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Why should we care about high temporal resolution monitoring of bioaerosols in ambient air?

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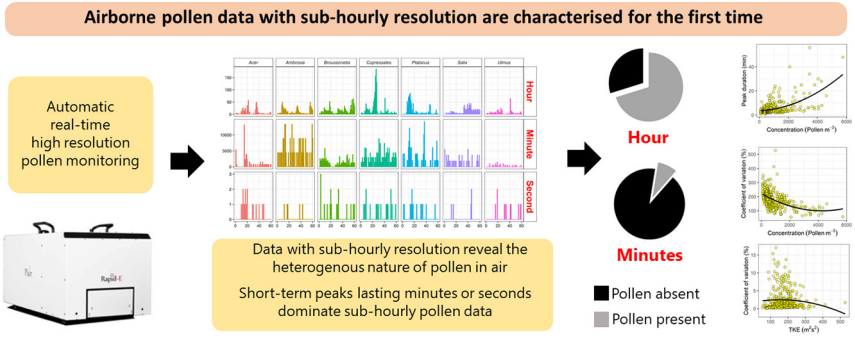
HIGHLIGHTS

- Hourly pollen concentrations for individual taxa recorded by automatic sampler
- Airborne pollen data with sub-hourly resolution are characterised for first time.
- Data with sub-hourly resolution reveal the heterogenous nature of pollen in air.
- Short-term peaks lasting minutes or seconds dominate sub-hourly pollen data.

GRAPHICAL ABSTRACT



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ABSTRACT

This is the first time that atmospheric concentrations of individual pollen types have been recorded by an automatic sampler with 1-hour and sub-hourly resolution (i.e. 1-minute and 1-second data). The data were collected by traditional Hirst type methods and state-of the art Rapid-E real-time bioaerosol detector. Airborne pollen data from 7 taxa, i.e. *Acer negundo*, *Ambrosia*, *Broussonetia papyrifera*, Cupressales (Taxaceae and Cupressaceae families), *Platanus*, *Salix* and *Ulmus*, were collected during the 2019 pollen season in Novi Sad, Serbia. Pollen data with daily, hourly and sub-hourly temporal resolution were analysed in terms of their temporal variability. The impact of turbulence kinetic energy (TKE) on pollen cloud homogeneity was investigated. Variations in Seasonal Pollen Integrals produced by Hirst and Rapid-E show that scaling factors are required to make data comparable. Daily average and hourly measurements recorded by the Rapid-E and Hirst were highly correlated and so examining Rapid-E measurements with sub-hourly resolution is assumed meaningful from the perspective of identification accuracy. Sub-hourly data provided an insight into the heterogenous nature of pollen in the air, with distinct peaks lasting ~5–10 min, and mostly single pollen grains recorded per second. Short term variations in 1-minute pollen concentrations could not be wholly explained by TKE. The new generation of automatic devices has the potential to increase our understanding of the distribution of bioaerosols in the air, provide insights into biological processes such as pollen release and dispersal mechanisms, and have the potential for us to conduct investigations into dose-response relationships and personal exposure to aeroallergens.

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1. Introduction

There have been a number of recent advances in monitoring of bioaerosols in ambient air, which has led to the launch of European COST Action CA18226 entitled "*New approaches in detection of pathogens and aeroallergens*" (ADOPT). The real-time sensing of bioaerosols has been reviewed in several publications, e.g. Huffman et al. (2019) and Maya-Manzano et al. (2020), and includes instruments that identify particles through the use of scattering and fluorescence signals from lasers operating at various wavelengths (Chappuis et al., 2020). These include the Waveband Integrated Bioaerosol Sensor (WIBS-4 and later version WIBS-NEO from Droplet Measurement Technologies) (O'Connor et al., 2014; O'Connor et al., 2015; Calvo et al., 2018; Fennelly et al., 2018), the PA-300 and more advanced Rapid-E developed by Plair SA (Crouzy et al., 2016; Huffman et al., 2019) and the Swisens Poleno (Sauvageat et al., 2020). The latter also takes holographic images in addition to fluorescence (Clot et al., 2020).

The WIBS, Plair and Swisens instruments provide rapid, on-line data with a resolution from minutes up to 3 h (Clot et al., 2020). Such short-term variations reveal something about the turbulent nature of the atmosphere and have value in explaining meteorological processes (Boubel et al., 1994). For instance, Chappuis et al. (2020) described some first insights from hourly concentrations of atmospheric pollen provided by the PA-300 in relation to manual measurements from the Hirst type trap (Hirst, 1952) and meteorological parameters. The authors suggested that the availability of such high temporal resolution (i.e. hourly) data would create new avenues of research, such as the possibility of examining relationships between airborne pollen and meteorological phenomena (Chappuis et al., 2020). In addition, high temporal resolution air monitoring data may facilitate studies into atmospheric stability, turbulence and emission sources as demonstrated in air pollution monitoring (Hagler et al., 2020; Tritscher et al., 2020). On the other hand, such a rapid-response record is not necessarily desirable for air quality studies as it contains a great deal of noise (Boubel et al., 1994). It is also important to note that the process of obtaining high-resolution data may increase the costs of bioaerosol monitoring, e.g. the lifetime of UV lasers used in instruments such as the Plair and WIBS have a limited number of shots and need to be replaced more often (Šikoparija et al., 2020).

