Grapevine Trunk Diseases. A review

Month : April

Year : 2016

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International Organisation of Vine and Wine
Intergovernmental Organisation

Grapevine Trunk Diseases. A review

Crédit Photo. Philippe Larignon, 2012.

Scope

Grapevine trunk diseases (GTD) are currently considered one of the most relevant challenges for the viticulture. These destructive diseases cause in vineyards several damages every year, and they are of rapidly growing concern in all wine producing countries. In 2006, the OIV established a resolution about some principal measures used to prevent or limit the proliferation of wood diseases (resolution OIV-VITI 2/2006), and minor references in CST 1/2008 and CST2/2011 OIV resolutions. Recently, a new initiative has arisen inside the OIV group "vine protection and viticultural technics (PROTEC)" concerning to the development and impact of main trunk diseases, and their different alternatives to control or mitigation of their spread and damage at an international level of the cultivated *Vitis* species.

The following document, which could be updated in the future on further editions, attempts to describe the state of art in this field, and was drafted in collaboration with the members of the International Council on Grapevine Trunk Diseases (ICGTD) by the following research group:

Crédit Photo. David Gramaje and Josep Armengol, 2011

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ISBN : 979-10-91799-60-7

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Introduction

Grapevine trunk diseases are considered the most destructive diseases of grapevine for the past three decades and are of rapidly growing concern in all wine producing countries. The worldwide economic cost for the replacement of dead grapevines is roughly estimated to be in excess of 1.5 billion dollars per year (Hofstetter *et al*., 2012). Vine trunk diseases are very harmful for the sustainability of the winemaking heritage because the pathogens responsible for these diseases attack the long-lasting organs, causing the death of vines on shorter or longer term. Esca, Eutypa and Botryosphaeria dieback are the leading players of these decay diseases. As well as mature vineyards being affected, those being planted as replacement can also be affected. Others like Petri disease or Black-foot disease (*Campylocarpon, Cylindrocladiella, Dactylonectria, Ilyonectria* and *Neonectria* spp.) are major diseases affecting young vineyards, reducing their productivity and longevity, thereby causing considerable economic loss to the industry (Gramaje and Armengol, 2011).

The general symptoms express themselves at the wood level through sectorial and/or central necrosis, by the presence of brown streaking or cankers, and at the foliar level by discoloration and drying, which can occur suddenly (Larignon *et al*., 2009, Mugnai *et al.,* 1999).

In young vineyards, external symptoms such as stunted growth, reduced vigor, retarded or absent sprouting, shortened internodes, sparse and chlorotic foliage with necrotic margins, wilting, dieback and death should appear due to black-foot or Petri disease affected vines (young vine decline), but they are frequently indistinguishable (Gramaje and Armengol, 2011). In addition, characteristic symptoms of vines affected by these diseases are sunken necrotic root lesions with a reduction in root biomass and root hairs.

The life cycle and epidemiology are very similar for all the known fungi that cause trunk diseases (Berstch *et al*., 2013). These diseases are cryptic and their symptoms usually take several years to develop. As such they are insidious, and difficult to observe. Pruning wounds are the main point of entry for fungal spores, but also invasion of mechanical and frost wounds are possible. They subsequently grow, decay the wood and slowly kill the vines. Fruiting bodies produced in dead wood and their spores are released in the presence of water, dispersed by wind, and finally, could infect fresh new wounds (Rolshausen and Kiyomoto, 2007).

This is probably the most relevant threat for vitivinicultural sector nowadays (Rubio and Garzón, 2011). For instance, on Spanish vineyards the degree of influence has grown from 1.8% in 2003 (the year of sodium arsenate prohibition in Spain), to 10.5% in 2007 (Rubio and Garzón, 2011).

Several factors (Rubio and Garzón, 2011) could be involved in recent trunk diseases development:

i) Ban of sodium arsenate, the only mean known to control Esca.

ii) Annual increase of the mortality rate from 4% to 5% starting from the fifth year in plots where the treatment by sodium arsenate has been stopped.

iii) Increased number of the contaminated asymptomatic stocks in vineyards.

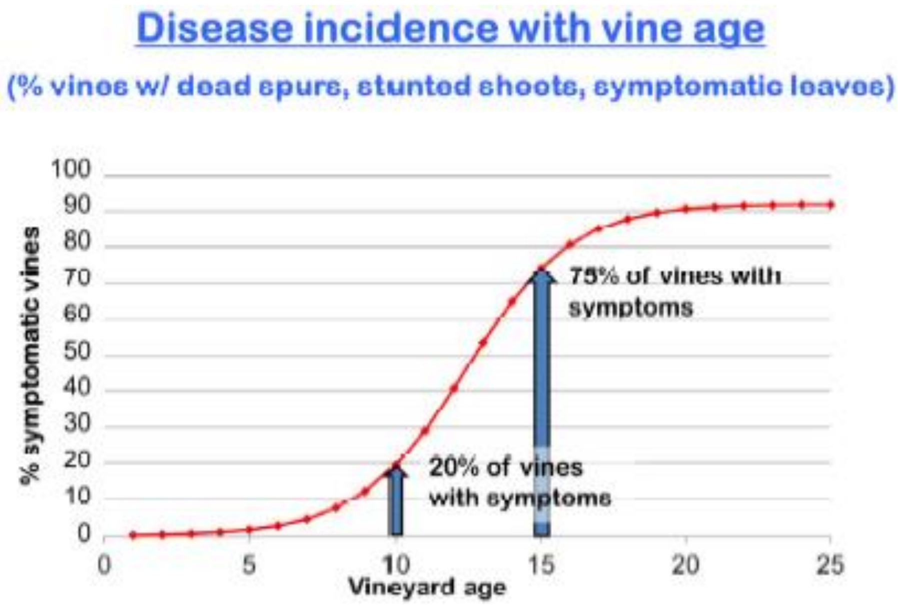
iv) Infections brought to vineyards by infected planting material.

