

Identification by plate culture method of the fungal pathogens causing the grapevine trunk diseases in Romanian vineyards

M. Comşa¹, L.C Tomoiagă¹, V. Botea¹, Al. Sîrbu¹, D. Dobromir²,
I. Vasiu¹ and V.S. Chedea^{1*}

¹Research and Development Station for Viticulture and Enology Blaj, Romania

²Research and Development Station for Viticulture and Enology Minis, Romania

*Corresponding author: chedeaveronica@yahoo.com

ABSTRACT

The aim of this study was the identification the pathogenic fungi causing the grapevine trunk diseases (GTD) in some Romanian vineyards. Twenty-one samples of biological material (bark, canes, and trunk wood) were collected from Tarnave, Aiud, Minis and Sarica Niculitel vineyards. Petri dish culture method was used to grow the fungi, inoculated at the central point on the Sabouraud medium. Fungal species were identified by macroscopic and microscopic examination based on the morphological characteristics of fungal colonies developed on medium: the type and color of mycelian hyphae, the shape, color and size of spores. In the studied Romanian vineyards GTD were caused by: *Phaeoacremonium aleophilum*, *Phaeomoniella chlamydospora*, *Stereum hirsutum*, *Diplodia seriata*, *Cadophora luteo-olivacea*, *Diatrypaeae sp.*, *Phomopsis viticola* and *Eutypa lata*, being associated with diseases, such as: Esca, Petri, excoriosis and Eutypa, identified by plate culture method and microscopic examination.

Keywords: Esca complex, Petri disease, excoriosis, Eutypa dieback, Transilvania vineyards, Minis and Sarica Niculitel vineyards

INTRODUCTION

Since the end of the 20th century, the grapevine trunk diseases had an economic impact which became a limiting factor in the production of grapes in many countries (Armengol *et al.*, 2001). In mature vineyards, diebacks such as Eutypa, Esca and *Botryosphaeria* are considered to be the most destructive (Dubos, 2002; Mugnai *et al.*, 1999; Úrbez-Torres, 2011). Generally characterized, these diseases develop a specific inner wood rot of the grapevine and are caused by pathogenic fungi that colonize and infect the plants. Esca symptoms on leaves are associated with the development of a variety of pathogenic fungi in the grapevine wood (Dubos, 2002; Chiarappa, 1959; Mugnai *et al.*, 1999; Arnaud and Arnaud, 1931). A cross section cut of the trunk of infected grapevines shows damaged tissue with varying stages of decay (Larignon and Dubos, 1997; Larignon, 2004; Maher *et al.*, 2012). Older grapevines present a white to yellow rotten mass inside of the trunk (Fischer, 2002; Fischer and Kassemeyer, 2003). The trunk damage continues further and the volume of dead tissue increases and in severe cases apoplexy can appear. The trunk apoplexy is the phenomenon

of sudden wilting and death of the vegetative organs and in the end, of the entire plant (Mugnai *et al.*, 1999).

In the last three decades, studies on grapevine wood inhabiting fungi increased, these studies highlighting that grapevines with Esca syndrome, generally show that many phytopathogenic fungi species cohabitate on the same plant, such as: the mitosporic fungi *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and the frequent *Fomitiporia mediterranea* (Armengol *et al.*, 2001; Aroca *et al.*, 2006; Larignon and Dubos, 1997; Luque *et al.*, 2009; Mugnai *et al.*, 1996; Péros *et al.*, 2008; Rumbos and Rumbou, 2001). Romania is an important region for grapevine cultivation ranking in 2019 the 10th place from 98 countries in terms of harvested grapes (<http://www.fao.org/faostat/en/#data/QCL>).

Due to this fact, grapevine diseases are a very important factor affecting the Romanian vineyards in terms of production and financial inputs. For instance, in Transylvania, GTD affect up to 5% of the vineyards, being also vineyards where the incidence of the attack exceeds 15% (Tomoiağă and Chedea, 2020). For this reason, the current study aims to identify the fungal pathogens causing the observed GTD in some Romanian vineyards.

MATERIALS AND METHODS

Twenty-one samples of damaged wood tissue were collected from Tarnave, Aiud, Minis and Sarica Niculitel vineyards in the year 2020. The description of the samples (county and site of collection, as well as the age of the vineyards and the cultivar) is presented in Table 1.

