

## MICROSCOPIC FILAMENTOUS FUNGI OCCURRENCE IN PLANT POLLEN FROM NONTRADITIONAL PLANT SPECIES

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### ABSTRACT

This is the first report about the occurrence of microscopic filamentous fungi in plant pollen. In our study was analyzed plant pollen of dogwood common (*Cornus mas*), ray mountain (*Secale strictum* spp. *strictum*), pumpkin rape (*Cucurbita pepo* var. *styriaca*) and vines (*Vitis vinifera*). Thirty two samples of plant collected pollen were gathered from trees and plants. The samples were examined for the concentration and identification of microscopic fungi able to grow on Malt and Czapek-Dox agar. The microscopic fungi isolated from dogwood common pollen ranged from 3.12 to 4.93 log CFU.g<sup>-1</sup>. The microscopic fungi isolated from ray mountain pollen ranged from 0.00 to 3.51 log CFU.g<sup>-1</sup>. The number of microscopic fungi from pumpkin rape pollen ranged from 0.00 to 3.00 log CFU.g<sup>-1</sup> and in vines pollen ranged from 2.52 to 4.34 log CFU.g<sup>-1</sup>. In plant pollen samples we found the representation of 7 genera and 19 species of microscopic fungi. From plant pollen samples, 138 isolates were recovered. The most frequently species in the plant pollen samples was *Alternaria alternata* (19 isolates), *Mucor racemosus* (11 isolates), *Mucor mucedo* (33 isolates) and *Cladosporium cladosporoides* (10 isolates).

**Keywords:** Plant pollen, nontraditional plant species, microscopic filamentous fungi

### INTRODUCTION

In plants reproduction, pollen grains carry the sperm cells to the pistil, ultimately to the ovules through the pollen tubes, to achieve fertilization. Successful fertilization is a key factor for many crop species. Among the different aspects related to pollen fertility and functioning, the relevance of the carbohydrates metabolism has been demonstrated by a number of studies (Pressman *et al.*, 2002; Firon *et al.*, 2006; Nashilevitz *et al.*, 2009). Although the male function can vary naturally along the plant life, especially in perennials (Bellani *et al.*, 1985a,b; Dag *et al.*, 2000), the studies of the carbohydrates metabolism in pollen have been generally done in standardized conditions, and eventual natural variations have been overlooked. Regarding soluble carbohydrates, even if they can be variable among species (Aloni *et al.*, 2001; Castro and Clément, 2007), it has not yet been investigated if the soluble carbohydrates content in the pollen of a species is constant or if it can fluctuate along the blooming period.

Pollen is a rich source of vitamins, especially the water-soluble B vitamins that have a vital role in honeybee larval nutrition. The following vitamins have been found in pollens: pro-vitamin A, thiamine, ascorbic acid, cyanocobalamin, pyridoxine, biotin, niacin, riboflavin, folic acid, and pantothenic acid. Pantothenic acid is of particular interest since it occurs in royal jelly in concentrations about 17 times the average value found in pollen found a positive correlation between the loss of ascorbic acid with age of the pollen and lowered nutritional value. They concluded that the autooxidation of ascorbic acid may cause it to be one of the most limiting vitamins in honeybee nutrition. In contrast, found that ascorbic acid did not decrease in concentration in the larval food produced by nurse bees of differing ages fed different diets (Loper *et al.*, 1980). Fungi colonize the terrestrial environment successfully and utilize solid substrates efficiently by growing over their surfaces and penetrating into their matrices. Many fungi produce mycotoxins that can cause acute or chronic intoxication and damage to humans and animals after ingestion of contaminated food and feed (Marasas and Nelson, 1987; Moss, 1996). Among the mycotoxins, aflatoxins and ochratoxin A (OTA) occupy especial places due to its high occurrence and toxicity. The European Commission has established maximum allowable limits for these toxins in some food products (Commission Regulation, 2001, 2002a,b, 2005).

Taking into account that plant pollen is collected in countries with moderate/cold temperate and humid climates during flowering season the major aims of our research were the evaluation of the mycobiota occurring in plant pollen. This is the first report about the occurrence of microscopic filamentous fungi in bee pollen.