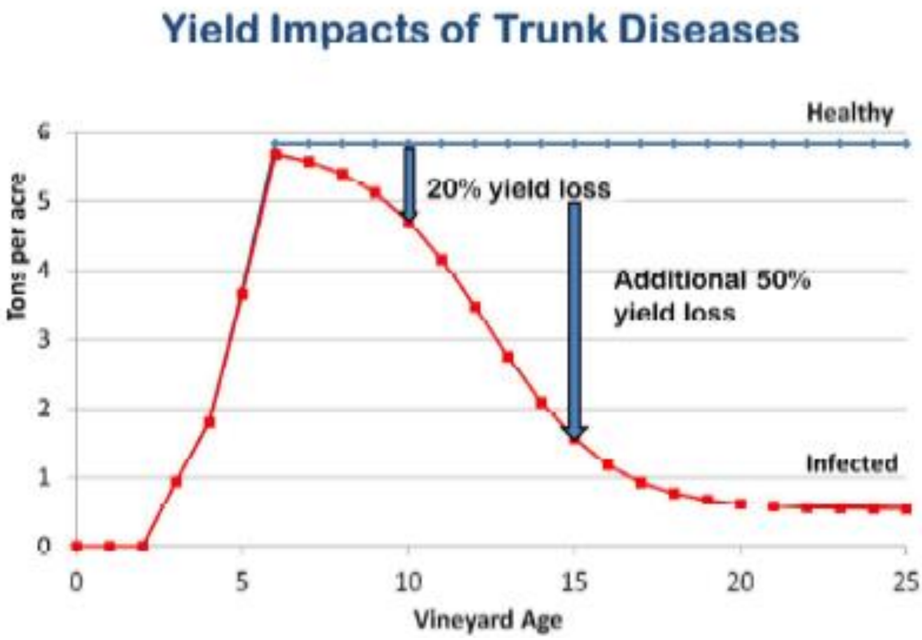
v) Cultural practices in vineyards. These usually focused to have grape yield during the first years and, giving a poor pruning wound protection.

Finally, other relevant problem is the assumption that the involved fungi are endophytic, which implies that they may live asymptomatically a part of their life in a plant, but should then, at some point and associated with plant stress, modify their behavior and becomepathogenic, thereby leading to the expression of the disease symptoms (Hofstetter *et al*., 2012).

GTD Impact around the world

Up until some decades ago, trunk diseases normally damaged mainly old plants, and the substitution of affected vines with healthy ones was a common and efficient way of restricting the dissemination of the disease (Bruno and Sparapano, 2007). Recently, these diseases have been observed to be in rapid extension and to affect even 2- or 3-year-old plants. Nowadays, trunk diseases of grapevines appear often in vineyards that are over 7-year-old (Díaz and LaTorre, 2013). Figures show the average percentage of incidence cumulate in vine and the disease evolution and also the yield impact along its productive life (Munkvold *et al*., 1984 in Baumgartner *et al*., 2014).





Hereby, some data from different countries about the impact of these diseases:

ESP: Since 2003 (year of the sodium arsenate prohibition in Spain), it has grown up from 1.8% of degree affections in vineyards to 10.5% in 2007 (Rubio and Garzón, 2011).

FRA: Close to 13 % of French vineyard is now affected by trunk diseases according to the survey led by the DGAL in 2012 (Grosman and Doublet, 2012). Therefore, it is a major concern of wine growers since the sodium arsenate was banned in 2001, which was the only effective molecule against the esca. Diseases known as esca, Botryosphaeria dieback and Eutypa dieback lowered potential wine production by 13% in France in 2014, according to the agriculture ministry and French Wine Institute (IFV). The increase of symptoms was investigated and the incidence reached values higher than 10% for Botryosphaeria dieback and 25% for Eutypa dieback in French vineyards (Bruez *et al*., 2013). The diseases are costing France the equivalent of 1bn euros ($1.14bn) annually in lost wine production, IFV said and means more than 100,000 hectares of vineyard was lost in 2014 and between 10 to 15% of potential production was lost last year. InterLoire also estimated that at least 5% of Europe's vineyards were affected by GTDs.

ITA: The use of sodium arsenate banned from 1970 onwards; however, under very limited use. GTD are a growing problem for many vineyards in all regions. Usually symptoms occur from 8 -10 years; only just occasionally they are evident on younger vines. It was I note that the incidence of the disease depend on the varietal susceptibility: on plants of 15-18 -year average incidence may fluctuate respectively around 12 to 19 % for white grapes, around 8 to 10 % for the black grapes. In some regions under extreme conditions of central and southern Italy where epidemiological studies have been carried out (such as Tuscany, Marche, Abruzzi, Apulia, and Sicily), esca incidence has reached 60% to 80% in some old vineyards (Romannazzi *et al*., 2009).

PRT: In Portugal, GTDs are also widely spread all over the grape growing regions, most noticeably in Vinhos Verdes, Douro, Dão and Alentejo regions. Botryosphaeria dieback and Esca are the major diseases of adult grapevines and they cause considerable damages and economic losses. Eutypa dieback incidence is low when compared with other neighbor countries. Surveys of young vine decline showed that, at the rootstock, black foot disease and Petri disease were dominant but other wood diseases like Botryosphaeria dieback were also present. From the tissues above the graft union, Botryosphaeria dieback was prevailing but black foot and Petri disease were also present (Rego *et al*., 2005).

ARG: In 30% of old vineyards (more than 10 years and representing 81% of total vineyard area) the "*hoja de malvón*" (a disease with different symptoms but usually compare to Esca in Europe) was detected during a national study in 2008 (Van den Bosch *et al*., 2011).

TUR: The rate of esca is very low in Turkish regions. For instance, it has been determined to be 2.61% in the vineyards of Tekirdag, Marmara Region (Ari, 2000).

USA: California, annual yield losses due to Eutypa dieback and Botryosphaeria dieback, two widespread wood-canker diseases of grape (trunk diseases), account for 14% of the gross producer value of CA wine grapes. In California, economic losses of at least US$260 million per year have been attributed to trunk diseases (Siebert, 2001), and some studies indicate that early intervention is necessary to restrict disease spread and loss of income (Smart, 2015). Esca means a loss of about 2.000-3.000$ per hectare per year to Californian viticulturist (Vazquez 2007, in Rubio and Garzón, 2011).

