WINETWORK

European Knowledge Transfer

THE SCIENTIFIC BASIS FOR A MORE EFFICIENT CONTROL OF GTDs FROM NURSERY TO VINEYARD

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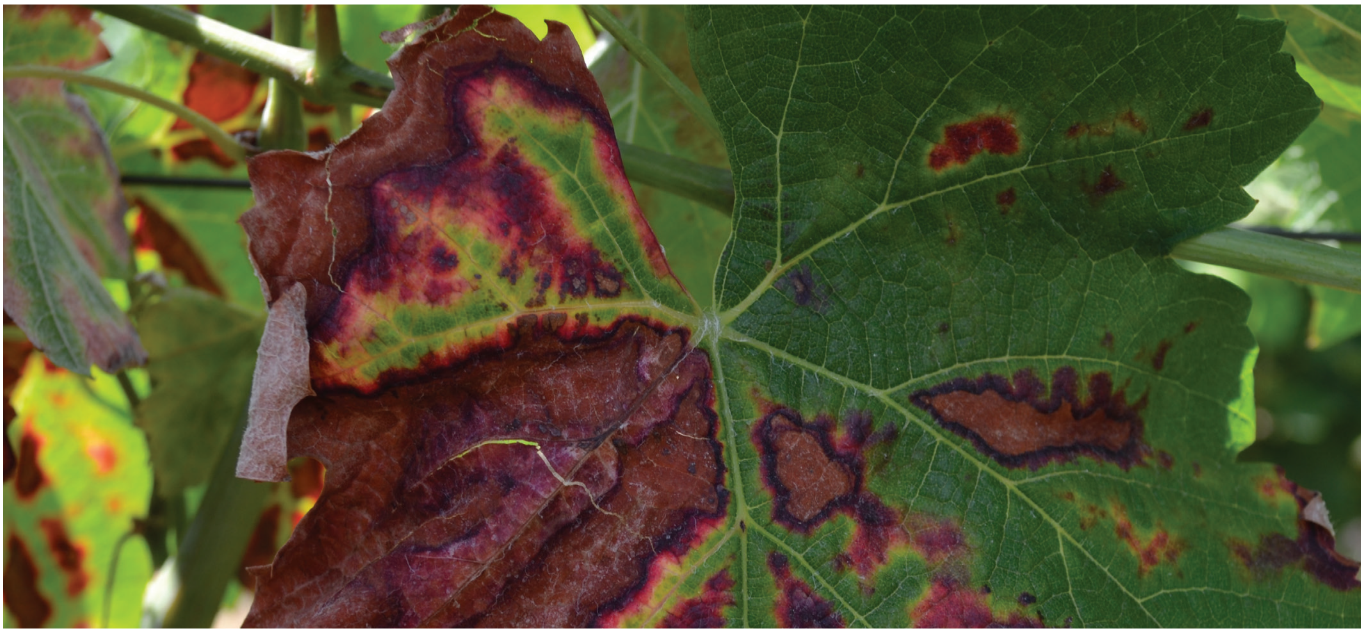

Results of the scientific litterature review made by the Winetwork scientific working group on Grapevine Trunk Diseases (GTDs)

Network for the exchange and transfer of innovative knowledge between European wine growing regions

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Introduction

GTDs is the acronym used for "Grapevine Trunk Diseases", a group of old and/or emerging diseases caused by fungal pathogens, belonging to different genera and able to colonize woody tissues. Nowadays, GTDs represent a serious problem in all the wine-growing countries worldwide. According to the recent OIV publication[1] the estimated economic cost due to the replacement of dead vines caused by the three more common GTDs, Esca complex, Botryosphaeria dieback and Eutypa dieback all over the world is more than 1.5 billion dollars per year. Other data show how the GTDs presence in vineyard is responsible for their unproductiveness, with loss percentages that could reach 30% (fig. 1). GTDs are an economic problem also in nurseries, since trunk pathogens could interfere with the plant production processes, leading to grafting failures, poor sanitary quality plants and, worse, to plants that could arrive to vineyards already infected.

The recrudescence of well known GTDs (Esca) and the increased incidence of the more recent ones (Eutypa dieback and Botryosphaeria dieback) recorded in the last thirty years in adult vineyards worldwide are related to different factors. The intense expansion of wine-growing areas has determined a high request of plant material for new plantation. This, together with the lack of knowledge on GTDs epidemiology, which has determined a wrong approach in the management strategies (no pruning wound protection, high-stressing vineyard management, etc), could have determined the unaware GTDs spread in field. Currently, the lack of efficient chemical control methods is exacerbating the situation; no active ingredient (AI) seems to control these diseases. Efficient and viable strategies to control GTDs are presently urgently needed.

As consequence of this emerging threat, several studies were conducted worldwide, both to in-depth the knowledge on the GTDs and to find suitable practices able to control GTDs and friendly for the environment.
This article, even if not exhaustive, wants to update the readers on the main achievements obtained by the scientific community worldwide on GTDs and that could represent the

