Airborne sub-pollen particles from rupturing giant ragweed pollen

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Abstract Ragweed pollen is a prevalent allergen in late summer and autumn, worsening seasonal allergic rhinitis and asthma symptoms. In the atmosphere, pollen can osmotically rupture to produce sub-pollen particles (SPP). Because of their smaller size, SPP can penetrate deeper into the respiratory tract than intact pollen grains and may trigger severe cases of asthma. Here we characterize airborne SPP forming from rupturing giant ragweed (*Ambrosia trifida*) pollen for the first time, using scanning electron microscopy and single-particle fluorescence spectroscopy. SPP ranged in diameter from 20 nm to 6.5 μm . Most SPP are capable of penetrating into the lower respiratory tract, with 82% of SPP < 1.0 μm , and

are potential cloud condensation nuclei, with 50% of SPP < 0.20 μm . To support predictions of the health and environmental effects of SPP, we have developed a quantitative method to estimate the number of SPP generated per pollen grain (n_f) based upon the principle of mass conservation. We estimate that one giant ragweed pollen grain generates 1400 SPP across the observed size range. The new measurements and method presented herein support more accurate predictions of SPP occurrence, concentration, and air quality impacts that can help to reduce the health burden of allergic airway diseases.

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Graphic abstract Rupturing ragweed pollen releasing cellular components (right), viewed by an inverted light microscope.

Keywords Ambrosia · Sub-micronic · Paucimicronic · Bioaerosol · Pollen fragment

1 Introduction

When exposed to high humidity or water, pollen grains can become engorged with water and osmotically rupture to release sub-pollen particles (SPP) to the atmosphere (Miguel et al., 2006; Schappi et al., 1997; Taylor et al., 2002, 2004). SPP have been detected in atmospheric particles much smaller than the diameter of intact pollen, especially on days with rain by measurements of allergens (Habenicht et al., 1984; Schappi et al., 1999) and chemical tracers (Hughes et al., 2020; Rathnayake et al., 2017). SPP have been observed to occur at the Earth's surface concurrent with precipitation and persist in the air for several hours after the rain ends, with peak concentrations occurring in convective thunderstorms (Hughes et al., 2020). Pollen rupturing is hypothesized to be enhanced by thunderstorms with strong winds, updrafts that carry pollen grains aloft to higher humidity where they rupture, and downdrafts that carry SPP to the surface (Taylor & Jonsson, 2004). SPP can deliver allergens deep into the respiratory tract and trigger severe asthma attacks (Schappi et al., 1999; Suphioglu et al., 1992). SPP are believed to be the major cause of “thunderstorm asthma” epidemics documented worldwide (D’Amato et al., 2019), including a catastrophic event in Melbourne, Australia, in 2016 in which a gust front storm in the midst of ryegrass pollen season triggered 9000 presentations

at emergency rooms for severe and non-fatal asthma (Hew et al., 2019). SPP are also hygroscopic and can serve as cloud condensation nuclei (CCN) (Steiner et al., 2015), decreasing the frequency and intensity of precipitation (Wozniak et al., 2018).

We characterized the SPP released by giant ragweed, a prevalent and persistent allergen indigenous to North America that has spread to Europe and Asia. As ragweed's geographic range expands, so has its growing season (Oswalt & Marshall, 2008; Ziska et al., 2011) and atmospheric load in the USA (Ziska et al., 2019). While intact ragweed pollen grains are 15–20 μm in diameter, field studies have documented a paradox in which ragweed pollen antigens are observed in substantially smaller ambient aerosol particles (Busse et al., 1972; Solomon et al., 1983; Habenicht et al., 1984). For example, common ragweed (*Ambrosia artemisiifolia*) antigen (*Amb a1*) was observed in particles < 5 μm in the Midwestern USA (Busse et al., 1972) and particles 2.5–10 μm in Poland (Grewling et al., 2016). Similarly, 27% ragweed DNA in particles < 10 μm in Germany was observed in particles < 2.5 μm (Muller-Germann et al., 2017). Our light microscopy reveals the ability of ragweed pollen to rupture to release cellular components upon exposure to water (TOC graphic).

