents the current knowledge of: (i) symptomatologies in trunks, leaves and berries; (ii) the characteristics of the disease-associated fungi; (iii) host–pathogen interactions; and (iv) disease management strategies. It also focuses on recently developed experimental tools which help to convey a better understanding of both host–pathogen interactions and the mechanisms involved in symptom expression.

Eutypa dieback

Fungi implicated

Eutypa lata (Rappaz, 1984) (Ascomycota, Diatrypaceae) is the causal agent of eutypa dieback (Carter, 1988), also referred to as eutypiosis, and could also be associated with processes leading to the degradation characteristics of esca (white rot) as a pioneer fungus (Larignon & Dubos, 1997). It is frequently found in vineyards that receive more than 250 mm of rainfall per year (Carter, 1988). Eutypa lata has a wide host range, occurring on more than 80 woody host species (Carter, 1991). This fungus produces perithecial stroma on diseased grapevine wood (Carter, 1988). Ascospores are released throughout the entire year (Pearson, 1980; Trese et al., 1980) and are disseminated with each rainfall >0.5 mm (Moller & Carter, 1965). Their liberation begins 2–3 h after the onset of rain and stops 24 h after the rain stops (Pearson, 1980). Ascospores penetrate the plant by infecting susceptible pruning wounds during winter dormancy. Studies of genetic variability suggest that E. lata has reproduced only in its sexual form (Péros et al., 1997).

Associated with eutypa dieback, Eutypella vitis (synonym E. aegilolinaris) was first described in Michigan (Jordan & Schilder, 2007). Other diatrypaceous species have been observed on eutypa dieback-affected plants, including Diatrype stigma, Diatrype whitmanensis, Cryptosphaeria pullmanensis and Cryptotovalsa ampelina (Trouillas & Gubler, 2010; Trouillas et al., 2010). Recently, new species have been described in Australia (Eutypella microtheca, Eutypella citricola and Diatrypella vulgaris; Trouillas et al., 2011) and in Chile (Eutypella leprosa; Díaz et al., 2011).

Disease

Symptoms are characterized by stunted shoots with shortened internodes, and small, chlorotic, cupped, tattered leaves with marginal necrosis and dead interveinal tissue (Fig. 1a,b; Moller et al., 1974). Foliar symptom expression is mainly detected during the spring. Most flowers dry before opening, and berries that develop from an infected spur position usually appear small and straggly. After infection in the pruning wounds and colonization of the trunk vascular tissues and cordon, a brown, wedge-shaped necrosis usually develops (Moller et al., 1974; Fig. 1c). The type of wood decay that is caused by E. lata is classified as a soft rot (Rudelle et al., 2005; Rolshausen et al., 2008).

Anatomical studies on the leaves of E. lata-infected grapevines showed changes in tissue ultrastructure including chloroplast degradation, lengthened thylakoids, cytoplasm lysis, bulked plastoglobules and endomembrane breakdown in severely affected leaves (Philippie et al., 1992; Valtaud, 2007). The decline of the photosynthesis system could be responsible, at least in part, for plant death. In addition, E. lata infection leads to both a decrease in leaf water content and an accumulation of abscisic acid. These changes may reduce the membrane permeability of the plant cell and, as a consequence, modify exchanges with the environment, which could intensify the dehydration of developing affected leaves (Koussa et al., 2002). The limitation of gas exchanges results in stomatal closure, higher concentrations of abscisic acid in the guard cells and effects on plant vascular tissues. Rifai et al. (2005) observed the capability of E. lata to affect polyamine metabolism, suggesting that the decline of specific free polyamines in the leaves of Eutypa-infected grapevines could be involved in the expression of foliar symptoms.

The degradation of the wood has been characterized by the death of vessel-associated cells (Rudelle et al., 2005). Analyses of naturally and artificially infected wood
revealed that non-structural (mostly stored starch) and structural (hemicellulosic) glucans are the primary targets of *E. lata* (Rudelle *et al.*, 2005; Rolshausen *et al.*, 2008). Woody tissues often contain stored starch reserves, which in grapevines are stored in xylem parenchyma cells and rays (Rudelle *et al.*, 2005). Moreover, the results from *in vitro* tests showed the complete depletion of starch reserves after 18 months of fungal activity (Rolshausen *et al.*, 2008).

A transcriptomic study on Cabernet Sauvignon leaves was performed to improve the knowledge of grapevine responses to *E. lata*. In response to the host–pathogen interactions, genes involved in carbon and amino acid metabolism were up-regulated, while several genes involved in lipid metabolism were down-regulated (Camps *et al.*, 2010). Another important part of this study identified genes that were more specifically associated with the asymptomatic phase of eutypa dieback. The most abundant genes that were regulated during the symptomless phase were associated with energy metabolism, especially with the light phase of photosynthesis (Camps *et al.*, 2010). The up-regulation of these genes suggests that the plant efficiently prevents the appearance of eutypiosis symptoms by stimulating chloroplast electron transport.

Others studies on the changes in physiological processes (e.g. the reduction of energy charge through the inhibition of photosynthesis and respiration or the

Figure 1 Typical symptoms of eutypa dieback, esca and botryosphaeria dieback in leaves and wood of Chardonnay grapevines. (a–c) Eutypa dieback; (a, b) typical symptoms of *Eutypa lata*, including stunted shoots; (c) wood cross-section showing a wedge of discoloured tissue. (d–f) Esca; (d) typical tiger-like necrosis and chlorosis; (e) apoplectic (severe) form, characterized by dieback of one or more shoots and leaf drop; (f) trunk cross-section showing white rot. (g–k) Botryosphaeria dieback; (g) yellowish-orange spots on the margins of the leaves; (k) leaf desiccation and fall accompanied by (j) desiccated fruits; (h) brown streaking under bark; (i) wood cross-section showing a grey rotted sector. All pictures were taken from Sauvignon grapevine except for h, from Cabernet-Sauvignon grapevine (This figure is available in colour online at wileyonlinelibrary.com).
decrease of assimilate uptake) showed that the dwarf shoots and leaf symptoms are caused by the presence of *Eutypa* toxins (Deswarte et al., 1996; Octave et al., 2006).

**Secondary metabolites isolated from *Eutypa lata***

*Eutypa lata* produces secondary metabolites, mainly acetylenic and heterocyclic compounds (Fig. 2). Eutypine 1,4-hydroxy-3-(3-methylbut-3-ene-1-ynyl) benzaldehyde, which is secreted by *E. lata*, possesses an unusual five-carbon acetylenic side chain. Eutypine was isolated and identified from a strain of *E. lata* (Renaud et al., 1989) and was determined to be the main phytotoxin produced by this fungus based on bioassays performed on excised leaves and leaf protoplasts (Tey-Rulh et al., 1991). Several structurally related metabolites bearing a pentenyne side chain ortho to the hydroxyl group were also isolated from *in vitro* cultures of *Eutypa* species, mainly eutypinol, siccayne, eutypinic acid, their cyclization products, the epoxidized chromanones and eutypoxide B (Fig. 2) (Renaud et al., 1989; Jiménez-Teja et al., 2006). The phytotoxicity of *E. lata* probably results from this suite of structurally related compounds, with each compound having a different level of toxicity and different molecular targets within the plant cell (Molyneux et al., 2002).

Eutypine exhibits weak acid properties and a marked lipophilic character. The toxin penetrates cells through a passive diffusion mechanism and tends to accumulate in the cytoplasm as a result of an ion-trapping mechanism that is related to the ionization state of the molecule (Amborabé et al., 2001). In grapevine cells, eutypine is metabolized into eutypinol with no protonophoric activity through enzymatic reactions (Colrat et al., 1999). It is believed that eutypine uncouples mitochondrial oxidative phosphorylation and decreases the ADP/O ratio in grapevine cells by increasing proton leaks, which it accomplishes by means of a cyclic protonophore mechanism (Deswarte et al., 1996).

Recently, it was demonstrated that a polypeptidic compound secreted by *in vitro* cultures of *E. lata* acts at various sites of plant cells through the modification of ion fluxes and the inhibition of H⁺-ATPase at the plasmalemma through the inhibition of respiration and photosynthesis, the induction of NADH oxidase and the inhibition of phenylalanine ammonia lyase (PAL) (Octave et al., 2006).

