



# Study Regarding the Status of Fungal Grapevine Trunk Diseases in some Romanian Vineyards

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## RESEARCH ARTICLE

### Abstract

Romania is a wine-producing country, with a rich and long tradition in the field of viticulture. Field evaluation of the land and grapevines from a phytosanitary point of view is essential for establishing the most effective control methods for grapevine diseases and pests. Grapevine trunk diseases (GTD) are currently considered one of the biggest threats to vineyard sustainability. In this study, GTD symptoms were investigated by direct field observations in vineyards from Blaj, Bujoru, Murfatlar and Miniș in 2020 throughout the vegetation period. In 2021 samples of the grapevines, showing signs of decline in the previous year were analyzed following traditional methods based on macroscopic examinations. A total of 69 samples were analyzed and symptoms of four GTDs were identified: Esca disease; Eutypa dieback; Phomopsis dieback and Petri disease. Out of these 55 % of the samples presented Esca disease symptoms. More than 80 % of the samples presented symptoms of 2 or more diseases at the same time.

**Keywords:** grapevine decline; Esca disease; Eutypa dieback; Phomopsis dieback; Petri disease; field observations.

## INTRODUCTION

Field-cultivated plants are, naturally, subject to multiple stress factors (biotic and abiotic, frequently in combination). These stresses may negatively impact the health status of plants and reduce yields (Atkinson & Urwin, 2012; Suzuki et al., 2014). Among them, the grapevine is an economically important crop considering the 6,95 Mha of harvested areas globally (FAOSTAT 2020). Romania is a wine-producing country, with a rich and long tradition in the field of viticulture. (ANF 2016; Muntean et al., 2022). Nowadays, grapevine trunk diseases (GTDs) are considered a major biotic stress of this crop due to the reduction of both yield and lifespan of the vineyards, thus leading to substantial economic losses for the grape and wine industry worldwide (Chacón-Vozmediano et al., 2021; Gramaje et al., 2018; Songy et al., 2019). All grapevine-growing countries are affected by GTDs. Bruez et al. (2014) estimate that 73% of the French vineyards are infected by GTD pathogens, leaving 13% of them unproductive. In Spain, a GTDs incidence of 10.5% was reported by Bruez et al. (2014) in 2007. In Italy, GTDs incidence ranged between 8 - 19% in 15–18-year-old grapevines and 60–80% in many old vineyards (Fontaine et al., 2016; Romanazzi et al., 2009). In British Columbia,

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symptoms of GTDs were present in 90% of the vineyards, an incidence of up to 54% being reported in some individual vineyards (Songy et al., 2019; Úrbez-Torres et al., 2013).

GTDs are characterized as slow-progression diseases caused by several wood-inhabiting fungi with similar life cycles and epidemiology (Patanita et al., 2022). They are more complex than other grapevine diseases such as powdery mildew and downy mildew, therefore managing them presents a challenge for winegrowers, nurserymen, technicians, and scientists (Kenfaoui et al., 2022). The reasons that make these diseases so complex and hard to identify and eradicate are the simultaneous presence of multiple trunk pathogens in a single plant together with the inconsistent GTDs symptoms expression, their isolation in asymptomatic plants, and the absence of effective treatments (Muntean et al., 2022; Patanita et al., 2022). The most important, fascinating and controversial element of GTDs in field trials, is their undefined latency period (Bertsch et al., 2013; Kenfaoui et al., 2022). Symptoms may appear in one year and not in the next one, on the same grapevine, due to environmental, climatic, and cultural factors (Kenfaoui et al., 2022; Murolo & Romanazzi, 2014; Sosnowski et al., 2011), which leads to an underestimation of the true incidence of GTDs in the vineyards (in any of the given year). This is a problem because, during the many processes of plant production, infected asymptomatic cuttings might cross-contaminate GTD pathogens, making latent infections harmful in the propagation process. If these infections are not managed, the unnoticed spread of diseased plants may occur, first in the nursery and subsequently throughout the vineyards (Gramaje & di Marco, 2015; Kenfaoui et al., 2022)

The term GTDs includes nowadays six specific diseases: Petri disease, Black foot disease, *Botryosphaeria dieback*, *Phomopsis dieback*, *Eutypa dieback*, and Esca, (Muntean et al., 2022; Patanita et al., 2022). Black foot and Petri diseases usually occur in young grapevines (under five years), while *Botryosphaeria dieback*, *Eutypa dieback*, *Phomopsis dieback* and Esca, are usually found in older grapevines (Claverie et al., 2020; Hrycan et al., 2020; Patanita et al., 2022). General symptoms of GTDs include leaf chlorosis, wood discoloration and necrosis, delayed bud-break, stunted growth, reduced vigor, canker formation, dieback, and the eventual death of symptomatic grapevine (Patanita et al., 2022). Sometimes symptoms take several years to appear after infection, making early detection quite difficult (Hrycan et al., 2020; Patanita et al., 2022).

