Hungarian University of Agriculture and Life Sciences

INVESTIGATION OF THE FUNGAL COMMUNITY OF GRAPEVINE (VITIS VINIFERA), AND THE FACTORS INFLUENCING THIS COMMUNITY IN THE TOKAJ AND EGER VINE REGION

DOI: 10.54598/005190

Theses of doctoral dissertation

GEIGER ADRIENN

GÖDÖLLŐ

2024

Doctoral school:	Doctoral School of Environmental Sciences
Discipline:	Environmental sciences
Head of doctoral school:	Csákiné Prof. Dr. Michéli Erika Doctor of the Hungarian Academy of Sciences, university professor, DSc, Hungarian University of Agriculture and Life Sciences, Institute of Environmental Sciences, Department of Soil Science
Scientific supervisor:	Dr. Geml József Group leader, Scientific advisor, PhD Eszterházy Károly Catholic University Food and Wine Research Center HUN-REN-EKKE Lendület Environmental Microbiome Research Group
Scientific Co-Supervisor:	Dr. Táncsics András Head of department, Scientific advisor, PhD Hungarian University of Agriculture and Life Sciences Department of Molecular Ecology

....

Csákiné Prof. Dr. Michéli Erika Dr. Geml József

.....

Dr. Táncsics András

Backgrounds and objectives

One of the most severe fungal diseases affecting grapevine is Grapevine Trunk Diseases (GTD), several fungi has been described as GTD pathogen. The occurrence of GTD is increasing worldwide due to the lack of effective defense strategies. The functional role of many fungal species associated with GTD is largely unknown. There are still numerous unanswered questions regarding the course of the disease. Currently, there is no effective defense method as pesticides and fungicides are ineffective since the fungi collonize the inner part of woody tissues. In recent decades, cultivation-independent techniques have emerged to explore the microbiome of different plants. Next-generation sequencing provides an opportunity for more detailed examination of GTD and to uncover which factors may influence the epidemiology of the disease.

My research involves the taxonomic and functional characterization of microorganisms found in bark and woody tissues of grapevines (*Vitis vinifera*), as well as in the soil. The central topic of the research is the grapevine's pathobiome, with particular attention on GTD pathogens and interactions between the mycobiome of soil and plants. Throughout our work, we examined the influence of terroir, microhabitat, and health state on plant pathogens, specifically GTD pathogens, in the Tokaj wine region. Additionally, we investigated the effects of cultivar, microhabitat, seasonality, and vintage on the mycobiome of healthy and GTD symptomatic plants in the Eger wine region.

The isolation and characterization of microorganisms traditionally involve time-consuming and labor-intensive processes, but these are essential steps. However, culturing a particular microorganism in pure culture offers the best opportunity for comprehensive characterization in terms of phylogenetics, taxonomy, and functionality. In addition to culture-independent DNA metabarcoding techniques, we investigated the fungi that can be cultured from healthy and diseased grapevine wood using traditional isolation methods to determine the dominant species.

I wanted to continue the topic I had started in my master thesis. We characterized *Clonostachys rosea* as a potential biocontrol agent against GTD pathogens under *in vitro* conditions. We plan to implement *in planta* tests because it is crucial to determine whether a biocontrol agent, which performs well under laboratory conditions, is capable of doing so in less controlled environments. In summary, my objectives are as follows:

1. Examination of the influence of terroir, microhabitat, and health state on plant pathogens, particularly GTD pathogens, in the Tokaj wine region

2. Investigation of the effects of different grapevine cultivars, microhabitat, seasonality, vintage, and health state on the mycobiome in the Eger wine region

3. Isolation of fungi from grapevine woody tissue using traditional culturing methods alongside next-generation techniques, followed by their identification and comparison with data from next-generation sequencing

4. *In planta* evaluation of the biocontrol agent *Clonostachys rosea* against some GTD pathogens

Materials and methods

Sample collection, DNA-extraction

In Eger wine region, at the experimental vineyard of Eszterházy Károly Catholic University, we examined four grapevine cultivars (kékfrankos, leányka, cabernet Sauvignon, chardonnay). We selected two vines showing symptoms of Esca disease and two healthy vines from each cultivar, from which we collected bark and wood tissue samples, as well as soil samples from the surrounding soil. Sampling was conducted in February and August of 2020 and 2021. In the Tokaj wine region, we examined three vineyards and selected two healthy and two Esca symptomatic plants per vineyard, then conducted sampling at the same time as in the Eger wine region. The samples were standardized, lyophilized, homogenized, and DNA was extracted. Subsequently, the extracted DNA was sent for next-generation sequencing, which was performed on the Illumina MiSeq platform. The ITS2 rDNA region (ribosomal DNA) was sequenced using the fITS7 (Ihrmark et al., 2012) and ITS4 (White et al., 1990) primers.

