Plan of Study – Version 4

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Comparison of Atmospheric Pollen Measurements in Ambient Air and Wet Deposition Samples to Traditional Pollen Monitoring Methods

Prepared by: National Atmospheric Deposition Program, Aeroallergen Monitoring Science Committee

Contacts: Gregory Wetherbee, U.S. Geological Survey, Denver, Colorado (wetherbe@usgs.gov)
David Gay, University of Wisconsin, Madison, Wisconsin (dgay2@wisc.edu)
Andrew Johnson, Maine Department of Environmental Quality (Andy.Johnson@maine.gov),
Daniel Coates, Aerobiology Research Laboratories, Ottawa, Ontario, CN (daniel@aerobiology.ca)
Landon Bunderson, PollenSense, Salt Lake City, Utah (landon@pollensense.com)
Mark Moss, University of Wisconsin, Madison, Wisconsin (mhm@medicine.wisc.edu)

Introduction

Millions of people suffer from illnesses related to allergic reactions to biological allergens in ambient air (aeroallergens). Detection of the beginnings and peaks of seasons during which plants are emitting pollen to the air is commonly done in selected population centers by clinicians and researchers, with most of the data for the United States historically controlled by the National Allergy Board (NAB). Since 2017, the National Atmospheric Deposition Program (NADP) has convened the Aeroallergen Monitoring Science Committee (AMSC) to find ways to share aeroallergen data, promote national aeroallergen monitoring, especially in rural areas, and devise studies to compare data for traditional pollen sampling, identification, and counting to ambient air-quality measurements and washout particulates collected in atmospheric wet-deposition samples.

Purpose and Scope

The goals of this study are to: 1) evaluate the potential for NADP to make reasonable and consistent measurements of pollen concentrations in wet-deposition samples; 2) identify the genes and species present in these samples, and 3) evaluate the correlation between pollen measurements from traditional sampling methods, NADP samples, and real-time continuous automated monitoring data. This study is designed to acquire data for traditional pollen samples, ambient air and wet-deposition samples, and real-time automated monitors from March 1 – October 31, 2021 at 3 NADP National Trends Network (NTN) sites. The data obtained will be evaluated graphically and statistically to determine how well these traditional and new sampling methods relate to one another. Additionally, the dates of the onsets and peaks of the pollen seasons at the genus, and perhaps the species levels will be compared among the methods. The results will inform decisions about the viability and design for a new National Aeroallergen monitoring Network (NAN) using one or, more likely, a combination of these selected methods.

Field Sampling and Measurements

Field sampling will occur at three locations in the United States. The primary monitoring site for the study is NADP NTN site WI93 at the University of Wisconsin-Madison (Fig. 1). Two additional sites will be at the UT01 site (Utah State University State Agricultural Experiment Station), and the NC30 Duke Forest site (US EPA).

