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# Grapevine Trunk Diseases: A Review of Fifteen Years of Trials for Their Control with Chemicals and Biocontrol Agents

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Grapevine trunk diseases (GTDs) are a group of diseases of grapevine caused by several fungal pathogens (Table 1) that live in and colonize the wood of the perennial organs causing wood necrosis, wood discoloration, vascular infections, and white decays (Bertsch et al. 2013; Mugnai et al. 1999). Affected vines show, externally, a general and progressive decline (delayed budburst, dead buds, dieback, stunted development, chlorosis, apoplexy, etc.) often associated with specific foliar symptoms according to the different diseases, that initially can cause loss of productivity and eventually death of the vine (Fig. 1).

One of the oldest of these diseases, known as “Esca,” was the white rot caused by basidiomycetes, to which several symptoms in the crown had been linked. In the early 1990s, this was shown to be just one manifestation of a larger problem, a complex of different diseases known as the Esca complex. Many actors are involved: different pathogens, causing different wood symptoms and interacting with the vine, the environment, and each other in different and still not clear ways (Box 1). These different diseases overlapping in the same vine or developing at different stages of the vine life were soon recognized as forming one of the most important problems in grapevine growing areas. Due to the various names often used in the literature during these 15 years, here we use “Esca complex” to generally indicate the target pathogens of the tests, when not differently specified and addressing to the relative papers for further and more specific information.

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\*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary file is published online.

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Since the 1970s, *Eutypa dieback* (formerly *Eutypa dead arm*) has been recognized as causing damage in some regions, especially in Australia, France, and California (Carter 1991; Carter and Price 1977; Munkvold et al. 1994; Péros and Berger 1994). When attention and research on GTDs and on their agents increased (Chiappara 2000), the importance of other canker agents, causing black dead arm (BDA, caused by *Diplodia mutila*) or *Botryosphaeria dieback*, became widely recognized (Phillips 2002; Urbez-Torres 2011). Nowadays, GTDs are seen as a serious threat to viticulture worldwide. A recent International Organisation of Vine and Wine (OIV) publication reported that in Italy, depending on the cultivar, on 15- to 18-year-old plants, incidence of GTDs ranged from 8 to 19% and around 10% in Spain. In France alone, approximately 13% of French vineyards are unproductive, with losses estimated at around €1 billion in 2014. In California, losses of at least US \$260 million per year have been attributed to trunk diseases, while in Australia, mainly *Eutypa dieback* is responsible for damages of AU \$8.3 billion to the wine industry (Fontaine et al. 2016a). Different causes could be considered responsible for the increasing incidence of GTDs (Lecomte et al. 2012; Surico et al. 2004) and among these, changes in cultural practices are surely a major inciting factor (Graniti et al. 2000): the lack of effective strategies and means to control these diseases surely contributed to their becoming widespread in all vine-growing areas.

In some countries in Europe, such as France, Portugal, and Spain, the sole product available to reduce the foliar symptoms within the Esca complex diseases (GLSD, Esca proper) was, for some decades, sodium arsenite. Despite this, incidence of the disease increased throughout Europe starting from the end of the 1980s. After the definitive ban of sodium arsenite in Europe in 2003, due to its high risks for humans and the environment (Larignon et al. 2008; Spinosi et al. 2009), no other control measure or any active ingredient (a.i.) was available to reduce the impact of the Esca complex. Meanwhile, the control of *Eutypa* and *Botryosphaeria diebacks* was based on pruning wound protection by treatments with carbendazim and benomyl (Magarey and Carter 1986; Ramsdell 1995). The banning of the two latter substances in most viticultural areas, especially in Europe, also put these two GTDs in the same situation as Esca complex. During the last decades, a large portion of research efforts was devoted to

selecting a.i.s and biological control agents (BCAs) able to limit or control GTDs by reducing wood infections.

This review does not cover the complexity of control strategies for GTDs, but aims to explain the main achievements of the scientific community worldwide in the search for products, i.e., a.i.s and BCAs, to control GTDs. After a short introduction on the problematic aspects of GTD control, the first part will be focused on a.i.s (organic and inorganic, natural or synthetic), and the second part on the trials with BCAs tested in the last 15 years. For each one, the methodology used and the results obtained against *Botryosphaeria* dieback, Esca complex, and *Eutypa* dieback in both vineyard and nursery are reported (Fig. 2). In the meantime, their efficacy and their sustainability for the winegrowers and the environment are discussed.

### The Complexity of GTDs in Brief

The control of GTDs represents a big challenge for winegrowers, nurserymen, technicians, and scientists, mainly because of their complexity compared with other grapevine diseases such as powdery and downy mildew. In field trials, one of the intriguing and problematic aspects of GTDs is related to their undetermined latency period (asymptomatic status). All these diseases are linked to several wood pathogens able to infect the vines through wounds in the nursery or through pruning wounds in the field, causing wood discoloration and necrosis (Bertsch et al. 2013; Mugnai et al. 1999). Some of them also

cause visible foliar symptoms. When the first external foliar symptoms occur, the wood may already be severely compromised (Calzarano and Di Marco 2007), giving few chances for viticulturists to reduce disease impact in the vineyard. This problematic aspect is confounded by the “erratic” behavior of the foliar symptoms displayed, especially for diseases in the Esca complex (GLSD, Esca proper). In the same vine, symptoms may appear in year *n* and not in year *n*+1, under the influence of environmental, climatic, and cultural factors (Marchi et al. 2006; Murolo and Romanazzi 2014; Sosnowski et al. 2011; Van Niekerk et al. 2011a) and that can lead to an underestimation of the real incidence in the vineyard in any given year. Latent infections are also dangerous in the propagation process since infected asymptomatic cuttings can transfer GTD pathogens by cross-contamination during the several steps of plant production (hydration, cold storage, grafting, callusing, etc.). If these infections are not controlled, an unsuspected spread of infected plants first in the nursery and then in the vineyard could occur (Aroca et al. 2010; Gramaje and Armengol 2011; Gramaje and Di Marco 2015).

The studies on GTDs conducted in several wine-growing areas worldwide have shown the large number of fungal genera and species associated with the diseases in the Esca complex, *Eutypa* and *Botryosphaeria* dieback, which are often different based on climate and geographical areas (Fischer 2006; Mostert et al. 2006; Van Niekerk et al. 2011a). These studies have also demonstrated how

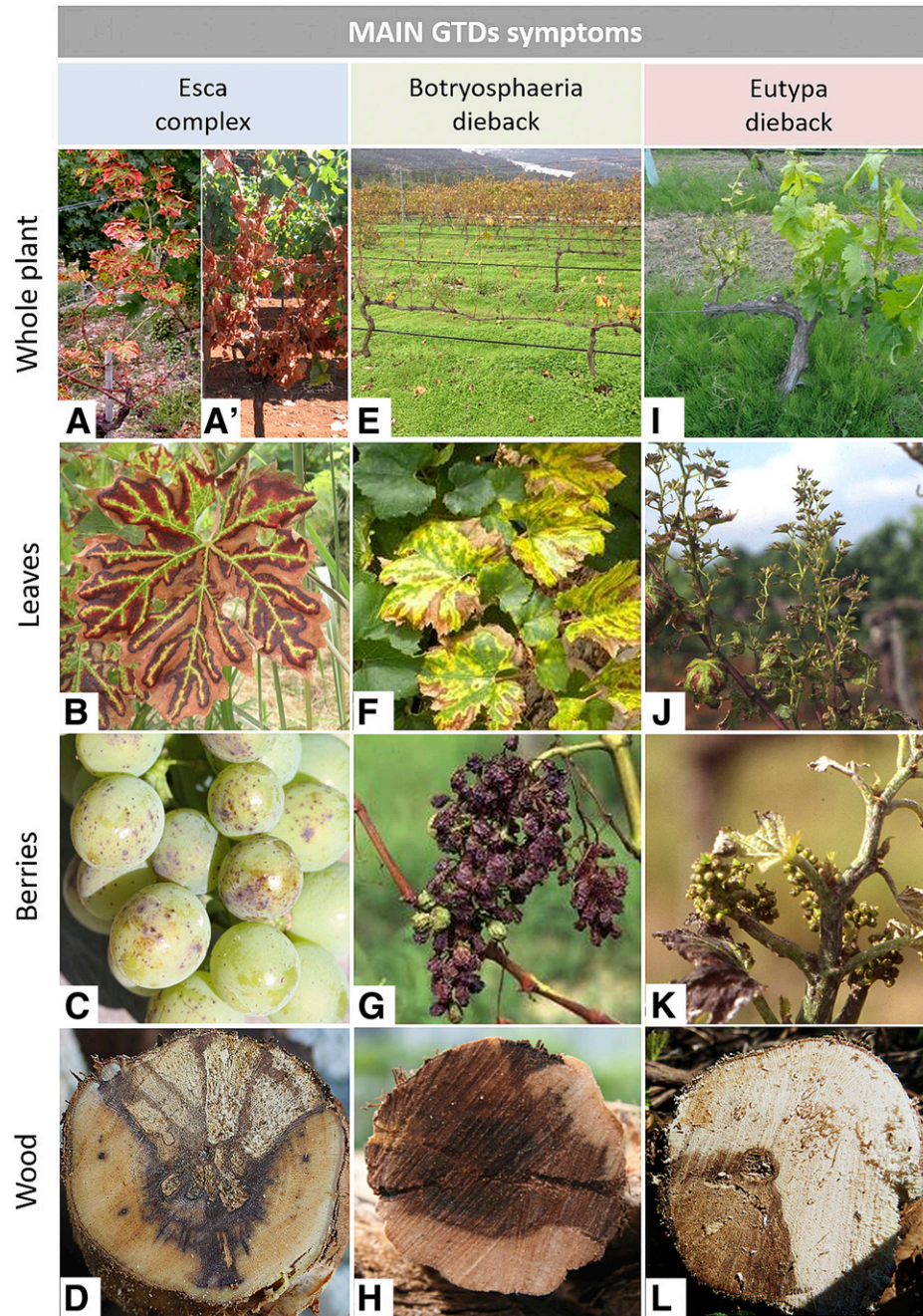
**Table 1.** Fungal wood pathogens isolated from wood showing discoloration, necrosis, or decays of grapevines affected by major grapevine trunk diseases (Esca complex diseases, *Botryosphaeria* dieback, or *Eutypa* dieback). Species that proved pathogenic, at least as discoloration or necrosis agents, by artificial inoculations are indicated in bold (revised and adapted from Carlucci et al. 2015; Cloete et al. 2014; Úrbez-Torres 2014).

Family	Genus	Species
Botryosphaeriaceae	<i>Botryosphaeria</i>	<b><i>B. dothidea</i></b>
	<i>Diplodia</i>	<b><i>D. corticola</i>, <i>D. mutila</i>, <i>D. seriata</i></b>
	<i>Dothiorella</i>	<b><i>D. americana</i>, <i>D. iberica</i>, <i>D. sarmentorum</i>, <i>D. vidmadera</i></b>
	<i>Lasidiplodia</i>	<b><i>L. citricola</i>, <i>L. crassipora</i>, <i>L. exigua</i>, <i>L. mediterranea</i>, <i>L. missouriana</i>, <i>L. theobromae</i>, <i>L. viticola</i></b>
	<i>Neofusicoccum</i>	<b><i>N. australe</i>, <i>N. luteum</i>, <i>N. macroclavatum</i>, <i>N. mediterraneum</i>, <i>N. parvum</i>, <i>N. ribis</i>, <i>N. viticlavatum</i>, <i>N. vitifusiforme</i></b>
Phaeomoniellaceae	<i>Phaeobotryosphaeria</i>	<b><i>P. porosa</i></b>
	<i>Spencermartinsia</i>	<b><i>S. viticola</i></b>
	<i>Phaeomoniella</i>	<b><i>P. chlamydspora</i></b>
Togniniaceae	<i>Phaeoacremonium</i>	<b><i>P. minimum</i>, <i>P. angustius</i>, <i>P. alvesii</i>, <i>P. argentiniense</i>, <i>P. armeniacum</i>, <i>P. australiense</i>, <i>P. austroafricanum</i>, <i>P. canadense</i>, <i>P. cinereum</i>, <i>P. croatiense</i>, <i>P. globosum</i>, <i>P. hispanicum</i>, <i>P. hungaricum</i>, <i>P. inflatipes</i>, <i>P. italicum</i>, <i>P. iranianum</i>, <i>P. krajdenui</i>, <i>P. mertoniae</i>, <i>P. occidentale</i>, <i>P. roseum</i>, <i>P. scolyti</i>, <i>P. sicilianum</i>, <i>P. tuscanum</i>, <i>P. venezuelense</i>, <i>P. viticola</i></b>
Helotiales	<i>Cadophora</i>	<b><i>C. luteo-olivacea</i>, <i>C. melinii</i></b>
Hymenochaetaceae	<i>Fomitiporia</i>	<b><i>F. australiensis</i>, <i>F. capensis</i>, <i>F. mediterranea</i>, <i>F. polymorpha</i>, <i>F. punctata</i></b>
	<i>Fomitiporiella</i>	<b><i>F. vitis</i></b>
Stereaceae	<i>Inocutis</i>	<b><i>I. jamaicensis</i></b>
	<i>Phellinus</i>	<b><i>P. ignarius</i></b>
	<i>Stereum</i>	<b><i>S. hirsutum</i></b>
Diatrypaceae	<i>Eutypa</i>	<b><i>E. lata</i>, <i>E. laevata</i>, <i>E. leptoplaca</i>, <i>Eutypa</i> sp., <i>E. citricola</i></b>
	<i>Eutypella</i>	<b><i>E. cryptovalsoides</i>, <i>E. microtheca</i>, <i>E. vitis</i>, <i>Eutypella</i> spp.</b>
	<i>Cryptosphaeria</i>	<b><i>C. lignyota</i>, <i>C. pullmanensis</i></b>
	<i>Cryptovalsa</i>	<b><i>C. amplelina</i>, <i>C. rabenhorstii</i></b>
	<i>Diatrype</i>	<b><i>D. brunneospora</i>, <i>D. oregonensis</i>, <i>D. stigma</i>, <i>D. whitmanensis</i>, <i>Diatrype</i> sp.</b>
Pleurostomataceae	<i>Diatrypella</i>	<b><i>D. verrucaeformis</i>, <i>D. vulgaris</i></b>
	<i>Pleurostomophora</i>	<b><i>P. richardsiae</i></b>
Diaporthaceae	<i>Diaportha</i>	<b><i>D. amplelina</i>, <i>D. eres</i></b>



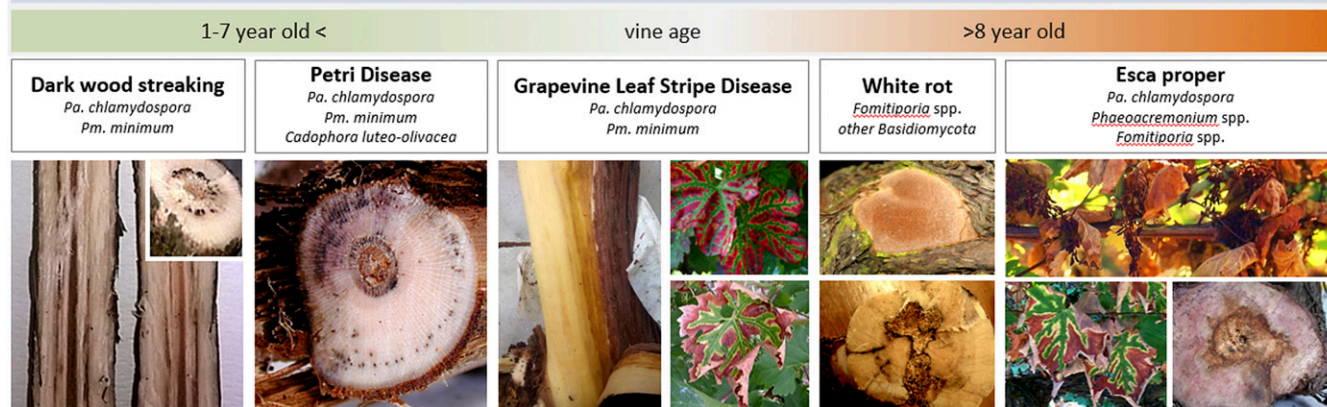
their life cycles are strictly connected with some cultural practices typical of viticulture, especially dormant pruning. Pruning wounds are the main point of entry for GTD pathogens into the vines. Depending on the pathogen, wounds can remain susceptible to

infection by GTD pathogens for up to 2 to 4 months (Eskalen et al. 2007a; Rolshausen et al. 2010; Serra et al. 2008; Van Niekerk et al. 2011b). As the wood of diseased vines can be simultaneously infected by various pathogens typically associated with different



**Fig. 1.** Symptomatology of the main grapevine trunk diseases (GTDs) occurring in adult vineyards. The different diseases included in the Esca complex have different symptoms but some wood symptoms in common. In the vineyard, grapevine leaf stripe disease (GLSD) or Esca proper symptoms are visible in summer and the whole vine can show: **A**, leaf stripe symptoms; **A'**, sudden wilting of the whole plant or part of the plant, i.e., apoplectic symptoms or apoplexy, a nonspecific but frequent symptom in older vineyards. **B**, A detail of the typical tiger-stripe pattern of symptomatic leaves. The external symptoms of GLSD can also include minute black spots called "black measles" on berries (**C**). The wood of vines affected by GLSD shows black spots, vascular streaking, discoloration, necrosis, and especially in vines more than 8 years old, can also show white decay (giving the complete Esca proper syndrome including all wood and foliage symptoms associated with Esca complex) (**D**). The first symptoms of Botryosphaeria dieback (often confused with black dead arm [BDA] by *Diplodia mutila*, and synonym of Botryosphaeria canker) are visible from budburst in spring and consist of dead spurs/buds and in a stunted or delayed growth (**E**). It can also appear in a severe form similar to Esca apoplexy. Foliar symptoms, when present, can be similar to chlorosis or to Esca (**F**). Botryosphaeriaceous fungi could determine also bunch rot on grapes (**G**). The wood of Botryosphaeria-infected plants shows the presence of brown necrotic sectors, typically arc-shaped and sectorial, wedge shaped necrosis, (**H**). The external symptoms of Eutypa dieback (formerly Eutypiose) appear early in the vegetative season, with stunted shoots with shortened internodes (forming witch's broom symptoms) (**I**). Leaves are small, chlorotic, cupped, and deformed (**J**); the grapes on the affected canes do not form at all or are small, poorly developed, and straggly (**K**). The internal wood of Eutypa-infected vines shows wedge-shaped grayish necrotic sectors, indistinguishable from Botryosphaeria dieback cankers (**L**). Simultaneous presence of pathogens of different GTDs in the same vine can result in overlapping symptoms. (Esca pictures: **A**, **B** courtesy of Institute of Agriculture and Tourism, Poreč, Croatia; **A'** courtesy of Maurizio Gily, Italy; **C** courtesy of DRL Rheinpfalz, Germany; **D** courtesy of Plant pathology sector SAF dept., University of Palermo, Italy. Botryosphaeria dieback pictures: **E**, courtesy of Feuga, Spain; **F** and **G**, courtesy of P. Larignon, France; **H** courtesy of Plant pathology sector SAF dept., University of Palermo, Italy. Eutypa dieback pictures: **I**, courtesy of ADVID, Portugal; **J**, **K** courtesy of P. Larignon, France; **L**, courtesy of Eger Food and Wine Research Center, Hungary.)

