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FILAMENTOUS FUNGI ON GRAPES IN CENTRAL SLOVAK WINE REGION

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ABSTRACT

The concern about filamentous fungi in the vineyards has traditionally been linked to spoilage of grapes due to fungal growth. The aims of this study were to monitor the mycobiota in Central Slovak wine region. The Central Slovak wine region is divided into seven different subregions. In this work we had ten grape samples from seven various wine growing subregions and eight different villages. Five of these samples were from white grape berries and five were from red grape berries. The sample nr. 7 was without chemical protection (interspecific variety) and three samples (nr. 8, 9, 10) were from bio-production. In the samples were determined exogenous contamination (direct platting method) and endogenous contamination (surface-disinfected grapes). The exogenous mycobiota was determined by the method that each sample of 50 grape berries without visible damage was direct plated on to a DRBC agar medium. In exogenous contamination was detected 17 different genera *Alternaria, Arthrinium, Aspergillus, Bipolaris, Botrytis, Cladosporium, Cunninghamella, Epicoccum, Fusarium, Geotrichum, Chaetomium, Mucor, Penicillium, Phoma, Rhizopus, Sordaria, Trichoderma* and group *Mycelia sterilia* in which we included all colony of filamentous fungi that after incubation did not create fruiting bodies necessary for identification to genera level. By the endogenous contamination was each sample of 50 grape berries was surface-disinfected with sodium hypochlorite solution (1%) for 1 min, rinsed in sterile distilled water three times and plated onto a DRBC (Dichloran Rose Bengal Chloramphenicol medium, Merck, Germany). The plates were incubated at 25±1 ºC for 7 days in the dark. By the endogenous plating method was identified 15 different genera from all ten samples *Alternaria, Arthrinium, Aspergillus, Botrytis, Cladosporium, Epicoccum, Fusarium, Geotrichum, Gelasinospora, Chaetomium, Mucor, Penicillium, Phoma, Rhizopus, Trichoderma* and *Mycelia sterilia.*

Keywords: mycobiota, grapes, Central Slowak vine region

INTRODUCTION

Grapevine can be attacked by a number of fungi which affect the berries and cause loss of quality and influence the taste of the wine. Some pathogens directly destroy the fruit tissue enzymtically; others impede ripening, and a number of fungi produce off flavours or myctoxins. Grapevine diseases can spread rapidly under favourable conditions and cause more or less severe epidemics. Besides the pathogenic fungi causing grapevine diseases, berries are also colonized by ubiquitous epiphytic fungi which use sugar and amino acids leaking out of berries as nutrient source **(König *et al*., 2009)**.
Molds are ubiquitous with various genera commonly found on grapes. Common examples include *Aspergillus*, *Botrytis*, and *Penicillium*, and, to a lesser extent, *Phythophthora*, *Moniliella*, *Alternaria*, and *Cladosporium* **(Rosa *et al*., 2002)**.
In general grapevine pathogens can be sub-divided into main pathogens of high economic importance which are pre-dominant, like downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator*) and bunch rot (*Botrytis cinerea*) and those which occur only locally or temporary **(König *et al*., 2009)**.
The concern about filamentous fungi in the vineyard has traditionally been linked to spoilage of grapes due to fungal growth. The main fungus responsible for grape rot is *Botrytis cinerea*, a pathogen that damages the berries and has a detrimental effect on the organoleptic properties. Nevertheless, other saprobic fungi can cause rot in grapes and in addition produce mycotoxins. Two main genera are responsible for mycotoxin production in grapes: *Aspergillus* and *Penicillium*. The mycotoxin production is characteristic of the species and therefore by identifying the species one can predict potential mycotoxin hazards **(Serra *et. al.* 2006)**.
Grapes have a complex microbial ecology including filamentous fungi, yeasts and bacteria with different physiological characteristics and effects upon wine production. Some species are only found in grapes, such as parasitic fungi and environmental bacteria, while others have the ability to survive and grow in wines, constituting the wine microbial consortium **(Barata *et al*., 2012)**.
Mold growth plays an important role in the physical and chemical stability as well as the sensory properties of the future wine. For example, uncont rolled proliferat ion of mold on grapes just prior to harvest rapidly leads to growth of secondary contaminants (yeasts and bacteria), which, in turn, leads to a deteriorative state called "rot" **(Fugelsang and Edwards, 2007)**.
The aim of this work was to evaluate the mycobiota of healthy Slovak grapes from Central Slovak wine region destined for commercial winemaking at harvest time.