DNA metabarcoding data analysis, statistical analysis

The data analyisis was only performed from the first sampling in case of the Tokaj wine region, while in case of the Eger wine region, we analyzed the data from all four samplings together. The raw sequences were processed using the *dada2* (CALLAHAN et al., 2016) package in the R environment (R Development Core Team 2013). Subsequently, the sequences were trimmed, denoised, merged, and sorted into amplicon sequence variants (ASVs). The taxonomic classification of fungi was made using the USEARCH program (version 11, Edgar, 2010) based on all fungal species reference sequences available in the UNITE database. The ASVs were assigned into different functional groups based on the list provided by TEDERSOO et al. (2014). Among these ASVs, we highlighted the genera that are associated with GTD according to the literature. All analyses were conducted in the R environment.

Samples from both sampling sites containing few sequences (<1000) were excluded from the analysis. Then, the matrices were normalized, in case of Tokaj, we continued working with plant pathogens, while in Eger, we focused on several functional groups like GTD pathogens non-GTD pathogens, mycoparasites, generalist saprotrophs, GTD-associated wood saprotrophs and wood saprotrophs. To test the effects of categorical variables such as microhabitat (wood tissue, bark, soil), terroir, seasonality, vintage, and health state, we compared diversity and abundance among samples using one-way ANOVA models. Significant differences were identified using the Tukey HSD test. The diversity and abundance of ASVs were visualized using boxplots with the *ggplot2* (WICKHAM, 2016) package for Tokaj based on the two-way microhabitat-terroir model, while for Eger, it was based on microhabitat alone. The compositional differences between samples were visualized

using non-metric multidimensional scaling (NMDS) with the *vegan* package. Permutational multivariate analysis of variance (PerMANOVA) was performed using the *adonis* function in the *vegan* package to estimate the variation explained by terroir, microhabitat, vintage, seasonality, and health state. We also conducted NMDS and PerMANOVA on the Eger matrix divided into isolation sources. The distribution of plant pathogen ASVs was visualized as a network using the sna package (BUTTS, 2008) for different isolation sources from both the Tokaj and Eger datasets.

Isolation from woody tissue using culture dependent method

In case of Eger fungal isolates were cultured from woody tissue using culture dependent method, from all four sampling times, while from Tokaj, only the year 2021. Wood chips were individually placed onto potato dextrose agar (PDA) supplemented with oxitetracycline-hydrochloride (10 μg/ml Sigma-Aldrich), with 5 chips per dish, and 3 replicates per trunk. The plates were monitored for 4 weeks, and pure cultures were obtained from emerging fungi. DNA was extracted from the samples, and the Internal Transcribed Spacer (ITS) region commonly used for fungal identification was amplified using PCR (Polymerase Chain Reaction). Subsequently, the amplified fragments were sent for Sanger sequencing to Eurofins Genomics (Ebersberg, Germany). Raw sequences were filtered using Geneious Prime bioinformatics software. Taxonomic classification of the fungi was performed similarly to sequences identified via DNA metabarcoding technique.

***In planta* greenhouse experiment with the potential biocontrol candidate *Clonostachys rosea* strain 19B1**

We tested the efficacy of the potential biocontrol agent *Clonostachys rosea* 19B/1 in an *in planta* greenhouse experiment. Mycelial discs approximately 3 mm in diameter were cut from strains of *Eutypa lata*, *Botryosphaeria dothidea*, and *Phaeomoniella chlamydospora*, then inoculated into the xylem of wounded cabernet sauvignon cuttings. Five cuttings were used for each pathogen-soil combination. The cuttings were planted in untreated soil or soil supplemented with a suspension of conidia of *C. rosea* 19B/1 (10^4 spores/gram). The plants were incubated in the greenhouse under ambient light conditions. After a 90-day incubation period, the cuttings were removed from the soil with roots intact. After removing the bark, they were longitudinally cut, and the length of necrotic lesions was measured. Attempts were made to re-isolate *C. rosea* 19B/1 from the soil and from three different points of the inoculated soil-grown cuttings (base, middle of internode, wound). For those fungi morphologically similar to *C. rosea*, a small amount of mycelium was transferred to PDA agar to obtain pure cultures. The fungi were identified based on morphological characteristics and the ITS region.

Results and discussion

Results obtained from the DNA metabarcoding dataset of the Tokaj wine region, and their evaluation

The abundance of GTD and non-GTD pathogens, as well as the total plant pathogen abundance, differed among the microhabitats, but not among health states. The total plant pathogen abundance was highest in the wood, GTD pathogens' abundance was highest in the wood tissue, while non-GTD pathogens' abundance was highest in soil. The richness of all plant pathogens differed among microhabitats and among terroirs as well. Non-GTD pathogens were the most diverse in the soil, while GTD pathogens showed higher diversity in the wood tissue, followed by bark, and exhibited lower diversity in the soil.

