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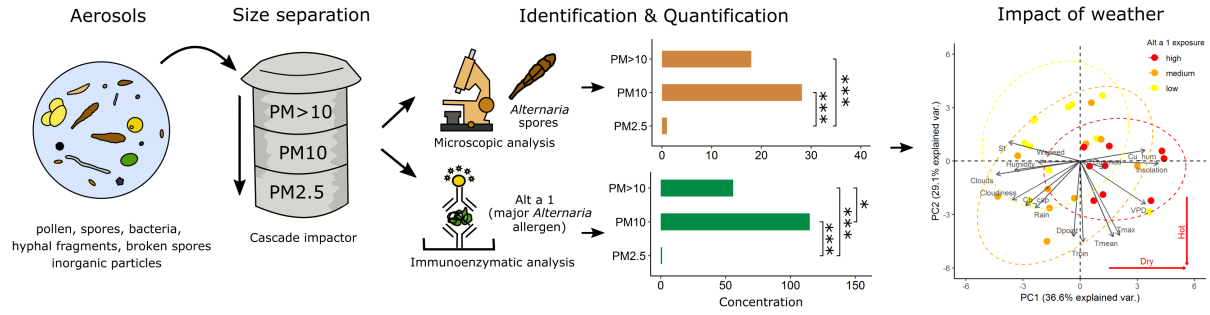
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MANUSCRIPT

Particle size distribution of the major *Alternaria alternata* allergen, Alt a 1, derived from airborne spores and subspore fragments.

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1 Abstract

2 Fungal fragments are abundant immunoreactive bioaerosols that may outnumber the
3 concentrations of intact spores in the air. To investigate the importance of *Alternaria*
4 fragments as sources of allergens compared to *Alternaria* spores, we determined the levels of
5 *Alternaria* spores and Alt a 1 (the major allergen in *Alternaria alternata* spores) collected on
6 filters within three fractions of particulate matter (PM) of different aerodynamic diameter: (1)
7 $PM_{>10}$, (diameter $>10\mu\text{m}$); (2) $PM_{2.5-10}$ (2.5-10 μm); (3) $PM_{2.5}$ (0.12-2.5 μm). The airborne
8 particles were collected using a three stage high-volume ChemVol cascade impactor during
9 the *Alternaria* sporulation season in Poznań, Poland (30 days between 6 July and 22
10 September 2016). The quantification of Alt a 1 was performed using the enzyme-linked
11 immunosorbent assay. High concentrations of Alt a 1 were recorded during warm and dry
12 days characterized by high sunshine duration, lack of clouds and high dew point values.
13 Atmospheric concentrations of *Alternaria* spores correlated significantly ($r=0.930$, $p<0.001$)
14 with Alt a 1 levels. The highest Alt a 1 was recorded in $PM_{2.5-10}$ (66.8% of total Alt a 1), while
15 the lowest in $PM_{2.5}$ (<1.0%). Significantly more Alt a 1 per spore (>30%) was observed in
16 $PM_{2.5-10}$ than in $PM_{>10}$. This Alt a 1 excess may be derived from sources other than spores,
17 e.g. hyphal fragments. Overall, in outdoor air the major source of Alt a 1 are intact *Alternaria*
18 spores, but the impact of other fungal fragments (hyphal parts, broken spores, conidiophores)
19 cannot be neglected, as they may increase the total atmospheric Alt a 1 concentration.

20 Keywords: fungal allergy; bioaerosols; hyphal fragments; ELISA; cascade impactor.

21

22

23 **Highlights**

- 24 1. Alt a 1 (major allergen of *Alternaria*) was quantified in different air fractions
- 25 2. *Alternaria* spores and Alt a 1 levels correlated significantly ($r=0.930$, $p<0.001$)
- 26 3. The highest Alt a 1 level was detected in $PM_{2.5-10}$, while the lowest in $PM_{2.5}$
- 27 4. Significantly more Alt a 1 per spore (31.3%) was observed in $PM_{2.5-10}$ than in $PM_{>10}$
- 28 5. Spores are the main source of Alt a 1, but the impact of hyphae cannot be neglected

29 **Introduction**

30 Fungal aerosols include generative and vegetative particles of different size and shapes,
31 fragmented, whole or aggregated, that can be passively or actively released into the air
32 (Afanou et al. 2014; Despres et al. 2012; Green et al. 2006). Airborne fungal particles are very
33 common both in indoor and outdoor environment comprising a large proportion of total
34 aerosol particle mass (Elbert et al. 2007; Frohlich-Nowoisky et al. 2009; Womiloju et al.
35 2003). The size of reproductive fungal propagules (spores, conidia) varies from approximately
36 1µm to over 100µm (Lacey and West 2006). Airborne fragments of vegetative mycelium may
37 be even smaller, reaching submicron dimensions (Green et al. 2006). Spores, conidiophores
38 and hyphal fragments could be released simultaneously, but the releasing mechanism is
39 different and depends on factors such as fungal species, weather conditions, mechanical
40 disturbance (e.g. action of animals) as well as the texture, moisture and the vibration of the
41 substrate (Afanou et al. 2014; Afanou et al. 2015; Frankel et al. 2014; Górny et al. 2002;
42 Green et al. 2005a; Green et al. 2005b; Green et al. 2006; Madsen et al. 2016). Furthermore,
43 morphological differences between intact spores and fungal fragments suggest that the
44 atmospheric behaviour of these particles, e.g. deposition velocity, may vary substantially. For
45 instance, measurement of fungal aerosols during aerolisation experiments have shown that
46 counts of fungal fragments do not always correlate well with spore concentrations (especially
47 in low air velocity) (Górny et al. 2002).

48 Fungal particles contain potentially harmful substances (mycotoxins and allergens)
49 that may cause serious health problems (Rick et al. 2016). Development, persistence, and
50 severity of allergic rhinitis and asthma have been associated with mold sensitivity, and it is
51 estimated that around 6.5 million people worldwide have severe asthma with fungal
52 sensitizations (Andersson et al. 2003; Denning et al. 2006; Knutsen et al. 2012). Allergenic
53 proteins have been detected both in fungal spores (that are traditionally linked with allergic

54 reactions) (Twaroch et al. 2015) and in hyphal fungal fragments (Green et al. 2005c; Levetin
55 et al. 2009). The degree that these fragments function as sources of allergens and contribute to
56 adverse health effects has not, however, been determined (Green et al. 2006). On the one
57 hand, spore fragments (fragments of hyphae and conidiophores, and broken spores) are
58 abundant bioaerosols and due to their small size may stay airborne longer and penetrate into
59 the lower regions of the respiratory tract more easily than larger intact spores (Cho et al. 2005;
60 Lacey and West, 2006; Pady and Gregory 1963; Pulimood et al. 2007). Also, as fragments
61 have large surface area relative to their mass (in comparison to larger particles) they may
62 show higher biological activity (Frankel et al. 2014). For instance, a positive association has
63 been observed between asthma admission and the level of broken spores (but not hyphae)
64 during thunderstorms (Pulimood et al. 2007). On the other hand, fungal allergens have
65 predominantly been localized in the cell wall of mature spores, as shown for *Alternaria* sp. -
66 one of the most clinically important fungal taxa (Burbach et al. 2009; Twaroch et al. 2012).
67 Also, significant positive correlations have been found between atmospheric levels of the
68 major *Alternaria alternata* allergen (Alt a 1) and both *Alternaria* spore concentrations
69 (Agarwal et al. 1983; da Silva et al. 2019) and allergy symptoms (Feo Brito et al. 2012)
70 suggesting close dependencies between intact spores, allergens, and symptoms.

71 Intact spores, due to the action of unfavourable weather conditions or mechanical
72 disturbances, may be additionally fragmented to enrich the spore fraction of fungal
73 aerosols (China et al. 2016; Pulimood et al. 2007). Also, many fungal spores are hygroscopic
74 and can absorb water from the surrounding atmosphere. Eventually, due to osmotic shock,
75 spores may burst in humid conditions releasing submicronic size fragments (China et al.
76 2016; Pasanen et al. 1991). Whether these fragments contain allergens remains unclear, but
77 pollen-orientated studies revealed high amounts of pollen allergens in cytoplasmic content
78 derived from fragmented pollen grains (Buters et al. 2015; Hoidn et al. 2005; Schäppi et al.

79 1997b; Schäppi et al. 1999; Taylor et al. 2002). The bursting of hyphal tips in water has also
80 been documented (Bartnicki-Garcia and Lippman 1972). This process resembles the abortive
81 pollen germination in rainwater, when allergens are expelled from the tip of the pollen tube
82 (Grote et al. 2003; Schäppi et al. 1997b). A recent study showed a positive correlation
83 between Alt a 1 and *Alternaria* spores was only observed in buildings that had high relative
84 humidity (da Silva et al. 2019). In general, moisture is essential in fungal spore germination
85 (Dagno et al. 2011; Hatzipapis et al. 2002; Vloutoglou et al. 1996), and it has been shown that
86 *Alternaria* spores in extraction liquid release allergens in just 15 minutes (Sweeney et al.
87 1985). Germinating spores were also observed in the warm and moist environment of the
88 nasal cavity, and this mechanism was postulated as an additional source of allergens (Green et
89 al. 2003; Sercombe et al. 2006).

90 This study aims to determine whether subspore fragments of *Alternaria* can be a
91 significant source of Alt a 1 in ambient air (in comparison to intact *Alternaria* spores) and to
92 investigate the relationship between allergen content and environmental conditions, especially
93 those related to moisture. This was achieved by examining the levels of Alt a 1 allergen
94 released from airborne particles collected on filters within three fractions of particulate matter
95 (PM) and relating these to various weather parameters.