**Esca disease complex**

**Fungi implicated**

The esca disease complex commonly comprises five syndromes (Surico et al., 2008). Its main causal agents are considered to be the tracheomycotic agents *Pa. chlamydospora* (Chaetothyriales, Herpotrichiellaceae) and *Pm. aleophilum* (Diaporthales, Togniniaceae), and several basidiomycetes species (Fischer, 2006), among which the most common is *Fomitiporia mediterranea*, which was previously named *Phellinus punctatus* and *F. punctata*. In addition to *Pm. aleophilum*, several other *Phaeoacremonium* species could be involved in the aetiology of the esca disease complex (Dupont et al., 2000; Mostert et al., 2006; Essakhi et al., 2008; Gramaje et al., 2009). Moreover, *E. lata* and *Stereum hirsutum* could also play roles in the esca disease complex (Larignon & Dubos, 1997; Reisenzein et al., 2000; Armengol et al., 2001). The sexual stages of *Pa. chlamydospora* are unknown, while *Togninia minima* was identified as the teleomorph of *Pm. aleophilum* (Mostert et al., 2003). *Phaeomoniella chlamydospora* and *Pm. aleophilum* are widely distributed in many grape-growing regions worldwide (Edwards et al., 2001;
Groenewald et al., 2001; Essakhi et al., 2008; Gramaje et al., 2010), while *F. mediterranea* is especially common in Europe (Fischer, 2002). Furthermore, *Pm. aleophilum* has been isolated from a large number of woody hosts, such as *Salix* sp., *Prunus penstemonica*, *Actinidia chinensis* (Hausnet et al., 1992; Di Marco et al., 2004a) and *F. mediterranea* from *Corylus avellana*, *Olea europaea*, *Lagerstroemia indica*, *Actinidia chinensis*, *Acer negundo* (Fischer, 2002) and *Citrus* spp. (Kalomira et al., 2006) (Farr & Rossman, 2011). Fischer & Kasseneyer (2003) reported that several different fungal species have been associated with wood rot in grapevine, including *Pleurotus pulmonarius*, *Trametes hirsuta*, *Trametes versicolor*, *Fomitiporia polymorpha* (Fischer & Binder, 2004) in North America and *Fomitiporia australiensis* (Fischer et al., 2005) in Australia. These fungi have also been isolated from wood rot of grapevines without foliar symptoms (Fischer, 2006).

Because *Pa. chlamydospora*, *Pm. aleophilum* and *F. mediterranea* are considered the main causal agents of the esca complex, several studies focusing on their life cycles have been conducted. *Phaeonomoniella chlorom dorspora* and *Pm. aleophilum* are characterized by their aerial dispersal (Larignon & Dubos, 2000; Eskalen & Gubler, 2001). The spore liberation of *Pa. chlamydospora* is correlated to rainfall, while for *Pm. aleophilum* it occurs during the vegetative period without any link to rainfall (Larignon & Dubos, 2000; Eskalen & Gubler, 2001). Spores of *Pa. chlamydospora* and *Pm. aleophilum* penetrate the plant through pruning wounds (Larignon & Dubos, 2000; Eskalen et al., 2007a; Serra et al., 2008). The sources of inoculum and pycnidia for *Pa. chlamydospora* and perithecia for *Pm. aleophilum* have been observed on protected wood surfaces inside deep cracks (Edwards et al., 2001; Rooney-Latham et al., 2005). *Phaeonomoniella chlorom dorspora* and *Pm. aleophilum* can also be spread through vine propagation material (Larignon & Dubos, 2000; Fourie & Halleen, 2002; Halleen et al., 2003; Whitman et al., 2007). In nurseries, the presence of *Pa. chlamydospora* has been confirmed in hydration tanks by PCR detection analyses and on grafting tools and the substrates used for callusing (Ridgway et al., 2002; Retief et al., 2006; Edwards et al., 2007a; Aroca et al., 2009). It has also been detected in infected commercial plants (Bertelli et al., 1998; Giménez-Jaime et al., 2006).

Regarding genetic variability, *Pa. chlamydospora* populations show low genetic variability (Péros et al., 2000; Comont et al., 2010; Smithem et al., 2010). With *F. mediterranea*, genetic variations were found within a single vineyard and among different vineyards (Jamaux-Despréaux & Péros, 2003). Variation within species may be related to the geographic location of the isolates. It has been suggested that *F. mediterranea* spreads by means of airborne basidiospores and regularly outcrosses in nature. In *Pm. aleophilum*, several genotypes can be found within a single vineyard (Borie et al., 2002). These studies indicate that *F. mediterranea* and *Pm. aleophilum* reproduce sexually; therefore, basidiocarps and perithecia, respectively, may represent sources of inoculum in the field (Cortesi et al., 2000; Borie et al., 2002; Jamaux-Despréaux & Péros, 2003; Rooney-Latham et al., 2005).

### Disease

The five described syndromes of esca complex are brown wood streaking (mostly affecting rooted cuttings), Petri disease, young esca, esca and esca proper (Surico et al., 2008). *Phaeonomoniella chlamydospora* and *Pm. aleophilum* are associated with brown wood streaking, Petri disease and young esca, whereas esca (white rot occurring in the trunk and branches of mature standing vines; Fig. 1f) is caused by *F. mediterranea* and/or other basidiomycetes. Esca proper, usually encountered in mature vineyards, indicates the co-occurrence of young esca and esca on the same plant.

Symptoms associated with *Pa. chlamydospora* and *Pm. aleophilum* occur either only internally (wood symptoms), as in brown wood streaking, or both internally and externally (symptoms in the wood and on the crown), as in Petri disease and young esca. The most common wood symptoms (observable in mother vine stocks, rooted cuttings or the trunk and branches of standing vines) comprise several forms of discoloration, among which black streaking involving single or several xylem vessels and areas with darkened or brown necrosis circumscribing the pith are most commonly observed. No specific symptoms have been described in the roots (Surico et al., 2006). External symptoms of Petri disease, which affects young vines (from 1 year), include the complete cessation of growth, leaf chlorosis, loss of yield and a decline in vigour. External symptoms of young esca are characterized by spots that appear between the veins or along the edges of the leaves and that expand and become confluent to finally result in chlorotic and necrotic strips with only a narrow green stripe along the midrib (Fig. 1d). In most cases, the affected leaf finally assumes a ‘tiger stripe’ appearance (Surico et al., 2008). Characteristic spotting in the berry skin, described as ‘black measles’ in the USA, is also observed (Mugnai et al., 1999). Foliar symptoms of young esca are not directly associated with those in the wood (Surico et al., 2008). Indeed, they usually appear several years after a grapevine has become infected and the wood symptoms have already developed. Moreover, even after their first appearance, foliar symptoms do not develop systematically and cannot be predicted from year to year, indicating that several factors are probably involved in their development.

A symptom that is often observed, especially on young esca- and/or esca-affected vines, is apoplexy, which is characterized by the dieback of one or more shoots and is accompanied by leaf drop and the shrivelling and drying of fruit clusters (Mugnai et al., 1999) (Fig. 1e). Healthy leaves can dry up within a few days. Usually, this violent event occurs in midsummer, particularly when dry, hot weather follows rainfall (Mugnai et al., 1999; Surico et al., 2006). After such an event, the affected vines can resume growth in the following season or even in the
current one, but they can also ultimately die. Because of its association with young esca and/or esca, apoplexy is regarded as a severe form of these diseases (Surico et al., 2008; Letousey et al., 2010). On the basis of data obtained by many research groups worldwide, some modifications of disease terminology have recently been proposed (Surico, 2009), including: (i) the replacement of the term ‘young esca’ with ‘grapevine leaf stripe disease’ (GLSD), which would lead to an association of the term ‘esca’ only with white rot (esca) and esca proper (i.e. esca sensu Viala; Surico, 2009); and (ii) grouping the three tracheomycotic syndromes (brown wood streaking, Petri disease and grapevine leaf stripe disease) under the name of phaeotracheomycotic complex to emphasize the involvement of the same fungi (Pa. chlamydospora and/or Pm. aleophilum) in the three symptomatically different diseases.