Predicting the health and climate effects of atmospheric SPP requires accurate modeling of pollen rupture, particularly the number of SPP generated per pollen grain (n_f) and their size (Steiner 2020; Wozniak et al., 2018). Prior to this study, a single value for n_f was reported by Suphioglu et al. (1992) for a single rye grass pollen grain, which formed 700 SPP greater than 0.6 μm in diameter upon rupturing. This value provides an incomplete description of SPP, as field and laboratory studies demonstrate high concentrations of SPP with diameters < 0.6 μm (Hughes et al., 2020; Taylor et al., 2002, 2004). Additionally, this single value does not account for variations in n_f across plant taxa, seasonal, or environmental conditions, which affects pollen grain shape, size, and composition. Herein, we characterize SPP released from giant ragweed (*Ambrosia trifida*) for the first time and develop and apply a new method to calculate n_f from measurements of SPP.

2 Materials and methods

SPP generated from fresh locally collected giant ragweed (*Ambrosia trifida*) pollen were characterized using our environmental chamber modeled after that of Taylor et al. (2002). Approximately five flowering stems of giant ragweed (20 cm) were attached to a steel rod and suspended inside a $25 \times 25 \times 30$ cm plexiglass chamber (Fig. S1). Intact pollen grains were dispersed by gently shaking the steel rod to simulate wind at 5-min intervals. SPP were generated by lightly misting the giant ragweed with 500 μ L of deionized water delivered by a mechanically modified glass syringe concurrent with gentle shaking at 5 min intervals over 40 min. A small fan was placed inside the chamber to simulate light wind and a digital hygrometer (Taylor model 1732) monitored temperature (22 °C) and relative humidity, which was 61% during dry (intact pollen) and control experiments and increased to 89% following the water injections. Before each experiment, the chamber was purged with pressurized air passed through a HEPA filter (PALL Life Sciences) and hydrocarbon trap (Restek) air at 10 L min⁻¹ for one hour.

Fluorescent particles with diameters 0.5–20 μ m were characterized online by a single-particle fluorescence spectrometer (Wideband Integrated Bioaerosol Sensor, WIBS-NEO, Droplet Measurement Technologies) for the intact pollen, SPP, and background experiments (Hughes et al., 2020). Single-particle measurements were averaged to 10 second intervals. SPP were collected by a 13-stage Nano-MOUDI (125R NanoMoudi-IITM, MSP Corporation) onto polycarbonate filters (37 mm, 0.2 μ m pore size, MilliporeSigma) or the Teflon after filter (47 mm, 0.2 μ m pore size, MilliporeSigma). Samples were collected over 2 h 10 min with 24 water injections. Approximately 9×9 mm of polycarbonate filter was mounted onto aluminum SEM stubs using carbon tape (Ted Pella Inc.). The samples were sputter coated with gold (K550 Emitech Sputter Coater) and imaged with an accelerating voltage of 5 kV using a scanning electron microscope (Hitachi S-4800).

Intact giant ragweed pollen grains were collected on microscope slides coated with a thin layer of stopcock grease (Lubrisal), mounted with glycerin jelly containing a minimal amount of basic fuchsin, and sealed (Covergrip). Slides were viewed with a light microscope (Olympus BX-61) at the

20 $\times$ objective. Micrographs were analyzed in ImageJ (Schindelin et al., 2012) to determine the intact pollen grain diameter, circularity, and aspect ratio. Giant ragweed pollen grains, after exposure to water, were also viewed with an inverted microscope (Olympus IX-81) as shown in the graphical abstract.