AUS: Eutypa dieback is a major disease of grapevines worldwide which causes considerable economic loss to the $8.3 billion Australian wine industry and is caused by the fungus *Eutypa lata* (Ridgway *et al.*, 2014). In Australia, yield losses of up to 1,500 kg/ha have been reported for Shiraz vineyards (Wicks and Davies, 1999 in Sosnowski *et al.,* 2013).

NZL: A national survey of symptomatic material from 43 vineyards showed that 88% had some degree of infection by *Botryosphaeriaceous* species (Ridgway *et al*., 2014).

Main diseases associated to trunk decay.

There are four major grapevine trunk diseases, all of them caused by different fungi. These diseases are called Esca, *Eutypa* dieback, *Botryosphaeria* dieback and *Phomopsis* dieback *"*excoriosis*"*. Esca is a major problem in Europe and *Eutypa* occurs around the world. *Botryosphaeria* is also global, but not so well understood nor recognized by many growers (Smart, 2015).

Established vineyards.

Esca Complex (Grapevine Leaf Stripe Disease and Apoplexy)

Phaeomoniella chlamydospora has usually been associated with grapevine decline called Esca (Díaz and LaTorre, 2013). Esca is a fungal disease and it is present in both hemispheres (Larignon *et al*., 2009). Recent studies have ascertained that the ascomycetes *Phaeoacremonium spp.* and *Phaeomoniella chlamydospora,* and the basidiomycete *Fomitiporia mediterranea* (M. Fisch.) are associated with the esca syndrome (Bruno and Sparapano, 2007). *Stereum hirsutum* is also involved, but less relevant. In South America and South Africa other different basidiomycetes (*Fomitiporia* species) have been also described associated with trunk diseases of grapevine (Cloete *et al.,* 2014). However, often *E. lata* and Botryopshaeriaceae are also present in the wood.

Two forms of Esca have been reported on older grapevines, but Esca could also cause decline and death of recently planted vines, which is usually associated with plant stress. Esca is also a problem on table grapes because the fruit clusters borne by the infected vine are unmarketable (Rolshausen and Kiyomoto, 2007).

Very likely, at least a part of the external and internal symptoms of Esca is caused by phytotoxic fungal metabolites produced in the discolored or decayed woody tissue, or by oxidation of some host response substances. Some chemicals produced as the consequence of fungal infection are toxic to vines. Particularly, α-glucans and two naphthalenone pentaketides called scytalone and isosclerone, are secondary metabolites of several fungi and were also produced in vitro by these fungi (Bruno and Sparapano, 2007). Esca is a complex disease whose symptoms may be due to the concomitant action of several factors (Andolfi *et al*., 2011; Bénard-Gellon *et al.,* 2015).

Phomopsis dieback.

Phomopsis viticola. Ravaz and Verge (1925) gave the name of excoriose and it comes from the verb meaning "to excoriate skin slightly". Recently, the most used named is Phomopsis dieback (Úrbez-Torres *et al*., 2013).

Phomopsis cane and leaf spot is more severe in grape-growing regions characterized by a humid temperate climate through the growing season. Crop losses up to 30% have been reported to be caused by Phomopsis cane and leaf spot (Úrbez Torres *et al*., 2013).

P. viticola can infect all green parts of the grapevine and its symptoms are present in all herbaceous organs (shoots, basal wood, leaves, stems or fruits). On the young shoots, the disease results in the first internodes with the presence of small black spots, that later develop into well-individualized blackish-brown crusts or brown lesions with strips of corky appearance like "chocolate". In branches, it could appear as a strangulation at their base, which can lead to breakage under certain conditions (wind, weight of the crop). During the dormant season, canes show a white appearance with black points at internode zones. Blackish necrotic spots may also be encountered along the main and secondary veins as well as the petioles. Some leaf portions can also turn to yellow, pale green and/or brown color. Severely infected leaves or leaves with heavily infected petioles may fall. On the other hand, the fruits turn brown and wither, with mummies or shriveled berries close to harvest (Larignon, 2012; Úrbez Torres *et al*., 2013).

Other associated fungi, like *Phomopsis theicola* and its symptoms are characterized by the mortality of the great part of a young plant. In the wood, particular sectorial necrosis and some punctuations of brown color are usually observed. Nowadays, this is not a big problem, but these decays are still present and described in Great Britain (Larignon, 2012).

Eutypa dieback.

Eutypa lata is an ascomycete (*Diatrypaceae*) and it is classified among the "soft decay" fungi, because it develops inside the secondary walls forming cavities (Larignon *et al*., 2009). Eutypa dieback (or eutypiosis), is caused not only by *E. lata* but also by other *Diatrypaceae* spp.

It shows its presence through the shriveling of shoots (fan leaf) which present chlorotic, wrinkled and ripped leaves with marginal necrosis, and can become widespread over the whole limb. Sometimes, eutypiosis can produce dried out inflorescences or clusters *millerandage*. Also, the death of the shoot can occur (dead arm).

In the trunk, a brown and hard sectorial necrosis with dark stripes or scratches is the main symptom (Larignon, 2012).

***Botryosphaeriae* dieback**

Botryosphaeriae dieback known for a very long time under the name of “slow stroke” *(D. seriata),* is produced by a family of fungi species called *Botryosphaeriaceae and* has been associated with Botryosphaeria dieback (Díaz and LaTorre, 2013). To date, several studies have allowed the identification of at least 21 different species in the Botryosphaeriaceae occurring in grapevines worldwide (Úrbez-Torres, 2011). Other fungi like *Lasiodiplodia theobromae, Neofusicoccum parvum* and *Botryosphaeria dothidea* are associated with this disease too.
Foliar symptoms are characterized by interveinal areas without yellow border at the first stages of appearance of symptoms in red cultivars, but with yellow border at the end similar to Esca (Lecomte *et al*., 2006; Reis *et al.* 2016). Some cultivars are more sensitive to this disease (Cabernet, Sauvignon, Ugni-Blanc, etc.) than others (Merlot).

