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From Field to Sky: Measurement and Modeling of Transgenic Switchgrass Pollen Dispersal in the Atmosphere

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Abstract

Accurate tracking and measurement of pollen dispersal in the atmosphere are essential for assessing cross-pollination risks, particularly in the case of genetically engineered (GE) crops. We conducted a series of unique release-recapture field studies with GE switchgrass in Oliver Springs, Tennessee, USA. Two hundred transgenic switchgrass plants (*Panicum virgatum* L. ‘Performer’) were planted at the center of a clear-cut field, with one block of 100 plants expressing orange fluorescent protein (OFP) under a maize ubiquitin promoter (PvUBI1) and another block of 100 plants expressing OFP driven by a maize pollen-specific promoter

(Zm13). Pollen was sampled from the atmosphere using fixed (ground-based) and mobile (drone-based) sampling devices at different distances from the source field, with Lagrangian Stochastic dispersal simulations run for sampling periods using high-resolution wind measurements. The pollen emission rate was estimated by combining simulated and measured pollen concentrations, and strong diurnal trends were observed. Diurnal emission rate trends were positively correlated with wind speed, temperature, and vapor pressure deficit, while negatively correlated with relative humidity. In low-wind meandering conditions, incorporating changing wind direction into the dispersal modeling improved pollen emission rate estimation and model-measurement comparisons. This study assesses the effectiveness of high and low volume pollen samplers in relation to source strength up to 1 km from the source, enhancing understanding of pollen measurement techniques. Additionally, it is a proof-of-concept for drone-based pollen sampling and GMO pollen tracking using fluorescence measurements. Results from our experiments have significant implications for cross-pollination risk assessment, prediction, and management of airborne allergens.

Keywords: switchgrass, transgenic, pollen dispersal, bioaerosol, drone sampling, pollen transport, fluorescence

1 Introduction

Accurate tracking and measurement of pollen dispersal in the atmosphere are important for assessing cross-pollination risks (Aylor et al. 2003; Nimmala et al. 2024), particularly in the case of genetically engineered (GE) crops. Wind-dispersed pollen is the primary method of gene flow in many grasses, including switchgrass (*Panicum virgatum*), an important bioenergy crop (Sofiev and Bergmann 2012). It is a perennial, warm-season C4 bunchgrass found across most of eastern North America—from northern Mexico to southern Canada. Originally adopted as a forage crop, it is now a leading candidate for large-scale lignocellulosic biofuel feedstock in the U.S. and beyond (Parrish and Fike 2005). There is increasing concern that the rapid growth and development of switchgrass as a biofuel could result in gene flow from GE switchgrass fields to nontransgenic fields (including wild populations), leading to both financial and ecological damage (Kwit and Stewart 2012; Millwood et al. 2017; Ahrens et al. 2011; Ecker et al. 2015; Stockdale and Millwood 2023). These changes could be compounded by the effects of climate change, where rising temperatures result in altered native switchgrass territory (Ahrens et al. 2014). Therefore, there is an urgent need for field experiments and modeling efforts to characterize the dispersal of airborne switchgrass pollen in relation to meteorological factors for regulation and risk management purposes.

There are limited experimental and modeling studies on switchgrass gene flow (Kwit and Stewart 2012); these model pollen dispersal with and without wind breaks (Auer et al. 2016), experimentally quantify the dispersal and cross-pollination of transgenic switchgrass (Millwood et al. 2017), and model transport in low and high-wind conditions (Ecker et al. 2013). In 2011, Millwood and colleagues conducted the first

regulated transgenic switchgrass field experiments in the U.S. (Millwood et al. 2017). A 3-year field experiment was performed in Oliver Springs, Tennessee, U.S.A. where 100 clonal switchgrass ‘Alamo’ plants transgenic for an orange-fluorescent protein (OFP) were used as the pollen source (whole plants, including pollen, were orange-fluorescent). To assess pollen movement, pollen traps were placed at 10 m intervals from the pollen-source plot in the four cardinal directions extending up 100 m from the field. Results showed that pollen-mediated gene flow is likely to occur over distances of at least 100 m (Millwood et al. 2017). This study provided important baseline data useful to determine isolation distances and other management practices, should transgenic switchgrass be grown commercially in relevant environments. Since switchgrass is an obligate outcrossing perennial grass, there are concerns about gene flow and the need for bioconfinement, especially for pollen (Kausch et al. 2010; Kwit and Stewart 2012; Stockdale and Millwood 2023). Moreover, since North America is the geographic center of switchgrass diversity, a better understanding of pollen movement in this species is needed (Kwit and Stewart 2012).

The spread of pollen through the atmosphere involves processes of liberation, drift, and deposition (Aylor et al. 2003; Isard and Gage 2001). Knowledge of these processes can help growers and producers make informed management decisions regarding pollen transport in seed production fields and neighboring farms (Isard and Gage 2001). Although atmospheric transport models can predict pollen movement, they often fail to incorporate actual measurements of pollen concentrations and viability. Various unmanned aircraft systems (UASs or drones) have previously been used to detect and monitor pollen movement over long distances in the lower atmosphere. Gottwald and Tedders pioneered the collection of pollen with UASs (Gottwald and Tedders 1985). They modified a remote-controlled biplane platform with two rotating drum samplers to collect pollen and plant pathogen spores over pecan and peach orchards. Their study demonstrated the significant potential for regional-scale transport of pollen and plant pathogens among orchards. Two decades later, Aylor and colleagues (Aylor et al. 2006) combined ground-based sampling devices with UASs to collect pollen within and above a cornfield. Over the past decade, Schmale and colleagues have integrated autonomous systems into UASs, enabling teams of vehicles to coordinate flight missions and perform complex atmospheric sampling tasks (Schmale III et al. 2008; Techy et al. 2010).

The allergen-management community needs a fast and reliable sensor network to measure airborne pollen concentrations to enable timely and accurate allergen reporting (Beggs 2024; Buters et al. 2024; Suanno et al. 2021; Tummon et al. 2021). Current allergen information reports only broad species group concentrations, typically at a daily resolution at best (Buters et al. 2018; Tummon et al. 2021). Future airborne pollen forecasts can be enhanced by integrating known pollen emissions with large-scale atmospheric models. Understanding diurnal pollen release patterns could aid in allergen treatment and improve emission source data for potential forecast models (Buters et al. 2018). To our knowledge, most airborne pollen field studies and corresponding allergen reports rely on Hirst-type samplers (Plaza et al. 2022). These sampling devices are constrained by a relatively low sampling rate of approximately 10 L/min (Adamov et al. 2024; Plaza et al. 2022), necessitating either high airborne

118 pollen concentrations or extended sampling durations to accurately characterize pollen
119 levels. The latter constraint contributes to the coarse temporal resolution of daily
120 allergen pollen reporting.

121 We hypothesized that (1) wind-dispersed pollen from switchgrass could be tracked
122 and quantified using orange fluorescent protein (OFP) expression, (2) a Lagrangian
123 Stochastic (LS) dispersal model could estimate pollen source strength in the field,
124 and (3) an array of novel samplers could serve as viable alternatives to standard
125 Hirst-type samplers. To test these hypotheses, we conducted a series of unique release-
126 recapture field studies using GE switchgrass in Oliver Springs, Tennessee, USA. Two
127 hundred plants from five transgenic lines of switchgrass (*Panicum virgatum* L. ‘Per-
128 former’) were planted at the center of a clear-cut field. One block consisted of 100
129 plants expressing OFP under the control of a maize ubiquitin promoter (PvUBI1),
130 while the other block contained 100 plants expressing OFP driven by a maize pollen-
131 specific promoter (Zm13). Pollen from the atmosphere surrounding these blocks of
132 transgenic switchgrass was collected using a series of fixed (ground-based) and mobile
133 (drone-based) sampling devices at various distances from the field center. The efficacy
134 of these various samplers was evaluated within 25 m of the source and up to 1 km
135 from the source. LS dispersal simulations were conducted for pollen sampling periods
136 using high-resolution wind measurements collected near the field. Pollen emission rates
137 were estimated by combining simulated concentrations with field concentration mea-
138 surements. By integrating high-resolution measurements and simulations, our study
139 evaluates the performance of emerging sampling technologies and highlights their
140 implications for biosecurity, allergen tracking, and ecological modeling.

141 2 Materials and Methods

142 Three field campaigns were conducted over the course of two calendar years (2021 and
143 2022) to sample airborne pollen around two blocks of transgenic switchgrass.

144 2.1 Field Site and Pollen Source

145 2.1.1 Field site

146 To assess the transport of wind-dispersed pollen from transgenic switchgrass plants,
147 a two-year field study was conducted under USDA APHIS BRS release permits
148 (21-094-103r and 124-86SS5F1). The experiments were carried out at the Tennessee
149 Agricultural Experiment Station, near the University of Tennessee’s Forest Resources
150 Research and Education Center at the Cumberland Forest Unit in Oliver Springs,
151 Tennessee, USA (36.0483147, -84.4811417).

