Phytopathologia Mediterranea (2018), 57, 3, 369−383
DOI: 10.14601/Phytopathol_Mediterr-23942

REVIEW - 10TH SPECIAL ISSUE ON GRAPEVINE TRUNK DISEASES

Management of grapevine trunk diseases: knowledge transfer, current strategies and innovative strategies adopted in Europe

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Summary. Since the early 1990s, grapevine trunk diseases (GTDs) have posed threats for viticulture. Esca complex, Eutypa- and Botryosphaeria- diebacks, mostly detected in adult vineyards, are currently responsible for considerable economic losses in the main vine-growing areas of the world. Other GTDs, such as Petri- (Esca complex) and Black-foot diseases, are emerging problems in grapevine nurseries (resulting in grafting failures and/or loss of saleable plants) and in young vineyards. The impacts of GTDs in modern viticulture depend on several factors, some related to their complexity, and others linked to host plant characteristics, changes in vineyard management and to the scarcity of simple tools for their control. For these reasons control of GTDs remains difficult, also depending on knowledge transfer from research to field and *vice versa*. This paper outlines the main preventive and curative techniques currently applied, scientifically tested or not that have resulted from the outcomes of "Winetwork", a European Union funded project with special emphasis on the promising and innovative approaches.

Key words: grapevine trunk diseases, control strategies, knowledge transfer, Europe, Winetwork project.

Introduction

Grapevine trunk diseases (GTDs), caused by several taxonomically diverse wood-colonizing fungi with similar symptomatology, epidemiology and economic impacts, are present in all the main vine-growing areas of the world. Esca complex diseases, Eutypa dieback and Botryosphaeria dieback are the main GTDs detected in adult vineyards (Úrbez-Torres, 2011; Bertsch *et al.*, 2013; Fontaine *et al.*, 2016; Gramaje *et al.*, 2018). "Esca" (Mugnai *et al.* 1999), a complex of diseases widespread in Europe, is also the oldest known GTD. Typical foliar symptoms were attributed to wood infections and described in the early 1900s. Until the 1980s, however, this complex was not considered a sanitary threat, since it was found only occasionally in mature vineyards in some countries and was seemingly controlled by sodium arsenite treatments. Eutypa dieback symptoms (formerly dying arm disease) observed in the 1970s in the USA,

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ISSN (print): 0031-9465
ISSN (online): 1593-2095

Australia, France, Hungary, Spain and later described by Moller and Kasimatis (1978) is now considered to be a recurrent disease especially in Australia, California and France. The contribution of Botryosphaeria dieback (including black dead arm caused by *Diplodia mutila*) to the decline of vineyards emerged only at the beginning of the 2000s (Phillips, 2002). This disease was soon recognized to be very damaging in many countries (Úrbez-Torres, 2011). Recently, Úrbez-Torres and collaborators (2013) confirmed the role of *Diaporthe ampelina* as causal agent of another GTD, the Phomopsis dieback in *Vitis vinifera*, previously described by Reddick in the early 1900s in the USA and often accompanied by other *Diaporthe* spp. (Guarnaccia *et al*., 2018). Other emerging GTDs, such as Petri- and Black foot- diseases, are responsible for grafting failures and/or loss of saleable plants in nurseries, and lead to grapevine decline in young vineyards (Edwards and Pascoe, 2004; Halleen *et al*., 2006; Gramaje and Armengol, 2011; Agustí-Brisach and Armengol, 2013).

The global increasing incidence of GTDs, constantly reported in vineyards worldwide during the last 30 years, is probably related to a sum of pathogen, host-plant, environmental (i.e. abiotic stresses) and cultural factors that interact synergistically (see Table 1). For example, GTD pathogens infect vines mainly through wounds and this is enhanced by the long period for which grapevine pruning wounds are susceptible (Eskalen *et al.,* 2007; Serra *et al.,* 2008; Rolshausen *et al.,* 2010; van Niekerk *et al.,* 2011) although with decreasing susceptibility (Kühn *et al.,* 2017). Furthermore, wound-producing cultural practices, such as winter pruning and desuckering, increase the number of wounds and thus the susceptibility of grapevines to infections by GTD pathogens. The sum of these predisposing, contributing and aggravating factors has shaped the current situation and wine-growing areas are now facing problems caused by GTDs (Surico *et al.,* 2004; Fontaine *et al*., 2016; Gramaje *et al*., 2018; Mondello *et al*., 2018).

Research towards greater understanding of GTDs began at the end of 1980s, with studies on Esca,

Table 1. The interacting factors related to GTDs and considered responsible for their spread in modern viticulture.

Factors	Pathogen		Host plant		Environment/cultural practices		Consequences
Predisposing	Wood colonizers, penetration through wound	+	Scarce ability in wound healing and long susceptibility period to GTD pathogens infections	+	Pruning practices performed to maintain the canopy system.	=	GTD infection risks due to both wounds and pathogens periodic presence in vineyard +
Contributing	Several genera/ species with different environmental needs	+	Long asymptomatic phase	+	Changes in cultivation techniques (higher plant density, training system with more pruning wounds). High demand of young plants for re-implant/ new implants	=	Increased GTD infections risks in vineyard. Possibility of unaware GTD spread of asymptomatic GTD-infected plants in new implants +
Aggravating	Life cycle synchronized with current cultural practices in vineyard	+	Sensibility to pathogens' toxins (only in grapevine)	+	No control means availability; Global warming and related pathogens spread in new areas	=	Increasing GTD incidence in field
	=		=		=		=
SUM	High virulence of GTD pathogens in vineyard /nursery		High susceptibility to GTD pathogen colonization and to their damages.		Increasing wine-growing areas with potential GTD problems.		More and more vineyards with GTDs and severe economic losses

