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Microscopic fungi and other contaminants on airborne pollen grains of ragweed (*Ambrosia artemisiifolia* L.)

D. Magyar · **B. Krasznai** · **M. D. Tóth**

Abstract Fungal particles were observed on the pollen grains of ragweed (*Ambrosia artemisiifolia* L.) in air samples collected in Nyíregyháza, Hungary. Microscopical observations showed the colonization of pollen grains by different fungal taxa. Concentration data were correlated with meteorological factors, day of the year (DOY), PM_{10}, $PM_{2.5}$, and spore levels. Pollen grains infected by fungi became common at the end of the pollen season. These particles correlated positively with wind speed and airborne spores, but a negative correlation was found with temperature. Pollen grains were most frequently infected with *Cladosporium* spp., but other fungi, such as *Alternaria*, *Aspergillus/Penicillium*, and yeasts were also found. A source of infected pollen grains was proven to be the plants' surface, where fungi colonized pollen grains, and subsequently, they were aerosolized by wind. Our results indicate that reaerosolization events can be identified by the closer examination of fungi found on pollen grains.

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1 Introduction

Common ragweed (*Ambrosia artemisiifolia* L.) is one of the most important allergenic and invasive weeds (Kazinczi et al., 2008; Mányoki et al., 2014). This plant is the second most common weed on arable lands in Hungary, especially on sunflower, maize and soybean fields (Novák et al., 2020), but it also invades a broad range of other habitats, like disturbed areas (waste lands, building operations), linear constructions (along road- and railway-sides), and riverbanks. This plant is dominant on haplic cambisols, sandy soils and on fluvisols (Kazinczi et al., 2008).

In Europe, the highest ragweed pollen counts were reported at the Carpathian Basin, in Serbia and Hungary. Amongst the monitoring stations working there, the highest concentrations were often measured in Nyíregyháza (Apatini et al., 2009).

Pollutants could exacerbate the allergenic effect of pollen grains (Behrendt et al., 1997). Particulate matter can affect allergy and asthma by adhesion onto pollen grains and formation of pollen-particle complexes (Schiavoni et al., 2017). Particles adhered on pollen grains could uptake proteins from pollen grains and interact with allergens (Solomon, 2002). In addition to health effects, the adhesion of particles on pollen grains may provoke a decrease in germination capability of pollen (Sénéchal et al., 2015). The deposition of pollutants on pollen grains can be explained by different processes (Visez et al., 2020). Pollen might be polluted directly on plants during

dehiscence. Airborne pollen grains and pollutants may collide during pollination. Once deposited on vegetation or soil, pollen may be polluted by particles deposited on it. However, particles can be deposited onto the surface of previously impacted pollen during sampling [this case is discussed in detail as air sampling artefact by Choël et al. (2020)]. Pollutants such as dust particles, bacteria and fungal spores were often observed on pollen grains during our routine aerobiological monitoring in Hungary. We hypothesize that these fungi—at least some of them—occur naturally on pollen grains and not as artefacts of air samplings.

It is known that fungi naturally occur on pollen grains, especially in environments with low nitrogen and phosphorus, where nutrients derived from pollen grains could be utilized by fungi (Stark, 1972; Wurzbacher et al., 2014). Pollen parasitism is known in many fungi, including Chytridiomycota, basidiomycetes and hyphomycetes (Goldstein, 1960; Chou & Preece, 1968; Warren, 1972; Hutchison & Barron, 1997; Huang et al., 1999; Rodrigues Marques et al., 2013). Apparently, some fungi are specialized to colonize pollen grains (*Hexacladium, Mycoceros*, *Rhizophidium* and *Retiarius*, see Olivier, 1978, 1983; Magyar et al., 2017a, 2017b; Skvarla & Anderegg, 1972), while others can grow non-exclusively on pollen but many different substrates as well. In this latter group, there are a number of different species of saprotrophic Basidiomycota (e.g. *Coprinus*, *Fomes* and *Pleurotus* spp., Hutchison & Barron, 1997). Some members of the non-specialized pollen-consuming fungi belong to well-known human- and plant pathogenic species such as *Botryis cinerea* (Kocsis et al., 2020), *Claviceps purpurea* (Williams & Colotelo, 1975) and *Fusarium verticillioides* (Duncan & Howard, 2010). During our filed studies in ragweed populations in Hungary, colonization of pollen grains with *Alternaria* and *Cladosporium* was observed (Magyar, 2010; Tóth & Burján, 2014; Tóth et al., 2009, 2014). As these fungi are well-known allergens (Kurup, 2003), and major constitutes of bioaerosol in Hungary (Grewling et al., 2019) co-exposure with pollen grains may have consequences on human health.

Our aim is to study fungi occurring on airborne ragweed pollen, the effect of meteorological factors on their concentration and seasonality, and compare their occurrence with other pollutants found on pollen grains.

2 Materials and methods

2.1 Outdoor air sampling

A 7-day recording Hirst-type air sampler (Hirst, 1952, Burkard 7-day recording volumetric spore trap, Burkard Manufacturing Co. Ltd. Rickmansworth, Hertfordshire, England) was used to record the daily concentration of airborne ragweed pollen. The samples were collected in 2016–2018. The sampling period was determined to cover pollen exposure times according to the recommendations of the European Academy of Allergy and Clinical Immunology (EAACI) (Pfaar et al., 2017). The sampler was located in Nyíregyháza (North-East Hungary in the northern Plain region, with a population of 116 799), placed at a height of 15 m on the top of a building in the city centre (4400 Nyíregyháza Árok street 41), aspirating in air at a rate of 10 l/min (Fig. 1). Airborne pollen grains and fungal spores were impacted on a tape (MELINEX tape) coated with a thin adhesive layer (Vaseline). The greased tape was mounted on a rotating drum within the trap, rotating at a 2 mm/h. The exposed tape was removed weekly and cut into 48-mm segments, thus representing 24-h periods. The segments were placed on microscopic slides and stained with basic fuchsine in mounting medium. To count pollen grains, two longitudinal transverses along the length of the slide were scanned (6–6 mm from the edge of the sample), at 200× magnification using a Leica BZ01 microscope. The annual pollen integral was calculated according to Galán et al. (2017). Pollen grains covered with large-sized (approx. > 5 μm) black and brown particles were counted separately. Black particles were defined as carbonaceous particles, forming soot clusters on pollen grains (Fig. 2a). Brown particles were amorphous, colloidal, soil-like particles often embedding pollen grains (Fig. 2b). Observations of other types of pollutants were also documented on microphotographs.