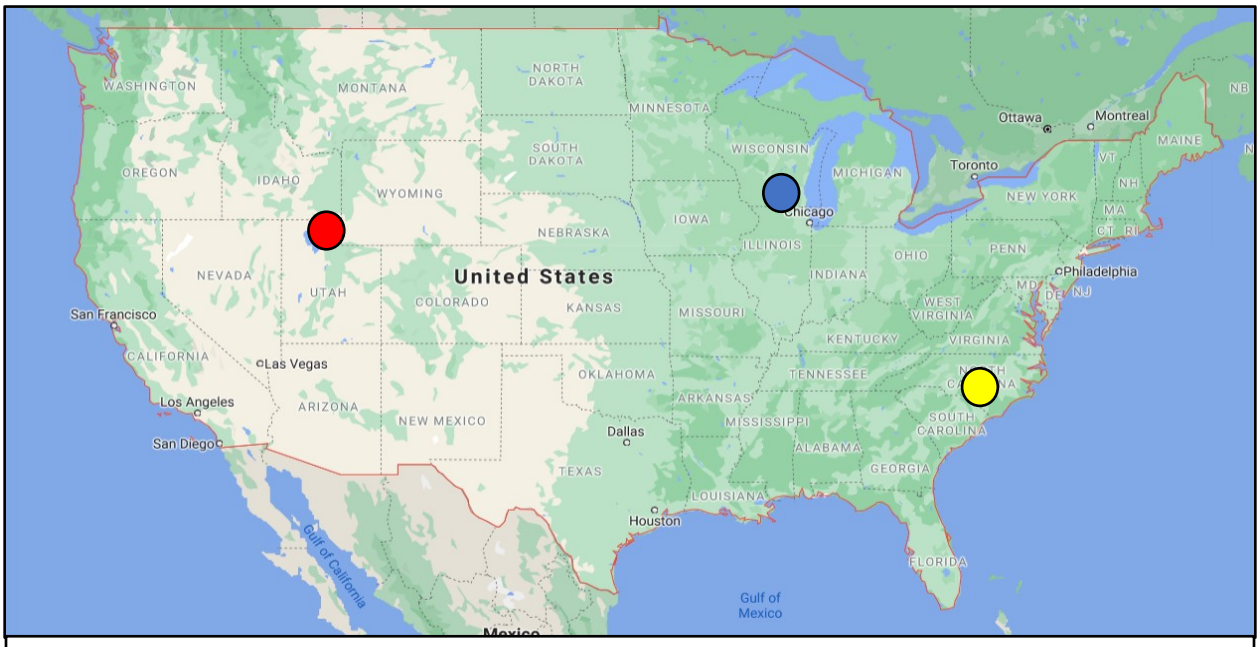

Figure 1: Site locations for this study: the NADP NTN site WI93 at the University of Wisconsin-Madison, Eagle Heights site (blue), UT01 (red), and NC30 (yellow).

A variety of sampling systems will be used to monitor for pollen at these three stations (see Table 1). Each site will have an NADP National Trends Network Site (NTN) to collect wet-deposition samples of pollutants. All liquid NTN samples are filtered prior to analysis of the water for chemical constituents. Normally, the filters are discarded unless they are saved for research purposes. For this study, the WI93, NC30, and UT01 filters will be saved for weekly determination of pollen counts on the filters and, if possible, genus and species identification. Pollen identification and counting for the NTN filter samples will be done at the Wisconsin State Laboratory of Hygiene (WSLH), in Madison, Wisconsin.

Daily pollen count and species identification data are currently being collected by the NAB atop the University of Wisconsin medical research building near the WI93 site. These samples are collected with the traditional Rotorod sampler, which collects samples by moving air past a sticky rod with atmospheric pollen collected by attachment. Pollen identification and counting is done manually by light microscopy. A separate Rotorod sampler will be loaned by Aerobiology Research Laboratories (ARL), Nepean, Ontario, Canada. Pollen samples will be collected weekly at NC30 by the NTN site operators and shipped to ARL. The Rotorod samples will be analyzed by ARL for genus, species, and total pollen counts. The ARL will not share its results with PollenSense or the WSLH to uphold the scientific integrity of the study.

A high-volume ambient air sampler will be installed at WI93 to collect total suspended particulate (PM) samples for pollen analysis. Pollen identification and counting for the PM samples will be done at the

WSLH. Selected samples from this collector and the NTN filters will be recounted by ARL for quality assurance.

PollenSense[1] sensors (https://www.pollensense.com/pages/automated-particle-sensors) are relatively new, continuous real-time pollen-data monitoring instruments. A PollenSense has been operating within Madison and will be relocated to the WI93 site. The PollenSense monitor in Logan, Utah will be relocated to the UT01 site, and a new PollenSense monitoring site will be established at NC30. The PollenSense instruments will collect hourly pollen count and species identification data.

The NC30 monitoring site will be co-located with the U.S. Environmental Protection Agency's Clean Air Status and Trends Network (CASTNet), which will provide a range of continuous air-quality measurements.

Table 1. National Atmospheric Deposition Program / Aeroallergen Monitoring Science Committee pollen monitoring study sites and expected data to be obtained.