firstly in France and Italy and then in South Africa and California (Chiarappa, 2000). To date, although knowledge obtained on GTD symptomatology, etiology and epidemiology has expanded the knowledge on the main and emerging GTDs, their control is still problematic. After the banning of some fungicides with control capability towards GTDs, trials have evaluated other active ingredients (a.i.s), natural compounds and biocontrol agents (BCAs) for control of GTDs (Calzarano *et al*., 2014; 2017a; Calzarano and Di Marco, 2018; Mondello *et al*., 2018). The scarcity of efficient a.i.s, complexity of the diseases and the high infection risks in nurseries and vineyards, suggest that adopting integrated strategies rather than single solutions will be the best approach to limit the incidence of GTDs (Armengol, 2014).

The European Winetwork project: filling the "*information divide*" between laboratory and field

Despite of the large amount of scientific information available, awareness of European viticulturists about GTDs and possible control strategies is often incomplete or divergent. For instance, some nurseries adopt no control measures to limit infections by GTD pathogens during plant production processes or do not use efficient a.i.s (Gramaje and Di Marco, 2015). The poor flow of information between research and end-users (nurserymen, winegrowers, technicians, viticulture consultants) could be considered as the main factor responsible for this "information divide". The Winetwork project (www.winetwork.eu), funded by the European Union through the ISIB-2-2014 call, intended to fill this gap by facilitating "*the exchange of existing knowledge on innovative approaches in agriculture, the supply chain, and rural areas"*. The project also aimed "*… to put existing research into practice and capture creative ideas from the grassroots-level*". Winetwork involved 11 wine-growing regions in seven European Union countries (Spain, Germany, Italy, France, Portugal, Croatia, Hungary) focusing on two grapevine diseases that can jeopardize production: grapevine trunk diseases (GTDs) and *Flavescence dorée.* For GTDs, a specific scientific working group (SWG) of European researchers belonging to the seven involved countries (Joško Kaliterna from Croatia; Florence Fontaine, Philippe Larignon and Vincenzo Mondello from France; Andreas Kortekamp from Germany; Kalman Vaczy from Hungary; Laura Mugnai from Italy; Cecilia Rego from Portugal and Josep Armengol from Spain) summarized the most relevant information arising from research and considered useful to be transferred to the field. Ten technical working groups (TWG), comprised of technicians, consultants and viticulturists, were responsible for updating the "picture" of both diseases in respective areas and collecting information on any conventional, unusual or innovative practices used for disease control. This included methods based on long-established field experience of winegrowers that may not have been scientifically verified. The key role of the project was the

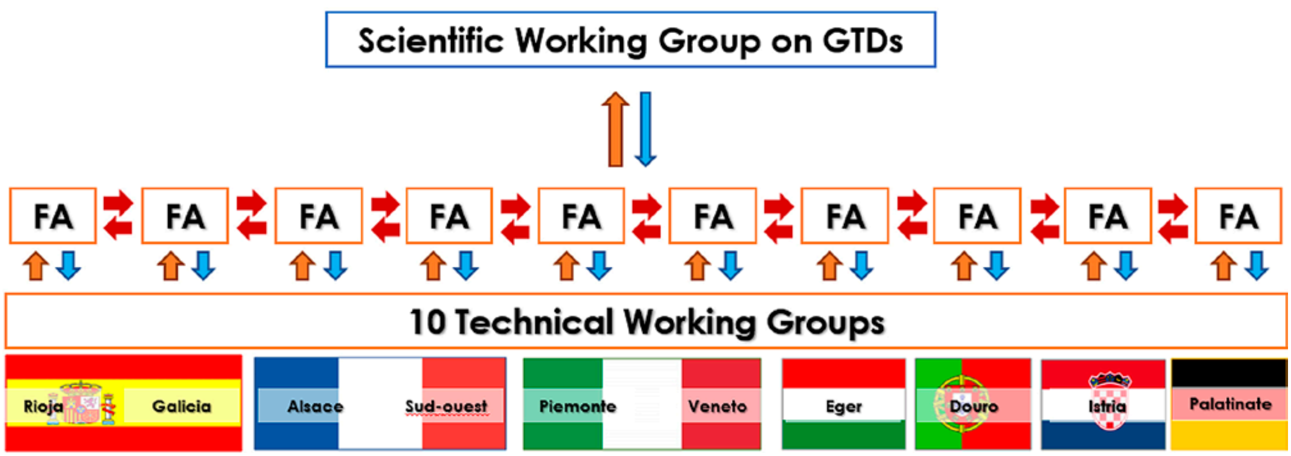

Figure 1. The working scheme of the Winetwork project generated two information flows: vertically between European end-users and scientists and horizontally among European FAs and Technical working groups.

Facilitator Agent (FA). For each viticultural area, the FA was the link between scientists, technical groups and the different countries. This approach generated two information exchange flows, vertical between scientific research and viticulture stakeholders and horizontally among the European wine-growing areas involved (Figure 1). Furthermore, FAs produced several outputs on the topic (short video, flyers, technical articles, etc.), useful to easily share an up-to-date information on GTDs. The Winetwork project is a natural continuation of the COST Action FA1303 but more devoted to the flux exchange with viticulturists. The aim of the present paper is to outline information acquired by the Winetwork project on GTDs, with a focus on the current strategies adopted for disease control in the main European viticultural areas.