Airborne pollen grains showing signs of fungal contamination were meticulously analysed under 500× magnification (Fig. 2c–f), to observe the different types of colonization. Thick-walled and melanized hyphae growing on or around the surface of the pollen grains were considered as a case when colonization was taking place before air sampling. Intensive melanization and development of thick wall is a common feature of fungi growing on habitats exposed to
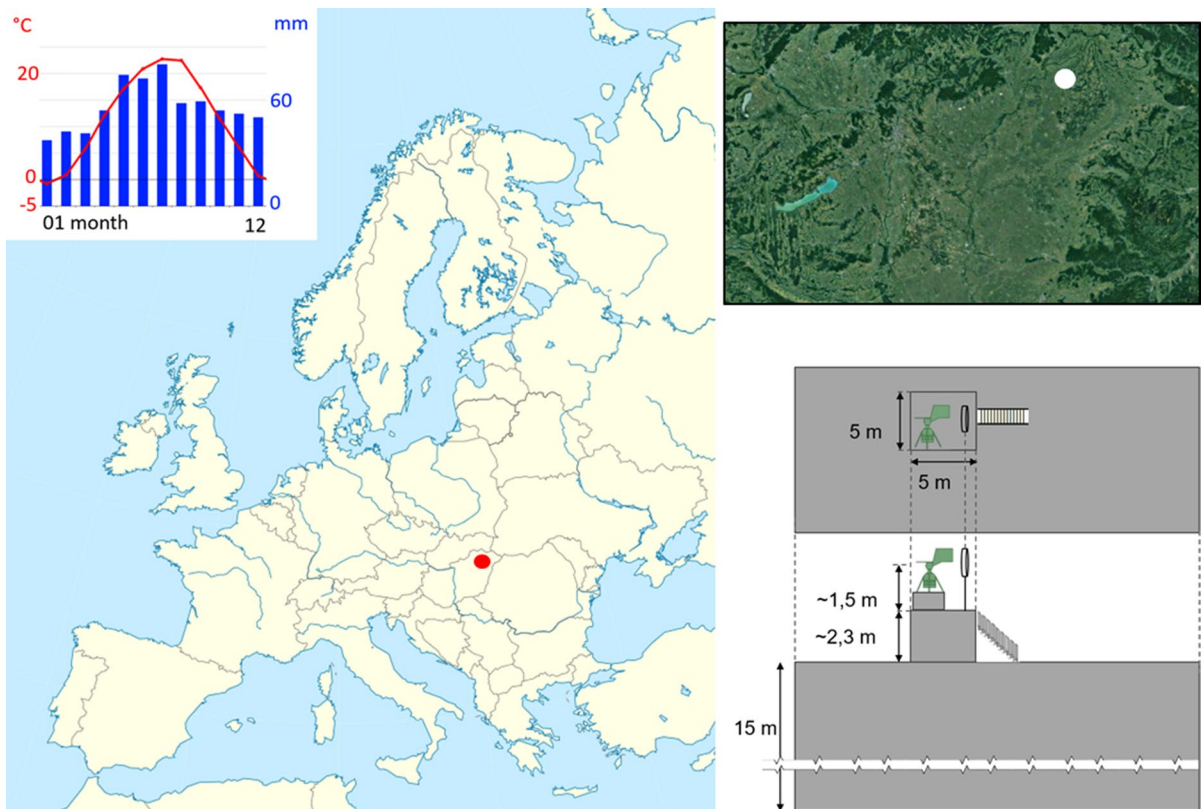
Fig. 1 Location of the aerobiological monitoring station in Nyíregyháza, Hungary. Climate data are from Climate-Data.org, collected between 1999 and 2019. Maps were modified from Wikipedia and Google Maps

ultraviolet radiation (Fuller et al., 2015). These particles were counted and included in the further statistical analysis (Fig. 2c, d). In some cases, spores tend to germinate after impaction on the Melinex tape, in humid conditions (Groth, 1996). Germ tubes originating from these spores show distinct morphology, being long, hyaline and thin-walled. These cases were excluded from the analysis, even if some of the spores were found closely to pollen grains, and their germ tubes grown towards and into a pollen grain (Fig. 2e). Long, thin-walled and hyaline germ tubes hardly remain intact during aerial transport; instead, they break or bend. Spores attached on pollen surface without hyphal growth were not accepted as a case of fungal colonization of pollen, as it may be a result of the co-deposition of spores and pollen (Fig. 2f).

Because fungi parasitizing pollen grains belonged mostly to the genus *Alternaria* and *Cladosporium*, airborne concentration of free conidia belonging to these genera was quantified. The spores were counted

in two 0.25×0.25 mm at twelve transverse traverses then summarized and multiplied by a factor (32) to obtain the daily concentration of spores per cubic meter of air. The presence of fungi on pollen grains other than ragweed was also detected, but not quantified.

2.2 Surface sampling

To test our hypothesis that pollen grains may be parasitized by fungi before aerosolization, deposited pollen grains were collected from aerial surfaces of ragweed plants (leaves, stems, calix and corolla) with pressure sensitive acrylic strips (MACbond B 1200, MACtac Europe S.A., Brussels). This sampling method enables direct observation of bioaerosol depositions (Magyar, 2008). The strips were placed onto a microscopic slide smeared with glycerine, then stained with Lacto-Cotton Blue (0.1 g