MATERIAL AND METHODS

Grape sampling

From each vineyard grape bunches apparently healthy, without visible fungal rot, were collected randomly diagonal transects. Grapes were transported to the laboratory in closed plastic bags in refrigerated boxes. The time between sample collection and laboratory analysis was less than one day.

Isolation of mycobiota

Exogenous fungal contamination

Exogenous fungal contamination was making by the method adapted from **Serra *et al.*** **(2006)**. Totally were tested 10 samples of the grape. Each sample of 50 grape berries without visible damage was direct plated on to a DRBC (Dichloran Rose Bengal Chloramphenicol medium, Merck, Germany) and incubated at 25±1 °C for 7 days in the dark (7-8 berries per bunch in each plate).

Endogenous fungal contamination

Endogenous fungal contamination was making by the method adapted from **Samson *et al.* (2002)**. Each sample of 50 grape berries was surface-disinfected with sodium hypochlorite solution (1%) for 1 min, rinsed in sterile distilled water three times and plated onto a DRBC (Dichloran Rose Bengal Chloramphenicol medium, Merck, Germany). The plates were incubated at 25±1 ºC for 7 days in the dark.

Identification of fungi

The spore-producing filamentous fungi detected were identified to genus level. As cultivation medium MEA (Malt Extract Agar) (**Tančinová *et al.*, 2012**) was used and cultivation at 25±1°C, 5-7 days in darkness was done. The identification of isolated strains was carried out according to special mycological literature by **Tančinová *et al.* (2012)**, **Pitt and Hocking (2009)**, **Samson and Pitt (2000)**, **Ramirez (1982).**

Statistics methods

The incidence of microscopic fungi in the samples can be expressed by means of frequency (Fr) and relative density (RD). Frequency and relative density of genera and species is calculated according to the following formulas **(González *et al.*, 1999)**:

$Fr(\%) = (ns/N) \times 100$ $RD(\%) = (ni/Ni) \times 100$

ns - number of samples in which the genus or species is detected, N - total number of samples, ni - number of isolates of the genus or species, Ni - total number of detected isolates.

RESULTS AND DISCUSSION

Vineyards

The Central Slovak wine region is area planted with grapevine, this area is divided into seven winegrowing subregions. These subregions are divided into villages which should have the same soil and climatic conditions for growing vines. The location of the selected vineyard and the grape varieties studied are listed in table 1.

Table 1 Collection point

Nr.	VILLAGE	SUBREGION	VARIETY	DATE OF ANALYSIS
1.	Vinica	Vinický	Blaufränkisch	1.10.2012
2.	Šahy	Ipeľský	Pearl of Zala	1.10.2012
3.	Sebechleby	Hontiansky	Saint Laurent	1.10.2012
4.	Čamovce	Fiľakovský	Pálava	17.10.2012
5.	Rimavská Sobota	Gemerský	Blaufränkisch	17.10.2012
6.	Kráľ	Tornaľský	Müller Thurgau	17.10.2012
7.	Hont. Moravce	Hontiansky	Konkordia	27.9.2011
8.	Veľký Krtíš	Modrokamenský	Pinot gris	17.10.2011
9.	Veľký Krtíš	Modrokamenský	Pinot noir	17.10.2011
10.	Veľký Krtíš	Modrokamenský	Sauvignon	17.10.2011

The geographical origin of grapes did not significantly influence the incidence of field fungi, but had a significant effect in the spoilage population, namely in *Aspergillus niger*, *Botrytis cinerea* and *Penicillium* species (**Serra *et al.*, 2006**).

Exogenous fungal contamination

The filamentous fungi identified from Central Slovak Wine region in years 2011 and 2012 by the exogenous fungal contamination are indicated in table 2. By the direct plating method was identified 17 different genera from all ten samples. The 5 most abundant genera found by descending order were *Alternaria*, *Cladosporium*, *Penicillium*, *Epicoccum* and *Fusarium*. Representatives of genera *Aspergillus* and *Penicillium* were in each sample.