Table 1. Description (region and site of the collection as well as the age of the vineyards and the cultivar) of the damaged wood tissue samples collected from Tarnave, Aiud, Minis and Sarica Niculitel vineyards in the year 2020

County	Site (Vineyard)	Age	Cultivar
Arad	Cuvin	10	Sultanina
Arad	Cuvin	20	Kober 5BB rootstock
Tulcea	Niculitel	2	Aligote
Alba	Ciumbrud	10	Traminer
Alba	Blaj	10	Codreanca
Alba	Blaj	10	Victoria
Alba	Blaj	13	S04-4 rootstock



Figure 1. Damaged wood samples collected from the studied cultivars: A- Codreanca; B- Sauvignon blanc; C- Aligote; D- Sultanina; E- Traminer roz; F- Kober 5 BB rootstock; G- S04-4 rootstock

Some of the damaged wood collected from vineyards and from which the primary cultures on the plate were made are presented in Figure 1.

Biological samples (old wood, bark, canes) were prepared for inoculation on culture medium, being cut in thin slices of 2-3 mm thick. Surface sterilization was done by immersion in a solution of sodium hypochlorite 3% concentration for 30 seconds and rapidly transferring the biological material to primary plate culture, 3 to 5 samples per Petri dish, every Petri dish containing material from the same sample. Primary plate culture on agar-agar medium and selective medium Sabouraud, was done from all these samples. To isolate the fungi, the Petri dish culture method was used and by the seeding method were inoculated at the central point on the Sabouraud medium the mycelium portions from every type of fungal growth in primary culture. Micelium transfer from a Petri dish to another was done with a scalpel, sterilized by burning after every transfer in order to avoid contamination with other microorganisms or fungal species. An attempt to identify the fungal species was done by macroscopic and microscopic (WETZLAR and OPTIKA) examination based on the morphological characteristics of the colonies developed on the medium: the type and color of mycelian hyphae, the shape, color and size of spores.

RESULTS AND DISCUSSIONS

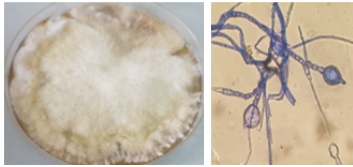
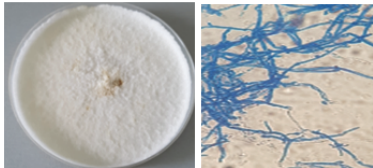
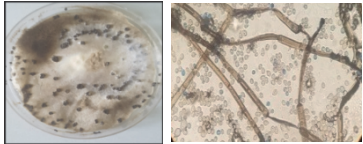
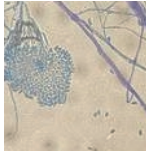
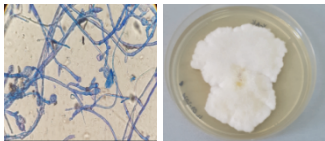
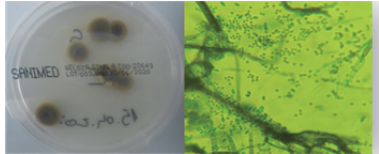
In order to determine the disease and then the fungal pathogens, the GTD symptoms of the grapevine trunk wood were examined. The wood symptoms were categorized according to their shape or discoloration as described by Larignon (2004) and Liminana *et al.* (2009). Shapes were punctuations, necrotic lines, central necrosis, sector shaped necrosis and mixed (central and sectorial) necrosis (Choueiri *et al.*, 2014). More specifically, these symptoms were: necrosis in V shape of dark color for Eutypa dieback (Comşa *et al.*, 2012), zones with degraded wood of light color for Esca complex, mixt necrosis with rottenness and also with hard dark colored parts for a combination of Esca and Eutypa dieback, dark spots and dots placed concentric in the centre of the canes as well as brown lesions for Petri disease and wood with excoriosis of different shapes and dimensions for Phomopsis caused excoriosis.

The macroscopic examination of the collected samples together with the fungi's colonies characteristics on the Sabouraud medium and with the microscopic examination of the isolates led us to identify some of the pathogens causing the GTD. The characteristics of these pathogens are presented in Table 2.