## The Esca Complex



### Box 1

#### The Esca complex of diseases.

The studies on Esca carried out from the 1980s have led to a deep revision of this trunk disease, on pathogens involved, and on symptom expression, in particular. Since then, researchers collected information and clues to explain the complexity not only in the internal symptoms but also in the association of the pathogens to different external symptoms, as only the wood symptoms could be reproduced by artificial infections, while the external symptoms could not be fully reproduced. The interpretation of the data collected led to an evolution in the description of the disease and a succession of different proposals. The more recent one revealed Esca as a “complex of diseases,” characterized by different diseases (in particular wood decay and a vascular disease) and different syndromes according to the stage of the vine life, to the type of wood and foliar symptoms, and/or to the pathogens infecting and acting into the vine. The old denomination of “Esca” as a wood white decay causing “apoplexy” (sudden wilting of the vine) and typical foliar symptoms is replaced by five different diseases/syndromes (dark wood streaking, Petri disease, grapevine leaf stripe disease [GLSD], white rot, and Esca proper) grouped under the name of “Esca complex.” These syndromes differ based on the age of the symptomatic vines and the pathogens involved (in white decay and Esca proper, basidiomycete decay agents are involved, while in all the others, vascular ascomycete species are the common factor, as *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., and more recently [in Petri disease], *Cadophora* spp.). The dark wood streaking is a wood symptom proving the presence of the pathogens in vines from the nursery, while the Petri disease is typical of very young vines, a decline developing in new plantations. The typical wood symptoms in both is the vascular discoloration, often coupled with the emission of dark tarry drops (see related pictures). The Petri disease could also determine foliar symptoms (chlorosis and decline, up to death of the vines). The GLSD syndrome is characterized by the typical tiger-stripe pattern on symptomatic leaves and is often associated with a partial or complete apoplexy in affected plants. It can be found in both young and adult vineyards, both in vines showing only vascular pathogens infections or those also showing wood decay. A longitudinal orange-brown stripe under the bark can usually be noticed removing the bark. The white decay is determined by basidiomycete fungi (in Europe, *F. mediterranea*), which determine only symptoms on the infected wood. Finally, the name Esca proper was retained to refer to the original name (Esca as decayed wood used as tinder), typical of adult vineyards, and characterized by the contemporary presence of the main vascular pathogens plus the basidiomycete species and the development of foliar symptoms (for more information, see Gubler et al. [2015] and Surico [2009]). Pictures: Dark wood streaking, V. Mondello; Petri disease, courtesy of Feuga (Spain); GLSD, courtesy of Plant pathology sector SAF dept., University of Palermo (Italy), DRL Rheinpfalz (Germany), and Maurizio Gily (Italy); white rot, upper picture courtesy of DRL Rheinpfalz (Germany); Esca proper, courtesy of Plant pathology sector SAF dept., University of Palermo (Italy), Feuga (Spain), and ADVID (Portugal).

GTDs, the different internal and external symptoms can overlap. Furthermore, some of the wood infections caused by GTD agents lead to foliar symptoms due to physiological factors (Lecomte et al. 2012), but also because of phytotoxins produced by the pathogens (Abou-Mansour et al. 2015; Andolfi et al. 2011; Sparapano et al. 2000; Tabacchi et al. 2000). In addition, grapevine cultivars differ in their sensitivity to the development of foliar symptoms in some GTDs even if no cultivar or species in the genus *Vitis* has been found to express complete resistance to GTDs (Bertsch et al. 2013; Guan et al. 2016).

Altogether, these factors are enough to complicate the linear relationship among symptom(s), disease diagnosis, and control strategy that exists in other plant diseases. For GTDs, external symptoms are not useful for rapid and early diagnosis. Diagnostic tools in terms of traditional, molecular, and serological methods may lead to identification of a group of associated pathogens with various virulence levels according to species or even to strains and that could eventually have different sensitivity to a specific a.i. or treatment. In this context, the possibility of an efficient control of GTDs has worsened, as reported before, by the lack of valid and simple control protocols.

### Toward New Chemicals Through the Experience of Sodium Arsenite

While the mode of action of the two banned benzimidazoles, benomyl and carbendazim, against fungi is well known (they bind to spindle microtubules, interfere with spindle formation, inhibit mitosis, and thus inhibit germ tube and mycelial growth), to date, no definitive mechanism has been identified to explain how sodium arsenite acts in suppressing foliar symptoms within Esca complex (GLSD, Esca proper) and has some, even if lower, efficiency against *Eutypa* and *Botryosphaeria* dieback (Larignon and Dubos 2001; Larignon et al. 2008). It can penetrate into woody tissues and move inside the vine and has been found in sap, leaves, bunches, and roots. It can remain within wood, especially rotten wood, explaining the persistent effect even in years without treatment. However, its concentration in sap (in the order of  $\mu\text{g/liter}$ ) is insufficient to inhibit fungal growth, which can be obtained in the laboratory only at  $\text{mg/liter}$  concentrations (Larignon et al. 2008; Santos et al. 2006). Some senescence effects (lower chlorophyll content and fluorescence levels) and lower growth rate in plantlets due to sodium arsenite have been reported (Santos et al. 2006), while no toxic concentrations were found inside bunches or roots. The suppression of GLSD foliar symptoms



by sodium arsenite could thus be related to a complex of interactions between the a.i., the plant microbiome, pathogen metabolism, and host metabolism. As a consequence of this hypothesis, no a.i. can be excluded a priori in the search for an alternative to sodium arsenite. Thus, the evaluation of either existing or novel a.i.s that can be used to reduce the upsurge of GTDs has been a major priority for industry and researchers during the last two decades. Although only one paper reports promising results with this type of winter application to the wood, applying a copper based product (Di Marco et al. 2011b), it does confirm that this type of applications warrants further exploration.

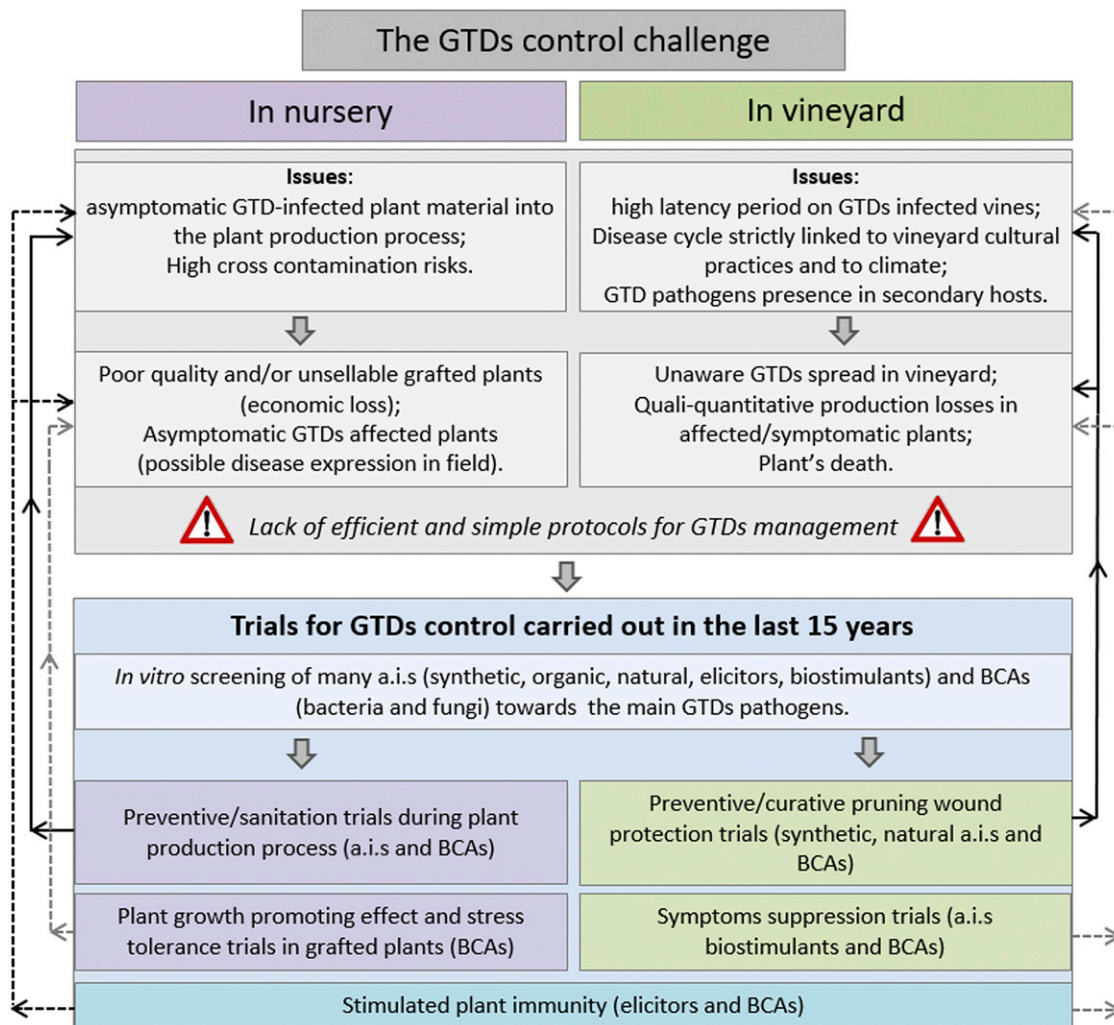
To date, the first barrier in this challenge is to identify the targets due to the lack of knowledge about the etiological agents of GTDs, except for diseases such as *Eutypa dieback* clearly related to *E. lata*, or cankers caused by *Botryosphaeriaceae* species. The second challenge is to manage GTDs at all stages from the production of new plants in nurseries to the growth of healthy vines in vineyards over the years and during a long period. Therefore, a.i.s have been tested by various researchers for preventive/sanitation protocols in the nursery, while they were tested in the field for their ability to reduce GTDs incidence by i) protecting pruning wounds, avoiding new infection; ii) suppressing foliar symptom expression and the related productivity and quality losses; and iii) improving or stimulating plant defenses against GTD pathogens.

### Main Strategies Used in Testing Active Ingredients Against GTDs

In the search for GTD control agents, particular attention has been devoted to detect antifungal compounds to be employed

against the main pathogens that have been revealed by research. This wide-range search has considered different organic and inorganic compounds, both synthetic and natural, and tested by various researchers (Tables 2 to 6).

Active ingredients have been assayed by different methods according to the kind of test (in vitro, in planta, and in the field), their usage (in nurseries, to reduce disease incidence, wound protection, symptom suppression, or improve plant defense mechanisms), and the specific GTD to manage. In vitro trials have been used to evaluate the efficiency of the a.i. in terms of its EC value (EC<sub>50</sub> and, sometimes, EC<sub>90</sub>) calculated either on percentage conidial germination or inhibition of mycelial growth of the pathogens on nutrient media amended with different concentrations of the a.i. (Bester et al. 2007; Cobos et al. 2015; Fleurat-Lessard et al. 2011; Gramaje et al. 2012; Halleen et al. 2010; Jaspers 2001; Martín and Martín 2013; Mazzullo et al. 2000; Nascimento et al. 2007; Pitt et al. 2012; Santos et al. 2006; Sosnowski et al. 2013). Some authors used both assays (Amponsah et al. 2012; Di Marco et al. 2011b; Rolshausen and Gubler 2005; Sosnowski et al. 2008, 2013) supplemented by studies on morphological modifications of the pathogen's mycelium (Fleurat-Lessard et al. 2011). In planta bioassays have also been useful to analyze other parameters on infected/treated plants such as wood colonization by GTD pathogens and plant mortality (Cobos et al. 2015; Nascimento et al. 2007), phytotoxic effects of the a.i. and its accumulation in host tissues (Di Marco et al. 2011b; Fleurat-Lessard et al. 2011), host plant growth (Cobos et al. 2015; Nascimento et al. 2007; Santos et al. 2006), and physiological



**Fig. 2.** The scheme followed by this review reflects the problems related to grapevine trunk disease (GTD) presence in both nursery and vineyard (in gray boxes), reporting the different strategies (colored boxes) set up by the research to solve the lack of suitable and efficient disease control protocols so far existing. a.i.: active ingredient; BCA: biocontrol agent.

changes (oxidative stress: Cobos et al. 2015; Santos et al. 2006; induction of defense mechanisms: Cobos et al. 2015; Di Marco et al. 2011a; Pierron et al. 2015; chlorophyll contents and fluorescence or leaf gas exchange: Di Marco et al. 2011a; Santos et al. 2006). The plant material used included plantlets, cuttings, and potted vines depending on the research aims, the trial timespan, and the a.i. selected for potential use against GTD pathogens. Plantlets were used for the evaluation of the a.i. impact on vine physiology by Santos et al. (2006) in

laboratory and controlled conditions, avoiding the influence of environmental biotic and abiotic factors that are normally difficult to control in greenhouse and field experiments. Bioassays with cuttings allowed the evaluation of a.i. efficiency after a relatively short period, ranging from 5 to 90 days after inoculation and mainly by pathogen reisolation, thus revealing the disease control capacity of an a.i. to reduce internal symptoms in terms of lengths of vascular discoloration (Bester et al. 2007; Cobos et al. 2015; Díaz and Latorre 2013).

**Table 2.** List of the synthetic organic compounds tested, singly or mixed in lab or as found in commercial products, against the three main grapevine trunk diseases in field and nursery

Chemical group	Active ingredient	Tested on			Tested in			
		Botryosphaeria dieback	Esca complex	Eutypa dieback	Lab	Vineyard	Nursery	
Anilinopyrimidine	Cyprodinil	x			x			
Anilopyrimidine	Pyrimethanil	x	x	x	x	x		
Arylamilopyrimidine	Fluazinam	x		x	x	x		
Benzimidazoles	Benomyl	x	x	x	x	x		
	Carbendazim	x	x	x	x	x	x	
	Thiophanate methyl	x	x	x	x	x	x	
Benzonitrile	Chlorothalonil	x		x	x			
Carboxamide	Boscalid	x			x			
Dicarboximide	Iprodione	x	x	x	x	x	x	
	Procymidone	x			x	x		
Disinfectans	Didecyltrimethylammonium chloride						x	
	Alcohol-phenol-iodine solution						x	
Dithiocarbamate	Mancozeb	x	x	x	x	x		
	Thiram		x		x		x	
	Ziram		x	x	x			
Phenoxyquinoline	Quinoxifen	x		x	x			
Hydroxianiline	Fenhexamid	x	x	x	x	x		
Imidazole	Prochloraz	x	x	x	x	x	x	
	Imazalil			x	x	x	x	
Morpholine	Dimethomorph			x	x			
Nitrile	Dithianon	x			x			
Phenylpyrrole	Fludioxonil	x			x		x	
Phthalimide	Captan	x	x		x		x	
Pyrimidine	Fenarimol	x	x	x	x	x		
Quinoline	Hydroxyquinoline sulfate	x	x		x		x	
Spyroketalamine	Spiroxamine	x	x	x	x		x	
Strobilurins	Azoxystrobin	x		x	x		x	
	Kresoxym-methyl	x	x	x	x			
	Pyraclostrobin	x	x	x	x	x		
	Trifloxystrobin			x	x	x		
Sulphamid	Tolyfluamid	x	x		x			
Triazoles	Cyproconazole		x		x	x	x	
	Difenoconazole		x			x		
	Flusilazole	x	x	x	x	x	x	
	Myclobutanil	x		x	x	x		
	Penconazole	x	x	x	x	x		
	Propiconazole		x	x		x		
	Tebuconazole	x	x	x	x	x	x	
	Tetraconazole	x	x	x	x	x		
	Thiabendazole		x		x	x		
	Triadimenol	x	x	x	x	x		
	Commercial mix	Boscalid + pyraclostrobin			x	x	x	
		Carbendazim + flusilazole		x		x		
Carboxine + thiram			x				x	
Cyproconazole + iodocarb		x	x	x		x		
Cyprodinil + fludioxonil		x	x	x	x	x	x	
Mancozeb + metalaxyl-m				x	x			
Prothioconazole + tebuconazole				x				
Pyraclostrobin + metiram		x	x				x	
Tebuconazole + boric acid + octhilinone			x		x			
Organic salts	Copper ammonium acetate	x		x	x			
	Copper bis(ethoxy-dihydroxy-diethylamino) sulfate						x	
Triazoles lab mix	Fosethyl-al	x	x			x		
	Propiconazole + thiabendazole		x			x		
	Difeconazole + tebuconazole		x			x		

Tests in the vineyard have focused mainly on wound protection from GTD pathogens or reduction of GTD incidence, permitting the evaluation of alternative a.i.s selected under standard conditions, since the a.i. action was tested under the influence of cultural practices and geographical and climatic variables during the test lifetime. Usage has been focused mainly on wound protection against GTD

pathogens or reduction of GTD incidence and/or severity. The methodology developed is closer to the hypothetical practical use, utilizing the usual agricultural equipment for a.i. distribution (Halleen et al. 2010; Sosnowski et al. 2013), or trying different application tools such as systemic a.i. pole injection in soil (Di Marco et al. 2000) or directly into vines by trunk injection (Calzarano et al.

**Table 3.** Inorganic compounds tested against the three main grapevine trunk diseases in field and nursery

Chemical group	Active ingredient	Tested on			Tested in		
		Botryosphaeria dieback	Esca complex	Eutypa dieback	Lab	Vineyard	Nursery
Inorganic acid	Boric acid	x		x	x	x	
	Phosphorous acid		x	x	x		x
Inorganic salt	Calcium polysulfides		x				x
	Phosphonic acid salt		x	x	x		
	Iron sulfate	x	x	x	x		
	Sodium arsenite	x	x	x	x		
	Copper oxychloride	x	x		x	x	
Inorganic base	Copper hydroxide	x	x	x	x		
Inorganic element	Sulfur		x	x	x		

**Table 4.** Natural compounds tested against the three main grapevine trunk diseases in field and nursery

Group	Natural compound	Tested on			Tested in		
		Botryosphaeria dieback	Esca complex	Eutypa dieback	Lab	Vineyard	Nursery
Organic extract	<i>Allium sativum</i>	x	x	x	x		
	<i>Evernia prunastri</i> lichen	x	x	x	x		
	Green coffee	x	x	x	x		
	Lemon peel	x	x	x	x		
	<i>Melaleuca alternifolia</i> oil			x	x		
Organic	Chitosan	x	x	x	x		
	Honey			x	x		
	Lactoferrin			x	x		
	Propolis	x	x	x	x		
	Saponins	x			x		
	Vanillin	x	x	x	x		
	Potassium bicarbonate			x	x		
Inorganic	Hydrogen peroxide	x	x	x	x		x
	Ozonated water		x		x		
	Allium + chitosan + vanillin	x	x	x	x	x	
Lab mix	Seaweed extract + CaCl <sub>2</sub> + Mg(NO <sub>3</sub> ) <sub>2</sub>		x			x	

**Table 5.** Plant-defense stimulating compounds tested against the three main grapevine trunk diseases in field and nursery

Group	Active ingredient(s) (commercial name)	Tested on			Tested in		
		Botryosphaeria dieback	Esca complex	Eutypa dieback	Lab	Vineyard	Nursery
Commercial products	Aluminum lignin sulfate, gluconic acid, microelements (Brotomax)		x	x	x	x	
	Amino acids, peptides, peptones (Fitostim)		x		x	x	
	Glutathione, oligosaccharine (Marvita)		x		x	x	
	<i>Aschophyllum nodosum</i> extract (Kendal)		x		x	x	
	2-hydroxybenzoic acid		x	x	x		
Plant-defense elicitor	Benzothiodiazole		x		x		
	Seaweed extracts + Ca + Mg		x			x	
	Resveratrol		x	x	x		
Phytoalexins	Pterostilbene		x	x	x		
	P-coumaric acid		x	x	x		
	Pterostilbene + resveratrol		x	x	x		
Lab mix	Copper oxychloride + gluconates		x		x	x	
	Resveratrol + phosphorous acid		x	x	x		



2004; Darrieutort and Lecomte 2007; Dula et al. 2007), as well as manual applications for wound protection (Díaz and Latorre 2013; Sosnowski et al. 2008).

