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Technical article

Global strategy for the control of Grapevine Trunk Diseases

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Grapevine trunk diseases (GTDs) are caused by a complex of pathogenic fungi. Such diseases are on the increase since the prohibition of a number of treatments - chemical products such as sodium arsenite for Esca, and benzimidazole-based products (benomyl and carbendazim) and Sterol Biosynthesis Inhibitors (cyproconazole and flusilazole), for Eutypa dieback. As no effective treatment exists, eradication of these diseases is not simple. Thus, in order to avoid the spread of GTDs, prophylactic methods should be employed from the moment of planting, both in nurseries and in vineyards.

Main symptoms caused by GTD fungi

GTDs affect the 'vital organs' of the vine, causing the death of the plant over the long term. There are two forms that are found regularly in vineyards - chronic and severe, also called apoplectic. The chronic form causes a loss of vitality and stunting leading to the destruction of parts of the vine and subsequently to the death of the entire plant. In the apoplectic form, the vine can die very quickly from necrosis of the vital tissues. Foliar symptoms are characterised by necrotic areas (staining or tabby appearance) or stunting. The inflorescences and fruits may dry out or take on a 'pumpkins and peas' appearance. GTDs cause necrosis and degradation in the wood by blocking the vessels or forming cankers. The current article focuses primarily on the following three trunk diseases: Esca, Botryosphaeria dieback and Eutypa dieback.

Symptoms and causal agents of Esca

Esca is a complex disease caused by various pathogenic fungi such as *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*. *Fomitiporia mediterranea* is responsible for "white-rot". This disease has both an apoplectic form and a chronic form. The rapid (apoplectic) form is characterised by accelerated drying of a part or of the entire vine. The chronic form is characterised by inter-nervous staining, yellow for white grape varieties and red with a yellow border for black grape varieties. Foliar necrosis then evolves towards leaf desiccation, whereas the grapes may dry out with the inevitable crop loss. In the slow form, the grapes may simply be less mature. Two types of necrosis characteristic of Esca can be observed in the wood - a clear central necrosis comprising several zones (white rot surrounded by a black border separating dead parts from healthy parts) and a more sectorially-placed necrosis, brown and hard at the start becoming lighter and tender in the central area.

Pictures : Characteristic symptoms of Esca : foliar symptoms on Cv Cabernet. Sauvignon (1), Sauvignon B. (2) central necrosis (3) and symptoms on fruits (4) .

Symptoms and causal agents of Botryosphaeria dieback

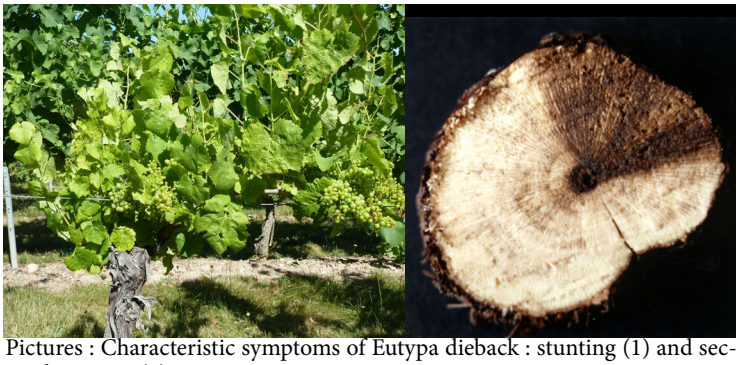
Botryosphaeria dieback, caused by fungi of the genus Botryosphaeria (*Diplodia seriata* and *Neofusicoccum parvum*), has long been mistaken for Esca, as the symptoms may be similar. This disease can be observed in both chronic and severe forms. Between the veins, leaves are initially stained red for red varieties and yellow/orange for white varieties and then become necrotic. After removing the bark, a brown band surrounded by a yellow/orange zone or a brown/black sectorial necrosis, which can extend from the shoot to the rootstock, is visible. The severe form is characterised by the rapid drying of the canes (which begins at the apex) and the destruction

of the inflorescences and fruits. Furthermore, Botryosphaeria dieback may give rise to stunted development and also apoplexy.

