



The ability of automated fluorometry and holography to discern airborne grass pollen beyond family level

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Abstract Grass pollen is the major outdoor allergen source, inducing allergic rhinitis in susceptible people. In Australia, the considerable prevalence of allergic rhinitis (23.8%) and asthma (11%) is creating a major health and economic burden. Subtropical grass species exhibit distinct ecological and phylogenetic differences compared with temperate grasses, including variations in pollen allergen composition and immune response. Traditional light microscopy, however, cannot differentiate between airborne grass pollen of various subfamilies. This study hypothesizes that automated holography and fluorometry sensing can discern grass pollen beyond family level (Poaceae). This project applied automatic sensing to five

aerosolized dried grass pollen samples from Panicoideae; *Paspalum notatum* (Bahia grass) and *Sorghum halepense* (Johnson grass), Chloridoideae; *Cynodon dactylon* (Bermuda grass), and Pooideae; *Lolium perenne* (Ryegrass) and *Phleum pratense* (Timothy grass). Datasets were examined for differences in distributions of holography-derived particle features and emitted fluorescence spectra. In three repeated measurement campaigns of the same dry *L. perenne* pollen sample, distributions of particle features, and relative fluorescence intensity values suggested that reliability and reproducibility of automatic sensors remain a challenge. Data from five grass pollen types, even within the same subfamily, showed distinct morphological and fluorescence profiles, particularly at excitation 280 nm with 357 nm emission. The automated monitoring was also capable of distinguishing dried and freshly collected local *P. notatum* pollen. To translate these new insights for improved respiratory disease management, further research with broader data collection and validation with key locally relevant pollen taxa will be required for more precise real-time monitoring of grass pollen exposure.

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1 Introduction

The prevalence of allergic rhinitis (Hay Fever; AR) (23.8%) and asthma (11%) is considerable in Australia, resulting in significant health and economic impacts (National Health Survey, 2022; Simunovic et al., 2020). Grasses (family Poaceae) dominate ecosystem and cover 31–43% of the Earth's land surface (Gibson, 2009). Grasses are major outdoor trigger of pollen allergies (Andersson & Lidholm, 2003; Davies et al., 2012; García-Mozo, 2017). It has been reported that family Poaceae and family Cupressaceae make up over 50% of the total airborne pollen in Australian urban environments, followed by *Betula*, Myrtaceae, Oleaceae, Pinaceae, *Casuarina*, and some herbaceous important taxa like *Plantago* and *Rumex* (Haberle et al., 2014). There are around 11,000 species, 12 subfamilies and 700 hundred genera found in grasses (Shaw et al., 2011; Soreng et al., 2022). The most common causes of grass pollen allergy are thought to be about 20 species from five subfamilies (Tonnel et al., 2001). More than 95% of grass species that cause allergies are from three subfamilies: Pooideae, Chloridoideae, and Panicoideae (Davies, 2014; García-Mozo, 2017).

Research studies have demonstrated the presence of diverse allergens in different grass species, for example fourteen allergenic components are identified in subtropical *Cynodon dactylon* (Bermuda grass) (Shen et al., 1988). Allergen components, Sor h 1 and h 13 are reported in *Sorghum halepense* (Johnson grass) (Campbell et al., 2015), and the main allergens of *Paspalum notatum* (Bahia grass) are Pas n 1 and Pas n 13. In *Lolium perenne* (Ryegrass) pollen, Lol p 1 and Lol p 5 allergen are major allergen components (Davies et al., 2011; Suphioglu, 2000). Other studies demonstrated that patient IgE profiles show region dependent reactivity with subtropical or temperate grass pollen (Kailaivasan et al., 2020).

Different grass species occupy different ecoregions according to their mode of nutrition and carbon dioxide fixation. Photosynthetic pathways (C3 and C4) play an important role in determining the adaptability and distribution of plants species across different biogeographic zones (Crous et al., 2022). Research studies have suggested that subtropical grass species like *C. dactylon* (subfamily Chloridoideae) and *P. notatum* (subfamily Panicoideae) have distinct ecological and

phylogenetic variation as compared with temperate grasses (Davies, 2014). Although grass pollen is the major outdoor allergenic pollen source, each location has its own biodiversity influenced by local environmental and climatic conditions, and therefore the timing and magnitude of grass pollen differ between locations (Davies et al., 2022; Medek et al., 2016; Xie et al., 2022).

In Australia, all active pollen monitoring sites follow the interim standard and protocols that provides the requirements and recommendations for all the steps involved in pollen and spore monitoring (Beggs et al., 2018). All recent and most previous pollen studies in Australia have used the Hirst style manual sampling (Davies et al., 2022; Haberle et al., 2014). However, this method has significant uncertainty (Adamov et al., 2024; Addison-Smith et al., 2020), requires a significant amount of physical effort and lacks precision due to technical and pollen identification/quantification variations (Milic et al., 2020; Oteros et al., 2015b). Moreover, by light microscopy, which is used for pollen identification and quantification, airborne pollen of grass cannot be distinguished beyond the family level (Salih et al., 1997). Automated pollen monitoring has a great potential for workload reduction and can provide rapid online data to researchers as well as allergic patients (Oteros et al., 2015a).

Recent studies have reported different automated bioaerosol monitoring instruments (Clot et al., 2024; Gallardo et al., 2024; Oteros et al., 2020; Tummon et al., 2024), involving technologies such as digital microscopy, elastic light scattering, light induced fluorescence, refractive index, disperse emission spectra, laser-induced spectroscopy, laser-diode, holography, and fluorometry (Huffman et al., 2020; Maya-Manzano et al., 2023; Šaulienė et al., 2019; Sauvageat et al., 2020). Devices and technologies, coupled with machine learning algorithms, have been demonstrated to achieve over 96% accuracy in identifying major allergenic pollen species in central Europe, even with only holographic imaging (Sauvageat et al., 2020). Recent study (Erb et al., 2024) reported that combining fluorometry with holography leads to a substantial increase in identification performance, but that study only examined one species from temperate grass subfamily

Pooideae; *Cynosurus cristatus*. Another study used automated sensors with neural networks including subfamily Pooideae species; *Dactylis*, *Festuca*, and *Secale*, and suggested that clustering of fluorescence data might be the best technique to identify the pollen beyond the taxonomic family level (Daunys et al., 2021). Pollen identification efficiency can be affected by particle size in some instruments, e.g., WIBS-NEO showed highest efficiency 90% to 0.9 μm diameter of the particles, while Rapid-E (Plair, Switzerland) perform best for larger particles with diameter of 10 μm or greater at an efficiency of 58% (Lieberherr et al., 2021). However, no study has tested the capacity for these technologies to discern pollen from different Poaceae subfamilies. Furthermore, these technologies that use holography and fluorometry have not been applied in subtropical regions of the globe.

In this study, we have examined whether holography, fluorometry, and combined holography and fluorometry, can effectively distinguish airborne grass pollen beyond the family level. In this research study, *P. notatum* and *S. halepense* (Panicoideae), *C. dactylon* (Chloridoideae), and *L. perenne* and *Phleum pratense* (Pooideae) grass pollen samples were analyzed. The outcomes of this research will contribute to the development and implementation of advanced automated pollen monitoring methods, by addressing the current limitations in differentiating grass pollen subfamilies.

2 Material and methods

2.1 The automated holography and fluorescence

This project utilized the real-time bioaerosol monitor Poleno Jupiter (Swisens SA, Switzerland). This automatic sensor was installed in the agricultural field of a peri-urban area of Rocklea, a central suburb of Brisbane in Queensland, Australia (27.5594° S, 152.9988° E). Poleno Jupiter is an airflow cytometer with an optical measurement system that provides automatic real-time measurement and identification of each aerosol particle that passes through the measuring system. Poleno Jupiter combines digital online holography and laser-induced fluorescence

techniques, with machine learning, as described by Lieberherr et al., (2021).