For the protection of pruning wounds, the duration of the trials reported in literature is variable, since the trials could be repeated identically or with some changes in a.i. and/or pathogen application time (Amponsah et al. 2012; Díaz and Latorre 2013; Halleen et al. 2010; Pitt et al. 2012; Rolshausen et al. 2010; Sosnowski et al. 2008, 2013). In the trials for disease incidence reduction in GTD-affected vineyards, the duration was highly variable, ranging from 1 to 9 years, with the last 1 to 2 years sometimes devoted only to the observation of the treatment residual effects (Calzarano et al. 2004; Darrieutort and Lecomte 2007; Di Marco and Osti 2009; Di Marco et al. 2000, 2011a, b; Dula et al. 2007).

Due to the diversity of fungal species associated with GTDs, several different pathogens have been used in the in vitro assays, while in both in planta and field tests the most used species are *Phaeoconiella chlamydospora* and several *Phaeoacremonium* species for Esca complex, *D. seriata*, *D. mutila*, *L. theobromae*, and *N. parvum* for Botryosphaeria dieback, and *E. lata* for Eutypa dieback (Amponsah et al. 2012; Díaz and Latorre 2013; Halleen et al. 2010; Pitt et al. 2012; Rolshausen et al. 2010; Sosnowski et al. 2008, 2013).

### The Most Efficient Active Ingredients Against GTDs

According to our literature review, more than 90 a.i.s have been tested from 2000 until now against the Esca complex diseases, Botryosphaeria dieback and Eutypa dieback. Results are reported in Tables 7 to 10 and grouped according to their main characteristics (synthetic organic, inorganic, natural, biostimulants, and plant defense elicitors). For each a.i., efficiency in relation to the tests (in vitro, in planta, in the field), the specific aim of the study (wound protection, symptom suppression, GTD control in nurseries), and the targeted GTD pathogens are reported, taking into account the geographical area of the test.

**Synthetic organic active ingredients and compounds tested.** Most of the assayed a.i.s (55 out of 93 in Table 7) are synthetic organic compounds, used both singly (44) or in mixtures (11). Triazolones (10 a.i.s), strobilurins (4 a.i.s), and benzimidazoles (3 a.i.s) represent the chemical groups most often tested. The now banned

benzimidazoles benomyl and carbendazim were used as positive controls in several tests. Eighteen a.i.s were tested against all three GTDs considered in this review and 19 against only one GTD. Considering the kind of test, the number of a.i.s tested decreased moving from in lab tests (in vitro and in planta) to the in field tests (42 and 31 a.i.s, respectively). Only nine synthetic organic compounds were tested in both field and nursery.

Among the benzimidazoles, the most efficient a.i.s were benomyl, carbendazim, and thiophanate-methyl, which always showed high efficiency in both lab and field for pruning wound protection and in nurseries, irrespective of the geographical area or GTD targeted (Amponsah et al. 2012; Díaz and Latorre 2013; Gramaje et al. 2009; Groenewald et al. 2000; Halleen et al. 2010; Jaspers 2001; Pitt et al. 2012; Sosnowski et al. 2008, 2013). Their efficacy toward the main GTDs was certainly due to their broad-range fungicidal activity, their persistence, and their systemic activity. In the in vitro tests, they showed high capability in reducing both mycelial growth and conidial germination in all species except *E. lata*. For pruning wounds, benzimidazoles protected vines from new infection mainly as preventive treatments and, to a lesser extent, in curative ones. Thus, Sosnowski et al. (2008) reported the long-lasting preventive effect of benomyl in protecting wounds from *E. lata* infections (14 days) and the slight curative effect of benomyl and carbendazim, observed only if the a.i.s were applied 1 day after inoculation with *E. lata*. Benzimidazoles sprayed or painted on pruning wounds showed similar efficiency. To date, only thiophanate-methyl is approved for use in agriculture and it is currently applied to control *Botrytis cinerea* in grapevine. A disadvantage of benzimidazoles is that fungi can develop resistance to this class of compounds, as observed in *Phaeoacremonium minimum* toward carbendazim (Martín and Martín 2013). Consequently, benzimidazoles in use should be mixed with another a.i. or used alternately with other a.i.s. In nurseries, benzimidazoles successfully reduced the presence of vascular Esca complex pathogens. For example, benomyl and carbendazim decreased the amount of pathogen inoculum in grafted plants when applied during hydration or before grafting (Fourie and Halleen 2006; Gramaje et al. 2009), while soaking the scions in thiophanate-methyl before grafting (Serra et al. 2011) followed by hot water treatment (Eskalen et al. 2007b) successfully controlled Esca complex associated pathogens.

**Table 6.** Grapevine trunk disease (GTD) pathogens employed in the active ingredient (a.i.) and biocontrol agent (BCA) evaluation trials. The most used species are in bold.

GTD	Target pathogen used in a.i.s tests	Target pathogen used in BCAs tests
Botryosphaeria dieback	<i>Botryosphaeria dothidea</i> <b><i>Diplodia mutila</i></b> <b><i>Diplodia seriata</i></b> <i>Dothiorella viticola</i> <b><i>Lasiodiplodia theobromae</i></b> <i>Neofusicoccum australe</i> <i>Neofusicoccum luteum</i> <b><i>Neofusicoccum parvum</i></b>	<i>Diplodia mutila</i> <i>Diplodia corticola</i> <b><i>Diplodia seriata</i></b> <b><i>Lasiodiplodia theobromae</i></b> <i>Lasiodiplodia mediterranea</i> <i>Neofusicoccum australe</i> <i>Neofusicoccum luteum</i> <i>Neofusicoccum mediterraneum</i> <b><i>Neofusicoccum parvum</i></b>
Esca complex	<i>Fomitiporia mediterranea</i> <b><i>Phaeoconiella chlamydospora</i></b> <b><i>Phaeoacremonium minimum</i></b> <i>Phaeoacremonium angustium</i> <i>Phaeoacremonium parasiticum</i> <i>Pleurostomophora richardsiae</i> <i>Togninia minima</i> <i>Stereum hirsutum</i>	<i>Cadophora luteo-olivacea</i> <i>Fomitiporia mediterranea</i> <b><i>Phaeoconiella chlamydospora</i></b> <i>Phaeoacremonium minimum</i>
Eutypa dieback	<i>Cryptovalsa ampelina</i> <i>Diatrypella vulgaris</i> <b><i>Eutypa lata</i></b> <i>Eutypa leptoplaca</i> <i>Eutypella citricola</i> <i>Eutypella microtheca</i>	<b><i>Eutypa lata</i></b>

Triazoles were the most numerous group within the synthetic compounds, with 10 a.i.s tested. They are currently used in vineyards to protect vines from several diseases (powdery mildew, botrytis bunch rot, etc.). For this reason and due to their systemic properties, triazoles have also been tested to manage GTDs. In vitro assays revealed a high efficiency in controlling the mycelial growth of GTD pathogens and sometimes inhibit conidial germination (Gramaje et al. 2009). Some of these positive in vitro effects were confirmed by in planta bio-assays. Differences in efficiency of triazoles were recorded in similar tests carried out by different laboratories, probably due to the differences in protocols, pathogen and strains tested, and grapevine cultivars used. When tested for pruning wound protection, triazoles showed different behaviors according to the GTD. Apart from tebuconazole, they were ineffective against *Botryosphaeria dieback* pathogens, while flusilazole, penconazole, tebuconazole and, to a lesser extent, triadimenol, showed good results when tested on *E. lata*. Only tebuconazole and triadimenol were tested against vascular Esca complex pathogens, but with unsatisfactory results. For suppression of foliar symptoms, cyproconazole, penconazole, and thiabendazole gave some positive results only in the younger vines, with initial GLSD symptoms and no or limited wood decay (Di Marco et al. 2000). Difenoconazole and propiconazole showed contrasting results in France (Darrietort and Lecomte 2007) and Hungary (Dula et al. 2007), and tetraconazole was inefficient. Propiconazole was also tested to suppress Eutypa dieback foliar symptoms, unsuccessfully. In the nursery, two triazoles were studied to control Esca complex vascular pathogens during the plant production process, with promising results. Flusilazole combined with carbendazim (see in commercial mix, Table 12) controlled the development of *Pa. chlamydospora* and *Pm. minimum*, and cyproconazole limited the incidence of *Pa. chlamydospora* but only when coupled with a hot water treatment (Serra et al. 2011).

Strobilurins are currently used to control downy and powdery mildew in vineyards. They were especially tested for pruning wound protection. The most efficient against GTD pathogens was pyraclostrobin, in both in vitro and wound protection trials. Other strobilurins were studied only in vitro and showed different efficiencies according to the considered GTD pathogen as reported by Sosnowski et al. (2008), Gramaje et al. (2009), Halleen et al. (2010), and Amponsah et al. (2012) (see Table 7).

In the arylamylopyrimidine family of compounds, fluazinam, which is especially used in vineyards to control botrytis bunch rot and downy mildew, was tested on both *Botryosphaeria* and Eutypa dieback pathogens. Being a broad-spectrum, contact fungicide with high activity in blocking conidial germination, it was tested in vitro directly on pathogens and for wound protection, showing a high level of efficiency when applied after pruning (Gramaje et al. 2012; Pitt et al. 2012; Sosnowski et al. 2013).

Among the imidazoles, prochloraz, currently not registered for the use in vineyards, blocked conidial germination of all the tested GTD pathogens in vitro, but this trend was not always confirmed in planta (Table 7). Bester et al. (2007) reported its efficiency in protecting wounds from infection of *D. seriata*, *L. theobromae*, *N. australe*, and *N. parvum*. On the contrary, Pitt et al. (2012) obtained high reisolation percentages of the pathogen 6 months after artificial inoculation of *N. luteum* onto pruning wounds treated with prochloraz.

Seven commercial products were tested mainly for wound protection and in nursery applications. A cyproconazole+iodocarb mix, registered as wound protectant in some countries against Eutypa dieback, also successfully controlled *Botryosphaeria dieback* and pathogens associated to GLSD and Esca proper in the Esca complex (Rolshausen et al. 2010). The other commercial mixtures showed better results in nursery trials. The mix of carbendazim+flusilazole reduced the inoculum of *Pm. minimum* and *Pa. chlamydospora* when applied in the cutting soaking water before cold storage (Gramaje et al. 2009). Similarly, cyprodinil+fludioxinil and pyraclostrobin+metiram mixes controlled some *Botryosphaeriaceae* in the nursery when diluted in the soaking water before grafting (Rego et al. 2009).

Two experimental lab mixtures of triazoles, propiconazole+thiabendazole and difenoconazole+tebuconazole, gave a significant

reduction in Esca foliar symptoms expression, more evident for the difenoconazole+tebuconazole mix (Dula et al. 2007).

The dithiocarbamates ziram and thiram, tested in soaking water before grafting, showed good efficiency in controlling the presence of Petri disease pathogens in the nursery (Eskalen et al. 2007b), even if Santos et al. (2006) reported ziram as inefficient in their in vitro test toward *Pm. angustius* and *Pa. chlamydospora*. Mancozeb gave satisfactory results for wound protection toward *N. luteum* (Amponsah et al. 2012).

For the other a.i.s tested, fosetyl-Al (organic salt) was able to reduce Esca complex foliar symptoms (Esca proper), and also to reduce infections by *D. seriata* and Esca complex vascular pathogens in wounds (Díaz and Latorre 2013; Di Marco et al. 2011a).

The phthalimide captan, thanks to its broad-spectrum activity, is currently used in nurseries in some countries to limit fungal infection in wood tissues. Its efficiency was validated on Esca complex vascular pathogens (Fourie and Halleen 2006; Gramaje et al. 2009). Some organic disinfectants (didecyldimethylammonium chloride, usually used in nurseries as biocides) showed positive effects in vitro toward *Botryosphaeria* and Esca vascular pathogens that were confirmed in the nursery (Fourie and Halleen 2006; Gramaje and Armengol 2011; Gramaje et al. 2009). Moreover, these products coupled a high efficiency with a low impact on grafted plant viability (Fourie and Halleen 2006).

*Inorganic active ingredients tested such as boron, copper, sulfur, and phosphorus.* Among inorganic compounds (Table 8), acids, bases, and salts of well-known elements in plant disease control, such as boron, copper, sulfur, and phosphorus, have been tested. Sodium arsenite, even if banned, was tested in France and Portugal to understand more about its mode of action in order to find other a.i.s that could mimic the same or similar mechanisms (Larignon et al. 2008; Santos et al. 2006). Boron, known for its ability to affect some wood-rotting fungi, is used in timber and wood industries. On pruning wounds, boron showed good protection toward all the tested GTD pathogens (Rolshausen et al. 2010), although no significant improvement toward *E. lata* infections was reported by Sosnowski et al. (2008), when boron was added in the sealing paste. A possible phytotoxic effect of boron-based treatments resulting in bud failure was hypothesized (Rolshausen and Gubler 2005).

**Trying a new sustainable road: tests with natural active ingredients.** The concerns related to the massive use of chemicals and the attention toward environmentally friendly production models have given a boost to the study of natural a.i.s in agriculture, especially in viticulture. Consequently, several natural a.i.s were evaluated to manage GTDs. To date, 14 natural organic or inorganic substances, ranging from simple molecules such as hydrogen peroxide (Fourie and Halleen 2006; Sosnowski et al. 2013) to complex mixtures like propolis (Cobos et al. 2015) have been studied (Table 9).

Chitosan is a nontoxic, biocompatible, and biodegradable polymer, known for its fungistatic and fungicide properties and for its capability to stimulate plant defense systems through the induced systemic resistance (ISR) mechanism. Chitosan is able to penetrate fungal conidia and hyphae causing membrane disorganization and the loss of the cellular content (Palma-Guerrero et al. 2008; Park et al. 2002). When tested against GTD pathogens in vitro, chitosan inhibited mycelial growth of *Botryosphaeriaceae*, Esca complex fungi, and to a lesser extent, Eutypa dieback pathogens. This potential was confirmed for wound protection. Moreover, Cobos et al. (2015) reported that low-molecular weight chitosan was more effective than medium- and high-molecular weight ones.

Extracts of *Allium sativum* were assayed toward GTD pathogens, giving good results both in vitro and in wound protection (Cobos et al. 2015; Sosnowski et al. 2013). When combined with chitosan and vanillin, it was more effective than the individual a.i.s in reducing the infections of *D. seriata* and *Pa. chlamydospora* artificially inoculated onto treated pruning wounds (Cobos et al. 2015). Furthermore, and according to the authors, the lower mortality rate of vines and low percentages of reisolation of the pathogens recorded in the field trials could be related to a putative synergism among the

**Table 7.** Synthetic organic compounds tested toward the three main grapevine trunk diseases (GTDs). Reported for each active ingredient are: the efficiency<sup>a</sup> recorded in the relative test (in vitro, in planta, and in field); the results when tested for wound protection (WP), symptom suppression (SS), or for nursery use (NU); the pathogens used in tests; and in superscript brackets, the country in which the studies were carried out.<sup>b</sup> The number in superscript brackets on an efficiency result indicates the country in which the test was carried out (see supplementary file for references regarding a specific a.i.).

Chemical group	Active ingredient	Botryosphaeria dieback							Pathogen <sup>c</sup>	
		In vitro	In planta	In field	WP	SS	NU			
Anilinopyrimidine	Cyprodinil	--	nt	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>	
		nt	nt	nt	nt	nt	+++	nt	<i>Botryosphaeria</i> spp. <sup>(11)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(1)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(1)</sup>	
Anilopyrimidine	Pyrimethanil	--	nt	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(1)(10)</sup>	
		--	- + <sup>(3)</sup>	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(3)(10)(12)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(1)(12)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>N. australe</i> <sup>(10)(12)</sup>	
Arylamilopyrimidine	Fluazinam	--	nt	nt	nt	nt	nt	nt	<i>N. luteum</i> <sup>(10)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)(12)</sup>	
		+++	nt	nt	+++	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>	
		+++	nt	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(1)</sup>	
		+++	nt	nt	+++	nt	nt	nt	<i>D. seriata</i> <sup>(1)</sup>	
		+++	nt	nt	+++	nt	nt	nt	<i>L. theobromae</i> <sup>(1)</sup>	
Benzimidazole	Benomyl	+++ <sup>(12)</sup>	+++ <sup>(3)</sup>	nt	+++ <sup>(12)</sup>	nt	nt	nt	<i>D. seriata</i> <sup>(3)(12)</sup>	
		+++	nt	nt	+++	nt	nt	nt	<i>L. theobromae</i> <sup>(12)</sup>	
		+++	nt	nt	+++	nt	nt	nt	<i>N. australe</i> <sup>(12)</sup>	
		+++	nt	nt	+++	nt	nt	nt	<i>N. parvum</i> <sup>(12)</sup>	
		+++	nt	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>	
		+++	nt	nt	+++ <sup>(1)</sup>	nt	nt	nt	<i>D. mutila</i> <sup>(1)(10)</sup>	
	Carbendazim	Carbendazim	+++	nt	nt	+++	nt	nt	nt	<i>D. seriata</i> <sup>(1)</sup>
			+++	nt	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(1)</sup>
			+++	nt	nt	nt	nt	nt	nt	<i>N. australe</i> <sup>(10)</sup>
			+++	+++	nt	+++	nt	nt	nt	<i>N. luteum</i> <sup>(10)</sup>
			+++	nt	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)</sup>
			+++	+	nt	+++	nt	nt	nt	<i>B. dothidea</i> <sup>(15)</sup>
			+++	+	nt	+++	nt	nt	nt	<i>D. mutila</i> <sup>(10)</sup>
			+++	nt	nt	+++	nt	nt	nt	<i>D. seriata</i> <sup>(3)(13)(15)</sup>
			+++	nt	++	+++	nt	nt	nt	<i>D. viticola</i> <sup>(15)</sup>
Benzonitrile	Chlorothalonil	- +	- + <sup>(10)</sup>	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(4)(15)</sup>	
		+	- + <sup>(10)</sup>	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(10)</sup>	
		++	nt	nt	nt	nt	nt	nt	<i>N. australe</i> <sup>(10)</sup>	
		- +	- +	nt	nt	nt	nt	nt	<i>N. luteum</i> <sup>(10)</sup>	
		- +	- +	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(10)</sup>	
		++	nt	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(1)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(1)(12)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(1)(12)</sup>	
Dicarboximide	Iprodione	--	nt	nt	nt	nt	nt	nt	<i>N. australe</i> <sup>(12)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>N. luteum</i> <sup>(10)</sup>	
		--	nt	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)(12)</sup>	
		- +	nt	nt	nt	nt	nt	nt	<i>Botryosphaeria</i> sp. <sup>(3)</sup>	
		- +	nt	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>	
		- + <sup>(1)</sup> /+++ <sup>(10)</sup>	nt	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(1)(10)</sup>	
		- + <sup>(1)(12)</sup>	nt	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(1)(10)(12)</sup>	
Dicarboximide	Procymidone	+++ <sup>(10)</sup>	nt	nt	+	nt	nt	nt	<i>L. theobromae</i> <sup>(1)(12)(15)</sup>	
		- +	nt	nt	nt	nt	nt	nt	<i>N. australe</i> <sup>(10)(12)</sup>	
		+++	+++ <sup>(10)</sup>	nt	+++ <sup>(10)</sup>	nt	nt	nt	<i>N. luteum</i> <sup>(10)</sup>	
		- +	nt	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)(12)</sup>	
		- +	nt	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>	
		- + <sup>(1)</sup>	+ <sup>(10)</sup>	+ <sup>(10)</sup>	nt	nt	nt	nt	<i>D. mutila</i> <sup>(1)(10)</sup>	
		+++ <sup>(10)</sup>	nt	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(1)</sup>	
		- +	nt	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(1)</sup>	
Disinfectants	Didecyldimethylammonium chloride	+++	+	+	nt	nt	nt	nt	<i>N. australe</i> <sup>(10)</sup>	
		+++	+	+	nt	nt	nt	nt	<i>N. luteum</i> <sup>(10)</sup>	
		- +	nt	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)</sup>	
		nt	nt	nt	nt	nt	++	nt	<i>Botryosphaeria</i> sp. <sup>(12)</sup>	
Disinfectants	Alcohol-phenol-iodine solution	nt	nt	nt	nt	nt	--	nt	<i>Botryosphaeria</i> sp. <sup>(12)</sup>	
		nt	nt	nt	nt	nt	--	nt	<i>Botryosphaeria</i> sp. <sup>(12)</sup>	

(Continued on next page)

<sup>a</sup> Efficiency: -- ineffective; - + less effective; + moderately efficient; ++ efficient; +++ highly efficient; nt, not tested.