152 The field site was selected to satisfy the primary requirements for regulatory trans-
153 genic pollen dispersal experiments: isolation and traceable source attribution. It was
154 situated on patch of recently cleared forest land, with felled trees forming a rough glade
155 area. The site provided sufficient open area (~1.5 ha) for switchgrass cultivation and
156 sampler deployment, while heavily forested borders served as a natural barrier that
157 reduced the likelihood of cross-contamination with nearby wild or cultivated switch-
158 grass and limited off-site pollen transport. The field location was intentionally chosen

in a remote, concealed area beyond a secured access point, ensuring restricted visibility and access. The field plot measured 13.7 m $\times$ 9.1 m, enclosed within a protective 15.2 m $\times$ 19.8 m fenced perimeter, as shown in Figure 1A. The outer fence was locked to prevent animal intrusion.

2.1.2 Transgenic line generation, analysis, and selection

Transgenic switchgrass plants expressing OFP were created by genetically engineering embryogenic callus derived from switchgrass seeds obtained from Ernst Conservation Seeds, Inc. (Meadville, Pennsylvania, USA). This was achieved through *Agrobacterium*-mediated transformation (*Agrobacterium tumefaciens* strain EHA105) as detailed in Li and Qu (2011), using one of two binary plasmid constructs.

The first plasmid, pANIC10A-OFP (Mann et al. 2012), contained the hygromycin phosphotransferase (hph) selectable marker gene under the control of the switchgrass ubiquitin 2 (PvUbi2) promoter as well as an orange fluorescent protein (OFP) gene pporRFP under the control of the switchgrass ubiquitin promoter (PvUBI1). This promoter drives the expression of OFP in leaves, stems, and pollen.

The second plasmid, PSYBIN1a-Zm13-pporRFP, also contained the OFP gene pporRFP under the control of a maize pollen-specific promoter (Zm13). This promoter drives the expression of OFP in the pollen. This construct also contained a second OFP gene mOrangeER under the control of the cauliflower mosaic virus (CaMV) 35S promoter which enable the expression of this OFP in callus and green tissues. In addition, the plasmid also contained the hygromycin phosphotransferase (hph) selectable marker gene under the control of the PvUBI2 promoter. Several transgenic OFP-expressing shoots were recovered from hygromycin selection media (100 mg/L), and once rooted, plants were grown in an environmental-controlled growth room (16/8 h day/night and 24/22°C day/night).

To confirm the presence of the OFP gene in the transgenic plants, PCR-screening was performed using primers (forward primer: GCAAAGTGGGGTCAAAGATG; reverse primer: CACCTTCAAGCCCTTCTTTG) designed to amplify a 556 bp fragment of the pporRFP gene. PCR-confirmed transgenic plants were moved to a greenhouse and grown (16/8 h day/night and 28/22°C day/night) until flowering. To identify transgenic events expressing OFP in pollen, visual analysis of OFP fluorescence was conducted on pollen grains from each event using epifluorescent microscopy as described by Rice et al. (2013). Transgenic lines in which all pollen grains exhibited OFP expression were propagated in the greenhouse and subsequently used in field experiments.

2.1.3 Planting

The planted area, less than 0.1 ha, consisted of 20 rows with 20 switchgrass plants per row, totaling 400 transgenic switchgrass plants arranged in a randomized design. These plants were hand-transplanted in the field at 76.2 cm intervals on three different dates. On July 20, 2021, 100 pANIC10A-OFP switchgrass plants from five transgenic events (20 clonal replicates per event) were transplanted. On August 26, 2021, another 100 PSYBIN1a-Zm13-pporRFP plants from five transgenic events (20 clonal replicates

per event) were transplanted. Lastly, on June 20, 2022, an additional 200 pANIC10A-OFP switchgrass plants from ten transgenic events (20 clonal replicates per event) were transplanted in the field plot. These last 200 plants were not mature enough to produce pollen during the field experiments. Figure 1B illustrates the locations of these plants and their ages in weeks during the August 2-3, 2022 field experiment. This experimental design was structured to monitor and analyze the dispersal from transgenic pollen over time and distance.

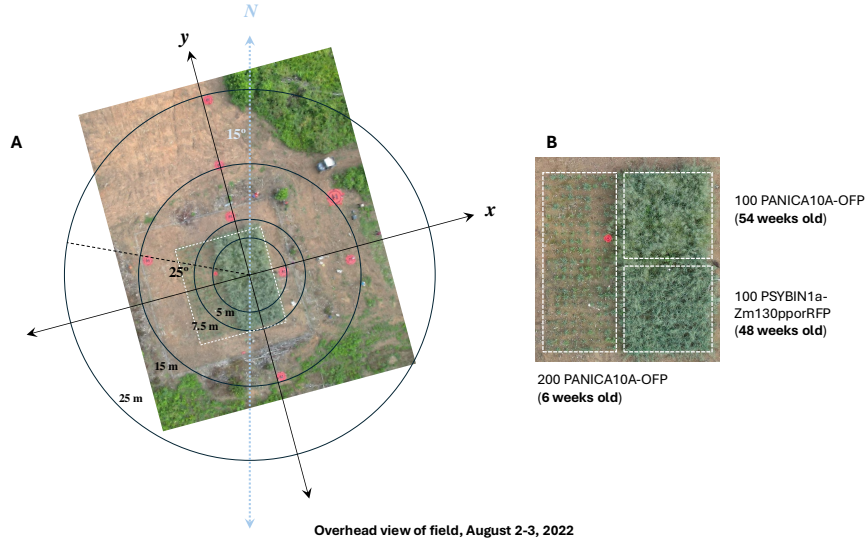


Fig. 1 Top-down drone image of the field during field experiments conducted on August 2-3, 2022. (A) The field of GE switchgrass is outlined by a white dotted square and enclosed by a perimeter fence, as required by the APHIS BRS permits. Reddish-orange circular pads mark the locations of pollen sampling devices, positioned at increasing distances from the center of the source field. (B) A close-up view of the 13.7 m × 9.1 m field of GE Switchgrass, showing the locations of both strains of OFP-expressing switchgrass plants. Labels indicate the plant positions and ages during the August 2-3, 2022, field campaign.

2.2 Sampling Methods

Four types of volumetric particle samplers, shown in Figure 2, were used to capture GE switchgrass pollen and estimate the concentration of airborne pollen at various times and distances from the source field. Each sampler had different sampling rates and sensing capabilities. To optimize equipment placement, wind forecasts and local conditions were assessed before the first sampling period. The samplers were strategically positioned based on prevailing and predicted wind directions, ensuring placement downwind along the expected pollen dispersal path. Figure 3 illustrates the placement of samplers around the field on each sampling day. The samplers were placed on reddish-orange circular drone landing pads to mark their locations and enhance

visibility in overhead footage, as shown in Figure 1A for the August 2-3, 2022 field campaign.

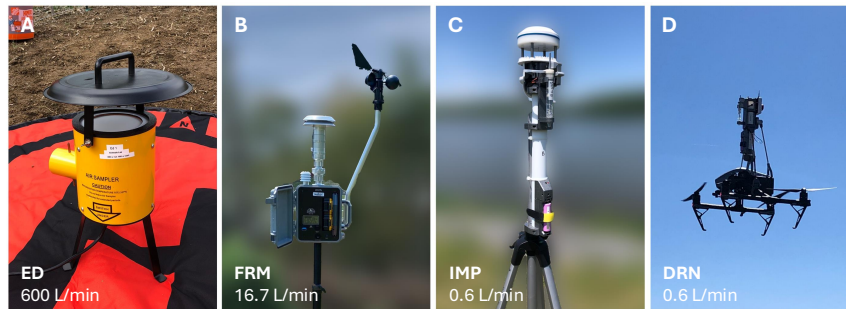


Fig. 2 All sampler units used during the field campaigns. (A) The ED (Science First 2007), a ground-based high-volume sampler (600 L/min). (B) The FRM, a ground-based medium-volume volumetric sampler (16.7 L/min). (C) The IMP, an impinger-based ground-based low-volume sampler (0.6 L/min). (D) The DRN, a drone-based sampler flown at a height of 10 meters above ground level (0.6 L/min).

2.2.1 Ground-based high-volume samplers (ED)

In anticipation of low pollen emission rates, several high-volume filter-based samplers (Science First #15000, Yulee, FL) (Science First 2007) were deployed during the campaign (Figure 2A). Originally designed for educational use in schools, these samplers are referred to as “ED” samplers throughout the manuscript.

The barrel-shaped ED samplers draw air through a filter surface at an initial flow rate of 600 L/min (Science First 2007). Cellulose filters with a pore size of 11 μm and a diameter of 125 mm were used to collect airborne pollen and other atmospheric particles at 0.432 m above ground level. The ED samplers’ volumetric sampling rate is 1000 times that of the IMP and DRN samplers, and 35 times that of the FRM sampler. This significant increase in sampling capacity allowed for improved detection of airborne pollen, particularly in cases of low pollen emission rates.

2.2.2 Ground-based medium-volume sampler (FRM)

A single near-Federal Reference Method (FRM) sampler (ARA Instruments, Eugene, Oregon) was deployed during the field experiments, shown in Figure 2B. This battery-operated device samples air at a flow rate of 16.7 L/min. The unit is equipped with a filter sampler (PM₁₀ filters were used in this study), meteorological sensors, and a particle counter. Additional details about this instrument are available on ARA’s website (ARA Instruments 2016).

