Using the WIBS-4 (Waveband Integrated Bioaerosol Sensor) technique for the on-line detection of pollen grains.

David J O'Connor*, David A Healy, Stig Hellebust, Jeroen T M Buters[2] and John R Sodeau*

Department of Chemistry and Environmental Research Institute

University College Cork

Cork, Ireland

[2]ZAUM – Center of Allergy and Environment, Helmholtz Zentrum München/Technische Universität München, Munich, Germany

Corresponding authors*

Email: j.sodeau@ucc.ie and doc@umail.ucc.ie

Abstract:

Primary biological aerosol particles (PBAP) such as pollen and fungal spores can induce allergenic responses and affect health in general. Conditions such as allergenic rhinitis (hay fever) and asthma have been related to pollen concentrations. Likewise some pollen have been shown to induce ice nucleation and cloud condensation at higher temperatures than those associated with some chemical species thereby affecting planet Earth's albedo and overall radiative balance. Hence the near real-time (on-line) monitoring of airborne pollen and other PBAP using a variety of spectroscopic and light scattering techniques represents an area of growing development and consequence.

In this study, two separate field campaigns (one at a rural site in Ireland and the other at an urbanized location in Germany) were performed to detect and quantify pollen releases using a novel on-line fluorescence spectrometer (WIBS-4). The results were compared with results obtained using more traditional Hirst-type impactors. Size, "shape" and fluorescent characteristics of ambient particles were used to determine the concentrations and identity of PBAP likely to be pollen grains.

The concentration results obtained for both methodologies at both the Irish and German sites correlated very well, with R^2 values >0.9 determined for both campaigns. Furthermore the sizing data available from the WIBS-4 approach employed in Ireland indicated that pollen grains can be identified in appropriate conditions. Hence Yew pollen was sampled both in the laboratory and at the rural site: the results indicated almost identical size ranges. In fact Yew pollen is generally reported to be between 25-27 μm in diameter but the measurements reported here are the first of their type providing data on the size of *in-flight* Yew pollen.

Introduction:

Primary biological aerosol particles (PBAP) such as pollen and fungal spores can induce allergenic responses and affect health in general. Conditions such as allergenic rhinitis (hay fever) and asthma have been related to elevated pollen concentrations [1, 2]. Likewise some pollen have been shown to induce ice nucleation and cloud condensation at higher temperatures than those associated with some chemical species [3, 4] thereby affecting planet Earth's albedo and overall radiative balance. However while the direct and indirect environmental implications of large airborne concentrations of pollen have begun to be realised, methodologies to achieve their real-time analysis have lagged behind.

Currently employed methods for pollen detection have remained off-line and traditional in their approach: they commonly employ simple impactors such as the Hirst type volumetric trap and the whirling arm traps [5, 6]. These instruments capture coarse airborne particles on a suitable substrate before they are taken to a laboratory for analysis using optical microscopy [6]. This dual approach is quite laborious and requires highly skilled operators for accurate determinations of number and identities due to the sheer variety of PBAP species present in the environment.

Newer, molecular methods of analysis have been used more recently, for example ELISA (enzyme-linked immunosorbent assay), a technique which is used, principally, for the identification of aeroallergens associated with allergenic pollen [7, 8].

Monitoring intrinsic fluorescence (autofluorescence) is another technique used for the identification of PBAP particles and has been used previously in fluorescent microscopes and spectrometers [9-17]. One development of this approach, in recent times, is the use of fluorescence for detection in on-line instrumentation such as the waveband integrated biological sensor (WIBS), the UV-APS and the automated pollen counter [18-23]. These instruments use combined fluorescence and optical scattering, as a simple diagnostic to differentiate between fluorescent and non-fluorescent particles present in the atmosphere. This technique offers the potential for rapid number/concentration determination of the target sample while being non-destructive and reagent free. PBAP are autofluorescent because of their chemical and biochemical contents. Hence structural components such as tryptophan, NAD(P)H, cellulose, chitin, lignin and "sporopollenin" as well as secondary metabolites such as phenols and terpenoids all can be excited and subsequently emit over a number of wavelength ranges [24-28].

Instrumentation like the WIBS employed here and the UV-APS in particular have been used for PBAP detection in both laboratory [29-34] and ambient environments recently [35-38]. They have been shown to provide both concentration and limited characterisation data. However the field campaigns have, as of yet, not compared the use of either of these instruments to more traditional techniques or focused on the identification of specific pollen.

In this study the conventional method generally employed for PBAP sampling and analysis (automatic volumetric trap) was co-located with a WIBS-4 instrument at both a rural site and an urban location. Results from both instrumental methods were compared and contrasted. The sets of data obtained here represent the first that employ the WIBS approach for the detection of biological particles in the super coarse size range 10-30 μm. Furthermore by comparison with the results obtained using traditional impact/optical analysis it is shown that the evaluation of ambient pollen concentrations can be obtained reliably in near real-time.