Each particle that enters the Poleno's measurement channel is first detected by trigger lasers. After the triggering, a particle is exposed to two orthogonal lasers, which generate greyscale holograms of the same particle. The holography measurement is followed by the fluorescence measurement, where a particle is exposed to three different excitation light sources (with center wavelengths around 280 nm, 365 nm and 405 nm). The resultant fluorescence intensity signal and lifetime are measured across five detector windows (with center wavelengths around 357 nm, 435 nm, 483 nm, 562 nm and 676 nm) (Erb et al., 2024). The relative fluorescence used in this study is a normalized fluorescence obtained by dividing the absolute fluorescence by channel, which is the sum of fluorescence across all channels for the same excitation wavelength. The value taken as a reference is 0–1, which is the sum of all channels together for each excitation. The final measurement in the Poleno system is a polarized time-resolve measurement of scattered light. Currently the system applies an integrated machine learning (ML) model using the classifier that assigns each particle to one of the 14 pollen classes. It is important to emphasize that the ML model used in this study was developed by MeteoSwiss using holographic pollen observations from Europe (Crouzy et al., 2025). This study will focus on examining particle properties from structural (holographic) images and reported relative fluorescence intensity for different grass pollen samples.

2.2 Grass pollen samples

Five non-defatted dry grass pollen samples (water content < 4.5%) were purchased and imported from Stallergenes Greer (Lenoir USA). Dry pollen was stored at $-20\text{ }^{\circ}\text{C}$ upon receipt. To minimize moisture ingress during storage, the container lid was sealed with parafilm, ensuring the gap between the lid and container remained airtight. For each experiment, the pollen container was retrieved from $-20\text{ }^{\circ}\text{C}$ and allowed to equilibrate to room temperature for 20–30 min to ensure complete thawing before use. After the required aliquot was measured, the remaining pollen was immediately resealed with parafilm

and returned to $-20\text{ }^{\circ}\text{C}$. A fresh *P. notatum* pollen sample was collected in the vicinity of the pollen monitoring site at Rocklea, during the 2023/24 grass pollen season. Pollen grains were collected by flicking the grass flowers, in the early morning hours, into paper bags. Shaking the bags helped the pollen settle down. Large particles and unwanted debris were eliminated with tweezers, and by using a stainless-steel 25 cm sieve with an aperture size of $63\text{ }\mu\text{m}$ (Impact Test Equipment Stevenston Industrial Estate, Stevenston, Ayrshire, Scotland KA20 3LR). After sieving, the pollen was collected into metal cuvette. After about 30 min from the collection, fresh pollen was introduced to the instrument with the atomizer.

2.3 Measurement campaigns

Eight measurement campaigns were conducted using dry and fresh grass pollen samples (Table 1). All measurement campaigns were conducted in the field using automatic holography and fluorescence sensor and the atomizer, with commercially available grass pollen from three subfamilies and one fresh pollen sample. The Atomizer (version 01 2020, Swisens) was used for sample aerosolization and Poleno Jupiter for holography and fluorometry measurements. The Atomizer is a small electric device used to aerosolize and introduce pollen grains into the Poleno's airflow cytometer through an air inlet. A grass pollen sample in a metal cuvette mounted on the acoustic speaker of the atomizer is aerosolized by the speaker's vibrations, and entrained into the airflow of the Poleno Jupiter. The aerosolization is controlled by changing vibration amplitude and frequency, and blower speed. In order to have sufficient particle event rate detected by the instrument sensors (120/min), the Atomizer

was set for frequency between 60 and 75 Hz, vibration amplitude of 20–35%, and blower speed of 20–30%. Each grass species pollen sample was tasted separately on different days. To avoid cross contamination, after every measurement campaign the atomizer was cleaned by compressed air to remove any residual particles. The instrument was also manually cleaned by 80% ethanol using lint-free laboratory wipes, before and after each measurement campaign. Repeated measurements for the same dry pollen samples of *L. perenne* were conducted multiple times to test the reliability and reproducibility of the automated sensor and pollen samples. The fresh *P. notatum* pollen was an opportunity to compare fresh versus dry pollen sample.

2.4 Dataset cleaning and statistical analysis

Each reconstructed holographic image was analyzed by Jupiter's software using the algorithms provided by scikit-image Python package (Scikit-image, 2024; Van der Walt et al., 2014), to determine particle properties. Important particle features include eccentricity, which indicates the deviation from a circle of the ellipse fitted to the particle (on a scale of 0–1, where 0 represents a perfect circle), the major and minor axis lengths, which represents the longer and shorter side of the ellipse fitted to the particle, respectively, and the equivalent area diameter, which refers to a diameter of a circle with the same area as the particle. Of the two holographic images captured, the maximum value of each feature is reported.

Each dataset generated in the measurement campaigns was visualized, explored, and cleaned using the Swisens DataExplorer, a browser-based software system acting as an analysis tool and

Table 1 Poleno Jupiter measurement campaigns, raw events - pollen with debris, and clean events - only pollen of Poaceae samples

Species scientific name	Subfamily name	Common name	Sample type	Measurement date	Raw events	Clean events
<i>Lolium perenne</i> (1)	Pooideae	Ryegrass	Dry	18-11-2022	2970	616
<i>Cynodon dactylon</i>	Chloridoideae	Bermuda grass	Dry	18-11-2022	3389	881
<i>Sorghum halepense</i>	Panicoideae	Johnson grass	Dry	16-02-2023	6986	2008
<i>Paspalum notatum</i>	Panicoideae	Bahia grass	Dry	21-03-2023	7024	2922
<i>Lolium perenne</i> (2)	Pooideae	Ryegrass	Dry	21-03-2023	7733	2140
<i>Lolium perenne</i> (3)	Pooideae	Ryegrass	Dry	26-10-2023	8350	1869
<i>Phleum pratense</i>	Pooideae	Timothy grass	Dry	13-06-2023	10,561	2796
<i>Paspalum notatum</i>	Panicoideae	Bahia grass	Fresh	19-12-2023	8780	795

interface to the Poleno's data. Different iterative techniques were used in the cleaning of each of the datasets. The UMAP plots were used to explore events that were clustered separately to Poaceae pollen clusters. Examples of these clusters were events other than pollen that were mostly debris. Similarly, in the parallel coordinate plots, events that were well outside of the feature range, where most events were clustered, were examined and considered for removal. Both plots were used to eliminate unwanted events including events with no image available, with blurred holographic images, and events other than pollen (i.e., debris and fragments). The percentage analysis was done to compare the mean values of one pollen sample feature to the mean value of the same feature of another pollen sample. The percentage difference tells us how different a sample is from other sample for the same holography or fluorescence feature.

Cleaned datasets were tested for normality using the Shapiro–Wilk test (R-Studio, version “2024.4.2.764”) and the common libraries like ComplexHeatmap, circlize, and grid. Percentage analysis generally relied on base functions, such as table(), sum(), and prop.table(). Moreover, for data plotting, dplyr, tidyr, and ggplot2 were used. The data were then analyzed using a nonparametric statistical test, Kruskal–Wallis test (GraphPad prism 10.4.1) to assess differences among five dried pollen samples from three subfamilies, as well as for three repeated measurement campaigns. Additionally, the nonparametric statistical Mann–Whitney U test was applied in data analysis of fresh versus dry *P. notatum* pollen.

2.5 Distribution of fluorescence on *Paspalum notatum* Pollen grain surface

To evaluate the distribution of fluorescence properties of dry and fresh *P. notatum* pollen, fluorescence microscopy was used. Non-defatted dried and fresh *P. notatum* pollen were suspended in phenol red-free Dulbecco's Eagle Medium (DMEM, 21063029, Gibco) at a concentration of 2 mg/ml. The pollen suspension was distributed into a 24-well plate (500 µL per well) and visualized using a Nikon Eclipse TS2 inverted microscope. Autofluorescence was induced with an excitation wavelength of 470 nm, and

emission was detected at 534/55 nm. The image was captured using a TrueChrome Metrics camera.