GTDs in the seven "Winetwork countries": situation and perception

The Winetwork TWG reports indicated, as expected, an increased occurrence of the three main GTDs in the "Winetwork countries", with an overall average of 15–20% of vines affected. Esca is the most widespread GTD, followed by Eutypa- and Botryosphaeria-diebacks, although there are area-specific differences for particular diseases. Beyond the production losses and the costs for replacing dead plants, occurrence of the diseases in "controlled designation of origin" areas, could also determine the downgrading of individual vineyards if a certain percentage of vines is missing (20%, for French *Appellation d'origine protegée* areas), thus aggravating the economic impact of GTDs. In Germany it was demonstrated that about one percent of vines dies each year leading to economic loss of approx. 50 million euros per year.

Despite the common and widespread presence of GTDs, according to the results of the Winetwork interview of about 200 European viticulturists facing GTDs in their vineyards, knowledge on GTDs remain variable and often quite poor. Although some winegrowers were well-informed of GTD symptomatology, epidemiology and control, others showed either no or incomplete knowledge, especially on diagnosis of diseases. In these cases, GTDs are globally identified as "Esca", often used as a synonym for Trunk Disease.

Eutypa dieback symptoms are easy to recognize when they are accompanied by the typical symptoms (shortened internodes, small and cupped leaves, often with necrotic margins) and easy to distinguish from Botryosphaeria dieback. On the other hand, symptoms of grapevine leaf stripe diseases (GLSD, Esca complex), associated either with vascular pathogens or with canker agents, could be confused with nutrient deficiency or water stress, leading to incorrect diagnosis to unsuitable disease management methods. Other surveys also suggested that viticulturists tend to prefer the use of remedial-oriented control practices rather than preventive ones (Osti *et al.*, 2017).

The interviews also identified the control methods used for GTDs in the Winetwork wine-growing areas. Some growers know and use practices to limit the spread of GTDs in vineyards but complain about the poor efficacy of these methods. Motivated by the increasing incidence of GTDs in their vineyards and by the poor results so far obtained with "conventional" control methods, some viticulturists decided to try alternative techniques to verify their potential for GTD control. Therefore, the actual background of the GTD control is characterized by both science-based and empirical approaches, which are driven by different information sources (scientists, technicians, chemical companies and viticulturists). The result is a framework in which is difficult to identify the most useful agronomic, chemical and biological practices among those proposed for GTD control if the prerequisite of scientific validation is not pursued. The data collected by the TWGs on GTD control strategies used in the seven "Winetwork countries" revealed which methods are currently adopted, highlighting both the common and different traits. These data have provided information on the empirical control attempts, though currently not validated by scientific trials, which could stimulate research in validating, refining or rejecting these approaches.

The most common preventive and post-infection management practices adopted for control of GTDs in European vineyards

The most common practices used for GTD control can be grouped into preventive or post-infection methods according to whether the target vines are not, or are already affected. Here are reported the practices actually applied by growers even if, as indicated, some are not validated by experimental data.

Preventive practices for GTDs control in vineyards

Innovative approaches to vine training

The Guyot-Poussard and the sap flux-respect pruning

The relevance of how vines are pruned for their ability to maintain efficient and uncolonized wood tissue was pointed out by the modified Guyot pruning method proposed by Poussard at the beginning of the 20th Century (Geoffrion and Renaudin, 2002; Lecomte *et al.*, 2012). This method for pruning vines is becoming increasingly popular (SICAVAC, 2008, 2013; Simonit, 2014). The technique was proposed to avoid the decline of grapevine and it is becoming clear that unhindered sap flow is related to reduced desiccation zones in the heads of the trunk. The aim of this pruning method is to avoid disconnection of sap flow by pruning only the upper side of each trunk head or cordons and to build up bipolar growth of two arms with production of shoots only at the end of each arm. Since this approach has been applied in many vine-growing areas for several years, its efficiency could soon become evident on a large scale.

The Guyot-Poussard training system, developed in France at the beginning of the 20th century (Lafon, 1921), is nowadays considered as an "innovative" technique in modern viticulture in Croatia, France, Germany, Italy, and Portugal. It ensures an optimal sap flux because it concentrates pruning wounds in the upper part of the vine arms, leaving a non-altered portion where the sap flow is uninterrupted. As a result, annual wounds from cane removal will also be on the upper part of the arms. The Guyot-Poussard pruned arms also develop horizontally (Figure 2). Currently, two pruning methods are in use: a "mixed" Guyot-Poussard (two spurs in a long cane and the "double" Guyot-Poussard (two spurs and two long canes).

The use of this pruning method to limit the Esca incidence in vineyard was promoted by viticulture advisors in the early 2000s, following the ban of sodium arsenite in France (Geoffrion and Renaudin, 2002, SICAVAC, 2008), but was unfounded. No experiments comparing different pruning systems on single plots have been officially reported to definitely demonstrate effectiveness in disease control. When Lafon (1921) reported the impacts of different pruning systems on apoplectic and declining vines, the data were not taken into sufficient account since GLSD foliar symptoms were not included in the Esca concept at that time. It was only later that Marsais (1923) and Viala (1926) included GLSD in the Esca symptomatology.

