Airborne fungal spore monitoring: between analyst proficiency testing

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Abstract

This study presents the results of a Europe-wide training and Quality Control (QC) exercise carried out within the framework of the European Aerobiology Society's QC Working Group. The main aim of this exercise was to examine the feasibility of carrying out a QC exercise for fungal spore monitoring in Europe, using a similar methodology to the one previously used for pollen. The QC survey was conducted in two parts: (1) Coordinators of national and regional aerobiological networks in Europe involved in the monitoring of atmospheric fungal spores were invited to complete a questionnaire survey related to their network and asked whether they were interested in taking part in an external inter-laboratory QC exercise; (2) Participating networks performed an inter-laboratory ring test with the same sample slide in order to determine the reproducibility of identifying and counting two fungal spore taxa (*Alternaria* and *Epicoccum*) in air samples collected by a Hirst-type volumetric spore trap. Participants were instructed to read five separate longitudinal transects in the "effective collecting area" of the slide. Reproducibility of analysis was determined following the method previously used in the European Aerobiology Society's QC exercises for pollen. Thirty-two counters from 16 national or regional networks in Europe participated in the QC exercise. Coefficients of Variation (CV%) ranged from 23.0 to 22.5 when reading one transect and from 14.0 to 16.0 when reading five

transects for *Alternaria* and *Epicoccum*, respectively. Considering a CV% of 30 as the limit for fungal spores, no significant differences were observed between the absolute errors from two, three, four and five transects. The only significant difference was between one and five transects. We recommend that fungal spore analysis should be carried out on about 5% of the slide (two transects in this study) because results were not significantly different to five transects.

1. Introduction

Volumetric spore traps of the Hirst (1952) design are traditionally used by most aerobiological monitoring networks across the world (Buters et al. 2018). The samples are analysed by optical microscopy, which is both time consuming and requires a high degree of training in order to accurately count and identify airborne pollen and fungal spores. Guaranteeing the quality of environmental data is crucial for ensuring the applicability and scientific value of those data, and so the Quality Control and Quality Assurance of aerobiological data is seen as a priority of many established national aerobiological monitoring networks (e.g. www.ilpolline.it, www.pollens.fr/, www.uco.es/rea/ - correct as of 30-08-2020). This interest in ensuring the quality of aerobiological data has produced several publications on the topic, e.g. Oteros et al. (2013) for the Spanish Aerobiology Network (REA), Smith et al. (2019) for the QC of pollen data collected during the construction of the automatic pollen monitoring network (ePIN) in Bavaria, Germany, and the recent pilot study presented by Milic et al. (2020) for the AusPollen Aerobiology Collaboration Network (AusPollen) in Australia.

The European Aerobiology Society (EAS) is one of the largest worldwide interdisciplinary scientific societies (http://www.eas-aerobiology.eu) focused on the topic of aerobiology. The EAS Working Group on Quality Control (QC) was created in 2008 and since then it has undertaken several initiatives including the Minimum Requirements for setting up of new pollen monitoring stations (Galán et al. 2014) and the implementation of several large-scale interlaboratory proficiency tests for bio-monitoring networks in Europe (Galán et al. 2014, Šikoparija et al. 2017, Smith and Šikoparija 2020). Such large-scale proficiency tests can be problematical to implement in terms of logistics and can take a long time to conduct from beginning to end, e.g. the QC exercise carried out for *Ambrosia* pollen described by Šikoparija et al. (2017) took a total of 531 days. For this reason, several studies have examined how the QC process can be expedited, such as Smith et al. (2019) who proposed an abbreviated method for the QC of pollen counters involved in short term projects and Smith and Šikoparija (2020) who

recently conducted a feasibility study for carrying out interlaboratory proficiency tests in aerobiology using virtual slides.

This international interest in the Quality Control and Quality Assurance of aerobiological data, and the need for comparable data between networks, has led to further international cooperation and the publication of the norm EN 16868:2019 (CEN, 2019) for *Ambient Air, Sampling and analysis of airborne pollen grains and fungal spores for networks related to allergy - Volumetric Hirst method*. This norm focuses on the need for a higher quality level of analysis and for a standardization of procedures, presenting performance requirements, i.e.: repeatability; performance recommendations, reproducibility and accuracy; and sensitivity and specificity.