Fig. 2 Particle types associated with ragweed pollen (rp): **a**: black particles on airborne rp; **b**: brown particles on airborne rp; **c**: melanized hyphae on airborne rp; **d**: *Alternaria* on airborne rp. Signs of fungal colonization occurring before aerosolization: melanized hypha originating from the spore (*) which grows on the pollen surface (+); **e**: *Cladosporium* spore germinating after air sampling, near rp; **f**: *Cladosporium* spores deposited on and around rp in an air sample, without any sign of colonization. **g–i**: fungi growing on rp grains on plant surface; **g**: *Alternaria*; **h**, **i**: *Cladosporium*; **j**: fungi growing on airborne plant tissue with rp grains on it. **a–h**, **j**: light microphotographs, pollen coloured with basic fuchsine; **b** and **d**: fungi coloured with cotton blue; **i**: SEM photo. Bar = 20 μm
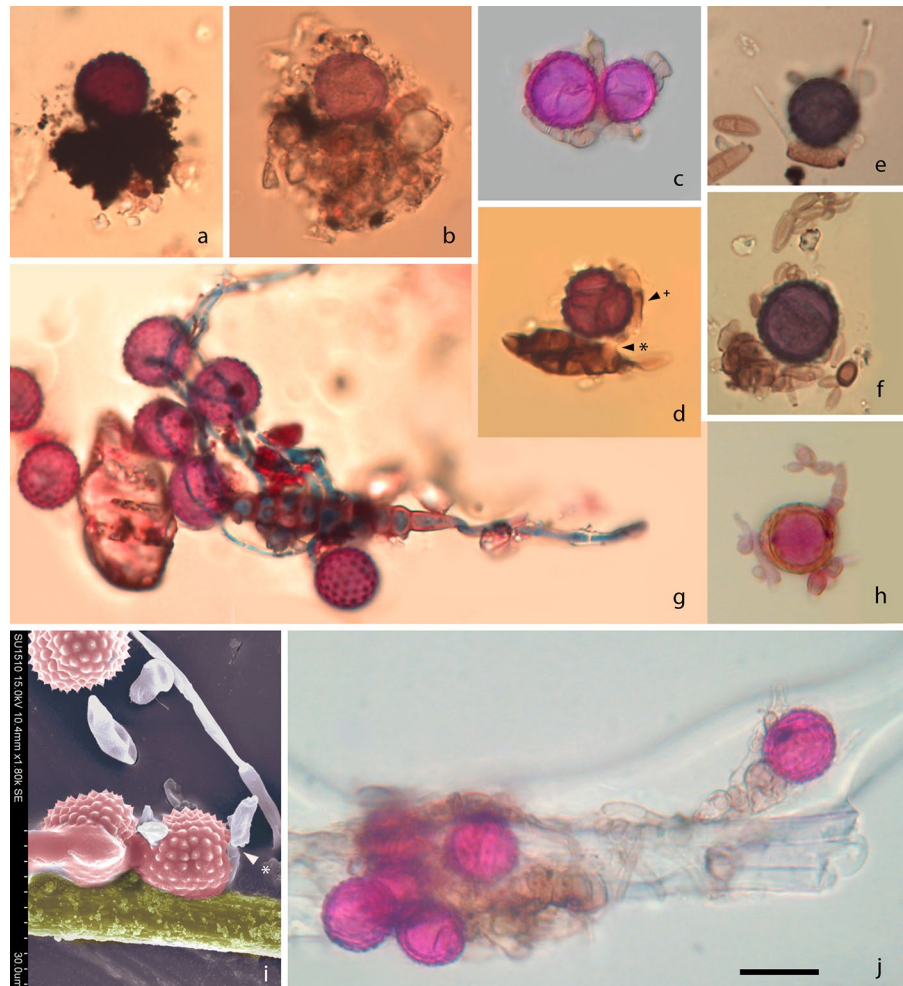
Cotton blue, 25 ml 85% lactic acid, 50 ml Glycerol, 25 ml distilled water) and incubated at 37 °C in a thermostat for one hour and then stained with a basic fuchsine solution (0.09 g basic fuchsin in 1000 ml of a 3:2:4 mixture of 96% ethanol, 87% glycerine and distilled water). The stained samples were covered by a cover slip and analysed under 500 × magnification. For scanning electroscopy, pollen grains were collected by touching plant surfaces with the heads of nail-shaped sample holders, covered with glue (Loctite Super Bond Power Flex Gel, Henkel AG & Co. KGaA, Germany). Samples were prepared by gold steaming and photographed with a Hitachi SU 1510 electron microscope. The colorization of SEM pictures and anaglyphs were prepared using Adobe Photoshop.

2.3 PM and meteorological data, statistical analysis

PM_{10} and $PM_{2.5}$ data were provided by the Hungarian Meteorological Service National Air Pollution Monitoring Network (OMSZ-OLM), in accordance with standard MSZ EN 16,450:2017: Ambient air: the automated measuring systems for the measurement of the concentration of particulate matter' based on the use of β-ray attenuation method. Meteorological data (daily mean, minimum and maximum temperature, precipitation, daily mean wind speed, and relative humidity) of the actual and the previous day were obtained from the + 3 h and + 6 h forecast files of the GDAS FNL (Global Data Assimilation System- Final) dataset through the NCAR Research Data Archive (NOAA, 2015). Meteorological conditions were markedly different in the studied years. In 2016

a relatively dry August and September was followed by a cold and wet October. In 2017, summer was dry and hot, while autumn was mild, with a moderate precipitation. 2018 was characterized by a hot and wet August, a moderate September and a relatively warm and dry October.

To determine if the data set is normal, a Kolmogorov–Smirnov Z test was performed. Since this statistic was significant (P 0.001), the hypothesis that the respective distribution is normal was rejected. Then, the variables were analysed with the nonparametric Spearman's rank correlation method to clarify the connection between concentration data and weather variables, using the IBM SPSS Statistic programme (Version 22.0.0.0, IBM Corp.)

2.4 Laboratory tests

According to Choël et al. (2020), particles can be deposited onto the surface of previously impacted pollen during sampling (as an artefact of air samplings). In order to test if fungi can be deposited onto the surface of previously impacted pollen during sampling, the following experiment was performed. Ragweed pollen was collected in Nyíregyháza in 20 September 2021 and stored at 7 °C. 0.05 g pollen was placed into a $120 \times 120 \times 60$ mm chamber connected to the 14×2 mm slit of a portable Hirst-type pollen trap (VPPS 1000, Lanzoni s.r.l., Bologna, Italy). Pollen was aerosolized in the chamber with an air current of 2 m/s provided by a pump (ELITE 799, Rolf C. Hagen Ltd., Castleford, UK). The trap impacted pollen onto a microscopic slide smeared with petroleum jelly. This slide, covered with airborne pollen, was used for a second air sampling to see if fungi can be deposited onto the surface of previously impacted pollen as a sampling artefact. For this test, *Cladosporium* species were used, because these fungi were found to be common both in the air and on pollen grains (Magyar, 2010; Tóth & Burján, 2014; Tóth et al., 2009, 2014). To collect airborne *Cladosporium* 100 L of air were aspired with Eco® microbial sampler (EMD Millipore, Merck KGaA, Darmstadt, Germany) onto Malt Extract Agar medium (MEA; 30 g l^{-1} malt extract, 5 g l^{-1} peptone, 15 g l^{-1} agar) containing 0.1 g l^{-1} chloramphenicol. Air sampling was conducted outdoors, in Budapest in 8 November 2021. The air sample was incubated at 25 °C for 5 days,

and then, one of the appearing *Cladosporium* colonies was isolated as a pure culture (strain number T844). To perform the experiment, this strain was transferred to a sterile 47 mm absorbent paper pad (Albet® Filtration & Separation Technology, Spain). The pad was placed onto MEA and saturated with sterile water and incubated at 25 °C for 4 days and then was removed and dried out in an exsiccator for 24 h. The dry pad was placed into the test chamber and gently rubbed on its surface by a metal needle before closing the lid of the chamber. The air was aspired at 10 L per minute to reach at least tenfold air exchange of the chamber where the pad was exposed to air currents provided by a pump (see above). The microscopic slide (previously covered by pollen, then by spores) was analysed under $400 \times$ magnification.