The studies of Lafon did not separate Eutypa dieback symptoms, unknown at that time, from those of plants showing other decline symptoms. The widespread of Eutypa dieback (Dubos *et al.*, 1980; Le Gall and Le Gat, 1994) was described only at the end of the 1970s in France (Dubos *et al.*, 1980). The differences observed by Lafon on effects of different pruning systems on apoplectic and declining vines could have also been related to this disease, as shown by recent studies (Dumot *et al.*, 2004; Cahurel, 2009). As for the apoplectic forms, they can also be explained by the implication of this disease, the apoplectic form being a non-specific symptom (Larignon, 2016). The use of Guyot-Poussard vine training to limit esca is not yet fully justified because of the lack of evidence of efficacy in proper experimental trials, but the role of the trellising systems has recently been reported to have a strong, statistically significant influence on symptoms development and vines death (Lecomte *et al.*, 2018).

Multiple trunk training

The multiple trunk technique, that is applied in Virginia USA and other countries to counteract damage caused by crown gall, is also a feasible technique to maintain the advantages of maintaining old root system and replacing the affected trunks with better performing shoots (Smart, 2015; Morton L., personal communication).

Innovative/empirical pruning strategies

Early vs late pruning

It is widely accepted that the infection of vine pruning wounds by aerial inoculum has primary roles on trunk diseases epidemiology (Mugnai *et al.*, 1999; Rolshausen *et al.*, 2010; van Niekerk *et al.*, 2011). Studies on the susceptibility of pruning wounds to GTD pathogens have shown that fresh wounds are more susceptible than older ones (Larignon and Dubos, 2000; Eskalen *et al.*, 2007; Serra *et al.*, 2008; van Niekerk *et al.*, 2011; Elena and Luque, 2016). Nevertheless, there is no agreement about what is the best period for pruning to reduce the risk of infection by GTD pathogens. Spore release periods of these fungi vary according to the pathogen and geographical location, so the risks of pruning wound infection will depend mainly on climatic differences among grapevine regions and pruning times (Gramaje *et al.*, 2018).