The affected plants are characterized by dead branches with weakened vegetative development, sometimes still alive but with low percentage of bud break. It is not usual to detect characteristic foliar symptoms, but sometimes chlorosis weaknesses or some deformations of leaves (Larignon, 2012) can be observed. The main symptom in the trunk is a typical sectorial necrosis with vascular discoloration.

Young vineyards.

Two fungal trunk diseases are associated to young vineyards decline: Petri disease and Black foot disease. Environmental factors and host stress such as malnutrition, poor drainage, soil compaction, heavy crop loads on young plants, planting of vines in poorly prepared soil and improper plant holes also play an important part in the development of black-foot and Petri diseases (Gramaje and Armengol, 2011).

Petri disease

The name of Petri disease was given to this decay during the second Congress IWGTD (Lisbon 2001) in honor of Petri, a phytopathologist (1912) who had observed on vines the process of decay of the vascular tannings, in which he found two species of *Cephalosporium* and one of *Acremonium* (Larignon, 2012).

The main fungal agents associated with this disease are *P. chlamydospora* and *Phaeoacremonium* spp. (Gramaje and Armengol, 2011), but it also related with *Cadophora luteo-olivacea* and *Pleurostoma richardsiae (current correct name)*.

External symptoms are expressed on aerial organs level with the presence of weakened vegetation or less developed vegetation, chlorotic leaves with necrotic borders and an undersized trunk. These symptoms can lead to the death of a plant.

Inside the trunk, it could be observed a typical brown streaking and brown red/brown necrosis, which is a result of tyloses, gums, and phenolic compounds formed inside these vessels by the host in response to the fungus growing in and around the xylem vessels (Gramaje and Armengol, 2011).

Especially at the grafted level, some brown or black spots appear, when the cutting is transversely performed. This sap flux originates often from those necrosis and it is popularly called "*black goo*" (Larignon, 2012).

Recently, the role of *Cadophora* spp. (*Pleurostoma richardsiae*) in the decline of grapevine has been questioned, based on species reports from California, South Africa, Spain, Uruguay, and Canada. In particular, *C. luteo-olivacea* has been isolated from both asymptomatic and symptomatic grapevine wood, in nursery and field plants showing black streaking of xylem vessels, the typical internal symptoms of Esca and Petri disease, or from decayed and discolored wood observed at the graft union of declining *V. vinifera* 'Syrah' plants and rootstocks (Travadon *et al.,* 2015).

Black foot.

Black foot disease of grapevines is a well-documented disease in various countries and it was previously reported as caused by *Cylindrocarpon* spp. and *Campylocarpon* spp. (Gramaje and Armengol, 2011), but now it is known to be associated with fungal species from the following genera; *Dactylonectria, Ilyonectria Campylocarpon, Cylindrocladiella* or *Neonectria* (Lombard *et al.,* 2014).

Characteristic symptoms of black-foot disease include a reduction in root biomass and root hairs with sunken and necrotic root lesions (Agustí-Brisach and Armengol, 2013). In some cases the rootstock diameter of older vines is thinner below the superficial (second) tier. To compensate for the loss of functional roots, a second crown of horizontally growing roots is sometimes formed close to the soil surface.

Black foot also expresses at aerial organ level either by an absence of breaking bud, or by a presence of weakened vegetation, which mostly dries out during the season (Larignon, 2004). It should be noted that the roots at the first level are necrotic, showing an intoxicated color between black and grey (according to the degree attack). The plant shows a reduced vigour with small-sized trunks, shortened internodes, uneven wood maturity, sparse foliage, and small leaves with interveinal chlorosis and necrosis (Agustí-Brisach and Armengol, 2013). The black foot is identified by a black necrosis which starts at the bottom and goes up affecting most of the rootstock wood.

Current methods to control and mitigation:

Currently proposed methods are not curative (fungicides, chemical products and biological stimulators, etc.) so, merely preventive methods are frequently applied to the vineyard.

Nursery measures before planting.

A healthy vine is fundamental to the successful beginning and sustainability of all grape vineyards (Gramaje and Armengol, 2011), being the first point in the production chain. There are many opportunities for infection by trunk disease pathogens during propagation processes: wounds at every stage of production or improperly healed graft unions are some examples to infection in the nursery, and if the vines survive, after planting in the vineyard.

Consequently, good hygiene and wound protection are of the utmost importance (Gramaje and Armengol, 2011). Even so, research on the management of black-foot disease and Petri diseases as well as *Botryosphaeriaceae* dieback (main species in mother fields, nurseries, and open root field nurseries or young vineyards) are being carried out in different areas.

Several studies have led to the conclusion that planting material can be already infected in young vineyards, either systemically from infected mother vines (Ridgway *et al.,* 2002; Halleen *et al.,* 2003; Gramaje and Armengol, 2011) or by contamination during the propagation process (Giménez-Jaime *et al.,* 2006; Larignon *et al*., 2009; Vigues *et al*., 2009; Gramaje and Armengol, 2011). The ratios of the infections could increase from 40% before cuttings up to 70% after nursery processing (Gramaje and Armengol, 2011). Hence, detection prior to planting is critical to assure longevity of newly established vineyards (Urbez-Torres *et al*., 2015).

Some practices such as dipping the bottom of the grafts in a fungicide, act like a protection against pathogen attack (Rego *et al*., 2009), cultivate rootstocks in a trellis system instead of sprawl in the soil or not use flood irrigation systems could help to control these diseases, but they are not a universal practice (Gramaje and Armengol, 2011).

Hot water treatment

In quality of planting material, disinfection of nursery propagating materials and control programs with Hot Water Treatments (HWT) are frequently used for obtaining commercial plants in good sanitary conditions. HWT is generally performed at 50ºC for 30 min, but it is stressful for the plant (Waite *et al*., 2013); if not applied correctly, it can result in the loss of the plant material. *Vitis vinifera* varieties have different degrees of sensitivity to HWT, which can be affected by the temperature experienced during the prior cutting growing season.