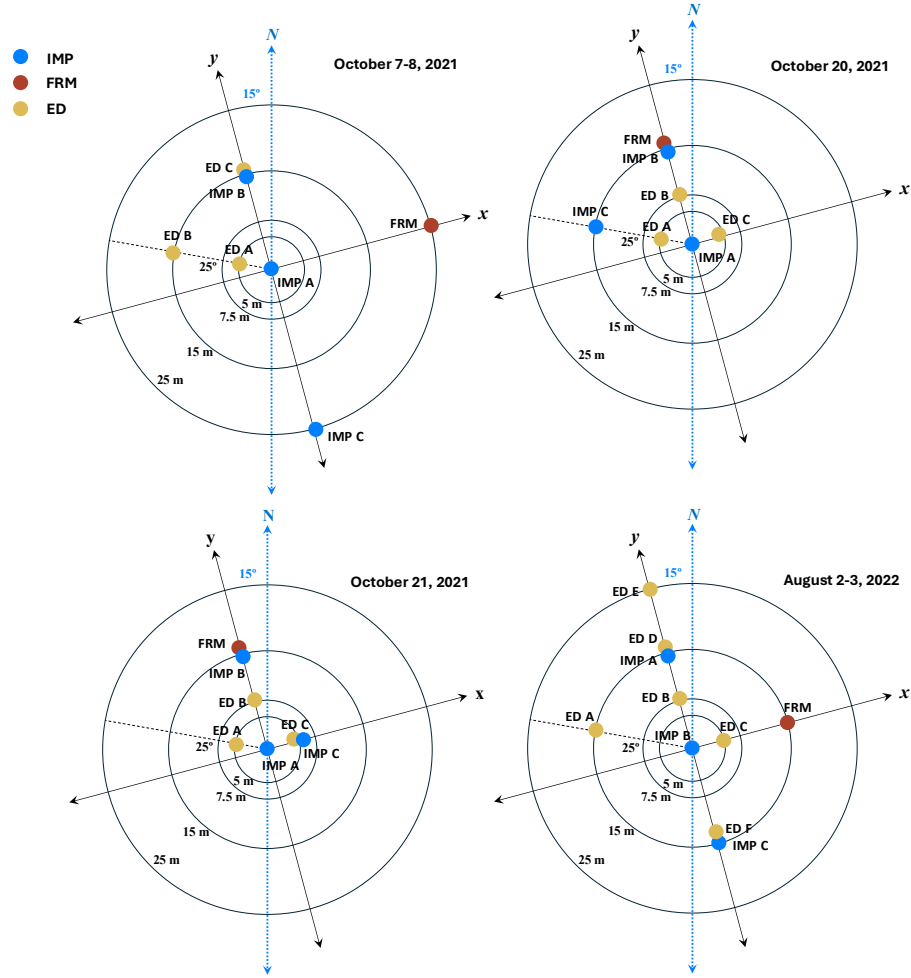


Fig. 3 Ground-based sampler locations for each collection day. Yellow, blue, and red denote placement of ED, IMP, and FRM samplers, respectively, positioned at radial distances of 5, 7.5, 15, and 25 meters from the center of field.

2.2.3 Ground-based low-volume samplers (Impingers or IMP)

To evaluate the effectiveness of impinger-type samplers, three custom-designed impinger packages were deployed during the field campaign (Figure 2C) . These sampling packages are referred to as “IMP” throughout the manuscript.

The IMPs were constructed from high-density polyethylene, following the design specifications outlined in Powers et al. (2018). The 3D printing (.stl) files for the impinger units are publicly available online (Powers, C. 2018). These files were modified to accommodate a 15 mL polypropylene conical collection vial (Corning #CLS430791) and a stainless-steel tube with a 4 mm diameter opening.

248 The IMP samplers were mounted on a tripod 2 m above ground level to approxi-
249 mate the height of the switchgrass panicles, the open flower structures that produce
250 pollen. The IMPs sampled airborne particles at a rate of 0.6 L/min, with collected
251 particles entrained in sterile 15 mL conical tubes containing 2 mL of sterile deionized
252 water.

253 2.2.4 Drone-based low-volume sampler (DRN)

254 To measure airborne pollen concentration at different altitudes above and downwind of
255 the field, we used a drone-based sampling system consisting of the IMP unit mounted
256 on a DJI Inspire 2 drone platform (Figure 2D). The system is described in detail in
257 Bilyeu et al. (2022).

258 A key design feature of the drone system is the positioning of the IMP sampler high
259 enough above the propellers, which ensures that the sampled air remains free from
260 propeller-induced turbulence, commonly known as downwash. The drone was flown at
261 a fixed altitude of 10 meters during each sampling interval, a height selected to prevent
262 propeller downwash from disturbing the switchgrass canopy during stable hovering.

263 Due to drone battery limitations and the need for safe flight and landing operations,
264 each sampling interval was restricted to 10 minutes. The IMP unit on the drone
265 operated at the same volumetric sampling rate as its ground-based counterpart (0.6
266 L/min). However, because the drone sampler was only flown for 10 minutes per flight,
267 its total sampling capacity was significantly lower than the ground-based IMP units,
268 which collected for 30 to 90 minutes during the field campaign. Hereafter, the IMP-
269 equipped drone system is referred to as “DRN” throughout the manuscript.

270 2.3 Meteorological Data

271 A weather station was installed near the field to collect meteorological data throughout
272 each sampling day. The station consisted of a Campbell Scientific CSAT3 three-
273 dimensional sonic anemometer, mounted at a height of 1.5 m above ground level, which
274 measured high-resolution wind velocity in three dimensions and sonic temperature at
275 a frequency of 10 Hz. In addition, a Campbell Scientific HMP45C probe recorded tem-
276 perature and relative humidity every 30 seconds. Meteorological data were recorded
277 with the Campbell Scientific CR3000 datalogger. To minimize interference from the
278 tripod pole, the sonic anemometer arm was positioned perpendicular to the antici-
279 pated dominant wind direction before each collection day. The wind velocities in the
280 u and v directions (relative to the sonic anemometer arm) were then rotated into the
281 cardinal coordinate system for analysis.

282 2.4 Processing of pollen samples

283 2.4.1 Sample preparation

284 Filters from the ED samplers were processed as shown in Figure 4A. Briefly, the 125
285 mm “ED” collection filters were removed with forceps and transferred to separate
286 150 mm petri dishes (Fisher #FB0875714) in the field immediately following each
287 sampling period. For each filter, 25 mL of 25% EtOH was added in the petri dish,

the filter was gently agitated with a sterile cell spreader, and then rinsed a total of 8 times. Each rinsate was transferred by a pipettor to a vacuum filtration unit (Thomas Scientific #300-4100) containing a 47 mm Isopore polycarbonate 10 μm filter (Millipore Sigma #TCTP04700). The sample was cleared through the filter using the vacuum from a hand pipetting bulb. Using forceps, the Isopore filter was then moved to a 60 mm petri dish (Genesee 32-105) and rinsed 6 times with 2 mL 25% EtOH. The resulting rinsate was transferred to an Ultrafree 5 μm PVDF centrifugal filtration tube (Millipore Sigma UFC40SV25) and centrifuged for 2 minutes at 2,500 rpm in a swinging bucket centrifuge (IEC Clinical Centrifuge). The concentrated sample was then resuspended from the 5 μm filter surface with 200 μL 25% EtOH and moved to a 1.5 mL Eppendorf tube and stored at 4 °C for further analysis.

Filters from the FRM sampler were processed as shown in Figure 4B. Briefly, the Isopore filter was removed from the FRM unit sampling cartridge using forceps and transferred to a 60 mm petri dish (Genesee 32-105) in the field immediately following each sampling period. For processing the sample, the Isopore filter was then rinsed 6 times with 2 mL 25% EtOH, and the resulting rinsate was moved to an Ultrafree 5 μm PVDF centrifugal filtration tube and centrifuged for 2 minutes at 2,500 rpm in a swinging bucket centrifuge (IEC Clinical Centrifuge). The concentrated sample was then resuspended from the 5 μm filter surface with 200 μL of 25% EtOH and moved to a 1.5 mL Eppendorf tube and stored at 4 °C for further analysis.

The fluid from the IMP and DRN samplers was processed as shown in Figure 4C. Samples from the IMPs and DRN were transferred by pipette to an Ultrafree 5 μm PVDF centrifugal filtration tube and centrifuged for 2 minutes at 2,500 rpm in a swinging bucket centrifuge (IEC Clinical Centrifuge). The concentrated sample was then resuspended from the 5 μm filter surface with 200 μL 25 EtOH and transferred to a 1.5 mL Eppendorf tube and stored at 4 °C for further analysis.

2.4.2 Pollen counting

Switchgrass pollen was counted in each concentrated sample by pipetting the samples into individual wells of a 96-well plate (Grenier Bio One 7000124). Samples were allowed to sediment for 15 minutes and then were observed using an Olympus CKX53 inverted microscope equipped with the Olympus EP50 digital camera and associated software. Following the quantification of the switchgrass pollen in each of the tubes, the samples were transferred back into their respective 1.5 mL Eppendorf storage tubes and held at 4 °C for transport and further analysis.