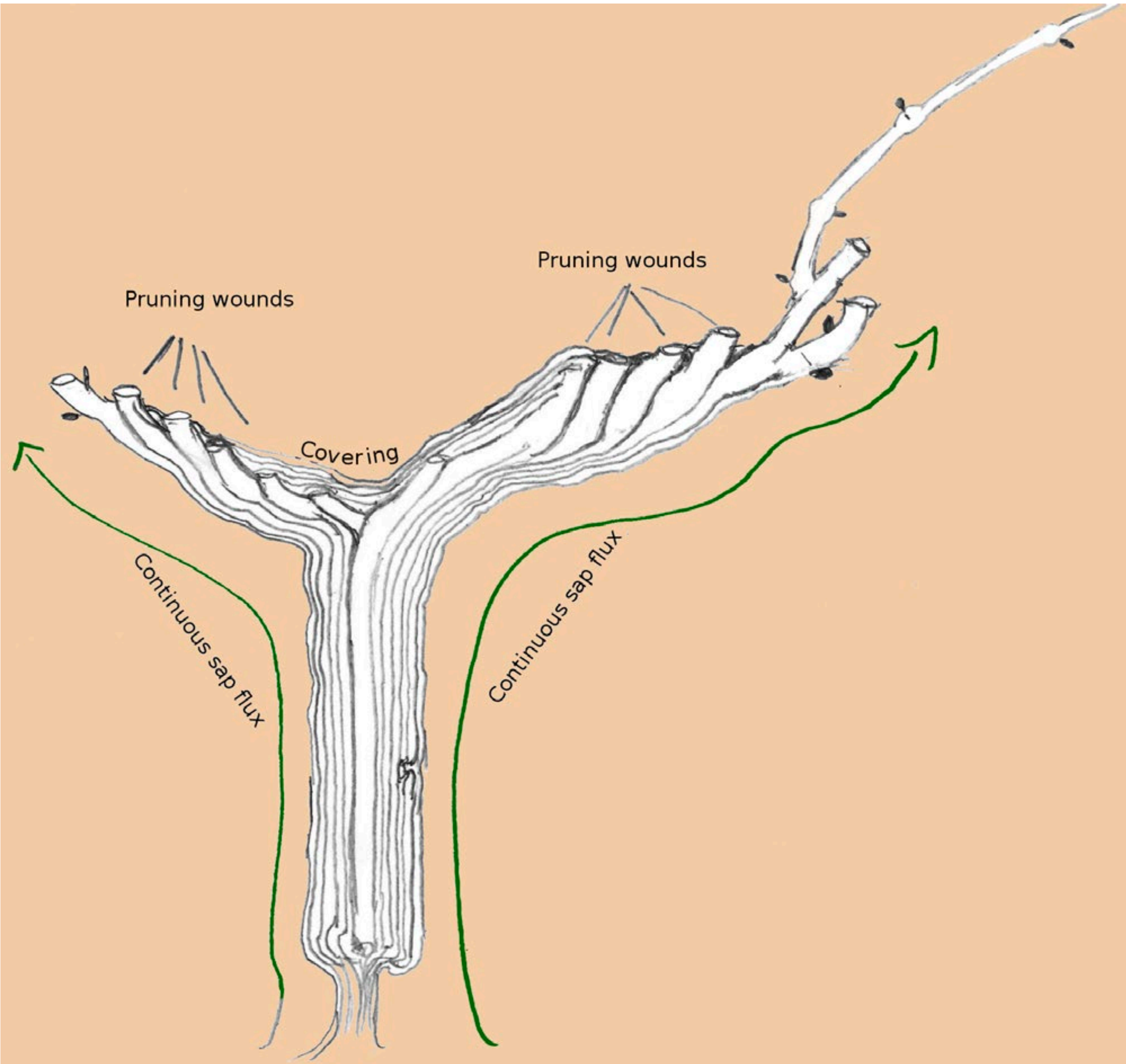

Figure 2. The Guyot-Poussard pruning system determines the upper position of dead wood following pruning and the respect of sap flux in the lower part. Its most important feature is the location of the spurs that are always placed below the vine arms. As a result, annual wounds resulting from the removal of long canes from the previous year are still on the top of the arms. This pruning avoids the elevation of the vines and the arms lengthen horizontally. This system has the following characteristics: establishment of the stem about 10 cm centimeters below the first wire and installation on this stem, two short arms that carry either two spurs in a long cane (Guyot Poussard mixed), or two spurs and two long cane (Guyot-Poussard double). (*Picture courtesy of Marceau Bourdarias,* https://marceaubourdarias.fr/taille-douce-de-vigne/).

Nevertheless, vines should be pruned during periods when inoculum is less prevalent and wound healing is more rapid (Gramaje *et al.*, 2018). Late (Úrbez-Torres *et al.*, 2010; Úrbez-Torres and Gubler, 2011) or early (Elena and Luque, 2016) prunings have been recommended as effective cultural practices to reduce GTD pathogen infections, but further research is needed to adjust the appropriate time of pruning in each wine region and for each pathogen.

Double pruning

Double pruning is a commonly used viticultural technique for speeding up the pruning process. This approach can also be used as a support to control method against GTD pathogens and for other purposes. The double pruning method consists of two parts: mechanical pruning in the winter that trims canes to 30–45 cm above the spur positions, followed by hand pruning before bud break.

This technique may help to limit GTDs since infections occurring in the wound produced by winter mechanical pruning are eliminated by the following manual pruning performed in spring. This allows for better managed pruning by qualified operators aiming to reduce infection risks by selecting the final pruning time when infection risk is low. Removal of pruning debris from vineyards will reduce the potential inoculum load. However, some GTD pathogens can remain viable in vine debris for up to 48 months (Elena and Luque, 2016).

Double pruning is applicable in cordon trained and spur-pruned vineyards, but is not appropriate for cane-pruned vineyards, because of the need to retain long 1-year-old canes for adequate fruit production (Weber *et al.*, 2007). Recent studies have described other benefits of this method as a management tool to delay berry ripening and also control yields (Palliotti *et al*., 2017).

Double pruning is favored by growers to speed up the pruning operations, but the costs of mechanical and hand pruning, together with the scarcity of registered products for use against GTD pathogens, makes this technique too expensive in low-value vineyards (Bertsch *et al.,* 2013). A case study in California showed that double pruning as a preventative technique was the least common practice and the costs for this approach are much greater than for the "delayed pruning" method (Hillis *et al.,* 2017).

The efficacy of double pruning combined with wound treatments for prevention of infection by GTD pathogens is unquestionable, but currently its application depends on the decision of winegrowers. These are usually determined by the value of both their wine or grapes, and their vineyards.

Ongoing studies are aimed at evaluating the role of annual infections in development and expression of the different GTDs. These studies could give useful information to assist evaluation of the effects of alternative pruning methods applied in GTD control strategies.

Wound protection

Pruning wounds are the most important points of entry for wood colonizing fungal pathogens (van Niekerk *et al*., 2011; Mutawila *et al*., 2015). In trees, wound protection proved to be inefficient as plants protects themselves by compartmentalization in efficient ways (Shigo, 1984). However, in grapevines, which are creeping plants with different wound responses (Sun *et al*., 2006), wound protection is essential to reduce infections. Different fungicides have been reported to be effective (Mutawila *et al*., 2015; Mondello *et al.,* 2018) but their efficacy in protecting pruning wounds is short-lived and the wounds remain susceptible for a period of 4–16 weeks depending on the time of pruning (Larignon and Dubos, 2000; van Niekerk *et al*., 2011). New formulations have been proposed and are under development (Diaz *et al*., 2013), including chemical and physical protection, some of which are registered in several European countries and are being applied routinely by growers (Aloi *et al*., 2017; Boulisset *et al*., 2017; Kühn *et al*., 2017), They are all aimed at providing long-lasting protection. This is, in fact, an essential characteristic for all proposed wound protection products.

Biocontrol agents (BCAs) that prevent fungal colonization of fresh vine wounds may offer increased protection, although the length of time needed for wound colonisation may create a short period of susceptibility to GTDs infection (Mondello *et al.,* 2018). *Fusarium lateritium*, *Chaetomium* spp. and *Trichoderma* spp. have been evaluated as BCAs against GTD pathogens (Compant and Mathieu, 2017). *Trichoderma atroviride* (strains SC1 and USPP-T1) and *T. harzianum* have shown good efficacy for the protection of pruning wounds (Pertot *et al.,* 2009; Halleen *et al.,* 2010; Kotze *et al*., 2011). In France, Pajot *et al.* (2012) and Mounier *et al*. (2016) also reported promising results from *T. atroviride* strain I-1237 (Esquive WP®) against canker diseases of grapevine. In Italy *T. asperellum* and *T. gamsii* (Remedier ®) have given good results against GLSD of the esca complex and to reduce vine mortality (Reggiori *et al.,* 2014; Bigot *et al*., 2015). In Portugal, *Trichoderma atroviride* (strain I-1237) reduced both incidence and severity of disease caused by *P. chlamydospora* and *N. parvum* (Reis *et al.*, 2016). Although biofungicides may not have the instant protective effect of chemical fungicides, the optimal conditions for the GTDs development are the same as those for the efficacy of these biofungicides (Mutawila *et al.*, 2015). A strong correlation between temperatures after pruning and pruning wound colonisation rate by naturally occurring epiphytes, which may act as competitors in wound colonization, has also been reported (Munkvold and Marois, 1995).