In a second experiment, *Cladosporium* was cultured on pollen and exposed to air currents to see if pollen grains infected by fungi can be aerosolized. For this test, 0.05 g of pollen and *Cladosporium* T844 were transferred to an absorbent pad and incubated together and then treated as described above. The presence of pollen-fungi complexes on the pad was tested by pressure-sensitive acrylic tape technique (see above).

In a third experiment, ragweed plants were placed into the test chamber and sampled for aerosols as described above, to see if pollen-fungi complexes can be aerosolized from a natural substrate. Ragweed plants were collected in Csákvár, in 25 September 2021. To verify the presence of *Cladosporium* on pollen, sterilized Wironit needles was used to collect pollen from anthers and placed onto MEA. The presence of pollen-fungi complexes on leaves and anthers was tested by the pressure-sensitive acrylic tape technique (see above).

To see if pollen-fungi complexes can be aerosolized from a deposition surface common in the urban environment, a $50 \times 50 \times 20$ mm block of asphalt was cut out from street pavement, washed, surface-sterilized with 90% ethanol and dried out. In this experiment, *Cladosporium cladosporioides* (strain number KMMG2758) was used and this fungus was isolated from ragweed pollen in Gödöllő in 19 August 2016. Pollen grains and *Cladosporium* were incubated together on the asphalt block placed in a plastic bag with 5 ml sterile water for 4 days at 25 °C, and then, it was removed and dried out in an

exsiccator for 48 h. The dry block was placed into the test chamber, and particles were aerosolized as described above.

At least 200 pollen grains per experiment were analysed for the presence of fungi. Between samplings, the test chamber and the trap's orifice were wet-cleaned and dried and air samples were collected as a control.

3 Results

3.1 Outdoor samplings

Weather conditions were different in the studied years (in 2016, August and September were cold and dry followed by a rainy October; in 2017, August and September were cold and wet but October was dry; and in 2018, August and September were warm and wet but October was dry again). Ragweed pollen seasons started at the end of July and lasted until October (26 July–04 October 2016, 25 July–10 October 2017, and 29 July–27 October 2018). The highest concentrations were measured in August and September (26 August 2016: 364 pollen/m³, 2 September 2017: 611 pollen/m³, 2 September 2018: 087 pollen/m³). The annual pollen integral was the highest in 2018, but only half was measured in 2016 (Table 1). The concentration of pollen grains infected by fungi was low in August and September and then remarkably increased up to 23.16% at the end of the season (Fig. 3). Infected pollen grains correlated positively with wind speed, as well as the airborne concentration of *Alternaria* and *Cladosporium* spores, but a negative correlation was found with temperature (Table 2). A positive correlation was shown with DOY. Pollen grains were most frequently infected with *Cladosporium* spp., but other fungi, such as *Alternaria*, A*spergillus/Penicillium*, and yeasts were also found (Table 3). Approximately 20% of filamentous fungi was represented by hyphae without attached conidia; thus, they cannot be identified. According to the position of fungal structures,

Table 1 Descriptive statistics of air sampling results in Nyíregyháza, Hungary

| Particles	Year	API, ASI	Avg	Max	St. dev
Ambrosia pollen[a] (pollen/m³)	2016	4996	70.4	364	92.6
	2017	7662	98.2	611	124.2
	2018	10,673	117.3	807	172.5
Ambrosia pollen + black particles[a] (pollen/m³)	2016	1953	30.0	200	42.8
	2017	2779	39.1	339	60.9
	2018	2823	32.1	243	51.5
Ambrosia pollen + brown particles[a] (pollen/m³)	2016	1249	19.2	116	24.8
	2017	2573	36.2	169	36.5
	2018	5642	64.1	467	93.9
Ambrosia pollen + fungi[a] (pollen/m³)	2016	27.3	0.4	2.5	0.7
	2017	60.9	0.8	6.7	1.3
	2018	n.d	n.d	n.d	n.d
Cladosporium[b] (spore/m³)	2016	497,024	6540	30,016	4498
	2017	541,024	7841	27,680	5439
	2018	496,800	6537	28,928	3981
Alternaria[b] (spore/m³)	2016	19,776	260	1120	228
	2017	27,456	398	1248	278
	2018	30,496	401	1408	278
PM$_{10}$[c] (µg/m³)	2016	1895	25	69.0	11.7
	2017	2054	28	66.0	12.0
	2018	2544	33	61.0	11.9
PM$_{2.5}$[c] (µg/m³)	2016	n.d	n.d	n.d	n.d
	2017	1037	14.8	40.4	7.1
	2018	1181	15.5	31.9	5.9

The airborne concentration of pollen grains contaminated with black and brown particles and colonized by fungi are shown in separate rows