The composition of the plant pathogenic fungal community was depicted using two-dimensional NMDS. Microhabitat exerted the greatest influence on the plant pathogenic fungal community, explaining 19.25% of the variation, followed by terroir with 7.78%, and health state with 1.86%. Network analysis confirmed the dominance of GTD pathogens in the wood tissue and bark, while non-GTD pathogens dominated in the soil, with several dominant ASVs appearing in multiple samples. According to network analysis, the pathobiome of healthy and diseased plants did not differ significantly, but dominant GTD genera in the bark and wood tissue did. Soil samples were nicely separated by terroirs, with terroir-specific ASVs alongside those found in every vineyard.

The pattern of plant pathogenic fungal community examined in the Tokaj wine region was greatly influenced by the isolation source, primarily by differences in the distribution of GTD and non-GTD pathogens. GTD pathogens were found in the wood tissue and bark, while they were completely absent from some soil samples or showed very low frequencies. This statement is in a disagreement with the results of NERVA et al. (2019), suggesting that soil is a significant inoculum source for GTD pathogens. It is important to mention that the diversity of microhabitats was influenced by non-GTD pathogens, while GTD pathogens had more influence on abundance values based on our normalized matrix. This implies that although less diverse, GTD pathogens are predominantly major members of the grapevine pathobiome, especially in the woody tissue, displacing non-GTD pathogens. According to our network analysis, species of *Phaeomoniella*, *Seimatosporium*, and *Diplodia* contribute to the aforementioned abundance surplus in the woody tissue, with *Seimatosporium* and *Diplodia* species in the bark. The high number of *Phaeomoniella* species in wood and their low frequency in the bark is likely due to the preference of *P. chlamydospora* for the xylem of wood tissue in terms of colonization (LANDI et al., 2012; FLEURAT-LESSARD et al., 2014).

Despite the relatively close proximity of sampling areas in the Tokaj wine region (<10 km), we found significant compositional differences in the plant pathogenic fungal community composition between terroirs. This was particularly evident for soil-borne pathogens and to a lesser extent for pathogens in the woody tissue, indicating a kind of environmental filtering driven by mesoclimatic and edaphic factors. Terroir had a greater influence on the composition of plant pathogenic fungal communities in the bark than in the wood tissue, likely because the bark is more exposed to environmental factors than the wood.

Our results emphasize the complexity of GTD, indicating that these pathogens are general members of the microbiome even in healthy plants, and their presence is not directly associated with the appearance of foliar symptoms. As noted by HOFSTETTER et al. (2012), most fungi considered as GTD pathogens are rather endophytic and/or latent pathogens, which can act opportunistically in weakened grapevines due to cold, drought, intense pruning, or other factors. We found that fungi associated with GTD are almost exclusively present in the bark and perennial woody tissue, with few exceptions in the soil. This supports the theory that GTD pathogens are primarily saprophytes and secondarily opportunistic pathogens.

Results and evaluation of the DNA metabarcoding dataset from the Eger wine region

Non-GTD pathogens dominated in soil, while GTD pathogens were prevalent in wood tissue, followed by the bark. Generalist saprotrophs were common in both soil and bark, while saprotrophs associated with non-GTD pathogens occurred in all three isolation sources, with many outliers in bark and wood tissue. Saprotrophs associated with GTD were present in bark and woody tissue with numerous sequences, while mycoparasites were mainly found in bark. The soil was the most diverse microhabitat for non-GTD pathogens, generalist saprotrophs, saprotrophs associated with non-GTD pathogens, and mycoparasites. Saprotrophs associated with GTD and GTD pathogens were the most diverse in the woody tissue and bark. In contrast to Tokaj, the abundance and species richness of GTD pathogens were higher in symptomatic plants. For abundance, the sequence numbers of *Botryosphaeria* and *Eutypella* species were higher in symptomatic plants, while for richness, the numbers of *Botryosphaeria*, *Eutypella*, and *Fomitiporia* species were higher. As suggested by several researchers, changes in the abundance of GTD pathogens may underlie the appearance of symptoms, a hypothesis that we can confirm with the dataset from Eger. It is likely that we could not detect the difference in Tokaj because we analyzed only one sampling. This is evidenced by MOTA LEAL et al. (2024), who found differences in the abundance and richness of plant pathogens between healthy and symptomatic plants in the Tokaj wine region by analyzing all four samplings. In the Eger wine region, the abundance and richness of several functional groups significantly decreased in 2021 compared to the year 2020, likely due to the drier

vintage. Overall, microhabitat was the most influential factor in shaping the abundance and richness values of the examined functional groups.

The fungal community composition was visualized using non-metric multidimensional scaling (NMDS) for all samples. According to PerMANOVA, microhabitat, vintage, cultivar, and seasonality showed significant correlations with the fungal community structure. Microhabitat explained 3.8% of the variation, vintage 2.5%, cultivar 1.3%, and seasonality 1.2%. Health state did not correlate significantly with community structure when all samples were analyzed together. Fungal community composition was also visualized using two-dimensional NMDS for different microhabitats, revealing the effects of other variables. For example, in the case of bark, the effect of cultivar was evident, as samples from chardonnay and leányka cultivars were completely separated.