The management of GTD-affected vineyards in the "Winetwork countries"

Remedial surgery

The remedial surgery, in its different typologies (cordon/spurs- or trunk renewal, trunk surgery) is applied for GTDs control in all the "Winetwork countries", as a current or innovative practice. The term indicates the techniques that bring to the elimination by pruning of symptomatic woody parts from affected vines, until healthy wood is left. The advantage of remedial surgery is related to the fast growth of the new vine parts that could reach the qualitative and quantitative production levels of the healthy ones within a few years, thanks to the support of the mature root system. This technique could prolong the productive life of plants affected by GTDs but is sometimes too expensive and time-consuming since other agronomic, chemical or structural intervention may be required. For these reasons remedial surgery could be applied where its advantages justify the economic effort as, for example, in highly remunerative vineyards, or to preserve a clone variety (Creaser and Wicks, 2004; Úrbez-Torres, 2011; Sosnowski *et al.*, 2011).

Affected cordon or spurs removal

According to the training systems and to the disease severity, the remedial surgery could lead to eliminate only affected cordons or spurs, and to re- train the treated vine using a healthy spur to re-form the eliminated parts. This approach has been successfully applied and scientifically studied for Eutypa dieback control since the 1990s (Creaser and Wicks, 2004; Sosnowski *et al.*, 2011; Sosnowski *et al.*, 2013; Savocchia *et al.*, 2014) and could also be used in vines affected by Botryosphaeria dieback, provided that all infected parts are removed (Savocchia *et al.*, 2014).

Trunk renewal

If the most of the vine trunk shows internal GTDs symptoms, the technique coincides with the elimination of the vine trunks. Among remedial surgery techniques, the trunk renewal is the most invasive. It consists of the substitution of a GTD-affected trunk with a new healthy one. This goal could be reached with different approaches, depending on the disease, the infection level and the presence of grafted or ungrafted vines. The easiest way to renovate the trunk of a GTD-affected vine is, after cutting the trunk to eliminate the infected part, to select a watershoot to obtain a new trunk (Úrbez-Torres, 2011; Savocchia *et al.*, 2014). For efficient removal of the infected wood, it is suggested to cut at least 10 cm below the first healthy wood observed for Botryosphaeria dieback-affected vines and 20 cm for those with Eutypa dieback (Sosnowski *et al.*, 2007; 2016a, b).

When the symptomatic wood is too close to the grafting point, the possibility of obtaining a watershoot from the scion could be very low. Furthermore, there are variety-specific differences regarding the capability of producing watershoots emerging from "resting buds". Thus, "trunk renewal" could be no longer useful. The main solution used in the ""Winetwork countries"" is the elimination of the diseased vine followed (not always) by the planting of new and younger ones. However, depending on the expected useful vine life, trunk renewal is an inexpensive way to maintain the economic value of a vineyard, and homogenous quality of the harvested grapes (Becker, 2018).

Successful application in Esca complex-affected vines is linked to the degree of infection, since Esca complex pathogens can also colonize rootstocks, so renovation of the upper parts of Esca-affected plants would be useless (Calzarano *et al.*, 2004; Gramaje and Armengol, 2011). However, rootstocks are probably less affected, since pathogens and symptom incidence are less in vine rootstocks than in the scions (Haustein *et al.*, 2016).

Trunk surgery or "curetage"

Another practice within the remedial surgery and utilized in some "Winetwork countries" (Spain, France, Italy and Portugal) is the removal of the rotten (i.e. degraded by white decay basidiomycete agents) tissues in the parts of vines where large wounds had been applied, a "trunk surgery", already known in some countries by the French name "*curetage*" or the Italian one "*slupatura*". The technique consists in the removal of the rotten wood tissue (white rot) in the trunk of GTD-affected vines using electric handsaws, and leaving only the hard and not yet degraded wood tissues. Expanding use of trunk surgery among viticulturists is probably related to the reported, but scientifically unproven, observation that treated vines have greater likelihood of not showing symptoms in the years following the treatment. This technique was only applied in Esca complex diseased vines in which white rot is formed and associated to GLSD foliar symptoms in the following years, but no data are available. The efficacy evaluation needs to take into account that vines infected by GLSD may not express foliar symptoms for several years after a growing season with visible symptoms on the canopy (Calzarano *et al*., 2018). From a scientific point of view, surgery applied to Esca-affected vines removes only rotten woody tissues affected by basidiomycetes, leaving vascular pathogens, such as *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*. The latter are the only ones capable of producing the toxins considered to be linked to GTDs foliar symptoms (Tey-Rulh *et al*., 1991; Mugnai *et al*., 1999; Tabacchi *et al*., 2000; Andolfi *et al*., 2011; Abou-Mansour *et al*., 2015; Burruano *et al*., 2016 Calzarano *et al*., 2016; 2017b). Calzarano and Di Marco (2007) could not detect correlations between the presence of white rot in the trunk and foliar symptoms in Esca-affected vines. The suppression of GTDs foliar symptoms attributed to this technique could be related to other factors, such as temporary increased oxygenation of the wood following the surgery, the action of elicitors that induce host resistance or the possible interaction of other saprobes on foliar symptom expression. In the sense of an increased exposure of infected tissues to oxygen, the practice of trunk surgery is similar to the ancient technique of putting a stone in the middle of the trunk, previously cut longitudinally. This was used on Esca-affected vines in Europe (Mugnai *et al*., 1999; Pérez Marín, 2000) and it is still used by some growers.