Eukaryotic organisms belonging to the Fungi Kingdom commonly produce spores that are transported by the wind in very high concentrations. Fungi are more diverse than seed plants and there is a lack of knowledge concerning the taxonomic diversity of the Kingdom, which makes it difficult to estimate the number of species. As a result, the morphology of airborne fungal spores is more variable than pollen grains (Mueller & Schmit 2007). This diversity is related to size, wall characteristics and shape, as well as the number of cells, type of septation, attachment scars, and spore colour. Consequently, fungal spores are much more difficult to identify than pollen and so fewer sites routinely monitor their presence in outdoor air. For pollen, a minimum of 10% of the sample produced by traditional Hirst type traps has been recommended (Galán et al. 2014). However, due to the high atmospheric concentrations and morphological diversity, counters usually examine a much lower percentage of the slide when examining fungal spores.

There is currently a dearth of literature concerned with studies examining the quality of fungal spore data. Therefore, the main aims of this study are to examine the feasibility of carrying out a QC exercise for fungal spore monitoring in Europe, using a similar methodology previously used for pollen, and to define the requirements necessary for ensuring a minimum of quality of the data (e.g. the minimum subsampling area of standard Hirst samples). This was achieved by: (1) Conducting a questionnaire survey of monitoring networks involved in the monitoring of atmospheric fungal spores; (2) Performing an inter-laboratory ring test with the same sample slide in order to determine reproducibility of identifying and counting of two fungal spore taxa (i.e. *Alternaria* and *Epicoccum*) present in air samples collected by the Hirst-type trap. This study represents the results of a Europe-wide training and Quality Control (QC) exercise for fungal spores, carried out within the framework of the European Aerobiology Society's Working Group on Quality Control.

2. Materials and methods

2.1 Questionnaire survey

Coordinators of different national or regional aerobiological networks in Europe were contacted and invited to answer the following questions:

(a) What is the number of laboratories in your network working on fungal spores?
(b) Is your network following the minimum requirements proposed by the QC EAS working group?
(c) If the answer to question "b" was "NO", please specify which part of the minimum requirements are not followed?
(d) Are you interested in carrying out an external internetwork QC survey for fungal spore counting and identification?
(e) Is your network involved in internal QC surveys for fungal spore counting and identification?
(f) If yes, what kind of QC survey do you undertake?
(g) Would you be interested in certification from the EAS QC working group?

A questionnaire was subsequently sent to all networks interested in participating in an external internetwork QC survey for fungal spore counting and identification with general questions related to: (a) Name of spore counter(s); (b) Sampling height above ground level (m); (c) Sampling height above sea level (m); (d) List of fungal spores identified; (e) Expected annual sampling period: start and end dates.

Further information was requested concerning sample preparation and counting (Galán et al. 2014): (a) Adhesive medium; (b) Mounting medium; (c) Possession of a written manual for sample preparation and pollen analysis; (d) Microscope magnification; (e) Sampling time per slide (e.g. 0 - 24h, 8 - 8h); (f) Counting method (e.g. horizontal or vertical transects); (g) Daily, hourly or bi-hourly counts; (h) Number of lines; (i) Field of view; (j) Area counted per slide; (k) How unidentified spores are dealt with.

2.2 Between participant reproducibility

A QC exercise examining between participant reproducibility was conducted, for which two fungal spore types were selected: *Alternaria* and *Epicoccum*. Both fungal spore types commonly occur in the atmosphere in concentrations that are not excessively high, as very high airborne concentrations can generate errors when counting (Galán et al. 2014, Šikoparija et al. 2017). Although common, *Alternaria* spores are notoriously variable in terms of morphology and so this QC exercise also examined how accurately they could be identified. The slide used for the

between participant reproducibility test was from Szczecin, Poland (28/07/2017). The selected slide was considered to contain ‘‘moderate’’ concentrations of both *Alternaria* and *Epicoccum* spores, i.e. no more than a daily average of 300 spore/m^3, as described by Galán et al. (2014) in the previous QC exercise for pollen.

In order to aid identification, participants received training material describing similar airborne fungal spore types seen in the atmosphere, a spore identification key and slides with spores from pure cultures of *Alternaria* and *Epicoccum*. The training material was provided by the Department of Air Hygiene and Aerobiology, National Public Health Centre, Budapest, Hungary; the Institute of Botany, University of Szczecin, Poland; and the Department of Biology and Environmental Protection, University of Rzeszow, Poland.

A maximum of three counters per network were invited to read the slide using the following protocol:

- Read 5 longitudinal (not vertical) transects separately.
- The 5 longitudinal transects must be counted in the “effective collecting area” which is in the middle of a 14mm tape.
- Report every transect separately in the table provided.
- Fill in all variables that appear in the table provided: (a) volumetric counts of fungal spores per cubic meter; (b) field of view; (c) % of the slide examined *per* transect; (d) conversion factor (bi-hourly); (e) conversion factor (daily).