A Rapid-E instrument has been operational in Novi Sad, Serbia, since 2019 as part of the integrated RealForAll system that combines the real-time monitoring of aeroallergens and the dissemination of information for allergy sufferers (Tešendić et al., 2020). Here we describe variations in airborne pollen recorded by the Rapid-E at different temporal resolutions as part of the system development - daily, hourly, and, for the first time, sub-hourly temporal resolution (i.e. 1-minute and 1-second data). We also investigate the influence of meteorological conditions on pollen cloud heterogeneity at 1-minute resolution.

2. Material and methods

2.1. Airborne pollen data

Airborne pollen data were collected on the roof of the University of Novi Sad Faculty of Sciences (20 m a.g.l.) by Hirst-type (Section 2.1.1) and Rapid-E (Section 2.1.2) devices. Novi Sad, situated on the left bank of the Danube river, is the capital of the Autonomous Province of Vojvodina. According to local administration data (https://www.nsinfo.co.rs/cyr/broj-stanovnika-po-naseljima) Novi Sad, together with its surroundings, is inhabited by ~411,000 people. The aerobiological measurements are deemed representative for the Pannonian biogeographical region where the usual European period of seasonal allergies is extended by the weed pollen season that is characterised by large quantities of highly allergenic *Ambrosia* sp. pollen that is commonly present in the atmosphere from July to the end of October (Šikoparija et al., 2018a).

Daily average, hourly and 1-minute airborne pollen concentrations are expressed as Pollen m^{-3} (Galán et al., 2017). The limits of the Main Pollen Season (MPS) were defined retrospectively using the 95% method (Goldberg et al., 1988) whereby the season starts when 2.5% of the cumulative catch for the year was reached and ends when 97.5% has been achieved. The Seasonal Pollen Integral (SPIn) is expressed as Pollen * day m^{-3} (Galán et al., 2017).

2.1.1. Manual Hirst type measurements

Air was sampled by Lanzoni VPPS2000 volumetric pollen and spore trap of the Hirst (1952) design. Sampling and analysis followed the standardised method EN16868:2019 (CEN, 2019) and the minimum requirements for pollen monitoring described by Galán et al. (2014). Air is continuously sucked into the trap at a rate of 10 l min^{-1} through a 2 mm × 14 mm orifice oriented towards the direction of the wind. Particles entrained in the airflow are impacted onto an adhesive coated, transparent plastic tape that is mounted on a rotating drum that moves past the orifice at 2 mm/h. Following its removal from the trap, the tape is divided into segments corresponding to 24-h periods (48 mm in length) and each segment is mounted on a microscope slide and analysed by light microscope at x400 magnification. Pollen were counted along three horizontal transects corresponding to 11.57% of the slide. Hirst data are available with daily average (00:00–24:00) and hourly resolution.

2.1.2. Automatic high temporal resolution measurements

Automatic high temporal resolution bioaerosol measurements were performed using the Rapid-E instrument designed and produced by Plair SA. The device continuously samples 2.8 l min^{-1} of air through an upward oriented two-layered Sigma-2 inlet (VDI 2119 2013). Each sampled particle interacts with the laser light sources resulting in scattered light and fluorescence (Šaulienė et al., 2019). The Rapid-E was operated in the "smart pollen mode" (Šikoparija et al., 2020) in which particles >8 μm in optical diameter are measured. The device is designed to save a JASON file containing scattered light, fluorescence spectrum and lifetime properties of each particle sampled in 1-minute making this the finest temporal resolution of the measurements (Tešendić et al., 2020). However, each detected particle has a timestamp, including year, month, day, hour, minute, second, and millisecond of the recording, allowing for examination of sub-minute temporal resolution data.

The collected signal is expected to provide information about particle morphology and chemical composition of each detected particle (Kiselev et al., 2011, 2013). In order to be able to discriminate pollen from other aerosols the classification algorithm, a multi-input one-output convolutional neural network (CNN) that combines multiple inputs from the Rapid-E has been developed (Šaulienė et al., 2019). The model was trained with a signal obtained from measurements when there was no pollen recorded in the atmosphere and by exposing the device to 23 pollen types commonly present in the atmosphere and class 'other_pollen' that contains a mix of pollen that is occasionally detected in small quantities (Tešendić et al., 2020).