96

97 **Materials and Methods**

98 *Fungal aerosol collection and quantification*

99 Airborne *Alternaria* particles were collected using a high-volume (400 l/min) ChemVol
100 cascade impactor (Butraco Inc., Son, Netherlands) (Buters et al. 2012; Demokritou et al.
101 2002). The ChemVol contains three impaction stages for collecting particles of different
102 aerodynamic cut-off diameters i.e. (1) $>10\mu\text{m}$ ($\text{PM}_{>10}$); (2) $2.5\text{-}10\mu\text{m}$ ($\text{PM}_{2.5\text{-}10}$); (3) $0.12\text{-}2.5$

103 μm ($\text{PM}_{2.5}$). *Alternaria* spores are large with the aerodynamic diameters ranging from about
104 10 to 30 μm (McCartney et al. 1993). The ChemVol sampler therefore seemed a suitable
105 collecting device for separation intact *Alternaria* spores from smaller fungal fragments
106 (hyphal parts and dissected spores). *Alternaria* particles were collected in Poznań, during the
107 main *Alternaria* sporulation season (30 days between 6 July and 22 September 2016, Tab.
108 1S). The highest daily *Alternaria* spore concentrations in Poznań are observed from the end of
109 June to the middle of September, with the seasonal peak recorded usually in the beginning of
110 August (Grewling et al. 2019; Kasprzyk et al. 2013). A previous study showed that the mean
111 seasonal *Alternaria* spore concentration in Poznań was the highest among other cities in
112 Central and Eastern Europe (Kasprzyk et al. 2015), which is related to the fact that the city is
113 located in the agricultural region of Western Poland. Field crops are considered the main
114 source (host plants) of various *Alternaria* species. In the studied area the predominant species
115 are *Alternaria alternata*, *A. brassicicola*, and *A. brassicae* as they infect oilseed rape fields
116 that are abundant in Western Poland (Jajor et al. 2012; Baranowski et al. 2015). In addition,
117 other crop pathogens are locally common, such as *A. solani* (infecting potatoes), *A. porri*
118 (pathogen of onion), and *A. dauci* that infects carrots (Gawińska-Urbanowicz and Kapsa
119 2013, Ogórek et al. 2011). However, it should be stressed that spores of different *Alternaria*
120 species are morphologically similar and so, when airborne samples are investigated,
121 *Alternaria* spores cannot be identified to species level and are therefore grouped to genus
122 level. The ChemVol was located at roof level (18 m a.g.l.) in the northern part of Poznań
123 (52.46°N, 16.92°E) (Fig. S1). The sampling time was 24 hours, from 12:00 to 12:00 of the
124 next day, but is described as a “daily average” throughout. The collection substrates were
125 polyurethane foam filters. Each filter (three filters per day) was cut into three equal pieces and
126 extracted in the dark (4 hours in 0.1 M ammonium bicarbonate buffer). After filter extraction
127 the content was centrifuged (10 min at 1699g). The supernatant was used for quantification of

128 the major *Alternaria alternata* allergen (Alt a 1), while sediment was used for *Alternaria*
129 spore calculation. The quantification of Alt a 1 in each air fraction was performed using the
130 enzyme-linked immunosorbent assay (ELISA) following the protocol described in Grewling
131 et al. (2019). Due to a high degree of structural similarity between Alt a 1 homologues
132 proteins (Saenz-de-Santamaria et al., 2006; Hong et al. 2005, Amado et al. 2016), fungal
133 species closely related to *A. alternata* could also be detected by ELISA. Therefore, when
134 describing the Alt a 1 concentration in the air the results are not limited only to *A. alternata*,
135 but may also refer to other *Alternaria* species. Other fungal genera, e.g. *Urocladium* and
136 *Stemphylium* that show close phylogenetic relationship to *Alternaria* (Gutierrez-Rodriguez et
137 al., 2011), should not markedly affect the obtained results as concentrations of their spores in
138 the air are often very low (Bednarz and Pawłowska 2016; Šcevkova and Kovac 2019). The
139 daily mean Alt a 1 concentration was expressed as pg/m^3 . The number of spores extracted
140 from filters was calculated using a method adopted from the estimation of pollen production
141 (Bogawski et al. 2016). After centrifugation, the sediment with spores was diluted in 200 μl of
142 distilled water. This was vortexed to obtain a homogenous solution and 25 μl was transferred
143 to a microscope slide and gently spread within 1.5 x 1.5 cm area. The spores were counted
144 under a light microscope (magnification 200x) in three horizontal lines. Taking into account
145 the microscope field of view and number of lines the total examined area was 49.5 mm^2 , i.e.
146 22% of total slide area (225 mm^2). This procedure was repeated three times and the number of
147 spores was averaged. The obtained value was used to calculate the mean spore concentration
148 in 1 μl of solution. We decided to not express the spore concentration in 1 m^3 of air due to the
149 uncertainty in estimating the total number of spores collected on filters (this value cannot be
150 precisely calculated using proposed method of spore extraction).

151 To validate the correctness of spore enumeration from filters, the results were compared with
152 the mean “daily” level of spores (from 12:00 to 12:00 of the next day) obtained by routine

153 methods used in aerobiology, i.e. volumetric Hirst spore trap (Hirst, 1952), located next to the
154 ChemVol impactor. The Hirst (1952) spore trap is a impaction type sampler where air is
155 sucked at a rate of 10 l/min through a 2 mm ×14 mm orifice. Behind the orifice the air flows
156 over a rotating drum that moves past the inlet at 2 mm/h and is covered with an adhesive
157 coated, transparent plastic tape. Airborne spores impact on the tape to give a time related
158 sample. Following its removal from the trap, the tape is divided into segments corresponding
159 to 24-h periods (48 mm in length). Each segment is mounted between a glass slide and cover
160 slip, and the samples are examined by light microscopy (×400 magnification). Spores were
161 counted along two longitudinal transects following the method described in literature (Maya-
162 Manzano et al. 2016). The correlation between both datasets was positive and statistically
163 significant (Pearson correlation coefficient, $r = 0.930$, $p < 0.05$) ensuring that selected methods
164 give comparable results. The usefulness of the ChemVol impactor for bioaerosols collection
165 has also been validated during the EU funded HIALINE project (Buters et al. 2015).

166 *Weather data collection*

167 Weather data were retrieved from the official weather station of the National Institute of
168 Meteorology and Water Management located at the Poznań Ławica airport (app. 5 km
169 southwest from aerobiological station) (Fig. S1). The temporal resolution of weather data was
170 adjusted to the Alt a 1 collection time (12:00-12:00). The following meteorological
171 parameters were analysed: daily mean, minimum and maximum air temperature (°C), dew
172 point (°C), vapour pressure deficit - VPD (kPa), relative humidity (%), rainfall (mm),
173 sunshine duration (h), wind speed (m/s), cloudiness (unit 1/8), daily fraction of specific cloud
174 types (%): *Cumulus humilis* (*Cu hum*)/*Cumulus fractus* (*Cu fra*), *Cumulus mediocris* (*Cu*
175 *med*)/*Cumulus congestus* (*Cu con*), *Stratocumulus* (*Sc*), *Cumulonimbus capillatus* (*Cu cap*),
176 and daily fraction of all types of clouds (%).

177 *Statistical analysis*

178 The concentrations of Alt a 1 and *Alternaria* spores collected in three air fractions were
179 compared by the Kruskal-Wallis H test and Dunn's procedure for multiple pairwise
180 comparison. *P*-values have been adjusted using Benjamini-Hochberg correction.
181 Relationships between Alt a 1 levels and *Alternaria* spore concentrations in selected air
182 fractions were checked by simple linear regression analysis. Daily mean concentration of Alt
183 a 1 in every stage of ChemVol sampler has been correlated (by Pearson correlation
184 coefficient) with meteorological parameters. Also, the ratio in the level of Alt a 1 (or spores)
185 between three investigated air fractions has been correlated with meteorological parameters.
186 Data that were right (positively) skewed have been transformed (log+1). The multivariate
187 principal component analysis (PCA) was performed to select the major weather conditions
188 affecting the daily Alt a 1 concentration. Days with Alt a 1 concentration were divided into
189 three groups based on two cut points estimated via probability quantiles (33.3% and 66.6%),
190 i.e.: "low" (daily Alt a 1 levels < 1.98 pg Alt a 1/m³, n=10), "medium" (1.98-6.71 pg Alt a
191 1/m³, n=10) and "high" concentration (>6.71 pg Alt a 1/m³, n=10). Before PCA analysis, the
192 data were Box-Cox transformed, scaled and centred. All statistical analysis has been
193 performed using computing environment R (R Core Team 2018) and packages: FactoMineR
194 (Lê et al. 2008), corrplot (Wei and Simko 2017), caret (Kuhn 2008), and factoextra
195 (Kassambara and Mundt 2017).