Materials and methods

Sampling site 1: Killarney National Park, Ireland

Particulate sampling was performed in Killarney National Park (KNP), Kerry, Ireland (N 52°01.263' W 09°30.553'), towards the eastern perimeter of Reenadinna Woods between 24/02/10 and 4/03/10. The Reenadinna Woods area is the most extensive location for Yew Trees in Ireland and covers ~60 acres (25 hectares).The canopy in this stand is typically strongly dominated by *Taxus baccata* (Yew) along with *Corylus avellana* (common Hazel), *IIex aquifolium* (European Holly) and *Fraxinus excelsior* (Ash). A list of typical species found in Reenadinna Woods is outlined elsewhere [39].

This site offered fairly ideal conditions for the preliminary testing of the WIBS-4 instrument because of its almost pristine air conditions and the dominance of Yew pollen at the time of year selected. There are no sources close to the campaign site of anthropogenic materials, such as diesel particles, which are fluorescent. Specifically there are no landfills, road traffic, animal housing or waste treatment plants in the vicinity of the sampling site. However even if some interfering airborne chemicals were present, their known size range (< 1μm) would preclude them from the WIBS analysis procedure. The chosen campaign location was also safe and secure.

All instruments were housed in a purpose-built mobile laboratory trailer unit which was positioned adjacent to a lawn area in front of "Arthur Vincent House", KNP, from where a 220 V power supply was obtained. Sample inlets were positioned 2.5–3 m in height above ground level and located ~4 m from the nearest tree.

Sampling site 2: Technical University of Munich (TUM), Germany

Sampling was performed at the grounds outside the Centre of Allergy and Environment, Munich, TUM, Germany (N 48° 9' 51.7314", W 11° 35' 32.7834") between 27/4/10 and 10/05/10. In contrast to KNP, the environment here would be expected to provide a much more complex ambient composition of airborne particulates because the site is located in an urban area. "The English Gardens" are also situated less than 1 km from the site. Hence this chosen campaign site was set up at a place in which aerosol sources from traffic, domestic heating and vegetation are likely present.

Volumetric traps:

A continuous volumetric spore trap (SporeWatch-Buckard Scientific) was used for the KNP campaign as a traditional method of PBAP capture. Prepared, silicone-coated sample substrates/tape (Lanzoi) were mounted on the drum within the sampler. The sampler was then set to run for a 7 day period at 10 L/min. The adhesive sampling tape was changed on completion of the 7 day sampling period. Upon completion, the tape was dissected into seven 48 mm segments representing 24 h time intervals. Mounting of these segments involved placing them on microscope slides before using a Gelvatol mounting medium, stained with basic fuchsin to adhere a cover lip permanently to the slide. The slides were analysed at 400x magnification under an optical microscope using the 12-traverse method. Thus a 2 h time resolution for the campaign was employed for the KNP campaign. A similar volumetric trap was used in the TUM campaign although the longitude method of counting was used.

Ozone monitoring:

A Thermo-Scientific, Model 49i, Ozone Monitor was used for the determination of ambient ozone concentrations during the KNP campaign.

Waveband integrated bioaerosol sensor, Model 4:

The waveband integrated biological sensor (WIBS-4) is a single particle, on-line fluorescence spectrometer with the capability to determine the size, "shape" and fluorescent characteristics of ambient particles at a microsecond time resolution. It achieves this through the use of two xenon flash lamps set to excite at 280 nm and 370 nm respectively to gauge the fluorescent intensities of individual particles. It also uses a 635 nm diode laser to establish their size and "shape" in terms of an asymmetry, AF, factor. The AF values are obtained by using the ratio of scattered light falling on a four quadrant detector, more detailed descriptions of this process have been discussed elsewhere [34, 35]. Upon excitation the fluorescence of an individual particle is evaluated using three detector channels, termed FL1, FL2, and FL3. These channels record the total fluorescence over two wavelength ranges, namely, FL1 = 310-400 nm and both FL2 and FL3 = 420-650 nm. Each particle is excited at both 280 nm (FL1 and FL2) and 370 nm (FL3). The WIBS-4 device used for the campaigns discussed here is similar to those also been described previously [19, 34, 35, 40]. The WIBS-4 instrument does differ in important respects from its predecessor (WIBS-3) as noted in those reports [34], especially as WIBS-4 allows the user to designate the size fraction of the ambient air upon which to focus sampling and subsequent data analysis. This feature is related to two sensitivity settings in WIBS-4: High Gain (HG) and Low Gain (LG). HG allows particles between 0.5 and 12 μm to be evaluated whilst LG allow particles from 3 to 31 μm to be analysed [34, 40]. Given that the target particles (pollen) for the KNP and TUM campaigns described here, are generally considered to be of sizes >15 μm the LG mode was selected. These sizes are also, of course, far larger than chemical particulates that are known to emit light because they contain fluorescing components such as PAH (Poly Aromatic Hydrocarbons) [41].