### MATERIAL AND METHODS

#### Biological material

In our study was analyzed plant pollen of dogwood common (*Cornus mas*, n=3), ray mountain (*Secale strictum* spp. *strictum*, n=12), pumpkin rape (*Cucurbita pepo* var. *styriaca*, n=10) and vines (*Vitis vinifera*, n=7). Thirty two samples of plant collected pollen were gathered from trees. There were respected qualitative criteria for gathering, drying and storage of plant pollen:

- Plant pollen was obtained from selected trees.
- The pollen was collected by special pollen traps.
- Pollen was harvested daily and in the shortest time placed to a freezer (-18 to -20 °C) for prevention of spoilage and for preservation of a maximum quality.
- Purification of frozen pollen pellets from different impurities was done most efficiently by air with special constructed purifier.
- The frozen and purified pollen was dried as the gentlest way as possible to keep high nutritional value of pollen. Firstly, pollen was defrosted 2 – 3 hours in room conditions. Time of drying in a drying-oven was 6 – 8 hours. The maximum temperature was 35-40 °C. The pollen was dried until humidity was 10 – 11%.
- Dried pollen was stored in cool conditions (around 8°C), in sterilized containers.
- During all stages of manipulation with pollen were kept as sterilized conditions as possible to avoid contamination.

#### Isolation and morphological characterization of fungi

For determination of fungi colony-forming units (CFU) was 1 g of plant pollen samples soaked in 99 ml sterile tap-water containing 0.02% Tween 80 and then

30 min. shaken. Dilutions were prepared (from  $10^{-2}$  to  $10^{-5}$ ) in sterile tap-water with 0.02 % Tween 80 and 1 mL aliquots were inoculated on each of three plates of Czapek-Dox agar with streptomycin (to inhibit the bacterial growth). Petri dishes were inoculated using the spread-plate technique and incubated at 25 °C in the dark. Total fungi CFU.g<sup>-1</sup> counts in samples were determined after 5 days of incubation. Malt agar (MA) and Czapek-Dox agar (CDA) were used to isolate and identify individual genera and species. Incubation was carried out at 25 °C for 5-10 days. After isolation, or in the some cases monosporic isolation, individual species were identified on the basis of their macro- and micromorphology in accordance with other scientific reports (de Hoog et al., 2000; Klich et al., 2002; Samson et al., 2002).

**RESULTS AND DISCUSSION**

The microscopic fungi in different plant pollen are shown in figure 1-4. The microscopic fungi isolated from dogwood common pollen ranged from 3.12 to 4.93 log CFU.g<sup>-1</sup>. The microscopic fungi isolated from ray mountain pollen ranged from 0.00 to 3.51 log CFU.g<sup>-1</sup>. The number of microscopic fungi from pumpkin rape pollen ranged from 0.00 to 3.00 log CFU.g<sup>-1</sup> and in vines pollen ranged from 2.52 to 4.34 log CFU.g<sup>-1</sup>.

The total number of microscopic fungi in study Kačaniová et al. (2011) ranged from  $2.98 \pm 0.02$  in frozen sunflower bee pollen to  $4.06 \pm 0.10$  log cfu.g<sup>-1</sup> in sunflower bee pollen after UV radiation. In this study, 449 isolates belonging to 21 fungal species representing 9 genera were found in 45 samples of bee pollen. The total isolates were detected in frozen poppy pollen 29, rape pollen 40, sunflower pollen 80, in dried poppy pollen 12, rape pollen 36, sunflower 78, in poppy pollen after UV radiation treatment 54, rape 59 and sunflower 58. The most frequent isolates of microscopic fungi found in bee pollen samples of all prevalent species were *Mucor mucedo* (49 isolates), *Alternaria alternata* (40 isolates), *Mucor hiemalis* (40 isolates), *Aspergillus fumigatus* (33 isolates) and *Cladosporium cladosporioides* (31 isolates). The most frequently found isolates were detected in sunflower bee pollen frozen (80 isolates) and the lowest number of isolates was observed in poppy bee pollen dried (12 isolates).