Based on network analysis of the first sampling from Eger, similar to Tokaj, non-GTD pathogens dominate in soil, while an increasing number of GTD pathogens appear in bark. In woody tissue, GTD pathogens outcompete non-GTD pathogens. Among GTD pathogens, *Phaeomoniella* and *Diplodia* species dominate in the wood tissue, just like in Tokaj. Interestingly, *Diaporthe* species are only present in the soil in the Eger wine region, and GTD pathogens are present with more ASVs in the soil in Eger than in Tokaj.

Based on our results from Eger, the fungal community composition differs more between soil and the examined plant parts than among the studied seasons, vintages, grapevine cultivars, or health states. The variability in the composition of the soil mycobiome correlates most strongly with abiotic factors (vintage, seasonality), while the influence of biotic factors (cultivar, health state) is more pronounced in the fungal community directly associated with the grapevine.

When analyzing all samples together, the soil fungal community composition significantly differed from that of bark and wood tissue, while the plant parts exhibited similar mycobiome composition. This indicates that the examined microhabitats harbor distinct fungal communities, as we also observed in case of plant pathogens in the Tokaj wine region. SWIFT et al. (2021) reached the same conclusion. When analyzing the dataset from Eger based on isolation sources, we found that vintage had the greatest impact on fungal community composition, especially in the soil, while the effects of seasonality, cultivar, and health state varied depending on the microhabitat.

In the vintage of 2021, significant changes were observed in the mycobial community composition of soil, affecting the diversity of several examined functional groups. In 2021, diversity values decreased significantly, likely due to the drier vintage. The decrease in diversity may explain the changes in community composition, as drought-tolerant species may have survived unfavorable conditions. PAPADOPOULOU et al. (2022) also investigated the effect of vintage and found

that vintage has a greater impact on the diversity and composition of epiphytic fungi on grapevine than seasonality. Based on our data from the Eger wine region, we can also conclude that vintage had a greater impact on fungal abundance, richness, and community composition than seasonality.

The results of culture dependent isolation from woody tissue

In the Eger wine region, traditional methods successfully yielded fungal cultures from wood tissue in all four sampling periods. In the year 2020, GTD pathogens were isolated in the highest numbers, both in winter and summer. Among GTD pathogens, *Diplodia* species predominated in the winter, along with *Phaeomoniella* and *Phaeoacremonium* species. In summer, in addition to *Diplodia* species, several *Seimatosporium* species were isolated, and *Fomitiporia* species also appeared. The proportions of functional groups were roughly similar in the two seasons. Common grapevine species were also isolated, such as *Aureobasidium*, *Cladosporium*, and *Alternaria* species. Among the mycoparasites, *Angustimassarina* species appeared in the winter period. It may be interesting to investigate the lifestyle of these species on grapevines in the future.

In the year 2021, a higher percentage of GTD pathogens were isolated from the Eger wine region, with over 60% of isolates identified as GTD pathogens during the summer season. Similar to 2020, *Diplodia* species constituted a large portion of GTD pathogens in 2021 as well. *Seimatosporium* species were only isolated during the summer in 2021. Both in winter and summer, *Cladosporium* and *Alternaria* species were isolated. The *Vishniacozyma* species isolated in winter has already been identified from grapes using next-generation techniques (YANG et al., 2023). *Vishniacozyma victoriae* has been shown to have antagonistic effects against numerous fungi when used as a biocontrol agent against pear storage diseases (GORORDO et al., 2022). Based on next-generation sequencing, *Seimatosporium* species were not identified from any of the sampling in the Eger wine region, while several *Seimatosporium* species were identified during cultivation, both in 2020 and 2021. In 2021, fungi were also isolated from the wood tissue in the Tokaj wine region. GTD pathogens comprised the majority of samples, accounting for about 40-50% in both seasons. Alongside *Diplodia* species, a large number of *Phaeomoniella* and *Seimatosporium* species were isolated among GTD pathogens. *Alternaria* species appeared in both seasons, as well as *Epicoccum*, *Cladosporium*, and *Aureobasidium* species.

Through culture-based techniques, we were able to identify significantly fewer fungi compared to next-generation sequencing (avg, 80 isolates/sampling time), however, it is evident that we succeeded in isolating species through cultivation that were not able to detect in the sequencing data. In the years 2020 and 2021 in the Eger wine region, *Seimatosporium* species were identified, and in 2021, we also isolated a species of *Uzbekistanica*, which was not found in the next-generation sequencing datasets used for analysis. Similarly, STEFANI et al. (2015) found differences in the

microbial community of hydrocarbon-contaminated soil between cultivation-independent and cultivation-based techniques, as we did.

Results of the *in planta* test with *Clonostachys rosea* strain 19B/1

Deep necrotic lesions developed in the wood tissue of the infected cuttings for *Eutypa lata* and *Botryosphaeria dothidea*, while *Phaeomoniella chlamydospora* necrotized the wood tissue directly under the bark. The length of necrotic lesions caused by *E. lata* and *P. chlamydospora* significantly decreased in the soil treated with *C. rosea*, whereas the severity of the disease did not change in plants infected with *B. dothidea*.