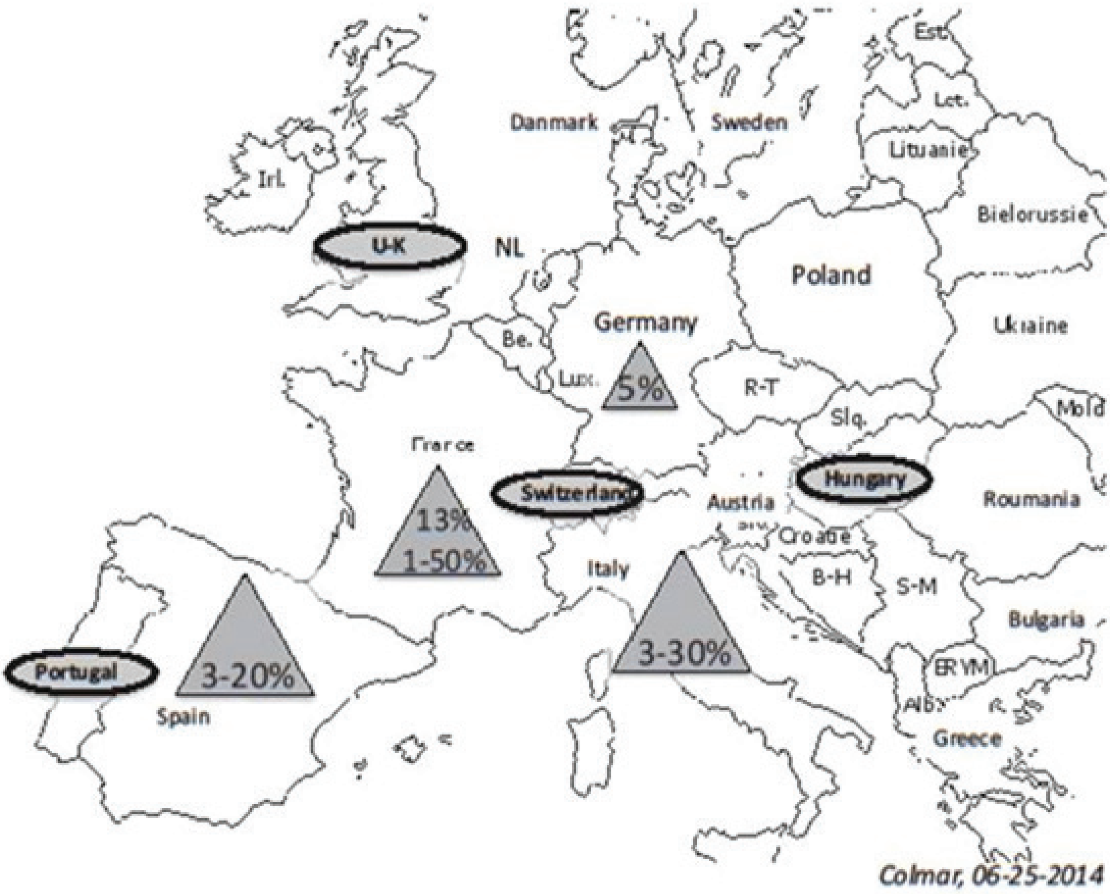


Figure 1: Percentages of unproductive vineyards due to GTDs presence in Europe. (F. Fontaine, COST Action FA1303

[1] Fontaine, F.; Gramaje, D.; Armengol, J.; Smart, R.; Nagy, Z.A.; Borgo, M.; Rego, C.; Corio-Costet, M-F. Grapevine trunk diseases. A review. ©OIV publications, 1st Edition: May 2016 (Paris, France). 2016.

base to a more efficient control of GTDs. The information here reported is part of the scientific literature revision by the members of the scientific working group on GTDs within the European project WINETWORK (www.winetwork.eu). In particular, the scientific working group focused its attention on a) the early detection of the disease; b) the AIs and c) bio-control agents (BCAs) tested until now to control GTDs in both vineyard and nursery; d) the influence of the current practices applied in vineyard and nursery on the disease spread and development.

What have we learnt meanwhile?

Disease and pathogen identification.

One of the main problems in controlling GTDs is the correct identification of the disease in vineyard. Botryosphaeria dieback, Esca and, to a lesser extent, Eutypa dieback are probably confused one another or with nutrient deficiency, due to the similar and/or overlapping symptomatology. Recently, several studies on their epidemiology have led to better define both the single GTD symptomatology and the associated pathogens, together with their life cycle in vineyard. As results, while external symptoms remain still not useful for a precise diagnosis (except for Eutypa dieback) to the non trained eye, the knowledge on the pathogens associated to the different GTDs, reported in Tab. 1, allows to discriminate among the different diseases. This could help in finding the best control strategy for the different GTDs within the available ones.

Thus, in symptomatic plants the exact GTDs diagnosis could requires laboratory analyses addressed to identify the associated pathogens. Beside the traditional and time-consuming methods, molecular ones allow to detect the pathogens through their DNA. To decrease the cost of these analyses, especially for massive ones, different solutions are ongoing, like the European "Mycorray" (still under development), a PCR-based method able to detect in the same sample up to 11 different GTDs pathogens or the macroarrays set up by some researchers in Canada, able to discriminate the presence of up to 61 different fungal species, thirty of which related to the three GTDs considered in this article.

The early detection of GTDs pathogens could be recommended especially in nurseries, both in mother plant fields and at the end of the plant production process. This analysis could avoid the introduction of infected material in the plant production process, since cross contaminations among infected and healthy plant material are attested by several authors. In vineyard, according to the ascertained presence of GTDs pathogen inoculum also in secondary hosts, it is even more important to set up a preventive plant protection than the mere diagnosis. Furthermore, the presence of GTDs pathogens inside the vine woody tissues in the field is not necessarily related to GTDs symptoms expression. More studies are needed to understand in-depth the mechanisms involved in the "step change" from latency to symptoms expression.

Botryosphaeria dieback	Eutypa dieback	Esca complex
Botryosphaeria dothidea	*Eutypa lata*	*Phaeomoniella chlamydospora*
Diplodia corticola	*Eutypa laevata*	*Phaeoacremonium minimum*
Diplodia mutila	*Eutypa leptoplaca*	*Phaeoacremonium angustius*
Diplodia seriata	*Eutypella citricola*	*Phaeoacremonium alvesii*
Dothiorella iberica	*Eutypella cryptovalsoidea*	*Phaeoacremonium argentiniense*
Dothiorella americana	*Eutypella microtheca*	*Phaeoacremonium armeniacum*
Dothiorella vidmadera	*Eutypella vitis*	*Phaeoacremonium australiense*
Lasiodiplodia crassispora	*Cryptosphaeria lygniota*	*Phaeoacremonium austroafricanum*
Lasiodiplodia exigua	*Cryptosphaeria pullmanensis*	*Phaeoacremonium canadense*
Lasiodiplodia mediterranea	*Cryptovalsa ampelina*	*Phaeoacremonium cinereum*
Lasiodiplodia missouriana	*Cryptovalsa rabenhorstii*	*Phaeoacremonium croatiense*
Lasiodiplodia theobromae	*Diatrype brunneospora*	*Phaeoacremonium globosum*
Lasiodiplodia viticola	*Diatrype oregonensis*	*Phaeoacremonium hispanicum*
Neofusicoccum australe	*Diatrype stigma*	*Phaeoacremonium hungaricum*
Neofusicoccum luteum	*Diatrype whitmanensis*	*Phaeoacremonium inflatipes*
Neofusicoccum macroclavatum	*Diatrypella verrucaeformis*	*Phaeoacremonium krajdenii*
Neofusicoccum mediterraneum	*Diatrypella vulgaris*	*Phaeoacremonium mortoniae*
Neofusicoccum parvum		*Phaeoacremonium occidentale*
Neofusicoccum ribis		*Phaeoacremonium roseum*
Neofusicoccum viticlavatum		*Phaeoacremonium scolyti*
Neofusicoccum vitifusiforme		*Phaeoacremonium sicilianum*
Phaeobotryosphaeria porosa		*Phaeoacremonium tuscanum*
Spencermartinsia viticola		*Phaeoacremonium viticola*
		Cadophora luteo-olivacea
		Cadophora melinii
		Fomitiporia australiensis
		Fomitiporia capensis
		Fomitiporia mediterranea
		Fomitiporia polymorpha
		Fomitiporia punctata
		Phellinus igniarius
		Stereum hirsutum