Indeed, characterizing the impact of esca in grapevine physiology represents a key step in obtaining accurate knowledge of physiological mechanisms that lead to disease development and the appearance of symptoms. In vineyards, leaf photosynthesis is greatly altered in cases of grapevine leaf stripe disease (Petit et al., 2006). Compared to leaves of symptomless canes, foliar symptoms are associated with: (i) a decrease in CO₂ assimilation; (ii) a significant increase in intercellular CO₂ concentration; (iii) a significant drop in both the maximum fluorescence yield and the effective photosystem II quantum yield; and (iv) a reduction in chlorophyll fluorescence and photosynthesis-related gene expression in leaves were also observed 7 days before the apoplectic event (Letousey et al., 2010).

Canes of plants with symptoms reduce their carbohydrate reserves during the winter rest, whether they exhibit symptoms of GLSD or not (Petit et al., 2006). During the first year of symptom development, the decrease in CO₂ assimilation may reduce the synthesis of carbohydrate and also its export to sink organs (Calzarano et al., 2001). The lower pool of reserves might contribute to a significant decrease in plant development and vigour during the subsequent year.

Secondary metabolites isolated from esca pathogens

Several secondary metabolites have been reported from Pm. aleophilum and Pa. chlamydospora (Evidente et al., 2000; Tabacchi et al., 2000; Abou-Mansour et al., 2004; Andolfi et al., 2011) (Fig. 2). Scytalone and isosclerone, the two main naphthalenic pentaketides that have been isolated, along with related naphthoquinine compounds, are precursors that result from the secondary pathway of DHN-melanin and are found in a number of pathogens (Wheeler & Stipanovic, 1985).

Scytalone, isosclerone and pullulan, a polysaccharide polymer of maltotriose units, are produced in culture by Pm. aleophilum and Pa. chlamydospora and have been extensively studied. Their toxic effects in detached leaves have previously been reported (Bruno & Sparapano, 2006a,b; Bruno et al., 2007). It has been hypothesized that these types of metabolites may intervene in the development of the disease, although their mode of action at the cellular level has not yet been accurately determined. No research using reliable analytical methods has reported the isolated compounds in infected tissues. However, the absence of these phytotoxic compounds is not surprising considering their high chemical reactivity and their strong tendency to undergo further oxidation, reduction or enzymatic reaction in vivo.

A recent study reported a polypeptide fraction secreted by Pa. chlamydospora and Pm. aleophilum that triggered the death of grapevine 41BT cells in culture, induced the membrane depolarization of cells, induced the activation of plant secondary metabolism, predominantly anthocyanin synthesis, and acted on key enzymatic reactions that are known to participate in the elicitation process, namely NADPH oxidase and phenylalanine ammonia

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lyase (PAL). This led to the hypothesis that the toxic polypeptides of the two fungi modified the plant cell metabolism through different pathways (Luini et al., 2010).

In addition to phytotoxins, many phytopathogenic fungi secrete enzymes that degrade macromolecules of the host plant tissues. Valtaud et al. (2009a) showed that *Pm. aleophilum* possessed all of the extracellular enzyme activities implicated in the degradation of polysaccharides, such as xylanase, exo- and endo-β-1,4-glucanase and β-glucosidase. However, no ligninase activity was observed. In contrast, *Pa. chlamydospora* showed none of these enzyme activities. Chemical analysis in damaged wood fragments 6 months after inoculation with *Pm. aleophilum in vitro* showed that the fungus preferentially modified cellulose and hemicellulose, whereas it degraded lignin poorly. Oxidative enzymes are of primary importance because of their ability to catalyse the oxidation of phenols into phytotoxic quinones and to inactivate plant proteins and hormones. Laccase enzymes, predominantly produced by wood rot fungi, oxidize and decompose lignin (Lindeberg & Holm, 1995). Mugnai et al. (1999) did not find laccase activity in *Pa. chlamydospora* and *Pm. aleophilum* in culture, but they did discover it in *F. mediterranea*. In contrast, Santos et al. (2006b) detected such activity in the solid growing medium of *Pa. chlamydospora* and *Bruno & Sparapano* (2006a) induced laccase production by the addition of resveratrol to the culture medium. Finally, a 60-kDa laccase that was able to oxidize several natural phenolic and polyphenolic compounds was isolated from a culture of *F. mediterranea*, the main causal agent of white rot in grapevines (Abou-Mansour et al., 2006; Gramaje & Armengol, 2011). The impacts on secondary metabolites of these oxidative enzymes that are secreted by the successive invading fungi remain a crucial issue to be investigated.

**Botryosphaeria dieback**

**Fungi implicated**

Among the 21 different species in the Botryosphaeriaceae (Ascomycota) that are presently associated with botryosphaeria dieback (Urbez-Torres, 2011), the most common species isolated from grapevine-growing regions worldwide are *Diplodia seriata* (teleomorph *Botryosphaeria obtusa*; Shoemaker, 1964) (Cristinzio, 1978; Rovesti & Montemini, 1987; Castillo-Pando et al., 2001; Larignon et al., 2001; Phillips et al., 2007; Savocchia et al., 2007; Urbez-Torres et al., 2008), *Diplodia mutila* (teleomorph *Botryosphaeria stevensii*; Shoemaker, 1964) (Lehoczky, 1974; Taylor et al., 2005), *Neofusicoccum parvum* (Crous et al., 2006) (teleomorph *Botryosphaeria parva*; Pennycook & Samuels, 1985), *Neofusicoccum australe* (Crous et al., 2006) (teleomorph *Botryosphaeria australis*; Slippers et al., 2004a), *Neofusicoccum luteum* (Crous et al., 2006) (teleomorph *Botryosphaeria lutea*; Phillips et al., 2002), *Botryosphaeria dothidea* (Cesati & De Notaris, 1863; Slippers et al., 2004b) (anamorph *Fusicoccum aesculi*; Corda, 1829) and *Lasiodiplodia theobromae* (Griffon & Maublanc, 1909; Punithalingam, 1976) (teleomorph *Botryosphaeria rubida*na) (Phillips, 2002; Luque et al., 2009; Urbez-Torres, 2011). Among these, the first three species have been commonly isolated in France (Larignon et al., 2001; Larignon, 2010). In addition to grapevine, they infect several varieties of fruit trees, inducing a large number of decays (Slippers & Wingfield, 2007; Slippers et al., 2007; Farr & Rossman, 2011; Urbez-Torres, 2011).

Little information is available about the life cycle of Botryosphaeriaceae. Pycnidia develop on infected wood or on pruning shoots. Airborne inoculum is present, especially during rainfall (van Niekerk et al., 2010; Urbez-Torres et al., 2010a) or during overhead sprinkler irrigation (Urbez-Torres et al., 2010a). Thus, aerial inoculum was observed during the winter in California (Urbez-Torres et al., 2010a), while it was mostly detected during the vegetative period in France (Kuntzmann et al., 2009). Nevertheless, spore dissemination may occur without rainfall, suggesting that other environmental factors are also involved (van Niekerk et al., 2010; Urbez-Torres et al., 2010a).

The method these fungi use to penetrate the grapevine remains unclear, but the most obvious approach appears to be through pruning wounds in plants (Urbez-Torres & Gubler, 2009). The susceptibility of pruning wounds is highest when inoculations were conducted immediately after pruning and decreased significantly as the interval between pruning and inoculation increased (Urbez-Torres & Gubler, 2011). These fungi are also propagated by infected mother plants or during propagation processes in the nurseries (Halleen et al., 2003; Giménez-Jaime et al., 2006; Gramaje & Armengol, 2011).