196

197 **Results**

198 *Distribution of Alt a 1 in different fraction of particulate matter*

199 The highest amount of Alt a 1 was detected in the 2.5-10 µm (PM_{2.5-10}) air fraction (Fig. 1)
200 and was significantly higher (*p*<0.05) than in two other air fractions (Fig. 2). The Alt a 1
201 concentration was extremely low (<1% of total Alt a 1) in the PM_{2.5} air fraction that contained

202 the smallest particles (i.e. $<2.5 \mu\text{m}$). A similar pattern was observed in relation to *Alternaria*
203 spores, as the highest spore level was observed in $\text{PM}_{2.5-10}$, while the lowest in $\text{PM}_{2.5}$.
204 Airborne concentrations of Alt a 1 were significantly ($p<0.001$) related to *Alternaria* spore
205 levels collected in $\text{PM}_{>10}$, $\text{PM}_{2.5-10}$ and $\text{PM}_{2.5}$ ($R^2=0.801$, $R^2=0.819$, and $R^2=0.454$,
206 respectively) (Fig. 3). The correlation between total Alt a 1 and *Alternaria* spores was positive
207 and significant ($R^2=0.865$, $p<0.001$). Significantly more Alt a 1 per spore (31.3%) was
208 observed in $\text{PM}_{2.5-10}$ than in $\text{PM}_{>10}$ ($p=0.015$).

209 *Impact of weather on Alt a 1 distribution*

210 There were significant positive correlations ($p<0.05$) between daily *Alternaria* spore
211 levels collected in the Hirst type trap and daily mean, maximum and minimum temperature,
212 VPD, dew point, and sunshine duration. Similar relationships were recorded between daily
213 levels of Alt a 1 (collected by ChemVol sampler) and weather conditions (Fig. 4). For
214 instance, the Alt a 1 in every air fraction correlated positively with daily maximum ($r>0.387$,
215 $p>0.05$) and mean temperature ($r>0.384$, $p>0.05$). Furthermore, statistically significant
216 positive correlations were recorded between Alt a 1 in the $\text{PM}_{2.5}$ fraction and fair weather
217 *Cumulus humilis* clouds ($r= 0.584$, $p<0.001$), sunshine duration ($r= 0.567$, $p=0.001$), and VPD
218 ($r= 0.505$, $p=0.004$).

219 On the other hand, humidity ($r= -0.447$, $p=0.013$), the occurrence of all types of clouds
220 ($r= -0.552$, $p=0.001$) and cloudiness ($r= -0.495$, $p=0.005$) all had significant negative
221 associations with levels of Alt a 1 in the $\text{PM}_{2.5}$ fraction. In addition, there was a significant
222 negative correlation between Alt a 1 and wind speed ($r= -0.372$) in the larger fractions. There
223 were no significant relationships between Alt a 1 concentrations and rainfall or
224 *Cumulonimbus capillaris* clouds. Finally, no significant relationships have been observed
225 between *Alternaria* spores and meteorological parameters related to “humid conditions”, such

226 as cloudiness, the occurrence of *Stratocumulus* and all types of clouds, rainfall and increased
227 humidity.

228 Considering the impact of meteorological conditions on the ratio of Alt a 1 (or spores)
229 recorded in different air fractions, only two significant correlations have been observed: (1)
230 Alt a 1 in PM_{2.5} to Alt a 1 in >PM_{2.5} was negatively correlated with humidity ($r = -0.411$,
231 $p = 0.024$); (2) Spores in PM_{2.5} to spores collected in >PM_{2.5} correlated significantly with
232 sunshine duration ($r = -0.413$, $p = 0.023$) (Fig. 5).

233 PCA supports results of correlation analysis, i.e. higher Alt a 1 concentrations were
234 generally recorded during warm and dry days characterized by high sunshine duration (PC1),
235 dew point and daily mean, maximum and minimum temperatures (PC2) (Fig. 6, Fig. S2 &
236 S3). In addition, the occurrence of *Stratocumulus* clouds and all types of clouds (PC1) showed
237 strong negative relationship with Alt a 1 concentrations. The first two principal components
238 explained 65.7% of variability in the dataset (Fig. S2).

239

240 Discussion

241 Sources of Alt a 1

242 Our study demonstrates that the daily levels of *Alternaria* spores correlated significantly with
243 Alt a 1 ($r = 0.930$, $p < 0.001$), and we can therefore assume that the majority of atmospheric Alt
244 a 1 was derived from intact *Alternaria* spores. Spores take part in the infection of plants and
245 Alt a 1 is a protein involved in plant pathogenesis, i.e. interacts with plant defence proteins
246 such as PR5 (Garrido-Arandia et al. 2016). In other words, spores need Alt a 1 to block plant
247 defences and to favour fungal entry into the plant. In view of these findings, it is not
248 surprising that Alt a 1 was located in the highest concentrations in the cell walls of old and

249 germinating spores (Mitakakis et al. 2001; Twaroch et al. 2012) because the allergen is
250 located exactly where it is most needed. The length of *Alternaria* spores vary from
251 approximately 20 μm to as much as 200 μm (Simmons 2007) and are many times larger than
252 micron sized fungal fragments. In addition, only part of the hyphal fragments are
253 immunoreactive, as around 25% of all hyphae expressed detectable allergens (Green et al.
254 2005c). Hundreds of fragments are therefore needed to exceed the allergen load of a single
255 spore, and comparative studies showed that the differences between the levels of airborne
256 hyphal fragments and spores are not in fact as high. According to Green et al. (2005c) fungal
257 hyphae concentrations surpassed spores only by around 2-3 times. Higher differences
258 (exceeding even 300-fold) have been observed in aerosolization experiments (Górny et al.
259 2002), but the mean difference between the number of hyphal fragments and spores was much
260 lower (varying from 10 to 60-fold depending on air velocity). In addition, in a study
261 conducted in two US cities, fungal fragments were present on 99% of all days, although
262 spores rather than hyphae predominated in the air (Levetin et al. 2009). What is more, it
263 should be stressed that in environmental samples, it is extremely difficult to morphologically
264 distinguish the hyphae of different fungal species (immunostaining and DNA extraction
265 techniques may be a solution) (Green et al. 2005b; Rittenour et al. 2012). In some of the
266 studies mentioned previously (Green et al. 2005c; Levetin et al. 2009), only total hyphae
267 fragments were counted (without species recognition). This approach, although valuable, does
268 not allow for the direct comparison between spores and hyphae of particular fungal species, so
269 the contribution of mycelial fragments could be overestimated.

270 The highest level of *Alternaria* spores (and Alt a 1) was observed in the $\text{PM}_{2.5-10}$ air
271 fraction. This is surprising as, according to previous studies, the aerodynamic diameter of
272 *Alternaria* spores exceeds 10 μm (McCartney et al. 1993; Yamamoto et al. 2014). Most of the
273 spores should therefore be deposited in the $\text{PM}_{>10}$ air fraction, as it was presented in pollen-

274 oriented studies (based on the same experimental setup) where around 90% of pollen
275 allergens (and therefore also pollen grains) were detected in PM_{>10} fraction (Buters et al.
276 2012; Buters et al. 2015; Galan et al. 2013; Grewling et al. 2016). It is worth noting, however,
277 that *Alternaria* colonies were also isolated from air samplers with particle diameter lower than
278 10µm (Kim et al. 2010; Sayer et al. 1969). Furthermore, DNA barcoding analysis (Yamamoto
279 et al. 2012) showed that the concentration of *Alternaria* DNA was also very high in the PM_{2.5-}
280 ₁₀ fraction (although DNA might originate from both fungal spores and fragments).
281 Presumably, the high number of *Alternaria* spores in the PM_{2.5-10} fraction derived from their
282 characteristic elongated club shape (in contrast to spherical pollen grains). McCartney et al.
283 (1993) stressed that because of the shape of *Alternaria* spores, the mass is not uniformly
284 distributed along their length, and thus it is difficult to predict their aerodynamic
285 characteristics. In addition, the aerodynamic diameter of fungal spores cannot be accurately
286 estimated solely based on the physical diameter but needs additional information, e.g. on the
287 density of the spores and ambient air humidity (Reponen et al. 2001). For instance, it has been
288 shown that high humidity may increase the diameter of *Cladosporium* spores by as much as
289 180% (Pasanen et al. 1991).

290 Our study revealed that the proportion of Alt a 1 to spores in the PM_{2.5-10} fraction was
291 around 30% higher than in PM_{>10}. Presumably, the 30% excess of Alt a 1 in PM_{2.5-10} derived
292 from subspore hyphal fragments. However, it should be noted, that we did not quantify the
293 level of *Alternaria* fungal fragments (based on their morphology) in air samples in this study
294 (only Alt a 1 derived from fragments). When interpreting the peculiarities in allergen
295 concentrations in the air, one should also remember about high variation in the allergenicity of
296 fungal spores (Grewling et al. 2019; Mitakakis et al. 2001). Grewling et al. (2019) revealed
297 differences of up to eightfold in day-to-day variations in *Alternaria* spore allergenicity that
298 could be linked to varying species composition during the sporulation season. Spores of

299 different *Alternaria* species vary in their aerodynamic properties (McCartney et al. 1993), and
300 so species-specific variations in the aerodynamic behaviour of spores (with different Alt a 1
301 content) may also affect the amount of Alt a 1 recorded in different fractions of particulate
302 matter.

303

304 *Impact of weather on Alt a 1 concentration*

305 The results of PCA and correlation analysis showed that the highest Alt a 1 levels were
306 recorded during sunny, warm and dry days, when weather conditions favoured the upward
307 movement of air currents (high temperature, sunshine duration, dew point and VPD, and
308 presence of *Cumulus* clouds). These conditions are known to positively affect the daily
309 concentrations of *Alternaria* spores (Grinn-Gofroń and Bosiacka 2015; Hjelmroos 1993;
310 Stennett and Beggs 2004; Troutt and Levetin 2001). It is striking to note that the strength and
311 direction of correlations between meteorological factors and both Alt a 1 (especially in $PM_{>10}$
312 and $PM_{2.5-10}$ fractions) and *Alternaria* spore concentrations were generally very similar (see
313 Fig. 4), which supports the idea that the main sources of Alt a 1 are *Alternaria* spores.