Very large data sets were created during the two field campaigns undertaken in this on-line pollen detection study. Thus various filters were applied to the data sets in order to allow the particles of interest to be more easily recognised and counted. The limits of detection for each of the three fluorescence channels FL1, FL2 and FL3 of the WIBS-4 were obtained by placing the WIBS-4 in forced trigger mode. This causes the xenon flash lamps to discharge in the absence of a triggering particle. The WIBS-4 was allowed to sample in this mode until a test data set with 1000 points was established. Mean values plus three times the standard deviation from the test data from each of the three fluorescent channels was then used as the

threshold between particles that were deemed to be biological or non-biological. This procedure is similar to that described previously [34, 35].

Weather station

Meteorological parameters were collected using a Casella Nomad Weather Station at 5 min time resolution. The data were subsequently averaged to 2 h intervals for more appropriate comparison with the results obtained from both the counting/identification methods used in the KNP Campaign.

Results and Discussion

KNP Campaign

Using WIBS-4, a large number of fluorescent, spherical particles (>15 μm) were counted throughout the KNP campaign. This information alone is suggestive that pollen was indeed sampled given the size, shape and fluorescent data obtained. In general it was found that the PBAP detected, in flight, were 24-27 μm in diameter and to be spherical in morphology. Daily concentration comparisons using results obtained from both the traditional volumetric trap and the WIBS-4 were clearly in full agreement as shown in Figure 1. The plot shows excellent daily correlations, with a computed R^2 correlation value, 0.93.

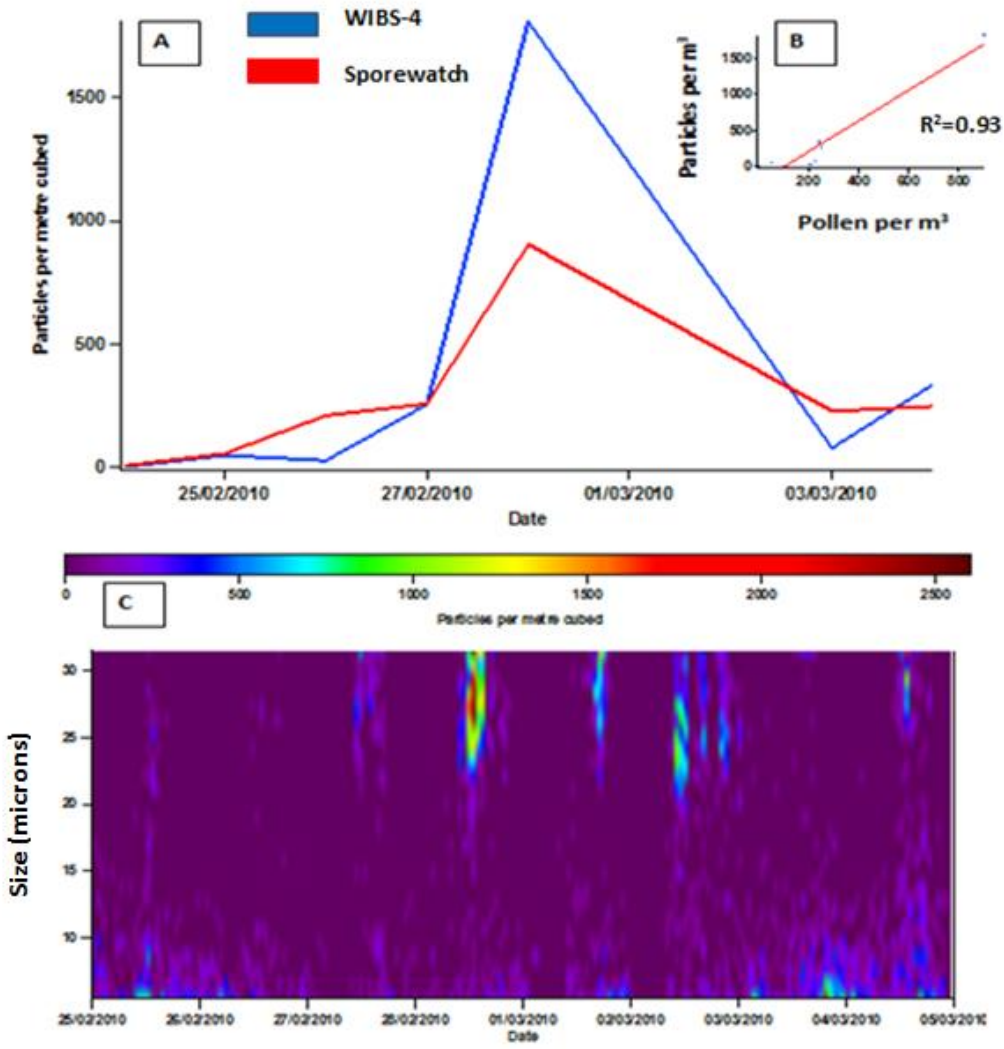

Figure 1. (A): Daily concentration data for the pollen and fluorescent particles (m^{-3}) obtained with the WIBS-4 (blue line) and the SporeWatch/microscope (red line). (B): Correlation plot for the daily concentrations obtained using the two methodologies. (C): Image plot obtained from the WIBS-4 data showing the size distribution for all particles sampled > 5 μm in size for the campaign with 1 h resolution. The colour bar indicates fluorescent particles (m^{-3}).