Table 1: Fungal species currently associated with the three main Grapevine trunk diseases.
Retrieved from i) http://managtd.eu/images/uploads/content/125/Urbez Torres.pdf and ii) Cloete, M., Fischer, M., Mostert, L., & Halleen, F. (2014). A novel Fomitiporia species associated with esca on grapevine in South Africa. Mycological Progress, 13(2), 303–311.

Chemical compounds tested for the control of GTDs

The search for a valid alternative to the forbidden chemicals (i.e sodium arsenite, benomyl and carbendazim) previously used, with some results, for the GTDs control has involved scientists worldwide. From 2000 to 2015 more than 70 AIs, both synthetic and natural, were tested in both lab and operative condition (field and nursery) to a) avoid GTDs infection during the plant production process in nursery; b) protect pruning wounds and c) reduce the GTDs incidence.

According to the results in scientific literature (tab. 1), the most efficient AIs belong to both synthetic and natural compounds, while all the tested “bio-stimulant” products resulted ineffective when used in field leading, in some cases, to an increase of GTDs foliar symptoms expression.

In general, systemic AIs (Benzimidazoles, Triazoles) were shown to be more efficient than translaminar or contact ones, especially when tested in field to reduce GTDs incidence. To date, only tetraconazole and tropiconazole (triazoles) have shown to be able to reduce Esca foliar symptoms, but only in young vines when injected in trunks.

Tests conducted in nurseries, where several steps of the plant production process are considered in danger of GTDs infection/cross-contaminations, have permitted to individuate different AIs able to limit this risk. In particular, general disinfectants and wider spectrum fungicides like, thiophanate-methyl, dithiocarbammates (ziram, thiram), pthalimides

Chemical group	Active ingredient	Botryosphaeria dieback					Esca complex					Eutypa dieback				
		in lab	in field	WP	SS	NU	in lab	in field	WP	SS	NU	in lab	in field	WP	SS	NU
		++	*nt*	+++	*nt*	+++						+++	*nt*	++	*nt*	*nt*
BENZIMIDAZOLES	Thiophanate methyl	+++	*nt*	+++	*nt*	*nt*	+++	*nt*	+++	*nt*	++	*nt*	*nt*	+++	*nt*	*nt*
DICARBOXIMIDE	Iprodione	+	*nt*	++	*nt*	*nt*	- -	*nt*	*nt*	*nt*	*nt*	- -	*nt*	*nt*	*nt*	*nt*
DISINFECTANTS	Didecyldimethyl-ammonium -chloride	*nt*	*nt*	*nt*	*nt*	++	++	*nt*	*nt*	*nt*	++					
	alcohol-phenol-iodine solution	*nt*	*nt*	*nt*	*nt*	- -	*nt*	*nt*	*nt*	*nt*	++					
DITHIOCARBAMMATE	Mancozeb	++	*nt*	+++	*nt*	*nt*						+	*nt*	*nt*	*nt*	*nt*
	Ziram						- -	*nt*	*nt*	*nt*	++	- -	*nt*	*nt*	*nt*	*nt*
	Thiram						++	*nt*	*nt*	*nt*	++					
IMIDAZOLE	Prochloraz	++	*nt*	+++	*nt*	*nt*	+++	*nt*	*nt*	*nt*	*nt*	+++	*nt*	*nt*	*nt*	*nt*
	Imazalil											++	*nt*	+	*nt*	*nt*
ORGANIC SALT	Fosethyl-Al	*nt*	*nt*	- +	*nt*	*nt*	*nt*	*nt*	+	+	*nt*					
PHTHALIMIDE	Captan						- +	*nt*	*nt*	*nt*	++					
	Hydroxyquinoline sulphate (chinosol)	nt	nt	nt	nt	- -	- +	*nt*	*nt*	*nt*	- -					
STROBILURINS	Pyraclostrobin	+++	*nt*	+++	*nt*	*nt*	*nt*	*nt*	+++	*nt*	*nt*	+	*nt*	*nt*	*nt*	*nt*
	Trifloxystrobin											- +	*nt*	- -	*nt*	*nt*
TRIAZOLE	Cyproconazole						++	+	*nt*	++	++					
	Difenoconazole						*nt*	*nt*	*nt*	++	*nt*	*nt*	*nt*	- -	*nt*	*nt*
	Flusilazole	+++	*nt*	+ -	*nt*	*nt*	+++	*nt*	*nt*	- -	*nt*	+++	*nt*	+	*nt*	*nt*
	Penconazole	+++	*nt*	- -	*nt*	*nt*	*nt*	*nt*	*nt*	+		+++	*nt*	+++	*nt*	*nt*
	Propiconazole						*nt*	*nt*	*nt*	++		*nt*	*nt*	*nt*	- -	*nt*
	Tebuconazole	+++	*nt*	+++	*nt*	*nt*	+++	*nt*	- +	*nt*	*nt*	+++	*nt*	+++	*nt*	*nt*
	Thiabendazole						+++	*nt*	*nt*	+++	*nt*					
	Tetraconazole	*nt*	*nt*	- -	*nt*	*nt*	*nt*	*nt*	*nt*	- -	*nt*	+++	*nt*	- -	*nt*	*nt*
	Triademenol	- -	*nt*	*nt*	*nt*	*nt*	+	*nt*	- -	*nt*	*nt*	+++	*nt*	- +	*nt*	*nt*
COMMERCIAL MIX	Boscalid+Pyraclostrobin											++	*nt*	- -	*nt*	*nt*
	Carbendazim+Flusilazole						*nt*	*nt*	*nt*	*nt*	++					
	Cyproconazole+Iodocarb	*nt*	*nt*	+++	*nt*	*nt*	*nt*	*nt*	++	*nt*	*nt*	*nt*	*nt*	+++	*nt*	*nt*
	Cyprodinil+Fludioxionil	*nt*	*nt*	- +	*nt*	++	+++	*nt*	*nt*	*nt*	*nt*	+++	*nt*	+	*nt*	*nt*
	Prothioconazole+Tebuconazole											+++	*nt*	*nt*	*nt*	*nt*
	Pyraclostrobin+metiram	*nt*	*nt*	*nt*	*nt*	++										
INORGANIC ACID	Boric acid	- -	*nt*	++	*nt*	*nt*	*nt*	*nt*	+++	*nt*	*nt*	++	*nt*	++	*nt*	*nt*
INORGANIC SALT	Calcium polysulfides						*nt*	*nt*	*nt*	*nt*	++					
NATURAL COMPOUNDS	Chitosan	+++	*nt*	+++	*nt*	*nt*	+++	*nt*	+++	*nt*	*nt*	- +	*nt*	*nt*	*nt*	*nt*
	Allium sativum extract	+++	*nt*	+++	*nt*	*nt*	+++	*nt*	+++	*nt*	*nt*	++	*nt*	- +	*nt*	*nt*
	Hydrogen peroxide	*nt*	*nt*	*nt*	*nt*	- -	*nt*	*nt*	*nt*	*nt*	- -	- -	*nt*	*nt*	*nt*	*nt*
	Vanillin	++	*nt*	*nt*	*nt*	*nt*	++	*nt*	*nt*	*nt*	*nt*	++	*nt*	*nt*	*nt*	*nt*
BIOSTIMULANTS	*Brotomax*						- -	*nt*	*nt*	- -	*nt*					
	Fitostim						- -	*nt*	*nt*	- -	*nt*					
	Marvita						- -	*nt*	*nt*	- -	*nt*					
	Kendral						- -	*nt*	*nt*	- -	*nt*					