314 In contrast to findings from pollen-oriented studies where humid conditions increased
315 the fraction of allergens related to small fragments (Buters et al. 2015; Schäppi et al. 1997a),
316 our experiment showed an opposite situation. The ratio of Alt a 1 in $PM_{2.5}$ to Alt a 1 in larger
317 fractions increased with decreasing relative humidity. This result concurs with previous
318 findings (Madsen 2012) showing that the fraction of the fungal particles being of respirable
319 size was the highest for particles aerosolized at low relative humidity. We suspect that the
320 different behavior of pollen and fungal spores arises from differences in the wall structure and
321 the role of water in pollen/fungal spore germination. The release of subpollen allergens
322 through pollen wall bursting (due to high humidity) was mainly described for pollen grains

323 characterized by a delicate and thin pollen wall like grasses (Poaceae) (Schäppi et al. 1999,
324 Taylor et al. 2002; Buters et al. 2015). It has not, however, been documented in pollen with
325 thicker walls like mugwort (*Artemisia* sp.). In this species the concentration of subspore
326 particle does not show positive relationship with air humidity (Grewling et al. 2020). Water
327 has an adverse effect on pollen longevity and viability and so plants developed certain
328 protective mechanisms (e.g. specific floral morphology, production of germination inhibitors)
329 to prevent pollen from water damaging or undesirable germination (outside the stigma)
330 (Eisikowitch and Woodell, 1974; Mao and Huang 2009). In contrast, water is essential for
331 fungal spore germination, and spores of some *Alternaria* species only germinate at 100%
332 relative humidity (Dickinson and Bottomley, 1980; Hatzipapas et al., 2002). Fungal spores are
333 therefore adapted to moisture conditions and likely more resistant to rupturing by osmotic
334 shock than thin-wall pollen grains. The recent study by Lawler et al. (2020) documented the
335 occurrence of fungal nanoparticles in the air, which peaked around 1.5 days after the rainfall.
336 Similar behavior was also observed in the Amazon, where daily increase in fungal particles
337 was related to high nighttime relative humidity (China et al. 2016). This suggests that post-
338 rain processes related to fungal spore germination may play a role in the release of
339 nanoparticles (Lawler et al. 2020). Considering the fragmentation of *Alternaria* spores, the
340 mechanical damage to spores (e.g. observed during grass moving or harvesting (Pulimood et
341 al. 2007)) seems to be more important than osmotic rupture.

342 In addition, our study showed that fine fungal fragments were more likely, than larger
343 particles, to become airborne when *Cumulus humilis* clouds were observed. This type of cloud
344 indicates unstable atmospheric conditions below the clouds, especially during their formation.
345 Such turbulent conditions could occur during intense sunshine duration preceding *Cumulus*
346 *humilis* formation and/or in the presence of *Cu hum* clouds (Stull 1985). Indeed, *Cu hum* and
347 sunshine duration are highly positively correlated with the amount of Alt a 1 in smallest air

348 fraction (see Figure 4). Consequently, higher numbers of small, immunoreactive fungal
349 particles may occur during weak or moderate convection (e.g. warm air ascending with
350 velocity 2-5 m s⁻¹). Larger fungal fragments (>2.5µm) therefore seem to be more loosely
351 connected with weak convection, probably because they require stronger air movements to
352 overcome gravity and drag.

353 Deposition velocity of airborne particles is the lowest (<0.03 cm s⁻¹) for particles of
354 aerodynamic diameter between 0.1-1.0µm. For larger particles (>5.0µm) deposition velocity
355 strongly increases (>1.0 cm s⁻¹) and sedimentation becomes a predominant atmospheric
356 process of particle removal (Nicholson 1995). According to Woo et al. (2018) *Alternaria*
357 spores with aerodynamic diameter of 10µm had a deposition velocity of 0.63 cm s⁻¹. Fine
358 hyphal fragments (0.12-2.5µm) might hypothetically gain in importance in indoor
359 environments (Górny et al. 2002) or during specific weather conditions, e.g. thunderstorm
360 events when many particles are uplifted, mixed and damaged (D'Amato et al. 2017; Pulimood
361 et al. 2007). In our study, we investigated the effect of “storm-like” conditions, e.g. presence
362 of *Cumulonimbus* clouds on Alt a 1 concentrations, but no significant relationships have been
363 observed. Also, episodes of rain were uncommon during the study period and so we could not
364 test the hypothesis linking occurrence of light rainfall (<1 mm) with increased level of
365 allergens (Schäppi et al. 1997a; Schäppi et al. 1997b).

366

367 *Alt a 1 in finest air fraction (PM 0.12-2.5 µm)*

368 Previous studies have shown that the concentration of the smallest fungal fragments (~1µm),
369 in comparison to spores, can be relatively high in the air (Reponen et al. 2007, Adhikari et al.
370 2009, Lee and Liao 2014). In these studies, the detection and enumeration of fungal fragments
371 was mainly based on the concentration of (1→3)-β-D-glucan, i.e. polysaccharide abundant in

372 fungal cell walls (Rylander 1999). Such methods do not, however, allow fragments belonging
373 to specific fungal taxa to be identified, so all fungal fragments were grouped and counted
374 together. When a molecular technique was applied, no sign of *Alternaria* DNA was found in
375 the PM_{2.5} fraction (Yamamoto et al. 2012). In the current study, we used a family-specific
376 allergen that occurs in *A. alternata* and other members of Pleosporaceae family (as Alt a 1
377 homologs) (Hong et al. 2005; Sáenz-de-Santamaría et al. 2006). Based on this detection
378 method, the total level of Alt a 1 in 0.12-2.5 µm air fraction was extremely low (1% of total
379 Alt a 1). This result concurs with pollen allergen studies where no allergens was found in the
380 PM_{2.5} fraction (Buters et al. 2010; Buters et al. 2012). Green et al. (2005b) postulated that the
381 amount of allergens released from a hyphal fragment might be a function of the critical
382 fragment size, which is the minimum size at which a fungal fragment remains viable. In the
383 study conducted by Górny et al. (2002) it was shown that immunoreactive fungal fragments of
384 *Aspergillus*, *Penicillium* and *Cladosporium* might be as small as 0.3µm. The critical sizes for
385 *Alternaria* species have not, however, been established. In addition, Buters et al. (2010)
386 showed that airborne pollen allergens of micronic size are easily absorbed by diesel soot
387 particles. It was postulated that this phenomenon could be responsible for the lack of pollen
388 allergens in micron-sized air fraction (de Weger et al. 2013). These studies and our results
389 suggest that the vast majority of immunoreactive airborne *Alternaria* particles belongs to
390 spores and larger fungal fragments.

391

392 **Conclusions**

393 Our study showed that the main source of airborne Alt a 1 in the outdoor environment are
394 intact *Alternaria* spores (app. 80%), which are deposited in both the PM_{>10} and PM_{2.5-10} air
395 fractions. The possible contribution of other fungal particles is the most visible in PM_{2.5-10},
396 where fungal fragments may be responsible for more than 30% of total Alt a 1. The amount of

397 allergen related to the finest fungal fragments (PM_{2.5}) is very low, almost negligible from
398 clinical and epidemiological point of view. This is important news, as the quantification of
399 *Alternaria* spores in the air (without mycelial fragments) is currently a routine practice in
400 many aerobiological laboratories. Our results suggest that such information could be used as a
401 relevant approximation of exposure to airborne Alt a 1 (based on very strong correlation
402 between spores and Alt a 1). Nevertheless, high variations in allergen content between
403 individual spores should also be considered to fully evaluate the exposure level to *Alternaria*
404 allergens.

405

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410

411 **Conflict of Interest**

412 The authors report no conflicts of interest. The authors alone are responsible for the content
413 and the writing of the paper.

414

415 **References**

416 Adhikari A, Jung J, Reponen T, Lewis JS, DeGrasse EC, Grimsley LF, Chew GL,
417 Grinshpun SA, 2009. Aerosolization of fungi, (1→3)-β-D-glucan, and endotoxin from

- 418 flood-affected materials collected in New Orleans homes. *Environmental Research*
419 **109(3)**:215-224.
- 420 Afanou KA, Straumfors A, Skogstad A, Nilsen T, Synnes O, Skaar I, Hjeljord L, Tronsmo A,
421 Green BJ, Eduard W, 2014. Submicronic Fungal Bioaerosols: High-Resolution
422 Microscopic Characterization and Quantification. *Applied and Environment*
423 *Microbiology*, **80(22)**: 7122-7130.
- 424 Afanou KA, Straumfors A, Skogstad A, Skaar I, Hjeljord L, Skare O, Green BJ, Tronsmo A,
425 Eduard W, 2015. Profile and Morphology of Fungal Aerosols Characterized by Field
426 Emission Scanning Electron Microscopy (FESEM). *Aerosol Science and Technology*,
427 **49(6)**: 423-435.
- 428 Agarwal MK, Swanson MC, Reed CE, Yunginger JW, 1983. Immunochemical quantitation of
429 airborne short ragweed, *Alternaria*, antigen E, and Alt-I allergens: a two-year
430 prospective study. *Journal of Allergy and Clinical Immunology*, **72(1)**: 40-45.
- 431 Amado M and Barnes C, 2016 Allergenic Microfungi and Human Health: A Review on
432 Exposure, Sensitization, and Sequencing Allergenic Proteins. *In Biology of*
433 *Microfungi*, Springer, pp. 429-449.
- 434 Andersson M, Downs S, Mitakakis TZ, Leuppi J, Marks G, 2003. Natural exposure to
435 *Alternaria* spores induces allergic rhinitis symptoms in sensitized children. *Pediatric*
436 *Allergy and Immunology*, **14**: 100-105.
- 437 Baranowski P, Jedryczka M, Mazurek W, Babula-Skowrońska D, Siedliska A, Kaczmarek J,
438 2015. Hyperspectral and thermal imaging of oilseed rape (*Brassica napus*) response to
439 fungal species of the genus *Alternaria*. *PLoS ONE*, **31(10(3))**: e0122913.
- 440 Bartnicki-Garcia S, Lippman E, 1972. The Bursting Tendency of Hyphal Tips of Fungi:
441 Presumptive Evidence for a Delicate Balance between Wall Synthesis and Wall Lysis
442 in Apical Growth. *Journal of General Microbiology*, **73**: 487-500.