Re-grafting

When GTDs wood symptoms are too close to the grafting point of a vine, an alternative to replanting is the re-grafting, which overcomes the problem of obtaining healthy watershoots from scions. This technique is recorded as a common practice for GTDs management only in the two French areas involved in the WINETWORK project. This was set up and proposed by the French Service Interprofessionnel de Conseil Agronomique de Vinification et d'Analyses du Centre (SICAVAC) to prolong the economic life of Esca-affected plants. The technique used is the "cleft graft", which consists of splitting each rootstock with a blade and putting two V-shaped scions in one end of this open cut. Similar to trunk renewal, empirical observations indicate that in 2–3 years treated plants recover in both quality and quantity of grape production, due to the mature root systems. As outlined above, the useful application of this method is linked to the sanitary status of the rootstock, susceptible to infection by some GTD pathogens (Fourie and Halleen, 2004; Aroca *et al*., 2010). The empirical experiences of SICAVAC suggests that re-grafting vines is evaluable, even if the rootstocks show, inner GTD-like symptoms in cross sections but healthy external wood (SICAVAC, 2013). On the contrary, scientific trials conducted in Italy and France demonstrated the resurgence of decline symptoms in renewed vines with symptomatic rootstocks (Calzarano *et al*., 2004; Larignon and Yobregat, 2016). No scientific data are available to confirm the reported high efficiency of re-grafting when applied to Esca-affected plants or to other GTDs. These data could be very useful for evaluating benefits of the technique in management of GTDs in the field, due to the high economic impacts on vineyard management.

Pruning of symptomatic canes

Another practice utilized in the ""Winetwork countries"" is pruning of symptomatic canes. In Piedmont, Veneto and Friuli (Italy), Palatinate region (Germany) and Eger area (Hungary) this is considered a common practice while in France and in Galicia (Spain) it is utilized as an innovative method for control of GTDs. The approach consists of removing the canes as soon as they show GTDs symptoms during the growing season. In Alsace (France) the technique eliminates symptomatic canes reducing them to two-bud spurs, even if in the full vegetative period. Winegrowers observed that treated vines could recover rapidly in the following season. According to the scientific knowledge so far acquired on GTDs, this method has no reasonable explanation. The removal of symptomatic canes does not remove the GTDs pathogens but simply hides the symptoms. It is also well-known that symptoms naturally fluctuate, and most of the vines that showed symptoms will not show them again in the immediately following years (Calzarano *et al*., 2018). Pruning of symptomatic canes has no scientific or practical justification as a GTDs control method.

Innovative tentative post-infection practices identified during the WINETWORK programme

The following are reports from trials carried out by growers or extension agents without scientific approaches. As is well known, satisfactory results for growers during efficacy trials on GTD management are usually made without inclusion of untreated experimental control. In Europe the main and most well-known GTD is GLSD within the Esca complex, the incidence of which fluctuates greatly from year to year, and there is no meaning in discussing results without comparing them with untreated controls. Scientifically validated results need several years of trials. Calzarano *et al.* (2014, 2017a; Calzarano and Di Marco, 2018) monitored GLSD foliar symptoms in vineyards of central Italy and calculated the incidence of GLSD in every year of the trial. By dividing the number of vines with visible symptoms by the total number of diseased vines (symptomatic for at least one of the years of survey), excluding the healthy vines therefore validating the efficacy of a control tool (seaweeds and nutrients application, Calzarano *et al*., 2014; 2017a; Calzarano and Di Marco, 2018). Furthermore often there is no knowledge of the previous disease distribution in the vineyards where the trials are sited. Nevertheless, this information can be useful to give an overview of the interest amongst the growers to find a solution, and some of the suggestions are made with regard to practical applicability.

Hydrogen peroxide trunk injections

The basic aim of trunk injections is to put active ingredients in contact with the vascular pathogens, through the grapevine conductive system to reach infected wood. This technique has been tested in GTD-affected vines in both Italy and France with triazoles, Fosetyl-Al and 2-hydroxybenzoic acid (Calzarano *et al*., 2004; Darrieutort et Lecomte, 2007; Dula *et al*., 2007). Trials carried out in South West France, Galicia (Spain) and in the Douro region of Portugal have injected 3–4 mL of hydrogen peroxide into a drill hole in the trunk of GTD-affected vine. Growers report good results, with no symptoms appearing in treated vines, but again this is based on observations with no scientific validation. Previous *in vitro* and in nursery scientific experiments that used hydrogen peroxide did not show any direct effects on GTD pathogens (Fourie and Halleen, 2006; Sosnowski *et al*., 2013). Furthermore, the presence of hydrogen peroxide, even if linked to a defense response in plants, boosted lignin degradation activities of fungal enzymes in plants infected by GTD pathogens as observed on grapevine infected by the Esca complex vascular pathogens *P. minimum* and *P. chlamydospora* (del Río *et al*., 2004; Fischer *et al*., 2016).