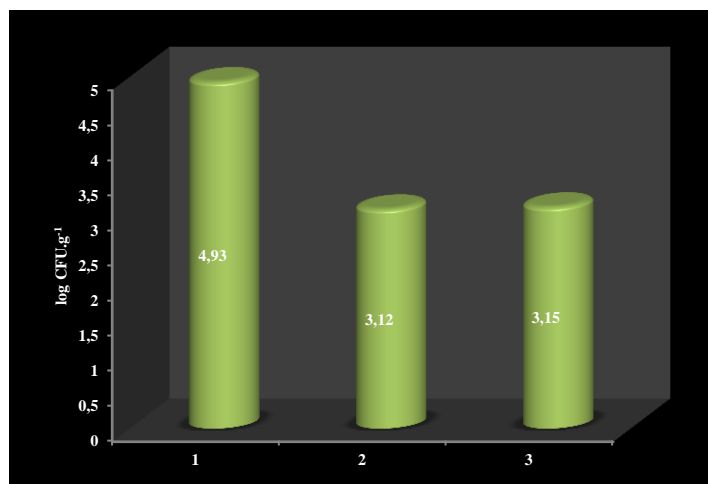


Figure 1 The number of microscopic fungi from dogwood common (DC) pollen

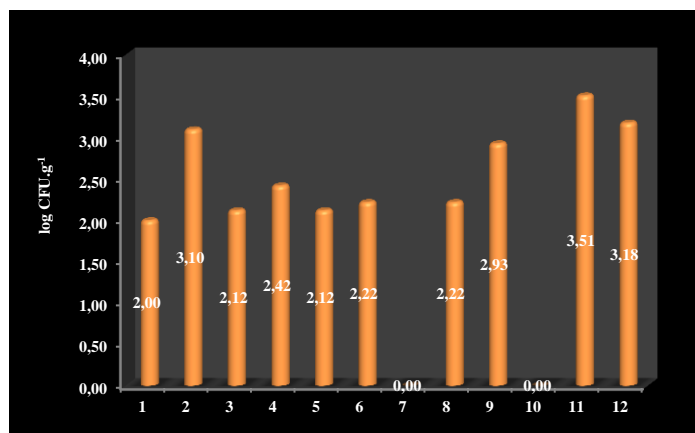


Figure 2 The number of microscopic fungi from ray mountain (RM) pollen

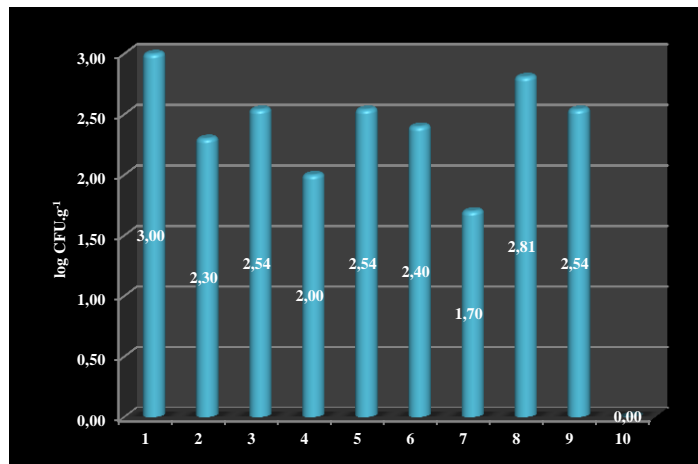


Figure 3 The number of microscopic fungi from pumpkin rape (PR) pollen

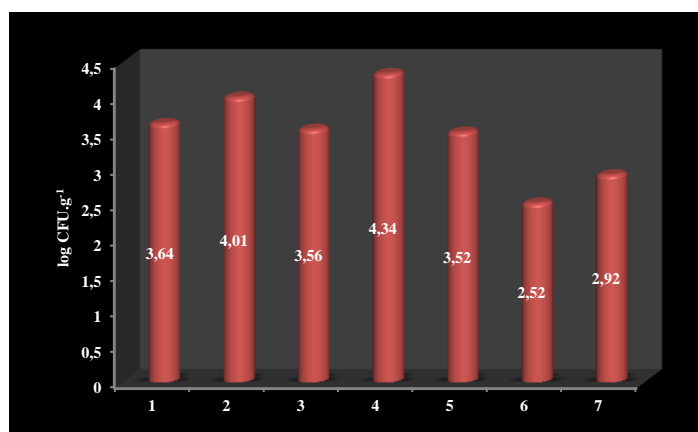


Figure 4 The number of microscopic fungi from vines (V) pollen

The results obtained with regard to the occurrence of the different fungal species in plant pollen samples and the number of positive samples are shown in Table 1. In plant pollen samples we found the representation of 7 genera and 19 species of microscopic fungi. From plant pollen samples, 138 isolates were recovered. The most frequently species in the plant pollen samples was *Alternaria alternata* (19 isolates), *Mucor racemosus* (11 isolates), *Mucor mucedo* (33 isolates) and *Cladosporium cladosporioides* (10 isolates).