2.5 Atmospheric dispersal modeling

2.5.1 Meteorological Inputs

Atmospheric dispersal simulations for each sampling interval were driven using time-averaged wind statistics collected during that interval. Most sampling intervals occurred under low-wind conditions (< 2 m/s), characterized by meandering winds with frequent directional shifts and intermittent lulls in wind speed. To better capture dispersal dynamics under these conditions, the wind data were processed using different averaging window sizes. Specifically, for the 45-minute sampling intervals on

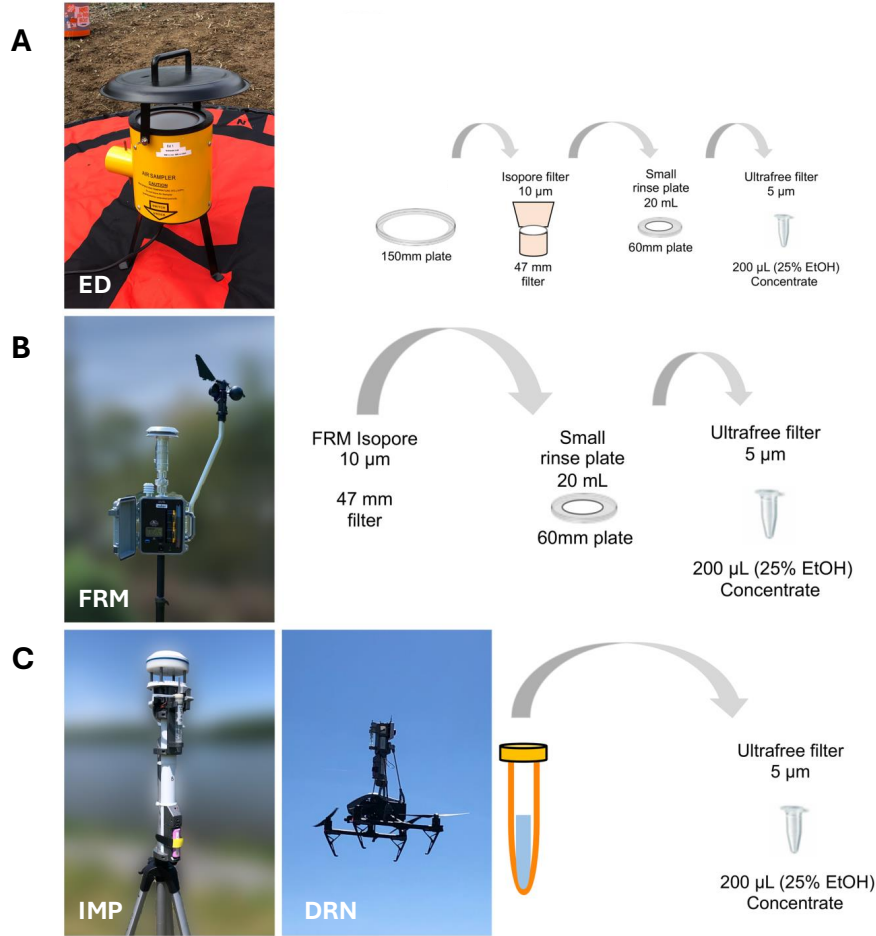


Fig. 4 Flow charts showing the processing of the filters from (A) the ED samplers, (B) the FRM sampler, and (C) the IMP and DRN samplers.

August 2-3, 2022, the following averaging windows were used: 45 one-minute averaging windows, 9 five-minute averaging windows, and 1 full 45-minute averaging window. This approach allowed for assessing how different temporal resolutions of wind averaging influenced the accuracy of dispersal simulations.

To compute turbulence statistics for each averaging window, the average downwind direction was determined and the wind velocity data were rotated into a downwind (u) and crosswind (v) coordinate system. For each sampling interval, the means, covariances, and variances were computed for these wind velocity components, as well as temperature. Mean temperature was computed from sonic temperature using the method described in Schotanus et al. (1983) and the relative humidity values. Heat

flux was estimated from sonic temperature and relative humidity using the Bowen ratio method from Schotanus et al. (1983). The Bowen ratio was determined using the simplified method of Lin et al. (2016), which requires only mean temperature and relative humidity. These turbulence statistics provided the necessary meteorological inputs for the dispersal simulation in each interval, specifically friction velocity (u_*) and the Monin-Obukhov length (L).

2.5.2 Pollen dispersal simulations

To simulate switchgrass pollen dispersal, we use the surface-layer Lagrangian Stochastic (LS) model described in Aylor and Flesch (2001) and expanded to three-dimensional transport in Aylor (2017). The LS model framework is based on Brownian motion theory, modeling turbulent diffusion by simulating the trajectories of thousands of particles through the air as random walks through the atmosphere. The movement of each particle is governed by turbulent wind statistics, and the ensemble average of these trajectories provides estimates of pollen concentration at any given location within the simulation domain.

LS models require turbulent wind statistics to be specified at every point in the simulation domain, including mean velocities, variances, and covariances of the wind components. Under the assumptions of stationarity and horizontal homogeneity, these wind field statistics remain constant over time within each averaging window but vary with height. To account for this height dependence, boundary layer scaling techniques are applied to generate vertical wind profiles based on measurable surface-level parameters, specifically the friction velocity (u_*) and the Monin-Obukhov length (L). The full model formulation and wind statistics used in the simulations are included in the code provided online.

These two parameters (u_* and L) were computed from the time-averaged meteorological measurements for each 45-minute sampling interval, using three different averaging approaches: one 45-minute averaging window; nine 5-minute averaging windows; and forty-five 1-minute averaging windows. A separate simulation was conducted for each averaging window in a 45-minute sampling interval, using the computed average wind direction, friction velocity (u_*), and Monin-Obukhov length (L). The resulting concentration fields from these simulations were then averaged to generate a single mean concentration field for each 45-minute sampling interval.

In each simulation, 100,000 particles (representing switchgrass pollen) are released from a point source at the center of the field at a height of 2 m, which approximates the height of most of the switchgrass panicles in the field experiment. Particles were removed from the simulation domain when they: traveled more than 50 m laterally from the source; rose above 100 m above ground level; or fell below 0.1 m above ground level. To simplify the simulation, pollen dispersion was modeled as if it occurred over a flat, rough surface, with estimated surface roughness of 0.01 m, consistent with values reported for level grassy plains and prairie in Hansen (1993). The settling velocity was estimated as **0.0371** m/s, based on an observed switchgrass pollen diameter of ~ 35 μm , using Stokes' law. Since switchgrass pollen was observed to be nearly spherical, Stokes' law provides a reliable approximation of its settling velocity.

2.5.3 Concentration estimation and source emission rate calculation

The pollen concentration estimation procedure in this study follows the approach described by Flesch et al. (1995) for a stationary LS model with a constant source. Pollen concentration is estimated by tracking the amount of time particles spend in each grid box, normalized by the total number of particles released (N_p) and the volume of the grid box ($V_{\text{box}} = 1 \text{ m} \times 1 \text{ m} \times 1 \text{ m}$), and then multiplied by the modeled emission rate (Q). Specifically, the concentration at a given grid point (i, j, k) is calculated as,

$$C(i, j, k) = Q \frac{1}{V_{\text{box}}} \frac{1}{N_p} \sum_{n=1}^{N_p} T_n(i, j, k), \quad (1)$$

where $T_n(i, j, k)$ represents the time particle n spends in the given grid box.

We employed a model-measurement fusion approach described in Aylor and Flesch (2001) to estimate pollen emission rate and concentration. For each sampling interval, the modeled pollen concentration at each grid point in the simulation domain is first computed under the arbitrary assumption of a constant release rate at the center of the field of $Q_{\text{model}} = 1$ particle per second. This yields a modeled relative concentration, which is proportional to the actual concentration at every point in the domain. To estimate the actual emission rate (pollen flux from the field), the ratio of the measured pollen concentration at each of the six ED samplers to the modeled relative concentration at the corresponding locations in the simulation domain was computed and used to update the value of Q . To obtain a single emission rate estimate for each sampling interval, the computed emission rates corresponding to calculations based on each of the six ED samplers were averaged. This estimated true emission rate was then used to update the modeled relative concentration to predict the actual concentration at every point within the simulation domain.

To investigate dispersal at greater distances—up to 1 km from the source—the same modeling procedure is conducted but with a coarser grid resolution of $V_{\text{box}} = 3 \text{ m} \times 3 \text{ m} \times 1 \text{ m}$ and an extended simulation domain covering $1000 \text{ m} \times 1000 \text{ m} \times 100 \text{ m}$. This coarser grid was selected to balance computational efficiency while maintaining consistency with the finer-resolution near-source grid.

To generate 2D dispersal kernels, which represent pollen concentration as a function of distance, concentrations at equal radial distances from the source are averaged, yielding average concentration as a function of radial distance from the source.