3 Results and discussion

3.1 Characterization of giant ragweed SPP

Pollen rupturing was induced by misting flowering sprigs of giant ragweed with a fine spray of water at five-minute intervals, as indicated by the instantaneous appearance of fluorescent sub-micrometer SPP (Fig. 1a). The peak SPP number concentrations in the chamber ($0.3\text{--}0.5\text{ cm}^{-3}$) were within the range of those observed in ambient air during convective thunderstorms ($0.3\text{--}1.3\text{ cm}^{-3}$) by the same instrument (Hughes et al., 2020), indicating that environmentally relevant concentrations of SPP were produced in the chamber. The SPP fluoresced in one or more of the following excitation/emission channels leading to their designation as A-, B-, and/or C-type particles: (A) 280/310–400 nm, (B) 280/420–650 nm, and (C) 370/420–650 nm (Savage et al., 2017). SPP primarily consisted of ABC, AB-, BC-, and B-type particles that increased in number with decreasing particle size (Fig. 1b). Additionally, SPP fluoresced in fewer channels compared to intact pollen, which results from a combination of the loss of fluorophores during fragmentation and lower fluorescence intensities for smaller particles (Hernandez et al., 2016; Savage et al., 2017). In comparison with intact pollen grains (Fig. 1c), SPP decreased significantly in diameter ($p < 0.001$, Table S1). Peak SPP number concentrations occur at the lower limit of the WIBS instrument and imply high number concentrations of SPP $< 0.5\text{ }\mu\text{m}$.

SPP observed by SEM ranged in diameter from 20 nm to 6.5 μ m (Fig. 2). The mean diameter of 147 SPP was 560 nm and median diameter was 210 nm (Table S2). Of the observed SPP, 82% of the observed SPP were $< 1.0\text{ }\mu\text{m}$, indicating that a large fraction of SPP are capable of penetrating the lower respiratory tract (Suphioglu et al., 1992). Additionally, 50% of observed particles were $< 0.20\text{ }\mu\text{m}$, such that they are likely to act as cloud condensation nuclei (CCN) and

Fig. 1 **a** Sub-micrometer giant ragweed SPP observed by WIBS immediately after misting flowers with water at 5 min intervals. **b** The fluorescent particle size distribution and signatures are given for SPP and **c** intact pollen grains

impact cloud formation and precipitation (Steiner et al., 2015; Wozniak et al., 2018).

Giant ragweed SPP consisted of individual and agglomerated starch granules and other cellular

Fig. 2 Frequency distribution of SPP generated by rupturing giant ragweed pollen determined by SEM ($n = 147$)

components (Fig. 3), consistent with prior studies of SPP (Grote et al., 2000, 2001; Taylor et al., 2004). Morphology of the particles varied with particle diameter (Fig. S2). Below 0.5 μm, SPP were rounder and more uniform, while above 0.5 μm they were increasingly elongated and irregular in shape. The observation of pollen fragments from ragweed is significant in that ragweed is a major allergen that has a wide geographic impact and long growing season, making it a potentially large source of SPP to the atmosphere.

3.2 Method for determining the number of pollen fragments generated per pollen grain (n_f)

Total SPP number (N) were summed over the SPP particle size range, with the upper limit excluding intact pollen grains.

$$N = \sum_{j=1}^k n_i \quad (1)$$

SPP mass (M ; μg) was estimated as the product of volume (V_i), and density (ρ_i), with SPP volume estimated as spherical particles having the measured area equivalent diameter ($V_p = \pi D_p^3/6$).

$$M = \sum_{j=1}^k V_i \rho_i \quad (2)$$

The mass per intact pollen grain (M_{grain} ; μg) was calculated from its volume using the diameter

Fig. 3 **a** Scanning electron micrographs of giant ragweed pollen and **b–i** SPP. The polycarbonate substrates have a pore size of 0.2 µm

determined by light microscopy. Applying the principle of mass conservation, the number of grains that ruptured ($N_{\text{grains, rup}}$) corresponds to

$$N_{\text{grains, rup}} = \frac{M}{M_{\text{grain}}} \quad (3)$$

The number of SPP generated per pollen grain is thus

$$n_f = \frac{N}{N_{\text{grains, rup}}} \quad (4)$$

In calculating n_f , we assume conservation of pollen mass upon rupturing, no size-dependent particle losses, and that aggregate SPP density is equivalent to intact pollen grains. The latter assumption

conveniently cancels density terms from M and M_{grain} when calculating n_f by Eq. 4. It is reasonable to assume condensed-phase materials comprising pollen grains do not change appreciably in volume (i.e., are non-compressible). However, it is plausible that some SPP are enriched in starch granules that are denser (1.5 g cm^{-3}) than intact ragweed pollen grains at 100% relative humidity (1.28 g cm^{-3}) (Harrington & Metzger, 1963). This maximum error that this assumption may introduce (corresponding to the case in which SPP are exclusively starch granules) is 17% (the percent different in densities), but is expected to be much smaller, because large SPP that dominate M appear to be comprised of mixtures of pollen constituents (Fig. 3). Rather, the accuracy of n_f