It can be seen that the counting data obtained from both approaches track each other well as shown in Figures 1A and 1B. However Figure 1C shows some of the added advantages for analysis that are apparent using the WIBS-4 technique. Thus particle (pollen) data is available at much lower time resolution than is traditionally possible because of the labour-intensive

nature of analysis. For example Figure 1C displays the data at an hourly resolution, although much shorter time scales are also possible to analyse. Specific features such as that shown for 28/02/10 are readily apparent from the size distribution colour-coded data. Clearly this date represents that on which the peak period of pollen release was found. Additional information such as the fact that large releases of fluorescent particles (>20 µm) occurred after midday on a number of occasions during the campaign, is also apparent. The phenomenon was investigated in more detail as outlined below.

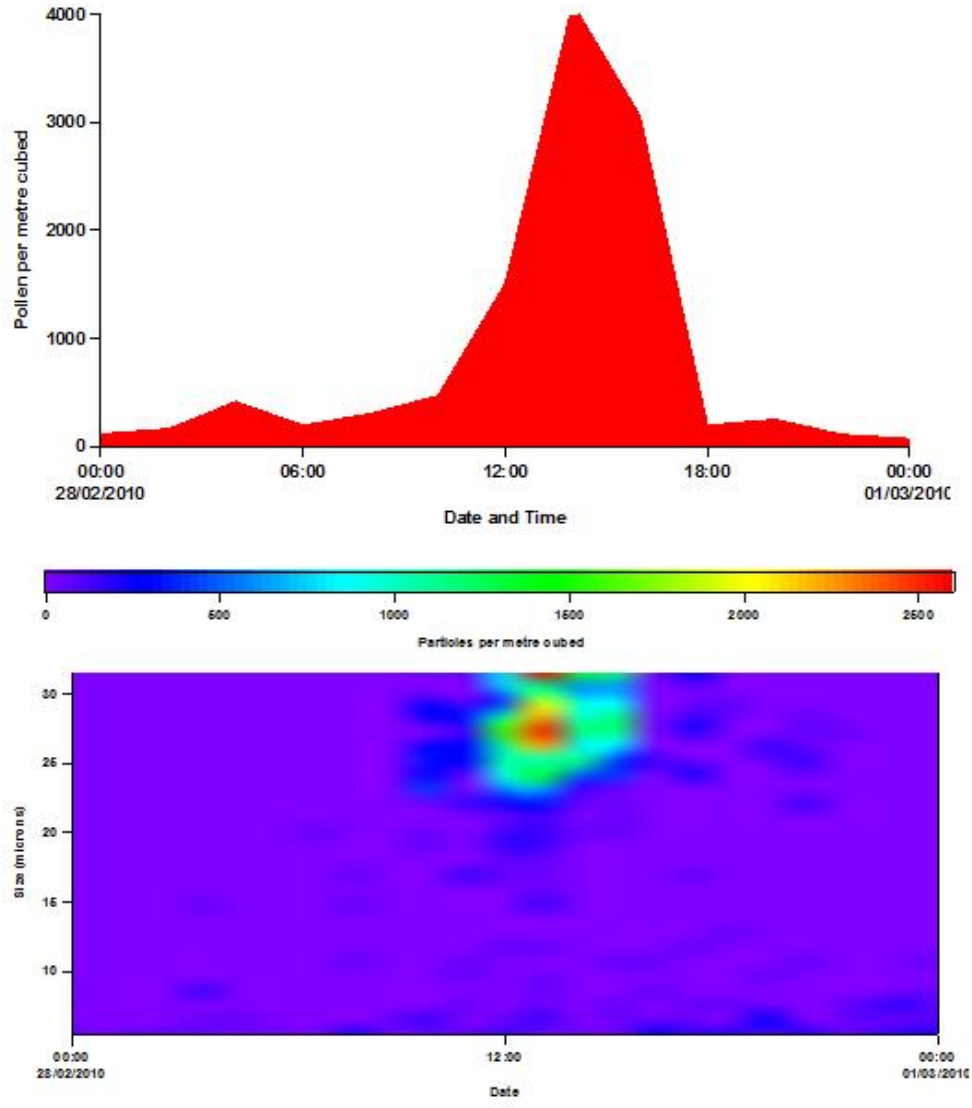

Figure 2: Comparison between the SporeWatch and WIBS-4 data obtained for 28/02/10 where in Figure 2A the pollen counts are expressed in grains per m^3 using the traditional method. In Figure 2B time-resolved number-size distribution profiles are

shown. (Note only fluorescent biological aerosol particles (FBAP) ≥4.9 µm are presented for the purpose of illustration).