<sup>b</sup> Countries: (1) Australia; (2) Austria; (3) Chile; (4) Egypt; (5) France; (6) Germany; (7) Greece; (8) Hungary; (9) Italy; (10) New Zealand; (11) Portugal; (12) South Africa; (13) Spain; (14) Switzerland; (15) U.S.A.

<sup>c</sup> Abbreviations used for pathogen species: *B.* = *Botryosphaeria*; *D.* = *Diplodia*; *L.* = *Lasiodiplodia*; *N.* = *Neofusicoccum*; *Pa.* = *Phaeoamoniella*; *Pm.* = *Phaeoacremonium*; *F.* = *Fomitiporia*; *S.* = *Stereum*; *P.* = *Pleurostomophora*; *T.* = *Togninia*; *C.* = *Cryptovalsa*; *Di.* = *Diatrypella*; *E.* = *Eutypa*; *Eu.* = *Eutypella*; *Li.* = *Libertella*.



Table 7. (Continued from previous page)

Esca complex						Eutypa dieback							
In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>	In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>
							--	nt	nt	nt	nt	nt	<i>C. ampelina</i> <sup>(13)</sup>
							--	nt	nt	nt	nt	nt	<i>Di. vulgaris</i> <sup>(13)</sup>
							--	nt	nt	+++ <sup>(1)</sup>	nt	nt	<i>E. lata</i> <sup>(1)(12)(13)</sup>
nt	- +	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(3)</sup>	--	nt	nt	nt	nt	nt	<i>E. leptoplaca</i> <sup>(15)</sup>
							--	nt	nt	nt	nt	nt	<i>Eu. citricola</i> <sup>(13)</sup>
							--	nt	nt	nt	nt	nt	<i>Eu. microtheca</i> <sup>(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>C. ampelina</i> <sup>(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>Di. vulgaris</i> <sup>(13)</sup>
							+++	nt	nt	++ <sup>(1)</sup>	nt	nt	<i>E. lata</i> <sup>(1)(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>E. leptoplaca</i> <sup>(15)</sup>
							+++	nt	nt	nt	nt	nt	<i>Eu. citricola</i> <sup>(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>Eu. microtheca</i> <sup>(13)</sup>
+++ <sup>(10)(12)</sup>	+++ <sup>(1)(3)</sup>	nt	nt	nt	+ <sup>(10)</sup>	<i>Pa. chlamydospora</i> <sup>(1)(3)(10)(12)</sup>	+++	nt	nt	+++	nt	nt	<i>E. lata</i> <sup>(1)(12)</sup>
nt	nt	nt	nt	nt	+ <sup>(10)</sup>	<i>Pm. minimum</i> <sup>(10)</sup>							
+++	+++	nt	nt	nt	+++	<i>Pm. minimum</i> <sup>(13)</sup>	+++	nt	nt	nt	nt	nt	<i>C. ampelina</i> <sup>(13)</sup>
+++	nt	nt	nt	nt	+++ <sup>(13)</sup>	<i>Pa. chlamydospora</i> <sup>(10)(13)</sup>	+++	nt	nt	nt	nt	nt	<i>Di. vulgaris</i> <sup>(13)</sup>
nt	nt	nt	nt	-- <sup>(9)</sup>	nt	for foliar symptom suppression <sup>(9)</sup>	+++	nt	nt	+++ <sup>(1)</sup>	nt	nt	<i>E. lata</i> <sup>(1)(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>E. leptoplaca</i> <sup>(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>Eu. citricola</i> <sup>(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>Eu. microtheca</i> <sup>(13)</sup>
nt	nt	nt	+++	nt	nt	<i>Pm. parasiticum</i> <sup>(15)</sup>	nt	nt	nt	+++	nt	nt	<i>E. lata</i> <sup>(15)</sup>
+++	nt	nt	+++ <sup>(15)</sup>	nt	++ <sup>(8)(13)</sup>	<i>Pm. minimum</i> <sup>(8)(10)(13)(15)</sup>							
+++ <sup>(10)</sup>	nt	nt	+++ <sup>(3)(13)(15)</sup>	nt	++ <sup>(4)(8)</sup> / + <sup>(9)</sup>	<i>Pa. chlamydospora</i> <sup>(3)(4)(8)(9)(10)(13)(15)</sup>							
nt	nt	nt	+++	nt	nt	<i>P. richardsiae</i> <sup>(15)</sup>							
nt	nt	nt	+++	nt	nt	<i>T. minima</i> <sup>(15)</sup>							
+++	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(12)</sup>	+	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
--	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(3)(10)(13)</sup>	--	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
--	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>							
--	nt	nt	nt	nt	nt	<i>Phaeoacremonium</i> sp. <sup>(3)</sup>							
++ <sup>(13)</sup>	nt	nt	nt	nt	++	<i>Pa. chlamydospora</i> <sup>(12)(13)</sup>							
++ <sup>(13)</sup>	nt	nt	nt	nt	++	<i>Pm. minimum</i> <sup>(12)(13)</sup>							
nt	nt	nt	nt	nt	++	<i>Pa. chlamydospora</i> <sup>(12)</sup>							
nt	nt	nt	nt	nt	++	<i>Pm. minimum</i> <sup>(12)</sup>							

(Continued on next page)

**Table 7.** (Continued from previous page)

Chemical group	Active ingredient	Botryosphaeria dieback						Pathogen <sup>c</sup>
		In vitro	In planta	In field	WP	SS	NU	
Dithiocarbamate	Mancozeb	++	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(10)</sup>
		++	nt	nt	nt	nt	nt	<i>N. australe</i> <sup>(10)</sup>
		++	+++	nt	+++	nt	nt	<i>N. luteum</i> <sup>(10)</sup>
	Ziram							
	Thiram							
Hydroxianiline	Fenhexamid	--	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>
		--	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(1)(3)</sup>
		--	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(1)</sup>
Imidazole	Prochloraz	--	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)</sup>
		+++	+	nt	nt	nt	nt	<i>Botryosphaeria</i> sp. <sup>(3)</sup>
		++	nt	nt	+++	nt	nt	<i>D. seriata</i> <sup>(12)</sup>
		++	nt	nt	+++	nt	nt	<i>D. seriata</i> <sup>(12)</sup>
		+++	nt	nt	+++	nt	nt	<i>L. theobromae</i> <sup>(12)</sup>
		+++	nt	nt	+++ <sup>(12)</sup>	nt	nt	<i>N. australe</i> <sup>(10)(12)</sup>
	+++	--	nt	--	nt	nt	<i>N. luteum</i> <sup>(10)</sup>	
	Imazalil	+++	nt	nt	+++	nt	nt	<i>N. parvum</i> <sup>(12)</sup>
Morpholine Nitrile	Dimethomorph Dithianon	--	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(10)</sup>
		--	nt	nt	nt	nt	nt	<i>N. australe</i> <sup>(10)</sup>
Phenylpyrrole	Fludioxonil	--	+	nt	+	nt	nt	<i>N. luteum</i> <sup>(10)</sup>
		+++	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>
		nt	nt	nt	nt	nt	+	<i>Botryosphaeria</i> spp. <sup>(11)</sup>
		+++	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(1)</sup>
		+++	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(1)</sup>
Phenoxyquinoline	Quinoxifen	+++	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(1)</sup>
		+++	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)</sup>
		+++	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>
		+++	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(1)</sup>
		+++	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(1)</sup>
Phthalimide	Captan	--	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(1)</sup>
		--	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)</sup>
		--	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)</sup>
		--	nt	nt	nt	nt	nt	<i>Botryosphaeria</i> spp. <sup>(12)</sup>
Quinoline	Hydroxyquinoline sulfate	nt	nt	nt	nt	nt	--	<i>Botryosphaeria</i> sp. <sup>(12)</sup>
Pyrimidine	Fenarimol	+	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>
		+	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(1)(10)</sup>
		+ <sup>(1)</sup> /+++ <sup>(12)</sup>	nt	nt	+	nt	nt	<i>D. seriata</i> <sup>(1)(12)</sup>
		+ <sup>(1)</sup> /+++ <sup>(12)</sup>	nt	nt	+	nt	nt	<i>L. theobromae</i> <sup>(1)(12)</sup>
		+ <sup>(10)</sup> /+++ <sup>(12)</sup>	nt	nt	+	nt	nt	<i>N. australe</i> <sup>(10)(12)</sup>
		+	+	nt	nt	nt	nt	<i>N. luteum</i> <sup>(10)</sup>
Spyroketalamine	Spiroxamine	+ <sup>(1)</sup> /+++ <sup>(12)</sup>	nt	nt	+	nt	nt	<i>N. parvum</i> <sup>(1)(12)</sup>
		- +	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>
		- +	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(1)</sup>
		- +	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(1)</sup>
		- +	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(1)</sup>
Strobilurin	Azoxystrobin	nt	nt	nt	++	nt	nt	<i>Botryosphaeria</i> spp. <sup>(11)</sup>
		--	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(10)</sup>
		--	nt	nt	nt	nt	nt	<i>N. australe</i> <sup>(10)</sup>
		--	nt	nt	nt	nt	nt	<i>N. luteum</i> <sup>(10)</sup>
	Kresoxym-methyl	--	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(12)</sup>
		--	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(12)</sup>
		--	nt	nt	nt	nt	nt	<i>N. australe</i> <sup>(12)</sup>
	Pyraclostrobin	--	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(12)</sup>
		+++ <sup>(1)</sup>	nt	nt	+++ <sup>(15)</sup>	nt	nt	<i>B. dothidea</i> <sup>(1)(15)</sup>
		+++ <sup>(1)</sup>	nt	nt	+++ <sup>(15)</sup>	nt	nt	<i>D. mutila</i> <sup>(1)(15)</sup>
nt		+ <sup>(3)</sup>	nt	+ <sup>(3)</sup> /+++ <sup>(15)</sup>	nt	nt	<i>D. seriata</i> <sup>(3)(15)</sup>	
nt		nt	nt	+++	nt	nt	<i>D. viticola</i> <sup>(15)</sup>	
	Trifloxystrobin	nt	nt	nt	+++	nt	nt	<i>L. theobromae</i> <sup>(15)</sup>

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Table 7. (Continued from previous page)

Esca complex							Eutypa dieback						
In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>	In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>
--	nt	nt	nt	nt	++	<i>Pa. chlamydospora</i> <sup>(12)</sup>	-- <sup>(1)</sup> + <sup>(12)</sup>	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)(12)</sup>
nt	nt	nt	nt	nt	++	<i>Pm. minimum</i> <sup>(15)</sup>	--	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
--	nt	nt	nt	nt	nt	<i>Pm. angustum</i> <sup>(11)</sup>							
-- <sup>(11)</sup>	nt	nt	nt	nt	++ <sup>(15)</sup>	<i>Pa. chlamydospora</i> <sup>(11)(15)</sup>							
++ <sup>(13)</sup>	nt	nt	nt	nt	++ <sup>(15)</sup>	<i>Pa. chlamydospora</i> <sup>(13)(15)</sup>							
++ <sup>(13)</sup>	nt	nt	nt	nt	++ <sup>(15)</sup>	<i>Pm. minimum</i> <sup>(13)(15)</sup>							
--	nt	nt	nt	nt		<i>Pm. chlamydospora</i> <sup>(3)</sup>	--	nt	nt	--	nt	nt	<i>E. lata</i> <sup>(1)(12)</sup>
+++	+ <sup>(3)</sup>	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(1)(3)(13)</sup>	+++	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
+++		nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>							
+++	+ <sup>(3)</sup>	nt	nt	nt	nt	<i>Phaeoacremonium</i> sp. <sup>(3)</sup>							
+++	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(13)</sup>	++	nt	nt	+	nt	nt	<i>E. lata</i> <sup>(1)</sup>
++	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>	+	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
nt	nt	nt	nt	nt	--	<i>Pa. chlamydospora</i> <sup>(11)</sup>							
							--	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
- + <sup>(13)</sup>	nt	nt	nt	nt	++ <sup>(12)</sup>	<i>Pa. chlamydospora</i> <sup>(12)(13)</sup>							
- + <sup>(13)</sup>	nt	nt	nt	nt	++ <sup>(12)</sup>	<i>Pm. minimum</i> <sup>(12)(13)</sup>							
+++ <sup>(2)</sup> /- + <sup>(13)</sup>	nt	nt	nt	nt	- + <sup>(2)</sup> /- <sup>(12)</sup>	<i>Pa. chlamydospora</i> <sup>(2)(12)(13)</sup>							
+++ <sup>(2)</sup> /- + <sup>(13)</sup>	nt	nt	nt	nt	-- <sup>(2)(12)</sup>	<i>Pm. minimum</i> <sup>(2)(12)(13)</sup>							
nt	+++	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(1)</sup>	+++	nt	nt	--	nt	nt	<i>E. lata</i> <sup>(1)(12)</sup>
+++	nt	nt	nt	nt	- +	<i>Pa. chlamydospora</i> <sup>(2)</sup>	+++	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)(12)</sup>
+++	nt	nt	nt	nt	--	<i>Pm. minimum</i> <sup>(2)</sup>							
+++ <sup>(13)</sup>	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(13)</sup>	--	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)(12)</sup>
+++ <sup>(13)</sup>	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>							
-- <sup>(3)</sup>	+ <sup>(1)</sup>	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(1)(3)</sup>	--	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(12)</sup>
nt	nt	nt	+++ <sup>(15)</sup>	nt	nt	<i>Pm. parasiticum</i> <sup>(15)</sup>	+	nt	nt	nt	nt	nt	<i>C. ampelina</i> <sup>(13)</sup>
nt	+ <sup>(2)</sup>	nt	+ <sup>(2)</sup> /+++ <sup>(15)</sup>	nt	nt	<i>Pa. chlamydospora</i> <sup>(3)(15)</sup>	+	nt	nt	nt	nt	nt	<i>Di. vulgaris</i> <sup>(13)</sup>
nt	nt	nt	+++ <sup>(15)</sup>	nt	nt	<i>P. richardsiae</i> <sup>(15)</sup>	+ <sup>(1)(13)</sup>	nt	nt	+++ <sup>(15)</sup>	nt	nt	<i>E. lata</i> <sup>(1)(13)(15)</sup>
nt	nt	nt	+++ <sup>(15)</sup>	nt	nt	<i>T. minima</i> <sup>(15)</sup>	+	nt	nt	nt	nt	nt	<i>E. leptoplaca</i> <sup>(13)</sup>
							+	nt	nt	nt	nt	nt	<i>Eu. citricola</i> <sup>(13)</sup>
							+	nt	nt	nt	nt	nt	<i>Eu. microtheca</i> <sup>(13)</sup>
							- +	nt	nt	-- <sup>(1)</sup>	nt	nt	<i>E. lata</i> <sup>(1)(12)</sup>

(Continued on next page)