Moreover, the range of temperatures used depends on the pathogens that need to be controlled.

Temperatures between 45–47ºC have been reported to eliminate *Pa. chlamydospora*, while temperatures of 51–53ºC are necessary to eliminate pathogens more resistant than the Petri disease ones (Bertsch *et al*., 2013). Hot water treatment could reduce the presence of *P. chlamydospora* (-78%) when treated at a temperature of 50ºC for 30 min (Larignon *et al.,* 2009).

Other results suggest that standard HWT protocols at 53ºC for 30 min or 50ºC for 45 min may be sufficient to control Petri or black foot pathogens in grapevine propagation material (Gramaje and Armengol, 2011; Agustí-Brisach and Armengol, 2013).

Vigues *et al.* (2009) concluded that HWT was the only practice among different control methods tested (chemical, biological, and technological methods) that showed promising results by reducing *B. dothidea*, *D. seriata*, and *Pa. chlamydospora* infections for several years in French nurseries.

Recently, Elena *et al*. (2015) concluded that HWT at 51°-53°C for 30 min was able to control eight species of Botryosphaeriaceae pathogenic to grapevine in the nursery grapevine propagation process.

On the practical and large-scale interventions, the risks should be evaluated on survival of the young plants treated with HWT.

Preventive culture measures in vineyard.

First of all, culture control methods are essential to limit the spread of inoculum by removing and burning branches, dead/dying vines, pruning residues, pruning dead arms, and trying to avoid dry periods, etc. Then it is also highly recommended to reduce and protect pruning wounds (plastics, mastic, oils, etc.) and to restore the dead shoots or branches if it is possible and if not, finally to replace the whole plant (VITI 2/2006 OIV resolution).

Alternatively, if vineyard soils constitute the main source of inoculum for grapevine infections, disease management practices based on soil disinfestation and amendments, plant-based resistance to infection, and prophylactic cultural practices should be investigated (Travadon *et al.,* 2015). The infected parts of a plant and the infected dead wood from soil should also be removed to lower inoculum loads in vineyards (VITI 2/2006 resolution).

Late pruning in the dormant season (as close as possible to budbreak) was also a recommended cultural practice, since the wounds heal faster with high degree-day temperatures. Nevertheless, recent studies revealed that the rate of natural infection of pruning wounds was lower following early pruning (autumn) than following late pruning (winter). The susceptibility of the wound is mostly influenced by the relative humidity and rainfall periods (Luque *et al.,* 2014). Double pruning or pre-pruning is enhanced by growers to speed up final pruning and to reduce disease incidence in spur-pruned vineyards. Sanitation methods are often complemented with the protection of pruning wounds from frost or biotic attack by the application of fungicides, biological formulations or both in rotation (Bertsch *et al*., 2013).

Prevention of wound infections coming from pruning should rely on strategies developed for other trunk pathogens of grape, giving that the timing for spore release is known for different viticulture regions. Rainfall encourages spore liberation. It has been demonstrated that fresh pruning wounds are the main infection route for fungal trunk disease pathogens (Díaz and LaTorre, 2013). Chemical protection of pruning wounds against infection by fungal trunk pathogens has been previously proposed to control *Eutypa lata* and some species of B*otryosphaeriaceae* in grapevines (Díaz and LaTorre, 2013). It is a major strategy to control trunk diseases.

Some studies have demonstrated that the infections in pruning wounds caused by *D. seriata, Inocutis sp*. and *Pa. chlamydospora* can be significantly reduced by using a single paste applications with a mixture of benomyl, pyraclostrobin, tebuconazole and thiophanate-methyl (Díaz y La Torre, 2013).

Furthermore, benomyl and thiophanate-methyl, two benzimidazole compounds having similar mode of action that inhibits cell mitosis, provided the best control in the field trials (Díaz and LaTorre, 2013). Anyway, in order to be effective, the products must be applied directly onto the wounds (Sosnowski *et al*., 2008).

Other relevant point is the better time for adopting the preventive culture measures. One experiment was carried out in an infected vineyard according to four treatments: no action is taken to manage trunk diseases, and when a practice with a level of 75% disease control efficacy (i.e., it protects 75% of pruning wounds) is adopted in a vineyard of ages 3, 5, or 10. Results shown, less yield loss the earlier the practice is adopted. Indeed, if adopted in year 3, the vineyard has annual yields similar to that of a healthy vineyard (Baumgartner *et al*., 2014).

Related to the visual inspections in vineyards, the efficiency of evaluation of some active principles and applied biological products (either curatively on sick vines or preventively since set-up of the vineyard) is based mainly on the visual observation of the symptoms on herbaceous parts (Larignon *et al*., 2009). This methodology is not appropriated for testing products such as mastic and paste for pruning wounds.

Finally, other cultural factors which could produce stress to the plant, should be taken into account. A study of the hydric balance carried out during three consecutive years in a Bordeaux vineyard shows that vine under hydric stress contribute to inhibition of the foliar expression of Esca disease. In addition, vine shoots composting allows however, the eradication of the fungi associated with trunk diseases

(Larignon *et al.,* 2009). An interesting fact from the past regarding Esca is that it was common to open the trunk with an axe and to insert a stone in it for drying off the fungi and lead to its death (Pérez Marin, 2000).

Training system and trunk renewal practices.

Systems such as two very short cordons in Double Guyot, usually move onto a Simple Guyot, which is one of the most probable factors to enhance the development of trunk diseases in the plant (Lecomte *et al*., 2012). In this point, pruning or training practices should be reconsidered in order to enhance the spurs' training, and to avoid the big wounds during mechanical pruning or others arising by the use of small electrical machines, that favors the initial focus to dry. Harvest is also to be controlled, due to the shaking produced during the harvest machine passing, which can frequently cause a foliar damage similar to apoplexy or "*folletage*".

The excessive simplification of training system (mechanical pruning, harvest, etc.) is probably, at present one of the most harmful reasons. This change of mind is often the response given to the need for increasing a low or minimal density per ha, without changing the vine material or distances. In this case, the distances are kept between rows, but the space decrease between vines (Lecomte *et al*., 2012). This choice leads to simple cordon formations, favoring the establishment, spread and development of trunk diseases.