API Annual pollen integral, *ASI* Annual spore integral. [a]In pollen season by EAACI definition; [b]between 1 August and 15 October, no data in 9–15 October 2017; [c]between 1 August and 15 October
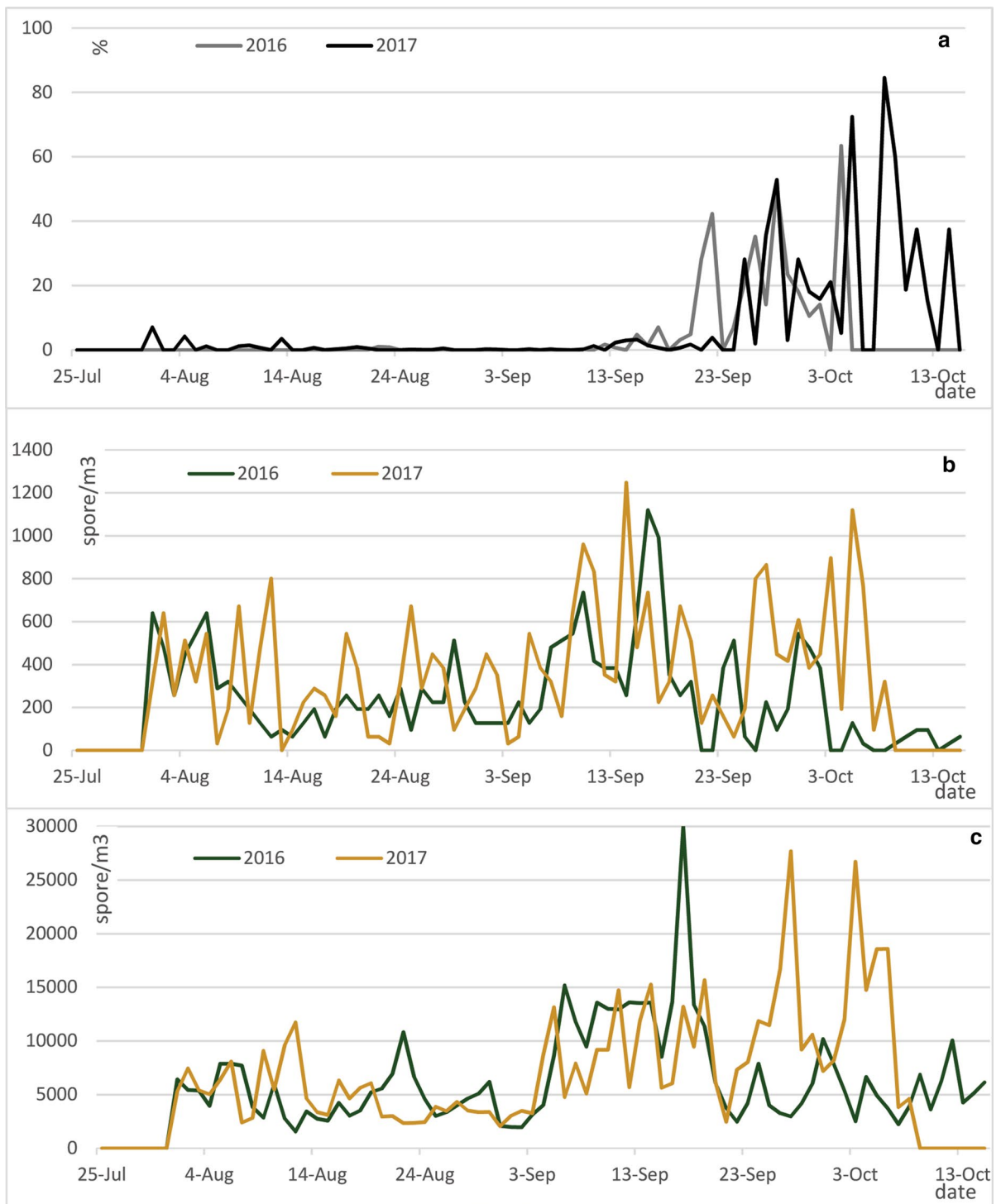
Fig. 3 a: Airborne *Ambrosia* pollen grains with fungal colonization in Nyíregyháza, Hungary. Data are shown in % of total *Ambrosia* pollen concentration. b: airborne concentration of *Alternaria* spores, c: airborne concentration of *Cladosporium* spores

Table 2 Spearman's correlation coefficients between relative abundance of airborne pollen grains of ragweed infested with fungi (*Ambrosia* pollen + fungi), and independent variables (day of the year (DOY), mean temperature (T_{avg}), minimum temperature (T_{min}), maximum temperature (T_{max}), relative humidity (r.h.), particulate matter (PM). Significant results are marked with asterisks ($T < 0.05$ *, $T < 0.01$ **)

| | *Ambrosia* pollen + fungi | |
	2016	2017
DOY	0.674**	0.270*
T_{avg} °C	− 0.337**	− 0.114
T_{min} °C	− 0.245*	− 0.080
T_{max} °C	− 0.337**	0.085
Precipitation mm	− 0.033	0.015
r.h. %	0.074	− 0.119
Wind speed m/s	0.148	0.258*
Alternaria spore/m^3	0.060	0.485**
Cladosporium spore/m^3	0.284*	0.350**

ecto- and endoparasitic fungi were observed on or inside pollen grains, respectively (Fig. 4). On surface samples of ragweed phylloplane, pollen grains infected by fungi were common, where the *Alternaria* and *Cladosporium* species dominated.

Pollen grains contaminated with black and brown particles were found abundantly in each year (Table 1), reaching 33.94 and 37.15 percent of the total ragweed pollen concentration, respectively (Table 4). Black pollutants correlated negatively with brown pollutants and day of the year (DOY) (Table 5). On the other hand, brown pollutants correlated positively with DOY, and negatively with temperature. Worth mentioning that crystalline particles were episodically observed on airborne ragweed pollen grains (Fig. 5).

3.2 Laboratory experiment

In the first laboratory experiment, sampling of airborne spores was conducted with a slide on which ragweed pollen has been previously deposited. Spore

Table 3 The relative abundance of airborne ragweed pollen grains colonized by fungi in Nyíregyháza, Hungary. Season end = last two weeks of the pollen season, determined by EAACI definition