The quality of pollen identification has been validated by comparing daily concentrations to measurements obtained using the standardised Hirst-type method EN16868:2019 (CEN, 2019). Total Pollen (the sum of all registered pollen types) and 7 pollen types with significant Spearman's correlations between daily average pollen concentrations recorded by the Hirst-type and Rapid-E methods during the MPS were further analysed at higher temporal resolution, i.e. *Acer negundo* L., *Ambrosia* L., *Broussonetia papyrifera* (L.) L'Hert. ex Vent., *Cupressales* (the families *Taxaceae* S.F.Gray *and Cupressaceae* Bartlett), *Platanus* L., *Salix* L., *Ulmus* L. For plotting time series the Rapid-E signal has been scaled linearly to make the quantity comparable to Hirst measurements. The scaling factor was obtained from the ratio of the SPIn for different taxa obtained by both measurement methods and is different for each taxa and for Total Pollen (Table 1 and Supplementary Fig. S1).

This study focusses on days with very high airborne pollen concentrations (>90% quartile) to ensure the data are statistically robust. The peak period within an investigated hour was deemed as starting after the last minute when no pollen were detected in the air, and ended the following minute without any pollen grains present. For sub-minute (i.e. 1-second) data, we focussed on detections and their distributions, i.e. yes/no measurements, rather than concentrations. For each pollen type we looked at, we

Table 1
Limits of airborne pollen seasons determined using the 95% method (Goldberg et al., 1988), the Seasonal Pollen Integrals (SPIn) and scaling for *Acer negundo*, *Ambrosia*, *Broussonetia papyrifera*, Cupressales, *Platanus*, *Salix*, *Ulmus* and Total Pollen measured by Hirst and Rapid-E devices.

Pollen type	Season characteristics[a]					
	Start [date]	End [date]	Length [days]	Hirst SPIn	Rapid-E SPIn	Scaling [Hirst/Rapid-E]
Acer negundo	7 Mar	29 Mar	23	1734	799	2.17
Ambrosia	5 Aug	18 Sept	45	14,368	1152	12.47
Broussonetia papyrifera	11 Apr	4 May	2431	2705	1502	1.80
Cupressales[b]	20 Feb	3 Apr	43	7277	1768	4.12
Platanus	31 Mar	26 Apr	27	3308	582	5.68
Salix	23 Mar	20 Apr	29	4057	792	5.12
Ulmus	21 Feb	21 Mar	29	616	447	1.38
Total Pollen	16 Feb	15 Oct	242	61,907	29,502	2.03

[a] The season limits for Total Pollen were the start and end of the measurement campaign in 2019 while the 95% method described by Goldberg et al. (1988) was used for determining season limits for specific pollen types. Season start, end and length refer to Hirst data.
[b] Taxaceae/Cupressaceae type.

examined the distribution of 1-second detections in the 1-minute periods with the highest number of records.

2.2. Meteorology data

Components of the wind speed u, v and w (in x, y and z direction, respectively) were measured at 10 Hz using 3D sonic anemometer YOUNG 8100 (Campbell Scientific, Inc.) installed besides the bioaerosols sampler. Turbulent kinetic energy (TKE, $m^2 s^2$) per unit mass was calculated at hour resolution as the sum of variances of all three wind components (Stull, 1988).

2.3. Data analysis

Daily average and hourly pollen concentrations collected by Hirst-type and Rapid-E methods were examined for normality using the Shapiro-Wilk test. Spearman correlation analysis was then used to determine whether daily average and hourly pollen concentrations collected by Hirst-type and Rapid-E devices were significantly related, and Mann–Whitney *U* tests were used to examine whether the magnitude of airborne pollen concentrations collected by the two methods differed significantly.

The relationships between mean hourly pollen concentrations, peak duration (minutes), and coefficient of variation (%) were investigated by linear regression analysis. In order to explore how turbulent kinetic energy (TKE) explains pollen cloud characteristics at the measurement location we have calculated the relationship between coefficient of variance of 1-minute records for all hours during the pollen season and TKE (Stull, 1988; Šikoparija et al., 2018b). TKE is a measure of turbulence, and we used it as a proxy for atmospheric stability that determines the way particles are suspended in the atmosphere (Dimotakis, 2005; Dupont et al., 2006; Šikoparija et al., 2018b). We assume that a higher TKE at the measurement location results in segregation of the particle cloud and greater heterogeneity of pollen in the air (Dimotakis, 2005). Data were analysed and visualised in Microsoft Excel, IBM SPSS Version 26, R (packages dplyr, ggplot2) and Python with open-source libraries (JetBains.com, 2019). Results were considered significant at $p < 0.05$.

3. Results

Both daily average and hourly concentrations of the selected pollen types recorded by Hirst and Rapid-E devices were significantly related (Supplementary Figs. S1 and S2 and Table S1). As a result, we assume that examining Rapid-E measurements with sub-hour resolution (i.e. 1-minute and 1-second resolutions) are meaningful at least from the perspective of identification accuracy. Pollen grains were observed most hours during the pollen seasons (ranging from 58.3% for *Ulmus* to 82.1% for *Broussonetia papyrifera*), but the distributions of hourly mean concentrations of airborne pollen grains were positively skewed indicating a predominance of low concentrations (Supplementary Fig. S3 and Table S2).