Pictures : Characteristic symptoms of Botryopshaeria dieback : foliar symptoms on black (1) variety, dessication of a shoot (2) and wood necrosis (3-4).

Symptoms and causal agent of Eutypa dieback

The fungus *Eutypa lata* is responsible for this disease. The internodes of diseased strains are shortened and the branches are stunted. The leaves are deformed and chlorotic with blackish necroses, with sagging or millerandage being observed on the berries. On the wood, the symptoms are in the form of a brown and hard sectorial necrosis, and the apoplectic form is also frequently encountered.



Pictures : Characteristic symptoms of Eutypa dieback : stunting (1) and sectoral necrosis (2) .

Factors influencing the expression of GTDs symptoms

Both biotic or abiotic factors may be active in the way GTDs develop in the field and on the types of symptom expressed. Another element that has been evoked as favouring GTD symptom development is dry weather causing water stress in the plant (Stamp, 2001). On the other hand, the presence of other microorganisms could interfere with GTDs, either limiting or even preventing their development. Mild and humid weather is favourable to the expression of the slow forms whereas dry and warm weather generally causes apoplexies (Surico G. et al. 2006).

The appearance of symptoms is variable. A vine may appear symptomatic one year but not the next, and symptoms may be more or less severe from one year to the next. The age of the vineyard and the particular cultivar clearly have an effect on symptoms, and the training and pruning system could also influence the expression of GTD foliar symptoms. The reasons behind this symptom variation are still not clear.

Preventive methods to avoid GTDs during the establishment of the vineyard

When planning a new vineyard or plot, a generally valid precautionary principle is to adhere to a set of good vineyard practices. The quality of grafted plants, the choice of cultivar and optimum planting conditions are all important elements to be considered if one wants to manage GTDs from the moment of planting. It has been pointed out that during the preparation of planting material, several stages (cold storage, hydration, disbudding, grafting, callusing, rooting) may predispose young plants to the development of GTD parasites (Gramaje & Armengol, 2011, Lecomte et al., 2008, Stamp 2001). In general, good quality plants, with a well-developed root system and with complete healing of the grafting point should be preferred for any new plantations, but even this may not be sufficient. Although not compulsory, some European nurseries have started to apply physical (Hot Water Treatment - HWT), chemical (fungicides: depending on the country) or biological methods (Trichoderma) to prevent GTD pathogen infections during the plant production process. Treatment with hot water can limit populations of P. chlamydospora and some strains of *Botryosphaeriaceae* (Larignon et al., 2009, Vigues et al., 2009, Elena et al., 2015). Acquiring high-quality, healthy, plants is therefore an important first step towards obtaining a satisfactory healthy basis for the vineyard and maintaining this in subsequent years. Some cultivars or rootstocks appear to be more sensitive to GTDs than others so, naturally, the choice should then be oriented towards the most resistant, depending on the country. Pinot Noir, Syrah and Cot tend to be less sensitive to Esca /BDA than Sauvignon, Ugni B, Gewurztraminer or Trousseau.

Planting in decompressed soil may be carried out between late autumn and early summer, during the vines' dormant period. Prior to planting, treatment with Trichoderma, cyprodinil and fludioxonil or metiram and pyraclostrobin products (according to the country) may be carried out by root dipping . Attention should be paid to the root system, ensuring it is undamaged and able to spread properly. During planting, watering of the vines is important to avoid water stress. It is advisable to tutor the plant in order to promote vertical growth of the trunk so that it will be less susceptible to damage related to

weeding tools. Thereafter, the choice of the vine formation system must be considered so as to allow a physiologically rational size, facilitating sap flows.

Soil management to prevent GTDs

The relationship between soil management and the development of GTDs is difficult to prove scientifically, but respecting best practices is clearly a good start. Generally, good soil management, where air and water circulate easily and where water does not become stagnant, may limit the stress on plants, limiting the risk of infection by GTDs. Excessive nitrogen-based fertilisation should be avoided as this promotes excessive vigour that could increase the appearance of symptoms. In this regard, the establishment of a permanent or temporary crop (peas or mustard, for example) in between rows could balance the availability of nutrients whilst, at the same time, also improving soil structure.