**Table 7.** (Continued from previous page)

Chemical group	Active ingredient	Botryosphaeria dieback								
		In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>		
Sulphamid	Tolyfluamid	- -	nt	nt	nt	nt	nt	<i>Botryosphaeria</i> sp. <sup>(3)</sup>		
Triazole	Cyproconazole									
	Difenoconazole	nt	nt	nt	++	nt	nt	<i>Botryosphaeria</i> spp <sup>(11)</sup>		
	Flusilazole		+	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>	
			+ <sup>(1)</sup> /+++ <sup>(10)</sup>	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(1)(10)</sup>	
			+ <sup>(1)</sup> /+++ <sup>(12)</sup>	nt	nt	- - <sup>(12)</sup>	nt	nt	<i>D. seriata</i> <sup>(1)(12)</sup>	
			+ <sup>(1)</sup> /+++ <sup>(12)</sup>	nt	nt	- - <sup>(12)</sup>	nt	nt	<i>L. theobromae</i> <sup>(1)(12)</sup>	
			+++	nt	nt	- - <sup>(12)</sup>	nt	nt	<i>N. australe</i> <sup>(10)(12)</sup>	
			+++	+++	nt	+++	nt	nt	<i>N. luteum</i> <sup>(10)</sup>	
			+ <sup>(1)</sup>	nt	nt	- - <sup>(12)</sup>	nt	nt	<i>N. parvum</i> <sup>(1)(12)</sup>	
			+++ <sup>(12)</sup>							
		Myclobutanil		+++	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>
				+++	nt	nt	- -	nt	nt	<i>Diplodia mutila</i> <sup>(1)</sup>
			+++	nt	nt	- -	nt	nt	<i>D. seriata</i> <sup>(1)</sup>	
			+++	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(1)</sup>	
			+++	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)</sup>	
	Penconazole		+++	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>	
			+++	nt	nt	- -	nt	nt	<i>D. mutila</i> <sup>(1)</sup>	
			+++	nt	nt	- -	nt	nt	<i>D. seriata</i> <sup>(1)</sup>	
			+++	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(1)</sup>	
			+++	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)</sup>	
Propiconazole										
Tebuconazole		+++ <sup>(3)</sup>	+ <sup>(3)</sup>	nt	++ <sup>(11)</sup>	nt	nt	<i>Botryosphaeria</i> sp. <sup>(3)(11)</sup>		
		+++	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>		
		+++	+++	nt	++ <sup>(1)</sup>	nt	nt	<i>Diplodia mutila</i> <sup>(1)(10)</sup>		
		+++ <sup>(1)(12)</sup>	+++ <sup>(3)</sup>	nt	+++ <sup>(1)(12)</sup>	nt	nt	<i>D. seriata</i> <sup>(1)(3)(12)</sup>		
		+++	nt	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(1)(12)</sup>		
		+++	nt	nt	nt	nt	nt	<i>N. australe</i> <sup>(10)(12)</sup>		
		+++	+++	nt	nt	nt	nt	<i>N. luteum</i> <sup>(10)</sup>		
		+++	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)(12)</sup>		
	Thiabendazole		+++	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(1)(12)</sup>	
			+++	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(1)</sup>	
		+++	nt	nt	- -	nt	nt	<i>D. seriata</i> <sup>(1)</sup>		
		- -	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(1)</sup>		
		- -	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(1)</sup>		
Carboxamide + strobilurin Anilide + dithiocarbamate Benzimidazole + triazole	Boscalid + pyraclostrobin <i>BASF516</i>									
	Carboxine + thiram <i>VitavaxT</i>			++			++	<i>L. theobromae</i> <sup>(4)</sup>		
	Carbendazim + flusilazole <i>Escudo</i>									
Triazole + carbamate	Cyproconazole + iodocarb <i>Garrison</i>	nt	nt	nt	+++	nt	nt	<i>B. dothidea</i> <sup>(15)</sup>		
		nt	nt	nt	+++	nt	nt	<i>D. mutila</i> <sup>(1)</sup>		
		nt	nt	nt	+++ <sup>(1)</sup> /+ <sup>(15)</sup>	nt	nt	<i>D. seriata</i> <sup>(1)(15)</sup>		
		nt	nt	nt	++	nt	nt	<i>D. viticola</i> <sup>(15)</sup>		
		nt	nt	nt	++	nt	nt	<i>L. theobromae</i> <sup>(15)</sup>		
Anilinopyrimidine + phenylpyrrole	Cyprodinil + fludioxonil <i>Switch</i>	nt	nt	nt	nt	nt	++	<i>Botryosphaeria</i> spp. <sup>(11)</sup>		
		nt	nt	nt	- +	nt	nt	<i>D. mutila</i> <sup>(1)</sup>		
		nt	nt	nt	- +	nt	++ <sup>(5)</sup>	<i>D. seriata</i> <sup>(1)(5)</sup>		
		nt	nt	nt	nt	nt	++	<i>N. parvum</i> <sup>(5)</sup>		
Dithiocarbamate + depsipeptides Triazoles	Mancozeb + metalaxyl-m <i>Ridomil Gold MZ</i>									
	Prothioconazole + tebuconazole <i>Prosaro</i>									
Strobilurin + dithiocarbamate	Pyraclostrobin + metiram <i>Cabrio top</i>	nt	nt	nt	nt	nt	++	<i>Botryosphaeria</i> spp. <sup>(11)</sup>		
		nt	nt	nt	nt	nt	++	<i>D. seriata</i> <sup>(5)</sup>		
Triazole + inorganic acid + thiazole	Tebuconazole + boric acid + octhilinone <i>Gelseal ultra</i>									
Organic salts	Copper ammonium acetate	- -	- -	nt	nt	nt	nt	<i>D. seriata</i> <sup>(12)</sup>		
		- -	- -	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(12)</sup>		
		- -	- -	nt	nt	nt	nt	<i>N. australe</i> <sup>(12)</sup>		
		- -	- -	nt	nt	nt	nt	<i>N. parvum</i> <sup>(12)</sup>		
	Fosetyl-al	nt	nt	nt	- +	nt	nt	<i>D. seriata</i> <sup>(3)</sup>		
		Copper bis (ethoxy-dihydroxy-diethylamino) sulfate								
Triazole	Propiconazole + thiabendazole									
Triazole	Difeconazole + tebuconazole									

(Continued on next page)

Table 7. (Continued from previous page)

Esca complex							Eutypa dieback						
In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>	In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>
- - <sup>(3)</sup>	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(3)</sup>							
- - <sup>(3)</sup>	nt	nt	nt	nt	nt	<i>Phaeoacremonium</i> sp. <sup>(3)</sup>							
++ <sup>(10)</sup>	nt	+ <sup>(9)</sup>	nt	++ <sup>(9)</sup>	++ <sup>(9)</sup>	<i>Pa. chlamydospora</i> <sup>(9)(10)</sup>							
nt	nt	nt	nt	- - <sup>(5)</sup>	nt		nt	nt	nt	- -	nt	nt	<i>E. lata</i> <sup>(5)</sup>
+++	nt	nt	nt	+++ <sup>(8)</sup>	nt	<i>Pa. chlamydospora</i> <sup>(13)</sup>	+++	nt	nt	- - <sup>(1)</sup>	nt	nt	<i>E. lata</i> <sup>(1)(12)</sup>
++ <sup>(9)</sup>	- - <sup>(13)</sup>	nt	nt	- - <sup>(9)</sup>	nt	<i>Pm. minimum</i> <sup>(9)(13)</sup>				+++ <sup>(12)</sup>			
							+ <sup>(1)</sup> +++ <sup>(12)</sup>	nt	nt	- - <sup>(1)</sup>	nt	nt	<i>E. lata</i> <sup>(1)(12)</sup>
nt	nt	nt	nt	+ <sup>(9)</sup>	nt	for foliar symptom suppression <sup>(9)</sup>	+++	nt	nt	+++	nt	nt	<i>E. lata</i> <sup>(1)</sup>
+++	nt	nt	nt	nt	+	<i>Pa. chlamydospora</i> <sup>(2)</sup>							
+++	nt	nt	nt	nt	+	<i>Pm. minimum</i> <sup>(2)</sup>							
nt	nt	nt	nt	- - <sup>(5)</sup>	nt		nt	nt	nt	nt	- - <sup>(5)</sup>	nt	
+++ <sup>(10)(13)</sup>	- + <sup>(1)</sup> +++ <sup>(3)</sup>	nt	- + <sup>(3)</sup>	+++ <sup>(8)</sup>	nt	<i>Pa. chlamydospora</i> <sup>(1)(3)(10)(13)</sup>	+++	nt	nt	nt	nt	nt	<i>C. ampelina</i> <sup>(13)</sup>
+++	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>	+++	nt	nt	nt	nt	nt	<i>Di. vulgaris</i> <sup>(13)</sup>
+++	+	nt	nt	nt	nt	<i>Phaeoacremonium</i> sp. <sup>(3)</sup>	+++	nt	nt	+++ <sup>(1)(10)(12)</sup>	nt	nt	<i>E. lata</i> <sup>(1)(10)(12)(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>E. leptoplaca</i> <sup>(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>Eu. citricola</i> <sup>(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>Eu. microtheca</i> <sup>(13)</sup>
+++	for phytotoxicity	nt	nt	nt	nt	<i>Pm. angustium</i> <sup>(11)</sup>							
+++	for phytotoxicity	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(11)</sup>							
nt	nt	nt	nt	+++ <sup>(8)</sup>	nt								
nt	nt	nt	nt	- - <sup>(9)</sup>	nt		+++	nt	nt	- -	nt	nt	<i>E. lata</i> <sup>(1)</sup>
+	nt	nt	- -	nt	nt	<i>Pa. chlamydospora</i> <sup>(1)</sup>	+++	nt	nt	- +	nt	nt	<i>E. lata</i> <sup>(1)</sup>
							++	nt	nt	- -	nt	nt	<i>E. lata</i> <sup>(1)</sup>
nt	nt	nt	nt	nt	++ <sup>(13)</sup>	<i>Pm. minimum</i> <sup>(13)</sup>							
++	nt	nt	nt	nt	++ <sup>(13)</sup>	<i>Pa. chlamydospora</i> <sup>(13)</sup>							
nt	nt	nt	- -	nt	nt	<i>Pm. parasiticum</i> <sup>(15)</sup>	nt	nt	nt	+++	nt	nt	<i>E. lata</i> <sup>(1)(15)</sup>
nt	nt	nt	++	nt	nt	<i>Pa. chlamydospora</i> <sup>(15)</sup>							
nt	nt	nt	+++	nt	nt	<i>P. richardsiae</i> <sup>(15)</sup>							
nt	nt	nt	+	nt	nt	<i>T. minima</i> <sup>(15)</sup>							
+++ <sup>(10)</sup>	++ <sup>(11)</sup>	nt	nt	nt	++ <sup>(5)</sup>	<i>Pa. chlamydospora</i> <sup>(5)(10)(11)</sup>	+++	nt	nt	+	nt	nt	<i>E. lata</i> <sup>(1)</sup>
							- -	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
							+++	nt	nt	nt	nt	nt	<i>C. ampelina</i> <sup>(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>Di. vulgaris</i> <sup>(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)(12)(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>E. leptoplaca</i> <sup>(13)</sup>
							- +	nt	nt	nt	nt	nt	<i>Eu. citricola</i> <sup>(13)</sup>
							+++	nt	nt	nt	nt	nt	<i>Eu. microtheca</i> <sup>(13)</sup>
nt	++	nt	nt	nt	nt	<i>F. mediterranea</i> <sup>(6)</sup>							
nt	++ <sup>(6)</sup>	nt	nt	nt	++ <sup>(5)</sup>	<i>Pa. chlamydospora</i> <sup>(5)(6)</sup>							
nt	++	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(6)</sup>							
							nt	nt	nt	+++	nt	nt	<i>E. lata</i> <sup>(1)(10)</sup>
							- -	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
nt	nt	nt	+	+	nt	<i>Pm. minimum</i> <sup>(9)</sup>							
nt	nt	nt	- + <sup>(3)</sup>	nt	nt	<i>Pa. chlamydospora</i> <sup>(3)(9)</sup>							
- +	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(13)</sup>							
- +	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>							
nt	nt	nt	nt	+	nt								
nt	nt	nt	nt	++ <sup>(8)</sup>	nt								

**Table 8.** Inorganics tested toward the three main grapevine trunk diseases (GTDs). Reported for each active ingredient are: the efficiency<sup>a</sup> recorded in the relative test (in vitro, in planta, and in field); the results when tested for wound protection (WP), symptom suppression (SS), or for nursery use (NU); the pathogens used in tests; and in superscript brackets, the country in which the studies were carried out.<sup>b</sup> The number in superscript brackets on an efficiency result indicates the country in which the test was carried out (see supplementary file for references regarding a specific a.i.).

Chemical group	Active ingredient	Botryosphaeria dieback						Pathogen <sup>c</sup>
		In vitro	In planta	In field	WP	SS	NU	
Inorganic acid	Boric acid	-- <sup>(1)</sup>	nt	nt	++ <sup>(15)</sup>	nt	nt	<i>B. dothidea</i> <sup>(1)(15)</sup>
		-- <sup>(1)</sup>	nt	nt	++ <sup>(15)</sup>	nt	nt	<i>D. mutila</i> <sup>(1)</sup>
		-- <sup>(1)</sup>	nt	nt	++ <sup>(15)</sup>	nt	nt	<i>D. seriata</i> <sup>(1)(15)</sup>
		nt	nt	nt	++ <sup>(15)</sup>	nt	nt	<i>D. viticola</i> <sup>(15)</sup>
		-- <sup>(1)</sup>	nt	nt	++ <sup>(15)</sup>	nt	nt	<i>L. theobromae</i> <sup>(1)(15)</sup>
		-- <sup>(1)</sup>	nt	nt	++ <sup>(15)</sup>	nt	nt	<i>N. parvum</i> <sup>(1)</sup>
	Phosphorous acid							
Inorganic salt	Phosphonic acid salt							
	Calcium polysulfides							
	Iron sulfate	- +	for phytotoxicity	nt	nt	nt	nt	<i>D. seriata</i> <sup>(5)</sup>
	Sodium arsenite	- +	for phytotoxicity	nt	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
	Copper oxychloride							
Inorganic base	Copper hydroxide	--	nt	nt	nt	nt	nt	<i>D. mutila</i> <sup>(10)</sup>
		--	nt	nt	nt	nt	nt	<i>N. australe</i> <sup>(10)</sup>
		--	+	nt	nt	nt	nt	<i>N. luteum</i> <sup>(10)</sup>
Inorganic element	Sulfur							

(Continued on next page)

<sup>a</sup> Efficiency: -- ineffective; - + less effective; + moderately efficient; ++ efficient; +++ highly efficient; nt, not tested.

<sup>b</sup> Countries: (1) Australia; (2) Austria; (3) Chile; (4) Egypt; (5) France; (6) Germany; (7) Greece; (8) Hungary; (9) Italy; (10) New Zealand; (11) Portugal; (12) South Africa; (13) Spain; (14) Switzerland; (15) U.S.A.

<sup>c</sup> Abbreviations used for pathogen species: *B.* = *Botryosphaeria*; *D.* = *Diplodia*; *L.* = *Lasiodiplodia*; *N.* = *Neofusicoccum*; *Pa.* = *Phaeomoniella*; *Pm.* = *Phaeoacremonium*; *F.* = *Fomitiporia*; *S.* = *Stereum*; *P.* = *Pleurostomophora*; *T.* = *Togninia*; *C.* = *Cryptovalsa*; *Di.* = *Diatrypella*; *E.* = *Eutypa*; *Eu.* = *Eutypella*; *Li.* = *Libertella*.

**Table 9.** Natural compounds tested toward the three main grapevine trunk diseases (GTDs). Reported for each active ingredient are: the efficiency<sup>a</sup> recorded in the relative test (in vitro, in planta, and in field); the results when tested for wound protection (WP), symptom suppression (SS), or for nursery use (NU); the pathogens used in tests; and in superscript brackets, the country in which the studies were carried out.<sup>b</sup> The number in superscript brackets on an efficiency result indicates the country in which the test was carried out (see supplementary file for references regarding a specific a.i.).

Chemical group	Active ingredient	Botryosphaeria dieback						Pathogen <sup>c</sup>
		In vitro	In planta	In field	WP	SS	NU	
Natural compounds	Ozonated water							
	Chitosan	++	nt	nt	nt	nt	nt	<i>Botryosphaeria</i> sp. <sup>(11)</sup>
		+++	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(13)</sup>
		+++	+++	nt	+++	nt	nt	<i>D. seriata</i> <sup>(13)</sup>
	<i>Allium sativum</i> extract	++	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(13)</sup>
		+++	+++	nt	+++	nt	nt	<i>D. seriata</i> <sup>(13)</sup>
	Honey							
	Hydrogen peroxide	nt	nt	nt	nt	nt	--	<i>Botryosphaeria</i> sp. <sup>(12)</sup>
	Lactoferrin							
	<i>Melaleuca alternifolia</i> oil							
	Potassium bicarbonate							
	Saponins	- +	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(5)</sup>
		- +	nt	nt	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
	Sard laundry powder							
	<i>Evernia prunastri</i> lichen extract	+++	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(13)</sup>
		++	- +	nt	nt	nt	nt	<i>D. seriata</i> <sup>(13)</sup>
	Green coffee extract	--	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(13)</sup>
		--	nt	nt	nt	nt	nt	<i>D. seriata</i> <sup>(13)</sup>
	Lemon peel extract	++	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(13)</sup>
		++	- +	nt	nt	nt	nt	<i>Diplodia seriata</i> <sup>(13)</sup>
Propolis	++	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(13)</sup>	
	++	+	nt	nt	nt	nt	<i>D. seriata</i> <sup>(13)</sup>	
Seaweed extract + CaCl <sub>2</sub> + Mg(NO <sub>3</sub> ) <sub>2</sub>								
Vanillin	+	nt	nt	nt	nt	nt	<i>B. dothidea</i> <sup>(13)</sup>	
	++	+	nt	nt	nt	nt	<i>D. seriata</i> <sup>(13)</sup>	
<i>Allium</i> + chitosan + vanillin	nt	++	nt	+++	nt	nt	<i>D. seriata</i> <sup>(13)</sup>	

(Continued on next page)

<sup>a</sup> Efficiency: -- ineffective; - + less effective; + moderately efficient; ++ efficient; +++ highly efficient; nt, not tested.

<sup>b</sup> Countries: (1) Australia; (2) Austria; (3) Chile; (4) Egypt; (5) France; (6) Germany; (7) Greece; (8) Hungary; (9) Italy; (10) New Zealand; (11) Portugal; (12) South Africa; (13) Spain; (14) Switzerland; (15) U.S.A.

<sup>c</sup> Abbreviations used for pathogen species: *B.* = *Botryosphaeria*; *D.* = *Diplodia*; *L.* = *Lasiodiplodia*; *N.* = *Neofusicoccum*; *Pa.* = *Phaeomoniella*; *Pm.* = *Phaeoacremonium*; *F.* = *Fomitiporia*; *S.* = *Stereum*; *P.* = *Pleurostomophora*; *T.* = *Togninia*; *C.* = *Cryptovalsa*; *Di.* = *Diatrypella*; *E.* = *Eutypa*; *Eu.* = *Eutypella*; *Li.* = *Libertella*.



**Table 8.** (Continued from previous page)

Esca complex						Eutypa dieback							
In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>	In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>
nt	nt	nt	+++ <sup>(15)</sup>	nt	nt	<i>Pa. chlamydospora</i> <sup>(15)</sup>	++ <sup>(15)</sup>	nt	nt	- - <sup>(1)</sup> +++ <sup>(15)</sup>	nt	nt	<i>E. lata</i> <sup>(1)(15)</sup>
nt	nt	nt	+++ <sup>(15)</sup>	nt	nt	<i>P. parasiticum</i> <sup>(15)</sup>							
nt	nt	nt	+++ <sup>(15)</sup>	nt	nt	<i>P. richardsiae</i> <sup>(15)</sup>							
nt	nt	nt	+++ <sup>(15)</sup>	nt	nt	<i>T. minima</i> <sup>(15)</sup>							
- - <sup>(9)</sup>	nt	nt	nt	nt	nt	<i>F. punctata</i> <sup>(9)</sup>	+++	nt	nt	nt	nt	nt	<i>L. blepharis</i> <sup>(9)</sup>
- - <sup>(9)</sup>	nt	nt	nt	nt	- + <sup>(12)</sup>	<i>Pm. minimum</i> <sup>(9)(12)</sup>							
- - <sup>(9)(10)</sup>	nt	nt	nt	nt	- + <sup>(12)</sup>	<i>Pa. chlamydospora</i> <sup>(9)(10)(12)</sup>							
- - <sup>(9)</sup>	nt	nt	nt	nt	nt	<i>S. hirsutum</i> <sup>(9)</sup>							
nt	nt	nt	nt	nt	++	<i>Pa. chlamydospora</i> <sup>(15)</sup>	- -	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
nt	nt	nt	nt	nt	++	<i>Pm. minimum</i> <sup>(15)</sup>							
+	for phytotoxicity	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(5)</sup>	+++	for phytotoxicity	nt	nt	nt	nt	<i>E. lata</i> <sup>(5)</sup>
+	for phytotoxicity	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>							
- -	- - <sup>(11)</sup>	nt	nt	nt	nt	<i>F. mediterranea</i> <sup>(5)</sup>	- -	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(5)</sup>
- -	- - <sup>(11)</sup>	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(5)</sup>							
- -	- - <sup>(11)</sup>	nt	nt	nt	nt	<i>P. angustium</i> <sup>(11)</sup>							
- -	- - <sup>(11)</sup>	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)(11)</sup>							
- +	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(9)(13)</sup>							
- +	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(9)(13)</sup>							
- -	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(10)</sup>	- -	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
- +	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(2)</sup>	- -	nt	nt	- -	nt	nt	<i>E. lata</i> <sup>(1)</sup>
- -	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(2)</sup>							

**Table 9.** (Continued from previous page)

Esca complex						Eutypa dieback							
In vitro	In planta	In field	WP	SS	NU	on Pathogen <sup>c</sup>	In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>
+++	+	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(5)</sup>							
++	nt	nt	nt	nt	nt	<i>F. mediterranea</i> <sup>(11)</sup>							<i>E. lata</i> <sup>(11)(13)</sup>
+++	+++	nt	+++ <sup>(13)</sup>	nt	nt	<i>Pa. chlamydospora</i> <sup>(11)(13)</sup>	- +	nt	nt	nt	nt	nt	
+++	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>							
+++	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(13)</sup>	+++ <sup>(1)</sup> + <sup>(13)</sup>	nt	nt	- + <sup>(1)</sup>	nt	nt	<i>E. lata</i> <sup>(1)(13)</sup>
+++	+++	nt	+++	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>							
nt	nt	nt	nt	nt	- -	<i>Phaeoacremonium</i> spp. <sup>(12)</sup>	- -	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
nt	nt	nt	nt	nt	- -	<i>Phaeoacremonium</i> spp. <sup>(12)</sup>	- -	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
							+++	nt	nt	- +	nt	nt	<i>E. lata</i> <sup>(1)</sup>
							+	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
							- -	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
++	- -	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(13)</sup>	- -	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
- +	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>	- +	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(13)</sup>
- -	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(13)</sup>	- -	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(13)</sup>
- -	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>							
++	- -	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(13)</sup>	++	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(13)</sup>
- -	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>							
++	+	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(13)</sup>	++	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(13)</sup>
++	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>							
nt	nt	+++	nt	+++	nt	for foliar symptoms reduction <sup>(9)</sup>							
++	++	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(13)</sup>	++	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(13)</sup>
++	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>							
nt	++	nt	+++	nt	nt	<i>Pa. chlamydospora</i> <sup>(13)</sup>							

**Table 10.** Biostimulants and plant elicitors tested toward the three main grapevine trunk diseases (GTDs). Reported for each active ingredient are: the efficiency<sup>a</sup> recorded in the relative test (in vitro, in planta, and in field); the results when tested for wound protection (WP), symptom suppression (SS), or for nursery use (NU); the pathogens used in tests; and in superscript brackets, the country in which the studies were carried out.<sup>b</sup> The number in superscript brackets on an efficiency result indicates the country in which the test was carried out (see supplementary file for references regarding a specific a.i.).