With respect to 1-minute temporal resolution, the frequency of detections was much lower and ranged from 6.3% to 14.3%, e.g. *Broussonetia* pollen grains were detected more than twice as often as *Salix* and *Platanus* pollen (Fig. 1 and Supplementary Table S3). Thus, during most of the recorded minutes, no pollen grains were observed at all. The Coefficient of Variation (CV%) of the 1-minute records for the analysed hours for each pollen type show a high degree of heterogeneity in pollen cloud characteristics (Fig. 2). This is exemplified by CV% > 100 that result from standard deviations greater than the mean. The highest CV% (>200%) were observed for *Ulmus* and *Platanus*, while the lowest were for *Broussonetia* and Cupressales type (Fig. 2 and Supplementary Fig. S4). The median duration of peaks (in sub-hour resolution) lasted from 3 min (for *Ulmus*, *Salix* and *Ambrosia*) to 8 min for *Broussonetia*. As one would expect, there was a clear positive relationship between duration of peak and pollen concentrations (Fig. 3A). In general, there were strong negative relationships between CV% and mean pollen concentrations, suggesting that CV% decreases with increased 1-minute concentrations (Fig. 3B). On the other hand, there were no clear trends in the effect of turbulence (TKE) on pollen plume heterogeneity, although there was a distinct negative relationship in the case of *Salix* (Fig. 3C).

For the majority of pollen types high Spearman's correlations ($r > 0.90$, $p < 0.05$) between 1-hour data (calculated by including all 60-minute periods) and averages of 1-minute data were achieved by the inclusion of ~30 randomly selected minutes of data. Thus, it is enough to consider only 50% of the total collection period (30/60 min) to achieve similar sampling accuracy (Fig. 4). Correlations are strongly influenced by sub-hourly

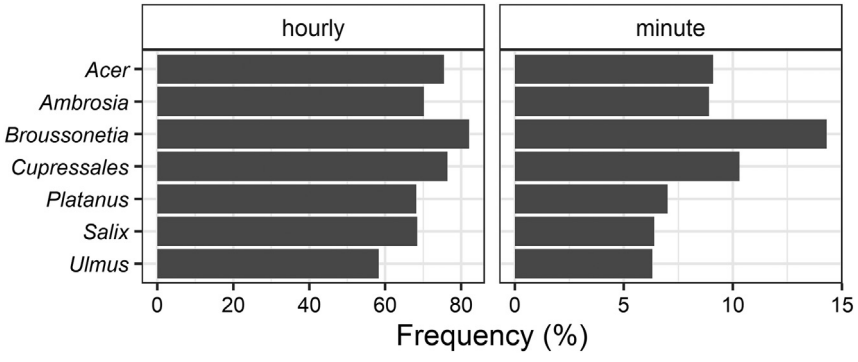

Fig. 1. Frequency (%) of pollen grains detection (>0 Pollen m^{-3}) with hourly and minute resolution for *Acer negundo*, *Ambrosia*, *Broussonetia papyrifera*, *Cupressales* (including pollen from the Taxaceae and Cupressaceae families), *Platanus*, *Salix*, and *Ulmus* (note: the frequency for minute resolution was calculated for hours when at least 1 pollen was detected).

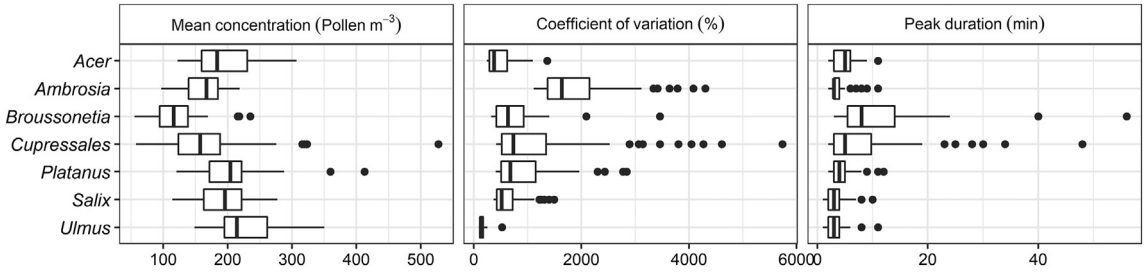

Fig. 2. Characteristics of sub-hourly (1-minute) temporal resolution measurements of airborne pollen of *Acer negundo*, *Ambrosia*, *Broussonetia papyrifera*, Cupressales (including pollen from the Taxaceae and Cupressaceae families), *Platanus*, *Salix*, and *Ulmus*. Mean concentrations (Pollen m^{-3}), coefficient of variation (%), and peak duration (minutes).

variations in pollen (CV%) but the relationships between both the magnitude of pollen concentrations and duration of pollen peaks and Spearman's correlations are less evident (Supplementary Fig. S5).