- 443 Bednarz A, Pawłowska S, 2016. A fugal calendar for the atmosphere of Szczecin, Poland.
444 *Acta Agrobotanica* **69(3)**:1-9.
- 445 Bogawski P., Grewling Ł., Frątczak A. 2016. Flowering phenology and potential pollen
446 emission of three *Artemisia* species in relation to airborne pollen data in Poznań
447 (Western Poland). *Aerobiologia* **32**:265-276.
- 448 Burbach GJ, Heinzerling LM, Edenharter G, Bachert C, Bindslev-Jenses C, Bonini S,
449 Bousquet J, Bousquet-Rouanet L, Bousquet PJ, Bresciani M, Bruno A, Canonica GW,
450 Darsow U, Demoly P, Durham S, Fokkens WJ, Giavi S, Gjomarkaj M, Gramiccioni C,
451 Haahtela T, Kowalski ML, Magyar P, Murakozi G, Orosz M, Papadopoulos NG,
452 Rohnelt C, Stingl G, Todo-Bom A, Von Mutius E, Wiesner A, Wohrl S, Zuberbier T,
453 2009. GA2LEN skin test study II: clinical relevance of inhalant allergen sensitizations
454 in Europe. *Allergy*, **64**: 1507-1515.
- 455 Buters J, Prank M, Sofiev M, Pusch G, Albertini R, Annesi-Maesano I, Antunes C, Behrendt
456 H, Berger U, Bandao R, Celenk S, Galan Soldevilla C, Grewling Ł, Jackowiak B,
457 Kennedy R, Rantio Lehtimäki A, Resse G, Sauliene I, Smith M, Thibaudon M, Weber
458 B, Cecchi L., 2015. Variation of the group 5 grass pollen allergen content of airborne
459 pollen in relation to geographic location and time in season. *Journal of Allergy and*
460 *Clinical Immunology*, **136(1)**: 87-95.e6.
- 461 Buters J, Thibaudon M, Smith M, Kennedy R, Rantio Lehtimäki A, Albertini R, Reese G,
462 Weber B, Galán C, Brandao R, Antunes C, Jäger S, Berger U, Celenk S, Grewling Ł,
463 Jackowiak B, Sauliene I, Weichenmeier I, Pusch G, Sarioglu H, Ueffing M, Behrendt
464 H, Prank M, Sofiev M, Cecchi L, 2012. Release of Bet v 1 from birch pollen from 5
465 European countries. Results from the HIALINE study. *Atmospheric Environment*, **55**:
466 496-505.

- 467 Buters JT, Weichenmeier MI, Ochs S, Pusch G, Kreyling W, Boere AJF, Schober W,
468 Behrendt H, 2010. The allergen Bet v 1 in fractions of ambient air deviates from birch
469 pollen counts. *Allergy*, **65**: 850-858.
- 470 China S, Wang B, Weis J, Rizzo L, Brito J, Cirino GG, Kovarik L, Artaxo P, Gilles MK,
471 Laskin A, 2016. Rupturing of biological spores as a source of secondary particles in
472 Amazonia. *Environmental Science and Technology*, **50(22)**: 12179-12186.
- 473 Cho S-H, Seo S-C, Schmechel D, Grinshpun SA, Reponen T, 2005. Aerodynamic
474 characteristics and respiratory deposition of fungal fragments. *Atmospheric*
475 *Environment*, **39**: 5454-5465.
- 476 D'Amato G, Annesi-Maesano I, Molino A, Vitale C, D'Amato M, 2017. Thunderstorm-
477 related asthma attacks. *Journal of Allergy and Clinical Immunology*, **139(6)**: 1786-
478 1787.
- 479 Dagno K, Lahlali R, Diourte M, Jijakli MH, 2011. Effect of temperature and water activity on
480 spore germination and mycelial growth of three fungal biocontrol agents against water
481 hyacinth (*Eichhornia crassipes*). *Journal of Applied Microbiology*, **110(2)**: 521-528.
- 482 Da Silva HA, Divjan A, Dannemiller KC, Acosta LM, Miller RL, Green BJ, Perzanowski M,
483 2019. Correlations between Alt a 1 concentrations, *Alternaria alternata* and
484 Pleasporaceae measured by molecular methods in New York City house dust. *Journal*
485 *of Allergy and Clinical Immunology*, **AB122** Abstracts
- 486 de Weger LA, Bergmann KC, Rantio Lehtimäki A, Dahl A, Buters J, Dechamp C, Belmonte
487 J, Thibaudon M, Cecchi L, Besancenot J-P, Galan C, Waisel Y, 2013. Impact of
488 Pollen. In: M. Sofiev and K.C. Bergmann (Editors), *Allergenic Pollen. A Review of the*
489 *Production, Release, Distribution and Health Impacts* Springer, Dordrecht.

- 490 Demokritou P, Kavouras IG, Ferguson ST, Koutrakis P, 2002. Development of a High
491 Volume Cascade Impactor for Toxicological and Chemical Characterization Studies.
492 *Aerosol Science and Technology*, **36**: 925-933.
- 493 Denning DW, O'Driscoll R, Hogaboam CM, Bowyer P, Niven RM, 2006. The link between
494 fungi and severe asthma: a summary of the evidence. *European Respiratory Journal*,
495 **27**: 615-626.
- 496 Despres VR, Huffman JA, Burrows SM, Hoose C, Safatov AS, Buryak G, Frohlich-Nowoisky
497 J, Elbert W, Andreae MO, Poschl U, Jaenicke R, 2012. Primary biological aerosol
498 particles in the atmosphere: a review. *Tellus B: Chemical and Physical Meteorology*,
499 **64**(15598).
- 500 Dickinson CH, Bottomley D, 1980. Germination and Growth of *Alternaria* and *Cladosporium*
501 in Relation to Their Activity in the Phylloplane. *Trans Br Mycol Soc* **74**: 309-319.
- 502 Eisikowitch D, Woodell SRJ, 1974. The Effect of Water on Pollen Germination in Two
503 Species of *Primula*. *Evolution* **28**: 692-694.
- 504 Elbert W, Taylor PE, Andreae MO, Poschl U, 2007. Contribution of fungi to primary biogenic
505 aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and
506 inorganic ions. *Atmospheric Chemistry and Physics*, **7**(17): 4569-4588.
- 507 Feo Brito F, Alonso AM, Carnes J, Martin-Martin R, Fernandez-Caldas E, Galindo PA,
508 Alfaya T, Amo-Salas M, 2012. Correlation between Alt a 1 levels and clinical
509 symptoms in *Alternaria alternata*-monosensitized patients. *Journal of Investigational*
510 *Allergology and Clinical Immunology*, **22**(3): 154-159.
- 511 Frankel M, Hansen EW, Madsen AM, 2014. Effect of relative humidity on the aerosolization
512 and total inflammatory potential of fungal particles from dust-inoculated gypsum
513 boards. *Indoor Air*, **24**(1): 16-28.

- 514 Frohlich-Nowoisky J, Pickersgill DA, Despres VR, Poschl U, 2009. High diversity of fungi in
515 air particulate matter. *Proceedings of the National Academy of Sciences of the United*
516 *States of America*, **106(31)**: 12814-12819.
- 517 Galán C, Antunes C, Brandao R, Torres C, Garcia-Mozo H, Caeiro E, Ferro R, Prank M,
518 Sofiev M, Albertini R, Berger U, Cecchi L, Celenk S, Grewling Ł, Jackowiak B, Jäger
519 S, Kennedy R, Rantio-Lehtimäki A, Reese G, Sauliene I, Smith M, Thibaudon M,
520 Weber B, Weichenmeier I, Pusch G, Buters JTM, 2013. Airborne olive pollen counts
521 are not representative of exposure to the major olive allergen Ole e 1. *Allergy*, **68**:
522 809-812.
- 523 Garrido-Arandia M, Silva-Navas J, Ramirez-Castillejo C, Cubells-Baeza N, Gomez-Casado
524 C, Barber D, Pozo JC, Melendi PG, Pacios LF, Diaz-Perales A, 2016. Characterisation
525 of a flavonoid ligand of the fungal protein Alt a 1. *Scientific Reports*, **6**: 3346.
- 526 Gawińska-Urbanowicz H, Kapsa J, 2013. Monitoring results of population *Alternaria* genus
527 in potato crops. *Progress in Plant Protection* **53(3)**:527-532.
- 528 Górny RL, Reponen T, Willeke K, Schmechel D, Robine E, Boissier M, Grinshpun SA, 2002.
529 Fungal fragments as indoor air biocontaminants. *Applied and Environment*
530 *Microbiology*, **68(7)**: 3522-3531.
- 531 Green BJ, Mitakakis TZ, Tovey ER, 2003. Allergen detection form 11 fungal species before
532 and after germination. *Journal of Allergy and Clinical Immunology*, **111(2)**: 285-289.
- 533 Green BJ, Schmechel D, Sercombe JK, Tovey ER, 2005a. Enumeration and detection of
534 aerosolized *Aspergillus fumigatus* and *Penicillium chrysogenum* conidia and hyphae
535 using a novel double immunostaining technique. *Journal of Immunological Methods*,
536 **307**: 127-134.