Table 2: The main active ingredients tested for the GTDs control. For each AI are reported: the efficiency recorded in the relative test (in lab and in field); the results when tested for wound protection (WP), symptoms suppression (SS) or for nursery use (NU). Legenda for efficiency: - - ineffective; - + less effective; + middle efficient; ++efficient; +++ highly efficient; *nt* not tested.

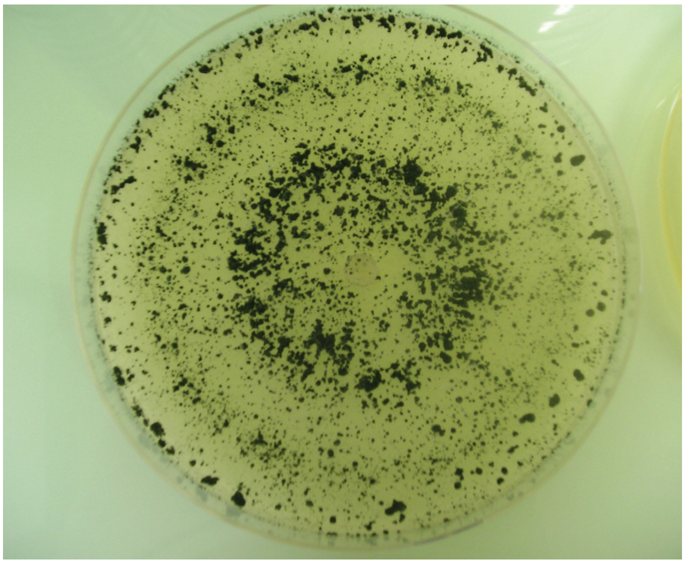
(captan) and triazoles were successfully tested to limit GTDs in nursery, especially during cuttings hydration. On the contrary, some tests highlighted the inefficiency of Chinosol, largely used in the nursery current practice to prevent Botrytis, in controlling GTDs pathogens.
Best results were recorded when AIs were tested for wound protection. In wound protection assays, both systemic (Triazoles, Benzimidazoles) and contact AIs (Strobilurins, Imidazoles) showed the ability in protecting the wounds from GTDs infections.

Within organic synthetic AIs, thiophanate-methyl, pyraclostrobin and tebuconazole showed high efficiency towards at least 2 out 3 GTDs, being able to protect pruning wound from GTDs pathogens' infection up to two weeks. Among inorganic compounds, boric acid showed the same efficiency while the "natural" vanilline-garlic extract-chinosol mix protected pruning wounds from GTDs infection, decreasing also the mortality rate on the tested vines.

Biocontrol agents

The increasing evidences of the environmental impact of chemicals used in agriculture have led to the request of more ecological and sustainable disease control methods. Biological control represents, thus, a valid alternative to the pesticides especially in organic farming or in Integrated Pest Management (IPM) strategies.

Contemporarily to tests with AIs, also bio-control agents (BCAs) were assayed since the early 2000s to evaluate their ability to control of GTDs.
According to some BCAs common characteristics, such as the broad spectrum activity towards different pathogens, the long lasting "protective" effect and the indirect beneficial effect on host plant defense capability, the BCAs use could help to limit GTDs infections. In particular, the broad spectrum activity fit well with the high number of pathogen's species associated to GTDs, overcoming the efficiency variability recorded by AIs when tested towards different pathogens associated with GTDs; the long lasting effect could help in protecting pruning wounds all along their susceptible period (2-4 months) whereas AIs need more applications to obtain the same results. Furthermore, BCAs could improve the host-plant resistance ability in limiting the disease effects as the external and internal GTDs symptoms incidence. The most of the tests were conducted with *Trichoderma* spp. a fungal genus known, since the early 1900, for the strong antagonistic activity towards several soil borne pathogens. Among Bacteria, only *Bacillus subtilis* was tested both in vineyard and nursery.