**Disease**

Black dead arm (BDA) was first described in 1974 in the Tokaj grape-growing region of Hungary as being associated with *D. mutila* (Lehoczky, 1974). However, in 1978 (Cristinzio, 1978) and later (Rovesti & Montemini, 1987; Larignon et al., 2001, 2009), other Botryosphaeriaceae species, namely *D. seriata* and *N. parvum*, were also shown to be associated with the disease. A number of taxa included in the Botryosphaeriaceae family (Crous et al., 2006) have been isolated from grapevine; thus, Urbez-Torres (2011) and Urbez-Torres et al. (2012) proposed the disease name botryosphaeria dieback to include all of the symptoms caused by Botryosphaeriaceae species on grapevine. To date, at least 22 Botryosphaeriaceae species are regarded as potential wood pathogens to *V. vinifera* (Luque et al., 2005; van Niekerk et al., 2006; Damm et al., 2007; Martin & Cobos, 2007; Urbez-Torres et al., 2007, 2010b, 2012; Aroca et al., 2009; Carlucci et al., 2009; Billones et al., 2010; Urbez-Torres, 2011).
The name BDA was coined by Lehoczky (1974) to distinguish the symptomatology associated with *D. mutila* from that of dead arm disease, which is attributed to *Phomopsis viticola*. The distinctive characteristic of BDA *sensu* Lehoczky is the wood necrosis of the trunk and arms of infected vines. Moreover, foliar symptoms associated with the disease have also been reported (Lehoczky, 1974; Cristinzio, 1978; Rovesti & Montermini, 1987; Larignon *et al.*, 2001). The form of BDA described by Larignon *et al.* (2001) is characterized by particular foliar symptoms that are reminiscent of those of young esca (Surico *et al.*, 2008). That similarity has generated some controversy, as many authors have considered it difficult to distinguish between the foliar symptoms of GLSD and those of BDA *sensu* Larignon *et al.* (2001) (Lecomte *et al.*, 2006; Surico *et al.*, 2008). However, the BDA foliar symptoms described by Larignon *et al.* are characterized by some peculiar features. Yellowish-orange (white cultivars) or wine-red (red cultivars) spots develop on leaf margins and the blade (Fig. 1g) well in advance of what is generally observed for young esca, usually from May to June instead of late June or early July in the northern hemisphere. As the disease progresses, these spots merge to finally form large interveinal necroses. Another symptom reported by Larignon *et al.* as typical of that form of BDA is a brown streaking on the wood under the bark (Fig. 1h). This symptom is often associated with a grey sector of rotted wood (Fig. 1i). Similarly to the symptoms observed in young esca- and/or esca-affected vines, BDA apoplexy is characterized by the dieback of one or more shoots and leaf drop (Fig. 1j,k). Moreover, the shrivelling and drying of inflorescences or fruit clusters are also observed.

Many published studies have investigated GLSD-affected grapevines, whereas few studies on BDA are available. This dearth of reports on BDA could be explained by the fact that the distinction between the two diseases is problematic. Nevertheless, anatomical studies on leaves with BDA symptoms revealed that affected cells have fewer starch grains than healthy ones and that those in vines that exhibit young esca symptoms (Valtaud, 2007).

**Secondary metabolites isolated from botryosphaeria dieback pathogens**

The production of phytotoxic metabolites by the *Botryosphaeriaceae* species that colonize grapevine wood has also been reported (Martos *et al.*, 2008; Djoukeng *et al.*, 2009; Evidente *et al.*, 2010; Andolfi *et al.*, 2011). A bioassay-guided fractionation of culture filtrate of *D. seriata* led to the isolation of four dihydroisocoumarins, namely mellein, *cis*- and *trans*-4-hydroxymellein, and the new 4,7-dihydroxymellein (Fig. 3; Djoukeng *et al.*, 2009).

In another study, five *Botryosphaeriaceae* species, namely *F. aesculi*, *D. seriata*, *Dothiorella viticola* (Luque *et al.*, 2005), *N. parvum* and *N. luteum*, were shown to produce phytotoxic metabolites, although the metabolites were not identified (Martos *et al.*, 2008). All of these fungi produced hydrophilic high-molecular-weight phytotoxins that were identified as exopolysaccharides in *N. parvum*. Additionally, *N. luteum* and *N. parvum* produced lipophilic low-molecular-weight phytotoxins. A recent study reported the identification and biological activity of four lipophilic phytotoxins that were produced by *N. parvum*, which were identified as *cis*- and *trans*-4-hydroxymellein isosclerone and tyrosol (Fig. 3; Evidente *et al.*, 2010). The complexity of the confrontation zones between *Eutypa lata* and *D. seriata*: *o*-methylmellein (18) and the hydroxy diastereoisomers (19–20).

**Grapevine defences against trunk diseases**

The perturbation of primary metabolism, such as photosynthesis disturbance, is often associated with the induction of defence reactions. For example, a down-regulation of photosynthesis-related genes and a simultaneous up-regulation of defence-related genes have been described for various plant–pathogen interactions, e.g. *Botrytis cinerea* in tomato plantlets (Berger *et al.*, 2004) and *Pseudomonas syringae* in *Arabidopsis thaliana* (Bonfig *et al.*, 2006). Little information is available on the responses of grapevines after xylotroph pathogens attack, although this knowledge is very important for elucidating the potential defence mechanisms that are developed by the plant against the wood-colonizing fungi.

During the infection of grapevines, the degradation of hemicellulose and lignin by *E. lata* has been reported.
Polyphenols are also able to scavenge reactive oxygen species (ROS) and thus protect the plant cells from oxidative stress after pathogen attack. Other inducible defence responses are characterized by the accumulation of ‘pathogenesis-related’ (PR) proteins. A fungitoxic activity has been described for many PR proteins (van Loon et al., 2006). The expression of PR proteins was shown to be up-regulated in the leaves of grapevines affected by eutypa dieback and GLSD (Valtaud et al., 2009b; Camps et al., 2010; Letousey et al., 2010; Magnin-Robert et al., 2011; Spagnolo et al., 2012). These PR proteins include PR1 (unknown function), osmotin, thaumatin, anionic peroxidase, chitinase, β-1,3-glucanase and ribosome-inactivating proteins (PR10). Moreover, genes encoding PR proteins were differentially regulated according to the kinetics of GLSD symptom development (Valtaud et al., 2009b; Letousey et al., 2010).

Early events during plant–pathogen interactions are characterized by the oxidative burst and the production of ROS, which could play a role in the induction of defence-related gene expression. ROS produced at the site of infection could contribute to the destruction of pathogens and induce lignin synthesis in the cell walls. Reactive oxygen production is also associated with various mechanisms that regulate and protect the plant cell against oxidative stress. Glutathione S-transferase (GST) and superoxide dismutase (SOD) are two important enzymes in detoxification processes and oxidative stress resistance (Bowler et al., 1992; Marrs, 1996). In symptomless leaves prior to the appearance of the apoplectic form, GST expression was induced, while SOD was clearly repressed (Letousey et al., 2010). The repression of SOD expression in the foliar tissues of GLSD-affected grapevines might indicate a lack of oxidative stress control by SOD enzymes, which could be lethal for the plant and consequently strengthen symptom expression (Letousey et al., 2010). Cellular glutathione status is important in relaying oxidative signals (Foyer et al., 1997; May et al., 1998), and glutathione (GSH) protects plant cells against oxidative stress (Maughan & Foyer, 2006). Valtaud et al. (2009b) showed that GLSD modified glutathione metabolism in a systemic way. The glutathione pool decreased in the leaves before the appearance of visible GLSD symptoms. Simultaneously, the expression levels of three genes encoding GSH-biosynthetic enzymes were successively strongly induced in symptomless leaves and repressed in leaves with symptoms (Valtaud et al., 2009b). Three other genes involved in the redox balance in leaves of eutypa dieback-affected grapevines: peroxiredoxin, thioredoxin peroxidase and glutaredoxin, were up-regulated (Camps et al., 2010). A proteomic analysis on green stem tissue showed the up-regulation of a GST phi-class protein and the repression of a SOD protein, respectively, in stems with symptoms on apoplectic and esca proper-affected vines (Spagnolo et al., 2012). Considering the relative perturbation of the antioxidant system; ROS regulation is critical during symptom expression and could be used as stress markers for infections by grapevine trunk disease agents.