Particles	Year	% of total *Ambrosia* pollen
Fungi on *Ambrosia* pollen (full season)	2016	0.5
	2017	0.8
	2018	n.d
Fungi on *Ambrosia* pollen (season end)	2016	24.1
	2017	22.2
	2018	n.d
		% of total fungi found on *Ambrosia* pollen
Cladosporium on *Ambrosia* pollen	2016	32.3
	2017	43.0
Alternaria on *Ambrosia* pollen	2016	7.7
	2017	9.3
Aspergillus/Penicillium on *Ambrosia* pollen	2016	0.0
	2017	8.6
Yeasts on *Ambrosia* pollen	2016	10.8
	2017	16.6
Hyaline hyphae on *Ambrosia* pollen	2016	33.8
	2017	11.3
Melanized hyphae on *Ambrosia* pollen	2016	15.4
	2017	11.3
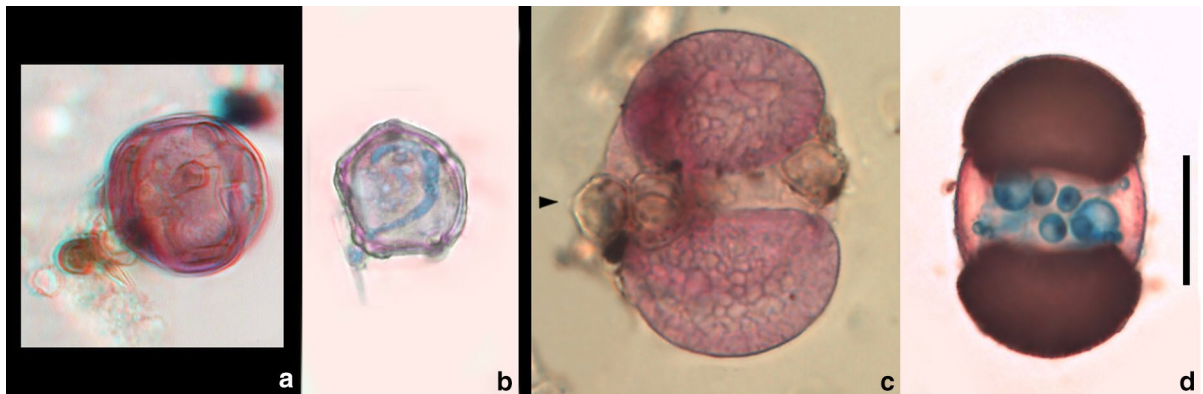
Fig. 4 Examples of obligate pollen parasitic fungi in air samples and their source in phyllosphere. **a**: airborne microsclerotia in an airborne pollen grain in Brufa, Italy. See this image in 3D using red-blue anaglyph glasses. **b**: development of a microsclerotium of *Mycoceros antennatissimus*, inside an *Alnus* pollen, on *Platanus* bark (Budapest, Hungary). **c**: *Olpid-* *ium*-like zoosporangia in the leptoma of an airborne *Pinus* pollen (Nyíregyháza, Hungary). Arrow shows a discharge pore. **d**: *Olpidium pendulum*-like zoosporangia in the leptoma of a *Pinus* pollen deposited on *Tamarix* bark (at Lake Vouliagmeni, Greece). Pollen coloured with basic fuchsine; **b** and **d**: fungi coloured with cotton blue Scale bar = 20 μm

Table 4 The relative abundance of ragweed pollen grains contaminated with black and brown pollutants in Nyíregyháza, Hungary

Particles	Year	% of total *Ambrosia* pollen
Black particles on *Ambrosia* pollen	2016	39.1
	2017	36.3
	2018	26.4
Brown particles on *Ambrosia* pollen	2016	25.0
	2017	33.6
	2018	52.9

deposition was observed on the surface of 95.5% of pollen grains. Deposited spores were loosely attached onto the pollen surfaces. No signs of fungal colonization (spore germination, hyphae growing on or around pollen grains) were observed in any of the cases. In the second experiment, *Cladosporium* was cultured on pollen placed onto a laboratory substrate (paper pad) for 5 days. This test showed that air currents aerosolized pollen and fungi. Signs of fungal colonization were detected on 85.7% of airborne pollen grains. On the 9.8% of pollen grains aerosolized from ragweed plants signs of colonization were also observed. In this air sample large complexes, i.e., fragments of plant tissues covered by pollen and hyphae were also

present. The analysis of the surface of the test plants showed that 2.0% of the pollen grains is colonized by fungi. On the plants, large fragments of plant tissues covered by pollen and hyphae were also found, similar to those present in air samples. Aerosols obtained from the asphalt block contained pollen grains of which 40.0% was showing signs of fungal colonization after 5 days of incubation.

4 Discussion

4.1 *Cladosporium* and *Alternaria*

The most common taxa on airborne pollen grains were *Cladosporium* and *Alternaria*. It seems to be unlikely that the relatively large conidia of *Alternaria* and *Cladosporium* contact and remain adhered to the pollen grains during their atmospheric transport. Apparently, spines of the ragweed pollen are too short to catch and hold these conidia in the air. According to our experiments, spores can deposit onto pollen grains during sampling as an artefact; however, these spores show no signs of fungal colonization. The laboratory tests showed that pollen-fungi complexes are formed during the development of hyphae on pollen grains laying on standard laboratory materials (paper pad), natural substrates (ragweed leaves and inflorescences) and urban surfaces (asphalt pavement).

Table 5 Spearman's correlation coefficients between parameters of airborne pollen concentration of ragweed (black particles %: percentage of pollen grains with black pollutants on surface; brown particles %: percentage of pollen grains with brown pollutants on surface), and independent variables (day of the year (DOY), mean temperature (T_{avg}), minimum temperature (T_{min}), maximum temperature (T_{max}), relative humidity (r.h.), particulate matter (PM). Significant results are marked with asterisks ($p < 0.05$*, $p < 0.01$ **)

	Black particles %			Brown particles %		
Years	2016	2017	2018	2016	2017	2018
DOY	0.110	−0.555**	−0.509**	0.015	0.408**	0.307**
T_{avg} °C	−0.015	0.608**	0.482**	0.354**	−0.379**	−0.352**
T_{min} °C	−0.199	0.560**	0.483**	0.300**	−0.375**	−0.360**
T_{max} °C	0.093	0.584**	0.503**	0.385**	−0.352**	−0.376**
Precipitation mm	−0.167	−0.079	0.008	−0.294*	−0.033	−0.020
r.h. %	−0.154	−0.396**	0.245*	−0.276*	0.114	−0.119
Wind m/s	−0.173	−0.143	0.016	−0.010	−0.104	0.023
PM_{10} µg/m³	0.301**	0.000	-0.040	0.284*	0.265*	0.003
$PM_{2.5}$ µg/m³	n.d	0.016	-0.090	n.d	0.291*	0.020
Black particles %	–	–	–	0.051	−0.543**	−0.567**
Brown particles %	0.051	−0.543**	−0.567**	–	–	–
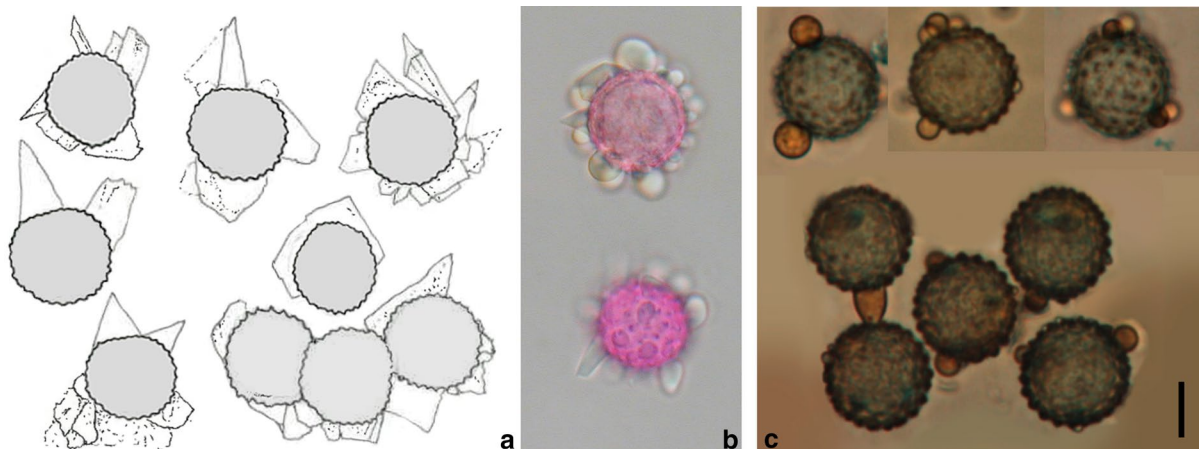
Fig. 5 **a**: Crystalline particles observed on airborne ragweed pollen grains. **b**: Semi-crystallised droplets on airborne pollen grains. **c**: Liquid droplets on fresh pollen grains, deposited on ragweed leaves, liquid is released after wetting