In the study Brindza et al. (2010) were identified 21 species of 13 genera of microscopic fungi totally in pollen samples. Most often there occurred species of *Mucor*, *Fusarium* (*Fusarium* sp.), *Rhizopus* (*Rhizopus arrhizus* A. Fischer, *Rhizopus nigricans* Ehrenb.) and *Aspergillus* (*Aspergillus clavipes*, *Aspergillus repens* (Corda) De Bary, *Aspergillus clavatus* Desm.). Over 62 % of the isolates were identified as species of *Mucor*, *Aspergillus*, *Alternaria*, *Rhizopus* and *Paecilomyces* genera.

Gilliam et al. (1989) study reported, the highest share belonged to two genera – *Mucor* and *Aspergillus*, while differently from the above cited authors on lower level was represented the genus *Penicillium*. Moulds detected on the pollen samples represent relative low number of species compared to other reports cited in the following sentence. Gilliam et al. (1989) isolated 148 different moulds from the almond pollen, Gonzales et al. (2010) found 116 fungal isolates in mixed pollen sample. Great majority of moulds isolated from pollen represented the fungal group of saprophytic microorganisms inhabiting soil and organic residues of plants indicating the provenience of these microorganisms from the microenvironment. This statement is supported by the fact reported by Burri (1947), that pollen is germ-free in blossoms that have not opened or in opened blossoms if uncontaminated by insects or wind. It was shown that honey and pollen left at ambient temperature for longer time could be overgrown by microorganisms when the air is humid (Lacey and Magan, 1991; Kačaniová et al., 2009).

**Table 1** Occurrence of fungi in plant pollen and number of isolates

Species of fungi	Type of plant pollen			
	DC	RM	PR	V
<i>Alternaria alternata</i> (Fr.) Keissl.	+ (5)	+ (2)	+ (4)	+ (8)
<i>Aspergillus clavatus</i>	+ (4)	+ (1)	-	-
<i>A. flavus</i> Link	+ (3)	-	+ (2)	+ (3)
<i>A. fumigatus</i> Fresen.	+ (2)	-	-	+ (5)
<i>A. niger</i> Tiegh.	+ (3)	+ (1)	-	-
<i>A. ochraceus</i> G. Wilh.	+ (2)	-	+ (2)	+ (2)
<i>A. parasiticus</i> Speare	+ (3)	+ (1)	-	+ (3)
<i>A. terreus</i> Thom	-	-	+ (1)	+ (2)
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	+ (3)	+ (2)	+ (2)	+ (3)
<i>Fusarium graminearum</i> Schwabe	+ (2)	-	+ (2)	+ (3)
<i>F. moniliforme</i> J. Sheld.	+ (1)	+ (1)	+ (4)	+ (3)
<i>F. oxysporum</i> E.F. Sm. & Swingle.	+ (2)	+ (1)	-	-
<i>Fusarium</i> sp.	-	-	+ (3)	+ (2)
<i>Mucor hiemalis</i> Wehmer	+ (2)	+ (1)	+ (1)	+ (2)
<i>M.ucedo</i> de Bary & Woron.	+ (3)	+ (1)	+ (6)	-
<i>M. racemosus</i> Bull.	+ (1)	+ (1)	+ (9)	-
<i>Rhizopus stolonifer</i> (Ehrenb.) Vuill.	+ (4)	+ (1)	-	-
<i>Penicillium</i> sp.	+ (1)	-	+ (2)	+ (2)
<i>Penicillium verrucosum</i> Dierck	+ (1)	+ (1)	+ (4)	+ (2)
<b>Total isolates</b>	<b>42</b>	<b>14</b>	<b>42</b>	<b>40</b>

**Legend:** DC – dogwood pollen, RM – ray mountain pollen, PR – pumpkin rape pollen, V – vines pollen

## CONCLUSION

Taking into account the food safety aspects, there are coming into question many possible interactions of different importance between microorganisms - plant pollen - bee nutrition - pollination - plant production for food and feed. It is generally accepted, that the human survival is tightly bound with the global cycles of elements enabling the restoration of substrates for synthesis of essential compounds, thus keeping vital processes in progress. Concerning the nutritional aspects, there are several components fulfilling the well-being of mankind including the interaction microorganisms – plants - animals - man as well.

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