It was recommended that the slide should be examined using 400X (40x10) magnification to aid the identification of fungal spores. However, counters could use different magnifications depending on the microscope being used, and they were asked to provide this information when presenting their results.

Between participant reproducibility of analysis was determined following the method previously used by the REA (Oteros et al. 2013) and by the EAS (Galán et al. 2014). The normality of the data distribution was studied by using the Lilliefors test (Starink and Visser 2010) and outliers were identified following ISO confidence levels for statistical outliers (ISO 5725-1:1994). Outliers were calculated by transforming the raw data to z scores and eliminating the outer 5% of the data.

Daily average fungal spore concentrations produced by counters were compared with an assigned value, considering the average data of central 95% and omitting outliers calculated by z scores. The confidence limits (CL) of the assigned value was defined as the “true value” assuming an acceptable error, i.e. the true value lies between the upper limit (UL) and the lower limit (LL) with 95% probability (Abraira 2002a, 2002b).

The coefficient of variation (CV) has been calculated considering the assigned value and the standard variation, and accepting the following criteria proposed by Galán et al. (2014) when referring to different pollen or spore concentrations: considering <15 in the case of an assigned value in the range 100-300 for *Alternaria*, and considering CV <20 in the case of assigned value in the range 25-100 for *Epicoccum*. Absolute Error (AE) and the Relative Error (RE) were calculated for each counter and separately for each identified spore type. We also considered other errors: the number of erroneous elements (NEE) as the number of counters that committed significant errors (it was considered a significant error when RE>30 % and AE>10); the percentage of erroneous elements (PEE) as the percentage of NEE with respect to the total; the average of relative error (ARE) as the average of the RE committed by each participant; the average of absolute error (AAE) as the average of the AE committed by every participant.

3. Results

3.1 Survey results:

A total of 17 invitations were sent to participating national or regional networks in Europe, with experience in identifying fungal spores. A total of 16 networks showed interest in carrying out an external inter-laboratory QC survey for fungal spore counting and identification. Only 3 networks conducted an internal QC exercise for fungal spore identification and counting; one during national courses and the others by checking slides. Fifteen networks have shown interest in a certification from the EAS QC working group. We did not receive a clear response from the networks regarding the number of laboratories *per* network involved on fungal spore analysis.

The following networks have participated in the QC external exercise: Austria (Austrian Pollen Information Service, APIS), Belgium (Belgian Aerobiological Surveillance Network, BASN), Croatia (Public Health Croatia, PHC), Estonia (Estonian Environmental Research Centre), France (National Network of Aerobiological Surveillance, RNSA), Germany (German Foundation Pollen Information Service, FGPIS); Hungary (National Public Health Center); Italy (Italian Aerobiological Monitoring Network, POLLnet and Italian Association for Aerobiology, AIA); Latvia (University of Latvia, LU), Lithuania (Environmental Research Group), Poland (Polish Aerobiological Network, PAN), Serbia (Laboratory for palynology, LP), Slovakia (Slovak Aerospore Information Service, SAIS), Spain (Spanish Aerobiology Network, REA), Turkey (Aerobiological Network of Turkey, ANT), Ukraine (Ukrainian Aerobiology Network).

Regarding the questionnaire sent to the networks interested in participating in this QC exercise (n = 16), results show that 7 networks (43.75%) did not follow the minimum