Picture 1: Trichoderma spp (IFV South-West)

The main aims of the tests with BCAs were i) the prevention of GTDs contamination in nursery, where several steps of the plant production process are in risk of GTDs infections and ii) their evaluation for a durable pruning wounds protection in field. Furthermore, nursery trials allowed evaluating the putative positive effects on plant growth, the induced and/or improved disease resistance and the global effect of host plant-BCA interactions in the development of more healthy and sound vines.

The most relevant results obtained with bacterial and fungal BCAs in the control of GTDs are reported in tab 2. In detail, *B. subtilis* showed high efficiency when used as wound protectant towards Botryosphaeria and Eutypa dieback. When tested on Esca, *B. subtilis* showed a lower protection degree, both in wound protection and in nursery tests.

Nursery tests with *Trichoderma* spp. were addressed mainly towards Esca pathogens. When used in the hydration steps before cold storage and before grafting, *Trichoderma* was able to reduce Esca pathogen infections, artificially inoculated at grafting. Grafted plants, treated with *Trichoderma* spp at rooting showed a lower incidence of Esca woody necrosis compared to the untreated ones. Furthermore, the presence of *Trichoderma* spp during roots development determined an increased production of root biomass, especially of hairy roots, responsible for the nutrients and water uptake. Some studies highlighted that the excessive use of *Trichoderma* spp. in all specific steps of the plant production (grafting, callusing) could determine losses in terms of quantity of good quality plants compared to those normally recorded in the untreated ones. This apparent negative result is however balanced by the higher percentages of saleable plants obtained at the end of the process. Furthermore, treated plants were more vigorous compared to the untreated ones, especially in terms of increased resistance to environmental stresses.

The more frequently tested *Trichoderma* species were *T. atroviride* and *T. harzianum*. In all the performed tests, they showed high efficiency in controlling the main GTDs pathogens, being able to protect from new infections both pruning wounds in the field and cuttings in nursery. Other *Trichoderma* species, like *T. gamsii* and *T. asperellum* were mainly tested towards Esca pathogens, showing the same efficiency. The great success of the *Trichoderma* products was shown in field applications in reducing the number of affected vines showing GLSD (Grapevine Leaf Stripe Disease - Esca complex) and the number of dead vines, making wound protection by *Trichoderma* (several producers) by an atomizer a really relevant and easily applicable GLSD and death of vines prevention tool.

Some tests attested the *Trichoderma* spp. ability in colonizing treated canes, staying viable up to one year in greenhouse and up to 8 months in the field conditions, with variations linked to the vine cultivar and/or to the *Trichoderma* species. Different authors observed a faster wood colonization when *Trichoderma* spp. was distributed in pruned canes close to the break dormancy stage (late winter) than during the dormancy period (winter), confirming the recommendation of *Trichoderma*-based product producers.

Bacteria

Genera	Species	Botryosphaeria dieback				Esca complex				Eutypa dieback			
		in lab	in field	WP	NU	in lab	in field	WP	NU	in lab	in field	WP	NU
Acinetobacter	A. radioresistens	- -	nt	nt	nt								
Bacillus	B. amyloliquefaciens					+							
	B. firmus	- -	nt	nt	nt								
	B. pumilus	- -	nt	nt	nt								
	B. subtilis	+++	nt	++	nt	+++	nt	+	- +	++	nt	++	nt
	B. thuringiensis									- +	nt	nt	nt
Brevibacillus	B. reuszeri	- -	nt	nt	nt								
Burkholderia	B. phytofirmans	 + -	nt nt	nt nt	nt nt								
Curtobacterium	Curtobacterium sp	nt	nt	nt	nt								
Enterobacter	Enterobacter sp	++	nt	nt	nt								
Erwinia	E. erbicola	- -	nt	nt	nt								
Paenibacillus	Paenibacillus sp	- -	nt	nt	nt								
Pantoea	P. agglomerans	+++	nt	nt	nt								
Pseudomonas sp.	Pseudomonas sp.									- +	nt	nt	nt
Streptomyces	Streptomyces spp									+	nt	nt	nt
Xanthomonas	Xanthomonas sp	- -	nt	nt	nt								
Bacterial mix	Azospirillum sp + Pseudomonas sp + Bacillus sp					nt nt	nt nt	nt nt	- - - -				

Fungi

Genera	Species	Botryosphaeria dieback				Esca complex				Eutypa dieback			
		in lab	in field	WP	NU	in lab	in field	WP	NU	in lab	in field	WP	NU
Pythium	P. oligandrum					++	nt	nt	nt				
Trichoderma	T. atroviride	+++	nt	+++	nt	+++	++	++	nt	+++	nt	+++	nt
	T. gamsii (ex T. viride)					+++	nt	nt	nt				
	T. hamatum					++	nt	nt	nt				
	T. harzianum	+++	nt	++	nt	+++	++	+++	++	++	++	++	nt
	T. koningii									nt	nt	++	nt
	T. longibrachiatum					++	nt	+++	++				
	T. polysporum					+++	nt	nt	nt	+++	nt	nt	nt
Genera mix	T. asperellum + T. gamsii					nt	++	++	nt				
	Trichoderma + Gliocladium					nt	nt	nt	++				

Table 3: The main biocontrol agents (BCAs) tested for the GTDs control. For each BCA are reported: the efficiency recorded in the relative test (in lab and in field); the results when tested for wound protection (WP) or for nursery use (NU). Legenda for efficiency: - - ineffective; - + less effective; + middle efficient; ++efficient; +++ highly efficient, *nt* not tested.

GTDs management in Nursery

Mother plants. In nursery, the strategy to limit and control GTDs must start from mother plant fields. Several guidelines recommend how to manage fertilization, watering, etc to have less-stressed plants, in order to obtain good and vigorous rootstock and scion cuttings. But this is not enough to limit the impact of GTDs in the following plant production processes.