Biostimulant	Active ingredient	Botryosphaeria dieback						
		In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>
Commercial	Aluminum lignin sulfate, gluconic acid, microelements (Brotomax)							
Elicitor	Amino acids, peptides, peptones (Fitostim)							
	Glutathione, oligosaccharine (Kendral)							
	<i>Aschophyllum nodosum</i> extract (Marvita)							
Phytoalexins	2-hydroxybenzoic acid							
	Benzothiadiazole							
	Resveratrol							
	Pterostilbene							
	P-coumaric acid							
Lab mix	Resveratrol + phosphorous acid							
	Pterostilbenes + phosphorous acid							
	Copper oxychloride + gluconates							

(Continued on next page)

<sup>a</sup> Efficiency: - - ineffective; - + less effective; + moderately efficient; ++ efficient; +++ highly efficient; nt, not tested.

<sup>b</sup> Countries: (1) Australia; (2) Austria; (3) Chile; (4) Egypt; (5) France; (6) Germany; (7) Greece; (8) Hungary; (9) Italy; (10) New Zealand; (11) Portugal; (12) South Africa; (13) Spain; (14) Switzerland; (15) U.S.A.

<sup>c</sup> Abbreviations used for pathogen species: *B.* = *Botryosphaeria*; *D.* = *Diplodia*; *L.* = *Lasiodiplodia*; *N.* = *Neofusicoccum*; *Pa.* = *Phaeoconiella*; *Pm.* = *Phaeoacremonium*; *F.* = *Fomitiporia*; *S.* = *Stereum*; *P.* = *Pleurostomophora*; *T.* = *Togninia*; *C.* = *Cryptovalsa*; *Di.* = *Diatrypella*; *E.* = *Eutypa*; *Eu.* = *Eutypella*; *Li.* = *Libertella*.

**Table 11.** List of the bacterial biocontrol agents tested, singly or mixed, against the three main grapevine trunk diseases both in field and nursery

Genus	Species	Tested on			Tested in		
		Botryosphaeria dieback	Esca complex	Eutypa dieback	Lab	Vineyard	Nursery
<i>Acinetobacter</i>	<i>A. radioresistens</i>	x	x		x		
<i>Bacillus</i>	<i>B. amyloliquefaciens</i>	x	x		x		
	<i>B. cereus</i>			x	x		
	<i>B. firmus</i>	x	x		x		
	<i>B. ginsengihumi</i>	x	x		x		
	<i>B. licheniformis</i>	x	x		x		
	<i>B. pumilus</i>	x	x		x		
	<i>B. subtilis</i>	x	x	x	x	x	x
	<i>B. thuringiensis</i>			x	x		
	<i>Bacillus</i> sp.	x		x	x		
	<i>B. reuszeri</i>	x	x		x		
<i>Burkholderia</i>	<i>B. phytofirmans</i>	x			x		
<i>Curtobacterium</i>	<i>Curtobacterium</i> sp.	x	x		x		
<i>Enterobacter</i>	<i>E. cowanii</i>	x	x		x		
	<i>Enterobacter</i> sp.	x			x		
<i>Erwinia</i>	<i>E. eribicola</i>	x			x		
<i>Paenibacillus</i>	<i>P. barengoltzii</i>	x			x		
	<i>P. illinoisensis</i>	x	x		x		
	<i>P. polymyxa</i>	x			x		
	<i>P. turicensis</i>	x			x		
	<i>Paenibacillus</i> sp.	x			x		
	<i>P. agglomerans</i>	x			x		
<i>Pantoea</i>	<i>P. agglomerans</i>				x		
<i>Pseudomonas</i>	<i>P. aeruginosa</i>			x	x		
	<i>P. fluorescens</i>			x	x		
	<i>Pseudomonas</i> sp.			x	x		
<i>Serratia</i>	<i>S. plymuthica</i>			x	x		
<i>Stenotrophomonas</i>	<i>S. maltophilia</i>			x	x		
<i>Streptomyces</i>	<i>Streptomyces</i> spp.			x	x		
<i>Xanthomonas</i>	<i>Xanthomonas</i> sp.	x				x	
Bacterial mix	<i>Azospirillum</i> sp. + <i>Pseudomonas</i> sp. + <i>Bacillus</i> sp.		x			x	

**Table 10.** (Continued from previous page)

Esca complex						Eutypa dieback							
In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>	In vitro	In planta	In field	WP	SS	NU	Pathogen <sup>c</sup>
+ <sup>(10)(13)</sup>	--	nt	nt	--	nt	<i>Pa. chlamydospora</i> <sup>(9)(10)(13)</sup>	nt	nt	+	nt	nt	nt	<i>E. lata</i> <sup>(1)</sup>
+	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(10)(13)</sup>							
nt	--	nt	nt	--	nt	<i>Pa. chlamydospora</i> <sup>(9)</sup>							
nt	--	nt	nt	--	nt	<i>Pa. chlamydospora</i> <sup>(9)</sup>							
nt	--	nt	nt	--	nt	<i>Pa. chlamydospora</i> <sup>(9)</sup>							
nt	nt	nt	nt	- + <sup>(7)</sup>	nt		nt	nt	nt	nt	- + <sup>(7)</sup>	nt	
nt	--	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(1)</sup>							
++	nt	nt	nt	nt	nt	<i>F. punctata</i> <sup>(9)</sup>	--	nt	nt	nt	nt	nt	<i>L. blepharis</i> <sup>(9)</sup>
--	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(9)</sup>							
- +	nt	nt	nt	nt	nt	<i>P. angustium</i> <sup>(11)</sup>							
-- <sup>(9)</sup> - + <sup>(11)</sup>	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(9)(11)</sup>							
++	nt	nt	nt	nt	nt	<i>S. hirsutum</i> <sup>(9)</sup>							
++	nt	nt	nt	nt	nt	<i>F. punctata</i> <sup>(9)</sup>	++	nt	nt	nt	nt	nt	<i>L. blepharis</i> <sup>(9)</sup>
++	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(9)</sup>							
++	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(9)</sup>							
++	nt	nt	nt	nt	nt	<i>S. hirsutum</i> <sup>(9)</sup>							
- +	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(13)</sup>	- +	nt	nt	nt	nt	nt	<i>E. lata</i> <sup>(9)</sup>
--	nt	nt	nt	nt	nt	<i>P. inflatipes</i> <sup>(13)</sup>							
- +	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(13)</sup>							
- +	nt	nt	nt	nt	nt	<i>S. hirsutum</i> <sup>(13)</sup>							
- +	nt	nt	nt	nt	nt	<i>F. punctata</i> <sup>(9)</sup>	+++	nt	nt	nt	nt	nt	<i>L. blepharis</i> <sup>(9)</sup>
+	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(9)</sup>							
+	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(9)</sup>							
- +	nt	nt	nt	nt	nt	<i>S. hirsutum</i> <sup>(9)</sup>							
--	nt	nt	nt	nt	nt	<i>F. punctata</i> <sup>(9)</sup>	+++	nt	nt	nt	nt	nt	<i>L. blepharis</i> <sup>(9)</sup>
+	nt	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(9)</sup>							
+	nt	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(9)</sup>							
+	nt	nt	nt	nt	nt	<i>S. hirsutum</i> <sup>(9)</sup>							
- +	nt	nt	nt	+	nt	<i>F. mediterranea</i> <sup>(9)</sup>							
- +	nt	nt	nt	+	nt	<i>Pm. minimum</i> <sup>(9)</sup>							
+	nt	nt	nt	+	nt	<i>Pa. chlamydospora</i> <sup>(9)</sup>							

different mechanisms of action of the three compounds. Similarly, foliar applications of a brown seaweed extract containing CaCl<sub>2</sub> + Mg(NO<sub>3</sub>)<sub>2</sub> to Esca-affected vines (GLSD) reduced the incidence and severity of foliar symptoms (Calzarano et al. 2014, 2017, 2018). In this case, it was suggested that the treatments acted by interfering with the mechanisms involved in the development of leaf symptoms, without any phytotoxicity or plant growth inhibition.

Some products belonging to the biostimulants and elicitors group were also tested (Table 10). This complex category, which is now under revision at EU regulation level, includes some commercial products with very different composition (Di Marco and Osti 2009) and others recognized in the scientific literature that are able to either stimulate plant defense system like phytoalexins (Mazzullo et al. 2000; Santos et al. 2006) or induce systemic acquired resistance as elicitors (Darrietort and Lecomte 2007; Santos et al. 2006). None of the assayed commercial products effectively reduced Esca complex foliar symptoms expression. Within elicitors, the application of 2-hydroxybenzoic acid, an aromatic compound able to stimulate systemic acquired resistance (SAR), seems to reduce foliar symptoms expression for both Esca complex diseases and Eutypa dieback (Darrietort and Lecomte 2007). The phytoalexins resveratrol, p-coumaric acid, and pterostilbene were tested in vitro only with the vascular pathogens involved in the Esca complex, giving different results according to the phytoalexin and the pathogen. For example, pterostilbene inhibited all of the tested Esca complex pathogens (Mazzullo et al. 2000), while resveratrol showed different results according to pathogen (Mazzullo et al. 2000; Santos et al. 2006).

### A Brief Introduction to Biological Control Agents (BCAs) Used for GTDs

Different reasons have led researchers to test BCAs to control GTDs beside chemical a.i.s. The most efficient a.i.s (benzimidazoles, for instance) are not able to protect pruning wounds throughout the entire period they are susceptible to GTD infections, which varies

from 2 to 4 months (Kotze et al. 2011). The use of potential BCAs in colonizing woody tissues and maintaining a broad spectrum activity against GTD pathogens for extended periods could be a strategy for wound protection in vineyards. Furthermore, the positive effects of some microorganisms in host-plant physiology, such as the resistance to biotic or abiotic stresses or systemic induced resistance (SIR), are well known (Berg 2009; Handelsman and Stabb 1996; Pal and McSpadden Gardener 2006). Consequently, the main aims of BCA trials were i) to prevent GTD pathogen contamination in nurseries, in which several steps of the plant production process are critical for the spread of GTD pathogens (Aroca et al. 2010; Gramaje and Armengol 2011; Gramaje and Di Marco 2015; Waite et al. 2013; Waite and Morton 2007), and ii) to evaluate BCAs for durable pruning wound protection in vineyards. In particular, nursery trials also allowed the evaluation of the putative BCA effects on plant growth, induced or improved disease resistance, and globally on the development of healthier and sound vines (Di Marco and Osti 2007; Fourie et al. 2001).

### Strategies and Main Aims of Potential BCA Tests Against GTDs

Similar to a.i.s, different BCAs have been tested in vitro, in planta, in the field, and during the nursery plant production process (Tables 11–12). Dual cultures in vitro are the primary means to detect antagonistic activity of a potential BCA (Di Marco et al. 2002; Haidar et al. 2016; Hunt et al. 2001; John et al. 2004; Kotze et al. 2011; McMahan et al. 2001; Mutawila et al. 2015; Schmidt et al. 2001). In vitro tests also allow us to decipher some aspects of the biocontrol mechanisms such as the detoxification of phytotoxins (e.g., eutypine, 4-hydroxybenzaldehyde, and 3-phenyllactic acid) considered to be involved in the expression of foliar symptoms in some GTDs (Christen et al. 2005; Tey-Rulh et al. 1991). BCAs have also been tested as wound protectants to limit annual contaminations in the field (Di Marco et al. 2002, 2004; Halleen et al. 2010; John et al. 2005; Kotze et al. 2011; Mutawila et al. 2011, 2015; Pitt

**Table 12.** List of the fungal biocontrol agents tested, singly or mixed, against the three main grapevine trunk diseases both in field and nursery

Genus	Species	Tested on			Tested in		
		Botryosphaeria dieback	Esca complex	Eutypa dieback	Lab	Vineyard	Nursery
<i>Aureobasidium</i>	<i>Aureobasidium</i> spp.		x		x		
<i>Epicoccum</i>	<i>Epicoccum</i> spp.		x		x		
	<i>E. purpurascens</i>	x			x		
<i>Fusarium</i>	<i>F. lateritium</i>		x	x	x	x	
	<i>F. lateritium</i> mutant Benzimidazole resistant			x	x		
	<i>F. proliferatum</i>	x			x		
<i>Pythium</i>	<i>P. oligandrum</i>		x		x		
<i>Trichoderma</i>	<i>T. atroviride</i>	x	x	x	x	x	x
	<i>T. atroviride</i> mutant Benzimidazole resistant	x	x	x	x	x	
	<i>T. gamsii</i> (ex <i>T. viride</i> )		x		x		
	<i>T. hamatum</i>		x		x		
	<i>T. harzianum</i>	x	x	x	x	x	x
	<i>T. harzianum</i> mutant Benzimidazole resistant	x	x	x	x		
	<i>T. koningii</i>			x		x	
	<i>T. longibrachiatum</i>	x	x		x	x	x
	<i>T. polysporum</i>		x	x	x		
	<i>Trichoderma</i> spp.	x					
	<i>T. asperellum</i> + <i>T. gamsii</i>		x	x		x	
Mix	<i>Trichoderma</i> + <i>Gliocladium</i>			x			x

**Table 13.** Bacterial control agents (BCAs) tested toward the three main grapevine trunk diseases (GTDs). Reported for each active ingredient are: the efficiency<sup>a</sup> recorded in the relative test (in vitro, in planta, and in field); the results when tested for wound protection (WP) or nursery use (NU); the pathogens used in tests; and in superscript brackets, the country in which the studies were carried out.<sup>b</sup> The number in superscript brackets on an efficiency result indicates the country in which the test was carried out (see supplementary file for references regarding a specific BCA).

Genera	Species	Botryosphaeria dieback					Pathogen <sup>c</sup>
		In vitro	In planta	In field	WP	NU	
<i>Acinetobacter</i>	<i>A. radioresistens</i>	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
<i>Bacillus</i>	<i>B. amyloliquefaciens</i>	+	nt	nt	nt	nt	<i>L. mediterranea</i> <sup>(9)</sup>
	<i>B. cereus</i>						
	<i>B. firmus</i>	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
	<i>B. ginsengihumi</i>	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
	<i>B. licheniformis</i>	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
	<i>B. pumilus</i>	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
	<i>B. subtilis</i>	+++ <sup>(12)</sup>	nt	nt	++ <sup>(12)</sup>	nt	<i>D. seriata</i> <sup>(12)</sup>
		+++ <sup>(12)</sup>	nt	nt	++ <sup>(12)</sup>	nt	<i>L. theobromae</i> <sup>(12)</sup>
		+++ <sup>(12)</sup>	nt	nt	+++ <sup>(12)</sup>	nt	<i>N. australe</i> <sup>(12)</sup>
		+++ <sup>(12)</sup>	nt	nt	+ <sup>(12)</sup>	nt	<i>N. parvum</i> <sup>(12)</sup>
	<i>B. thuringiensis</i>						
<i>Bacillus</i> sp.		nt	+	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
<i>Brevibacillus</i>	<i>B. reuszeri</i>	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
<i>Burkholderia</i>	<i>B. phytofirmans</i>		--	nt	nt	nt	<i>D. seriata</i> <sup>(5)</sup>
			+-	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
<i>Curtobacterium</i>	<i>Curtobacterium</i> sp.	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
<i>Enterobacter</i>	<i>E. cowanii</i>	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
	<i>Enterobacter</i> sp.	nt	+++	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
<i>Erwinia</i>	<i>E. herbicola</i>						
<i>Paenibacillus</i>	<i>P. barengoltzii</i>	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
	<i>P. illinoisensis</i>	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
	<i>P. polymyxa</i>	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
	<i>P. turicensis</i>	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
	<i>Paenibacillus</i> sp.	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
<i>Pantoea</i>	<i>P. agglomerans</i>	nt	+++	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
<i>Pseudomonas</i>	<i>P. aeruginosa</i>						
	<i>P. fluorescens</i>						
	<i>Pseudomonas</i> sp.						
<i>Serratia</i>	<i>S. plymuthica</i>						
<i>Stenotrophomonas</i>	<i>S. maltophilia</i>						
<i>Streptomyces</i>	<i>Streptomyces</i> spp.						
<i>Xanthomonas</i>	<i>Xanthomonas</i> sp.	nt	--	nt	nt	nt	<i>N. parvum</i> <sup>(5)</sup>
Bacterial mix	<i>Azospirillum</i> sp. + <i>Pseudomonas</i> sp. + <i>Bacillus</i> sp.						