3 Results

3.1 Field Experiments

3.1.1 First campaign (October 7-8, 2021)

The first field campaign took place on October 7-8, 2021. At this time, the pANIC10A and PSYBIN1a plants (Fig. 1) were only 11 and 6 weeks old, respectively, and the

420 youngest 200 pANIC10A plants had not yet been planted. The following ground-based
421 samplers were placed radially around the field at distances of 0, 5, 15, and 25 meters,
422 as shown in Figure 3: three ED samplers, three IMPs, and one FRM sampler. These
423 ground-based samplers operated in 30-minute intervals, while the drone-based sampler
424 (DRN) was flown at a 10-meter height for a duration of 10 minutes per sampling
425 interval. However, due to technical difficulties, the drone sampler was only deployed
426 on October 7th, and was not used on October 8th.

427 IMP A was placed at the center of the field (the midpoint of field 1 and 2) to esti-
428 mate pollen emission rate, but an insufficient amount of pollen was collected—at most
429 two pollen grains in each sampling interval, and often zero—which was not statistically
430 significant to estimate the pollen emission rate. IMP B and ED C were co-located to
431 verify alignment of their concentration estimates. However, due to their vastly differ-
432 ent sampling rates and the low pollen numbers collected, direct comparisons were not
433 feasible. On October 8th, slightly more pollen was captured, particularly by ED C,
434 but overall pollen collection remained low. Impingers showed an increase in measured
435 concentrations later in the afternoon, though data remained sparse. The IMP, FRM,
436 and DRN samplers captured negligible pollen amounts.

437 Pollen concentrations for each sampling interval and sampler are presented in Table
438 1. On October 7th, too few pollen grains were collected for meaningful analysis. Con-
439 centrations marked with a dagger[†]superscript denote cases where only 1-2 pollen grains
440 were sampled. On October 7th, all samplers captured 2 or fewer pollen grains. On
441 October 8th, 1-5 grains were collected per sampler. IMP B did not capture any pollen,
442 despite being placed alongside ED C, likely due to the overall low pollen counts.

443 3.1.2 Second campaign (October 20-21, 2021)

444 During the second field campaign on October 20-21, 2021, we increased ground-based
445 sampling intervals to 90 minutes to compensate for the low pollen counts observed
446 during the previous campaign’s 30-minute sampling intervals. To improve pollen cap-
447 ture, all samplers were moved within 15 meters of the field. The drone was still flown
448 for 10 minutes per sampling interval. On October 21st, heavy rainfall forced us to
449 shorten the final sampling interval of the day. Despite the longer sampling durations
450 and closer sampler placement, pollen counts remained negligible (at most two pollen
451 grains captured per interval) throughout this campaign. See Table 1 for details.

452 3.1.3 Third campaign (August 2-3, 2022)

453 The third and final campaign took place on August 2-3, 2022. Field and sampler
454 placements during this campaign are shown in Figure 1A and B, respectively. The
455 oldest pANIC10A plants were at peak pollen production during this campaign, but
456 the PSYBIN1a plants had not yet begun releasing pollen. The youngest pANIC10A
457 field, planted only six weeks prior, was too immature to release pollen. Given that ED
458 samplers were the most effective in previous campaigns, their number was increased
459 from three to six. In anticipation of prevailing winds directed toward north-northeast,
460 samplers were primarily aligned along the x and y axes in Figures 1 and 3. The drone
461 sampler was again flown for 10 minutes at 10 meters during all sampling intervals. ED
462 D and IMP A were co-located to compare concentration measurements. IMP B was

placed at the field center to estimate the pollen emission rate, if sufficient pollen was collected. To address diurnal trends, sampling intervals were kept consistent across both collection days. All ground-based samplers were operated for 45-minute sampling intervals. ED samplers collected significantly more pollen than in previous campaigns. A clear diurnal pattern emerged, with concentrations peaking between 2:00-2:45 PM time on both days. Concentrations began increasing around 1:00 PM, peaked at 2:00 PM, then declined after 3:00 PM. See Table 1 for details.

Table 1 Pollen concentrations (pollen/m³) sampled during specific time periods, organized by date and sampler type.

Date	Time	ED						FRM	IMP			DRN**
		A	B	C	D	E	F		A	B	C	
7 Oct 2021	11:30-12:00	0	0.17	0	-	-	-	0	0	-	0	0
	12:35-13:05	0	0	0.06 [†]	-	-	-	0	0	0	0	0
	13:45-14:15	0.06 [†]	0	0	-	-	-	0	0	0	0	0
	14:45-15:15	0.11 [†]	0	0.11 [†]	-	-	-	2.00 [†]	0	0	0	0
	15:50-16:20	0.17	0.06 [†]	0.11 [†]	-	-	-	0	0	0	0	0
8 Oct 2021	12:00-12:30	0	0	0.33	-	-	-	2.00 [†]	0	0	0	-
	13:00-13:30	0.17	0.17	0.06 [†]	-	-	-	0	0	0	0	-
	14:00-14:30	0	0	0.22	-	-	-	0	111.11 [†]	0	0	-
	15:00-15:30	0.11 [†]	0.17	0.33	-	-	-	0	0	0	0	-
20 Oct 2021	14:00-16:00*	0.02 [†]	0.02 [†]	0.02 [†]	-	-	-	0.67 [†]	0	0	0	0
	16:30-18:15*	0.02 [†]	0.02 [†]	0.02 [†]	-	-	-	0	0	0	0	0
21 Oct 2021	12:00-13:45*	0	0	0.02 [†]	-	-	-	0	0	0	0	0
	14:00-14:50	0	0	0.03 [†]	-	-	-	0	0	0	0	0
2 Aug 2022	10:00-10:45	0.37	0.48	2.48	0.15	0.04 [†]	0.04 [†]	0	0	0	0	0
	11:00-11:45	0.11	0.89	0.59	0.04 [†]	0.04 [†]	0.04 [†]	0	0	0	0	0
	12:00-12:45	0.07 [†]	3.19	1.04	0.37	0.22	0.11	0	0	0	0	0
	13:00-13:45	0.22	4.30	31.78	0.33	0.26	1.89	0	0	0	0	-
	14:00-14:45	0.37	41.44	19.74	4.67	1.00	1.59	15.97	74.07 [†]	37.04 [†]	0	166.67 [†]
3 Aug 2022	15:00-15:45	0.26	5.41	5.85	0.15	0.11	0.22	3.99	0	0	37.04 [†]	0
	10:00-10:45	0.04 [†]	0.15	0.33	0	-	0.07 [†]	0	0	37.04 [†]	0	333.33 [†]
	11:00-11:45	0	1.74	0.30	0.07 [†]	0	0.15	0	0	0	0	0
	12:00-12:45	0	0.48	0.85	0.04 [†]	0.15	0.07 [†]	0	0	0	0	0
	13:00-13:45	0.15	6.22	17.89	0.74	0.04 [†]	0.11	1.33 [†]	0	37.04 [†]	37.04 [†]	0
	14:00-14:45	15.11	23.44	29.26	9.67	1.70	3.19	10.65	0	444.44	74.07 [†]	500.00
	15:00-15:45	0.78	5.19	9.07	1.44	-	0.48	13.31	111.11	185.19	37.04 [†]	0

[†] Only 1-2 pollen grains were sampled.

* For these sampling intervals, sampling times for ED and FRM were limited to two 45 minute intervals with a break in between to prevent overheating, resulting in 90 minutes of total sampling time. Impingers sampled for the entire time.

** Drone was sampling for a total of 10 minutes in each sampling interval due to battery limitations.

3.2 Orange-Fluorescent Protein Expression

The primary source of pollen in the field experiments came from the first planted block of GE switchgrass, which contained 100 plants expressing OFP under the control of a maize ubiquitin promoter (PvUBI1). As shown in Figure 5, the OFP signal in pollen grains from these transgenic plants was difficult to distinguish from wild-type (WT) pollen exposed to the same OFP-inducing wavelength of light. In contrast, the OFP signal in pollen from the later planting of GE switchgrass (expressing OFP under a maize pollen-specific promoter, Zm13) was much stronger and easily distinguishable from WT pollen. However, these Zm13-expressing plants were smaller and did not produce sufficient mature panicles in time for the field experiments, limiting their contribution to the study.

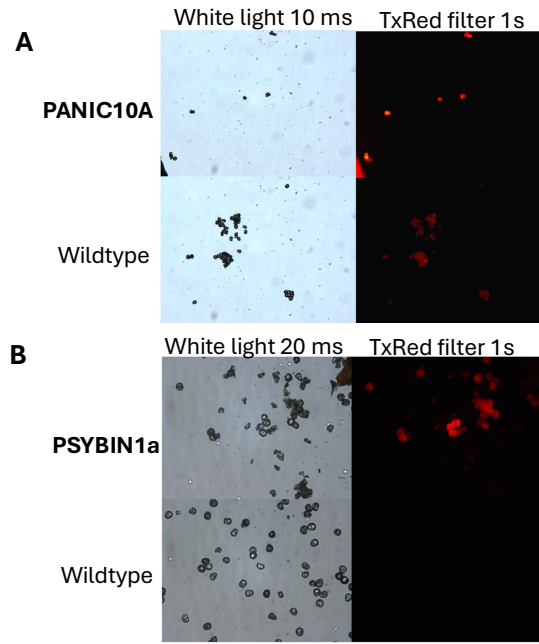


Fig. 5 Comparison of OFP signal between GE switchgrass pollen and wild-type. (A) Pollen from the pANIC10A strain (OFP expression throughout the entire plant) is not easily distinguishable from wild-type pollen under OFP-inducing light. (B) Pollen from the PSYBIN1a strain (OFP expression restricted to pollen) exhibits a strong, highly distinguishable OFP signal compared to wild-type pollen.