The analysis at sub-minute resolution showed that even in periods with the largest number of detections, mostly single pollen grains were recorded per second. The highest maximum number of pollen detections were observed for *Broussonetia papyrifera* (3 pollen/s). In comparison, the total number of other aerosols exceeded 25 detections/s. The 1-second data indicate lack of distinct continuous pollen "plumes" as most of single pollen grains were recorded at intervals of a few seconds (Table 2, Supplementary Fig. S6).

4. Discussion

Many aerobiological networks are still based on volumetric spore traps of the Hirst (1952) design, which were developed in the middle of the twentieth century (Buters et al., 2018). The longevity of the Hirst type trap is partly due to it being robust and relatively inexpensive to install and operate, although processing and analysis of samples is extremely time consuming and the identification of samples by light microscopy requires highly trained operators (Beggs et al., 2017) thereby increasing operating costs. There are also issues related to reproducibility, with differences reported between Hirst type traps located only a few meters apart (Tormo Molina et al., 2013) and difficulties with calibrating flow rates (Oteros et al., 2016).

The highest possible temporal resolution for Hirst type trap is 1 h (Galán et al., 2014) and a number of studies have examined diurnal variations in airborne pollen (Mahura et al., 2009; Peel et al., 2014; Grewling et al., 2016). Experiments have also been conducted above the pollen source, where measurements were taken at 7.5-minute resolution (Šikoparija et al., 2018b) as well as approximately 1.07-minute resolution for 12 h (Šikoparija et al., 2020). However, in reality, due to the fact that air passes through a 2 mm wide orifice before impacting on a drum moving at 2 mm an hour, the sample at any given point on the adhesive tape includes particles collected from the air before and after the sampling time and so represents the mean concentration of particles over one hour (BAF, 1995).

On the other hand, the advantage of so called third generation of automatic samplers (Oteros et al., 2019) is that the data are in real-time (or almost real-time) and can potentially identify the processes such as the release of pollen from the source that approximates puff release (Faegri and Iversen, 1992). With this in mind, the focus of the current study is to explore the behavior of airborne pollen grains at high temporal resolution by examining sub-hourly data.

4.1. Compatibility of measurements of airborne pollen collected by Hirst type and Rapid-E devices

The results for different pollen types show notable variations in SPIn produced by the Hirst and Rapid-E. Scaling factors are therefore required to make the quantitative data comparable, which is corroborated by the number of significant differences seen in the magnitude of airborne pollen concentrations recorded by the two devices (Supplementary Table S1). It is obvious from these results that it is not enough to simply use a scaling calculated using data for Total Pollen for different pollen types (i.e. 2.10) as there is an almost 13-fold difference between the Hirst and Rapid-E for *Ambrosia* (i.e. 12.94). There are several potential reasons for this, and it is probably a combination of differences in sampling (e.g. different flow rates and types of orifice) (Mimić and Šikoparija, 2021), the filtering out of particles with poor quality signals by the Rapid-E that could not be used for classification (Šaulienė et al., 2019), and uncertainties in automatic classification.

The effect that different orifice types have on sampling efficiency has been assessed in real life conditions for two Hirst type devices by Mimić and Šikoparija (2021). The tests indicated that replacing the mobile sampling head oriented towards the direction of the wind with a fixed sampling head with two-layered inlet like the one used in the Rapid-E device does not notably affect the detected quantity of pollen (Mimić and Šikoparija, 2021). However one should bear in mind that the notably lower flow rate of the Rapid-E is expected to produce different sampling efficiencies under the same wind conditions.

It is interesting to note that the scaling factors do not seem to depend on the size of pollen grains. For instance, *Broussonetia papyrifera* (~10 μm) and *Ulmus* (~30 μm) have similar scaling factors while *Ambrosia* (~20 μm) and *Acer negundo* (~25 μm) have notably different scaling factors suggesting sampling efficiency is not related to aerodynamic properties alone. On the other hand, particles with poor quality signals (e.g. low fluorescence intensity) are filtered out from the Rapid-E measurements prior to analysis (Šaulienė et al., 2019) and this is expected to severely affect detection efficiency for different pollen types. From the measurements of fluorescence signatures (Pöhlker et al., 2013) clear differences in fluorescence emission intensity after excitation at 337 nm can be seen for *Ambrosia artemisiifolia* L. and *Broussonetia papyrifera*, which corresponds to differences in their scaling factor. It seems that fluorescence emission signatures following excitation at 337 nm of the Rapid-E UV laser explains why losses are not the same for all pollen and so each detected pollen type should be scaled before calculating Total Pollen. Finally, the filtering out of particles with poor quality signals most likely exceeds any uncertainty involved in subsampling the microscope slide and the uncertainty involved in automatic classification is likely to be greater than the performance of trained counters.