The pathogen *Diplodia seriata* was determined as causing the Esca complex and the Petri disease on the cultivars Aligote, Sultanina and Victoria as well as on the rootstock Kober 5BB. On the Sabouraud medium the young mycelial colonies were fluffy with white color, later becoming gray. The microscopic prepareate shows thin hyaline hyphae with a single conidium on the top. *Stereum hirsutum* presents on the plate a white mycelium with fine grains, and at the microscop the branched mycelial filaments have also a single conidium on the top. On the Sabouraud medium, the pathogen *Phaeomoniella chlamidospora* was identified as green-olive color colonies. This fungus presents a filamentous mycelium with multiple ovoid conidia. *Phaeoacremonium aleophilum*, an Esca complex pathogen had on the medium white fluffy colonies with the centre of the colony of dark color and with a dark yellow reverse side. At the microscop circular conidia and hand like shape mycelial filaments were identified. Excoriosis or Phomopsis dieback is caused by *Phomopsis viticola* identified here as whitish mycelia colonies with black pycnidia. It also has a gelatinous mass out of which at the microscop there were observed the typical alfa and beta conidia. On the wood samples showing symptoms of Petri disease the pathogen *Cadophora luteo-olivacea* was identified as

presenting a cream-gray mycelium with greenish edge on the Sabouraud medium and branched mycelial filaments and ovoid conidia seen at the microscop.

Table 2. The fungal pathogens' localization and their identification based on the fungal colonies features and microscopic examination of their isolates

Potentially identified species	GTD caused	Sample's cultivar	Photos of Petri dish fungal colonies and microscopic prepare lamella
<i>Diplodia seriata</i>	Petri disease	Kober 5BB Aligote	
	Esca	Sultanina Victoria	
<i>Eutypa lata</i>	Eutypa dieback	Victoria	
<i>Diatrypaceae sp.</i>	Eutypa dieback	Codreanca	
<i>Phomopsis viticola</i>	Excoriosis	Traminer	
<i>Phaeoacremonium aleophilum</i>	Esca	Traminer	
		Sultanina	
		Aligote	
<i>Stereum hirsutum</i>	Esca	Traminer Codreanca	
<i>Cadophora luteo-olivacea</i>	Petri disease	Rootstock Kober 5 BB Rootstock SO4-4	
<i>Phaeomoniella chlamydospora</i>	Petri disease	Rootstock Kober 5 BB Rootstock SO4-4	

Two pathogens were determined as causing the Eutypa dieback: *Eutypa lata* and *Diatrypaeae* sp. On the medium both pathogens showed fine white mycelia colonies and allantoic ascospores.

Previous studies carried out in the European vineyards showed that *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum* and *Fomitiporia mediterranea* are the three fungi commonly associated with the Esca complex, but *Eutypa lata*, which causes Eutypa dieback and several Botryosphaeriaceae species (e.g. *Diplodia seriata* and *Neofusicoccum parvum*) are also frequently retrieved from the wood of vines showing GTD symptoms (Claverie *et al.*, 2020). Several fungal pathogens were associated with the suddenly collapsed grapevines in Czech vineyards, and the most frequent was *Phaeomoniella chlamydospora* (77% of analyzed grapevines), followed by *Eutypa lata* (62%), *Diaporthe ampelina* (31%), *Botryosphaeria dothidea* (15%) and *Fomitiporia mediterranea* (15%). Some other important and very common Botryosphaeria dieback-associated pathogens, such as *Neofusicoccum parvum* or *Diplodia seriata*, were not identified in the study of Baranek *et al.* (2018).

Choueiri *et al.* (2006) reported also that the fungi *Phaeomoniella chlamydospora* and *Diplodia seriata* (*Botryosphaeria obtusa*) besides *Fomitiporia* sp. were associated with diseases such as Esca. Another study from Lebanon (Choueiri *et al.* 2014) showed that the three fungi species associated with esca in Europe (*Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Fomitiporia mediterranea*) (Larignon and Dubos, 1997; Mugnai *et al.*, 1996) were consistently found. *Diplodia seriata* and *Neofusicoccum parvum* were the most often isolated species in the Libanese vineyards (Choueiri *et al.* 2014). *Eutypa lata* is less abundant in dry regions of the world, such as Spain (Luque *et al.*, 2009), or South Africa (van Niekerk *et al.*, 2006), but is an important pathogen in cold humid regions (Comşa *et al.*, 2012). *Phomopsis* sp. and *Ilionectria* sp. associated with Phomopsis dieback or excoriosis and black foot disease were rarely found in the libanese survey (Choueiri *et al.*, 2014). In Romania, Phomopsis was found in Valea Calugareasca, Odobesti, Cotesti – Vrancea, Pietroasele and Dragasani vineyards (Oprea and Podosu, 2008). Excoriosis caused by *Phomopsis viticola* was also reported for the Tarnave vineyards (Savu *et al.*, 2020, Botea *et al.*, 2020) together with other grapevine trunk diseases such as Eutypa dieback (Comşa *et al.*, 2012).