[NADP/NTN, National Atmospheric Deposition Program / National Trends Network; PM, particulate matter; CASTNet, Clean Air Status and Trends Network; NAB, National Allergy Bureau; ARL, Aerobiology Research Laboratories]

NADP / NTN SiteID	Location	Weekly NADP / NTN wet-deposition filters	Weekly PM filter samples	Continuous CASTNet air-quality data	Traditional Rotorod or Burkard pollen samples	PollenSense hourly automated pollen count and Identification
WI93	Madison, WI	X	X		NAB – daily	X
NC30	Duke Forest, NC	X		X	ARL – weekly	X
UT01	Logan, UT	X				X

Laboratory Pollen Analysis

The NADP Central Analytical Laboratory (CAL) is located within the WSLH, in Madison, Wisconsin. The CAL will filter the weekly wet-deposition samples collected at WI93, NC30, and UT01, and the filters will be saved for subsequent pollen analysis. In addition to the CAL, the WSLH has many other analytical resources and capabilities, including micro-organism identification expertise. The WSLH will analyze the NTN filters as well as the PM filters for pollen genus and species identification and total pollen count. The methodology used by WSLH will be well documented. The methodology is not specified herein because several techniques could be evaluated to determine which ones give the most reliable results. During the course of the study, the WSLH will not share its results with PollenSense or any other laboratories to uphold the scientific integrity of the data.

[1] Use of trade or firm names in this document does not constitute endorsement by the U.S. government or the University of Wisconsin.

Quality Assurance

Pollen identification and counting data will be quality assured using:

- Documented laboratory procedures;
- Certification of trained technical staff;
- Comparison of pollen identifications to reference standards; and
- Reanalysis of samples exchanged between WSLH and ARL.

The WSLH will select 5 PM samples and 5 NTN samples for reanalysis by ARL. The WSLH will relabel each sample to disguise its identity by assigning a reanalysis identification code to each sample. This way, ARL will be unable to correlate the Rotorod sample results with the WSLH samples. Likewise, ARL will relabel 10 Rotorod samples to hide their identity by assigning them with reanalysis identification codes. These will be labeled as follows.

WSLH Reanalysis Codes	ARL Reanalysis Codes
WSLH001, WSLH002, … WSLH010	ARL001, ARL002, … ARL010

Both laboratories will send their sample reanalysis coding keys to the USGS Precipitation Chemistry Quality Assurance Project (PCQA) to the attention of Greg Wetherbee. After each laboratory reanalyzes the 10 QC samples, they will send their results to the USGS/PCQA for independent sample identified and data comparison. This analysis will result in quantification of relative bias and variability, but it will not evaluate accuracy.

Accuracy between the laboratory counts will be implied by absolute percent differences (APD) between the two laboratories' pollen counts, calculated as:

$$APD = 100 \times [\,(|\text{WSLH count} - \text{ARL count}|) / ((\text{WSLH count} + \text{ARL count}) / 2)]$$

We will also compare the Differences in species determinations will also be compared. All quality assurance results will be reported in the final study report or journal article.

Results and Data Analysis

The maximum numbers of pollen samples to be collected by this study are summarized in Table 2.

Table 2. Maximum numbers of pollen samples to be collected by various methods at three National Atmospheric Deposition Program / National Trends Network sites for the pollen monitoring study.

[PM, particulate matter; WI93, Madison, Wisconsin; UT01, Logan, Utah; NC30, Duke Forest, North Carolina]

Method	Time Period	Site/Location	Pollen Genus & Species Counts	Total Pollen Counts
Wet Deposition	weekly integrated	WI93	30	30
		UT01	28	28
		NC30	32	32
PM	weekly	WI93 / Madison	34	34
RotoRod	weekly integrated	WI93	34	34
		NC30	34	34
PollenSence	hourly / weekly	WI93	hourly / weekly 5712 / 34	34
		UT01	5712 / 34	34
		NC30	5712 / 34	34
Total weekly measurements, all methods:			294	294

Wet Deposition Data

- Approximately 30 weekly NTN filter samples from each NTN site (WI93, NC30, and UT01) for pollen identification (genus or genus/species) and count data;
- 15-minute, daily, and monthly precipitation depth data;
- Precipitation intensity;
- Sample catch efficiency (Catch efficiency = 100 × (Sample depth / Precipitation depth); and
- Standard NTN solute concentrations, pH, and specific conductance.