4.2. Potential implications of high resolution monitoring of bioaerosols in ambient air

Chappuis et al. (2020) provided some initial observations of hourly data, but only for Total Pollen, and so this is the first time that atmospheric concentrations of individual pollen types have been recorded by an automatic sampler with 1-hour resolution. This is important because airborne pollen data measured with a temporal resolution of 1 h can be used to examine processes involved in the release and atmospheric dispersal of particular pollen types. For example, the significant correlations between Hirst and Rapid-E measurements for *Ambrosia* (Supplementary Fig. S2 and Table S1) show that the Rapid-E is able to identify the distinctive diurnal periodicity in *Ambrosia artemisiifolia* flowering (Bianchi et al., 1959) needed to examine the mechanisms involved in the long distance transport of *Ambrosia* pollen (e.g. Šikoparija et al. (2013)). Furthermore, the fact that daily

average and hourly measurements recorded by the Rapid-E are comparable to Hirst data opens the door to looking at even higher resolution data (i.e. 1 min or less). Unfortunately, it was beyond the scope of the present study to compare sub-hourly concentrations with Hirst data, but this might be a topic for future research using the Burkard Scientific SporeWatch samplers set to maximal temporal resolution as described by Šikoparija et al. (2020).

4.3. 1-Minute temporal resolution monitoring, pollen cloud heterogeneity and turbulence

Timerman et al. (2014) described how atmospheric turbulence causes resonant vibrations that release episodic bursts of pollen grains from *Plantago lanceolata* L. In a field study, Šikoparija et al. (2018b) examined high temporal resolution measurements of airborne *Ambrosia* pollen

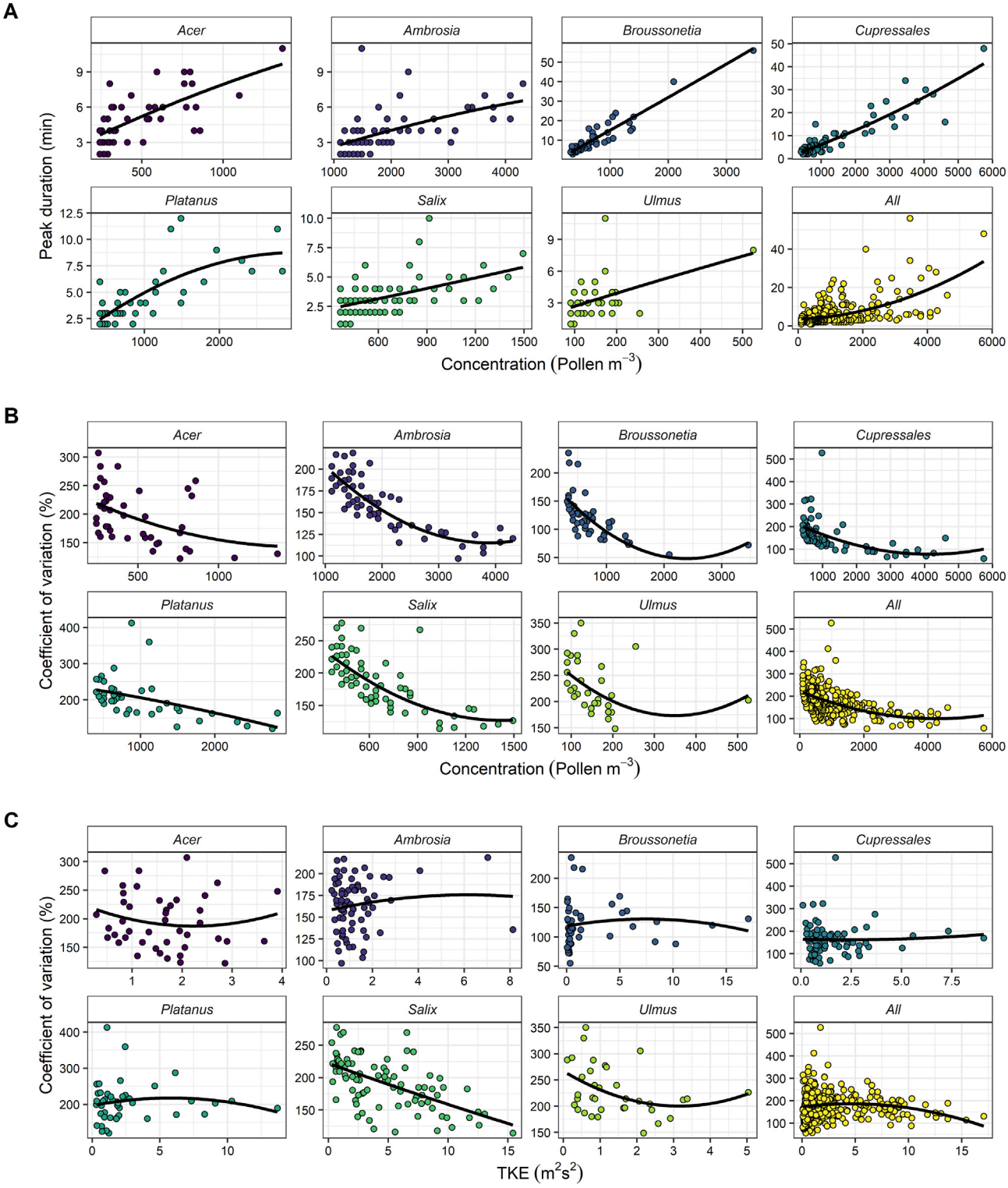

Fig. 3. Relationships for selected taxa between: (A) Peak duration and pollen concentrations; (B) Coefficients of Variation (CV%) and pollen concentrations; (C) CV% and turbulence (TKE).