Table 1 Summary of the number of SPP (n_f) generated per intact pollen grain by plant taxa, with the size range of SPP considered in the determination of n_f , conditions of pollen rupturing in the laboratory, diameter of the intact pollen grain, and references

Plant taxa	Conditions	D_{grain} (μm)	D_{SPP} (μm)	n_f (size range)	References
Giant ragweed	Wetting	17.7	0.018–6.5 0.6–6.5	1400 400	This study
Bermuda grass	Wet/dry cycle	–	0.01–1	NA	Taylor et al. (2002)
Birch	Wet/dry cycle	18.6	0.01–0.7	NA	Taylor et al. (2004)
Chinese elm	Wet/dry cycle	29.8	1.5–8	530 ^a	Miguel et al. (2006)
Ryegrass	Wetting	30.1	0.6–2.5	700	Suphioglu et al. (1992)

^aDenotes values determined by retrospective data analysis using the method for n_f presented herein; NA marks experiments in which n_f could not be reliably determined because SPP > 1 μm were not reported

determination depends heavily on that of N and M , which require consideration of the full size range of SPP, with SPP number highly influenced by particles < 0.5 μm and SPP mass heavily weighted by particles > 3 μm . While this method is applied to single-particle SEM measurements, it is readily applied to other types of single particle measurements and airborne size distributions (see Supporting Information), making it broadly applicable to many measurement approaches.

3.3 Determination of n_f for giant ragweed

Our n_f method was applied to SEM measurements (Fig. 3), which accessed the broadest SPP diameter range and allowed ready distinction between SPP and intact pollen grains. In calculating M , we assume SPP are spherical and used the area equivalent diameter to help control for variation in size and shape. In calculating M_{grain} , we assumed a spheroidal shape, and applied measured major diameter of $17.7 \pm 0.9 \mu\text{m}$ (Fig. S3, Table S3) and mean aspect ratio of 1.04 ± 0.03 ($n = 21$). Our resulting estimate of n_f indicates that one giant ragweed pollen grain ruptures into 1400 fragments ranging in diameter from 0.02–6.5 μm (Table 1). This n_f value is considered a lower limit, in case SPP < 200 nm are lost to substrate pores. Increasing the number of particles characterized will improve the accuracy of the estimated n_f .

In making this estimate, we cover a much larger particle size range than has been examined before. The only prior measurement of n_f in ryegrass pollen measured no SPP < 0.6 μm , and that a single ruptured ryegrass pollen released 700 starch granules ranging 0.6–2.5 μm in diameter (Suphioglu et al.,

1992). Considering the same lower limit, we estimate that a giant ragweed pollen grain produces 380 SPP > 0.6 μm . The magnitude of this estimate gives us confidence in its reasonableness. The smaller n_f for giant ragweed likely results from its fivefold smaller volume compared to ryegrass pollen. Our method was also applied to Chinese elm pollen, using the SPP size distribution reported by Miguel et al. (2006), and returned an n_f value of 540. In doing so, we considered that Chinese elm pollen grains were spherical with a diameter of 29.8 μm and SPP above 1.5 μm due to the lower limit of their aerodynamic particle sizer. The minimum diameters measured by Miguel et al. (2006) and Suphioglu et al. (1992) likely underestimate n_f (Table 1) as they exclude the smaller SPP that are demonstrated to form from pollen rupturing by us (Figs. 1, 2) and others (Taylor et al., 2002, 2004). The developed method and new value of n_f for giant ragweed will advance atmospheric predictions of SPP from this plant taxa, but emphasize the need for accurate determination of n_f for other prevalent and allergenic pollen types across the entire size range of SPP.

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Author contributions EAS designed, directed, and supervised the research, developed the method for n_f , analyzed data, and wrote the manuscript; CBAM conducted laboratory experiments, collected and analyzed SEM data, and wrote the manuscript; DDH conducted laboratory experiments, collected and analyzed WBS data, and wrote the manuscript; LMJ collected and analyzed light microscopy images and wrote the manuscript.

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