CONCLUSIONS

Based on the studies performed, using the macroscopic and microscopic examination, in the Romanian vineyards, the most commonly (potentially) detected fungal species were: *Stereum hirsutum*, *Eutypa lata*, *Phomopsis viticola*, *Phaeomoniella chlamydospora*, *Diplodia seriata*, *Phaeoacremonium* sp, *Cadophora luteo-olivacea* and *Diatrypaeae* sp. *Stereum hirsutum* caused Esca complex on the cultivars Codreanca in Blaj vineyard and Traminer from Ciumbrud vineyard. In the Ciunbrud vineyard was also found *Phomopsis viticola* causing the excoriosis on the Traminer cultivar.

Eutypa dieback was caused by *Eutypa lata* and *Diatrypaeae* sp. on the cultivar Victoria from Blaj. *Phaeomoniella chlamydospora* and *Cadophora luteo-olivacea* were linked to Petri disease symptoms on the rootstocks Kober 5BB from Cuvin vineyard and SO4-4 from Blaj vineyard. As a pathogen causing the Petri disease was identified *Diplodia seriata* on the rootstock Kober 5BB from Cuvin and Aligote cultivar from Sarica Niculitel. *Diplodia seriata* is also a pathogen of the Esca complex on the cultivars Sultanina from Cuvin and Victoria from Blaj vineyards. *Phaeoacremonium aleophilum* was found to infect the grapevines of the cultivars Traminer from Ciunbrud, Sultanina from Cuvin and Aligote from Sarica Niculitel vineyards causing the Esca complex dieback.

ACKNOWLEDGEMENTS

This work was supported by the Romanian Ministry of Agriculture and Rural Development (MADR), inside the project ADER 7.5.3.