Due to the adequate climate and fertile soils, Romania has a rich and long tradition in the field of viticulture (Chedea et al., 2021; Chiurciu et al., 2020; Muntean et al., 2022). Globally, in 2020, Romania was ranked in the 10th place having a total of 175,590 ha of grapevine cultivated areas and a production of 932,770 tons of harvested grapes (Muntean et al., 2022). More than 80% of the Romanian vineyards are cultivated with grape varieties used for wine production (Figure 1). Therefore, Romania is an important wine-producing country, that should be paying enough attention to GTDs because the pathogens that cause GTDs attack the long-lasting organs of the grapevines and the debilitating and drying of the grapevines have serious repercussions for the productivity and longevity of vineyards, making GTDs a serious threat to the sustainability of Romania's winemaking heritage. (Fontaine et al., 2016; Tomoiagă & Chedea, 2020).



**Figure 1.** Major grape producers by type of grape  
 [\*Source: OIV Statistical Report on World Vitiviniculture,  
 (Statistics Unit of the International Organisation of Vine and Wine, 2010)]

Knowledge regarding the distribution of GTDs and the factors associated with their development is essential to predict the GTDs spread and to improve disease management, in order to reduce their negative economic impact (Gramaje et al., 2018; Guerin-Dubrana et al., 2019; Mondello et al., 2018; Muntean et al., 2022). At the present time,

there is limited scientific information available regarding the status of GTDs in Romania. Studies on this matter are scattered and often inconsistent. This study aims to present the status of GTDs in vineyards from Blaj, Bujoru, Murfatlar and Miniș using field observations of the general GTD symptoms during the vegetation period and simple traditional methods based on macroscopic laboratory examinations in order to open a national platform in an international framework.

## MATERIALS AND METHODS

In this study, signs of grapevine decline such as leaf chlorosis, delayed bud-break, stunted growth, reduced vigor, wood discoloration, necrosis, lesions or cankers were observed in vineyards from Blaj, Bujoru, Murfatlar and Miniș. and further investigated in the Plant Protection laboratory of the Research Station for Viticulture and Enology Blaj. Grapevines showing signs of biological decline were marked and the symptomatic evolution was followed throughout the whole vegetation period of 2020. In the next year, 2021, samples of the marked grapevines were collected in plastic bags, tagged and sent to the Plant Protection Laboratory of the Research Station for Viticulture and Enology Blaj for further analysis and GTD diagnosis (Figure 2). The samples received at SCDVV Blaj were transferred to paper bags, stored at room temperature and analyzed as soon as possible. Fresh cross-sections and longitudinal cuts of the samples were made at the analyzing time for better observations.



**Figure 2.** Aspects from plantations regarding the sampling of the diseased grapevines

Blaj vineyard is located in the viticultural zone 1 (VZ 1) on the Transylvanian Plateau of Romania (Chedea et al., 2021; Muntean et al., 2022). From the environmental point of view, grapevines find here good microclimatic conditions and good growing seasons being cultivated on the slopes that separate the valleys of Târnava Mare and Târnava Mica rivers (Călugăr et al., 2018; Chedea et al., 2021; Donici et al., 2019; Iliescu et al., 2010). The microclimate is thus characterized by slightly low temperatures, relatively high amount of precipitation and fog which make Blaj vineyard known and appreciated for its quality white wines with a specific flavor and a good sugar/acidity balance (Chedea et al., 2021; Cudur et al., 2014; Donici et al., 2019; Iliescu et al., 2010). The training system used in Blaj vineyards is the one particular for Transylvanian vineyards, the demi-high Guyot system with periodic replacement arms. Samples were collected from 4 different grapevine plantations: Crăciunelu de Jos, Ighiu, Ciumbrud and Research Station for Viticulture and Enology Blaj (SCDVV Blaj) headquarters. The plantation from Crăciunelu de Jos, located at 46°17' N lat. and 23°85' E long., was established in 2010, it is placed at an altitude of 285 m, has a southern exposition and alluvial soil with sandy texture and clayey sand. The Ighiu plantation is located at 46°16' N lat. and 23°50' E long. at an altitude of 415 m, and it is the oldest plantation from this study being established in 1975. The plantation from Ciumbrud, located at 46°31' N lat. and 23°76' E long., was established in



2013, it is placed at an altitude of 322 m and has a south-eastern exposition. The plantation from SCDVV Blaj headquarters, located at 46°17' N lat. and 23°93' E long., was established in 2012, it is placed at an altitude of 244 m, has a southern exposition and eutricambosol with a clay texture.