Figure 2 shows a comparison between the SporeWatch and WIBS-4 data obtained for 28/02/10. In Figure 2A the pollen counts are expressed in grains per m^3 using the traditional method, whereas in Figure 2B time-resolved (30 minute) number-size distribution profiles are shown. The colour coding scheme further highlights one of the main advantages of the WIBS-4 technique: its far higher sampling time resolution capability. The major peak in the large fluorescent particle count arose at *ca.* 13:00 (red colour). This measurement correlates well with the concentration obtained with the traditional pollen sampling method, which also peaked at about this time, albeit at a lower, bi-hourly, resolution (Figure 2A). Indeed the pollen peak determined by the SporeWatch/microscopy method was identifiable with Yew pollen grains alone. The size and shape information obtained using the WIBS-4 is in full agreement with such identification and provides an indication of the potential for this on-line, automated technique.

Comparing the size profiles of the pollen grains sampled during the WIBS-4 field (ambient) campaign with that of Yew samples investigated in the laboratory [34] shows very little deviation between the two. The agreement is shown in Figure 3.

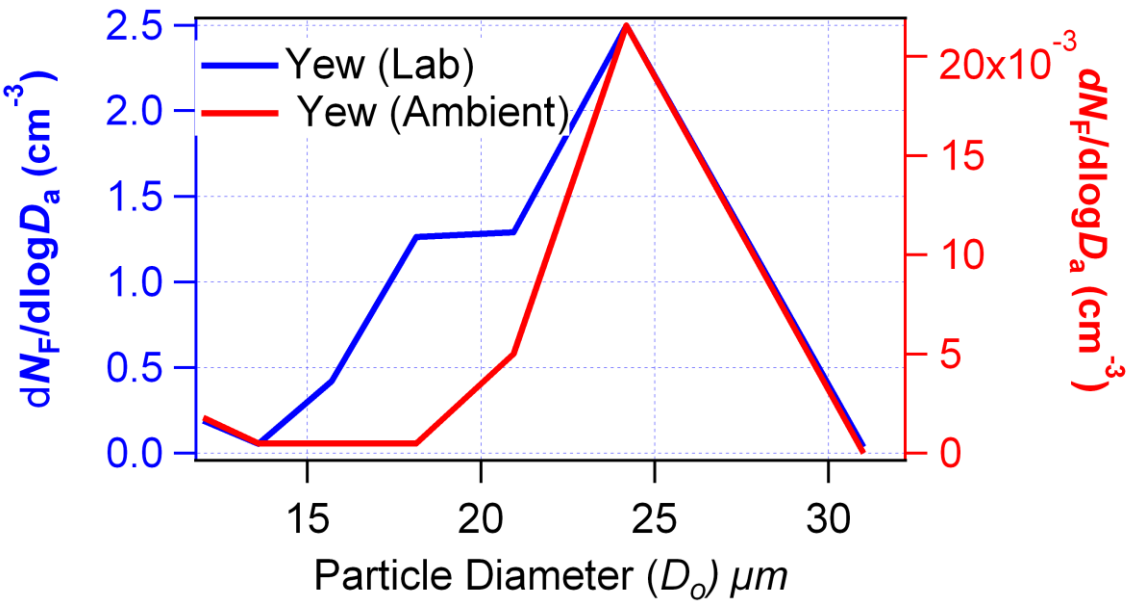

Figure 3: Average number-size distribution for Yew pollen introduced in the laboratory to the WIBS-4 (blue trace, left y-axis) as compared fluorescent particles detected in the KNP campaign (red trace, right y-axis).

Both the laboratory data (blue) and the ambient data (red) tracked well at the peak maximum. One significant difference between the two trends is the appearance of a "shoulder" for particles with a size D_o ~ 18-20 µm as sampled in the laboratory. However this slight divergence is most likely due to the break-up of source material due to the aerosolization process required for laboratory detection.

Given the extremely high time resolution of the WIBS-4 instrument, the potential for monitoring unique correlations between particle releases and meteorological parameters would also appear to be a distinct possibility. Figures 4A and 4B show treatments of this type where the average release of Yew pollen as a function of time and the corresponding meteorological parameters associated with dispersal are plotted.