REFERENCES

1. Armengol J., Vicent A., Torné L., García-Figueres F. and García-Jimenez J. (2001). Fungi associated with esca and grapevine declines in Spain: a three-year survey. *Phytopathol. Mediterr.* 40: 325-329.
2. Arnaud G. and Arnaud M. (1931). Esca, polypores et maladies fongiques diverses du tronc, In: *Traité de Pathologie Végétale - Encyclopédie Mycologique III*. Lechevalier et Fils, Paris: 428-444.
3. Aroca A., García-Figueres F., Bracamonte L., Luque J. and Raposo R. (2006). A survey of trunk disease pathogens within rootstocks of grapevines in Spain. *Eur. J. Plant Pathol.* 115: 195-202.
4. Baránek, M., Armegol, J., Holleínová, V., Pecenká J., Calzarano, F., Penázova E., Vachun M. and Einchmeier, A. (2018). Incidence of symptoms and fungal pathogens associated with grapevine trunk diseases in Czech vineyards: a first example from a north-eastern European grape-growing region. *Phytopathologia Mediterranea*, 57(3): 449-458.
5. Botea V., Tomoiagă L. L., Vasii I., Sîrbu A., Răcoare H-S., Chedea V. S. (2020). *Phomopsis viticola* management in Tarnave vineyards, Lucrari Șt. Seria Hort. 63 (2) /2020, USAMV IAȘI.
6. Chiarappa L., (1959). Wood decay of the grapevine and its relationship with black measles disease. *Phytopathology* 49: 510-519.
7. Choueiri E., Jreijiri F., Chlela P., Louvet G. and Lecomte P. (2006). Occurrence of grapevine declines and first report of Black Dead Arm associated with *Botryosphaeria obtusa* in Lebanon. *Plant Dis.* 90: 115.
8. Choueiri E., Jreijiri F., Chlela P., Mayet V., Comont G., Liminana J-M., Mostert L., Fischer M., Lecomte P. (2014). Fungal community associated with grapevine wood lesions in Lebanon. Vol. 48 No. 4: *Journal international des sciences de la vigne et du vin*, 48(4):293-302.
9. Claverie M., Notaro M., Fontaine F., Wery J. (2020) Current knowledge on Grapevine Trunk Diseases with complex etiology: a systemic approach. *Phytopathologia Mediterranea* 59(1): 29-53.
10. Comșa M., L., Cudur F., Cudur C., Cristea C. (2012). Research on some pathogenic fungi involved in the biological decline of the grapevine at the Blaj Viticultural Centre. *Lucrări Științifice, Universitatea de Științe Agricole și Medicină Veterinară "Ion Ionescu de la Brad" Iași, Seria Horticultură*, 55(2): 503-508.
11. Dubos B. (2002). *Maladies Cryptogamiques de la Vigne*. Editions Féret, Bordeaux.
12. Fischer M. (2002). A new wood-decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (Hymenochaetales). *Mycol. Prog.* 1: 315-324.
13. Fischer M. and Kassemeyer H. -H. (2003). Fungi associated with Esca disease of grapevine in Germany. *Vitis* 42: 109-116.
14. Larignon P. and Dubos B. (1997). Fungi associated with esca disease in grapevine. *Eur. J. Plant Pathol.* 103: 147-157.
15. Larignon P., (2004). Réflexions sur l'esca. *Phytoma* 576: 28-31.
16. Liminana J., M., Pacreau G., Boureau F., Menard E., David S., Himonnet C., Fermaud M., Goutouly J.P., Lecomte P. and Dumot V. (2009). Inner necrosis in grapevine rootstock mother plants in the Cognac area (Charentes, France). *Phytopathol. Mediterr.* 48: 92-100.
17. Luque J., Martos S., Aroca A., Raposo R. and García-Figueres F. (2009). Symptoms and fungi associated with declining mature grapevine plants in Northeast Spain. *J. Plant Pathol.* 91: 381-390.
18. Maher N., Piot J., Bastien S., Vallance J., Rey P. and Guérin-Dubrana L. (2012). Wood necrosis in esca-affected vines: types, relationships and possible links with foliar symptom expression. *J. Int. Sci. Vigne Vin* 46: 15-27.
19. Mugnai L., Surico G. and Esposito A. (1996). Microflora associata al mal dell'escavite in Toscana. *Inf. Fitopatol.* 46: 49-55.
20. Mugnai L., Graniti A. and Surico G. (1999). Esca (Black Measles) and brown wood-streaking: two old and elusive diseases of grapevines. *Plant Dis.* 8: 404-418.
21. Oprea M., Podosu A. (2008). Grape dieback in Romania induced by pathogenic lignicolous fungi. *Lucrari Științifice USAMV Bucuresti, Seria B-LII-2008*: 128-133.
22. Péros J. P., Berger G. and Jammaux-Despréaux I. (2008). Symptoms, wood lesions and fungi associated with esca in organic vineyards in Languedoc-Roussillon (France). *J. Phytopathol.* 156: 297-303.
23. Rumbos I. and Rumbou A. (2001). Fungi associated with esca and young grapevine decline in Greece. *Phytopathol. Mediterr.* 40: 330-335.
24. Savu, S., Tomoiagă, L.L. and Chedea, V.S. (2020). Ecological Microclimate Influence on Grapevine *Phomopsis viticola* Attack Frequency in Aiud-Ciurbrud Vineyards. *Bulletin UASVM Horticulture*, 77(2):64-70.
25. Tomoiagă L.L., Chedea V.S. (2020). Grapevine Trunk Diseases management in vineyards from Central Transylvania. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture*, 77(1): 117-121.
26. Úrbez-Torres J. R., 2011. The status of *Botryosphaeriaceae* species infecting grapevines. *Phytopathol. Mediterr.* 50: 5-45.
27. van Niekerk J. M., Fourie P. H., Halleen F. and Crous P.W. (2006). *Botryosphaeria* spp. as grapevine trunk disease pathogens. *Phytopathol. Mediterr.* 45: 43-54.
28. <http://www.fao.org/faostat/en/#data/QCL>