Trunk renewal is not a new practice in viticulture, because in nature vines have been multi-trunked and, of course, not trained. Pruning can be done to enhance the renewal of the old infected trunks and cordons with uninfected canes.

Multi-trunks is a practice used commercially in places with severe winters to replace cold-damaged trunks (e.g. in New York State), and it can be used to fight trunk disease too. Studies in Australia have shown that *Eutypa* disease can be controlled by taking healthy suckers from the base of the plant to replace the trunk, and this technique works with the other GTD as well (Smart, 2015).

Importantly, the vine root system if healthy can be saved. Timely Trunk Renewal (TTR, Smart 2015) depends on sucker presence arising from 'base' buds at prior node positions on the vine trunk. TTR can help slow the spread of disease, as fruiting bodies on the old framework can be removed in this process. There is, however, no guarantee that re-infection may not occur and pruning wounds should be protected.

The Timely Trunk Renewal protocol (Smart, 2015), establishes some guidelines before doing the renewal, such as: sucker training may precede trunk renewal to avoid crop loss; pre-harvest inspection to identify early stage symptomatic vines; severe winter prune and spring trunk removal to encourage suckers for trunk renewal etc..

Chemical control products.

There were certain products for the trunk diseases control in the past, but none at present. $NaAsO_2$, a toxic product that was employed to control fungi associated with trunk diseases, was capable of killing most of them through the xylem. In 2003, this product was forbidden in all the winemaking countries because of its toxicity for wine growers: the median lethal dose (LD_{50}) of sodium arsenate for humans is 150 mg /kg administered cutaneously and transdermally. In 1996, Escudo© (flusilazole and carbendazime) was formulated and already in 2010 retired from the market. Therefore, there is no fungicide in the market that is allowed for use as a chemical product against these diseases by the authorities (Rubio and Garzón, 2011).

Chemical control is based on preventive measures for protecting pruning wounds, usually with fungicides, to avoid grapevine infection and to limit fungal expansion in the plant. Chemical treatments that often contain more than one fungicide are frequently applied to the soil (injector pole), the trunk (trunk injections) and pruning wounds (painted pastes or liquid formulations). Sprayed or paintbrush applied formulations are usually the most practical (even if some may be easily washed off by rainfall), trunk injections are impractical and expensive practices (Bertsch *et al*., 2013).

Some substances like tebuconazole, flusilazole, benomyl, prochloraz (Rolshausen *et al*., 2010), prothioconazole+tebuconazol, fluazinam (Gramaje and Armengol, 2010), tyophanate methyl, mancozeb, fenarimol and procymidone (Amponsah *et al.,* 2012) have been showed a positive effects against GTD *in vitro*. Unfortunately, some of them were restricted because of health and safety concerns (Bertsch *et al.,* 2013).

In nurseries, the range of registered products is limited, their application can be difficult and expensive and also, they generally do not provide long-term wound protection or broad spectrum control (Gramaje and Armengol, 2011). Related to young vine infections, only benomyl and imazalil showed some effect to control these pathogens in semi-commercial field trials against black foot disease (Agustí-Brisach and Armengol, 2013).

In fact, only some preventive products like tebuconazol + synthetic resins or Esquive® WP (active substance *Trichoderma atroviride* I-1237), Folicur (tebuconazole), Shirlan (fluazinam) or Cabrio (pyraclostrobin) have demonstrated certain degree of GTD control in vineyards.

One application of Bion (acibenzolar-S-methyl) + Cuprocol (Cu oxiclorure) after pruning followed by one application of Bion + Score (difeconazole) at phenological stage C/D was the most efficient treatment to consistently reduce incidence and severity of Botryosphaeria and Phomopsis dieback. Also, the lowest number of dead plants, the highest yield per plant and the highest percentage value for plant vigour were achieved with the same combination of products/spray application timing (Rego *et al*., 2014).

Rolshausen and Gubler (2005) found that boron (applied as boric acid mixed in water) accumulated in shoots and leaves, and that bud failure occurred at the first node below the treated wound. South Australian trials have demonstrated that boron significantly reduces infection by *E. lata* (Sosnowski *et al*., 2008; Rolshausen *et al.,* 2010).

In addition, carried out by several laboratories in the world so far, the trials have not produced any satisfactory curative or preventive method of use of the chemical products to fight against Esca or other GTDs. The reason for this is that either the tested products are not effective or their application methods are not practical for vine-growers, and their success depends on several factors, such as the method and the number of applications on grapevines, the persistence of the product and the species of fungus treated (Bertsch *et al.,* 2013).

Future perspectives:

Breeding, propagation and clonal selection, traceability and certification.

Research on cultivars and clones is needed. For instance, Merlot cultivars seem to be more resistant to trunk diseases than other varieties (Pouzoulet and Rolshausen, 2014; Travadon *et al.,* 2014; Guan *et al.,* 2015). Two-year visual inspections of 10 different cultivars in Italy demonstrated that the incidence of Esca was higher in cultivars Cabernet Sauvignon, Sangiovese, and Trebbiano toscano, and lower in Montepulciano and Merlot (Quaglia *et al*., 2009). In similar way, many varieties have different susceptibility to Esca disease (Borgo, pers. com).

Sometimes, the degree of sensitiveness to the disease depends on the rootstock, such as differences regarding their free polyamine content. For instance, some rootstocks such as *Vitis riparia* 039-16 and Freedom had a good degree of resistance to black foot disease (Gubler *et al.,* 2004). Gramaje *et al.* (2010) suggest that grapevine rootstock crosses of *V. riparia* × *V. berlandieri* could be the least susceptible to Petri disease pathogens.