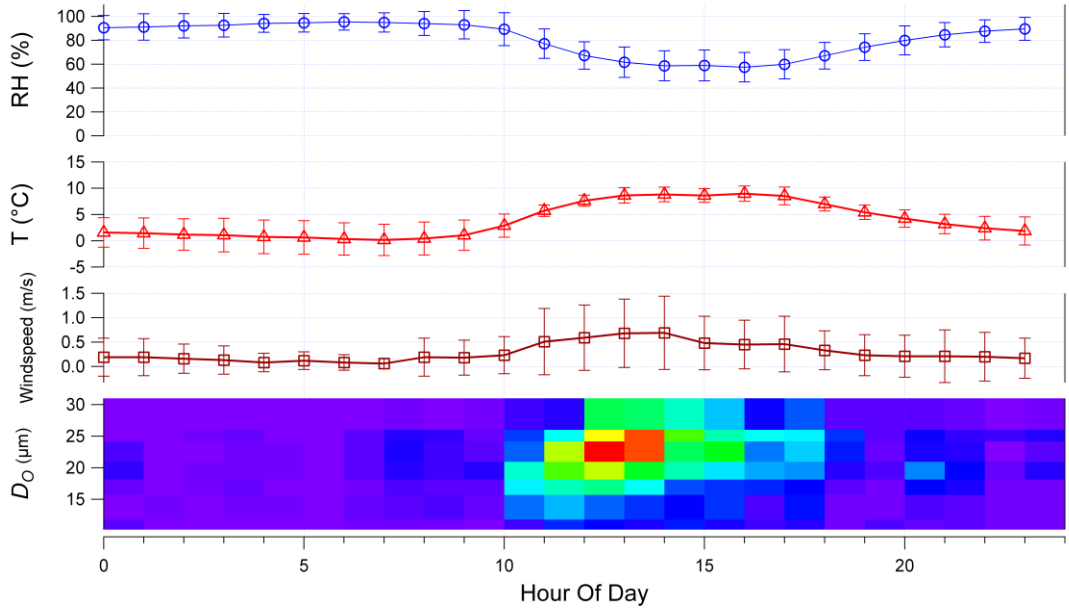

Figure 4: Diel cycles comparing, windspeed, temperature, relative humidity and Yew pollen release as detected by WIBS-4 (presented as hourly median values *vs*. local time of day).

From Figure 4A it is clear that associations between temperature, relative humidity (RH) and windspeed with Yew pollen release exist. In fact over the campaign period increasing in-flight Yew pollen concentrations were observed as RH values decreased Although not shown in Figure 4 it was also noted that atmospheric pressure had to be >995 mb for Yew pollen to

be detectable, in flight. Finally, mean temperatures of between 5 and 8 °C were shown to coincide with peak sampling of the Yew pollen as also indicated in Figure 4.

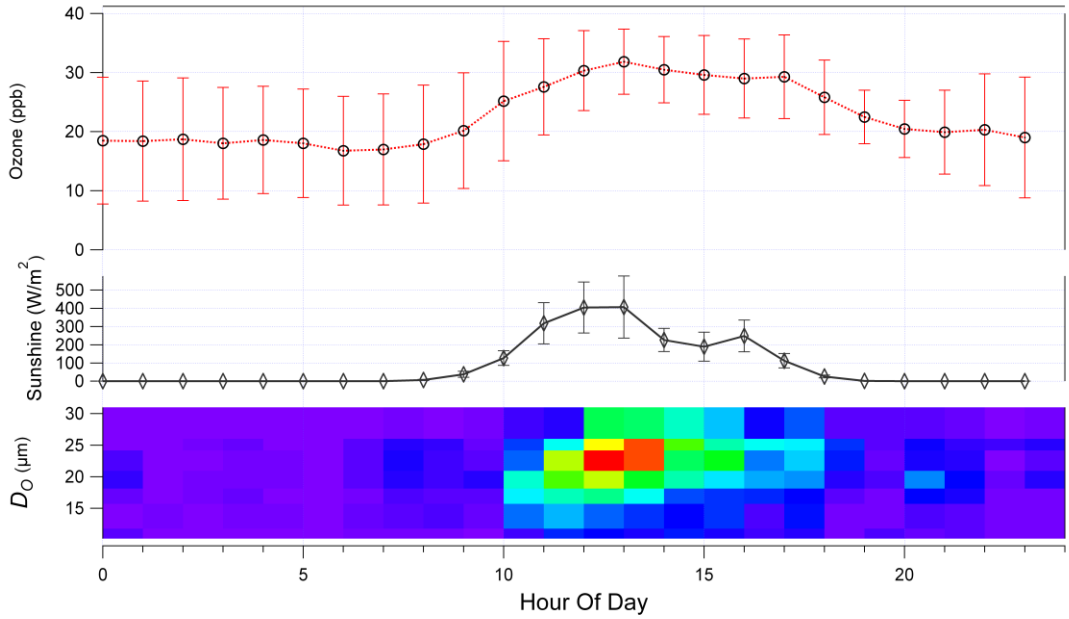

Figure (5): Diel cycles for ozone, light levels and Yew pollen release as detected by WIBS-4 (hourly median values *vs*. local time of day).

Figure 5 shows correlations between light levels experienced as well as ozone mixing ratios monitored with Yew pollen release. Again both solar radiation and ozone appear to peak in coincidence with pollen release and noted throughout the campaign. The ozone relationship is particularly interesting and requires further investigation. All that is stated here is that pollen release was observed only at ozone levels > 20 ppbv.

TUM Campaign

The campaign at TUM was undertaken in a more urbanised environment than that provided by KNP. Again a traditional Burkard/Hirst-type impaction sampler was co-located with the WIBS-4 and again very good daily correlations between the concentrations of PBAP monitored using both approaches was obtained. The results of the study are shown in Figure 6.