- 537 Green BJ, Schmechel D, Tovey ER, 2005b. Detection of aerosolized *Alternaria alternata*
538 conidia, hyphae, and fragments by using a novel double-immunostaining technique.
539 *Clinical and Diagnostic Laboratory Immunology*, **12(9)**: 1114-1116.
- 540 Green BJ, Sercombe JK, Tovey ER, 2005c. Fungal fragments and undocumented conidia
541 function as new aeroallergen sources. *Journal of Allergy and Clinical Immunology*,
542 **115**: 1043-1048.
- 543 Green BJ, Tovey ER, Sercombe JK, Blachere FM, Beezhold DH, Schmechel D, 2006.
544 Airborne fungal fragments and allergenicity. *Medical Mycology*, **44(Suppl 1)**: 245-
545 255.
- 546 Grewling Ł, Bogawski P, Jenerowicz D, Czarnecka-Operacz M, Sikoparija B, Skjøth CA,
547 Smith M, 2016. Mesoscale atmospheric transport of ragweed pollen allergens from
548 infected to uninfected areas. *International Journal of Biometeorology*, **60(10)**: 1493-
549 1500.
- 550 Grewling Ł, Nowak M, Szymańska A, Kostecki Ł, Bogawski P, 2019. Temporal variability in
551 the allergenicity of airborne *Alternaria* spores. *Medical Mycology*, **4**: 403-411.
- 552 Grewling Ł, Bogawski P, Kostecki Ł, Nowak M, Szymańska A, Frątczak A, 2020.
553 Atmospheric exposure to the major *Artemisia* pollen allergen (Art v 1): Seasonality,
554 impact of weather, and clinical implications. *Science of the Total Environment* **713**:
555 136611.
- 556 Grinn-Gofroń A, Bosiacka B, 2015. Effects of meteorological factors on the composition of
557 selected fungal spores in the air. *Aerobiologia*, **31(1)**: 63-72.
- 558 Grote M, Valenta R, Reichelt R, 2003. Abortive pollen germination: A mechanism of allergen
559 release in birch, alder, and hazel revealed by immunogold electron microscopy.
560 *Journal of Allergy and Clinical Immunology*, **111(5)**: 1017-1023.

- 561 Gutierrez-Rodriguez A, Postigo I, Guisantes JA, Sunen E, Martinez J, 2011. Identification
562 of allergens homologous to Alt a 1 from *Stemphylium botryosum* and *Ulocladium*
563 *botrytis*. *Medical Mycology* **49**:892–896
- 564 Hatzipapis P, Kalosak K, Dara A, Christias C, 2002. Spore germination and appressorium
565 formation in the entomopathogenic *Alternaria alternata*. *Mycological Research*,
566 **106(11)**: 1349-1359.
- 567 Hirst JM, 1952. An automatic volumetric spore trap. *The Annals of Applied Biology* **39**: 257-
568 265.
- 569 Hjelmroos M, 1993. Relationship between airborne fungal spore presence and weather
570 variables *Grana*, **32**: 40-47.
- 571 Hoidn C, Puchner E, Pertl H, Holztrattner E, Obermeyer G, 2005. Nondiffusional release of
572 allergens from pollen grains of *Artemisia vulgaris* and *Lilium longiflorum* depends
573 mainly on the type of the allergen. *International Archives of Allergy and Immunology*,
574 **137**: 27-36.
- 575 Hong SG, Cramer RA, Lawrence CB, Pryor BM, 2005. Alt a 1 allergen homologs from
576 *Alternaria* and related taxa: analysis of phylogenetic content and secondary structure.
577 *Fungal Genetic and Biology* **42(2)**: 119-129.
- 578 Jajor E, Kozłowska M, Wójtowicz M, 2012. Prevalence of fungi of the genus *Alternaria* on
579 rape siliques and seeds depending on weather conditions. *Progress in Plant*
580 *Protection* **52(4)**:1011-1015.
- 581 Kasprzyk I, Sulborska A, Nowak M, Szymańska A, Kaczmarek J, Haratym W, Weryszko-
582 Chmielewska E, Jędryczka M, 2013. Fluctuation range of the concentration of
583 airborne *Alternaria* conidiospores sampled at different geographical locations in
584 Poland (2010-2011). *Acta Agrobotanica* **66(1)**: 65-76.

- 585 Kasprzyk I, Rodinkova V, Šauliene I, Ritenberga O, Grinn-Gofroń A, Nowak M, Sulborska
586 A, Kaczmarek J, Weryszko-Chmielewska E, Bilous E, Jędryczka M, 2015. Air
587 pollution by allergenic spores of the genus *Alternaria* in the air of central and eastern
588 Europe. *Environmental Science and Pollution Research* **22**: 9260-9274.
- 589 Kassambara A, Mundt F, 2017. *Factoextra: Extract and visualize the results of multivariate*
590 *data analyses* (1.0.4 ed.). R package; CRAN. Retrieved from
591 <http://www.sthda.com/english/rpkgs/factoextra>
- 592 Kim KY, Kim YS, Kim D, 2010. Distribution characteristics of airborne bacteria and fungi in
593 the general hospitals of Korea. *Industrial Health* **48**: 236-243.
- 594 Knutsen AP, Bush RK, Demain JG, Denning DW, Dixit A, Fairs A, Greenberger PA, Kariuki
595 B, Kita H, Kurup VP, Moss RB, Niven RM, Pashley CH, Slavin RG, Vijay HM,
596 Wardlaw AJ, 2012. Fungi and allergic lower respiratory tract diseases. *Journal of*
597 *Allergy and Clinical Immunology*, **129(2)**: 280-91.
- 598 Kuhn M, 2008. Building predictive models in R using the caret package. *Journal of Statistical*
599 *Software* **28(5)**: 1-26.
- 600 Lacey ME, West JS, 2006. *The Air Spora. A manual for catching and identifying airborne*
601 *biological particles*. Springer, Dordrecht, The Netherlands.
- 602 Lawler MJ, Draper DC, Smith JN, 2020. Atmospheric fungal nanoparticle bursts. *Science*
603 *Advances* **6**: eaax9051.
- 604 Lê S, Josse J, Husson F, 2008. FactoMineR: An R Package for Multivariate Analysis. *Journal*
605 *of Statistical Software*. **25(1)**:1-18
- 606 Lee S-A, Liao C-H, 2014. Size-selective assessment of agricultural workers' personal
607 exposure to airborne fungi and fungal fragments. *Science of the Total Environment*
608 **466-467**:725-732.

- 609 Levetin E, Owens C, Weaver H, Davis W, 2009. Airborne fungal fragments: are we
610 overlooking an important source of aeroallergens? *Journal of Allergy and Clinical*
611 *Immunology*, **123(2)**: S231.
- 612 Madsen AM, 2012. Effects of Airflow and Changing Humidity on the Aerosolization of
613 Respirable Fungal Fragments and Conidia of *Botrytis cinerea*. *Applied and*
614 *Environment Microbiology*, **78(11)**: 3999-4007.
- 615 Madsen AM, Larsen ST, Koponen IK, Kling KI, Barooni A, Karottki DG, Tendal K, Wolkoff
616 P, 2016. Generation and Characterization of Indoor Fungal Aerosols for Inhalation
617 Studies. *Applied and Environment Microbiology*, **82(8)**: 2479-2493.
- 618 Magyar D, Vass M, Li DW, 2016. Dispersal Strategies of Microfungi. In: Li DW. (eds)
619 *Biology of Microfungi*. Fungal Biology. Springer, Cham.
- 620 Mao Y-Y, Huang S-Q, 2009. Pollen resistance to water in 80 angiosperm species: Flower
621 structures protect rain-susceptible pollen. *New Phytologist* **183**: 892-899.
- 622 Maya-Manzano JM, Munoz-Trivino M, Fernandez-Rodriguez S, Silva-Palacios I, Gonzalo-
623 Garijo A, Tormo-Molina R, 2016. Airborne *Alternaria* conidia in Mediterranean rural
624 environments in SW of Iberian Peninsula and weather parameters that influence their
625 seasonality in relation to climate change. *Aerobiologia* **32(1)**: 95-108.
- 626 McCartney HA, Schmechel D, Lacey ME, 1993. Aerodynamic diameter of conidia of
627 *Alternaria* species. *Plant Pathology*, **42(2)**: 280-286.
- 628 Mitakakis TZ, Barnes C, Tovey ER, 2001. Spore germination increases allergen release from
629 *Alternaria*. *Journal of Allergy and Clinical Immunology*, **107**: 388-390.
- 630 Nicholson KW, 1995. Physical Aspects of Bioaerosol Sampling and Deposition. In: Ch.S.
631 Cox and Ch.M. Wathes (Editors), *Bioaerosols Handbook*. Lewis Publishers, Boca
632 Raton.