Bujoru vineyard is located in the viticultural zone 2 (VZ 2) in Galați county on the hills surrounding Chineja Valley. The eco-pedoclimatic conditions particularities of this vineyard consist of air temperature extremes, pluviometric deficits, strong winds and a hilly-steppe relief having low altitudes. The predominant soil is levigated chernozem having a clayey sandy texture (Bora et al., 2016). The microclimate found in Bujoru vineyard is favorable for the culture of both white and red grapevine varieties (Antoce et al., 2013). The training system used is the semi-high (70cm) bilateral cordon system and grapevines are cultivated on espaliers having a distance of 2 m between rows and 1.2 m between vines. The samples for this study were collected from the Farm 2, 3 and 4 plantations, all being old plantations of around 40 years, located in Dealul Bujorului vineyard at 45°86' N latitude and 27°90' E longitude on the same plateau with a slight slope of 110 m altitude.

Murfatlar vineyard is part of the viticultural zone 6 (VZ 6) in Dobrogea Hills located in the south-eastern part of Romania. Grapevines are cultivated here on the gentle slopes guarding the Danube - Black Sea Canal. The physical-geographical particularities of this region are characteristic of the plateau landscape with loessic soil and a continental bioclimate of the Pontic steppe-silvosteppe, partially mitigated by the vicinity of the Black Sea and the water bodies of the Danube Basin and the Danube- Black Sea Canal. The climate is therefore a semiarid one characterized by higher temperatures, higher solar radiations with longer hours of sunlight and reduced amounts of precipitations. Overall, the eco-pedoclimatic conditions are favorable for grapevine cultivation, they allow the accumulation of high concentrations of sugar and the possibility of producing a wide range of white or red high-quality wines, from dry to natural sweet ones (Ranca & Filip I, 2008). The samples were collected from the grapevine plantations from the Research Station for Viticulture and Enology Murfatlar headquarters, located at 44°17' N latitude and 28°42' E longitude, at an altitude of 34.7 m, on a calcareous chernozem soil with an average texture and a humus content of 2.3%. The training system used in the studied plantations is the demi-high (70 cm) Guyot system with a bilateral cordon with two canes and spurs as fruiting elements. The age of the studied plantations ranges between 8 and 28 years.

Miniș vineyard is located in the viticultural zone 5 (VZ 5) in Arad County on the hills at the base of the Zarand mountains. The ecological features of the vineyard are characterized by higher temperature levels (especially in June, July and August), generally mild winters, a longer vegetation period, the SW exposure and the preluvosoil. The eco-pedoclimatic conditions allow the production of a wide range of wines with good favorability for the production of red wines. The training system used in the studied grapevine plantations is the Guyot system. Samples were collected from Cuvin's rootstock plantation, located at the following coordinates 46°11' N latitude and 21°35' E longitude having an altitude of 117m; the Covăsânt plantation established in 2009, located at 46°11' N latitude and 21°37' E longitude having an altitude of 162 m; SCDVV Miniș headquarters, plantation established in 1982 at the following coordinates 46°9' N latitude and 21°35' E longitude having an altitude of 177m; Sâmbăteni plantation located at the following coordinates 46°13' N latitude, 21°51' E longitude having an altitude of 112 m.

## RESULTS AND DISCUSSIONS

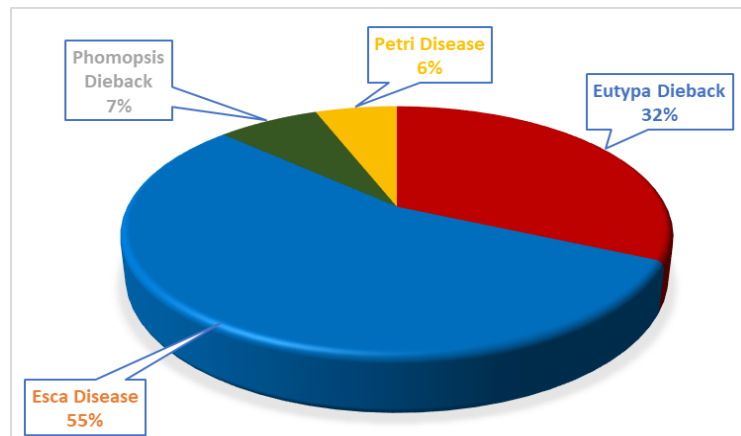
Throughout direct field observations, signs of biological decline such as delayed bud-break, stunted growth, reduced vigor, leaf chlorosis, wood discoloration, necrosis, lesions or cankers were easily identified in the studied vineyards (Figure 3).