3 Results

Across three repeated measurements, *L. perenne* pollen appeared to show consistent distribution of values for eccentricity, indicating consistent roundness of the particles in repeated measure of this sample (Fig. 1b, Figs. S1b, S7b). There were, however, some minor but statistically significant differences in size-related properties, e.g., maximum equivalent area diameter (Fig S7a) and maximum major-axis (Fig. 1a and Fig S1a, d, e). There was also a small, but statistically significant difference observed in solidity between the first and subsequent measurements (Fig. 1c, Figs. S1c, S7c). While the distribution of relative fluorescence of *L. perenne* pollen appeared visually similar between measurements, significant differences were detected (Fig. 1d, e, f, Figs. S2, S7d, e, f).

Pollen of all five grass species showed obvious differences in distribution of equivalent area diameter and axis length, while data for distribution of eccentricity and solidity appeared to be similar between grass species, although there were statistically significant differences detected (Fig. 2a, b, c, Figs. S3, S8a, b, c). The dried *C. dactylon* pollen showed the smallest median value for the maximum equivalent diameter (29 µm; IQR 28–30), followed by *P. notatum* (32 µm; IQR 31–46), *L. perenne* (34 µm; IQR 32–35), *P. pratense* (38 µm; IQR 34–37), and the *S. halepense* being the largest (45 µm; IQR 42–46) among these five grass species (Fig. 2a). All five species showed distinguishable distributions of their measured relative fluorescence spectra (Fig. 2d, e, f and Fig. S4). However, with the excitation/emission wavelengths of 280/357 nm there was more obvious differentiation between the pollen of these grasses; *L. perenne* showed the lowest median and IQR (0.29, 0.26–0.34) for relative fluorescence followed by *S. halepense* (0.34, 0.28–0.41), *P. pratense* (0.37, 0.29–0.44), *C. dactylon* (0.51, 0.46–0.55), and *P. notatum* (0.67, 0.62–0.71), respectively, (Fig. 2d) and this was further confirmed by percentage differences (Fig. S8d, e, f).

(1) *Lolium perenne* (18-11-2022), (2) *Lolium perenne* (21-03-2023), (3) *Lolium perenne* (26-10-2023)

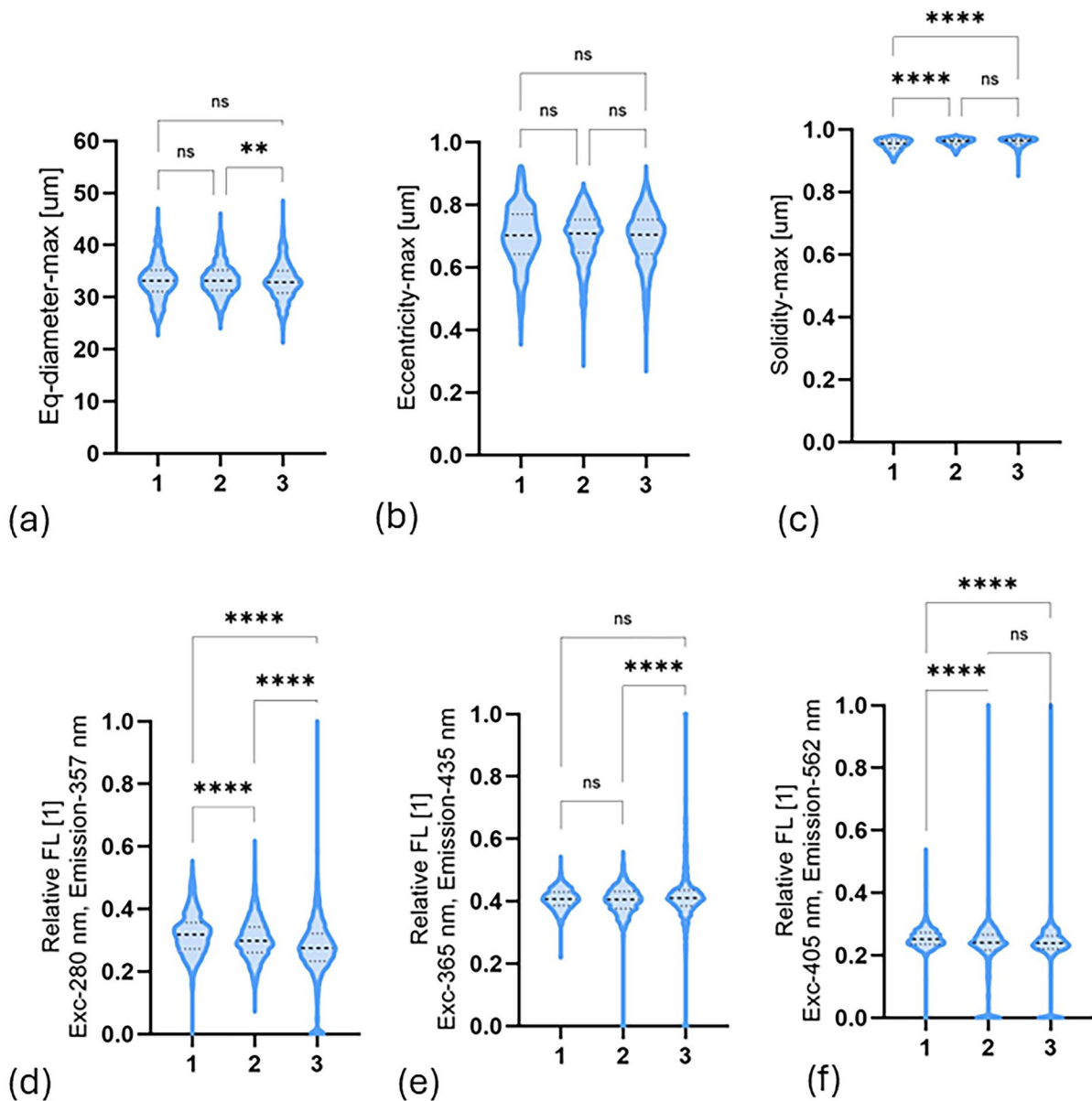


Fig. 1 Distribution of morphological and fluorescence features of *Lolium perenne* ryegrass pollen across three measurement campaigns. **a** Maximum equivalent area diameter **b** Maximum eccentricity **c** Maximum solidity **d** Measured relative fluorescence intensity with excitation of 280 nm and emission

of 357 nm, **e** excitation of 365 nm and emission of 435 nm, and **f** excitation of 405 nm and emission of 562 nm. *P* values below 0.05 were considered significant with values designated with asterisks; *p* < 0.05; *, *p* < 0.01; **, *p* < 0.001; ***, and *p* < 0.0001; ****

UMAP based on holography-derived structural properties, fluorescence data, and combined holography and fluorescence features, was also examined. Using only holography seems to be sufficient in

distinguishing between some but not all the grasses, with *S. halepense* being clearly different from others (Fig. 3a). The same was observed in case of fluorescence properties, with *P. notatum* grass having the

most distinct fluorescence signature (Fig. 3b). Combining the two data types, however, generated more accurate and distinct clustering of different species compared to using them separately (Fig. 3c).

The measurements for fresh *P. notatum* were compared with those from dried *P. notatum* pollen. The distributions of the equivalent area diameter and eccentricity, as well as each of the fluorescence measurements, appeared to differ between fresh and dry pollen (Fig. 4, Figs. S5, S6, S9). Fresh *P. notatum* pollen was larger than dry *P. notatum* pollen, with Eq-diameter-max median and IQR of 39.31, 36.22–42.78 and 31.54, 30.52–32.51, respectively (Fig. 4a). The same was observed in light micrographs of fresh *P. notatum* (Fig. 4j) and dry *P. notatum* (Fig. 4k). Fresh pollen also appeared to be rounder than dried *P. notatum* pollen (eccentricity-max of 0.6 μm and 0.7 μm , respectively) (Fig. 4b). Fresh and dry *P. notatum* pollen showed significantly different relative fluorescence at different excitation and wavelength channels. It was noted that both samples showed the highest relative fluorescence intensities when illuminated by the 280 nm source and detected by the 357 nm measurement channel (Fig. 4d). Notably, the intensity of autofluorescence of pollen increases in the vicinity of the pore (Fig. 4g).