requirements proposed by the QC EAS working group (Galán et al. 2014), as these networks examined less than 10% of the slide; 1 network did not answer. Only eight networks (50%) monitor the whole year. Seven networks (43.75%) identify $\leq$ 5 fungal spore types; five networks (31.25%) identify between 6 and 15 different fungal spore types; two networks (12.5%) identify more than 20 fungal spore types; and 2 networks did not answer to this question. Presenting the fungal spore list, we have included in brackets the identification frequency of each spore taxon as cited by the different networks. All networks that count fungal spores examine *Alternaria* (n = 16), the second most cited was *Cladosporium* (n = 14) and the third *Epicoccum* (n = 10). Other spores: *Arthrinium* (n = 1), *Agrocybe* (n = 1), *Ascobolus* (n = 1), ascospores (n = 2), *Aspergillus/Penicillium* (n = 3), *Asperisporium* (n = 1), basidiospores (n = 1), *Botrytis* (n = 4), *Bovista* (n = 1), *Caloplaca* or *Xanthoria* (n = 1), *Cercospora* (n = 1), *Chaetomium* (n = 1), *Coprinus* (n = 2), *Curvularia* (n = 2), Diatrypaceae (n = 1), *Didymella* (n = 4), *Drechslera* or *Helminthosporium* (n = 4), *Exosporium* (n = 1), *Fusarium* (n = 2), *Fusicladium* (n = 1), *Ganoderma* (n = 3), *Gliomastix* (n = 1), *Leptosphaeria* type (n = 2), *Melanospora* (n = 1), Myxomycetes (n = 1), *Nigrospora* (n = 2), *Oidium* (n = 5), *Oncopodiella* (n = 1), *Panaeolus* (n = 1), *Paraphaeosphaeria* (n = 1), *Periconia* (n = 3), *Peronospora* (n = 3), *Pestalotiopsis* (n = 1), *Pithomyces* (n = 4), *Pleospora* (n = 5), *Polythrincium* (n = 4), *Puccinia* (n = 1), *Stemphylium* (n = 6), *Sporormiella* (n = 1), *Tetraploa* (n = 1), *Tilletia* (n = 1), Telephoraceae (n = 1), *Torula* (n = 6), *Urocystis agropyri* (n = 1).

Concerning the questions regarding the preparation and counting of the samples, the networks provided the following information:

- Adhesive medium - 8 networks used vaseline and 8 silicon fluid;
- Mounting medium - 10 used glycerine-gelatine and 6 polyvinyl alcohol (e.g. Gelvatol or Mowiol);
- Protocols - All 16 networks count with a written manual for sample preparation and pollen analysis, but none have a specific manual for fungal spores;
- Magnification - All 16 used x40 magnification, one of them x40 and x25, and another x40 and x100;
- Sampling time - 12 networks examined slides 0:00-24:00, 2 examined slides 8:00-8:00, and 3 networks used both sampling times;
- 13 networks use horizontal transects as the counting method: almost half of them (n = 7) with 3 or 4 transects and the rest with 1-2 transects, but one of this last group read 4 transects after they have initially found low fungal spore concentrations <10-20 spore/m^3 on the slide;
- Two networks use vertical transects, one with 10 and one with 12 transects;
- 7 networks record bi-hourly counts, 6 daily counts, and 3 both bi-hourly and daily counts. 6 networks record the figure for unidentified spores, but the rest no count all spores.

3.2 QC Between participant reproducibility

Lilliefors test showed that in this Quality Control exercise for between participant reproducibility, the data were normally distributed, and outliers were identified following ISO (ISO 5725-1:1994). Figure 1 shows the absolute concentrations reported by each participant in the study. The dot-dashed orange line is the absolute value deviating 20% from the average (20% RE) and the red line is the absolute value deviating 30% from the average (30% RE).