Agitation (e.g. fluttering of leaves, rubbing steps on the asphalt, etc.) and air currents can aerosolize these pollen-fungi complexes. As our experiments demonstrated, pollen-fungi complexes are formed before aerosolization, and not as a sampling artefact. These pollen-fungi complexes show the following signs of fungal colonization: thick-walled and melanized hyphae growing on or around the surface of the pollen grains.

Our observations confirmed that plant surfaces are common habitats of *Alternaria* and *Cladosporium*. Especially anemophilous flowers, leaves, and herbaceous stalks are suitable habitats for growth and sporulation, but they also offer an optimal base for the aerosolization of large quantities of *Cladosporium* spores. This wind-exposed habitat may be one of the factors that explains the high atmospheric concentration of *Cladosporium*. What's more, this is an adaptation of cladosporia for a successful airborne dispersal strategy. On the same plant surfaces, pollen grains are also common, where fungal colonization can take place. It is

known that *Cladosporium* parasitize pollen grains in the phyllosphere (Fokkema, 1968). The correlation between the occurrence of infected pollen grains and airborne spore concentration may be explained by the similar growth period of fungi on pollen and other substrates. The kinetic forces of wind, fluttering or rubbing of plant parts could aerosolize *Cladosporium*-infected ragweed pollen grains from the phylloplane. Observation of aggregates composed of plant debris, pollen and mycelia in our air samples provides further evidence to the phyllosphere being a major source of airborne parasitized pollen grains. Large clusters of multiple pollen grains (up to 8) bound together by mycelia as well as pollen with mixed fungal composition (*Alternaria + Cladosporium* + yeasts) were also observed in the air samples. We suppose that a relatively high kinetic force of wind is needed to lift up such large particles or tear-off parasitized pollen grains, fastened by mycelia, from plant surfaces. Plant senescence and decay may facilitate this aerosolization process in the late season.

Wind speed and parasitized pollen grains correlated positively in the current study. This is in accordance with our previous study performed in Italy, where the concentration of parasitized pollen grains was correlated with wind speed and wind direction (Magyar, 2005). Dry periods affected their aerosolization positively, while precipitation on one or two days before sampling had a negative effect. A canonical correspondence analysis showed that parasitized pollen grains were present in air samples in dry weather (Magyar, 2005, Fig. 23: Points 23, 25, 139). This was the case in other airborne particles as well, like ecto- and endoparasitic fungi of pollen grains as well as parasitized plant tissues.

Correlations with DOY show that the main period of pollen colonization is the end of the vegetation season. Similar trends were found in Budapest, Hungary, where air samples were collected in a ragweed field for two years (Magyar, 2010). These results showed that three days after rainfall and subsequent fungal growth the detection of parasitized pollen grains in air samples would be expected.

Airborne *Alternaria* and *Cladosporium* have been reported to cause allergy. Sensitization to these fungi have been reported to be 3–30% in European countries (Bavbek et al., 2006; Wilken-Jensen & Gravesen,

1984). Modification of allergenicity of pollen grains infected with these fungi needs further studies.

4.2 Other fungi

Unidentified ectoparasitic mycelia and yeast cells may belong to common phylloplane fungi, like *Aureobasidium*, *Fusarium* and *Tripospermum.* These taxa were often observed near pollen deposits or inside flowers, as they are typical members of this habitat (Dickinson, 1976; Magyar et al., 2021; Ponette-González et al., 2020), but without distinct morphological characteristics, their identification is not possible in air samples.

In some cases, endoparasitic mycelia were observed inside the pollen grains, resembling those of the pollen parasitic *Mycoceros* and *Retiarius* spp. (Fig. 4). These fungi live on tree phyllosphere and trap pollen grains (2017b; Magyar et al., 2017a). Pollen grains are attacked and invaded though openings or the mycelia break through thin regions of the exine (e.g., leptoma of *Pinus* pollen). The external colonies are short-lived; however, the fungus survive desiccation inside the pollen grain by a thick-walled chlamydospore-like resting mycelium (Olivier, 1978). The airborne dispersal of these fungi in airborne pollen grains is highly plausible. The above-mentioned taxa has hyaline hyphae. Pollen-fungi complexes containing hyaline hyphae may belong to these fungi, or other non-melanized hyphomycetes. Another pollen parasitic fungus, *Olpidium* (possibly *O. pendulum*), was also observed in the air samples. This fungus is common on pollen grains deposited in water and on pool margins after rain (Czeczuga & Muszynska, 2001). To our knowledge, this is the first report of airborne *Olpidium*. The aerosolization of this pollen-fungus complexes may occur after drying out ponds. This fungus was also observed on pollen grains accumulated on bark fissures of a *Tamarix* tree (Fig. 4.), where ephemerial aquatic conditions could support the colonization of pollen grains. Thus, pollen-*Olpidium* complexes may be aerosolized from plant surfaces too.