4 Discussion

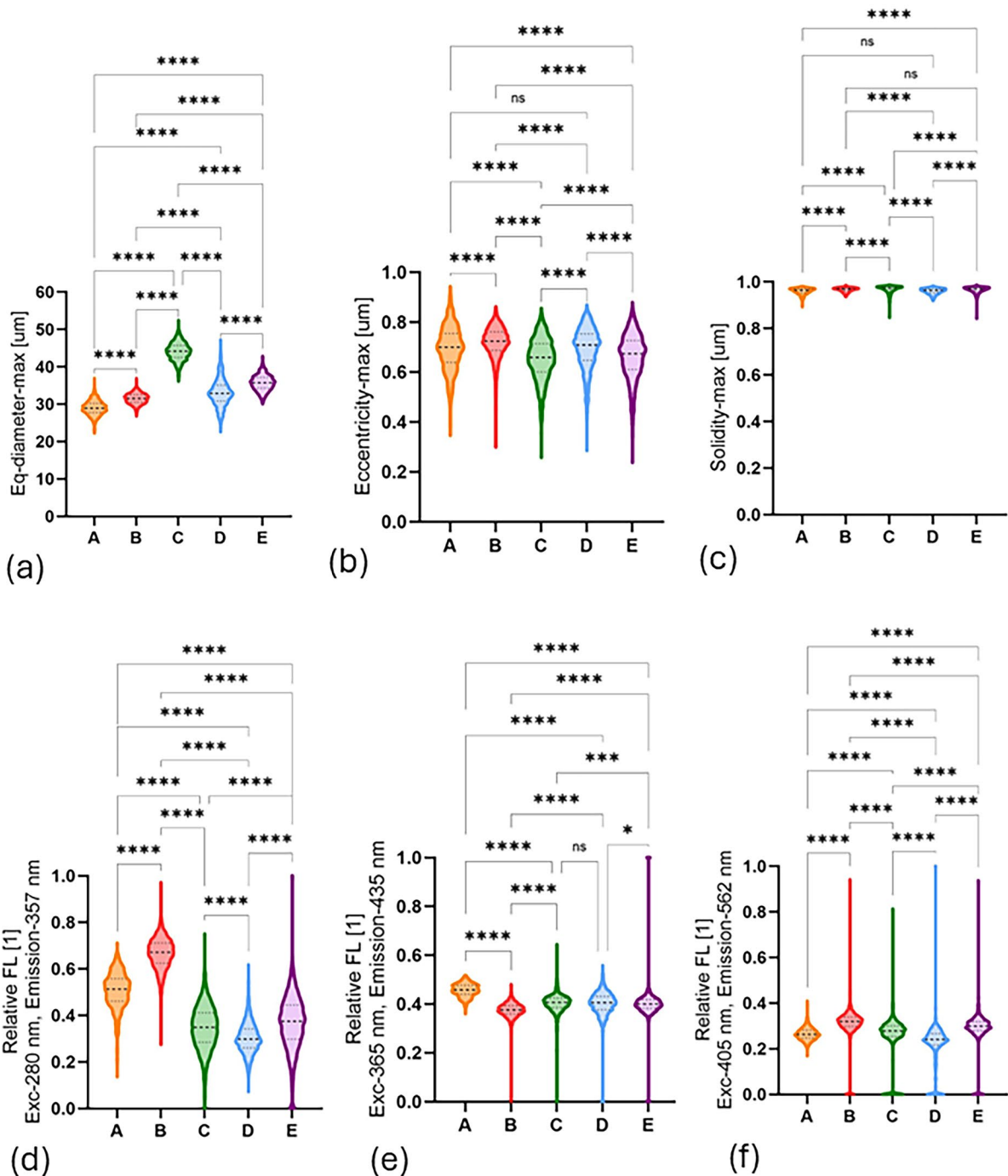
In this research study, for the first time, it was identified that morphological features and fluorescence profiles can be used to differentiate grass pollen beyond the family level, using automatic real-time pollen sensors. This research study included the same clustering technique of holography and fluorescence observations, previously applied by Daunys et al. (2021), which was able to distinguish grasses that belong to the same subfamily Pooideae, and suggested that combined techniques are promising method to classify grass pollen by genera level.

In repeated measurements of *L. perenne* pollen, while the structural features appeared to be very similar, statistical difference were observed for diameter between the second and third, and solidity between the first and subsequent measurements. These data suggested that reproducibility of automated sensors remains the primary challenge. The maximum eccentricity of *L. perenne*

was observed to be 0.63–0.78 μm . In contrast, a recently published study reported maximum eccentricity to be in the range of 0.40–0.55 μm for *C. cristatus* pollen from the same grass subfamily Pooideae (Erb et al., 2024). The relative spectra intensity appeared quite similar across these three experiments. The highest difference of approximately 14% was detected by relative fluorescence 280/357 (Fig. S7d). The detectors were highly sensitive and likely have picked up minor differences between testing of the same sample, that, while statistically significant, it is not clear if it is biologically meaningful. Evidence from dry and fresh *P. notatum* pollen fluorescence microscopy showed that fluorescence distribution is not uniform over the surface of pollen grain (Fig. 4g, Fig. S10b). Thus, since fluorescence is more intense adjacent to the pore, depending on the orientation of the airborne pollen relative to the laser and detector, grass pollen may show some variability in fluorescence measurements. This may partly explain why repeated measurements of the same sample displayed some differences. It is also important to note that the sample had been stored, and frozen and thawed, several times throughout these experiments, potentially enabling the pollen to absorb water, accounting for the slightly but significantly higher solidity measurements in the second and third measurement campaigns. The instrument sensitivity indicates its high precision and suggests possible minor changes either in the instrument's calibration over the course of the study, shifts in environmental conditions over the season, e.g., higher ambient temperature, or in the sample itself due to freeze-thaw cycles. These differences nonetheless appear to be minor.

Our results for five grass species demonstrated that automated holography and fluorometry sensors can discern grass pollen beyond the subfamily level, to genus, or even species level. Pollen of all five grasses, even from the same subfamily, showed obvious differences in particle features; particularly for maximum equivalent area diameter, while data for eccentricity and solidity appeared to be more similar between species, although statistically significant differences were detected. In our UMAP analysis, the data points showed overlap in holography and fluorometry clustering for some of the pollen, whereas there

(A) *Cynodon dactylon* (orange), (B) *Paspalum notatum* (red), (C) *Sorghum halepense* (green),
(D) *Lolium perenne* (sky blue), (E) *Phleum pratense* (purple)



was a very clear difference in the combined holography and fluorimetry. *L. perenne* and *P. pratense* both belong to the Pooideae subfamily and were

not distinguished by separate holography and fluorimetry UMAP. Similarly, in the combined holography and fluorimetry UMAP analysis,

Fig. 2 Distribution of morphological and fluorescence features for pollen of five grass species. **a** Maximum equivalent area diameter, **b** Maximum eccentricity, and **c** Maximum solidity **d** Measured relative fluorescence intensity with excitation of 280 nm with and emission of 357 nm, **e** excitation of 365 nm and emission of 435 nm, and **f** excitation of 405 nm and emission of 562 nm. A: *Cynodon dactylon* (orange) B: *Paspalum notatum* (red), C: *Sorghum halepense* (green), D: *Lolium perenne* (sky blue) and E: *Phleum pratense* (purple). *P* values below 0.05 were considered significant with values designated with asterisks; *p* < 0.05; *, *p* < 0.01; **, *p* < 0.001; ***, and *p* < 0.0001; ****

both species from the same subfamily clusters overlapped each other's, however, all the other three species: *C. dactylon* (Chloridoideae), and *P. notatum* and *S. halepense* (Panicoideae) were clearly discerned, indicating that combined use of holography and fluorometry offers improvements in pollen identification accuracy, consistent with the findings of others (Daunys et al., 2021; Erb et al., 2024). For the fresh versus, dry *P. notatum* pollen relative fluorescence-based features provided clearer and consistent separation as compared to holography and combined (holography and fluorometry features) UMAP analysis (Fig. S10, c, d, e). Holographic features provide detailed particle morphology data, while fluorescence features offer insights into biochemical properties, for instance lipid and exine composition. Merging these datasets improved the differentiation and classification of pollen from these Poaceae species, leading to meaningful and more precise identification.