The re-isolation of *C. rosea* strain 19B/1 from non-infected cuttings showed a decreasing trend from the base of the cuttings upwards. From the base of the cuttings, the strain was successfully re-isolated from three out of five plants, from the middle of the internode once out of five plants, the re-isolation was unsuccessful from wound. The quantity of the 19B/1 strain increased by an order of magnitude after 90 days of greenhouse incubation. Importantly, the soil treated with *C. rosea* did not cause any negative effects on wounded but non-infected cuttings.
Based on our results, it can be concluded that *C. rosea* exhibits strong antagonistic effects against certain GTD pathogens due to its antibiotic and mycoparasitic abilities. The results of the *in planta* confrontation test suggest that *C. rosea* can be effectively used in the soil to prevent the development of GTD symptoms in grapevines. Its application would be particularly advisable in nurseries, as if it is able to colonize the cutting and persist there long term, its effect against GTD pathogens would be effective even before vineyard establishment. Previous studies have already demonstrated that *C. rosea* exerts antagonistic effects against *P. chlamydospora* (SILVA-VADERRAMA et al., 2021), although antibiotic effect were not mentioned in this study. Our investigation is the first report demonstrating the antagonistic effect of *C. rosea* against *E. lata*.

Overall, it can be concluded that *C. rosea* has significant potential and can be an effective biocontrol agent against numerous GTD pathogens. Both previous *in vitro* findings and our *in planta* results suggest that the antifungal compounds produced by *C. rosea* are more important in practice than its mycoparasitism, although the latter could also be a promising long-term strategy if systematic colonization of *C. rosea* in grapevines can be achieved.

Conclusions and recommendations

The study conducted in Tokaj provides novel insights into the compositional dynamics of the grapevine mycobiome, highlighting the diversity, abundance, and distribution of plant pathogenic fungi in different grapevine (bark, perennial wood) and soil compartments and in plants of different cultivars with and without foliar symptoms of Esca disease. The data presented here show that there are large compositional differences in plant pathogenic fungi among soil and aboveground plant parts, with GTD-associated pathogens dominating the grapevine trunk and non-GTD pathogens dominating soil. GTD pathogens exhibit similar diversity, abundance, and distribution in asymptomatic and symptomatic plants, not only taxonomically but also in terms of genetic diversity. This could be due to the one time sampling in case of Tokaj. Terroir-specific environmental factors have a decisive influence on the composition of plant pathogenic fungal community in all three studied habitats; however, they have a greater impact on the composition of plant pathogens living in the soil than those in the wood.

Similar to the Tokaj wine region, we observed that different mycobial communities inhabit the soil and grapevine parts we examined in the Eger wine region. Due to examining multiple functional groups, we observed that not only the abundance, diversity, and composition of plant pathogens differed, but also several functional groups varied significantly across the analyzed microhabitats. Examining microhabitats individually led us to conclude that besides vintage, health state, and cultivar also influence the microbial composition. Environmental factors play a critical role, and extreme weather conditions, such as a dry year, can contribute to a decrease in microbiome diversity. In contrast to the results from the Tokaj wine region, we observed higher abundance and richness values of GTD pathogens in case of symtomatic plants in the Eger wine region, with *Botryosphaeria*, *Eutypella*, and *Fomitiporia* species contributing to this difference. This is likely due to the fact that we studied and analyzed the results of four sampling. As several researchers suggest, changes in pathogen abundance may underlie symptom development, a hypothesis that our data analysis from Eger also supports.

However, further studies are needed for a complete understanding of GTD symptom development. Exploring the process requires a holistic approach that examines the entire grapevine microbiome, as microbial interactions may play a key role in symptom development. Interactions between fungi-fungi, fungi-bacteria, fungi-virus, and microbiome-plant are all important. Modern "omics" techniques, such as genomics, transcriptomics, proteomics, and metabolomics, are suitable for studying these interactions, enabling detailed analysis of the grapevine microbiome and identification of key events and members contributing to GTD development.

In addition to omics techniques, traditional methods should not be overlooked. Firstly, because according to our results, there are fungi that we could not identify from the samples using DNA metabarcoding techniques, but were able to identify

through culture-dependent methods. Secondly, by producing pure cultures, we have the opportunity to conduct further experiments beyond confirming the presence of a given microorganism. In our case, for example, during isolation from wood, we found *Angustimassarina* and *Vishniacozyma* species, which can parasitize other fungi. From the Eger dataset, it is evident that these species were more abundant in healthy grapevines and the surrounding soil. It would be worthwhile to further investigate the lifestyle of these species on grapevines and characterize their potential biocontrol properties against fungi associated with GTD.