3.3 Modeling Results

For the dispersal modeling, we focused on the third field campaign (August 2-3, 2022), as sufficient pollen was captured on both days to allow for concentration measurements from the ED samplers. All sampling intervals during these days were 45 minutes long.

485 Sampling occurred at consistent times across both days, facilitating comparisons and
 486 enabling the identification of diurnal trends.

487 3.3.1 Near-source concentration and emission rate estimation

488 Dispersal simulations more accurately capture changing wind directions and pollen
 489 concentrations when shorter averaging periods are used for each sampling interval.
 490 Figure 6 presents ground level relative concentration contours and downwind wind
 491 roses for the August 2nd, 2:00-2:45 PM sampling interval, simulated using 1-minute,
 492 5-minute, and 45-minute averaging windows. This interval corresponded to the highest
 493 measured pollen concentrations by the ED samplers. The wind rose in Figure 6A was
 494 generated using wind data collected at a sampling frequency of 10 Hz. The wind roses
 495 in Figure 6B, C, and D were created using 1-minute, 5-minute, and 45-minute time-
 496 averaged wind data, respectively. Wind roses display the downwind direction. Yellow
 497 circles indicate the ED sampler locations, with their size proportional to pollen counts
 498 at each site.

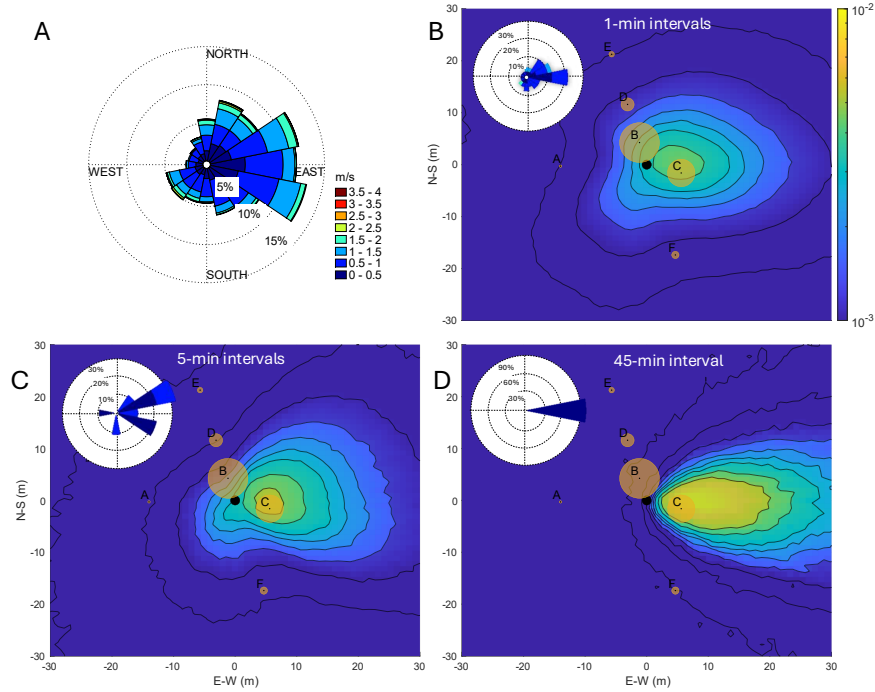


Fig. 6 Relative ground level concentration contours for the August 2nd, 2:00-2:45 PM sampling interval. (A) Wind rose for this sampling interval, indicating the downwind direction. (B) Contour plot averaging forty-five 1-minute simulations. (C) Contour plot averaging nine 5-minute simulations. (D) Contour plot generated using a single 45-minute simulation. Yellow circles indicate the ED sampler locations, with their size proportional to pollen counts at each site.

Comparison of averaging windows. The 45-minute plot (Figure 6D), based on a single LS simulation in the average downwind direction, fails to capture wind variability and lateral pollen spread, missing high pollen counts at ED sampler B due to a single eastward-directed plume. The 5-minute plot (Figure 6C), which averages nine LS simulations, shows some directional variation but lacks the detail seen in the 1-minute plot. The 1-minute plot (Figure 6B) provides the most accurate representation of dispersal dynamics. However, all three simulations share a common discrepancy: peak concentrations appear a few meters from the point source, indicating lateral transport before deposition. A field source, rather than a point source, may better address these inconsistencies, albeit at the cost of further model complexity.

Emission rate and diurnal pattern. The computed pollen emission rate from the field exhibits a clear diurnal trend. Figure 7 presents the mean, minimum, and maximum non-zero computed emission rates for each sampling interval on August 2-3, 2022 during the third field campaign. Emission rates were computed from Equation (1), based on the ratio of measured to modeled concentrations. Samplers with zero measured or modeled concentrations were excluded to prevent infinite or zero emission rate estimates. Emission rate calculations were performed for 1-minute, 5-minute, and 45-minute averaging windows. The range of estimated emission rates decreases with smaller averaging windows, indicating greater precision with shorter averaging periods. The pollen emission rate increased by approximately three orders of magnitude between 10 AM and 2 PM. Note that the emission rate is shown on a log scale in Figure 7. The log-transformed emission rate estimate is positively correlated with the horizontal velocity magnitude (Pearson's $R = 0.73$, $P = 0.01$), temperature ($R = 0.71$, $P = 0.01$), and vapor pressure deficit ($R = 0.69$, $P = 0.01$), while negatively correlated with relative humidity ($R = -0.63$, $P = 0.02$). These results indicate that higher pollen emissions occur under conditions of higher wind speed, temperature, and vapor pressure deficit, while increased relative humidity reduces pollen release.

Modeled concentration predictions improve dramatically when changing wind conditions are incorporated into the simulations. Figure 8 compares modeled concentrations, computed by multiplying the estimated emission rate by the relative concentration, with measured concentrations derived from ED sampler pollen counts. Although the figures directly compare modeled and measured concentrations, they are not intended as a formal model validation, as the measured concentrations were directly used to compute emission rate and modeled concentration (see Section 2.5.3). Instead, they highlight the substantial improvement in model performance as the averaging window is reduced. The Pearson's R -value increases from 0.19 for 45-minute windows to 0.71 for 5-minute windows to 0.84 for 1-minute windows. While reducing the averaging window from 45 minutes to 5 minutes requires nine times the computational power, it yields a 270% improvement in model performance (as measured by the R -value). In contrast, refining the resolution further from 5-minute to 1-minute windows results in only a 20% increase, suggesting that shorter windows may not always be computationally worthwhile beyond a certain threshold.

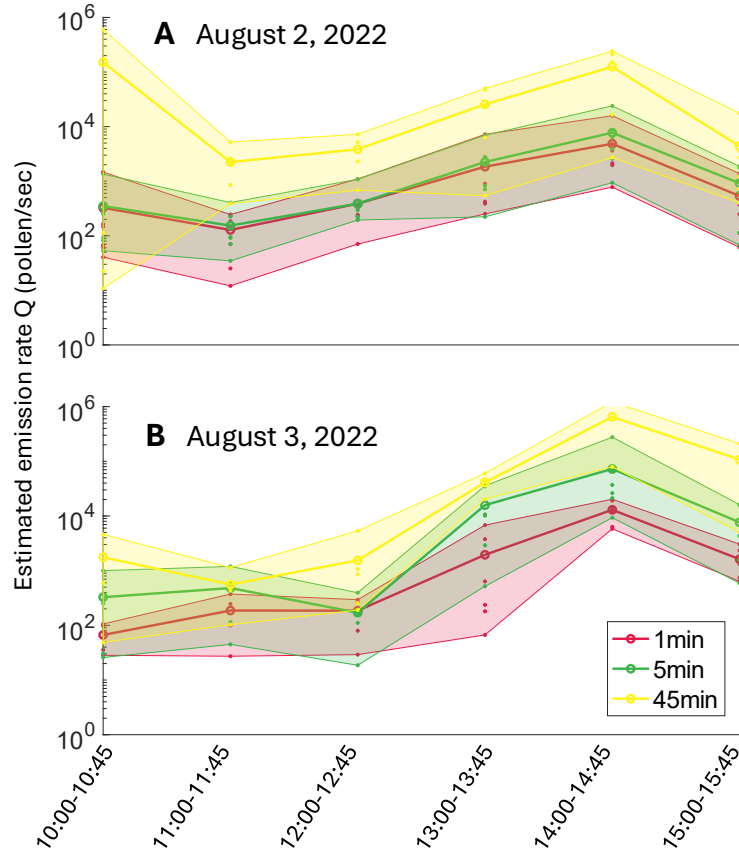


Fig. 7 Mean estimated pollen emission rates for each sampling interval using 1-minute, 5-minute, and 45-minute simulations. (A) Estimated emission rates for August 2, 2022 and (B) August 3, 2022. Non-zero emission rate estimates for each sampler are shown as solid points. Shaded regions indicate the range between the minimum and maximum non-zero emission rate estimates. The vertical axis is on a log scale.