The differences of holographically-derived particle features in dry and fresh *P. notatum* were reasonable to expect given that dehydration can impact pollen shape. However, it was somewhat unexpected and unclear as to why dry and fresh *P. notatum* fluorescence profiles differed. It can be speculated that this may be due to different environmental and geographical factors in the location where the fresh and dry pollen samples were sourced. Research studies have also shown that pollen morphology may vary within species due to sexual and floral diversity,

breeding systems, environmental influences, and polyploidy, which leads to inconsistent sculpturing, aperture size and shapes, and other diagnostic features (El Ghazali, 2025; Koti et al., 2005; Xiao et al., 2022). It is not known whether genotype or environmental conditions of the freshly collected, local *P. notatum* pollen are the same as the commercially available source. Moreover, grass pollen sampled at different times over the season, in the same geographic location, can show different composition of allergens within pollen. For example, *P. pratense* makes more Phl p 5 allergens inside pollen late in pollen season (Buters et al., 2015). The use of autofluorescence microscopy corresponds to previous studies (Pöhlker et al., 2013; Ronneberger et al., 2002), while the natural fluorescence of pollen grains has been used in the palynological analysis and detection of environmental changes in different pollen grains using fluorescence microscopy (Yeloff & Hunt, 2005). Analysis in this study indicates that pollen of the same grass species *P. notatum* in different conditions (fresh and dry) show different pollen structural and fluorescence features in real-time automated holography and fluorometry monitoring. In high humidity, pollen may remain more hydrated and become larger, which could affect its aerodynamic properties and how pollen is detected by sensors. Conversely, in dry, windy conditions, or during anemophilous transport, pollen can quickly desiccate. Proximity of the sensor to the source may mean airborne pollen is likely to be fresh as it is entrained in the automated pollen monitoring device, whereas pollen that has traveled further distances may become desiccated, which influences how pollen is detected by sensors in ambient conditions. Whether it is essential in future to use local fresh sources of pollen to calibrate holographic and fluorometric sensors is an important topic for ongoing discussion. Whilst these sensors can clearly discern differences between fresh and dry pollen of the same species, albeit from different origins, desiccation and rehydration of pollen

(A) *Cynodon dactylon* (orange), (B) *Paspalum notatum* (red), (C) *Sorghum halepense* (green), (D) *Lolium perenne* (sky blue), (E) *Phleum pratense* (purple)

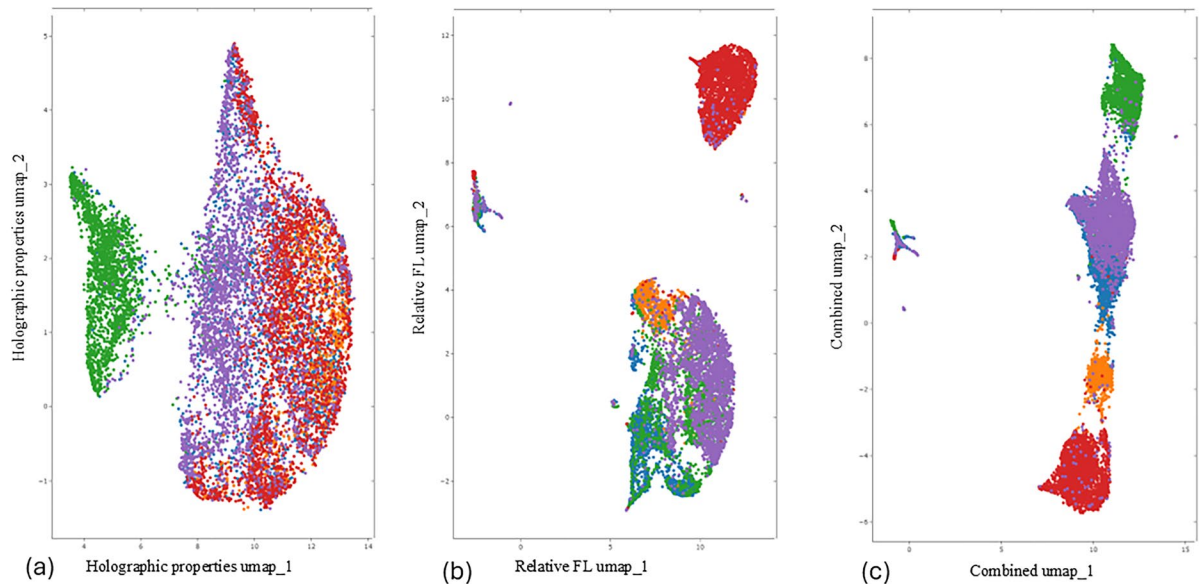


Fig. 3 Uniform approximation and projection (UMAP) analysis for pollen of five grass species. **a** UMAP of morphological properties **b** UMAP of fluorescence properties **c** UMAP of combined morphological and fluorescence properties. *Cyno-*

don dactylon (orange), *Paspalum notatum* (red), *Sorghum halepense* (green), *Lolium perenne* (sky blue) and *Phleum pratense* (purple)