PM Data

Approximately 34 weekly PM samples for pollen identification (genus or genus/species) and count data from NADP site WI93 only.

Traditional Pollen Monitoring Data

- NAB site daily and weekly pollen identification (genus or genus/species) and count data for Madison, Wisconsin.
- 34 weekly Rotorod samples collected per site from NC30.

Automated Continuous PollenSense Data

- PollenSense hourly, daily, and weekly pollen identification (genus or genus/species) and count data for:
    - NADP site WI93, Eagle Heights, Madison, Wisconsin,
    - NADP site UT01, Logan, Utah, and
    - NADP site NC30, Duke Forest, North Carolina.

Quality Assurance Data

- Pollen count relative differences and relative percent differences by genus and total counts calculated for the laboratory analyses.
- Pollen count absolute percent differences by genus and total counts.

Statistical Analyses

- Determine the interrelation between the methods by statistically correlating PM, wet-deposition, PollenSense measurements, and traditional Rotorod measurements.
- Comparison of wet-deposition, PM, and Rotorod pollen counts to estimate pollen burst rate in wet-deposition and collection efficiency.
- Estimated relative differences between all methods.
- Model the relations between measured concentrations by genus and total counts for each site as well as pooling of all data from the three sites.
- Evaluate statistical significance between measurement methods using Kruskal-Wallis nonparametric analysis of variance and other methods.
- Evaluate the representativeness of the wet-deposition filter data given that these samples only account for pollen deposition during precipitation events whereas other methods provide continuous measurements for all weather conditions.
- Calculation of a correction factor for estimating true concentrations will be attempted from relating the PM and wet-deposition measurements, and a literature search and interviews with researchers will also be done.

Assumptions/Potential Problems

• The wet-deposition measurements will result in total numbers of pollen grains in precipitation (#/Liter), but just for grains still intact or mostly intact. Many grains will decompose after becoming wet, perhaps as much as 80%. Calculation of a correction factor for estimating true concentrations from the PM and wet-deposition measurements might be necessary to account for pollen burst in water.

• This study relies on good performance of the PollenSense, NADP, and CASTNet monitoring instruments, the operations of which will be overseen by the NTN / CASTNet site operators with no previous experience with the PollenSense instrument. Operation of electronic instruments in the environment can be challenging, and interruptions in the data series from power outages, lightning, extreme weather, and biological vectors (birds, insects, rodents, etc.) would not be surprising.

• The null hypothesis for this study is: "No correlation between air-quality and traditional measurements exists." This may be true. However, given the data already obtained by the University of Maine and PollenSense, each by independent methods, it is reasonable to assume that most of the bioaerosols are in the lower atmosphere, and that precipitation washes out a representative sample of the lower atmosphere which NTN can measure with sufficient accuracy to signal the timing of pollen season onset and peak concentrations as well.

- Cost for this project are estimated with the following assumptions.
    - A PM sampler will be obtained from the WSLH as and in-kind collaboration.
    - PollenSense will cover all costs for deployment and(or) relocation of their instruments at NC30, UT01, and WI93.
    - NTN / CASTNet site operators will change out Rotorod samples and perform very minimal, weekly operation and maintenance of the PollenSense monitors as an in-kind contribution to the project.
    - NAB data for Madison, Wisconsin will be shared with NADP as an in-kind collaboration.
    - Aerobiology Research will loan a Rotorod sampler to the project at no cost.

Costs and In-kind Contributions

Table 3. Estimated costs for collection of pollen-monitoring samples and associated data for the National Atmospheric Deposition Program pollen monitoring study.

[PM, total suspended particulates]

Project Component	Cost
Wet Deposition Sample Pollen Analysis	In kind
High Vol PM Pollen Analysis	in kind
Weekly Rotorod sample analysis for NC30	$1,000
Student for field sampling and data collection	$3500
Sample and equipment shipping	$500
Cellular modem internet at UT01 and WI93	$600
Total:	**$5,600**

The national scales of the NADP, CASTNet, and PollenSense networks allows this study to be implemented economically, in three very different precipitation/climate and pollen emission regimes. In-kind support to the study from the collaborating networks will be invaluable to the study's success. Table 4 shows an estimation of the value of in-kind services and loaned equipment provided by each collaborator.