Major improvement efforts have been directed toward enhancing fungal-disease resistance in table and wine grape cultivars. A number of pathogenesis-related (PR) proteins were screened for their response to fungal pathogen infection. Genetically modified grapevines constitutively expressing rice chitinase genes exhibited enhanced resistance to anthracnose and powdery mildew. Enhanced resistance to *Eutypa lata* was observed in Richter 110 grapevines that constitutively expressed a *Vigna radiata* eutypinedetoxyfing gene (Vr-ERE), which converts eutypine toxin produced by the pathogen to non-toxic eutypinol. Stilbene synthase genes encoding resveratrol were isolated from several *Vitis* species and engineered for constitutive expression to improve fungal resistance. Other non-grapevine derived genes such as lytic peptides encoding magainin and polygalactouranase inhibiting proteins (PGIP) were demonstrated to improve fungal disease resistance (Gray *et al*., 2014).

On the other hand, in some nurseries, the analyses of trunk diseases detection with PCR have proved their presence in rehydration baths, grafts tools, substrates in pots (e.g. sawdust) as well as in water (Larignon *et al*., 2009; Cardoso *et al*., 2013; Gramaje and Di Marco, 2015). Therefore, it is still obligatory to preserve international standards and protocols with control and safety measures in order to provide grapevine material without propagative diseases.

Due to that, propagation process is a key point for the propagation of these diseases, but there are many differences amongst international protocols. It must also to be noted that the accumulation of several treatments on the same lot of plants may lower the biological status of the cuttings and thus compromise their viticulture soundness. Some guidelines seem to be expected in this area (ie. OIV Recommendations for Certification and Trading Material of Vine Plants/ VITI-PROTEC 14-565 Et3).

Bioagents.

Current research is increasingly concerned with the effect of microorganisms used for biological control, in particular *Trichoderma* species. *Trichoderma* are well known as fungi that exhibit antagonistic activity and hyper-parasitism in regard to other microorganisms (more precisely to those related to the soil), and it is used for biological control against several diseases. The trials with *T. harzianum* and *T. atroviride* have shown have shown a promising action controlling Esca, Botryosphaeria dieback and other common trunk disease pathogens (Larignon, 2004).

Trichoderma significantly improved root grown which would possibly make plants less sensitive to black-foot disease when subjected to stress (Fourie *et al*., 2001; Agustí-Brisach and Armengol, 2013). These treatments have decreased incidence of fungi involved in grapevine trunk diseases when applied in vitro or in nurseries. To extend their effect of protection, healthy vines should be inoculated with these fungi to colonize the woody tissues of the cordon and trunk to provide a 'vaccination effect' against pathogens. The effectiveness of protection based on *Trichoderma* spp. treatments depends on the ability of these fungi to colonize grapevine pruning wounds. They usually need a period of time for a complete colonization, during which the pruned grapevine is susceptible to infections and ⁄ or to washing off by rainfall.

Other biological agents (e.g. *Bacillus subtilis, Fusarium lateritium, Erwinia herbicola, Cladosporium herbarum, Aureobasidium pullulans* and *Rhodotorula rubra*) and natural molecules (e.g. chitosan and cysteine) have also been reported to be effective against grapevine trunk disease agents, alone or in combination with fungicides, although some of them have only been tested in vitro or in nurseries (Bertsch *et al.,* 2013). Nascimento *et al*. (2007) explored the *in vitro* and *in vivo* fungicidal effect of chitosan on some of the most important grapevine wood fungi. The results showed that chitosan was effective in reducing mycelial growth of all fungi and significantly improved plant growth and decrease diseased incidence compared with untreated plants.

Another example is the induction of grapevine defense systems using oomycetes against Esca. Necrosis was reduced by 50% when *Phytium oligandrum* colonized the root system of the Cabernet Sauvignon cuttings (Gerbore, 2103; Yacoub *et al.,* 2014, Yacoub *et al.,* 2016).

Finally, *Arbuscular mycorrhizal* fungi have been shown to increase tolerance of grapevine rootstocks to black foot disease caused by *Ilyonectria* spp., and changes in the function of the rhizosphere microbial community (Jones *et al.,* 2014). Petit and Gubler (2006) also indicated that grapevines inoculated with an arbuscular-mycorrhizal (AM) fungus, *Glomus intraradices* were less susceptible to black-foot disease than non-mycorrhizal plants.

Chemicals or other products.

Sodium arsenate is in focus again, but only for the research purposes. The research has two objectives: to understand its mode of action against trunk diseases, and try to find a substitute product or to set the principles which would feign its action. This work with a multidisciplinary approach, which includes the expertise of pathologists, physiologists and chemists, is being financed by the French Ministry of agriculture, agri-food and forestry and the CNIV.

One of the most interesting studies was carried out in several vineyards in France (Bertrand *et al.,* 2007) and one of its main results is that trunk disease rates depends highly on the vine variety as well as on the vine-growing region. They also shed light on the fact that Eutypa dieback is mainly linked to the age of the grapevine where higher Esca/BDA is present. Moreover, no grapevine taxa, either cultivated or wild, are known to be resistant to trunk diseases (Bertsch *et al.,* 2013).

During the infection of grapevines, the degradation of hemicellulose or lignin by the pathogen has usually a response correlated with these effects; such as tylose accumulation, accumulation of polysaccharides and phenolic compounds (gummosis), tannins in vacuoles or phytoalexins like the resveratrol, are also observed in Botryosphaeria dieback or in "grapevine leaf stripes diseases (GLSD)" diseased grapevines (Bertsch *et al*., 2013). Application of resveratrol showed a direct antifungal effect by inhibiting the in vitro growth of *E. lata, S. hirsutum* and *F. mediterranea*. Stilbenic polyphenols are also able to scavenge reactive oxygen species (ROS) and thus protect the plant cells from oxidative stress after pathogen attack. However, only specific stilbens as transpterostilbene or isohopcaphenol are efficient against many dieback pathogen *D. seriata*, *E lata*, *F. mediterranea*, *Pa. chlamydospora* (Lambert *et al.,* 2012).

Other inducible defense responses are characterized by the accumulation of 'pathogenesis-related' (PR) proteins. The expression of PR proteins was shown to be up-regulated in the leaves of grapevines affected by grapevine trunk diseases. A fungitoxic activity has been described for many PR proteins including PR1 (unknown function), osmotin, thaumatin, anionic peroxidase, chitinase, β-1,3-glucanase and (PR10) ribosome-inactivating proteins (Bertsch *et al.,* 2013).