C. rosea was also isolated in a previous study of ours when examining the mycobiome of cuttings using culture-based methods. We were able to identify several *C. rosea* strains from the cuttings. In the future, other strains of the same species or even the aforementioned species could be examined against GTD pathogens in both *in vitro* and *in planta* experiments, followed by field experiments with well-performing strains. However, this requires the isolation of fungi with mycoparasitic properties or even yeast and bacteria with antibiotic effects from grapevine.

Understanding microbial interactions and investigating the environmental effects on the microbiome is crucial. A unified approach encompassing plant protection practices, microbial ecology, and viticulture is important not only to understand GTD development and the crucial role of microbial interactions but also for sustainable grape cultivation and disease prevention and management. With a deeper understanding of the complex cooperation of these factors, grape growers and winemakers can work to preserve grape health, support microbial diversity, and ultimately produce wines that embody the unique characteristics of each vintage.

New scientific results

1. In the Tokaj wine region, I found that there is a greater difference in the pathobiome between plant parts and soil than among individual plants, whether they exhibit Esca symptoms or not. Additionally, the soil and plant have terroir-specific pathobiomes, which are more pronounced in the soil.

2. In the Eger wine region, the abundance and richness of the studied functional groups (non-GTD pathogens, GTD pathogens, generalist saprotrophs, non-GTD wood saprotrophs, GTD wood saprotrophs, mycoparasites), as well as the composition of the mycobiome, were most influenced by microhabitats (soil, wood tissue, bark). The abundance and richness of GTD pathogens were higher in symptomatic plants, driven by species such as *Botryosphaeria* and *Eutypella* for abundance and *Botryosphaeria*, *Eutypella*, and *Fomitiporia* for richness.

3. In the Eger wine region, among the studied factors – vintage, cultivar, seasonality, health state – vintage had the greatest impact on the mycobiome composition when analyzing microhabitats (soil, wood tissue, bark) collectively. When analyzing microhabitats individually, the influence of health state and cultivar was also evident. The variability of soil mycobiome composition correlated most strongly with abiotic factors (vintage, seasonality), while the impact of biotic factors (cultivar, health state) was more pronounced for the grapevine-associated fungal community.

4. *Clonostachys rosea* was successfully applied as a biocontrol agent against *Eutypa lata* and *Phaeomoniella chlamydospora* in an *in planta* experiment with cabernet sauvignon cuttings.

5. Our experiments confirmed for the first time that *Clonostachys rosea* exhibits antagonistic effects against *Eutypa lata*. *Clonostachys rosea* can be effectively used in soil to prevent the development of GTD symptoms in grapevines.

6. During the culturing process from the woody tissue, we isolated species that were not detected during next-generation sequencing, and we potentially identified biocontrol species such as *Angustimassarina* and *Vishniacozyma* against GTD pathogens.

Publications related to the topic of the dissertation

Publications in international peer-reviewed journals

In a foreign-language, impact factor journal

Leal, C. M., Geiger, **A.**, Molnár, A., Váczy, K. Z., Kgobe, G., Zsófi, Z., & Geml, J. (2023). Disentangling the effects of terroir, season, and vintage on the grapevine fungal pathobiome. Frontiers in Microbiology, 14.

Szabó, D; Molnár, N; **Geiger, A**; Karácsony, Z; Váczy, KZ. (2023) *In vitro* characterization of a *Bacillus velezensis* isolate as an antagonist of grapevine trunk disease pathogens. Acta phytopathologica et entomologica hungarica 58. 2 pp. 156-167., 12 p.

Geiger, A., Karácsony, Z., Geml, J., & Váczy, K. Z. (2022). Mycoparasitism capability and growth inhibition activity of *Clonostachys rosea* isolates against fungal pathogens of grapevine trunk diseases suggest potential for biocontrol. Plos one, 17(9), e0273985.

Geiger, A., Karácsony, Z., Golen, R., Váczy, K. Z., & Geml, J. (2022). The compositional turnover of grapevine-associated plant pathogenic fungal communities is greater among intraindividual microhabitats and terroirs than among healthy and Esca-diseased plants. Phytopathology®, 112(5), 1029-1035.

Molnár, A., Geml, J., **Geiger, A.,** Leal, C. M., Kgobe, G., Tóth, A. M., ... & Zsófi, Z. (2022). Exploring Relationships among Grapevine Chemical and Physiological Parameters and Leaf and Berry Mycobiome Composition. Plants, 11(15), 1924.

Papers published in congress proceedings

A one-page abstract in a foreign or Hungarian language based on a presentation or poster - in a scientific journal or its special issue:

Geiger, A; Leal, CM; Karácsony, Z; Golen, R; Váczy, KZ; Geml, J. DNA metabarcoding reveals that grapevine parts harbor different, niche-specific mycobiome In: Christoph, Hoffman; David, Gramaje Abstracts Book, International Organisation for Biological and Integrated Control – West Palaearctic Regional Section (IOBC-WPRS) Meeting of the Working Group “Integrated Protection in Viticulture (2023) p. 75.