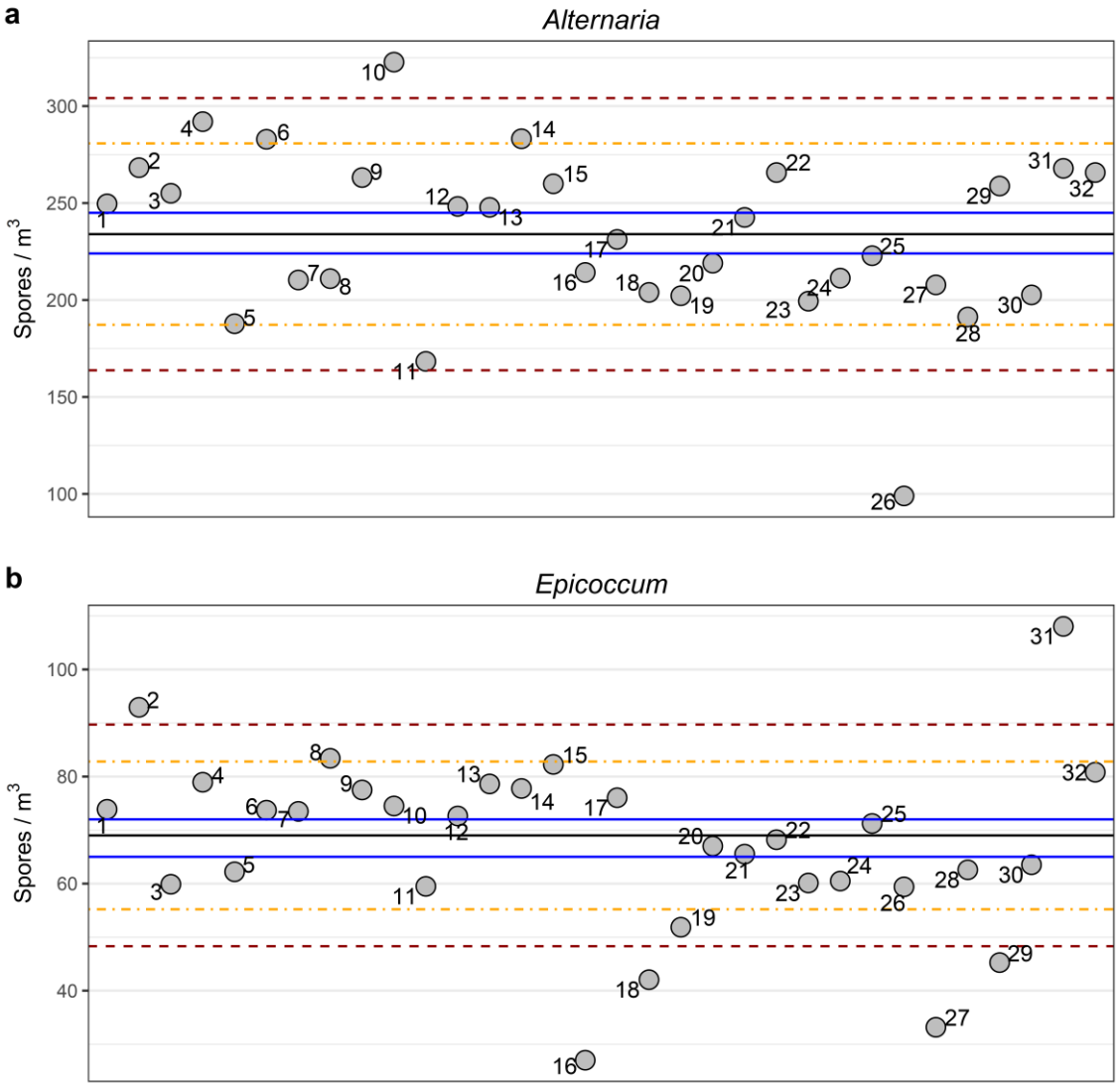

Figure 1. Absolute concentrations reported in the study for (A) *Alternaria* and (B) *Epicoccum* after analysing 5 transects. The black line signifies the average, while the blue lines the ± 5% confidence limits, the dot-dashed orange line is the absolute value deviating 20% from the average and the red line is the absolute value deviating 30% from the average.

The summary statistics obtained from all individual values are presented in Table 1 for *Alternaria* and *Epicoccum* spores.

Table 1. Summary parameters of *Alternaria* and *Epicoccum* spores when reading 1, 2, 3, 4, or 5 transects. N, size of sample (number of counters); X, assigned value; UL, upper limit; LL, lower limit; S', standard deviation for proficiency; VC, variation coefficient; ARE (%), average of relative error; NEE, number of erroneous elements; PEE, percentage of erroneous elements; ; Outliers were identified following ISO confidence levels. An element is erroneous when both conditions: RE (>30 %) and AE (>10).

spore	lines	N	X	UL	LL	S'	VC	ARE (%)	NEE	PEE (%)
Alternaria	1	32	243.1	260.8	225.3	55.8	23.0	20.3	5	15.6
	2	32	241.5	257.2	225.8	49.3	20.4	19.2	7	21.9
	3	32	245.3	258.4	232.1	41.4	16.9	16.8	4	12.5
	4	32	242.4	254.3	230.6	37.3	15.4	15.6	3	9.4
	5	32	234.5	244.9	224.1	32.8	14.0	14.7	2	6.3
Epicoccum	1	32	74.0	79.3	68.7	16.7	22.5	21.7	7	21.9
	2	32	72.1	77.1	67.2	15.5	21.5	22.1	6	18.8
	3	32	70.4	74.4	66.5	12.4	17.6	19.4	5	15.6
	4	32	69.5	73.0	65.9	11.2	16.2	18.2	5	15.6
	5	32	68.8	72.5	65.1	11.5	16.8	18.1	5	15.6

A total of 32 counters participated in the between participant reproducibility exercise for both *Alternaria* and *Epicoccum* spores. When considering the number of transects examined, the CV was similar for both spore types: 23.0 (*Alternaria*) and 22.5 (*Epicoccum*) when reading 1 transect; 14.0 (*Alternaria*) and 16.8 (*Epicoccum*) when reading five transects. Higher errors were observed when counting *Epicoccum* compared to *Alternaria* spores. We have considered both 20% and 30% for NEE and PEE. In the case of considering 20% as significant error, only 30% of participants would be within the limits of acceptable variation (Galán et al. 2014). In this case, the Relative Error produced by each counter was not related to the area (%) of the sample analysed when examining five transects (Fig. 2).