3.3.2 Estimating sensor capabilities in the far field

The intake rates of the samplers used in this study differ by multiple orders of magnitude, with 0.6 L/min for the low-volume IMP and DRN, 16.7 L/min for the medium-volume FRM, and 600 L/min for the high-volume ED. This disparity highlights the coarseness of concentration measurements for the low-volume IMP and DRN samplers, particularly when considering error estimates based on the Poisson distribution. The Poisson distribution models the error of discrete event counts, such as the number of pollen grains collected, as $\sqrt{N}$, where N is the number of pollen grains captured by a sampler (Addison-Smith et al. 2020; Lin et al. 2013). Lower intake

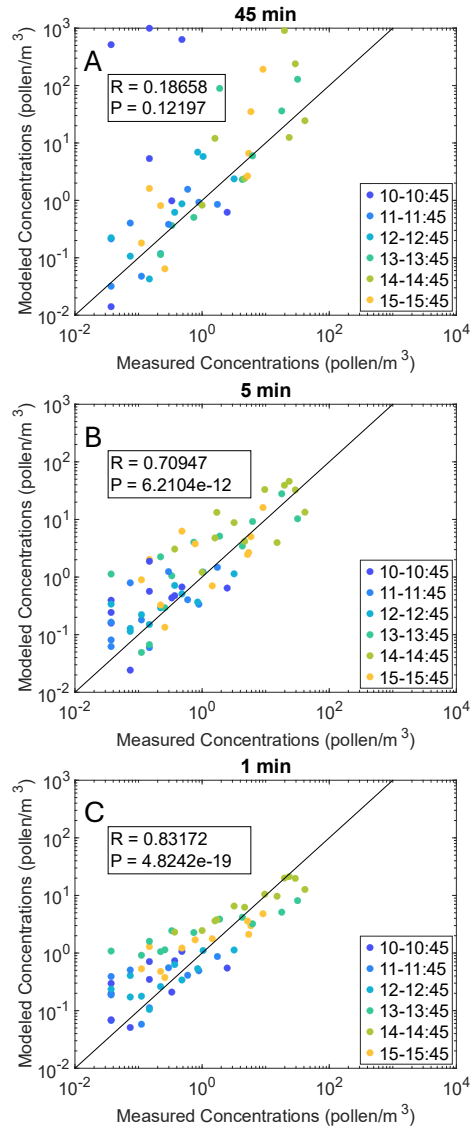


Fig. 8 Measured concentrations at the ED samplers compared with simulated concentrations for (A) 45-minute, (B) 5 minute, and (C) one-minute intervals. These plots are not intended as model validation, but rather to show that decreasing the averaging time for simulations greatly improves modeled concentrations. Note that the plots are on log-log scales.

550 rates necessitate proportionally higher aerial concentrations, and consequently, greater
 551 pollen emission rates for detection, leading to significantly higher measurement error
 552 compared to high-volume samplers.

553 To determine optimal sampler placement based on varying field emission rates, we
 554 combined sampler intake rates with long-distance dispersal simulations. Figure 9 plots
 555 the maximum distances at which each sampler could be placed to collect at least 100
 556 pollen grains (± 10) within a 45-minute sampling interval, as a function of the pollen
 557 emission rate. These estimates were generated using the long-distance 2D relative
 558 concentration dispersal kernels, computed separately for ground-based samplers (IMP,
 559 ED, FRM) at ground level and the drone-based sampler (DRN) at 10 m above ground
 560 level. Although the drone was only flown for 10 minutes per sampling interval in our
 561 field campaigns, we evaluated its performance over 45-minute intervals to account for
 562 potential future battery-life improvements or wire-tether power modifications. The
 563 threshold of 100 pollen grains corresponds to different effective concentrations across
 564 sampler types, with 3.7×10^3 pollen/ m^3 for IMP and DRN, 133 pollen/ m^3 for FRM,
 565 and 3.7 pollen/ m^3 for ED. This threshold was selected as it ensures a $\pm 10\%$ error in
 566 concentration estimates when modeled with the Poisson distribution.

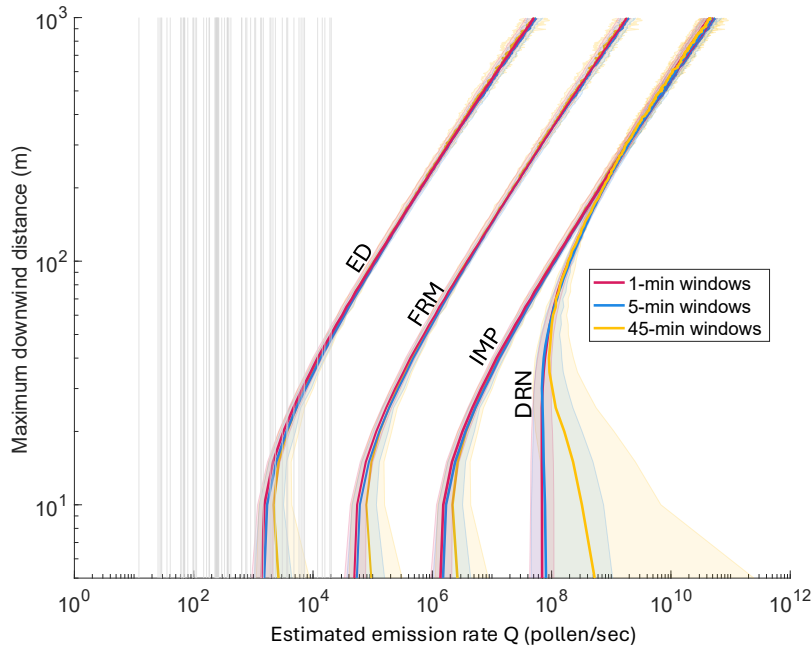


Fig. 9 Maximum distance at which ED, FRM, IMP, and DRN sensors should be placed from the source to capture at least 100 pollen grains for a given emission rate from the field. These are calculated using concentration curves derived from each sampling interval, distinguishing between estimates computed with 1-minute, 5-minute, and 45-minute averaging windows. Solid lines represent the median values, while the shaded regions indicate the range between the lowest and highest values observed across all sampling intervals. Gray vertical lines correspond to the estimated emission rates for each sensor during all sampling periods.

Figure 9 shows that, to collect at least 100 pollen grains, the ED samplers require 1.5 orders of magnitude less emission rate than the FRM and 3 orders of magnitude less than the IMP, across all distances from the field. This advantage allows ED samplers to be placed significantly farther from the field compared to IMP and FRM samplers. The DRN requires an even greater emission rate, due to lower concentrations at 10 meters altitude. However, at approximately 200 meters downwind, the DRN and IMP detection capabilities converge, as vertical concentration gradients become less pronounced further from the source.

The gray vertical lines in Figure 9 represent computed emission rate values at each ED sampler during our field campaign, which are also shown in Figure 7. These results indicate that only the ED samplers had a reasonable chance of collecting at least 100 particles during some sampling intervals. The remaining samplers were largely ineffective at detecting airborne pollen given the small emission rates observed in this study, even at close proximity to the source field. For a larger source, with an emission rate of 10^6 pollen/s (approximately 100 times larger than ours), the feasible placement of samplers would improve substantially. Under such conditions, impingers could be placed up to 10 meters from the field, the FRM samplers could be placed up to 100 meters from the field, and ED samplers could be placed up to 200 meters away. These adjustments would allow each sampler type to collect at least 100 pollen grains within a 45-minute sampling interval, enhancing measurement reliability and reducing uncertainty.

4 Discussion

In these field experiments, airborne pollen from two different strains of OFP-expressing switchgrass plants was captured and analyzed using the method described in (Rice et al. 2013). The PSYBIN1a strain exhibited strong fluorescence compared to wild-type pollen, whereas the pANIC10A strain was difficult to distinguish by fluorescence alone. However, since the PSYBIN1a plants did not release sufficient pollen during any of the field experiments, OFP expression was not used for pollen identification in this study. If we had successfully captured PSYBIN1a pollen from the younger field, it would have expedited the counting and sampling process. Despite this limitation, the study serves as a proof-of-concept that fluorescence tagging can be a valuable tool for pollen tracking. Though prior studies have tracked the movement of GE pollen in the atmosphere (Aylor et al. 2006; Aylor and Flesch 2001; Aylor et al. 2003; Millwood et al. 2017; Frisk et al. 2023), to our knowledge, this is the first detailed study to incorporate aerial and ground-based volumetric samplers to track the movement of GE pollen from a known source. Fluorescent tagging presents a unique opportunity to trace pollen dispersal and track its movement over long distances. Understanding switchgrass gene flow is particularly relevant as biofuel production increases, helping to mitigate ecological risks posed by invasive strains and unintended cross-pollination between transgenic varieties (Kwit and Stewart 2012). Additionally, fluorescence-tagged pollen could facilitate rapid and automated counting using instruments such as the Helmut Hund BAA500, Plair Rapid-E, Swisens Poleno, or WIBS-4 (Buters et al. 2024; O'Connor et al. 2014), which is of particular interest for allergen monitoring.