The correlation between the on-line and off-line techniques to determine pollen concentrations at TUM are remarkable, displaying an R^2 value, 0.98. Clearly possible chemical interfering signals caused by non-biological particles would appear to be negligible at this urbanized site. However the finding is not surprising as chemical pollutants, such as diesel particles and other secondary organic aerosol, SOA, particles, which are known to fluoresce upon excitation at wavelengths used by the WIBS-4 method are found airborne in, typically, the sub-micron size range and are therefore far smaller than pollen. Although of little apparent relevance to the TUM results discussed here, the prospect that airborne chemical particulate matter could adhere to the surface of pollen grains and thereby alter their size, shape and fluorescent properties remains a little explored phenomenon that deserves more experimental attention.

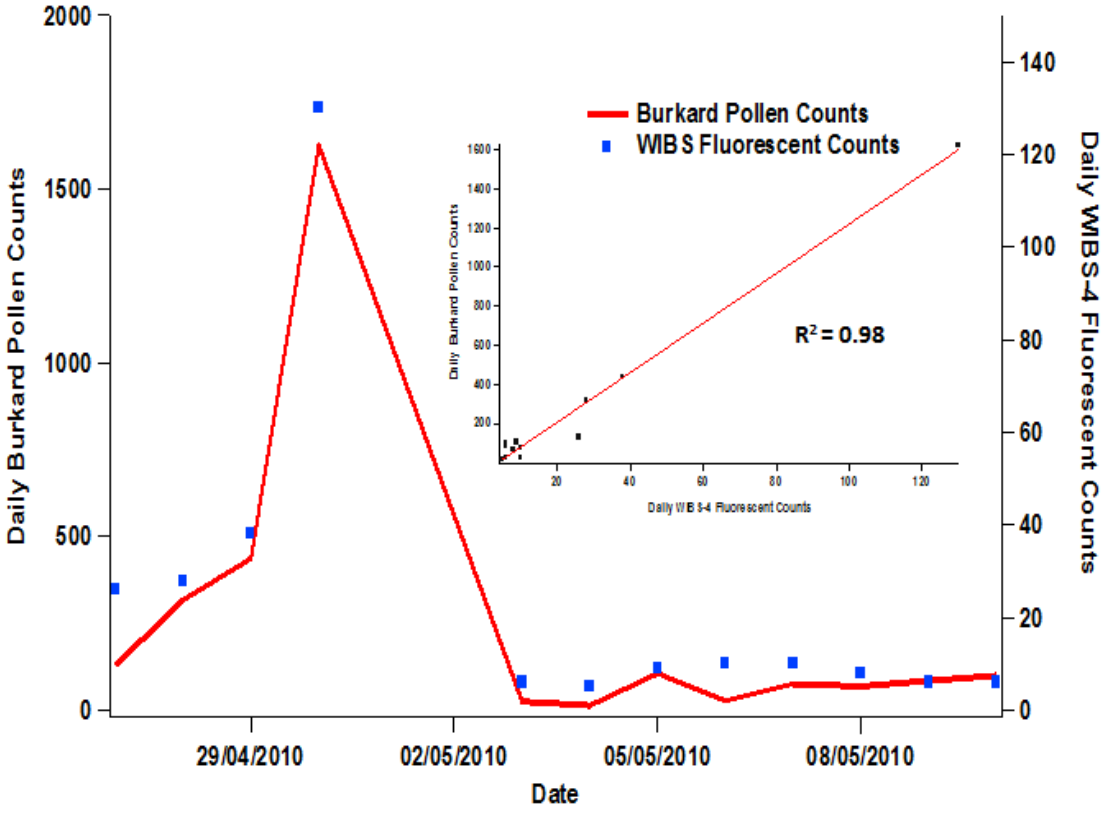

Figure 6: Daily Burkard volumetric impactor counts *vs* WIBS-4 "large" fluorescent particle counts.

Figure 7 shows box plots for the size and "shape" of likely pollen grains detected by the WIBS-4 instrument. It can be seen that the majority of the particles were designated >20 µm

in size while the interquartile spanned between 22 and 31 μm. Saturation of the WIBS-4 sizing detector occurs at 30-31 μm thus the 100th and 75th percentiles are observed at this value. Similarly the "shape" of the particles of interest exhibits a median value of 20 AF units. It has been shown previoulsy that AF< 30 can be considered to be roughly spherical in nature with the degree of roundness increasing at lower AF values. Given that Birch and Poplar were the most prominent pollen types seen by the impactor/microscopy technique over the course of the campaign, AF values in the 12 to 42 range but with a median value of 20 could be considered to be similarly assignable, by use of the on-line technique. Birch and Poplar are known to possess mainly globular morphologies.