The colonization of pollen has many benefits for fungi. Pollen grains are rich in nutrients to start an intensive fungal growth. According to Stanley and Linskens (1974), viable pollen grains become leaky after wetting, and nutritive compounds of the cytoplasm (vitamins, amino acids, carbohydrates, etc.)

can be detected in the leachates. This could provide a basis for the chemotropic attraction of the fungal hyphae (Hutchison & Barron, 1997). Pollen parasitism is often followed by the proliferation of fungi on plant surfaces and the attack of plant tissues. Pollen grains play a crucial role in the life history of several members of plant pathogenic fungi, supplementing the nutritional requirements after spore deposition and in the early stages of infection of the host plant.

Fungal infection was observed not exclusively on ragweed pollen, but it was common on other pollen taxa too, especially Asteraceae (including *Helianthus*), *Betula*, Chenopodiaceae, Pinaceae and Poaceae. Typically, these infected pollen grains appeared at the end or out of pollen season of the given taxa. In the latter case, their presence could be interpreted as an indicator of pollen reaerosolization. In preparing pollen information, it is important to have a closer look on pollen grains and consider if they are new (i.e., released from anthers) or old (i.e. reaerosolized pollen of the last year). The presence of new pollen could be considered to determine the start of the season, while using old pollen to determine the start of the season can result in misinterpretation as early flowering. Brown pollutants on the surface of the pollen may also be a sign of reaerosolization.

4.3 Black and brown particles on pollen grains

Meteorological conditions were markedly different in the studied years. The pollen season was shortened by rains in October 2016, but weather was optimal to observe late-season processes in 2017 and 2018. Pollen grains contaminated with black and brown particles were found to be one third of the total pollen concentration. The yearly sum of black pollutants on ragweed was similar, but those of brown pollutants showed remarkable differences, as their concentration was high in 2018, when a wet August and September were followed by a dry October. Correlations revealed further differences between black and brown pollutants. The relative abundance of black pollutants decreased by DOY, contrary to the brown pollutants, which showed an increasing tendency by time. In 2016 atmospheric PM_{10} concentrations had a strong correlation with black particles. Ribeiro et al. (2015) studied the composition of PM on pollen grains. The dominant particulates identified were Si-rich, organic-rich, SO-rich, rich in metals and oxides, and Cl. Visez

et al. (2020) proposed four processes to explain deposition of PM on the surface of pollen grains: the sticking of particles to anthers and pollen during dehiscence, the coagulation of airborne pollen-particles, wet and dry scavenging of pollen and particles, and the co-deposition of PM and pollen. Ragweed pollen is isopolar, tricolporate, 20–22 μm in diameter with an echinate surface (Feliziani, 1986). In case of $PM_{2.5}$ airborne coagulation seems to be plausible, and may be aided by the echinate sculpture and electrostatic field of pollen grains (Inchaussandague et al., 2018). Furthermore, Visez et al. (2020) draw attention to airborne residence time of pollen, which is in the order of hours, but some pollen may suffer several cycles of deposition/reaerosolization. Such pollen could accumulate pollutants leading to 'superpolluted' pollen. However, deposition of PM particles on pollen grains during sampling (as an artefact, discussed by Choël et al., 2020 is also probable. Positive correlations of PM10 concentrations and "inorganic" pollution in 2016 may indicate this artefact. The negative health effects of pollen grains carrying pollutants is well documented (Behrendt et al., 1997; Visez et al., 2020), but their description goes beyond the aim of the current study.

The frequency of pollens' brown pollutants increased with DOY. Temperature had a negative effect in 2017 and 2018, but positive in 2016, possibly due to the early end of the season in this year. The temperature in August and September may have different effects on the formation of these particles than in late October. A positive correlation between the occurrence of brown pollutants on pollen grains and the concentration of deformed ragweed pollen grains was also reported (D. Tóth M, unpublished data), which deserves further studies. Massive brown particle deposits often blocked the basic fuchsine uptake of pollen grains. We can only speculate about the origin of Brown pollutants on pollen grains. Their morphological appearance suggests soil deposition on pollen grains. Soil particles can be adhered to the pollen grains after deposition. Subsequent reaerosolization events may lift up the pollen-soil complex. Contact with soil and reaerosolization is probable on and from wind- or rain-exposed areas covered with loose soil or canopy-held soil. Aerial surfaces of trees are known to accumulate so-called crown humus (Jeník, 1973) or arboreal histosol (Nadkarni et al., 2002), composed

of intercepted (micro)litter, decaying bark and epiphytes and airborne particles, including pollen. However, the sources and formation mechanisms of airborne soil organic particles are poorly understood and typically neglected in atmospheric studies (Wang et al., 2016).

The origin of crystalline particles observed on airborne ragweed pollen grains is unknown. The crystals are opaque-yellowish, often broken or eroded; their length is up to 10 μm, sometimes ordered to one direction (Fig. 5a). Pollen grains cemented together by crystals were also caught. Two hypotheses were raised to explain their origin. (i) The mix of crystals and liquid droplets (Fig. 5b) suggests early crystallization. Similar liquid droplets, resembling pollenkitt were found on fresh pollen grains deposited on ragweed leaves. The droplets were not present on dry pollen, but after wetting, yellow liquid droplets bubbled out the colpi. It is speculated that fresh, aerosolized and wetted pollen grains can develop the crystal-like structures under certain meteorological circumstances (Fig. 5c). Angular pollenkitt, similar to the above-mentioned crystals was illustrated by Hesse et al., 2009 (pg. 217). (ii) Another possible explanation is that crystals are composed of calcium oxalate. Calcium oxalate crystals are common in plants, e.g. in the corolla, anther and tapetum cells of Asteraceae (Meriç, 2009; Meriç & Dane, 2004). Such crystals in the anther may assist pollen against predators, they are metabolic waste products (Horner 1977; Horner and Wagner 1980). Therefore, one can speculate that crystals are deposited on pollen grains before pollen release. This phenomenon merits further analyses, focusing on the origin and chemical composition of these particles.

5 Conclusions

The microscopical observation of pollutants on pollen grains could provide information on source of pollen grains. Fungi are common on pollen grains in air samples, and can be an artefact of air sampling or sign of colonization of pollen grains by fungi before aerosolization. In this later case, pollen grains show the following signs of fungal colonization: thick-walled and melanized hyphae growing

on or around the surface of the pollen grains. Our results indicate that reaeroslization events can be identified by the examination of infected pollen grains.

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