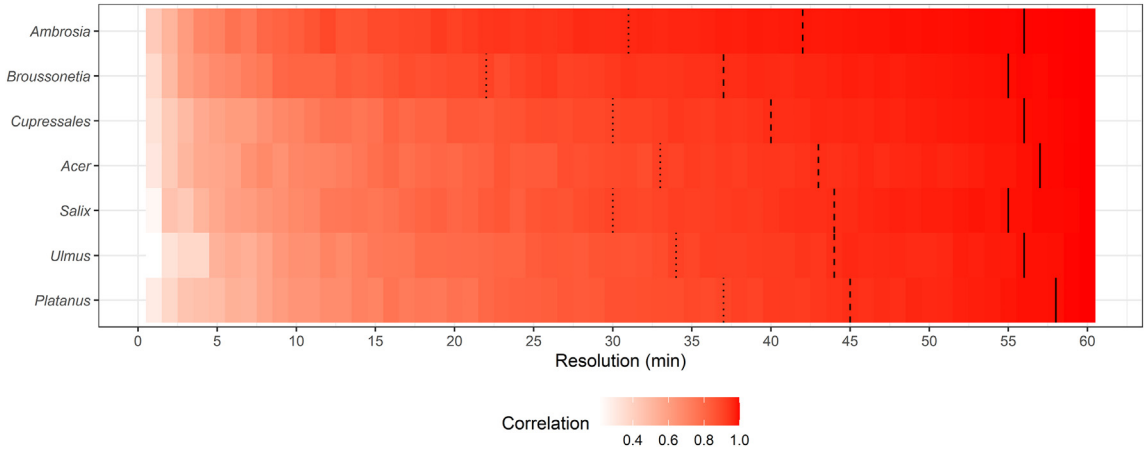

Fig. 4. Spearman's correlations between mean hourly pollen concentration (calculated by including all 60-minute periods) and mean 1-minute pollen concentrations during higher temporal resolutions periods starting from 1 min to 59 min in 1 min steps. The time periods were randomly selected through random number generator. All hours during the pollen seasons were considered (dotted vertical line $r > 0.90$, dashed vertical line $r > 0.95$, solid vertical line $r = 0.99$).

above the source and found that TKE of less than 0.1 $m^2 s^{-2}$ was sufficient to lift *Ambrosia* pollen into the air, but the authors found no consistency in the influence of TKE on airborne pollen and higher energy available for emission did not necessarily result in higher pollen release. These studies support a puff, rather than a continuous, mode of pollen release in the anemophilous species examined. Puff emission is likely to result in small pollen clouds near the source that become evident when examined at high temporal resolution. Although the overall effect might be lost in the presence of high amounts of pollen asynchronously released from a multitude of anthers.

In the current work, 1-minute concentrations of atmospheric pollen recorded at a sampling station located at roof level have provided an insight into the heterogenous nature of pollen in the air, with distinct peaks lasting ~5–10 min. As one would expect, there were clear, positive, relationships between overall amounts of airborne pollen and these 1-minute fluctuations in atmospheric pollen concentrations. However, the short term variations could not be wholly explained by TKE, which is in keeping with Šikoparija et al. (2018b). How atmospheric turbulence affects the distribution of pollen in the atmosphere cloud is not certain. As previously mentioned, it could be related to the mode of pollen release (Timerman et al., 2014; Šikoparija et al., 2018b). In addition, the intensity of turbulence alters the vertical velocity gradient of air movement and the vertical temperature profile, both of which collectively influence the dispersal of airborne pollen grains (Niklas, 1985). As a result, atmosphere instability and increased turbulence could segregate pollen clouds causing them to become increasingly heterogeneous (Dimotakis, 2005). Furthermore, Dupont et al., 2006 discussed the heterogenous distribution of particles in turbulent flow and described how locally high concentrations could be formed in regions of low flow vorticity, a process influenced by factors such as Stokes numbers (Dupont et al. (2006) and references therein).