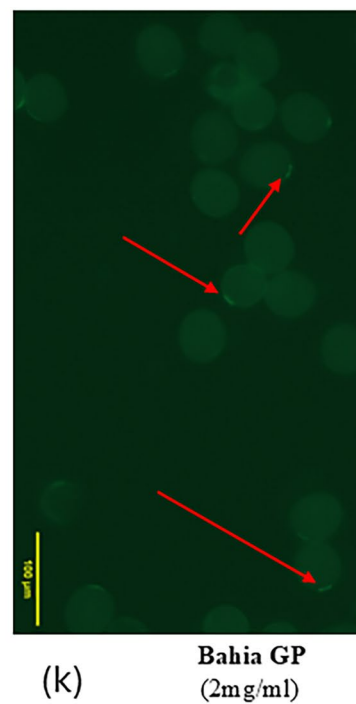
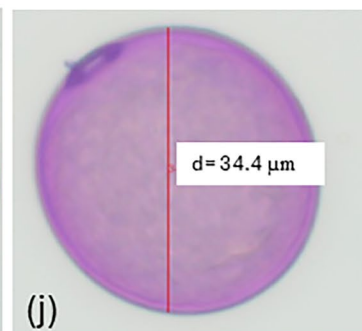
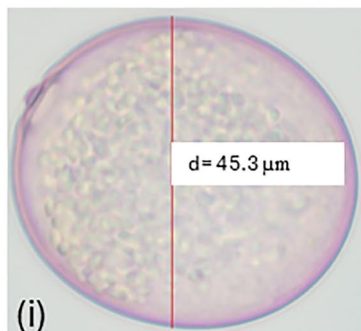
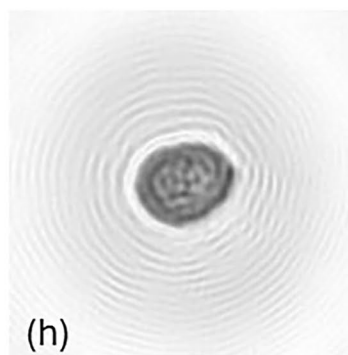
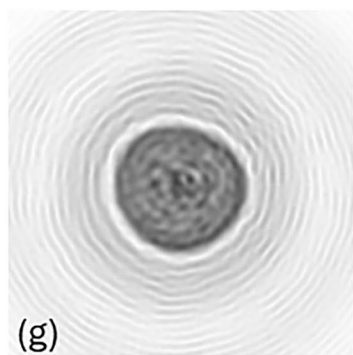
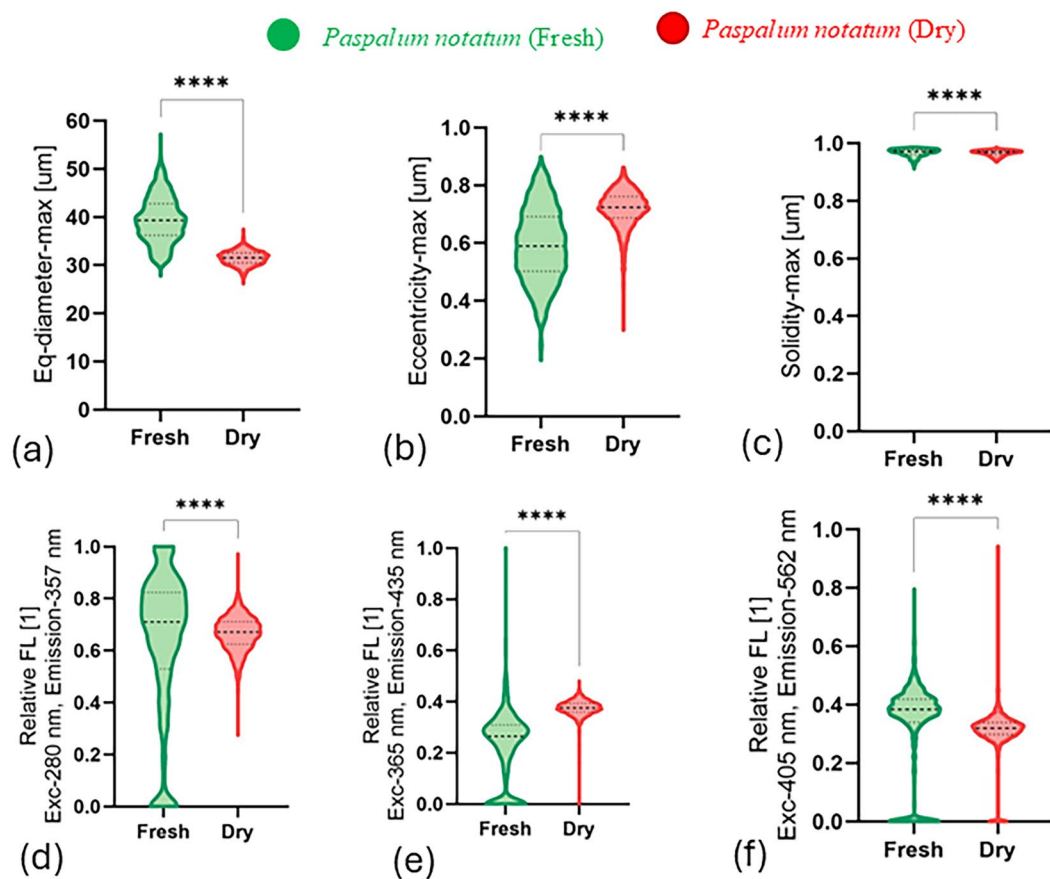
is a natural process of maturation, release, transport and fertilization.

This study elaborated the understanding of how automated sensors can distinguish grass species beyond the family level in subtropical Queensland environment, particularly those species that are associated with asthma and AR (Girgis et al., 1999). However, this system still needs to be updated to deal with environmental variability e.g. ruptured pollen (Lindstrom et al., 2017) and water droplets. In future, the same automatic sensors should be calibrated on more comprehensive list of species to train a broader model that can be validated using

eDNA metabarcoding records (Campbell et al., 2023; Frisk et al., 2023; Van Haeften et al., 2024), in field assessments of species diversity (Van Haeften et al., 2021), and with operational pollen monitoring data of the local environment.

These research findings have significant implications for the field of aerobiology, potentially providing a more precise approach for characterizing grass pollen concentration in the air. Discernment of airborne Poaceae pollen to subfamily, and/or genus taxonomic level, may lead to better grass pollen forecasting and public health interventions (Emmerson et al., 2022; Frisk et al., 2023), given differential response

Fig. 4 Distribution of morphological and fluorescence features of fresh versus dry pollen of *Paspalum notatum*. **(a)** Maximum equivalent area diameter **(b)** Maximum eccentricity **(c)** maximum solidity **(d)** Measured relative fluorescence intensity with excitation of 280 nm and emission of 357 nm, **(e)** excitation of 365 nm and emission of 435 nm, and **(f)** excitation of 405 nm and emission of 562 nm **g** Nikon eclipse TS2 microscopic image (200x) of dry pollen; red arrows show the intense fluorescence near the pores. Holographic image of fresh **(h)** and dry **(i)** pollen. Light micrograph (400x) of *P. notatum* dry **(k)** and fresh **(j)** pollen. *P* values below 0.05 were considered significant with values designated with asterisks; *p* < 0.05; *, *p* < 0.01; **, *p* < 0.001; ***, and *p* < 0.0001; ****



of subtropical and temperate grasses to environmental and weather factors.

5 Conclusions

This is the first study of combined morphological and fluorometry profiles of pollen from multiple Poaceae subfamilies, and the first use of sophisticated automated pollen monitoring in a subtropical environment of the Southern Hemisphere. Combining holography-derived morphological data with fluorescence spectra significantly enhanced the accuracy and precision of identification by distinguishing pollen grains to genus level. In field experiments, three repeated measurements of pollen from *L. perenne* indicated that reproducibility of automated sensors remains a primary challenge. Commercially grown and freshly collected *P. notatum* pollen had noticeably different morphological as well as fluorometry features. Variability in detection and intensity of fluorescence outputs may be related to the position of the pollen particle relative to the sensor, and more specifically the orientation of the pore, which appears to fluoresce more intensely. This research unlocks new possibilities for accurate and automated grass pollen monitoring in subtropical environments, and other regions with similar climate and biodiversity (Davies et al., 2021; Esterhuizen et al., 2023; Ramon et al., 2020). The outcomes will contribute to the development of advanced automated pollen monitoring methods, addressing the current limitations in differentiating airborne pollen of various grass subfamilies, and potentially aiding in pollen forecasting and public health interventions.

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Author contributions IZ (conducted the study, original draft writing and formal analysis). AM (conducted the study, formal analysis, supervision, and writing—review and editing) SH (conducted the study, writing—review and editing), BA-S (formal analysis, writing—review and editing), and DW (supervision, statistical formal analysis, and reviewing and editing).

JMD (supervision, conceptualization, formal analysis, writing—review and editing, and funding acquisition).

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Data availability The underlying research data and codes can be accessed upon request to the corresponding author (Janet M. Davies; [j36.davies@qut.edu.au] (mailto:j36.davies@qut.edu.au)).

Declarations

Competing interests Competing Interests On behalf of JMD, Queensland University of Technology has received a loan of pollen monitoring equipment from Swisens SA, Switzerland and services from Kenelec Australia, and an investigator-initiated grant from Bayer Healthcare LLC, USA outside the scope of submitted work. JMD is an inventor on patents owned by QUT; US PTO 14/311944 issued and PCT WO 2025/102125. Other authors have no relevant financial or non-financial interests to disclose.