The importance of pruning systems

Pruning wounds are the main entry point for pathogens causing GTDs. The wider and more numerous the wounds are, the larger the area where GTD pathogen spores can infect. In addition, pruning wounds may be correlated with the underlying necrosis (also known as desiccation or drying cone). Such necrosis could be infected by wood pathogens and/or deteriorate the sap flux, increasing physiological stress and thus the harmful effects of chronic and severe GTD forms. To avoid such necrosis and its effects, the residual length of the pruned spur should be at least 1.5 times its diameter. Therefore, the training system, the pruning time and wound protection are elements to be taken into account.

Late pruning during the dormant season and close to budbreak is recommended especially for controlling Eutypa dieback infections, since wounds heal better with mild temperatures. More generally, late pruning can be carried out in vine-growing areas with dry springs. Wound susceptibility is mostly influenced by relative humidity and rainfall (Luque et al., 2014). Precipitation and temperature have a direct effect on pathogen distribution. It is therefore recommended to prune vines during dry periods since the available inoculum is not dispersed.

The training system used would appear to play a role in the impact of GTDs, but the various information sources are not consistent. It is preferable to opt for less severe pruning, generating small wounds and fewer disturbances for the development of the plant. Long pruning should be favoured and many studies show that goblets and Guyot systems should be preferred. In Bordeaux, one study has shown that symptom expression was increased in Guyot (short canes). Eutypa dieback foliar symptoms were higher with short than with long pruning, but the mortality rate was lower in long-pruned vines. The latter, such as those in Guyot, are damaged by numerous wounds localised in the upper part of the trunk; on the contrary, short pruned vines (Cordon or goblet) had a larger total area of wounds.

The Guyot-Poussard pruning method, recently proposed to vine growers to limit GTDs, preserves the sap flux since pruning wounds are located on the upper part of the cordon. The wounds are smaller, less numerous and located on young wood that would be more resistant to infections than older wood. This type of training system could limit GTDs but it still requires to be scientifically assessed.

Even if scientifically proved, contamination through pruning scissors is quite irrelevant compared with that occasioned by pruning wounds. Consequently, it is not strictly necessary to disinfect tools to limit Esca and Eutypa dieback (Larignon, 2007).

Because pruning wounds are the gateway to GTD pathogens,

it is recommended to protect such wounds using fungicides, bio-control products or mastics, according to the products approved for such use in each country. These products must be applied locally to the wounds and as soon as possible after pruning. Some studies have shown that contamination by *D. seriata* and *P. chlamydospora* can be significantly reduced using a mixture of the fungicides benomyl, pyraclostrobin, tebuconazole and thiophanate methyl (Díaz and La Torre, 2013). Spraying is more convenient, faster and less expensive, but the product can easily be washed away by rain. *Trichoderma*-based products (Di Marco et al., 2000) or chitosan (Bertsch et al., 2013) have been shown to be effective when used to protect pruning wounds, as were mastics that act as a physical barrier to GTD contamination.

Destruction of inoculum sources

The GTD's inoculum is found on diseased and dead plants (leaves, grapes, and both necrotic wood and old wood). Pruning debris, symptomatic and dead vines are a potential source of new infections in the vineyard. Pruning debris, for example, could bring be a source of viable inoculum of Botryosphaeria

dieback pathogens up tofor as long as 42 months. Thus, as a preventative measure it is better to get rid ofremove pruning debris as quickly as possible (VITI 2/2006 resolution). To destroy potential inoculum, pruning woods may be crushed and buried in the soil, burned incinerated (if permitted by law), crushed and compacted (40 to 50 ° C for 6 months) or removed them from the parcel.vineyard.