Trichoderma-inoculated wood dowels inserted into grapevine trunk

An unusual attempt to limit Esca symptoms with *Trichoderma* was reported by the Spanish facilitator agent in Galicia. This consists of introducing wooden dowels previously inoculated with *Trichoderma* spp. into the trunks of Esca-affected vines These dowels are prepared by a New Zealand company and their use was originally suggested for control of *E. lata* and some *Botryosphaeriaceae*. In spring a small surface of the trunk (2–3 cm^2) of each diseased vine is peeled, drilled and then the dowels inserted. The technique requires three dowels to be inserted, one at the trunk base and two in the vine arms. Beside direct antagonistic effects of *Trichoderma* on pathogens the response is also related to the capability of *Trichoderma* spp. to induce host defense response as reported by several authors (Handelsman and Stabb, 1996; Harman, 2006; Pal and McSpadden Gardener, 2006). Previous trials conducted in the same way by Rod Bonfiglioli in New Zealand and Laura Mugnai in Italy (Bicchi, 2004) did not give disease reduction in Esca incidence or reduction of GLSD symptoms.

Copper nails in trunk

The use of copper nails placed in the scion parts of grapevine trunks has been proposed as a novel method to lower the progress of wood rot in infected plants. This method, found applied in the Galicia region (Spain) would provide a slow but constant release of copper, but has not yet been validated in any scientific trial. Growers who applied it report a distribution of copper upwards and downwards the vine, as seen by discolored in the areas surrounding each nail. Furthermore, white rot is suggested to be retarded or restricted when reaching the copper enriched wood. Current experiments may provide, beside showing a real efficacy of the method, more information regarding the number of nails needed in each vine without raising the risk of phytotoxicity, the optimal nail position, and optimum application time. Copper should also be evaluated for general capability to prevent GTDs, when it is not banned due to high eco-toxic potential.

Nursery GTDs control in "Winetwork countries"

Grapevine planting material produced in nurseries is very prone to infection by GTD pathogens due to the large numbers of wounds made during the different steps of the propagation processes (Gramaje and Armengol, 2011; Gramaje *et al.*, 2018). In 2015, Gramaje and Di Marco conducted a wide questionnaire survey in grapevine nurseries of 13 in European countries (plus Israel and Algeria). These covered all aspects of grapevine propagation including cultural and sanitation practices in mother blocks, at harvest and during transport of cuttings from field to nurseries, as well as grafting operations and field nursery management (Gramaje and Di Marco, 2015). This study identified the main risk factors that could increase infection by GTD pathogens during the vine propagation, and indicated clear requirements for further research to improve the management of GTDs in nurseries. No curative control measures are available in Europe to control GTDs in nurseries. Incorporation of multiple control measures, such as cultural practices and sanitation, chemical and biological control, hot-water treatments, and other strategies (e.g. ozonation) have been shown as the best approach to improve the phytosanitary quality of planting material (Gramaje and Armengol, 2011; Gramaje *et al.,* 2018). The progressive implementation of these measures in grapevine nurseries of the "Winetwork countries" is strongly recommended.

Several studies have recently focused on the use of BCAs to control GTD infections in nurseries especially with *Trichoderma* species (Fourie *et al.*, 2001; Di Marco and Osti 2007; Pertot *et al.*, 2016). Most of the nursery treatments are directed towards control of infections by the grey mold pathogen *Botrytis cinerea*. These treatments do not eliminate GTD pathogens from the wood and as a negative side-effect these pathogens may colonize the free niches. *Trichoderma* conidia applied directly after the treatment may fill this gap, and avoid the colonization by unwanted microorganisms. First results of *Trichoderma* use in nursery indicate positive effects such as increased callusing and root growth (Di Marco and Osti, 2007). Effects on GTD incidences must be validated. Furthermore, since *Trichoderma* application must be implemented as an additional step within the plant propagation process, the optimal treatment time must be evaluated o obtain maximum colonization rates and the possible negative interactions with other nursery-specific operations must be assessed.

Conclusions

The relationships between GTDs, grapevine decline and yield losses are currently well-known. These losses represent emerging problems among European winegrowers, and are increasing as climate change is putting vineyards under serious stress. The EU Winetwork project achieved all the proposed aims related to GTDs, especially those focused on sharing the state-of-art on disease knowledge and control strategies with viticulturists. The project gave an accurate and up-to-date picture of GTDs problems in the main European wine-growing areas. The studies carried out in the last 20 years worldwide have highlighted the complexity of these diseases, the role of pruning practices in the spread of GTDs in vineyards, and the risks of GTD pathogen infections during the plant propagation process in nurseries. Although a simple and efficient control method is still not available for these diseases, the scientists involved in the project were able to share the best available and scientifically proven strategies to limit GTDs with end-users, both in nurseries and vineyards, either for prevention or for limiting the damages of these diseases. Simultaneously, the practical attempts on control of GTDs coming from vineyard operators (the grass-roots-level) were collected, reported and were also critically evaluated and revised.

The Winetwork project has highlighted the crucial role of information exchange among the different viticulture operatives and between the researcher and practical viticulturalists, who are involved in finding efficient solutions for GTD control. The expected output of this exchange was more constant flow of high-quality information. Furthermore, the "open" and not-only-scientific approach, even if could not allowed a real efficiency comparison among the different described techniques, has highlighted to scientists other possible and alternative ways to manage GTDs, taking advantages of the empirical observation of winegrowers.

According to our results, the Winetwork working method could be usefully applied in the study of other complex plant diseases (Le May *et al.*, 2009; Muhammad *et al.*, 2009; Lamichhane and Venturi, 2015).

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Accepted for publication: November 5, 2018