(Continued on next page)

<sup>a</sup> Efficiency: -- ineffective; + less effective; + moderately efficient; ++ efficient; +++ highly efficient; nt, not tested.<sup>b</sup> Countries: (1) Australia; (2) Austria; (3) Chile; (4) Egypt; (5) France; (6) Germany; (7) Greece; (8) Hungary; (9) Italy; (10) New Zealand; (11) Portugal; (12) South Africa; (13) Spain; (14) Switzerland; (15) U.S.A.<sup>c</sup> Abbreviations used for pathogen species: *B.* = *Botryosphaeria*; *D.* = *Diplodia*; *L.* = *Lasiodiplodia*; *N.* = *Neofusicoccum*; *Pa.* = *Phaeoconiella*; *Pm.* = *Phaeoacremonium*; *F.* = *Fomitiporia*; *S.* = *Stereum*; *P.* = *Pleurostomophora*; *T.* = *Togninia*; *C.* = *Cryptovalsa*; *Di.* = *Diatrypella*; *E.* = *Eutypa*; *Eu.* = *Eutypella*; *Li.* = *Libertella*.

et al. 2012). Among these, *Trichoderma* species and strains present in registered products actually bring a clear reduction in the GLSD foliar symptoms, being at present the only tool for in field Esca complex reduction (GLSD) (Mounier et al. 2016). For BCA persistence within the trunk, the viability and colonizing capability of BCAs in grapevine woody tissues have been studied (Di Marco et al. 2002, 2004; Halleen et al. 2010; Hunt et al. 2001). Several BCA-based commercial products and different BCA strains have been tested, taking into consideration different grapevine cultivars and BCA distribution methods (Bourbos and Barboupoulos 2005; Di Marco et al. 2002, 2004; Halleen et al. 2010; John et al. 2005; Kotze et al. 2011; Mutawila et al. 2011, 2015, 2016).

In planta tests under controlled conditions have been carried out (Di Marco et al. 2002, 2004; John et al. 2005; Haidar et al. 2016; Yacoub et al. 2016) to study grapevine-BCA-pathogen interactions, especially the role of the BCA in systemic induction of resistance in grapevines.

Another useful application of BCA is in the nursery. Studies of BCA treatments often focus on their effects on the physiology of the plant such as root development (Di Marco et al. 2004; Fourie et al. 2001; Pertot et al. 2016), plant growth rate (Fourie and Halleen 2006; Fourie et al. 2001), and overall plant quality (Di Marco and Osti 2007; Fourie and Halleen 2004). The treatments are usually compared with a.i.s or other preventive/sanitation treatments, such as the hot water treatment (HWT) that is often used during the grapevine plant production process (Fourie and Halleen 2004, 2006; Pertot et al. 2016).

Similarly to the a.i. tests, the GTD pathogens most used in BCA tests were *D. seriata*, *L. theobromae*, and *N. parvum* for Botryosphaeria dieback, while *Pa. chlamydospora* and *E. lata* were used in tests on control of Esca complex and Eutypa dieback (see Table 6).

### The Most Efficient Biocontrol Agents Against GTDs: Antagonistic Bacteria and Fungi

Since 2000, more than 40 BCAs have been tested against Esca complex, Botryosphaeria dieback, and Eutypa dieback pathogens (Tables 13 and 14). For each BCA, the efficiency in relation to the kind of tests (in vitro, in planta, in the field), the application strategy (wound protection, control in nursery process), and the targeted GTD pathogens are reported in Tables 13 and 14, taking into account the geographical area where the tests were carried out.

Among bacterial BCAs, *Bacillus subtilis* was the most tested toward GTDs. Its ascertained in vitro efficiency against GTD pathogens was confirmed as wound protectant, with different biocontrol degrees according to both the GTD and the selected pathogens (Halleen et al. 2010; Kotze et al. 2011; Schmidt et al. 2001). In nurseries, *B. subtilis* reduced the incidence of the vascular pathogen associated to Esca complex (Petri disease), but the severity of internal symptoms increased (Fourie and Halleen 2004). However, no effects of the *B. subtilis* treatment on callus formation or on the percentage of certifiable plants have been reported. Within the other assayed bacterial genera, some strains of *Enterobacter* spp. and *Pantoea*

**Table 13.** (Continued from previous page)

Esca complex						Eutypa dieback					
In lab	In planta	In field	WP	NU	Pathogen <sup>c</sup>	In lab	In planta	In field	WP	NU	Pathogen <sup>c</sup>
- -	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>						
+	nt	nt	nt	nt	<i>F. mediterranea</i> <sup>(9)</sup>						
+	nt	nt	nt	nt	<i>Pm. minimum</i> <sup>(9)</sup>						
+	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(9)</sup>						
+++	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>	- +	nt	nt	nt	nt	<i>E. lata</i> <sup>(9)</sup>
++	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>						
+	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>						
- +	- -	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>						
nt	nt	nt	nt	- + <sup>(12)</sup>	<i>Phaeoacremonium</i> spp. <sup>(12)</sup>	- - <sup>(12)</sup> /+++ <sup>(6)(12)</sup>	++ <sup>(6)</sup>	nt	+ <sup>(1)</sup> /++ <sup>(12)</sup>	nt	<i>E. lata</i> <sup>(1)(6)(12)</sup>
+++ <sup>(12)</sup>	nt	nt	+ <sup>(12)</sup>	- + <sup>(12)</sup>	<i>Pa. chlamydospora</i> <sup>(12)</sup>						
						- +	nt	nt	nt	nt	<i>E. lata</i> <sup>(6)</sup>
						- +	nt	nt	nt	nt	<i>E. lata</i> <sup>(6)</sup>
+	++	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>						
- -	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>						
- +	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>						
++	+++	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>	+++	nt	nt	nt	nt	<i>E. lata</i> <sup>(6)</sup>
						+++	nt	nt	nt	nt	<i>E. lata</i> <sup>(6)</sup>
+	++	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>						
++	+++	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>						
- +	++	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>						
						- +	nt	nt	nt	nt	<i>E. lata</i> <sup>(6)</sup>
						- +	nt	nt	nt	nt	<i>E. lata</i> <sup>(6)</sup>
						- +	nt	nt	nt	nt	<i>E. lata</i> <sup>(6)</sup>
						+	nt	nt	nt	nt	<i>E. lata</i> <sup>(6)</sup>
						- -	nt	nt	nt	nt	<i>E. lata</i> <sup>(6)</sup>
						+	nt	nt	nt	nt	<i>E. lata</i> <sup>(6)</sup>
nt	nt	nt	nt	- -	<i>Phaeoacremonium</i> spp. <sup>(12)</sup>						
nt	nt	nt	nt	- -	<i>Pa. chlamydospora</i> <sup>(12)</sup>						



agglomerans reduced necrotic lesions when coinoculated in planta with *N. parvum* (Haidar et al. 2016).

Regarding fungi, the majority of the reviewed trials were carried out with *Trichoderma* spp. (Di Marco and Osti 2007; Di Marco et al. 2004; Fourie and Halleen 2004, 2006; Fourie et al. 2001; Pertot et al. 2016). Less-tested were some antagonistic and nonpathogenic *Fusarium* spp. and *Pythium oligandrum* within the Oomycetes. Within the eight *Trichoderma* species, tested singly or in mixtures, *T. atroviride* and *T. harzianum* were the most studied and are currently present in several commercial products. Besides competition for space and nutrients, *Trichoderma* spp. were able to produce both volatile (Hunt et al. 2001; John et al. 2004) and nonvolatile antibiotics, with various effects on fungal hyphae (lysis, degeneration, mycoparasitism, etc.) (Hunt et al. 2001; John et al. 2004; Kotze

et al. 2011; Mutawila et al. 2015). *Trichoderma* spp. generally showed high efficiency in wound protection toward all GTD pathogens, were able to colonize the wood of pruned canes and remained viable for up to one year in the greenhouse (Di Marco et al. 2002) and up to 8 months under field conditions (Di Marco et al. 2002, 2004; Halleen et al. 2010; John et al. 2008). Intensity of colonization was more related to the grapevine cultivar than to the strain tested (Mutawila et al. 2011). Moreover, the influence of vine physiological status on *Trichoderma* colonization ability was reported and dormancy break is regarded as the best time for applications (John et al. 2005; Mutawila et al. 2016). Interestingly, a *T. asperellum* and *T. gamsii* mix (Remedier) as well as *T. atroviride* strain I-1237 (Mounier et al. 2016) reduced the incidence of and mortality in Esca complex affected vineyards (GLSD, Esca proper) starting from the

**Table 14.** Fungal control agents tested toward the three main grapevine trunk diseases (GTDs). Reported for each active ingredient are: the efficiency<sup>a</sup> recorded in the relative test (in vitro, in planta, and in field); the results when tested for wound protection (WP) or nursery use (NU); the pathogens used in tests; and in superscript brackets, the country in which the studies were carried out.<sup>b</sup> The number in superscript brackets on an efficiency result indicates the country in which the test was carried out (see supplementary file for references regarding a specific a.i.).

Genus	Species	Botryosphaeria dieback					Pathogen <sup>c</sup>
		In vitro	In planta	In field	WP	NU	
<i>Aureobasidium</i>	<i>Aureobasidium</i> spp.						
<i>Chaetomium</i>	<i>Chaetomium</i> spp.	++					<i>D. seriata</i> <sup>(5)</sup>
<i>Epicoccum</i>	<i>Epicoccum</i> spp.	++					<i>N. parvum</i> <sup>(5)</sup>
<i>Fusarium</i>	<i>E. purpurascens</i> <i>F. lateritium</i>	++	nt	nt	nt	nt	<i>L. theobromae</i> <sup>(9)</sup>
<i>Pythium</i>	<i>F. lateritium</i> mutant Benzimidazole resistant						
<i>Trichoderma</i>	<i>P. oligandrum</i>	++	nt	nt	nt	+	<i>B. dothidea</i> <sup>(10)</sup>
	<i>T. atroviride</i>	++	nt	nt	nt	+	<i>B. stevensii</i> <sup>(10)</sup>
		++	++	nt	nt	nt	<i>D. corticola</i> <sup>(13)</sup>
		+++	nt	nt	+++	+(10)	<i>D. seriata</i> <sup>(10)(12)</sup>
		+++ <sup>(12)</sup>	nt	nt	+++	nt	<i>L. theobromae</i> <sup>(12)</sup>
		+++ <sup>(12)/++<sup>(13)</sup></sup>	nt	nt	+++	nt	<i>N. australe</i> <sup>(12)(13)</sup>
		++	++	nt	nt	+(10)	<i>N. luteum</i> <sup>(10)(13)</sup>
		++	++	nt	nt	nt	<i>N. mediterraneum</i> <sup>(13)</sup>
		+++ <sup>(12)/++<sup>(13)</sup></sup>	nt	nt	+++	+(10)	<i>N. parvum</i> <sup>(10)(12)(13)</sup>
	<i>T. atroviride</i> mutant Benzimidazole resistant	+++ <sup>(12)</sup>	nt	nt	nt	nt	<i>D. seriata</i> <sup>(12)</sup>
		+++ <sup>(12)</sup>	nt	nt	nt	nt	<i>N. parvum</i> <sup>(12)</sup>
	<i>T. gamsii</i> (ex <i>T. viride</i> )						
	<i>T. hamatum</i>						
	<i>T. harzianum</i>	++	nt	nt	nt	nt	<i>B. stevensii</i> <sup>(10)</sup>
		+++ <sup>(12)</sup>	nt	nt	+(1)/+++ <sup>(12)</sup>	nt	<i>D. seriata</i> <sup>(1)(12)</sup>
		+++ <sup>(12)</sup>	nt	nt	++ <sup>(12)</sup>	nt	<i>L. theobromae</i> <sup>(12)</sup>
		+++ <sup>(12)</sup>	nt	nt	++ <sup>(12)</sup>	nt	<i>N. australe</i> <sup>(12)</sup>
		+++ <sup>(12)</sup>	nt	nt	++ <sup>(12)</sup>	nt	<i>N. parvum</i> <sup>(12)</sup>
	<i>T. harzianum</i> mutant Benzimidazole resistant	+++ <sup>(12)</sup>	nt	nt	nt	nt	<i>D. seriata</i> <sup>(12)</sup>
		+++ <sup>(12)</sup>	nt	nt	nt	nt	<i>N. parvum</i> <sup>(12)</sup>
	<i>T. koningii</i>						
	<i>T. longibrachiatum</i>	+++	nt	nt	nt	nt	<i>D. seriata</i> <sup>(8)</sup>
	<i>T. polysporum</i>						
	<i>Trichoderma</i> spp.	+++	nt	nt	nt	nt	<i>D. seriata</i> <sup>(8)</sup>
	<i>T. asperellum</i> + <i>T. gamsii</i>						
Genera mix	<i>Trichoderma</i> + <i>Gliocladium</i>						

(Continued on next page)

<sup>a</sup> Efficiency: - - ineffective; - + less effective; + moderately efficient; ++ efficient; +++ highly efficient; nt, not tested.

<sup>b</sup> Countries: (1) Australia; (2) Austria; (3) Chile; (4) Egypt; (5) France; (6) Germany; (7) Greece; (8) Hungary; (9) Italy; (10) New Zealand; (11) Portugal; (12) South Africa; (13) Spain; (14) Switzerland; (15) U.S.A.

<sup>c</sup> Abbreviations used for pathogen species: *B.* = *Botryosphaeria*; *D.* = *Diplodia*; *L.* = *Lasiodiplodia*; *N.* = *Neofusicoccum*; *Pa.* = *Phaeomonilla*; *Pm.* = *Phaeoacremonium*; *F.* = *Fomitiporia*; *S.* = *Stereum*; *P.* = *Pleurostomophora*; *T.* = *Togninia*; *C.* = *Cryptovalsa*; *Di.* = *Diatrypella*; *E.* = *Eutypa*; *Eu.* = *Eutypella*; *Li.* = *Libertella*.

second or third year of a multiple-year treatment of a vineyard. This result was mainly attributed to the wound protective effect of *Trichoderma* against new infections by GTD pathogens.

In the nursery, *Trichoderma* species are applied exclusively for the prevention of infections by vascular pathogens associated with Esca complex. Grafted plants previously treated with a *Trichoderma*-based product showed a reduction in the incidence of vascular Esca pathogens comparable to that obtained with quintozone and procymidone applications (Fourie et al. 2001). Similarly, Di Marco and Osti (2007) and Di Marco et al. (2004) reported a significant reduction of *Pa. chlamydospora* wood necrosis in grafted plants treated with *Trichoderma* (Trichodex, Rootshield) at the rooting stage, linking this reduction more to a stronger defense reaction in the treated plants than to the direct effect of *Trichoderma* on the pathogen. Recently, Pertot et al. (2016) reported that use of the selected *T. atroviride* strain SC1 (Vintec) during the hydration steps before cold storage and grafting were the best treatments to prevent *Pm. minimum* and

*Pa. chlamydospora* infections compared with the use of cryptonol (8-hydroxyquinoline sulfate) or iprodione during the same steps. On the contrary, Fourie and Halleen (2004, 2006) considered the exclusive use of *Trichoderma* (Trichoflow-T) not suitable for efficient GTD control in nurseries. As a matter of fact, in none of the nursery trials was the preventive *Trichoderma* application able to fully avoid infections, as expected, but it reduced them strongly. In addition to the antagonistic effect, a significant increase of root biomass was reported in grafted plants treated with *T. harzianum* or *T. longibrachiatum* (Di Marco and Osti 2007; Di Marco et al. 2004; Fourie et al. 2001). On the other hand, Pertot et al. (2016) observed no differences in the root systems between plants treated with a strain of *T. atroviride* and the untreated control. Overall plant quality may be also influenced by *Trichoderma* spp. (Di Marco and Osti 2007; Fourie and Halleen 2004). In previous trials with a different *Trichoderma* strain, the use of *Trichoderma* in all first steps of production (grafting, calusing, rooting) led to more plant growth failures, but also to a higher

**Table 14.** (Continued from previous page)

			Esca complex			Eutypa dieback					
In vitro	In planta	In field	WP	NU	Pathogen <sup>c</sup>	In vitro	In planta	In field	WP	NU	Pathogen <sup>c</sup>
					<i>Cadophora luteo-olivacea</i> <sup>(13)</sup>						
					<i>Pa. chlamydospora</i> <sup>(13)</sup>						
					<i>Pm. minimum</i> <sup>(13)</sup>						
++					<i>Pa. chlamydospora</i> <sup>(5)</sup>						
++					<i>Pm. minimum</i> <sup>(5)</sup>						
					<i>Cadophora luteo-olivacea</i> <sup>(13)</sup>						
					<i>Pa. chlamydospora</i> <sup>(13)</sup>						
					<i>Pm. minimum</i> <sup>(13)</sup>						
+++	nt	nt	nt	nt	for detoxification <sup>(14)</sup>	nt	nt	nt	+	nt	<i>E. lata</i> <sup>(1)</sup>
						+++	nt	nt	nt	nt	for detoxification <sup>(14)</sup>
						nt	+++	nt	nt	nt	<i>E. lata</i> <sup>(15)</sup>
nt	++	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(5)</sup>						
+++	nt	nt	nt	nt	for detoxification <sup>(14)</sup>	+++	nt	nt	nt	nt	for detoxification <sup>(14)</sup>
+++ <sup>(12)</sup>	nt	nt	++ <sup>(12)</sup>	+++ <sup>(9)</sup>	<i>Pm. minimum</i> <sup>(9)(12)</sup>	+++ <sup>(12)</sup>	nt	nt	-- <sup>(5)</sup> /+++ <sup>(12)</sup>	nt	<i>E. lata</i> <sup>(5)(12)</sup>
+++ <sup>(12)</sup>	nt	nt	++ <sup>(12)</sup>	+++ <sup>(9)</sup>	<i>Pa. chlamydospora</i> <sup>(9)(12)</sup>						
nt	nt	++ <sup>(12)</sup>	nt	nt	for viability <sup>(12)</sup>						
+++ <sup>(12)</sup>	nt	nt	++ <sup>(12)</sup>	nt	<i>Pa. chlamydospora</i> <sup>(12)</sup>	+++ <sup>(12)</sup>	nt	nt	nt	nt	<i>E. lata</i> <sup>(12)</sup>
+++	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(9)</sup>						
++	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(9)</sup>						
+++ <sup>(12)</sup>	++ <sup>(9)</sup>	nt	++ <sup>(6)</sup> /+++ <sup>(9)(12)</sup>	++ <sup>(9)(12)</sup>	<i>Pa. chlamydospora</i> <sup>(6)(9)(12)</sup>	+++ <sup>(12)</sup>	++ <sup>(1)</sup>	nt	-- <sup>(5)</sup> /+++ <sup>(1)(7)(12)</sup>	nt	<i>E. lata</i> <sup>(1)(5)(7)(12)</sup>
+++ <sup>(12)</sup>	nt	nt	+ <sup>(6)</sup> /+++ <sup>(12)</sup>	nt	<i>Pm. minimum</i> <sup>(6)(12)</sup>	nt	nt	++ <sup>(12)</sup>	nt	nt	for viability <sup>(12)</sup>
nt	+++ <sup>(9)</sup>	++ <sup>(9)(12)</sup>	nt	nt	for viability <sup>(9)(12)</sup>	+++	nt	nt	nt	nt	for detoxification <sup>(14)</sup>
+++	nt	nt	nt	nt	for detoxification <sup>(14)</sup>						
+++ <sup>(12)</sup>	nt	nt	nt	nt	<i>Pa. chlamydospora</i> <sup>(12)</sup>	+++ <sup>(12)</sup>	nt	nt	nt	nt	<i>E. lata</i> <sup>(12)</sup>
nt	++ <sup>(9)</sup>	nt	+++ <sup>(9)</sup>	++ <sup>(9)</sup>	<i>Pa. chlamydospora</i> <sup>(9)</sup>	nt	nt	nt	++	nt	<i>E. lata</i> <sup>(10)</sup>
nt	+++ <sup>(9)</sup>	++ <sup>(9)</sup>	nt	nt	for viability <sup>(9)</sup>						
+++	nt	nt	nt	nt	for detoxification <sup>(14)</sup>	+++	nt	nt	nt	nt	for detoxification <sup>(14)</sup>
nt	nt	++ <sup>(9)</sup>	++ <sup>(9)</sup>	nt	<i>Pa. chlamydospora</i> <sup>(9)</sup>						
nt	nt	nt	nt	++ <sup>(12)</sup>	<i>Phaeoacremonium</i> spp. <sup>(12)</sup>						
nt	nt	nt	nt	++ <sup>(12)</sup>	<i>Pa. chlamydospora</i> <sup>(12)</sup>						

percentage of certifiable plants at the end of the process, especially for the vines treated at the rooting stage (Di Marco and Osti 2007). Furthermore, *Trichoderma*-treated plants were more vigorous compared with the untreated ones, in terms of increased resistance to environmental stresses and to *Pa. chlamydospora* artificial inoculations.