**Figure 3.** Signs of grapevine biological decline monitored during the vegetation period of 2020

A total of 69 samples were analyzed following traditional methods based on macroscopic examinations (Appendix 1). The results regarding the GTDs diagnosis as well as the main laboratory observations, along with representative photos of the samples are presented in Appendix 2.

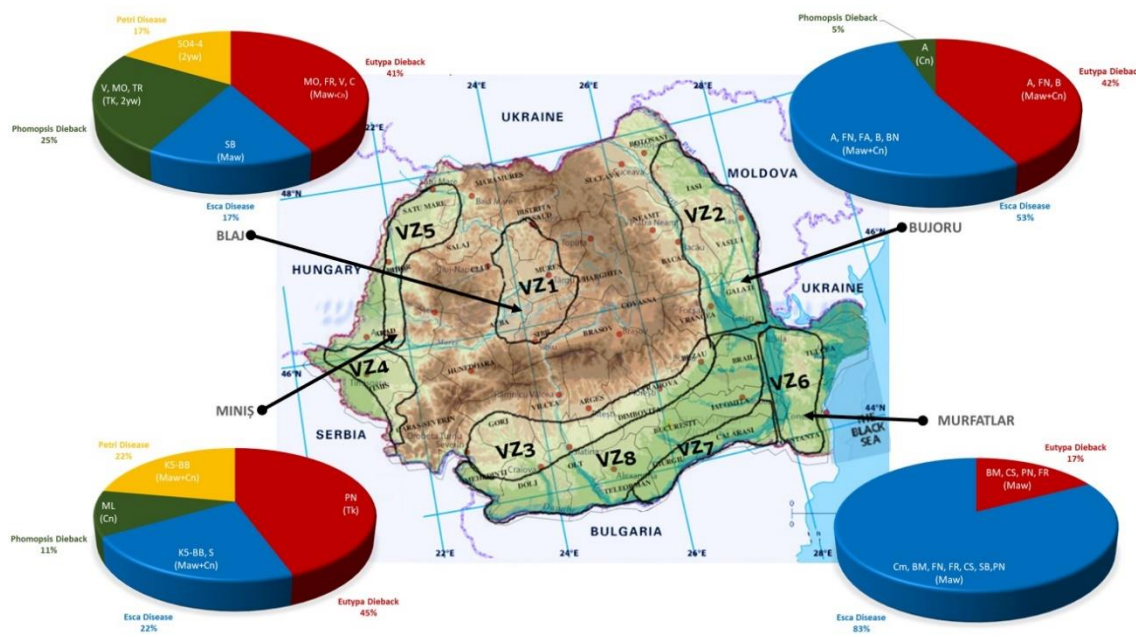
There were 4 GTDs diagnosed in this study: Esca disease, Eutypa dieback, Phomopsis dieback and Petri disease. Esca disease and Eutypa dieback were the main GTDs identified in all of the studied vineyards. Esca disease was identified in 55% of the samples and Eutypa dieback was diagnosed in 32% of the samples. Phomopsis dieback and Petri disease were identified in a lesser extent, only 7% of the samples were diagnosed with Phomopsis dieback and 6% of the samples were diagnosed with Petri disease (Figure 4).



**Figure 4.** Results regarding the diagnosed GTDs

Out of the 69 analyzed samples, 56 of them representing more than 80%, presented symptoms for more than one disease (Appendix 2). Besides the overlapping of GTDs, Crown Gall disease symptoms were observed in 10% of the samples and also symptoms of Downy mildew were observed in 1.5% of the samples.

In Blaj vineyard, 41% of the samples were diagnosed with Eutypa dieback as the main disease, 25% of the samples were diagnosed with Phomopsis dieback, 17% of the samples were diagnosed with Esca disease and 17% of the samples were diagnosed with Petri disease. Eutypa dieback was identified on multiannual woods and canes of Muscat Ottonel, Fetească regală, Victoria and Codreanca cultivars. Phomopsis dieback symptoms were observed on the trunks and two years wood of Victoria, Muscat Ottonel and Traminer cultivars. Esca disease symptoms were identified on the multiannual wood of the Sauvignon blanc cultivar and Petri disease was diagnosed on the rootstock SO4-4 cultivar (Figure 5).