The visual screening of mother plants and the external GTDs symptoms absence are not indicative of the real phytosanitary status of the plants. Several studies have shown, in fact, no difference in the incidences of GTDs pathogens between symptomatic and asymptomatic vines, both in vineyard and in mother field plants. For the latter, a solution to limit the presence of GTDs could be the reduction of their productive life. Currently, mother plant fields are renewed after 15-25 years and during this time, plants could be infected and GTDs pathogens could spread all along the plants. If this time is shorten, the possibility of having large and spread GTDs infections could be reduced, limiting the number of infected cuttings used in the plant production processes. Furthermore, it is most favorable to maintain mother plants in a trellised system, avoiding the direct contact of the canopy with the soil.

A recent European survey on nursery practices[2] have highlighted that the most of nurserymen don't protect wounds in mother plant fields, either with chemical or biological products, after harvesting the cuttings. According to the ascertained presence of GTDs pathogens in the environment, also on secondary hosts, this represents a critical gap for the management of GTDs in nursery, since wounds are the preferred way of penetration for GTDs pathogens.

To limit the inoculum sources, pruning debris should be also removed in mother plant fields.

Nursery. After the harvest, rootstock and scion cuttings enter in the plant production process (fig. 2). Several steps of this process (hydration, cold storage, disbudding, grafting, rooting) are considered critical for GTDs cross-contamination between healthy and infected material. Scientific studies ascertained the presence of GTDs pathogens in hydration tanks and in the used water, in the sawdust used for callusing, etc. Despite this ascertained presence, nurseries utilize different approaches to limit GTDs infections. While few nurseries (according to the above mentioned survey) still don't use any treatment to avoid GTDs infections during all the production process, the most of the surveyed use fungicides against grey mould two or three times. Only a low percentage uses fungicides at each step. The most used fungicides are beltanol (8-hydroxyquinoline sulphate) thiophanate methyl, captan, mancozeb, thiram, cyprodinil+fludioxonil and metiram + pyraclostrobin. Scientific results for Chinosol, the most commonly fungicide utilized, indicate it as not suitable for the GTDs control, since it was not able to limit the growth of the GTDs pathogens tested.

Even if the use of BCAs in nursery is still limited, scientific trials have demonstrated the positive effect of *Trichoderma* when used in the plant production process. When used as drench in hydration, in the sawdust during callusing or for rooting, *Trichoderma* was able to protect from GTDs infection and to improve the development and the quality of the root system of the treated plants. So the use of *Trichoderma*-based products should be encouraged.

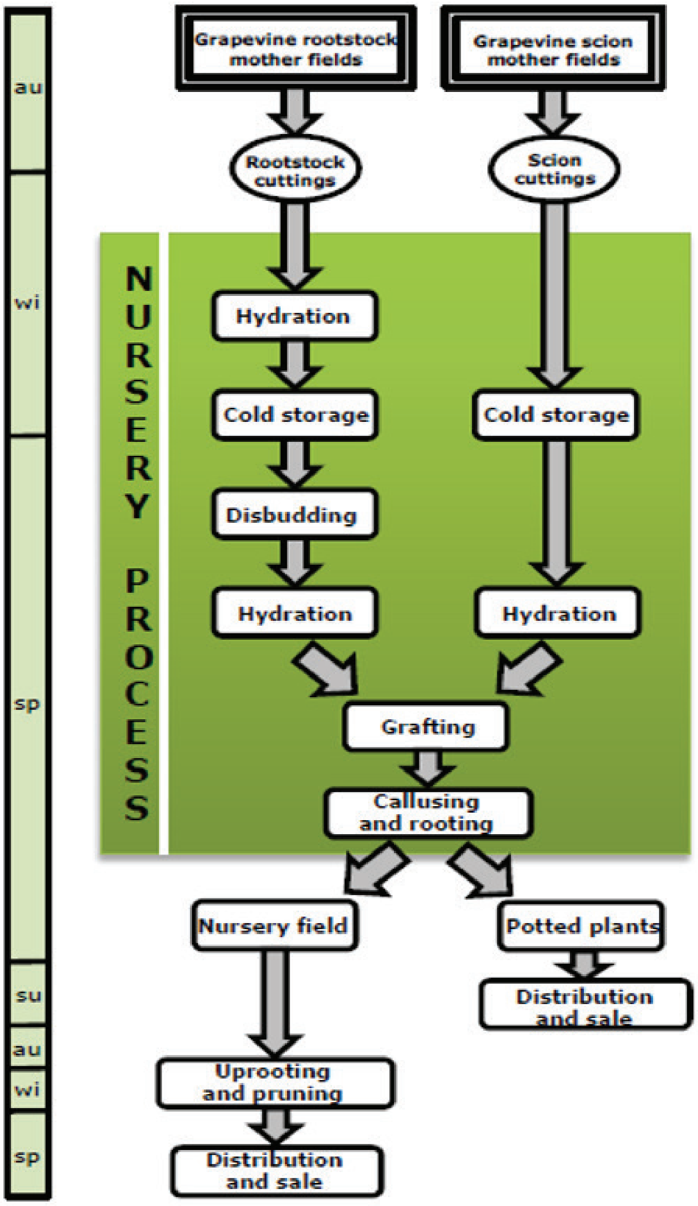


Figure 2. Diagram representing the propagation process of grafted plants in grapevine nurseries. Abbreviations: fa, fall; wi, winter; sp, spring; su, summer. from : Gramaje, D., & Armengol, J. (2011). Fungal Trunk Pathogens in the Grapevine Propagation Process: Potential Inoculum Sources, Detection, Identification, and Management Strategies. Plant Disease, 95(9), 1040–1055.