Table 2 Fungi identified by exogenous contamination in Central Slovak wine region

Genera/Nr. of sample	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Alternaria	71*	85	75	118		78	17	61	55	64
Arthrinium			1			1			1	
Aspergillus	5	9	12	10	3	6	1	2	5	3
Bipolaris										1
Botrytis						3		15	4	8
Cladosporium	9	55	60	95		27	36	25	51	14
Cunninghamella										1
Epicoccum	4	8	10	2		1	10	6	18	6
Fusarium	6	8	11	6		5	16	2	3	1
Geotrichum					3					
Chaetomium										1
Mucor							4			
Mycelia sterilia	2	2	1	3			1	18	15	3
Penicillium	11	8	12	30	79	141	6	8	21	4
Phoma	2	1					1			
Rhizopus	11	9	11	3		1	4	3	3	8
Sordaria									1	
Trichoderma	2	2	2	1			5	3		

*-number (quantity) of colony forming units (CFU) total count per 50 grape berries

Only two genera *Aspergillus* and *Penicillium* had 100% frequency of occurrence (table 3). *Alternaria*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Rhizopus* and *Trichoderma* had frequency of occurrence more than 50% and other detected genera *Arthrinium*, *Bipolaris*, *Botrytis*, *Cunninghamella*, *Geotrichum*, *Chaetomium*, *Mucor*, *Phoma* and *Sordaria* had less than 50% of occurrence. The three genera with highest relative density were *Alternaria* (37.68%), *Cladosporium* (22.46%) and *Penicillium* (19.32%), four genera *Aspergillus*, *Epicoccum*, *Fusarium* and *Rhizopus* had relative density between 2 – 4% and all other genus had relative density less than 2% (Table 3).

Table 3 The frequency of occurrence and relative density of each genera by exogenous contamination

Genera	Frequency of occurrence (Fr)	Relative density (RD)
Alternaria	90%	37.68%
Arthrinium	30%	0.18%
Aspergillus	100%	3.38%
Bipolaris	10%	0.06%
Botrytis	40%	1.81%
Cladosporium	90%	22.46%
Cunninghamella	10%	0.06%
Epicoccum	90%	3.93%
Fusarium	90%	3.50%
Geotrichum	10%	0.18%
Chaetomium	10%	0.06%
Mucor	10%	0.24%
Mycelia sterilia	80%	2.72%
Penicillium	100%	19.32%
Phoma	30%	0.24%
Rhizopus	90%	3.20%
Sordaria	10%	0.06%
Trichoderma	60%	0.91%

In a general way, as maturation advances, the incidence of field fungi (e.g., *Alternaria*, *Epicoccum*, *Fusarium*, *Stemphyliumand* and *Ulocladium*) decreases, while active spoilage agents such as *Aspergillus*, *Penicillium* and *Rhizopus* increase in grapes. Conditions are more favourable for fungal invasion at harvest time, when more damage to the berries is likely to occur (**Serra *et al.*, 2005**).

Endogenous fungal contamination

By the endogenous (surface-disinfected) plating method were identified 15 different genera from all ten samples (Table 4). The 3 most abundant genera found by descending order were *Alternaria*, *Penicillium* and *Cladosporium*. Only representatives of genus *Alternaria* was in each sample.

Table 4 Fungi identified by endogenous contamination in Central Slovak wine region

Genera/Nr. of sample	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Alternaria	54*	64	53	72	52	33	20	66	56	63
Arthrinium		1								2
Aspergillus	4		2	1	1					
Botrytis	1			1		8		7	8	7
Cladosporium	23	20	12	28		8	19	17	5	17
Epicoccum	5		2					5	4	10
Fusarium	1	8	2	11	4	5	10	1	2	
Geotrichum					3					
Gelasinospora								1	2	
Chaetomium										1
Mucor							1			
Mycelia sterilia	4	1		3			11	10	2	2
Penicillium	3	6	4	9	150	51	1			2
Phoma		1								
Rhizopus	7	1		1	3	1	9			
Trichoderma	3			2	1			1	3	

*-number (quantity) of colony forming units (CFU) total count per 50 grape berries

The genus with 100% frequency of occurrence in endogenous contamination of grapes was only *Alternaria*, followed by *Cladosporium* and *Fusarium* with 90% of frequency of occurrence.
The three genera with highest relative density were *Alternaria* (48.68%), *Penicillium* (20.64%) and *Cladosporium* (13.61%), four genera *Fusarium, Botrytis, Epicoccum*, and *Rhizopus* had relative density between 1–5% (Table 5). All other genera had relative density less than 1%, *Arthrinium* 0.27%, *Aspergillus* 0.73%, *Geotrichum* 0.27%, *Gelasinospora* 0.27%, *Chaetomium* 0.09%, *Mucor* 0.09%, *Phoma* 0.09% and *Trichoderma* 0.91%.