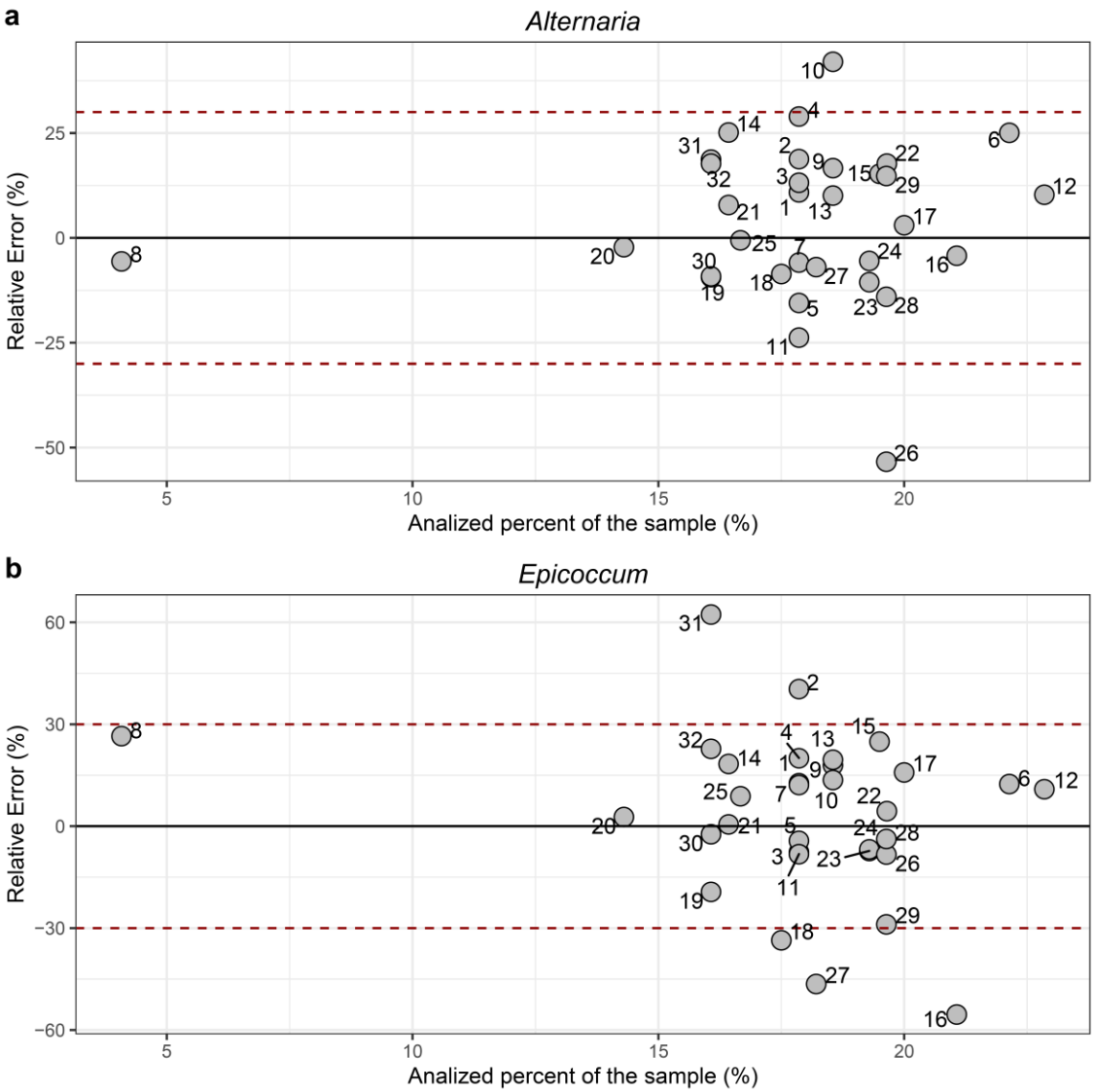

Figure 2. Relative Error of each counter relating to the area (%) of the sample analysed with 5 transects for (a) *Alternaria* and (b) *Epicoccum*.