[2] Gramaje, D., & Di Marco, S. (2015). Identifying practices likely to have impacts on grapevine trunk disease infections: a European nursery survey. Phytopathologia Mediterranea, 54(2), 313–324. http://doi.org/10.14601/Phytopathol)

The hot water treatment (HWT), used as sanitation method towards the phytoplasmosis Flavescence dorée is nowadays utilized by some nurseries also towards GTDs pathogens. It consists in soaking plant material (cuttings or grafted plants) in hot water 50-53° C for 30-45'. The protocol used varies according to the nurseries and the countries. This sanitation treatment devitalizes the most of GTDs pathogens, even if the complete sanitation is not guaranteed. The use of this practice towards GTDs is controversial, since negative effects on cutting and grafted plants, observed by several authors as delayed callusing and rooting, buds death etc., could occur if the technique is not well and professionally handled. Furthermore, studies are needed to evaluate the heat-sensibility level of the different cultivars.

According with the possible infection risks along the plant production, the most suitable times for HWT could be those at the end of the process, before the sale. The early HWT could help in stopping GTDs infections at the beginning of the process but requires a high hygienic level in all the following steps.

The results of the studies focused on the presence and effects of GTDs pathogens in nursery have clearly attested their presence during all the production process and their negative economic impact in the plant production. So, as a general recommendation, nurseries should pay great attention to hygiene, avoiding contaminations in the most critical steps. In mother plant field, plant should be trellised and renewed each 10-15 years at maximum, and pruning wounds should be treated with fungicides or BCAs. In nursery, hydration should be done using water and fungicides or suitable

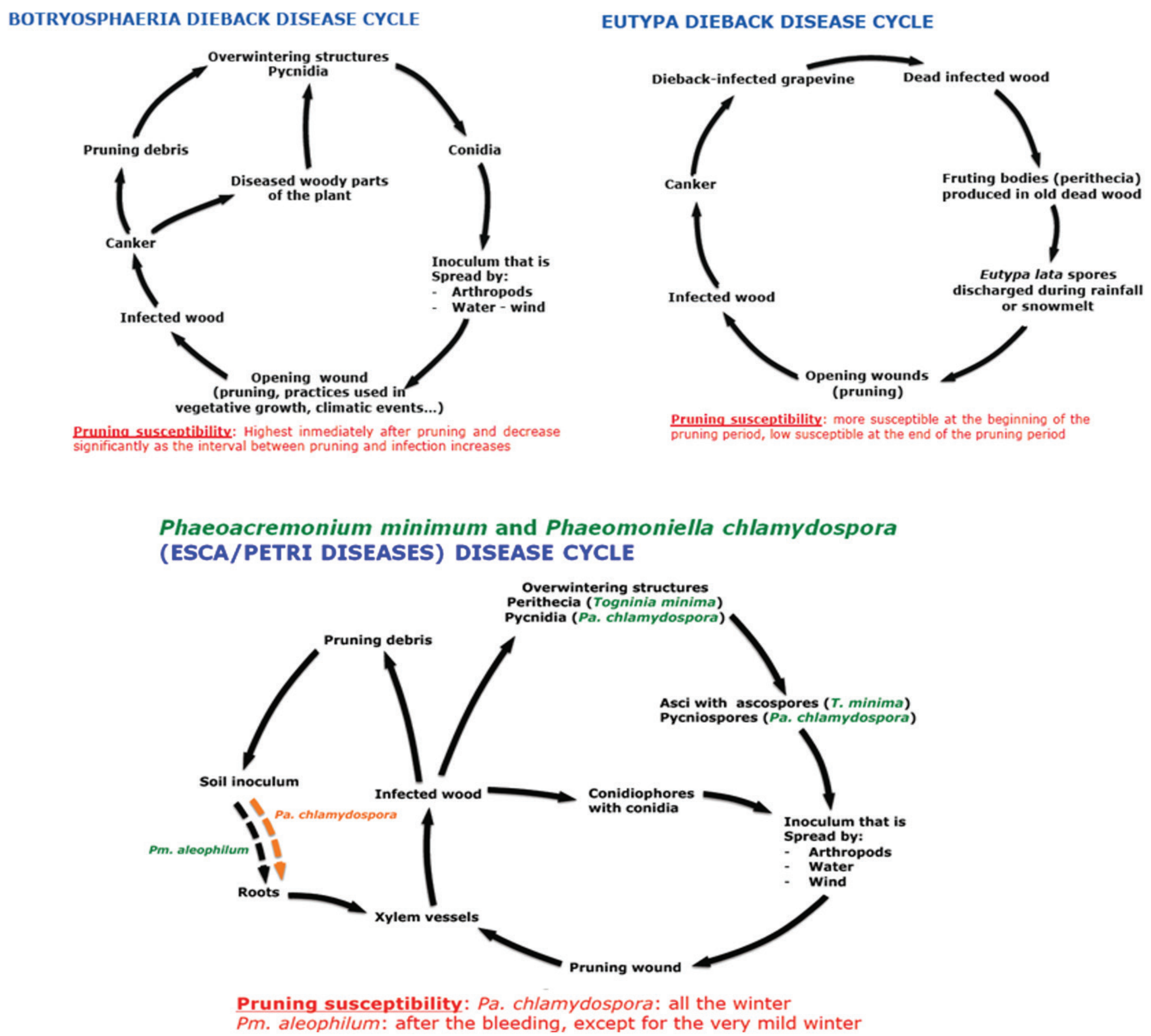


Figure 3. The disease cycle of the three main GTDs. (courtesy of D. Gramaje and P. Larignon, COST Action FA1303)

BCAs, avoiding re-using the same water for different bulks. Hydration tanks, pruning shears, grafting machines, callusing boxes etc, should be often cleaned and sterilized with disinfectants. All these measure, however, has an economic impact, especially for small nurseries.

Vineyard management and GTDs

Several epidemiological studies on Botryosphaeria dieback, Esca and Eutypa dieback conducted in several wine-growing areas worldwide showed the high number of fungal genera and species associated to these trunk diseases. Furthermore, these studies also demonstrated how the life cycle of these fungi is strictly connected with some cultural practices typical of viticulture, especially the pruning (Fig. 3).

Pruning wounds are the preferred way of penetration into the wood for GTD pathogens in the vineyard, and wounds can remain susceptible to GTD infections up to 2-4 months, or even longer, after bleeding, for instance.