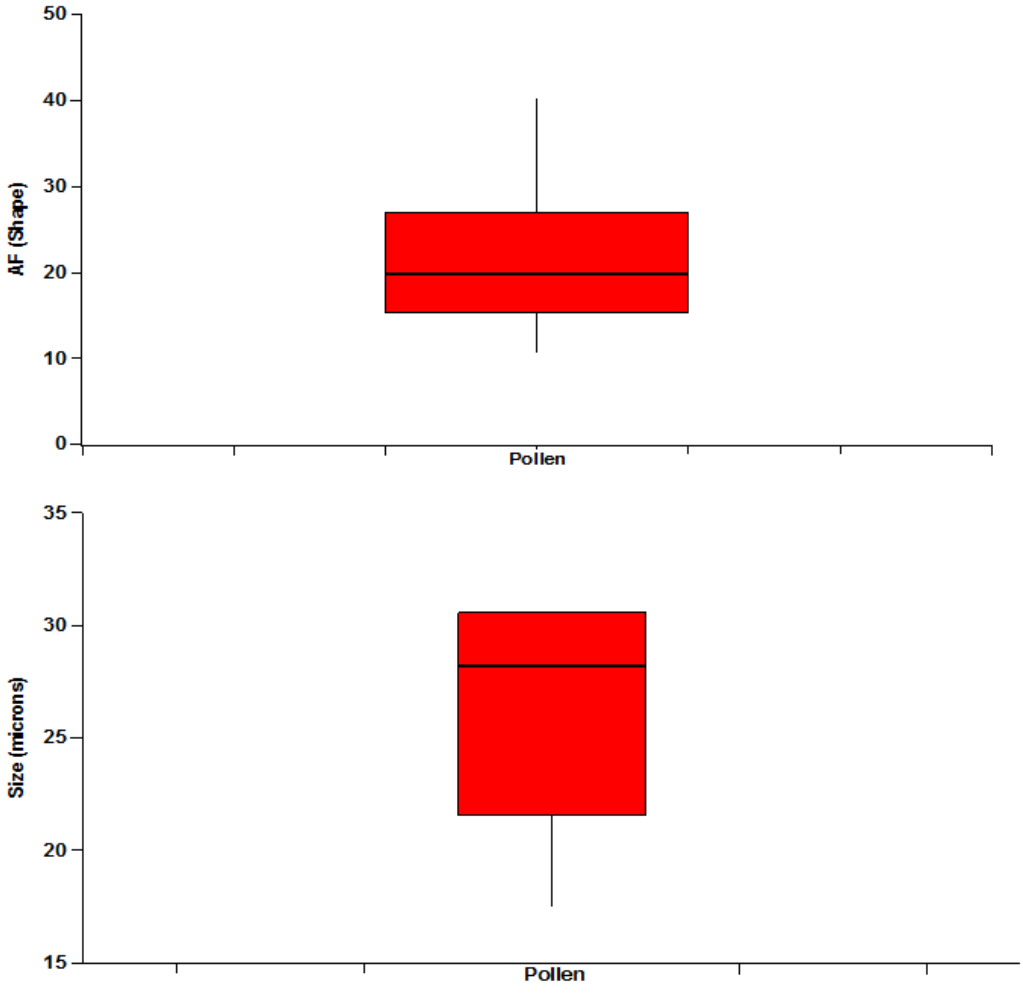

Figure 7: Box plots for the size and "shape" of the large fluorescent particles sampled by the WIBS-4 during the TUM campaign.

In summary both the size and "shape" information obtained by WIBS-4 regarding the in flight PBAP detected in the TUM campaign is consistent with the size ranges and morphologies that are known for Birch and Poplar pollen.

Conclusion

The WIBS-4 instrument was sited at two contrasting locations, one rural and the other urbanized in order to evaluate its potential for detecting, counting and identifying pollen. In order to obtain meaningful information the traditional impaction/optical microscopy techniques of pollen collection and analysis were also employed. The concentration results correlated very well, with R^2 values >0.9 determined at both campaign sites. Furthermore the sizing data available from the WIBS-4 approach employed here indicates that pollen grains can be identified in appropriate conditions. Hence Yew pollen was sampled both in the laboratory and in the field at KNP for this study: the results indicated almost identical size ranges. In fact Yew pollen is generally reported to be between 25-27 μm in diameter but the measurements reported here are the first of their type providing data on the size of *in-flight* Yew pollen. The number, size and "shape" ranges of PBAP determined by use of the WIBS-4 in the TUM campaign were also in good agreement with the results obtained using the traditional off line technique.

The campaigns performed were only of a relatively short duration but they do show that the counting ability of the near real-time WIBS-4 method provides excellent agreement with the labour intensive, time-consuming impaction/microscopy methodology. Additionally because of the high time resolution of the WIBS-4 instrument, correlations between pollen release levels and parameters such as ozone levels and RH can be readily visualized as virtual "snapshots".

The overall study should be taken as a proof of principle of the WIBS-4 approach to pollen counting. More detailed assessments would require comparison studies to be performed over full pollen seasons and the potential interference posed by some of the larger spores such as Alternaria, Epiccocum, and Polythrincium should be investigated In fact few of these fungal spore types were found to be present during both the KNP and TUM campaigns but their contributions later in the calender year may become more apparent.

Acknowledgements

We would like to thank the Irish EPA for financial support with two separate grants: 2007 CCRP Project 4.4.6.b. (BioCheA) and a Doctoral Scholarship to DJOC.

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