4.4. Operational consequences of high temporal resolution monitoring of bioaerosols

High resolution data, although extremely useful, can markedly increase the cost of monitoring, and thus the most effective methods of aerosol monitoring are sought. Šikoparija et al. (2020) showed that, in order to save resources, intermittent recordings can replace continuous sampling for the assessment of hourly pollen concentrations. According to the authors, the total pollen collection time of ~15 min in every hour (25% of total period) was enough to capture both the magnitude and the trends of hourly *Ambrosia* pollen concentrations (Šikoparija et al., 2020). The study was only conducted for one pollen type (*Ambrosia*), at heights just above the source (0.5 m and 5.5 m a.g.l.) for a limited period of time (2 days). We therefore decided to investigate the effect of reducing sampling time (i.e. reducing the number of 1-minute periods) for 7 pollen taxa recorded for whole pollen seasons at roof level by Rapid-E. Our analysis revealed that decreasing detection frequency by 3–8% caused almost no loss of information ($r > 0.99$, $p < 0.05$) (Fig. 4 and Supplementary Fig. S5). In long-term monitoring, a reduction in sampling time of ~5% can result in considerable savings.

4.5. Further steps — future research and sub-minute pollen data

The new real-time, high temporal resolution devices for aerosol monitoring could be a considered a game changer in terms of increasing our knowledge of bioaerosols in ambient air. For example, our knowledge of the processes involved in the release pollen grains is extremely limited. Wind–stamen interactions are poorly understood, as are the specific forces that deliver pollen grains into airflows (Timerman et al., 2014). The effects of varying micrometeorological conditions, such as turbulence, on the rate of pollen release is also poorly studied (Timerman et al., 2014; Šikoparija et al., 2018b). As discussed, traditional measurements using Hirst-type

Table 2
Character of the detections at 1-second temporal resolution for selected minutes with largest number of pollen detected for *Acer negundo*, *Ambrosia*, *Broussonetia papyrifera*, Cupressales, *Platanus*, *Salix* and *Ulmus*.

Pollen type	Period	Total number of detections	Maximum detections per second
Acer negundo	13-03-2019 08:15:00–08:15:59	17	2
Ambrosia	24-08-2019 06:06:00–06:06:59	5	1
Broussonetia papyrifera	26-04-2019 06:25:00–06:25:59	17	3
Cupressales[a]	04-03-2019 00:49:00–00:49:59	18	2
Platanus	09-04-2019 11:10:00–11:10:59	14	2
Salix	05-04-2019 08:18:00–08:18:59	5	2
Ulmus	07-03-2019 10:13:00–10:13:59	11	2

[a] Taxaceae/Cupressaceae type.

methodology provide, at best, data with 1-hour temporal resolution that contain a great deal of uncertainty. High-speed cameras have been used in some experimental setups for observing pollen (and spore) release, which are very precise but have a limited operational time and are mainly suitable for laboratory conditions (Edwards et al., 2005; Timerman et al., 2014; Gallenmüller et al., 2018).

As well as examining data with 1-minute resolution, this study also provided some first insights into sub-minute airborne pollen data. The 1-second data obtained for minutes with the highest pollen concentrations revealed that the number of pollen detections per second were strikingly low (in comparison to other airborne particles), and never exceeded 3 pollen/s. Moreover, 1-second measurements of airborne pollen data were discontinuous in nature with a predominance of single pollen grains recorded seconds apart. Such data have mouth-watering possibilities for examining biological processes such as validating current hypothetical models describing the aerodynamic basis of pollen release like turbulence-initiated wind-pollination mechanisms (Urzay et al., 2009).

Kitinoja et al. (2020) found that 'short-term' exposure to airborne pollen, defined as 1-day, increases the risk of allergic and asthmatic manifestations. Even shorter periods of exposure have been replicated in provocation tests, either directly using allergen extracts (Schumacher and Pain, 1979) or pollen grains (Lebel et al., 1988), or by spending various lengths of time (e.g. 2 h (Horak et al., 2009) or 6 h (Horak et al., 2001)) being exposed to airborne pollen in a challenge chamber. The results of this study provide further evidence into the usefulness of automatic pollen sampling, and we concur with Chappuis et al. (2020) who suggested that research into high temporal resolution monitoring of bioaerosols could be used to help define levels of critical exposure and aid individuals in avoiding exposure and planning their medication.

In conclusion, the new generation of automatic devices have the potential to increase our understanding of the distribution of bioaerosols in the air, provide insights into biological processes such pollen release and dispersal mechanisms and, from a human health perspective, high temporal resolution monitoring of bioaerosols in ambient air will allow us to conduct investigations into dose-response relationships (Erbas et al., 2012) and personal exposure (Peel et al., 2013) to aeroallergens.

CRediT authorship contribution statement

Matt Smith: Conceptualization, Writing – original draft, reviewing and editing; **Branko Šikoparija**: Conceptualization, Investigation, Formal analysis, Writing - original draft, Funding acquisition; **Gordan Mimić**: Formal analysis, Data curation; **Predrag Matavulj**: Formal analysis; **Lukasz Grewling**: Formal analysis, Writing – reviewing and editing.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

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Declaration of competing interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154231.

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