A microscopic examination of grapevine wood infected by Pa. chlamydospora showed that the fungus...
spreads slowly in the wood tissues and requires 9 months to colonize up to 25–35 cm above the site of infection (roots, 10 cm from the root collar), moving mainly along the vessels (Lorena et al., 2001). This spread appears to be related to plant defence responses, including the production of tylose and the accumulation of phenols and stilbene-like substances in the cell wall surrounding the infected cells (Lorena et al., 2001). The relatively long latency periods encountered in GLSD, botryosphaeria and eutypa dieback could be an example of the power of pre-formed and inducible defences of grapevine to restrain the propagation of the pathogens in the wood tissues. Consequently, the invader remains in a nearly dormant stage or is restricted to a small number of host cells. It produces no obvious symptoms and can only be detected through cultivation or molecular techniques (Scheck et al., 1998; Spagnolo et al., 2011).

Inducible defence responses tend to strengthen the plant cell wall, maintain the osmotic and redox balance, destroy the fungal cell walls and resist pathogen infection. However, these defence responses are unable to prevent the pathogenic infection and the expression of disease symptoms because they are often expressed too late or at insufficient levels for an effective defence response, as reported in the works cited above.

**Experimental tools: reproduction of symptoms in in vitro and field experiments**

Although grapevine trunk diseases are relatively well described under natural conditions, accurate knowledge of host–pathogen interactions poses certain problems, including: (i) determining the seasonal influence of the homogeneity of field-collected data; and (ii) distinguishing pathogen effects in grapevines from effects in response to other biotic agents in the field. To gain a better understanding of the mechanisms involved in symptom expression, it has been artificially reproduced through individual or combined inoculations of pathogenic fungi or by the use of simplified grapevine models (e.g. cuttings, grapevine vitroplants, or cultivated grapevine cells) under controlled conditions.

Eutypa dieback symptoms, including the stunting of new shoots with small cupped, chlorotic and tattered leaves, were reproduced on greenhouse cuttings infected with *E. lata* ascospores or mycelium plugs (Petzoldt et al., 1981; Péros & Berger, 1994, 1999; Sosnowski et al., 2007a) and on field-grown grapevines (Müller & Kasimatis, 1978). Eutypa dieback symptoms also appeared 7 weeks after inoculation in grapevines in vitro (Campos et al., 2010). Symptoms on green stem and in the wood were also observed after *Eutypella vitis* infection, but the virulence was weak compared to *E. lata* infection (Jordan & Schilder, 2007). A significant reduction of growth was observed in grapevines inoculated in *vitro* with either *Pa. chlamydospora* or *Phaeoacremonium angustius* (Santos et al., 2005, 2006a) and in greenhouse plants inoculated with *Pa. chlamydospora* (Chiarappa, 2000). In addition, co-culturing these fungi in *vitro* with plantlets induced symptoms in leaves (Sparapano et al., 2001a).

The inoculation of detached healthy grape berries with *Pa. chlamydospora* and *Pm. aleophilum* also led to the appearance of typical GLSD lesions (measles) within 4–5 days (Gubler et al., 2004). In addition, because these fungi were inoculated individually or in combination, several symptoms, such as wood streaking and foliar chlorosis, were shown to be commonly produced by a group of four fungi (*Pm. aleophilum, Pa. chlamydospora, E. lata* and *Pm. angustius*), while others are characteristically induced by just one class, e.g. black goo and black measles induced by ascomycetes (i.e. *Pa. chlamydospora/Pm. aleophilum*) and white rot by basidiomycetes (i.e. *F. mediterranea/S. hisutum*) (Larignon & Dubos, 1997; Sparapano et al., 2000b, 2001b). The capacity of *F. mediterranea* to induce wood rot has already been studied in the field by inoculating both adult and young healthy grapevines with *F. mediterranea* via wounds. Wood decay symptoms, including white rot, developed within 2 years of inoculation, but the first signs of wood rot (spongy wood) were observed as soon as 6 months after inoculation on both tested cultivars ( cvs Sangiovese and Italia) (Sparapano et al., 2000a). Regarding the pathogenic fungi involved in botryosphaeria dieback, some discoloration of woody tissues and canker formations are commonly observed in cuttings, detached woody shoots or field-grown grapevine shoots that have been inoculated with *D. seriata* (Castillo-Pando et al., 2001; Larignon et al., 2001; van Niekerk et al., 2004; Savochia et al., 2007). Some discoloration of woody tissues was also observed in cuttings inoculated with *D. mutila* (Taylor et al., 2005; Whitelaw-Weckert et al., 2006) and *N. pareum* (Phillips, 1998; van Niekerk et al., 2004; Luque et al., 2009; Urbéz-Torres & Gubler, 2009).

In *vitro* grapevine models (e.g. plantlets, calli and liquid-cultured cells) are also used to determine the accurate physiological or molecular changes that take place during the plant–pathogen interaction. In *vitro* cultures are excellent tools for studying host–pathogen interactions, as the organisms are grown in well-controlled conditions. Co-culturing grapevine calli with *Pa. chlamydospora, Pm. aleophilum, Pm. angustius* and *F. mediterranea* has been shown to reduce callus growth, increase plant cell lipid peroxidation, and induce browning and necrosis (Sparapano et al., 2000c, 2001a; Santos et al., 2005, 2006b; Bruno & Sparapano, 2006a). As with calli, both reductions in growth and increases in lipid peroxidation were observed in grapevine plantlet leaves in response to *Pa. chlamydospora* and *Pm. angustius* (Santos et al., 2005; Oliveira et al., 2009). Infections by GLSD fungi also reduced chlorophyll content and fluorescence in plantlet leaves (Santos et al., 2005; Oliveira et al., 2009). In parallel, a decrease in osmotic potential, loss of membrane integrity, perturbations in macronutrient accumulation (K, P, Ca, Mg) and nutritional disorders (such as reductions in total sugars, glucose and uronic acids) were observed in the leaves of *in vitro* *Pa. chlamydospora*-infected grapevine (Oliveira et al., 2009). Santos et al. (2005) showed that the fungal strain most virulent
to in vitro plants was also the most virulent to calli, revealing a similarity in the pattern of responses between cultured and plants in these grapevine genotypes. The accumulations of total and recurring phenols were analysed in calli and in the leaves of various grapevine genotypes in response to infections by Pa. chlamydospora, Pm. aleophilum and F. mediterranea. The ability to produce phenolics appeared to be correlated with a lower susceptibility to GLSD (Bruno & Sparapano, 2006a,b). Cultured grapevine cells were previously used as a model to study biochemical changes during the first stages of interaction between the plant and the pathogenic fungi. Co-culturing stages of interaction between the plant and the pathogenic fungi. Co-culturing Pa. chlamydospora with cultured cells showed the presence of a biphasic oxidative burst that was dependent on Ca$^{2+}$ influxes and was associated with NADPH oxidase and peroxidase activities (Lima, 2009). Under the same conditions, the expression of seven defence-related genes encoding the PR proteins PAL, STS and lipoxygenase was induced with a biphasic pattern. Moreover, the infection of cultured grapevine cells with Pa. chlamydospora induced the production of three phenolic compounds, namely $\varepsilon$-viniferin-2-glucoside, $\varepsilon$-viniferin-glucoside and a polymer that consisted of two $\varepsilon$-viniferin molecules (Lima, 2009).

**Disease control**

The control of esca and botryosphaeria dieback is difficult because sodium arsenite, the sole effective fungicide, was banned because carcinogenic effects in humans and high toxicity to the environment were reported (Decoin, 2001; Bisson et al., 2006; Larignon et al., 2008; Spinosi & Févotte, 2008). Consequently, a wide range of methods of control, including chemicals, biological control agents, natural molecules and sanitation methods, have been tested against grapevine trunk diseases. Despite these efforts, the effectiveness of a single method of control seems to be limited, and management strategies that combine two or more of these methods must be applied to reduce disease incidence.

Several authors have compiled all the research data that have been published until now on management and control of fungal grapevine trunk pathogens. They describe in detail the potential stages of grapevine trunk disease propagation. These potential stages should be carefully monitored in nurseries to improve the quality of the planting stock that will be delivered to grape producers (Stamp, 2001; Hunter et al., 2004; Waite & Morton, 2007; Gramaje & Armengol, 2011). In 1998, the European and Mediterranean Plant Protection Organization (OEPF/EPPO, 2008) established a standard that describes the production of pathogen-tested materials of grapevine varieties and rootstocks.