Trunk renewal to replace vines

To rejuvenate the trunk of GTDs- affected vines, the trunk must be cut at a level above the rootstock and below any necrosis. The vine will then produce a sucker that will have to be tutored in order to obtain a new trunk. This simple technique has provides good results with vigorous sturdy varieties. This The technique has been shown to be effective in controlling Eutypa dieback (BNIC, Chamber of Agriculture, 1989, BNIC, INRA Bordeaux, 1989, Mette et al., 2004, Sosnowski et al., 2011). It seems that it is also could be also be useful for Esca and BDA, but its efficacy dependings on the sanitary status of the remaining part (if the trunk below the cut has GTDsis infectioninfecteds, vines could express GTDs symptoms again in the followinglater years (Calzarano et al., 2004), (Larignon & Yobregat, 2016).) It is possible to anticipate prepare for the trunk renewal by keeping a sucker several years in advance. ThereforeThus, when cutting the vine, the new one is already formed. If the renewal is done during the winter, it is preferable to protect the wound in order to limit infections by GTDsS. (Smart, (2015) established aA guideline (The Timely Trunk Renewal protocol) has been produced to do theperform this type of renewal (Smart, 2015). As part of the Winetwork project, a survey carried out in south-western France estimated the cost of this operation to be between 225 and 275 €/ ha for renewing 250 vines in a plot planted withat 4500 vines/ha with a proportion of 250 vines.

Re-grafting to limit the repercussions of GTD effects on vines

An alternative method to manage GTD-s diseased vines is the re-grafting. The This operation consists in grafting again a diseased vines starting from its healthy rootstock. It seems that slotted grafting is the most appropriate method for regenerating diseased vines (SICAVAC source). This grafting method can be carried out in spring or autumn, when the sap is in circulation but not very active, and during dry periods. It is necessary to cut the vine and to verify that at the cut's location it is healthy at the incision point (free from decay, or otherwise the graft will also be diseased). Sur-grafted vines produce a halfsemi-harvest the year after the graft with a normal and harvest returning generally to normal two years later. The advantage is that the root system, and therefore the age of the original vine, is preserved, giving a grape production with the same quality of as that of the healthy ones. This technique is quite complex but the recovery rate could should be in the region ofreach up to 80 - 90%. Re-grafting can be carried out in service provision or by the winegrower himself or contracted out to a service company. The cost of the service can range from 1.35 to 2.05 € HT (without before VATtaxes) per vine, depending on the surfaceacreage. In other words, the cost for the purchase of supplies, for the use of equipment, workforce and fixed charges for the first two years is estimated at around 12, 000€/ha (Source: Chambre d'agriculture, Loir-et-Cher). For such an operation, It one needs to plan on havingis necessary to contract an employee for 3 months full-time to sustain grafted-vines for some 4500 vines (IFV).

Trunk cleaning to restore vines

Trunk cleaning is a kind of surgical cleansing surgery, which could may be effective to in limiting Esca and BDA. It consists in removing the rotten white wood from the trunk, as a dentist could might do for carieswhen scraping teeth. Trunk cleaning can be performed as soon as the first symptoms of GTDs occur appear and when the presence of rotten wood is observed. If done early in the season, the harvest of the year can be preserved. To clean, it is necessary to locate the pieces areas of rotten and spongy wood that are affected whilst preserving the h. Healthy wood and sap flux should be preserved.
In Sancerre, France, onea winegrower has observes observed that 99% of the cured diseased vines treated in this way are now free from Esca (Source SICAVAC). Similarly, a trial carried out in Alsace shows the non-treatedcured vines, compared to cured, express exhibit more of the and fatally GTD symptoms of GTDs. The same test indicates indicated that it takes an average of 5-20 minutes to cure clean diseased vines, but this also depends also on the kind of pruning system used, the reached level of vines and the winegrower's experience (Source IFV). These studies need to be extended to determine understand how long the vines are remain free from GTDs.

Use of phytosanitation for limiting GTDs symptoms

Since the prohibition of sodium arsenite and Escudo®, no fungicide is allowed to be used for combatting against GTDs. For this reason,S several studies have been, or are were and are currently, focusing ed oin finding other efficient effective alternatives. Systemic fungicides such as N-carboxymethyl-3-cyano-4-

(2,3-dichlorophenyl) pyrrole can circulate in the phloem (Chollet et al., 2004, Jousse, 2004) and have an effect on Eutypa lata. Other studies have been conducted with Fenpiclonil (Jousse, 2004) to control Esca. GTDs could be controlled by preventive products like tebuconazol + synthetic resins or Esquive® WP (T. atroviride I-1237), Folicur (tebuconazole), Shirlan (fluazinam) or Cabrio (pyraclostrobin). An application of Bion (acibenzolar-S-methyl) + Cuprocol (Cu oxiclorure) then offollowed by Bion + Score (difeconazole) seems to reduce Botryosphaeria dieback incidence and severity. Boron is capable ofto reducinge infection by E. lata (Sosnowski et al., 2008; Rolshausen et al., 2010). Several trials have also been conducted with natural defectivesantidotes, Trichoderma or fertilizersfertilisers, as preventive or curative agents, but no significant results have been obtained are significant. Foliar application of a mixture of calcium chloride, magnesium nitrate and fucal algae extract for over several years has resulted in a significant reduction in symptoms in the vines thus treated vine.