Geiger, A; Leal, CM; Karácsony, Z; Golen, R; Váczy, KZ; Geml, J. Microhabitats show stronger impact on grapevine mycobiome more than cultivar, season or health

In: Szabó, A; Piwosz, K; Kormas, K; Panosyan, H Book of Abstracts Central and Eastern European Symposium on Microbial Ecology (2023) p. 27 Paper: 20.

Geiger, A; Leal, CM; Karácsony, Z; Golen, R; Váczy, KZ; Geml, J. DNA metabarcoding study reveals greater effect of microhabitat and vintage on grapevine

mycobiome than cultivar, season or health state In: Verdier, V 12th International Congress of Plant Pathology (2023) Paper: 21.

Geiger, A; Leal, CM; Karácsony, Z; Váczy, KZ; Geml, J. Microhabitats drive grapevine mycobiome more than cultivar, season or health state In: Julia, Kerrigan Annual Meeting of the Mycological Society of America 2023 (2023) Paper: 87.

Geiger, A; Karácsony, Z; Váczy, KZ. Fist report of mycoparasitism in *Diaporthe* species associated with grapevine trunk diseases. In: K., MÁRIALIGETI; O., DOBAY (szerk.) Abstracts of the 19th International Congress of the Hungarian Society for Microbiology (2023) 38 p. p. 64.

Geiger, A; Leal, CM; Karácsony, Z; Golen, R; Váczy, KZ; Geml, J. Comparison of the grapevine-associated plant pathogenic fungal community among different microhabitats, cultivars and between healthy and Esca-diseased plants In: Ales, Eichmeier 12th International Workshop on Grapevine Trunk Diseases

(2022) p. 54.

Geiger, A; Karácsony, Z; Golen, R; Váczy, KZ;Geml, J. The compositional turnover of grapevine-associated plant pathogenic fungal communities are greater among intraindividual microhabitats and terroirs than among healthy and Esca-diseased plants. In: 16th Congress of the Mediterranean Phytopathological Union

(2022) Paper: 31.

Geiger, A; Karácsony, Z; Geml, J; Váczy, KZ. Evaluation of the biocontrol capabilities of *Clonostachys rosea* against grapevine trunk diseases. In: 16th Congress of the Mediterranean Phytopathological Union (2022) Paper: 25.

Geiger, A; Leal, CM; Molnár, A; Lepres, L; Muller, M; Kgobe, G; Váczy, KZ; Geml, J. A szőlő növénypatogén gombaközösségére ható tényezők - mikroélőhely, fajta, egészségi állapot, évjárat, szezonalitás – vizsgálata In: Márialigeti, Károly; Dobay, Orsolya (szerk.) Magyar Mikrobiológiai Társaság 2022. évi Nagygyűlése (2022)

Geiger, A; Karácsony, Z; Golen, R; Váczy, KZ; Geml, J. Egészséges és Esca-tünetes szőlő növénypatogén gombaközösségének vizsgálata a tokaji borvidéken, különös tekintettel a GTD kórokozókra In: Haltrich, Attila; Varga, Ákos (szerk.) 68. Növényvédelmi Tudományos Napok Magyar Növényvédelmi Társaság (2022) 74 p. p. 29.

Geiger, A; Karácsony, Z; Golen, R; Váczy, KZ; Geml, J. The compositional turnover of grapevine-associated plant pathogenic fungal communities are greater among intraindividual microhabitats than among healthy and Esca-diseased plants

ACTA MICROBIOLOGICA ET IMMUNOLOGICA HUNGARICA 68: Supplement 1 p. 68 (2021)

Geiger, A; Karácsony, Z; Golen, R; Geml, J; Váczy, KZ. A *Clonostachys rosea* biokontroll tulajdonságainak felmérése *in planta* konfrontációs tesztek segítségével a szőlő korai elhalását okozó gombás betegségek ellen. In: Haltrich, Attila; Varga, Ákos (szerk.) 67. Növényvédelmi Tudományos Napok konferenciakiadványa: Növényvédelmi Tudományos Napok 2021 (2021) 76 p. p. 62.

Geiger, A; Karácsony, Z; Golen, R; Váczy, KZ; Geml, J. Különböző fajtájú Esca tünetes és tünetmentes szőlők növénypatogén gombaközösségének vizsgálata az Egri borvidéken In: Haltrich, Attila; Varga, Ákos (szerk.) 67. Növényvédelmi Tudományos Napok konferenciakiadványa: Növényvédelmi Tudományos Napok 2021 (2021) 76 p. p. 30

Geiger, A; Karácsony, Z; Golen, R; Váczy, KZ; Geml, J. Esca tüneteit mutató szőlő növénypatogén gombaközösségét befolyásoló tényezők vizsgálata = Investigation Of Factors Influencing The Plant Pathogenic Fungal Community Of Grapevines Showing Symptomss Of Esca In: Bíró, Andrea Barbara; Tuza-Matyus, Judit; Hegyi, Ádám István; Váczy, Kálmán Zoltán (szerk.) Növényegészségügyi jó gyakorlatok az Eszterházy Károly Egyetem szőlő-bor kiválósági központjában: konferencia (2020) pp. 7-8., 2 p.