**Chemical control**

Chemical control is based on protecting pruning wounds, usually with fungicides, to avoid grapevine infection and to limit fungal expansion in the plant. Chemical treatments that often contain more than one fungicide are frequently applied to the soil (injector pole), the trunk (trunk injections) and pruning wounds (painted pastes or liquid formulations) (Table 1). However, these applications can be expensive, impractical and/or washed off by rainfall (Calzarano et al., 2004; Sosnowski et al., 2004; Rolshausen & Gubler, 2005).

Sprayed formulations are usually the most practical, but they are easily washed off by rainfall. Paintbrush applications and trunk injections are impractical and expensive, but are cost-effective when applied in high-value vineyards (Di Marco et al., 2000; Rolshausen et al., 2010). Applications of fungicides in vitro, in the greenhouse or in the field have been reported to reduce mycelial growth and/or conidial germination of grapevine pathogens. Nevertheless, their efficacy in reducing pathogen incidence is very variable and species-dependent (Bester et al., 2007; Rolshausen et al., 2010; Amponsah et al., 2012). Experiments in vitro and on rooted grapevine cuttings were performed by Bester et al. (2007), who tested efficacy of fungicide wound dressings against several Botryosphaeriaceae species. These experiments showed that tebuconazole, flusilazole, benomyl and prochloraz reduced pathogen incidence. In other experiments in vitro, Gramaje & Armengol (2011) reported an inhibition in the mycelial growth of E. lata and other Diatrypaceae species associated with grapevine trunk diseases by carbendazim, tebuconazole, prothioconazole + tebuconazole and fluzanam. Amponsah et al. (2012) tested 16 fungicides in order to determine their inhibitory effect on mycelial growth and conidial germination of N. australie, N. luteum and D. mutila; carbendazim, procymidone, iprodione, flusilazole and mancozeb were effective in all cases, but flusilazole was the most effective against pathogen recovery when some of the fungicides were tested on vineyards of 12-year-old cv. Chardonnay grapevines artificially infected by N. luteum. Other fungicides that were reported to be effective to a lesser degree in this experiment were carbendazim, tebuconazole, thiyophanate methyl, mancozeb, fenarimol and procymidone. The authors concluded that the results of in vitro and field experiments seemed to corroborate each other.

Another issue is the effectiveness of these treatments under different conditions. Rolshausen et al. (2010) tested a thiyophanate-methyl treatment (Topsin M®), a wound-sealing paste with 5% boric acid (Biopaste®), a pyraclostrobin treatment (Cabrio EG) and a cyproconazole + iodocarb treatment (Garrison®) in the field. All these treatments showed effectiveness against grapevine pathogens, despite there being variations in efficacy between species. Topsin M® was overall the most efficacious fungicide. Until recently, commercial preparations with carbendazim (Bavistin®, Solucivre®) were quite effective against E. lata in the field (Bourbos & Barboupolou, 2003; Sosnowski et al., 2005, 2008). However, in 2010 the use of carbendazim on grapevines was restricted in Australia and in Europe because of health and safety concerns (http://www.apvma.gov.au/news_media/chemicals/carbendazim.php).
### Table 1 Chemical control of grapevine trunk diseases in field

<table>
<thead>
<tr>
<th>Treatment and results</th>
<th>Esca</th>
<th>Eutypa dieback</th>
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<tr>
<td>Foliar treatment</td>
<td>Fosetyl-Al foliar treatment. Results on esca-infected vineyards have been unsatisfactory (S. Di Marco, Istituto di Biometeoro logia, Bologna, Italy, personal communication)</td>
<td>Benomyl, fenamidol, flusilazole, myclobutanil and triadimefon. Benomyl and flusilazole were the most effective (90% wound reduction) (Munkvold &amp; Marois, 1993a)</td>
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<td>Foliar treatment</td>
<td>Foliar fertilization using bioactivators and nutrients: iron-humate, microelement-humate ‘S’ activator, Ca-Mg-B solution, ‘S’ bioactivator. All of these treatments had negative effects (Calzarano et al., 2007)</td>
<td>Bavistin 50 WP (carbendazim), Ohayo 50 SC (fluazinam) and the biological product Promot (Trichoderma harzianum and T. koningii). All of the tested products were effective (i.e. reduced incidence of sections of infected wood) but in different conditions: Bavistin was applied once or twice. Ohayo was applied twice and Promot was applied twice in combination with the fungicides (Bourbos &amp; Barbopoulou, 2005)</td>
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| Foliar treatment and trunk injections | Commercial formulations of fosetyl-Al in combination with mancozeb and cymoxanil and/or copper oxychloride. In field experiments fosetyl-Al treatments reduced incidence of esca and mortality of vines (Di Marco & Osti, 2005) | Foscyl-A, flusilazole + thiabendazole, carboxin, mancozeb + carboxin, Teldor (fenhexamide) and Topas® (penconazole) were less effective. Bavistin® (carbendazim), Solucuivre® (copper and carbendazim), Garrison® (cyproconazole and iodocarb in paste) and ATCS Tree Wound Dressing (acrylic paint) were more effective. (Sosnowski et al., 2005). In field trials, benomyl (Benlate®) was effective in preventing infection, but has been withdrawn from the market. Bavistin® (carbendazim) was the most effective. Shirlan® (fluazinam), Scala® (pyrimethanil) and Cabrio® (pyraclostrobin) were less effective.