Methods that might avoid harmful effects of GTDs

Layering: this This process can be done used to renew the any missing vine trunk and maintain the production level of a particular plot. This It consists in burying a branch from the mother plant and bringing ihaving the end of the branch showingt out of the ground at the end. In this way, the buried part takes root and creates a plant with the same characteristics as the mother plant. The mother plant must be healthy and the marcotte must remain linked to the mother plant due to the risk of Phylloxera.

Copper nanoparticles: The injection of NP of Cu using a syringe is an a novelinnovative practice to fight Esca carried out in the Galician vineyards of Galicia in Spain to fight Esca. Two injections and 4 to 5 foliar treatments, of using a mixture of Cu NP and a porcine blood amino acid elicitor, are carried outrequired. For the moment, there are no results that can attest to the beneficialbe presented as to the effect of this technique on the Esca. Copper has a broad spectrum of action against bacteria and fungi and has been used as a pesticide longtime in the agricultural world for some timee as a pesticide. Recently, copper nanoparticles (particles between 10-7 and 10-9 meters) have become very popular due to their physical, chemical and antimicrobial properties, as well as their abundance (Betancourt et al., 2013). The Cu NPs are effective in vitro on pathogenic fungi and yeasts (Ren et al., 2009, Rupareli et al., 2009, Ramyadevi et al., 2012).

Copper nails: This technique consists in planting a copper nail in a diseased trunk. Copper is supposed to diffuse from the nail into the trunk through the sap and exerts a fungicidal effect on GTDs fungi. This practice has been tested by a one winegrower for the past 3 years in Germany but for the moment no scientific validation verification is available.

H2O2: Injecting H2O2 using a syringe is a novel innovative technique which has been applied on vines showing symptoms of Esca or BDA in southern France, Galicia in Spain and in the Douro vineyards in of Portugal, Galicia in Spain and Southern France in vines with symptoms of Esca or BDA. It would appear that the results are positive for GTDs, but these have not been scientifically evaluatedassessed. HoweverIn addition, some countries do not permit the use of H2O2is not always accepted by law in some countries. Plants, when subjected to biotic attack, produce H2O2. Reactive oxygen species (ROS) exert a direct toxic activity effect on microorganisms and often inhibit its their development. It is recognized recognised that ROS have a role in the gene expression of the hypersensitive response (Lamb & Dixon, 1997), in parietal reinforcements and in plant resistance, allowing the expression of defense defence genes and accumulation of Pathogenesis-Related proteins (Van Breusegem & Dat, 2006). Injection of H2O2using a syringe is an innovative technique applied in the Douro vineyards in Portugal, Galicia in Spain and Southern France in vines with symptoms of Esca or BDA. It would appear that the results are positive for GTDs, but have not been scientifically evaluated. However, H2O2is not always accepted by law in some countries.

Soaking in fungicides: Before planting, the grafted-welded plants are soaked in a fungicidal solution for 50 minutes. Another novel technique, not yet scientifically endorsed, but which has been tested in Portugal, is soaking in fungicides - before planting, the grafted-welded plants are soaked in a fungicidal solution for 50 minutes.

Conlusion

Overcoming wood diseases is not a simple task as the symptoms are influenced by many different factors. Indigenous microflora can interact with GTD fungi by limiting or preventing their development. The growing conditions and the pruning system can also be relevant factors in limiting GTDs. A deeper understanding of GTDs would help to comprehend better the way these diseases function. Many methods, already tried in European vineyards, seem to be promising but still need to be validated scientifically. The application of a single control method to manage GTDs is only partially effective. It seems important to try to combine different preventive techniques to have effective management and control over GTDs.

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