Literature cited

1. BUTTS, C. T. (2008). Social network analysis with sna. J. Stat. Softw., 24, 1-51.
2. CALLAHAN, B. J., MCMURDIE, P. J., ROSEN, M. J., HAN, A., W., JOHNSON, A. J. A., HOLMES, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. In: Nat. Methods, 13, 581-583.
3. FLEURAT-LESSARD, P., LUINI, E., BERJEAUD, J. M., ROBLIN, G. (2014). Immunological detection of *Phaeoacremonium aleophilum*, a fungal pathogen found in esca disease. In: Eur. J. Plant Pathol., 139, 137-150.
4. GORORDO, M. F., LUCCA, M. E., SANGORRÍN, M. P. (2022). Biocontrol efficacy of the *Vishniacozyma victoriae* in semi-commercial assays for the control of postharvest fungal diseases of organic pears. In: Curr. Microbiol., 79(9), 259.
5. HOFSTETTER, V., BUYCK, B., CROLL, D., VIRET, O., COULOUX, A., GINDRO K. (2012). What if esca disease of grapevine were not a fungal disease? In: Fungal Divers., 4, 51–67. https://doi.org/10.1007/s13225-012-0171-z
6. IHRMARK, K., BÖDEKER, I. T., CRUZ-MARTINEZ, K., FRIBERG, H., KUBARTOVA, A., SCHENCK, J., STRID, Y., STENLID, J., BRANDSTRÖM-DURLING, M., CLEMMENSEN, K.E., LINDAHL, B. D. (2012). New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. In: FEMS microbiology ecology, 82(3), 666-677.
7. LANDI, L., MUROLO, S., ROMANAZZI, G. (2012). Colonization of *Vitis* spp. wood by sGFP-transformed *Phaeomoniella chlamydospora*, a tracheomycotic fungus involved in esca disease. In: Phytopathology, 102(3), 290-297.

8. MOTA LEAL, C., GEIGER, A., MOLNÁR, A., VÁCZY, K. Z., KGOBE, G., ZSÓFI, Z., GEML, J. (2024) Disentangling the effects of terroir, season, and vintage on the grapevine fungal pathobiome. In: Front. Microb., 14, 1322559.
9. NERVA, L., ZANZOTTO, A., GARDIMAN, M., GAIOTTI, F., CHITARRA, W. (2019). Soil microbiome analysis in an ESCA diseased vineyard. In: Soil Biol. Biochem., 135, 60-70.
10. PAPADOPOULOU, E., BEKRIS, F., VASILEIADIS, S., PAPADOPOULOU, K. K., KARPOUZAS, D. G. (2022). Different factors are operative in shaping the epiphytic grapevine microbiome across different geographical scales: Biogeography, cultivar or vintage? In: JSAE., 1, 287-301.
11. SILVA-VALDERRAMA, I., TOAPANTA, D., MICCONO, M. D. L. A., LOLAS, M., DÍAZ, G. A., CANTU, D., CASTRO, A. (2021). Biocontrol potential of grapevine endophytic and rhizospheric fungi against trunk pathogens. In: Front. Microbiol, 11, 614620.
12. STEFANI, F. O., BELL, T. H., MARCHAND, C., DE LA PROVIDENCIA, I. E., EL YASSIMI, A., ST-ARNAUD, M., HIJRI, M. (2015). Culture-dependent and-independent methods capture different microbial community fractions in hydrocarbon-contaminated soils. In: PloS one, 10, e0128272.
13. SWIFT, J. F., HALL, M. E., HARRIS, Z. N., KWASNIEWSKI, M. T., MILLER, A. J. (2021). Grapevine microbiota reflect diversity among compartments and complex interactions within and among root and shoot systems. In: Microorganisms, 9, 92.
14. TEDERSOO, L., BAHRAM, M., PÕLME, S., KÕLJALG, U., YOROU, N. S., WIJESUNDERA, R., RUIZ, L.V., VASCO-PALACIOS, A.M., THU, P.Q., SUIJA, A., SMITH, M.E. (2014). Global diversity and geography of soil fungi. In: Science, 346, 1256688
15. WHITE TJ, BRUNS T., LEE S., TAYLOR J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: MA Innis, DH Gelfand, JJ Sninsky, TJ White (Eds) PCR Protocols: a Guide to Methods and Applications, pp. 315–322. Academic Press, New York.
16. YANG, H. C., RODRIGUEZ-RAMOS, J. C., HALE, L., NAEGELE, R. P. (2023). Grapevine leaf epiphytic fungal and bacterial communities are influenced more by spatial and temporal factors than powdery mildew fungicide spray programs. In: PhytoFrontiers, (ja).
17.