In order to determine the minimum percentage of the slide to be examined, Absolute Errors were examined in relation to the number of the transects examined, e.g. two transects represent a minimum of 5% of the slide. Here we assume that the result provided by five transects is the most accurate (closest to the unknown true value of the sample). There were no significant differences (by paired t-test) between the distribution of Absolute Errors by five transects and the distribution of Absolute Errors for two, three or four transects. The only significant difference was for AE between one and five transects (Figure 3). It is important to emphasise that we compared numbers of transects to make the interpretation easier, but the microscope's field of view and area of slide examined is of utmost importance.

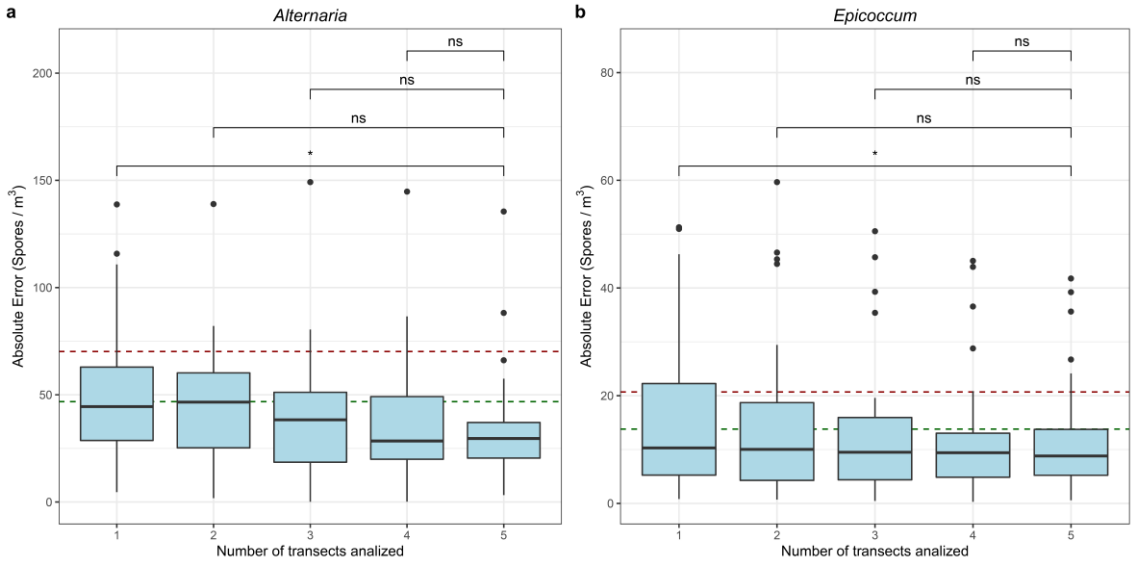

Figure 3. Distribution of absolute errors per number of transects analysed (a. *Alternaria* and b. *Epicoccum*), presenting the significance level of them (paired t-test). Ns, not significant; red dashed lines, average of 30%; green dashed lines, average of 20%. To interpret the results, one transects means a minimum of 2.5% of the slide subsampled.

Discussion

When monitoring airborne pollen and fungal spores, the standard protocols described by Galán et al. (2014) and the norm EN 16868 (CEN, 2019) are available for aerobiologists. Comparing the results of the questionnaire conducted here with those from Galán et al. (2014) it is evident that there is greater heterogenicity in the case of fungal spore monitoring. The greatest difference is related to reading of slides, because most networks (43.75%) count fungal spores over less than 10% of the slide surface.

When presenting the minimum requirements for counting slides it is important to present the percentage of the slide to be examined, rather than the number of transects. This is because the diameter of the field of view of a microscope varies depending on its make and model. For this reason, for counting pollen we required examining at least 10% of the slide (Galán et al 2014). However, in this study we require examining a minimum of 5% of the slide for counting fungal spores. We recognise that this requirement is based on just two fungal spore types, *Alternaria* and *Epicoccum*. However, due to the high diversity of fungal spore morphology and subsequent difficulties in identification, the majority of participating networks (43.75%) identify five or fewer fungal spore types, and only two networks (12.5%) identify more than 20

fungal spore types. For fungal spores, it is more difficult to know the real number of species or taxa represented by one spore type than for pollen. Some morphological differences are not well distinguished between some spores from different origin. For this reason, we have considered both 20% and 30% for NEE and PEE. A previous publication about pollen QC, 20% was considered to be the threshold for significant error (Galán et al. 2014). However, we do not have a precedent for fungal spores. Here we consider 30% as the threshold for significant error ending with a percent of erroneous elements (PEE) of around 15%. In the case of considering 20% as significant error, only 30% of participants would be within the limits of acceptable variation (Galán et al. 2014).