- 633 Ogórek E, Płaskowska E, Kalinowska K, 2011. Characteristics and taxonomy of *Alternaria*
634 *fungi*. *Mikologia Lekarska* **18(3)**: 150-155.
- 635 Pady SM, Gregory PH, 1963. Numbers and viability of airborne hyphal fragments in England.
636 *Transactions of the British Mycological Society* **46(4)**: 609-613.
- 637 Pasanen A-L, Pasanen P, Jantunen MJ, Kalliokoski P, 1991. Significance of air humidity and
638 air velocity for fungal spore release into the air. *Atmospheric Environment* **25A(2)**:
639 459-462
- 640 Pulimood TB, Corden J, Bryden C, Sharples L, Nasser S, 2007. Epidemic asthma and the role
641 of the fungal mold *Alternaria alternata*. *Journal of Allergy and Clinical Immunology*,
642 **120(3)**: 610-617.
- 643 R Core Team, 2018. *R: A language and environment for statistical computing*, Vienna,
644 Austria.
- 645 Reponen T, Grinshpun SA, Conwell KL, Wiest J, Anderson M, 2001. Aerodynamic versus
646 physical size of spores: Measurement and implication for respiratory deposition.
647 *Grana*, **40**: 119-125.
- 648 Reponen T, Seo S-C, Grimsley F, Lee T, Crawford C, Grinshpun SA, 2007. Fungal fragments
649 in moldy houses: a field study in homes in New Orleans and Southern Ohio.
650 *Atmospheric Environment* **41(37)**:8140-8149
- 651 Rick EM, Woolnough K, Pashley CH, Wardlaw AJ, 2016. Allergic fungal airway disease.
652 *Journal of Investigational Allergology and Clinical Immunology*, **26(6)**: 344-354.
- 653 Rittenour WR, Park J-H, Cox-Ganser JM, Beezhold DH, Green BJ, 2012. Comparison of
654 DNA extraction methodologies used for assessing fungal diversity via ITS sequencing.
655 *Journal of Environmental Monitoring*, **14(3)**: 766-774.
- 656 Rylander R, 1999. Indoor air-related effects and airborne (1→3)-β-D-glucan. *Environmental*
657 *Health Perspective* **107(suppl 3)**:501-503.

- 658 Saenz-de-Santamaria M, Postigo I, Gutierrez-Rodriguez A, Cardona G, Guisantes JA,
659 Asturias J, Martinez J, 2006. The major allergen of *Alternaria alternata* (Alt a 1) is
660 expressed in other members of the Pleosporaceae family. *Mycoses* **49(2)**:91-95
- 661 Sayer WJ, Shean DB, Ghosseiri J, 1969. Estimation of airborne fungal flora by the Andersen
662 sampler versus the gravity settling culture plate. *Journal of Allergy*, **44(4)**: 214-227.
- 663 Šcevkova A, Kovac J, 2019. First fungal spore calendar for the atmosphere of Bratislava,
664 Slovakia. *Aerobiologia* **35**: 343-356
- 665 Schäppi GF, Suphioglu C, Taylor PE, Knox RB, 1997a. Concentrations of the major birch
666 tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere. *Journal*
667 *of Allergy and Clinical Immunology*, **100(5)**: 656-61.
- 668 .
- 669 Schäppi GF, Taylor PE, Staff IA, Suphioglu C, Knox RB, 1997b. Source of Bet v 1 loaded
670 inhalable particles from birch released. *Sex Plant Reproduction*, **10**: 315-323.
- 671 Schäppi GF, Taylor PE, Pain MCF, Cameron PA, Dent AW, Staff IA, Suphioglu C, 1999.
672 Concentrations of major grass group 5 allergens in pollen grains and atmospheric
673 particles: implications for hay fever and allergic asthma sufferers sensitized to grass
674 pollen allergens. *Clinical and Experimental Allergy*, **29**: 633-641
- 675 Sercombe JK, Green BJ, Tovey ER, 2006. Recovery of germinating fungal conidia from the
676 nasal cavity after environmental exposure. *Aerobiologia*, **22**: 295-304.
- 677 Simmons EG, 2007. *Alternaria. An identification manual*. CBS Fungal Biodiversity Series,
678 Utrecht, Netherlands.
- 679 Stennett PJ, Beggs PJ, 2004. *Alternaria* spores in the atmosphere of Sydney, Australia, and
680 relationships with meteorological factors. *International Journal of Biometeorology*,
681 **49**: 98-105.

- 682 Stull RB, 1985. A Fair-Weather Cumulus Clouds Classification Scheme For Mixed Layer
683 Studies. *Journal of Climate and Applied Meteorology* **24**: 49-56
- 684 Sweeney M, Kroutil L, Recht M, Bush R, 1985. Kinetics of allergen release from alternaria
685 (ALT). *Journal of Allergy and Clinical Immunology*, **75(1)**: 118.
- 686 Taylor PE, Flagan RC, Valenta R., Glovsky MM, 2002. Release of allergens as respirable
687 aerosols: A link between grass pollen and asthma. *Journal of Allergy and Clinical*
688 *Immunology*, **109(1)**: 51-56.
- 689 Troutt C, Levetin E, 2001. Correlation of spring spore concentrations and meteorological
690 conditions in Tulsa, Oklahoma. *International Journal of Biometeorology*, **45**: 64-74.
- 691 Twaroch T, Arcalis E, Sterflinger K, Stoger E, Swoboda I, Valenta R, 2012. Predominant
692 localization of the major *Alternaria allergen* Alt a 1 in the cell wall of airborne spores.
693 *Journal of Allergy and Clinical Immunology*, **129(4)**: 1148-1149.
- 694 Twaroch T, Curin M, Valenta R, Swoboda I, 2015. Mold allergens in respiratory allergy: from
695 structure to therapy. *Allergy, Asthma & Immunology Research*, **7(7)**: 205-220.
- 696 Vloutoglou I, Fitt BDL, Lucas JA, 1996. Germination of *Alternaria linicola* conidia on
697 linseed: effects of temperature, incubation time, leaf wetness and light regime. *Plant*
698 *Pathology (Oxford)*, **45(3)**: 529-539.
- 699 Wei T, Simko V, 2017. R package “corrplot”: *Visualization of a Correlation Matrix* (Version
700 0.84). Available from <https://github.com/taiyun/corrplot>
- 701 Womiloju TO, Miller JD, Mayer PM, Brook JR, 2003. Methods to determine the biological
702 composition of particulate matter collected from outdoor air. *Atmospheric*
703 *Environment*, **37(31)**: 4335-4344.
- 704 Woo Ch, An Ch, Xu S, Yi S-M, Yamamoto N, 2018. Taxonomic diversity of fungi deposited
705 from the atmosphere. *The ISME Journal*, **12**: 2051-2060.

- 706 Yamamoto N, Bibby K, Qian J, Hospodsky D, Rismani-Yazdi H, Nazaroff WW, Peccia J,
707 2012. Particle-size distributions and seasonal diversity of allergenic and pathogenic
708 fungi in outdoor air. *The ISME Journal*, **6**: 1801-1811.
- 709 Yamamoto N, Nazaroff WW, Peccia J, 2014. Assessing the aerodynamic diameters of taxon-
710 specific fungal bioaerosols by quantitative PCR and next-generation DNA sequencing.
711 *Journal of Aerosol Science*, **78**: 1-10.

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712 Figures

713 Fig. 1. Distribution of Alt a 1 in three investigated air fractions. *Alternaria* spores
714 concentration (line curve) collected using Hirst type volumetric trap (samples description in
715 Table S1).

716 Figure 2. Ratio (%) between the concentrations of Alt a 1 (pg/m^3) and *Alternaria* spores
717 (spore/m^3) in selected air fractions to the total levels of Alt a 1 and total *Alternaria* spores.
718 The statistically significant differences between spores and Alt a 1 levels in three air fractions
719 are marked by asterisks, i.e. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

720 Figure 3. Correlations between Alt a 1 concentrations and *Alternaria* spore levels in selected
721 air fractions.

722 Figure 4. Correlations matrix showing relationships between daily weather parameters and
723 both *Alternaria* spores (collected by Hirst trap) and Alt a 1 in three investigated fractions of
724 particulate matter (statistically significant correlations with $p < 0.05$ are in bold).

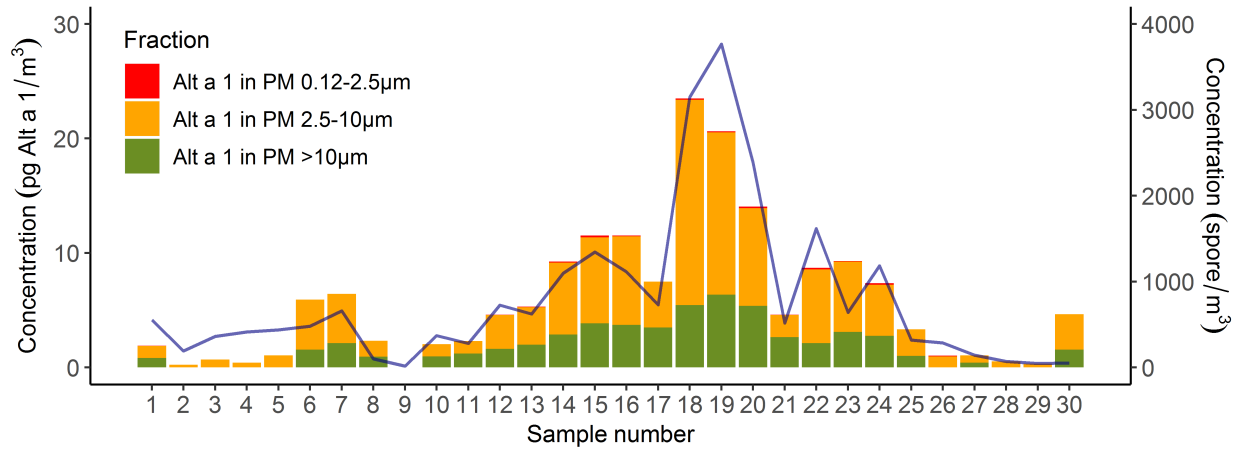
725 Figure 5. Correlations matrix showing relationships between daily weather parameters and
726 ratio in the level of Alt a 1 (or spores) between investigated air fraction of particulate matter
727 (statistically significant correlations with $p < 0.05$ are in bold).