Table 5 The frequency of occurrence and relative density of each genera by endogenous contamination

Genera	Frequency of occurrence (Fr)	Relative density (RD)
Alternaria	100%	48.68%
Arthrinium	20%	0.27%
Aspergillus	40%	0.73%
Botrytis	60%	2.92%
Cladosporium	90%	13.61%
Epicoccum	50%	2.37%
Fusarium	90%	4.02%
Geotrichum	10%	0.27%
Gelasinospora	20%	0.27%
Chaetomium	10%	0.09%
Mucor	10%	0.09%
Mycelia sterilia	70%	3.01%
Penicillium	80%	20.64%
Phoma	10%	0.09%
Rhizopus	60%	2.01%
Trichoderma	50%	0.91%

Comparison of our results with results from Spain shows a higher occurrence of *Aspergillus* spp., while the percentage of berries contaminated by nonochratoxin A (OTA) producing species such as *Alternaria* spp. And *Cladosporium* spp. is lower. *Penicillium verrucosum* and *P. nordicum*, the only confirmed *Penicillium* species that are able to produce OTA, were not isolated **(Bau *et al.* 2005)**.
The most commonly isolated fungal genera were *Alternaria*, with a decreasing percentage from setting to harvest, yeasts and *Aspergillus*, with increasing percentages from setting to harvest, and *Cladosporium*, *Rhizopus*, and *Penicillium*. Isolates belonging to the following genera: *Arthrinium*, *Botrytis*, *Dreschlera*, *Epicoccum*, *Fusarium*, *Humicola*, *Phoma*, *Staphylocotrichum*, *Trichoderma*, and *Ulocladium*, were occasionally isolated from the grapes **(Bellí *et. al.*, 2006)**.
The highest percentage (60%) of contaminated grape samples was found in the black seedless variety and the lowest (29%) was present in the black seeded. The levels of contamination (percent of contaminated berries per sample) ranged between 0–33% in black grapes and 0–80% in green seedless grapes. The mean contamination level, however, was relatively low ranging from 5% in black seeded and 15% in green seedless grapes. Very low contamination level of black (especially of black seeded) grapes can probably be explained by the fact that these varieties possess very hard, difficult to break skin, whereas the higher sensitivity of the green seedless grapes to fungal contamination could be due to their thinner, easier to invade epidermis **(Tournas *et al.*, 2005)**.

CONCLUSION

The Central Slovak wine region is divided into seven different subregions. In this work we had 10 grape samples from seven various wine growing subregions and eight different villages. Five of these samples were from white grape berries and five were from red grape berries. The sample number seven was without chemical protection (interspecific variety) and samples number eight; nine and ten were from bio–production.
In exogenous contamination was detected 17 different genera *Alternaria, Arthrinium, Aspergillus, Bipolaris, Botrytis, Cladosporium, Cunninghamella, Epicoccum, Fusarium, Geotrichum, Chaetomium, Mucor, Penicillium, Phoma, Rhizopus, Sordaria, Trichoderma* and group *Mycelia sterilia* in which we included all colony of filamentous fungi that after incubation did not create Fruiting bodies necessary for identification to genus level.
By the endogenous (surface-disinfected) plating method was identified 15 different genera from all ten samples *Alternaria, Arthrinium, Aspergillus, Botrytis, Cladosporium, Epicoccum, Fusarium, Geotrichum, Gelasinospora, Chaetomium, Mucor, Penicillium, Phoma, Rhizopus, Trichoderma* and *Mycelia sterilia.*
The most frequent genus was *Alternaria,* genera whit higher concentration were *Cladosporium* and *Penicillium* both of them in exogenous and endogenous contamination.

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