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References

- Adamov, S., Lemonis, N., Clot, B., Crouzy, B., Gehrig, R., Graber, M.-J., Sallin, C., & Tummon, F. (2024). On the

- measurement uncertainty of Hirst-type volumetric pollen and spore samplers. *Aerobiologia*, 40(1), 77–91.
- Addison-Smith, B., Wraith, D., & Davies, J. M. (2020). Standardising pollen monitoring: Quantifying confidence intervals for measurements of airborne pollen concentration. *Aerobiologia*, 36(4), 605–615.
- Andersson, K., & Lidholm, J. (2003). Characteristics and immunobiology of grass pollen allergens. *International Archives of Allergy and Immunology*, 130(2), 87–107.
- Beggs, P. J., Davies, J. M., Milic, A., Haberle, S., Johnston, F., Jones, P., Katelaris, C., & Newbigin, E. (2018). Australian airborne pollen and spore monitoring network interim standard and protocols.
- Buters, J., Prank, M., Sofiev, M., Pusch, G., Albertini, R., Annesi-Maesano, I., Antunes, C., Behrendt, H., Berger, U., & Brandao, R. (2015). Variation of the group 5 grass pollen allergen content of airborne pollen in relation to geographic location and time in season. *Journal of Allergy and Clinical Immunology*, 136(1), 87–95.
- Campbell, B. C., Gilding, E. K., Timbrell, V., Guru, P., Loo, D., Zennaro, D., Mari, A., Solley, G., Hill, M. M., & Godwin, I. D. (2015). Total transcriptome, proteome, and allergome of Johnson grass pollen, which is important for allergic rhinitis in subtropical regions. *Journal of Allergy and Clinical Immunology*, 135(1), 133–142.
- Campbell, B., Van Haeften, S., Massel, K., Milic, A., Al Kouba, J., Addison-Smith, B., Gilding, E., Beggs, P., & Davies, J. (2023). Metabarcoding airborne pollen from subtropical and temperate eastern Australia over multiple years reveals pollen aerobiome diversity and complexity. *Science of the Total Environment*, 862, Article 160585.
- Clot, B., Gilge, S., Hajkova, L., Magyar, D., Scheifinger, H., Sofiev, M., Büttler, F., & Tummon, F. (2024). The EUMETNET AutoPollen programme: Establishing a prototype automatic pollen monitoring network in Europe. *Aerobiologia*, 40(1), 3–11.
- Crous, K. Y., Uddling, J., & De Kauwe, M. G. (2022). Temperature responses of photosynthesis and respiration in evergreen trees from boreal to tropical latitudes. *New Phytologist*, 234(2), 353–374.
- Crouzy, B., Meurville, M.-P., Clot, B., Erb, S., Lbadaoui-Darvas, M., Tummon, F., & Lieberherr, G. (2025). Operational pollen classification using digital holography and fluorescence. *Aerobiologia*, 41(4), 841–846.
- Daunys, G., Šukienė, L., Vaitkevičius, L., Valiulis, G., Sofiev, M., & Šaulienė, I. (2021). Clustering approach for the analysis of the fluorescent bioaerosol collected by an automatic detector. *PLoS ONE*, 16(3), Article e0247284.
- Davies, J. (2014). Grass pollen allergens globally: The contribution of subtropical grasses to burden of allergic respiratory diseases. *Clinical & Experimental Allergy*, 44(6), 790–801.
- Davies, J. M., Berman, D., Beggs, P. J., Ramón, G. D., Peter, J., Katelaris, C. H., & Ziska, L. H. (2021). Global climate change and pollen aeroallergens: A southern hemisphere perspective. *Immunology and Allergy Clinics of North America*, 41(1), 1–16.
- Davies, J. M., Li, H., Green, M., Towers, M., & Upham, J. W. (2012). Subtropical grass pollen allergens are important for allergic respiratory diseases in subtropical regions. *Clinical and Translational Allergy*, 2, 1–10.
- Davies, J. M., Smith, B. A., Milic, A., Campbell, B., Van Haeften, S., Burton, P., Keaney, B., Lampugnani, E. R., Vicendese, D., & Medek, D. (2022). The AusPollen partnership project: Allergenic airborne grass pollen seasonality and magnitude across temperate and subtropical eastern Australia, 2016–2020. *Environmental Research*, 214, Article 113762.
- Davies, J. M., Voskamp, A., Dang, T. D., Pettit, B., Loo, D., Petersen, A., Hill, M. M., Upham, J. W., Rolland, J. M., & O’Hehir, R. E. (2011). The dominant 55 kDa allergen of the subtropical Bahia grass (*Paspalum notatum*) pollen is a group 13 pollen allergen, Pas n 13. *Molecular Immunology*, 48(6–7), 931–940.
- El Ghazali, G. E. (2025). Intraspecific pollen morphological variations and their importance to characterize species boundaries: A review. *Review of Palaeobotany and Palynology*, 333, Article 105248.
- Emmerson, K., Addison-Smith, E., Ebert, E., Milic, A., Vicendese, D., Lampugnani, E., Erbas, B., Medek, D., Huete, A., & Beggs, P. (2022). Evaluation of the performance of short-term curated daily airborne grass pollen forecasts in diverse biogeographical regions during the AusPollen Partnership project 2016–2020. *Atmospheric Environment: X*, 15, Article 100183.
- Erb, S., Graf, E., Zeder, Y., Lionetti, S., Berne, A., Clot, B., Lieberherr, G., Tummon, F., Wullschlegel, P., & Crouzy, B. (2024). Real-time pollen identification using holographic imaging and fluorescence measurements. *Atmospheric Measurement Techniques*, 17(2), 441–451.
- Esterhuizen, N., Berman, D. M., Neumann, F. H., Ajikah, L., Quick, L. J., Hilmer, E., Van Aardt, A., John, J., Garland, R., & Hill, T. (2023). The south African pollen monitoring network: Insights from 2 years of national aerospora sampling (2019–2021). *Clinical and Translational Allergy*, 13(11), Article e12304.
- Frisk, C. A., Adams-Groom, B., & Smith, M. (2023). Isolating the species element in grass pollen allergy: A review. *Science of the Total Environment*, 883, Article 163661.
- Gallardo, R., García-Orellana, C. J., González-Velasco, H. M., García-Manso, A., Tormo-Molina, R., Macías-Macías, M., & Abengózar, E. (2024). Automated multifocus pollen detection using deep learning. *Multimedia Tools and Applications*, 83, 1–16.
- García-Mozo, H. (2017). Poaceae pollen as the leading aeroallergen worldwide: A review. *Allergy*, 72(12), 1849–1858.
- Gibson, D. J. (2009). *Grasses and grassland ecology*. Oxford University Press.
- Girgis, M., Marks, G., Downs, S., Car, G., & Kolbe, T. (1999). Thunderstorm related asthma epidemic in inland New South Wales Australia: Who is at risk? *American Journal of Respiratory and Critical Care Medicine*, 159(3), A127–A127.
- Haberle, S. G., Bowman, D. M., Newnham, R. M., Johnston, F. H., Beggs, P. J., Buters, J., Campbell, B., Erbas, B., Godwin, I., & Green, B. J. (2014). The macroecology