A different alternative is the research of *two-way* molecules, for example, systemic phloem fungicides that can be distributed with the plant sap through the phloem after the foliar pulverization. Some acid molecules such as N-carboxymethyl-3-cyano-4-(2,3-dichlorophenyl) pyrrole, penetrate into the phloem wherein they circulate (Chollet *et al.,* 2004; Jousse, 2004) and exhibit some fungicidal activity on the pathogenic fungus *Eutypa lata*. This feature is a consequence of the physic-chemical properties of these compounds. Recent studies are being carried out with Fenpiclonil molecules (Jousse, 2004) against Esca.

In that sense, there are other active substances that have shown a certain degree of control: copper oxychloride and acibenzolar-S-methyl against *Phomopsis* and Botryosphaeria dieback (Rego *et al*., 2014); foliar fertilizers based on Ca chloride or Mg nitrate seaweeds on grapevine leaf stripe disease (GLSD) symptoms (Calzarano *et al.,* 2014).

On the other hand, some practices like the impact of ozonation on grapevine scion decontamination was evaluated in previous experiments, but not all of them showed conclusive results (Mailhac *et al.*, 2010), and others concluded that this oxidative agent did not control the fungi in nurseries (Vigues *et al*., 2010). On the contrary, recent studies revealed that fungicide properties of ozonated water and the absence of gene induction *in planta* make however ozonated water a promising candidate for limiting grapevine infection by *Pa. aleophilum* in nurseries (Pierron *et al.,* 2015).

In the same way, Di Marco and Osti (2009) evaluated the potential use of electrolyzed acid water in cutting hydration after the cold-stored period to control *P. aleophilum* and *Pa. chlamydospora*, showing that it was effective in reducing conidial germination of both pathogens without affecting plant growth and development in the nursery field.

Finally, the plant fortifiers (phytostrengtheners) or vegetal extract products are another recent alternative, but an interdisciplinary research is needed to open up new perspectives in this kind of alternatives (Chollet *et al.,* 2014). Products that stimulate mechanisms of defense in the plant like 2-hydroxybenzoic have a promising effect on Esca/BDA. Other organic products showed a reduction ratio of almost 30% of plant death by Esca/BDA (Sentenac *et al*., 2004). These products can be administrated by injections or foliar pulverization of plants.

Abou-Mansour *et al*. (2015) showed that *Neofusicoccum parvum* is able to produce a diverse variety of phytotoxins that confer high flexibility to the fungus that allows it to adapt to several environmental conditions, the evidence that genes for secondary metabolites are highly conserved in *N. parvum* of grapevine, the ability of the plants to respond to fungal toxins, and the presence of two of the toxins in grapevine wood from plants showing Botryosphaeria dieback symptoms.

Cobos *et al.,* (2015) showed that chitosan oligosaccharide, vanillin, and garlic extract have greater *in vitro* efficacy when tested on autoclaved grape wood assays against *D. seriata* and *Pa. chlamydospora*. In field trials, a significant decrease in plant mortality was observed after 2 years of growth in inoculated pruning wounds for plants treated compared to untreated plants.

Mustard biofumigant crops have potential to be incorporated into an integrated strategy for management of black foot in vineyards and nurseries (Barbour *et al.,* 2014; Whitelaw-Weckert *et al.,* 2014). Green crops of *Brassica* species such as mustard (*B. juncea* L.) and rape (*B. napus* L.) incorporated into the soil release volatile isothiocyanates, which are known to suppress pathogenic fungal species (Agustí-Brisach and Armengol, 2013). It appeared that mustard meal incorporated into infested soil was as good as growing the plants and incorporating the plant into the soil (Barbour et al. 2014).

Conclusions

During its life, the vine may be subject to different aggressors under several forms of expression. These when observed in the vineyard correspond to various disturbances in the metabolism of the plant when it faces the pathogen agent.

Apart from some exceptions (e.g. *Fomitiporia* spp.), fungal and other diseases can spread by trading plant material and thus, can be introduced in areas where they did not exist before.

Despite their presence in vineyards, diseases not necessarily externalize even though they exist. The fact that symptoms are not expressed on the grapevine may be due to various factors, of which the most important is the climate effect on the fungal development in vineyards and its expression of symptoms. Likewise, the indigenous microflora can be involved and play an important role, by limiting or preventing the development of pathogens and thereby inhibiting the onset of symptoms. Another relevant factor could be the growing conditions.

To conclude, the evolution of these diseases also depends on the climate change. It can lead to a nearly total disappearance of some disease, a sudden emergence of a new microorganism, or manifestation of the already present fungi that could become pathogenic for whatever reason (Larignon, 2012). New cutting edge lines and technologies like drone monitoring or others can be useful in the close future. A real effort for prevention and monitoring of these diseases will be required from all the members of the OIV.

For the purposes of prevention must be assess the genetic potential susceptibility and resistance in *V. vinifera* in respect of GTD. Precision breeding could be one possible solution because grapevine plants naturally contain lot of useful genetic material, which should be tested in the following years. Significant advancements in cell culture, gene discovery and gene insertion technologies were only recently merged to fully enable precision breeding for the genetic improvement of grapevine or their resistances. However, more wide spread and robust evaluations, as is the norm for conventional breeding, must occur to confirm the utility of cultivars produced by precision breeding (Gray *et al.,* 2014).

Based on all the previous research, an integrated management program that includes HWT, chemical, biological, or other control measures has been suggested to be the most interesting procedure to reduce infections by fungal trunk pathogens during the nursery stages (Gramaje and Armengol, 2011).

Finally, other promising alternatives like alternative chemical products or molecules, bioagents and plant fortifiers, monitoring plans or drones applications should be developed in the future in order to corroborate their effects in a long term. OIV should be vigilant concerning their evolution, risks, effects, real applicability and their spread in vitivinicultural sector.

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