Among the other fungal BCAs, *Fusarium lateritium* was able to degrade in vitro some phytotoxins involved in expression of GTD foliar symptoms (Christen et al. 2005). It was successfully tested as a wound protectant for the control of *E. lata* infections and the benomyl-resistant mutant strains were especially effective (John et al. 2005; McMahan et al. 2001). The nonpathogenic Oomycete *Pythium oligandrum* was able to stimulate host plant defenses when present in the rhizosphere of different plants (Benhamou et al. 2012) and reduced *Pa. chlamydospora* wood necrosis by a higher induction of the genes normally activated during pathogen infection (Yacoub et al. 2016).

## Discussion

The complexity of GTD management is increasingly evident as research deepens our knowledge on the topic. Agronomical, environmental, ecological, host plant, and pathogen factors are all involved in the development and expression of GTDs, giving few possibilities in building a conceptual model of GTDs effective in all viticulture situations. In view of this complexity, the main goal of this review was to attempt to synthesize the results obtained in the last 15 years by scientific testing in the search for efficient tools, active ingredients, and biocontrol agents as a support for the control of the three main GTDs. The review of scientific literature gave us the chance to report the different approaches used to test and select products and methods for control of GTDs.

Assayed a.i.s ranged from systemic to protectant, from broad-spectrum to selective, and from synthetic to natural. Most of the reviewed a.i.s were tested in vitro while only 50% were further tested in bioassays. This proportion could be partially due to the lack of activity obtained in vitro but also to the complexity in setting up and following a long-term field study, which is unavoidable given the “erratic” appearance of some GTD foliar symptoms. The variation in efficiency often recorded for the same a.i. toward the same GTD pathogens in different studies reflects the well-known complexity of GTDs, but also attests to how other factors (a.i. formulation used, time of application, climatic conditions, etc.) can influence activity. Furthermore, the often very different applied protocols could lead to an under- or overestimation in a.i. efficiency. For example, Bester et al. (2007) linked the low Botryosphaeriaceae incidence recorded after a prochloraz treatment to both the short time of the test and the low stress condition of the vines growing in a glasshouse. Such conditions could have limited the colonizing ability of the inoculated pathogens. Again, a.i. efficiency is surely underestimated in the case of artificial inoculations by mycelial plug since wood pathogens usually infect by spores. In the same way, the spore concentration used in artificial GTD inoculations could also affect the treatment evaluation. If significantly higher than what is normally expected in natural conditions, this high advantage for the pathogen could lead to an underestimation of the efficiency of the chemical or biological treatment. The results of Ayres et al. (2017), observing different efficiency levels for the same a.i. in wound protection trials when different spore concentrations of *E. lata* were used, are in agreement with this remark. Regarding the field trials, some protocols seem too far from extensive and practical application. For wound protection, the only application method already in use by growers and economically feasible is the atomizer application of *Trichoderma*-based commercial products soon after pruning. Most of the other a.i.s were applied directly on pruning cuts by local spraying or hand painting. These modes of a.i. application are economically feasible only in high-value vineyards and are suitable in large vineyards only if special tools are used to speed up the application. Similarly, the pole and trunk injection methods used to distribute a.i.s in some experimental tests become both expensive and time-consuming if applied on a large scale in a vineyard, especially if they do not have a long lasting effect. Thus, field trials that set up applicable and feasible protocols, limiting the variability only to the unavoidable

factors (pathogen, climate, cultivars, etc.), could have a better impact in setting up efficient tests.

Within the synthetic organic compounds, thiophanate-methyl was the most “flexible” a.i., showing good efficiency to control infections by GTD pathogens in vineyards and nurseries in Australia, Chile, New Zealand, and the U.S.A. Even if they do not reach the efficiency levels recorded for banned benomyl and carbendazim, thiophanate-methyl could be a valid substitute useful to manage GTDs in nurseries and to protect vines from new infections in the field. Different sensitivities toward some a.i.s, such as thiophanate-methyl, chlorotalonil, iprodione, procymidone, etc., recorded for Botryosphaeriaceae species and, in some cases, for the vascular Esca complex pathogens were highlighted. Such variability, which can limit the results of a treatment, could be overcome by the use of a.i. combinations with various different modes of action. As a consequence, tests with commercial products of two a.i.s have shown some promising results. Despite their large use in nurseries, some a.i.s were ineffective toward GTD pathogens. For example, 8-hydroxyquinoline sulfate showed inconsistent results when used toward a species of Botryosphaeriaceae, *Pa. chlamydospora* and *Pm. minimum* in soaking water (Gramaje et al. 2009).

Among natural compounds, the efficiency of a garlic+chitosan+vanillin mix for wound protection, and of an inorganic salt and seaweed extract mix in the reduction of GLSD symptoms indicate the possibility of finding tools, based on natural substances, to help and limit losses by GTDs in organic viticulture (Calzarano et al. 2014; Cobos et al. 2015). However, the results obtained up to now with biostimulants showed that they are largely inefficient in vineyards, sometimes leading to an increase in disease incidence, possibly because of faster and/or greater movement of fungal toxins to the leaves. Still, the biostimulants field is developing and it cannot be excluded that other products acting as biostimulants or defense inducers can bring different results and efficacy. The use of biostimulants in nurseries toward vascular pathogens appears to be promising, where they reduced the length of necrotic lesions in potted plants artificially infected with *Pa. chlamydospora* (Calzarano and Di Marco 2007; Di Marco and Osti 2007).

For BCAs, although several microorganisms were tested, currently only *Trichoderma* spp. have been shown to be the most suitable agent for biological control of GTDs, in both field and nursery. The reason for this supremacy probably stems from the synergistic action of different biocontrol mechanisms, in their ecological characteristics (saprotrophic, endophytic) and in the positive effects induced in the host plants (Handelsman and Stabb 1996; Harman 2006; Pal and McSpadden Gardener 2006). All these aspects allow *Trichoderma* spp. to be used in different environmental conditions with almost the same biocontrol efficiency observed in vitro, despite the recorded influence of biotic and abiotic factors. With regard to this, Mutawila et al. (2011, 2016) demonstrated how, following *Trichoderma* field application on different cultivars, the BCA incidence during the season could be highly variable and not related to its biocontrol efficiency toward GTD pathogens. Age of vineyard, cultivar, environmental conditions, the age of the vines of first application (as application at early stages is essential in a preventive control tool), and also phenological grapevine stages were considered to be the main factors responsible for either the variation in biocontrol efficiency or the inconsistent results obtained with *Trichoderma* and, more in general, for the low use of BCAs in vineyards for management of fungal diseases.

*Trichoderma* can be useful as a preventive and long lasting treatment for pruning wound protection. It is important to protect pruning wounds with *Trichoderma*-based treatments as soon as possible in young vineyards to avoid the increase of GTD infections. It also seems important to choose the most suitable *Trichoderma* strains according to the cultivar, the main GTD to control, and the climatic conditions. The contemporary use of different *Trichoderma* strains could reduce the variation in biocontrol levels due to environmental factors (John et al. 2008; Aloï et al. 2015). *Trichoderma* spp. also have several positive effects for nursery applications. Globally, grafted vines had a more developed root system able to support plants in stressful conditions such as transplantation in the vineyard.

Furthermore, *Trichoderma*-treated vines were more resistant to wood colonization by GTD pathogens and stimulate the general defense reaction of the vine.

Among other promising BCAs, the rhizospheric *P. oligandrum* showed high persistence in the root system and stimulated plant defenses against complex pathogens. As reported by Yaacoub et al. (2016), *P. oligandrum* promotes a particular physiological condition called priming, in which the plant is able to mobilize its defense reactions more intensely in response to infection by the Esca complex pathogens.

## Conclusions

All these studies clearly indicate that currently, there are many possible solutions even if none can be seen yet as a straightforward tool to add to the GTD management protocols. The control of Botryosphaeria dieback, the Esca complex diseases, and Eutypa dieback still represent a challenge for both end-users and scientists, even if the knowledge on GTDs so far acquired allowed the identification of some practices to limit the impact of GTDs in vineyards and nurseries (Fontaine et al. 2016a). These practices could widely benefit from the support of a.i.s and BCAs studied by scientists during recent years.

High infection risks in nurseries and vineyards and the lack of curative treatments have encouraged the idea of a transversal strategy for GTD control along the grapevine growing sector. According to Armengol (2014), any GTD control method could be useless if applied only in the nursery or in the vineyard. To date, the best strategy is to start the GTD control in the nursery to obtain healthier vines and to continue the control in the vineyard, limiting contamination by GTD pathogens. Recent studies support this strategy. For example, Larignon and Bruez (2016) demonstrated that after 15 years in a vineyard without any treatment for GTD control, nursery-GTD-treated plants were infected to a similar extent as those in the nursery-untreated group. Furthermore, the beneficial effect of early adoption of good practices to limit the economic impact of GTDs in vineyards was clearly reported by Baumgartner et al. (2014). According to that study, the use of highly efficient control methods could strongly limit the economic impact of GTDs up to its elimination, if the practices are adopted within 3 years of planting the vineyard. Another problem is that of knowledge transfer from scientists to end-users. To date, despite the broad knowledge obtained by research on GTDs, they are not well known in some countries and are often confused with one another by vine-growers, leading to underestimation or to the adoption of wrong practices and treatments. First results of the WINETWORK project (<http://www.winetwork.eu/>), funded by European Community and focused on the knowledge transfer on GTDs, are demonstrating that this transfer fault has boosted many empirical attempts to “solve” the problem, sometimes reporting a tool as “a cure” but without any scientific validation, or continuing to use inefficient a.i.s or practices. In this regard, a recent European survey on nursery practices attested how 11.6% of nurseries still use no fungicides or disinfectants during the entire production process and, when used, the most common one is based on an a.i. that was shown to be nonefficient for the control of GTDs when assayed in scientific trials (Gramaje and Di Marco 2015).

Trials on promising ways to develop tools to support GTD control are ongoing. For example, recent studies are focusing on the translocation of a.i.s inside plants. In particular, carboxylic acid function, methyl groups, amino-acids, or sugars can be added to a.i. molecules to increase their translocation in plant tissues or to transform nonsystemic molecules into a.i.s able to reach the pathogens in woody tissues (Chollet et al. 2004, 2005; Wu et al. 2015).

Regarding BCAs, they are at present the most efficient agents for sustainable disease management, both in the vineyard and nursery. They can be used not only in organic viticulture but also in integrated strategies, for example, by the use of benzimidazole-resistant *Trichoderma* mutants (Mutawila et al. 2015). The integrated a.i.-BCA approach could take advantage of the specific characteristics of both control methods toward GTDs, namely i) the immediate protective effect of the a.i. and ii) the broad-spectrum and lasting efficacy of

the BCA, coupled with their positive effects on plants. For instance, BCAs could limit the effect of biotic and abiotic stress that often leads to higher symptom expression levels (Sosnowski et al. 2011; Spagnolo et al. 2014, 2017; Van Niekerk et al. 2011c). The BCA could have a positive effect through actions on host plant metabolism changes and disorders determined by plant defense responses and fungal toxins, respectively (Abou-Mansour et al. 2015; Burruano et al. 2016; Fontaine et al. 2016b). The study of both the grapevine microbiome and its interaction with grapevine could represent a technological development in the control of these diseases, permitting the identification of new BCAs useful for GTD pathogens and/or the host plant defense system (Pinto and Gomes 2016). Other promising opportunities to limit GTD spread and damage could be linked to the transfer of resistance factors from wild species into new GTD-resistant varieties, as was done for other important grapevine diseases such as downy or powdery mildew (Eibach et al. 2007). In this direction and according to the putative genetic basis of *Vitis vinifera* that confers a different sensibility to GTDs, ongoing studies are addressed to find GTD resistance genetic factors within the Vitaceae family for transfer into cultivated ones. Some promising results have been obtained for Botryosphaeria dieback (Guan et al. 2016). Nevertheless, the introduction of genetically modified grapevine cultivars could show different issues, linked either to the prohibition of their use in some countries or to the required adjustment in terms of cultural and enological practices (Pedneault and Provost 2016).

In conclusion, the perspectives for efficient GTD control could take advantage of both the GTD knowledge reservoir so far built and the ongoing new insights. A holistic view of the problem could be the key to define winning strategies for the control of GTDs, whose model could also be applied for the control of other complex plant diseases.

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### Vincenzo Mondello

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### Aurélie Songy

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### Enrico Battiston

Enrico Battiston is a former Ph.D. student in plant pathology at the University of Florence (Italy) and in plant physiology at the University of Reims Champagne-Ardenne (URCA, France), where he undertook a joint Ph.D. project on the development of an innovative delivery system to enhance the biological activity of active substances for the control of grapevine fungal diseases. He graduated with a B.Sc. in viticulture and enology at the University of Udine (Italy) and a M.Sc. in the same fields at the Hochschule Geisenheim University (Germany). He was introduced to grapevine trunk disease management during his first degree, performing a study on the composting process of grapevine pruning residues, monitoring the survival of *Diaphorte neoviticola* spores in the composted material. Dr. Battiston is actively involved in the development of sustainable practices for plant protection in vineyard and grapevine propagation in nursery with particular regard to the aspects related to grapevine young decline.

### Catia Pinto

Catia Pinto is a research scientist at the Induced Resistance and Plant Bio-Protection (RIBP) research unit at the University of Reims Champagne-Ardenne (URCA, France). Her main research interest is currently focused on the study of grapevine-microbiome interactions and the development of new sustainable solutions for grapevine protection against grapevine trunk diseases. She received a master's degree in molecular genetics in 2011 from the University of Tras-os-Montes and Alto Douro (UTAD) and completed a double Ph.D. degree in biology and molecular genetics in 2017 by a European cotutelle network with

the University of Aveiro (UA, Portugal), University of Reims Champagne-Ardenne (URCA, France), and Biocant (Portugal). Her Ph.D. research was deep analysis of the dynamics of the natural microbial resources from vineyard until wine fermentation through NGS approaches, and to exploit potential beneficial microorganisms from grapevine to grapevine protection, namely to control Botryosphaeriaceae species associated with grapevine trunk diseases.

### Cindy Coppin

Cindy Coppin is a research scientist at the Induced Resistance and Plant Bio-Protection (RIBP) research unit at the University of Reims Champagne-Ardenne (URCA, France). She graduated with a master's degree in enology and viticulture in 2015 from the Institute of Grapevine and Wine Sciences (ISVV), Bordeaux (France). She is working on a network biocontrol project named Advantage that aims to develop new technical management tools for a global management of grapevine trunk diseases at both nurseries and vineyards. While completing her master's thesis at INRA (National Institute for Agricultural Research, Bordeaux), she was strongly involved in the phenotypic characterization of Botryosphaeriaceae species and their interactions with plants.

### Patricia Trotel-Aziz

Patricia Trotel-Aziz is associate professor in the research unit of Induced Resistance and Plant Bioprotection (RIBP) at the University of Reims Champagne-Ardenne (France). Her research activities within the general framework of plant responses to environmental constraints focus on plant-rhizobacterial interactions with pathogens and their virulence factors. She isolated the "PTA" beneficial bacteria and contributes to decipher their impacts on grapevine protection and tolerance against pathogens with different lifestyles through both direct- and indirect- effects (antagonism, detoxification, ISR, ...), and takes also advantage of *Arabidopsis* for functional approaches. She also explored the possibility to reduce the potential toxicity and diffused pollution due to organic pollutants introduced into soil and/or water in vineyards, by using plants associated with beneficial bacteria. She is currently mainly associated with the MALDIVE Chair 'Disease of grapevine wood' at RIBP, aiming to reduce the variability of plant protection in situ by using such beneficial microorganisms.

### Christophe Clément

Christophe Clément a research scientist at the Induced Resistance and Plant Bio-Protection (RIBP) research unit at the University of Reims Champagne-Ardenne (URCA, France). The goal of his research is to decipher plant immunity in response to both biotic and abiotic stresses. This knowledge is further used to trigger plant immunity previously to stress appearance by eliciting/priming plant defense mechanisms, using either beneficial microorganisms or the elicitors they may synthesize. Plant defense response is more deeply investigated in the context of global warming, especially drought and high temperatures.

### Laura Mugnai

Laura Mugnai is associate professor of plant pathology at the Department of Agrifood Production and Environmental Sciences at the University of Florence, where she runs the courses of grapevine plant pathology, diseases caused by fungal agents, and tropical plant pathology. She has conducted or collaborated in research on various aspects of plant pathology, first in forestry, where she focused on taxonomy and described a new intersterility group of *Heterobasidion annosum*, and later in plant pathology of agricultural crops, including olive and grapes. In the last 25 years, she has concentrated on wood diseases of grapevine, in particular the esca complex. On this subject, she has been an invited speaker in Italy and many other grape-growing countries worldwide. She has been responsible for and collaborated in several national and international projects, including EU funded projects. She was co-founder and, for 12 years, chairperson of the subject matter committee on grapevine trunk diseases within the International Society of Plant Pathology (ISPP), for which she organized or co-organized many workshops and



meetings. She was in the management committee as the Italian representative of the COST action on this subject at the European level (COST ManaGTD – FA1303 Sustainable control of grapevine trunk diseases). She is actively engaged in running the activities of the Mediterranean Phytopathological Union and, since 2009, she has been co-chief editor of the international journal *Phytopathologia Mediterranea*.

### Florence Fontaine

Florence Fontaine is a research scientist at the Induced Resistance and Plant Bio-Protection (RIBP) research unit at the University of Reims Champagne-Ardenne (URCA, France). She studied plant physiology and biology at URCA and earned her Ph.D. in biological sciences in 1999, investigating wood quality on oak trees. She has served as a teacher and researcher at URCA since 2002. Since 2002, she has been the head of the grapevine trunk disease (GTD) team, with

main topics of identification of the molecules linked to the aggressiveness of Botryosphaeriaceae, pathogens associated to *Botryosphaeria dieback*, the impact of GTDs on vine physiology including the primary and secondary metabolisms; and the focus on friendly strategies to manage GTDs. During the last 5 years, she was the chair of the COST Action FA1303 entitled “Sustainable control of grapevine trunk diseases” (2013–2017), which includes 120 people from 24 European countries and the leader of the scientific working group on GTDs in the WINETWORK project (2015–2017). She is an active member of the International Council for Grapevine Trunk Diseases and member of the International Organization of Vine and Wine (OIV) expert group “Vine Protection.” Starting in 2017, she is the leader of the Chaire MALDIVE, with a term through 2020, that suggests more research connected with private companies. She is currently teaching plant biology and physiology, plant pathology, and viticulture at the graduate level.

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