During the final campaign on August 2-3, 2022, a distinct diurnal pattern emerged in both measured pollen concentrations and computed pollen emission rate. Pollen emission rate increased steadily after the first sampling interval at 10 AM, peaked at 2 PM on both days, and declined during the final sampling interval at 3 PM. This diurnal pattern was consistent across both days and correlated with increasing wind velocity and temperature, as well as decreasing relative humidity. Such diurnal pollen release patterns are common in wind-pollinated species, where anther dehiscence is driven by drying conditions such as low humidity and rising temperatures (Sofiev and Bergmann 2012). Similar trends have been observed in previous switchgrass field studies, where peak pollen concentrations occurred in the late morning and early afternoon, followed by a decline around 3 PM (Auer et al. 2016). Comparable findings in maize have linked pollen release patterns to increasing vapor pressure deficit (Jarosz et al. 2005) and decreasing relative humidity combined with rising wind velocity (Marceau et al. 2011). This information can be used to better predict peak allergen concentrations and improve accuracy of large-scale air pollution models.

Throughout all sampling intervals and field campaigns, we observed very low wind velocities (< 2 m/s) and frequent shifts in wind direction. Under these meandering wind conditions, particle dispersal is primarily controlled by wind direction shifts rather than turbulence (Vickers et al. 2008; Anfossi et al. 2005). Standard dispersal models that assume a dominant downwind direction fail to account for this effect, often producing overly narrow plumes that underestimate lateral spread. This limitation is particularly characteristic of LS models (Jarosz et al. 2005; Anfossi et al. 1990) and Gaussian plume models (Sagendorf and Dickson 1974) which require a single downwind direction. Even more advanced modeling approaches, such as Large Eddy Simulations, do not fully incorporate changing wind directions (Chamecki et al. 2009). To address this, we reduced the averaging window for wind statistics from 45 minutes to 5 and 1 minute, then ran dispersal models for each of these smaller intervals and combined the resulting plumes. This approach dramatically improved the fit between modeled and measured concentrations, enhancing emission rate estimates. Similar techniques have been applied in Gaussian plume modeling with 2-minute intervals, yielding significantly better agreement with measured data (Sagendorf and Dickson 1974). Anfossi et al. (1990) Anfossi et al. (1990) also emphasized the importance of using short averaging windows for dispersal modeling, recommending intervals of only a few minutes. A maize dispersal study similarly attributed discrepancies between measured and modeled concentrations to wind direction variability and assumptions of a dominant wind direction (Jarosz et al. 2005). Future large-scale pollen forecasting and bio-confinement strategies should consider meandering wind conditions, which are not currently accounted for in large-scale models (Chamecki et al. 2009).

The highest concentration measurements in this study came from the high-volume ED samplers. The pollen source size—100 plants releasing pollen—was exceedingly small compared to previous dispersal experiments in switchgrass, which involved 3,200 plants (Auer et al. 2016), as well as studies in maize (Jarosz et al. 2005; Aylor et al. 2006; Marceau et al. 2011). High-volume ED samplers performed best under these small-source conditions, capturing spatial variations in concentration and diurnal patterns. To the best of our knowledge, this is the first pollen-trapping field study to

655 utilize these samplers. Their volumetric flow rate of 600 L/min is 60 times greater
656 than that of the traditional Hirst-type samplers, which operate at 10 L/min and are
657 comparable to the FRM sampler used in this study. Due to the small pollen source,
658 the FRM sampler did not produce usable data. As the ED samplers were originally
659 designed for educational purposes, they are inexpensive and lack pre-programming
660 and other advanced features found in commercial volumetric samplers. However, their
661 simplicity and affordability make them easily deployable, and they have strong poten-
662 tial for measuring concentrations from small sources and capturing high-resolution
663 pollen dispersal patterns even in small fields.

664 A novel impinger-type particle sampler (IMP and DRN) was used in this study
665 to collect pollen, marking the first application of this integrated system for pollen
666 tracking. While previously employed for airborne microbial sampling (Powers et al.
667 2018; Bilyeu et al. 2022), this study extends its use to pollen dispersal. The IMP
668 and DRN samplers successfully collected pollen in the field, demonstrating their fea-
669 sibility for tracking pollen movement. However, due to the small source size, limited
670 pollen production, and the relatively low sampling rate of 0.6 L/min, the collected
671 pollen quantities were insufficient for reliable concentration estimates. The differing
672 flow rates between the ED and IMP samplers further complicate direct comparisons,
673 as impinger samplers inherently capture fewer particles at high concentrations due
674 to their small intake volumes. The IMP system would likely perform more effectively
675 when sampling from much larger sources, at least 100 times the size of the field used in
676 our study (Figure 9). Similarly, the drone-mounted sampler, operating at 10 m above
677 ground-level, would require a significantly larger pollen source for effective deployment
678 at further distances and altitudes. Nevertheless, the drone platform remains a valu-
679 able tool for aerobiological research, offering future opportunities for prolonged and
680 spatially resolved sampling, particularly when paired with higher-volume sampling
681 technologies, including those incorporating filter-based collection systems. Moreover,
682 impinger samplers, which preserve particles in liquid, could prove especially useful for
683 future viability and molecular studies.

684 A primary constraint in regulated transgenic pollen dispersal studies is the feasi-
685 ble scale of the pollen source. From an agricultural perspective, a 100-plant source is
686 small relative to other agricultural dispersal studies. However, in the context of per-
687 mitted flowering GE pollen dispersal, it is substantial because it requires specialized
688 propagation, isolated siting, and a stringent regulatory permitting process. Our exper-
689 imental design therefore prioritized a small, but well-contained OFP-tagged source,
690 and we structured the sampling campaign to extract the most robust insights that
691 this rare setup could support. The value of the present study is therefore not that it
692 reproduces a large agricultural pollen release, but that it establishes what is measur-
693 able and how to model it under realistic constraints that are intrinsic to transgenic
694 field experimentation.

695 With more resources, the natural next step would be a scaled-up version of this
696 same experiment focused on validation: a large, well-established source containing only
697 PSYBIN1a to maximize the OFP signal, multi-year sampling on the same dates and
698 times to quantify repeatability of diurnal emission patterns, and colocated Hirst-type
699 gold-standard samplers deployed alongside the novel samplers to provide independent

concentration estimates for model validation and rigorous sampler inter-comparison. A substantially larger source would allow meaningful comparisons between low- and high-volume samplers, with one set used to estimate the particle release rate and another for validating modeled concentrations. It would also enable more effective use of impinger-type samplers (IMP and DRN), which could preserve pollen for downstream viability studies, although isolated siting requirements for transgenic work would continue to pose limits for long-distance tracking.

Focusing solely on PSYBIN1a switchgrass, with its stronger OFP fluorescence in pollen, could further enhance tracking accuracy via automatic fluorescence-based quantification. As shown in Figure 9, these methods could enable pollen detection up to 1 km or even tens of kilometers from the source. This fluorescence tagging technique could also be transferable to other crops of interest. For instance, hemp is known to produce copious amounts of pollen capable of long-distance dispersal, and its monitoring is increasingly relevant (Nimmala et al. 2024). If transgenic hemp lines become available, similar fluorescence-based tracking methods could be applied to study its pollen movement and gene flow in detail.

5 Conclusions

Three field campaigns were conducted to measure pollen concentrations around a small field of genetically modified switchgrass, utilizing both traditional and novel sampler types. The experiments also included a drone-mounted sampler, demonstrating the feasibility of airborne pollen sampling at 10 meters above the field as a proof-of-concept. Despite the exceedingly small source size, the high-volume ED samplers successfully collected sufficient pollen to analyze spatial variations in concentration and identify diurnal release patterns. This study evaluated the effectiveness of different sampler types for pollen collection under varying conditions. Among the three field campaigns, only the final campaign on August 2-3, 2022, produced sufficient concentration data for detailed analysis and modeling. During this campaign, a clear diurnal pattern was observed in the pollen concentration and, consequently, in the calculated emission rate. Persistent low-wind meandering conditions were recorded throughout the campaign, and reducing the averaging window for simulations significantly improved pollen emission rate estimations by better incorporating shifting wind directions. This study highlights the potential for drone-based pollen sampling and fluorescence-based GMO pollen tracking. The findings provide insight into the effectiveness of different sensor types with respect to source strength and sampling distance, advancing the understanding of pollen dispersal dynamics and measurement techniques. These results have important implications for allergen monitoring, cross-pollination risk assessment, and broader bioaerosol surveillance strategies.

737 Statements and Declarations

738 Data and code availability

739 All sampling data, modeling code, and simulation results are made available in the
740 Virginia Tech Data repository:
741 <https://figshare.com/s/54a308163b60865d55bf>.

742 Competing interests

743 The authors have no competing interests to declare.

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