### Table 1 continued

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<td>Bavistin 50 WP (carbendazim), Ohayo 50 SC (fluazinam) and the biological product Promot (Trichoderma harzianum and T. koningii). All of the tested products were effective (i.e. reduced incidence of sections of infected wood) but in different conditions: Bavistin was applied once or twice. Ohayo was applied twice and Promot was applied twice in combination with the fungicides (Bourbos &amp; Barbopoulou, 2005)</td>
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<tr>
<td>Spray-treated</td>
<td>Benomyl (5%), flusilazole (5.5%) and biological treatments: Bacillus subtilis, Trichoderma formononetin and T. harzianum (carbendazim and T. koningii). All of the tested products were effective (i.e. reduced incidence of sections of infected wood) but in different conditions: Bavistin was applied once or twice. Ohayo was applied twice and Promot was applied twice in combination with the fungicides (Bourbos &amp; Barbopoulou, 2005)</td>
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<td>Spray-treated</td>
<td>Liquid fertilizer Britomax™, which stimulates the synthesis of phenolic compounds, alleviated foliar symptoms and increased yield was applied to leaves and trunk by spray applications. A significant yield increase was noted, but foliar symptoms were not reduced (Sosnowski et al., 2007b)</td>
<td>Bavistin 50 WP (carbendazim), Ohayo 50 SC (fluazinam) and the biological product Promot (Trichoderma harzianum and T. koningii). All of the tested products were effective (i.e. reduced incidence of sections of infected wood) but in different conditions: Bavistin was applied once or twice. Ohayo was applied twice and Promot was applied twice in combination with the fungicides (Bourbos &amp; Barbopoulou, 2005)</td>
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<td>Spray-treated</td>
<td>One trial with artificial inoculation was performed. Biological products tested: Bacillus subtilis isolate EE, T. harzianum T77 (with and without Bio-Stabiliser), Trichoseal spray and Bio-Tricho. Chemical products tested: benomyl and flusilazole. Chemical products were the most effective. Another trial with natural infection was reported. Products tested: Vinevax® (Trichoseal spray) and Eco77 (T77). Both treatments reduced incidences of E. lata and other grapevine trunk disease pathogens (Hallen et al., 2010)</td>
<td>Bavistin 50 WP (carbendazim), Ohayo 50 SC (fluazinam) and the biological product Promot (Trichoderma harzianum and T. koningii). All of the tested products were effective (i.e. reduced incidence of sections of infected wood) but in different conditions: Bavistin was applied once or twice. Ohayo was applied twice and Promot was applied twice in combination with the fungicides (Bourbos &amp; Barbopoulou, 2005)</td>
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<td>Spray-treated (liquid) and paint-treated (paste)</td>
<td>BioShield (5% boric acid + suspension of Cladosporium herbarum) and Biopaste (5% boric acid + commercial paste). Both reduced disease in field trials. Boron did not accumulate in the leaves and shoots of treated vines, but they suffered some bud failure (Rolshausen &amp; Gubler, 2005)</td>
<td>Bavistin 50 WP (carbendazim), Ohayo 50 SC (fluazinam) and the biological product Promot (Trichoderma harzianum and T. koningii). All of the tested products were effective (i.e. reduced incidence of sections of infected wood) but in different conditions: Bavistin was applied once or twice. Ohayo was applied twice and Promot was applied twice in combination with the fungicides (Bourbos &amp; Barbopoulou, 2005)</td>
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<td>Paint-treated</td>
<td>Fungazor® (imazalil sulphate), Scala® (pyrimethanil), Cabrio® (pyraclostrobin), Bayfidan® (triadimenol), Teldor® (fenhexamide) and Topas® (penconazole) were less effective. Bavistin® (carbendazim), Solucuivre® (copper and carbendazim), Garrison® (cyproconazole and iodocarb in paste) and ATCS Tree Wound Dressing (acrylic paint) were more effective. (Sosnowski et al., 2005). In field trials, benomyl (Benlate®) was effective in preventing infection, but has been withdrawn from the market. Bavistin® (carbendazim) was the most effective. Shirlan® (fluazinam), Scala® (pyrimethanil) and Cabrio® (pyraclostrobin) were less effective.</td>
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<td>Spray-treated</td>
<td>Thiophanate-methyl and myclobutanil. Applied on grapevine pruning wounds was effective against Phaeomoniella chlamydosporia and Phaeomoniella chlamydosporia. Myclobutanil was also effective against E. lata (Herche, 2009)</td>
<td>Bavistin 50 WP (carbendazim), Ohayo 50 SC (fluazinam) and the biological product Promot (Trichoderma harzianum and T. koningii). All of the tested products were effective (i.e. reduced incidence of sections of infected wood) but in different conditions: Bavistin was applied once or twice. Ohayo was applied twice and Promot was applied twice in combination with the fungicides (Bourbos &amp; Barbopoulou, 2005)</td>
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<td>Trunk injections</td>
<td>Propiconazole, difenoconazole and the elicitor 2-hydroxybenzoic acid. Triazole fungicides had phytotoxic effects. No treatment had a sustaining effect. Results were unsatisfactory (Darrieutort &amp; Lecomte, 2007)</td>
<td>Bavistin 50 WP (carbendazim), Ohayo 50 SC (fluazinam) and the biological product Promot (Trichoderma harzianum and T. koningii). All of the tested products were effective (i.e. reduced incidence of sections of infected wood) but in different conditions: Bavistin was applied once or twice. Ohayo was applied twice and Promot was applied twice in combination with the fungicides (Bourbos &amp; Barbopoulou, 2005)</td>
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<tr>
<td>Botryosphaeria dieback</td>
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<td>Not specified</td>
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<tr>
<td>Chitosan was applied to control Botryosphaeraceae fungi and Phomopsis viticola.</td>
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<tr>
<td>Effectiveness was compared with that of the conventional fungicides azoxystrobin and</td>
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<td>pyraclostrobin + metiram used to control dead arm-like symptoms under vineyard</td>
</tr>
<tr>
<td>conditions (Rego et al., 2010)</td>
</tr>
<tr>
<td>Esca, eutypa dieback and botryosphaeria dieback</td>
</tr>
<tr>
<td>Grapevine rootstock and scion cuttings soaked in a product</td>
</tr>
<tr>
<td>Several products were tested: Trichoflow-T (Trichoderma), Bio-Steriliser (hydrogen</td>
</tr>
<tr>
<td>peroxide) and Chinosol (8-hydroxyquinoline sulphate). Results were inconsistent.</td>
</tr>
<tr>
<td>Benomyl, Sporekill (didecyltrimethylammonium chloride formulation) and Captan were</td>
</tr>
<tr>
<td>the best treatments (Fourie &amp; Halleen, 2006)</td>
</tr>
<tr>
<td>Painted-treated or spray-treated</td>
</tr>
<tr>
<td>Fungicides: 1% Cabrio EG (pyraclostrobin), 1% Topsin M (thiophanate-methyl), Biopaste</td>
</tr>
<tr>
<td>(5% boric acid in a polyvinyl paste) and Garrison (cyproconazole). Inefficient control</td>
</tr>
<tr>
<td>of the entire spectrum of pathogens was reported. Topsin M was overall the most</td>
</tr>
<tr>
<td>efficacious product (Rolshausen et al., 2010)</td>
</tr>
<tr>
<td>Painted-treated or spray-treated</td>
</tr>
<tr>
<td>Several fungicides and Vinevax® (Trichoderma spp.) tested: Folicur® (tebuconazole),</td>
</tr>
<tr>
<td>Shirlan® (fluazinam), Bavistin® (carbendazim) were more effective against</td>
</tr>
<tr>
<td>Botryosphaeraceae and E. lata (Pitt et al., 2010) than others</td>
</tr>
</tbody>
</table>

Table 1 shows other chemical products tested in the field for control of grapevine trunk diseases. This table regroups some treatments, their application type and field results. Some authors reported efficacy of the fungicide benomyl (Benlate®) in preventing and reducing incidences of fungal grapevine trunk diseases. However, this product was withdrawn from the market because of its toxicity and possible carcinogenic effects (Halleen & Fourie, 2005; Fourie & Halleen, 2006; Sosnowski et al., 2008).

Other products that can be effective treatments for reducing disease incidence are based on tebuconazole (Folicur, BacSeal, Greeenseal®), combinations of fosetyl-Al with otherfungicides, cyproconazol (Garrison®), formulations of didecyltrimethylammonium chloride (Sporekill®), N-trichloromethylthio-cyclohexene-1,2-dicarboximide (Captan®) and flusilazole. Nevertheless, their success depends on several factors, such as the mode and the number of applications on grapevines, the persistence of the product and the species of fungi treated (Di Marco & Osti, 2005; Halleen & Fourie, 2005; Sosnowski et al., 2005; Fourie & Halleen, 2006; Pitt et al., 2010; Rolshausen et al., 2010).

Control with biological agents and natural molecules

Trichoderma species have been tested to protect cut pruning wounds against pathogens of esca, BDA and eutypa dieback (Hunt et al., 2001; Di Marco et al., 2004b; John et al., 2004). As shown in Table 2, Trichoderma-based treatments have decreased incidence of fungi involved in grapevine trunk diseases when applied in vitro or in nurseries. To extend the effect of protection of Trichoderma spp., healthy vines should be inoculated with these fungi to colonize the woody tissues of the cordon and trunk to provide a ‘vaccination effect’ against pathogens. This was demonstrated by John et al. (2001), who found that Trichoderma harzianum AG1 from Vinevax® (a product registered as a wound protectant for eutypa dieback) can live in association with the pith parenchyma cells of healthy vine tissues (John et al., 2001; Hunt, 2004). Pitt et al. (2010) reported that Vinevax® reduced the incidence of colonization of D. seriata on 1-year-old canes of standing vines. The effectiveness of protection based on Trichoderma spp. treatments depends on the ability of these fungi to colonize grapevine pruning wounds (John et al., 2008). They usually need a period of time for a complete colonization, during which the pruned grapevine is susceptible to infections and/or to washing off by rainfall. However, these Trichoderma-based approaches still require more tests in the field in order to be accurately evaluated and could possibly be optimized by a combination of other management strategies (such as combination with other biological or chemical products, remedial surgery, reducing the number and size of pruning wounds and application of sanitation methods).

Other biological agents (e.g. Bacillus subtilis, Fusarium lateritium, Erwina herbicola, Cladosporium herbarum, Aureobasidium pullulans and Rhodotorula rubra) and natural molecules (e.g. chitosan and cysteine) have also been reported to be effective against grapevine trunk disease agents, alone or in combination with fungicides (Tables 1 & 2), although some of them have only been tested in vitro or in nurseries.