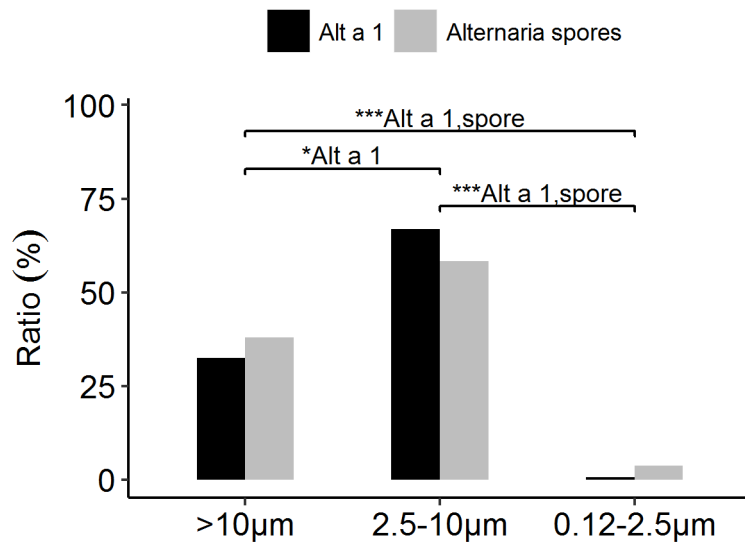
728 Figure 6. Principal component analysis, using weather data collected during sampling period,
729 for Alt a 1 concentration levels (ellipses represent the 90% confidence interval of selected
730 groups). Additional information of PCA analysis in Suppl. Materials, Fig. S2 & S3.

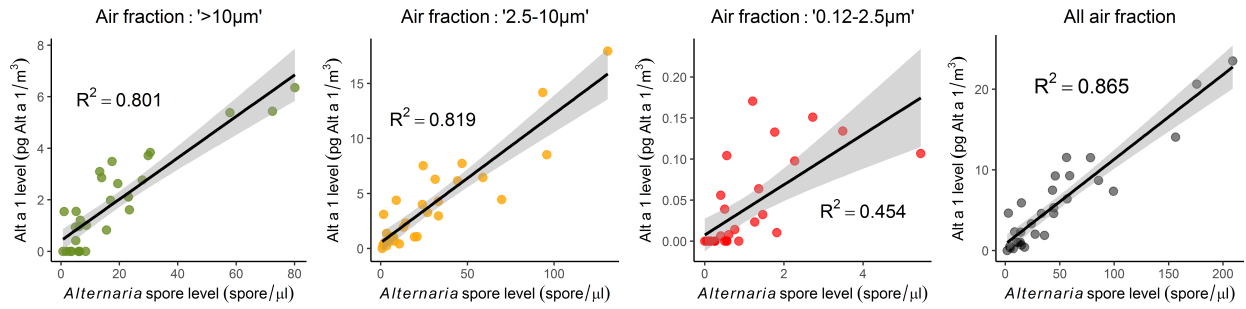
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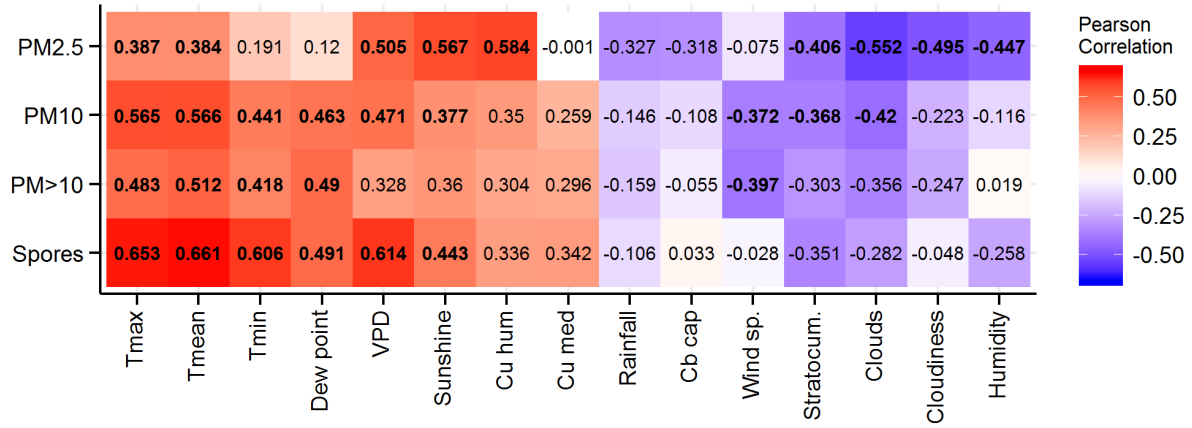
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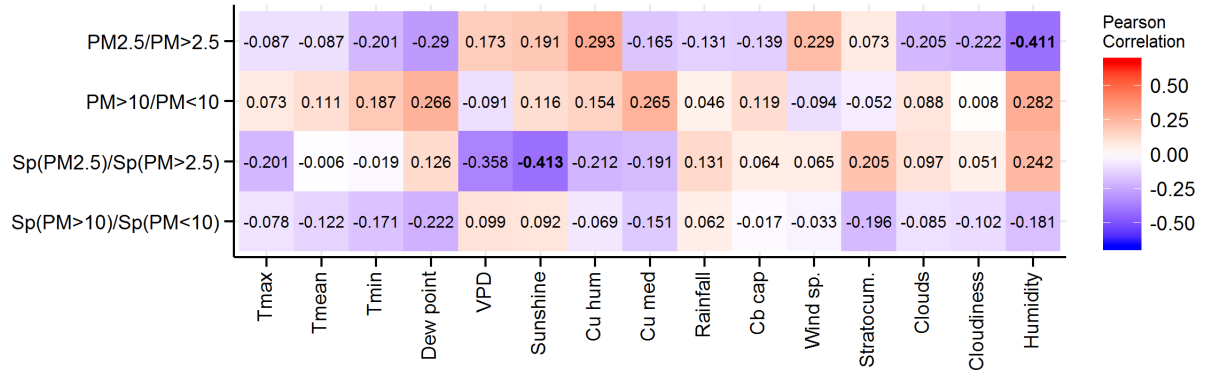


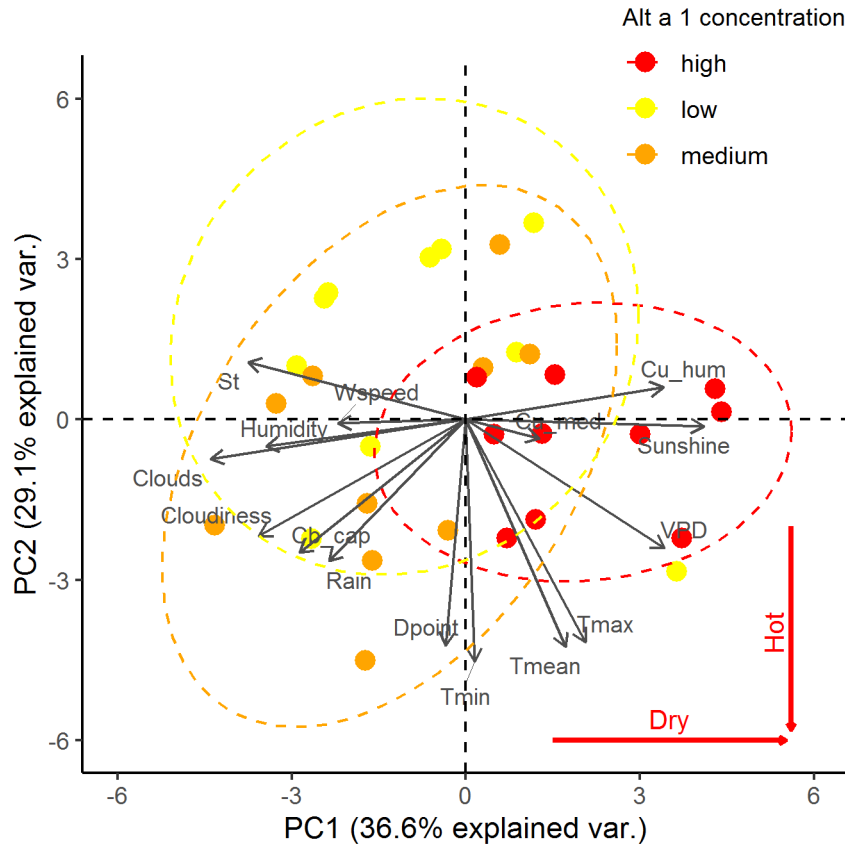




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Highlights

1. Alt a 1 (major allergen of *Alternaria*) was quantified in different air fractions
2. *Alternaria* spores and Alt a 1 levels correlated significantly ($r=0.930$, $p<0.001$)
3. The highest Alt a 1 level was detected in $PM_{2.5-10}$, while the lowest in $PM_{2.5}$
4. Significantly more Alt a 1 per spore (31.3%) was observed in $PM_{2.5-10}$ than in $PM_{>10}$
5. Spores are the main source of Alt a 1, but the impact of hyphae cannot be neglected