- of airborne pollen in Australian and New Zealand urban areas. *PLoS ONE*, 9(5), Article e97925.
- Huffman, J. A., Perring, A. E., Savage, N. J., Clot, B., Crouzy, B., Tummon, F., Shoshanim, O., Damit, B., Schneider, J., & Sivaprakasam, V. (2020). Real-time sensing of bioaerosols: Review and current perspectives. *Aerosol Science and Technology*, 54(5), 465–495.
- Kailaivasan, T. H., Timbrell, V. L., Solley, G., Smith, W. B., McLean-Tooke, A., van Nunen, S., Smith, P., Upham, J. W., Langguth, D., & Davies, J. M. (2020). Biogeographical variation in specific IgE recognition of temperate and subtropical grass pollen allergens in allergic rhinitis patients. *Clinical and Translational Immunology*, 9(2), Article e01103.
- Koti, S., Reddy, K. R., Reddy, V., Kakani, V., & Zhao, D. (2005). Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination, and tube lengths. *Journal of Experimental Botany*, 56(412), 725–736.
- Lieberherr, G., Auderset, K., Calpini, B., Clot, B., Crouzy, B., Gysel-Beer, M., Konzelmann, T., Manzano, J., Mihajlovic, A., & Moallemi, A. (2021). Assessment of real-time bioaerosol particle counters using reference chamber experiments. *Atmospheric Measurement Techniques*, 14(12), 7693–7706.
- Lindstrom, S. J., Silver, J. D., Sutherland, M. F., Treloar, A., Newbigin, E., McDonald, C. F., & Douglass, J. A. (2017). Thunderstorm asthma outbreak of november 2016: A natural disaster requiring planning. *Medical Journal of Australia*, 207(6), 235–237.
- Maya-Manzano, J. M., Tummon, F., Abt, R., Allan, N., Bunderson, L., Clot, B., Crouzy, B., Daunys, G., Erb, S., & Gonzalez-Alonso, M. (2023). Towards European automatic bioaerosol monitoring: Comparison of 9 automatic pollen observational instruments with classic Hirst-type traps. *Science of the Total Environment*, 866, Article 161220.
- Medek, D. E., Beggs, P. J., Erbas, B., Jaggard, A. K., Campbell, B. C., Vicendese, D., Johnston, F. H., Godwin, I., Huete, A. R., & Green, B. J. (2016). Regional and seasonal variation in airborne grass pollen levels between cities of Australia and New Zealand. *Aerobiologia*, 32(2), 289–302.
- Milic, A., Addison-Smith, B., Jones, P. J., Beggs, P. J., Erbas, B., & Davies, J. M. (2020). Quality control of pollen identification and quantification exercise for the AusPolen aerobiology collaboration network: A pilot study. *Aerobiologia*, 36, 83–87.
- National Health Survey. 2022. Retrieved from <https://www.abs.gov.au/statistics/health/health-conditions-and-risks/national-health-survey/latest-release>
- Oteros, J., Pusch, G., Weichenmeier, I., Heimann, U., Möller, R., Röseler, S., Traidl-Hoffmann, C., Schmidt-Weber, C., & Buters, J. (2015a). Automatic and online pollen monitoring. *International Archives of Allergy and Immunology*, 167(3), 158–166.
- Oteros, J., Weber, A., Kutzora, S., Rojo, J., Heinze, S., Herr, C., Gebauer, R., Schmidt-Weber, C. B., & Buters, J. T. (2020). An operational robotic pollen monitoring network based on automatic image recognition. *Environmental Research*, 191, Article 110031.
- Pöhlker, C., Huffman, J. A., Förster, J.-D., & Pöschl, U. (2013). Autofluorescence of atmospheric bioaerosols: Spectral fingerprints and taxonomic trends of pollen. *Atmospheric Measurement Techniques*, 6(12), Article 3369.
- Ramon, G. D., Vanegas, E., Felix, M., Barrionuevo, L. B., Kahn, A. M., Bertone, M., Reyes, M. S., Gaviot, S., Ottaviano, C., & Cherez-Ojeda, I. (2020). Year-long trends of airborne pollen in Argentina: More research is needed. *World Allergy Organization Journal*, 13(7), 100135.
- Ronneberger, O., Schultz, E., & Burkhardt, H. (2002). Automated pollen recognition using 3D volume images from fluorescence microscopy. *Aerobiologia*, 18, 107–115.
- Salih, A., Jones, A. S., Bass, D., & Cox, G. (1997). Confocal imaging of exine as a tool for grass pollen analysis. *Grana*, 36(4), 215–224.
- Šaulienė, I., Šukienė, L., Daunys, G., Valiulis, G., Vaitkevičius, L., Matavulj, P., Brdar, S., Panic, M., Sikoparija, B., & Clot, B. (2019). Automatic pollen recognition with the Rapid-E particle counter: Xxaaa? Xmltex\break?> the first-level procedure, experience and next steps. *Atmospheric Measurement Techniques*, 12(6), 3435–3452.
- Sauvageat, E., Zeder, Y., Auderset, K., Calpini, B., Clot, B., Crouzy, B., Konzelmann, T., Lieberherr, G., Tummon, F., & Vasilatou, K. (2020). Real-time pollen monitoring using digital holography. *Atmospheric Measurement Techniques*, 13(3), 1539–1550.
- Scikit-image. (2024). https://github.com/scikit-image/scikit-image/blob/main/skimimage/measure/_regionprops.py
- Shaw, R. B., Rector, B. S., & Dube, A. M. (2011). *Distribution of grasses in Texas*. BRIT Press.
- Shen, H. D., Wang, S., Tang, R., Chang, Z. N., Su, S., & Han, S. (1988). Identification of allergens and antigens of Bermuda grass (*Cynodon dactylon*) pollen by immunoblot analysis. *Clinical & Experimental Allergy*, 18(4), 401–409.
- Simunovic, M., Dwarakanath, D., Addison-Smith, B., Susanto, N. H., Erbas, B., Baker, P., & Davies, J. M. (2020). Grass pollen as a trigger of emergency department presentations and hospital admissions for respiratory conditions in the subtropics: A systematic review. *Environmental Research*, 182, Article 109125.
- Soreng, R. J., Peterson, P. M., Zuloaga, F. O., Romaschenko, K., Clark, L. G., Teisher, J. K., Gillespie, L. J., Barberá, P., Welker, C. A., & Kellogg, E. A. (2022). A worldwide phylogenetic classification of the Poaceae (Gramineae) III: An update. *Journal of Systematics and Evolution*, 60(3), 476–521.
- Suphioglu, C. (2000). What are the important allergens in grass pollen that are linked to human allergic disease? *Clinical & Experimental Allergy*, 30(10), 1335–1341.
- Tonnel, A., Gosset, P., & Tillie-Leblond, I. (2001). Characteristics of the inflammatory response in bronchial lavage fluids from patients with status asthmaticus. *International Archives of Allergy and Immunology*, 124(1–3), 267–271.
- Tummon, F., Bruffaerts, N., Celenk, S., Choël, M., Clot, B., Crouzy, B., Galán, C., Gilge, S., Hajkova, L., & Mokín, V. (2024). Towards standardisation of automatic pollen and

- fungal spore monitoring: Best practises and guidelines. *Aerobiologia*, 40(1), 39–55.
- Van der Walt, S., Schönberger, J. L., Nunez-Iglesias, J., Boulogne, F., Warner, J. D., Yager, N., Gouillart, E., & Yu, T. (2014). Scikit-image: Image processing in Python. *PeerJ*, 2, Article e453.
- Van Haeften, S., Campbell, B. C., Milic, A., Addison-Smith, E., Al Kouba, J., Huete, A., Beggs, P. J., & Davies, J. M. (2024). Environmental DNA analysis of airborne Poaceae (grass) pollen reveals taxonomic diversity across seasons and climate zones. *Environmental Research*, 247, Article 117983.
- Van Haeften, S., Milic, A., Addison-Smith, B., Butcher, C., & Davies, J. M. (2021). Grass gazers: Using citizen science as a tool to facilitate practical and online science learning for secondary school students during the COVID-19 lockdown. *Ecology and Evolution*, 11(8), 3488–3500.
- Xiao, F., She, Y., She, J., Wang, Y., Wu, F., Xie, P., & Chen, Q. (2022). Intraspecific pollen morphology variation and its responses to environmental factors of wild *Cathaya argyrophylla* chun et kuang endemic to China. *Forests*, 13(5), Article 651.
- Xie, Q., Huete, A., Hall, C. C., Medlyn, B. E., Power, S. A., Davies, J. M., Medek, D. E., & Beggs, P. J. (2022). Satellite-observed shifts in C3/C4 abundance in Australian grasslands are associated with rainfall patterns. *Remote Sensing of Environment*, 273, Article 112983.
- Yeloff, D., & Hunt, C. (2005). Fluorescence microscopy of pollen and spores: A tool for investigating environmental change. *Review of Palaeobotany and Palynology*, 133(3–4), 203–219.

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