**Figure 5.** Results regarding the proportions of the diagnosed GTDs in the studied vineyards

In Bujoru vineyard, Esca disease was the main GTD identified in 53 % of the samples. Eutypa dieback was diagnosed in 42 % of the samples and Phomopsis dieback in 5 % of the samples. Symptoms of Esca disease were identified on multiannual wood and canes of Aligote, Fetească neagră, Fetească albă, Burgund and Băbească neagră cultivars. Eutypa dieback symptoms were observed on multiannual wood and canes of Aligote, Fetească neagră and Burgund cultivars and Phomopsis dieback symptoms were identified on canes of Aligote cultivar (Figure 5). In Murfatlar vineyard, only two GTDs were diagnosed. Esca disease was the main GTD identified in 83 % of the samples and Eutypa dieback was diagnosed in 17 % of the samples. Symptoms of Esca disease were observed on multiannual wood of Columna, Burgund mare, Băbească neagră, Fetească neagră, Fetească regală, Cabernet Sauvignon, Sauvignon blanc and Pinot noir cultivars. Eutypa dieback symptoms were observed on multiannual wood of the Burgund mare, Cabernet Sauvignon, Pinot noir and Fetească regală cultivars.

Our findings are in agreement with the results of other GTDs-related studies from Romania: Eutypa dieback, Phomopsis dieback, and Esca were reported in all Romanian viticultural zones (Muntean et al., 2022). (Comşa et al., 2021) present the most common fungal GTD species identified in Romanian vineyards: *Stereum hirsutum*, *Eutypa lata*, *Phomopsis viticola*, *Phaeoemoniella chlamydospora*, *Diplodia seriata*, *Phaeoacraemonium* sp., *Cadophora luteo-olivacea*. Esca disease, Phomopsis dieback and Eutypa dieback are also reported as main GTDs in Transylvania vineyards by Tomoiagă and Chedea (Tomoiagă & Chedea, 2020).

## CONCLUSIONS

Symptoms of GTDs like delayed bud-break, stunted growth, reduced vigor, leaf chlorosis and then wood discoloration, necrosis, lesions or cankers were identified in all of the studied vineyards.

After detailed laboratory observations, the samples were diagnosed with 4 GTDs: Esca disease, Eutypa dieback, Phomopsis dieback and Petri disease (Figure 4). Wood symptoms were mainly characterized according to the necrosis shape, color and consistency as described by Comşa et al. (Comşa et al., 2021). Shapes identified include punctuations, necrotic lines, central necrosis, sectorial necrosis or mixed necrosis (central and sectorial). More specifically, in this study, the following wood symptoms were identified (Appendix 2):

- *V shape necrosis* of dark color and hard consistency for Eutypa dieback,
- zones with *degraded wood of light color* for Esca disease,
- mixt necrosis with white rot of spongy consistency but also dark colored zones of hard consistency when a combination of Esca disease and Eutypa dieback was diagnosed,
- *dark concentric spots* of the canes as well as *brown lesions* for Petri disease,
- wood with *excoriations of different shapes and dimensions* in the case of Phomopsis dieback.

Esca disease and Eutypa dieback were the main GTDs identified in all of the studied vineyards, with Esca disease being the diagnosis for 55% of the samples and Eutypa dieback being the diagnosis for 32% of the samples. Phomopsis dieback and Petri diseases were identified in a much lesser extent (< 10%). Phomopsis dieback was diagnosed in 7% of the samples in Blaj, Bujoru and Miniş vineyards and Petri disease was identified in 6 % of the samples in rootstock samples from Blaj and Miniş vineyards (Figure 5).

More than 80 % of the analyzed samples presented symptoms of more than one grapevine disease. Besides the overlapping of GTDs symptoms, some samples presented Crown Gall disease (*Agrobacterium tumefaciens* - 10 % of the samples) and Downy mildew (*Plasmopara viticola* - 1.5 % of the samples) symptoms.

In Romania, the grapevine decline phenomenon has been recognized as a problem for more than a century (Tomoiagă Liliana, 2006), but GTDs impact has increased significantly only in recent decades (Muntean et al., 2022). At the present time, in Romania, there is quite limited scientific information available regarding this topic, therefore the present study may contribute to the accurate identification of GTDs in vineyards which is very important in order to avoid significant infection rates and considerable losses of production or quality.

More specific information concerning GTDs are needed, because certain confusions regarding the typical symptoms of GTDs, the biology of the causal agents, and the mechanisms of their transmission can restrain vineyard protection efficiency. In order to maintain effective management and minimize the effect of the pathogens involved in GTDs, it is critical to better understand the circumstances and variables related to their development, as well as the biological and molecular mechanisms involved in the infection processes.

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### Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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