Sanitation methods

For many years, sanitation measures have remained the most widely used approach to controlling the spread of trunk diseases in the vineyard. Quality of planting material, disinfection of nursery propagating materials and application of hot water treatment (HWT) are crucial for obtaining commercial plants in good sanitary conditions. HWT is generally performed at 50°C for 30 min, but it is stressful for the plant; if not applied correctly, it can result
Table 2: Some biological agents reported in the literature for combating grapevine trunk diseases

<table>
<thead>
<tr>
<th>Systems</th>
<th>Name of treatment and results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esca, eutypa dieback, botryosphaeria dieback</td>
<td><strong>In vitro</strong></td>
</tr>
<tr>
<td></td>
<td><strong>In nurseries</strong></td>
</tr>
<tr>
<td></td>
<td><strong>In field</strong></td>
</tr>
<tr>
<td>Greenhouse</td>
<td>Cysteine Antifungal action on Eutypa lata (complete fungal inhibition at 10 μM) was observed, but with a lower efficiency against fungal species associated with other grapevine diseases (esca, black dead arm) (Octave et al., 2005)</td>
</tr>
<tr>
<td></td>
<td><strong>In vitro and nurseries</strong></td>
</tr>
<tr>
<td>Eutypa dieback</td>
<td><strong>In field</strong></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>In vitro</strong></td>
</tr>
<tr>
<td></td>
<td><strong>In field</strong></td>
</tr>
<tr>
<td></td>
<td><strong>In vitro</strong></td>
</tr>
</tbody>
</table>

in the loss of the plant material. *Vitis vinifera* varieties have different degrees of sensitivity to HWT. For example, in decreasing order of sensitivity, Pinot Noir is more sensitive than Chardonnay, Merlot and Riesling (moderately sensitive), Paulsen (sensitive) and Cabernet Sauvignon (least sensitive) (Waite et al., 2001; Crocker et al., 2002). Moreover, the range of temperatures used depends on the pathogens that need to be controlled. Temperatures of 45–47°C have been reported to eliminate *Pa. chlamydospora*, while temperatures of 51–53°C are necessary to eliminate pathogens more resistant than the Petri disease ones. Two different HWTs can also be performed: one at 54°C for 5 min to control external pests and pathogens and another at 50°C for 30–45 min to control internal pests and pathogens (Waite & Morton, 2007; Gramaje et al., 2009).

Double pruning or pre-pruning is favoured by growers to speed up final pruning and to reduce disease incidence in spur-pruned vineyards (Weber et al., 2007). Sanitation methods are often complemented with the protection of pruning wounds from frost or biotic attack by the application of fungicides, biological formulations or both in rotation. The infected parts of a plant and the infected dead wood from soil should also be removed to lower inoculum loads in vineyards (Carter, 1991; Di Marco et al., 2000).
All of the above-described treatments can lose effectiveness as a result of factors such as stress in extreme climatic conditions that could predispose the vines to an infection. For example, warm and rainy summers favour the expression of GLSD and BDA symptoms, while hot summers, strong winds and drought favour the apoplectic form of GLSD (Surico et al., 2000). Other factors include age, cultivar susceptibility (Table 3) and the stage and degree of the infection (Boyer, 1995; Di Marco et al., 2000). Although Table 3 shows different degrees of susceptibility of some grapevine cultivars, this classification can vary with region and year, so is not absolute (Mimia-gue & Le Gall, 1994). Moreover, the costs of hand/mechanical pruning, double pruning and the small number of registered products with their different ranges of action against pathogens can be expensive in low-value vineyards. Thus, multiple factors contribute to the fact that it is not possible to control grapevine trunk diseases effectively.

Conclusions

Over the past few decades, the incidence of grapevine trunk diseases, eutypa dieback, esca and botryosphaeria dieback has increased considerably worldwide. In 1999, the International Council on Grapevine Trunk Disease (ICGTD) was created to facilitate the exchange of useful data on pathogen identification, detection, host–pathogen interaction, epidemiology and disease management concerning grapevine trunk diseases.

In the research community, there is good overall knowledge of the symptomatologies in trunk, leaves and berries for eutypa dieback, esca and botryosphaeria dieback. The characteristics of the fungi associated with these diseases are also well documented. Host–pathogen interactions, especially grapevine defences against trunk diseases, have been described under natural conditions and by the use of simplified grapevine models under controlled conditions. Regarding host–pathogen interactions, the general response of grapevine organs affected by trunk diseases is characterized by a strong perturbation of primary metabolism associated with an induction of stress/defence reactions. The latter has been observed in foliar or lignified organs of grapevines infected by the fungal agents, but no scientific work has reported the response at the whole-plant level. Most knowledge concerns leaves and green stems, where the presence of the pathogenic fungi has not been reported. No hypothetical relationships have yet been proposed for the following aspects of grapevine–fungus interactions: the alteration of photosynthesis or gas exchange, the induction of detoxification system, the stimulation of defence response and the presence of fungal toxins. Apart from the accumulation of phenolic compounds and starch depletion in the wood, there is generally a lack of knowledge concerning the response of functional grapevine wood to trunk diseases. As grapevine trunk disease agents are lignicolous, particular attention must be paid to the responses of the infected woody tissues. In the future, bioinformatic analysis might be useful for comparing the expression of various sets of genes in infected woody tissues, including (i) biotic and abiotic stress-related genes involved in general plant response to pathogen infection, (ii) plant primary metabolism genes, and (iii) fungal genes required for pathogenicity. The combination of data on plant responses and fungal activity in compatible interactions could give important information about the mechanisms developed by the fungi to colonize grapevine and the protective responses induced by grapevine to limit fungal progression. Such work presents difficulties because grapevine is a perennial plant cultivated all around the world and in various environmental conditions. A priority is probably to optimize and validate a simplified model of artificial inoculation of grapevines under controlled conditions. With such a tool, understanding of the interactions between grapevine and trunk disease agents could progress, and such a model may represent a first step towards testing management solutions against these diseases.

Attempts to control these fungal diseases are currently based on the employment of biological agents, natural molecules, chemical compounds and sanitation methods, used alone or in combination. Nevertheless, they are not

<table>
<thead>
<tr>
<th>Disease</th>
<th>Susceptibility</th>
<th>Cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow form of esca (Graniti et al., 2000)</td>
<td>Susceptible</td>
<td>Cabernet Sauvignon, Cinsaut, Mourvèdre, Sauvignon blanc, Trousseau, Ugni blanc</td>
</tr>
<tr>
<td>Botryosphaeria dieback (Larignon &amp; Dubos, 2001)</td>
<td>Moderately susceptible</td>
<td>Carignane, Merlot, Pinot noir, Roussanne</td>
</tr>
<tr>
<td>Eutypa dieback (Dubos, 1999)</td>
<td>Susceptible</td>
<td>Cabernet franc, Cabernet Sauvignon, Sauvignon blanc</td>
</tr>
<tr>
<td></td>
<td>Moderately susceptible</td>
<td>Merlot</td>
</tr>
<tr>
<td></td>
<td>Highly susceptible</td>
<td>Cabernet Sauvignon, Chasselas, Chenin, Cinsaut, Mauzac, Muscadelle, Nérette, Sauvignon, Ugni blanc</td>
</tr>
<tr>
<td></td>
<td>Susceptible</td>
<td>Alicante Bouschet, Chardonnay, Chenin, Cinsaut, Gewürztraminer, Juraçon</td>
</tr>
<tr>
<td></td>
<td>Moderately susceptible</td>
<td>Cabernet franc, Carignane, Colombard, Duras, Gamay, Malbec, Mourvèdre, Pinot Meunier, Portugais bleu</td>
</tr>
<tr>
<td></td>
<td>Tolerant</td>
<td>Algolè, Merlot, Sémillon, Sylvaner, Groslot, Petit Verdot</td>
</tr>
</tbody>
</table>
yet completely effective. Therefore, control strategies are urgently needed to prevent and/or reduce incidence of grapevine trunk diseases, and worldwide, researchers are working to find means to eradicate this significant problem for the industry.

In conclusion, despite the fact that the relationship between wood necrosis and the presence of several fungi is well documented, the causes of the development of the typical foliar symptoms are still elusive. Fungal extracellular compounds, changes in vine behaviour, climate or microbiological equilibrium, and the presence of undiagnosed pathogens, are all thought to influence the expression of disease symptoms and remain to be investigated in depth.

Acknowledgements
This research was financed by the national programme CPER (Contract Project État-Région) of the Champagne-Ardenne region, the CASDAR programme (Compte d’Affectation Spéciale au Développement Agricole et Rural) and the Alsace region.

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