The airborne fungal spores most cited in the literature, and those more represented in networks that have participated in this external exercise are (in order of frequency): *Alternaria, Cladosporium* and *Epicoccum*. However, for this study we have only considered moderate values of *Alternaria* and *Epicoccum* spores. *Cladosporium* spores are usually detected in very high concentrations. These high concentrations result in an enormous effort on the part of the counters - counting *Cladosporium* spores by optical microscopy is a very slow and time-consuming process, which can increase the chance of personal error. In addition, regarding the *Alternaria* and *Epicoccum* spore identification, some staff members have shown higher ability to count one or the other spore type, probably depending on their familiarity with them because of the higher or lower presence in different biogeographical areas.

The relatively high error (30%) shows that morphological identification of *Alternaria* is difficult in air samples collected by Hirst-type traps. Due to the development of conidia and their cell structure (Cole and Samson 1979, Rotem 1994), morphological characteristics are less consistent and stable in *Alternaria* and *Epicoccum* than in other monitored airborne particles, i.e. pollen grains. *Alternaria* conidia tend to become airborne even when not fully developed. Consequently, *Alternaria* and *Epicoccum* are often present in the air in different developmental stages. Their morphology is changed by desiccation during aerial transport, especially in length of longisepta (Magyar 2007). In pollen grains, fully developed forms dominate the air and their morphology is hardly changed during aerial transport. These features make pollen grains to be good subjects of Hirst-type monitoring. In our test slide, typical morphological characteristics were missing in 22.5% and 14.7% of *Alternaria* and *Epicoccum*, respectively. A total of 18.8% atypical, but intact, *Alternaria* conidia were present, as young ones and non-beaked conidia. The latter group includes 'true' non-beaked *Alternaria* spp. (e.g. *A. radicina*), atypical conidia of usually beaked *Alternaria* spp. and *Ulocladium* spp. Recent DNA-based studies place *Ulocladium*

within the genus *Alternaria* (Woudenberg et al. 2013.). Thus, we recommend to include *Ulocladium* as well as non-beaked *Alternaria* conidia in *Alternaria* counts.

Conidia of *Stemphylium* and ascospores of some *Pleospora* species also resemble non-beaked *Alternaria* conidia in air samples. The sexual morph of *Stemphylium* is *Pleospora* (Woudenberg et al. 2017). The major allergen of *Alternaria*, Alt a 1, is abundant in *Stemphylium* and is expressed in other Pleosporaceae members as well (Agarwal et al. 1982, Sáenz-de-Santamaría et al. 2006, Gutiérrez-Rodríguez et al. 2011). Consequently, misidentification of *Stemphylium* and *Pleospora* as *Alternaria* leads to incorrect communication of *Alternaria* allergy risk. In scientific studies, databases containing misidentified data distort the results of analyses. Precise identification is especially important in *Pleospora*, because its aerial dispersal strategy is the opposite of that of *Alternaria*: ascospores of *Pleospora* become airborne in wet weather, contrary to *Alternaria*, which is a typical dry-weather fungus (Magyar et al. 2009). The percentage of error represented by *Pleospora* misidentification was low in the present study but may be higher in a test slide of an air sample collected in wet weather. Fungal spore data are extensively used, not only in the human health sector, but also in plant protection where the correct identification of fungal spores has major importance; see high error % by *Tilletia*, an important pathogen of wheat vs. *Epicoccum* (Magyar et al. 2009). It is therefore recommended that workers involved in fungal spore counting are trained in the correct identification of *Alternaria* and *Epicoccum* to minimise errors.

It is worth mentioning that *Alternaria* species are not only represented in the air by their conidia. In outdoor air the main source of the major allergen Alt a 1 are intact *Alternaria* spores, but also the quantity of other fungal fragments (broken spores, conidiophores, hyphal fragments, Pady and Kramer 1960, Green et al. 2005a). Consequently, the impact of these particles should not be overlooked, as they could increase the total atmospheric concentration of Alt a 1 (Green et al. 2005b, Grewling et al. 2020).

We have achieved the main aims of this study, which were to examine the feasibility of carrying out a QC exercise for fungal spore monitoring in Europe and to define the Minimum Requirements necessary for quality of the data. The high level of error in the morphological identification of *Alternaria* and *Epicoccum* spores during routine monitoring revealed by our study, shows the urgent need for the development of new methods based on spectral or molecular techniques for analysing mycological samples collected from the atmosphere. As *Alternaria* and *Epicoccum* comprise major allergens in the air, the continuation of Hirst-type monitoring is recommended until a more reliable method can accurately replace it.

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