Beside the "pruning wound protection" with AIs or BCAs (previously described and most relevant), today highly recommended to limit GTDs spread in vineyard, several empirical and alternative pruning methods were proposed and adopted with the same purpose of limiting the GTDs spread in vineyard. Works on different ways to manage vines and the ability to limit GTDs incidence in vineyard was extended also to training system, either with modifications of the traditional ones or with unusual training methods. Thus, several authors focused their attention on these practices.

Pruning methods. The "double pruning" is a pruning technique with two cutting periods: the first (pre-pruning), often made at the end of autumn or early winter, leaves long canes and could be mechanized. At the end of winter, a second cut eliminates the exceeding wood, leaving only two-bud canes. In this way, the potentially winter-infected canes will be eliminated, leaving only healthy short canes. This technique was evaluated for the control of Botryosphaeria and Eutypa dieback, giving good results in terms of infection control in vineyard in USA and in Australia. No differences between early and late pruning were observed on Esca and on Botryosphaeria dieback in France.

Another possibility used by winegrowers to limit GTDs infection in field is to adopt the "late" pruning method. This consists in a delay of the pruning, normally done in early winter, up to the end of winter or to spring, with the purpose of having susceptible wounds during the period of lower GTDs inoculum presence. Some authors consider the late pruning equivalent to the double pruning, with the advantage of a lower economic impact in the vineyard management. Several authors studied the pathogens' incidence in "late pruned" grapevines but the results were opposite. Some trials in North-East Spain, for instance, have identified the early pruning (mid autumn) as the best period to reduce GTDs infection. The difference in the result could be related to climate, especially to the rainfall.

The scientific community agrees that for maintaining low GTDs infection incidences in vineyard, it is very important to prune vines during dry periods, independently of the calendar, since the humidity plays an important role in the infection process.

Another technique supposed to be useful to control diseases in vineyard is the "minimal pruning". Vines are pruned during vegetative season only to reduce longer shoots. Each 2-3 years vines are pruned for shape maintenance. The theory supporting the minimal pruning links the reduced number of wounds with a lower presence of necrosis and pathogens. A recent study, addressed to compare the microbial community on vines trained differently, showed some differences between minimal- and normal-pruned vines. In particular, minimal-pruned vines globally showed a lower presence of necrosis and of wood-colonizing fungi, confirming the theory, even if no great differences were found on GTDs pathogen incidences.

Another pruning technique more and more popular, especially in France and Italy, is the "guyot Poussard" method. In this case, one of the main characteristic is the "sap flux respect" that must be maintained in vines. According to the results reported by technical services and private pruning consultants, this technique allows to have more healthy wood in vines, without necrosis along the main sap pathway in the trunk. Furthermore, it seems able to reduce the GTDs incidence and severity, compared to the traditional methods. Even if the technique is considered interesting by scientific community, since a more efficient "sap flux" (water, nutrients, etc.) could help vines to be more resistant to biotic and abiotic stresses, currently there is no scientific validation for this method.

Training system. Other studies were focused on the possible effect of the training system in the GTDs development. Vines are trained according to different schemes, but in general we have long arm vines (cordons) and short arm vines (guyot). Old and recent studies have evidenced an inverse relationship between internal and external GTDs symptoms. In detail in Guyot, to a higher presence of internal GTDs symptoms correspond to a lower incidence of foliar symptoms; the inverse relation was observed in cordon pruned vines. These differences could be related to the number of wounds realized in their management. In any case GTDs influence both vines sustainability and productivity, independently to the training system.

Since GTDs pathogens colonize woody tissues, one of the proposed methods to limit the economic impact of trunk disease in vineyard is the "trunk renewal". The trunk renewal could be applied in different ways.

The first method consists in re-grafting the rootstock of GTDs affected vines. The vine is cut up to reach healthy tissues and then re-grafted. According to some empirical experimentation, the treated vines could reach the productivity level of the non-treated ones in three years, thanks to the support of the "mature" root system. This technique, together with the over-grafting could prolong the productivity life of the diseased vines. Another trunk renewal method, proposed by Dr. Smart is the "timely trunk renewal". Differently to the previous methods, the future trunk (a chosen sucker/watershoot) is breed together with the main trunk. Independently of the GTDs presence in the vine, the main trunk will be periodically eliminated and substituted by a new one. The renewal time is chosen according to the "disease pressure" in the area/ vineyard. This interesting technique needs a scientific validation.

Management of diseased vines. Presently, old and new techniques are applied to the trunk of GTD affected vines, in order to extend their productive life. An increasing interest within viticulturists is the "trunk cleaning". This technique consists in the elimination of the wood degraded in a white rot by an electric handsaw. According to the reported results, the most of the treated vines don't show GTDs symptoms in the following years. The main observation towards this technique is related to the fact that the cleaning eliminates only rotten wood, thus leaving the trunk infected with the other vascular GTDs pathogens. Anyway, the Basidiomycetes eventually eliminated with the cleaning are the sole, among the GTDs associated pathogens, that don't produce toxins, that seem to be responsible for the GTDs foliar symptoms, so other hypothesis have to be formulated (as elicitors) as in the case of the "guyot Poussard" pruning methods, no scientific validation is currently available for this technique. On the contrary, the "remedial surgery, namely the elimination of diseased branches was successfully utilized, together with the "late pruning", to control Eutypa dieback in vineyard.

Debris management. Pruning debris was attested to be a long-lasting inoculum source, at least for Botryosphaeria dieback pathogens. In facts, a recent study ascertained the viability of D. seriata conidia in debris 42 months after pruning. Thus, the pruning debris removal could be recommended within a GTDs control strategy. According to the GTD, they could be buried in soil (not suitable for fungi producing durable spores as chlamydospores) or, better, utilized in compost production since the high temperature of the composting processed could devitalize the inoculum.

Conclusion

The results so far obtained have clearly demonstrated that the control of GTDs depends on a global strategy rather than a single solution. The scientific community agrees that the control of GTDs must start in mother plant fields, ending at the end of the vineyard productive life. Different studies showed, in fact, that GTDs-free young vines, if not protected in vineyard, after 5 years could be infected by GTDs pathogens, nullifying the advantage reached in nursery. On the contrary, an accurate management in vineyard, applying pruning wound protection for instance, could be less effective if plants are developing a GTD started in nursery.