Table 4. Estimated values of in-kind contributions from each collaborator for the National Atmospheric Deposition Program (NADP) pollen monitoring study.

[USGS, U.S. Geological Survey; USEPA, U.S. Environmental Protection Agency; NAB, National Allergy Bureau]

Collaborator and In-kind Contributions	Estimated Value of In-kind Contribution
USGS In-kind oversight, data management, data analysis, and report/article preparation	$10,000
NADP in-kind technical support	$10,000
PollenSense in-kind instrument setup, data collection, quality assurance, and other support	$20,000
Wisconsin State Laboratory of Hygiene in-kind instruments and technical support	$10,000
Aerobiology Research Laboratories in-kind loan of Rotorod pollen collector and discounted laboratory analysis services	$8,000
USEPA in-kind CASTNet data collection and NC30 cellular data link	$10,000
NAB data for Madison, Wisconsin site	$3,000
Total Value of In-kind Contributions:	**$68,000**

Study Personnel and Responsibilities

This study relies on the infrastructure of national monitoring networks, which are operated by hundreds of dedicated people in many institutions, agencies, nations, and companies across North America. The following individuals will have the primary responsibilities for study implementation.

Responsibilities	Principal Collaborators
Study coordination and implementation	Greg Wetherbee (USGS), Dr. David Gay (NADP), Andrew Johnson (MEDEQ)
Sample Collection and Instrument O&M	NADP Site operators at NC30, UT01, WI93
Scientific consultation, PM instrumentation, WSLH services coordination	Dr. James Shauer (WSLH, NADP)
PollenSense instrumentation and data collection	Landon Bunderson, Pollensense
Wet-deposition filter collection	Amy Mager, NADP Central Analytical Laboratory
Wet-deposition and PM pollen analysis	Terri Williams (WSLH)
Rotorod collector and sample analysis	Daniel Coates (Aerobiology Research Laboratories)
Madison NAB pollen data	Dr. Mark Moss, University of Wisconsin
Data compilation and management	University of Wisconsin student, TBD
Data analysis, interpretation, and reporting	Greg Wetherbee, David Gay, Daniel Coates, Landon Bunderson, Mark Moss

Final Products

1. As stated in the Introduction of this proposal, the ultimate goal of this study is to advance national pollen monitoring capabilities for expedient creation of the NAN using scientifically proven technologies. Another goal is to simultaneously intercompare all currently used methods to the PollenSence real-time data, and to evaluate correlations between the methods. These objectives shall not influence outcomes or harm the scientific integrity of the study. NADP studies are peer reviewed by its subcommittee members to protect NADP's long-standing reputation for producing high-quality scientific data products and monitoring networks. The NADP is committed to producing data freely available to researchers, policy makers, regulators, public health officials, and industry for the benefit of humankind.

2. NADP report or journal article – The data will be presented with statistical analysis and interpretation in either a peer-reviewed journal or a NADP report published on the NADP web site or both. If the data indicate positive correlations between the air-quality measurements, PollenSense data, and traditional pollen counting data, then a journal article will be preferred over the online NADP report.

3. Should the study be unexpectedly inconclusive or indicate that the air-quality measurements are not suitable for pollen season monitoring, then a NADP report will be completed in lieu of publication in the scientific literature. An inconclusive report will not be published on the web site. Instead, the NADP will issue a statement in their Executive Committee meeting minutes that the study was completed and was inconclusive.

4. PollenSense will have the ability to use the results to improve their instrumentation and network and for marketing purposes. However, the data shall not be used to imply endorsement by the U.S. government, the State of Wisconsin, or the NADP, and PollenSense may not use the NAB data independent of the journal article(s) and report(s). Should the NADP and PollenSense collaborate to expand the PollenSense network into a NAN, then mutually beneficial language that accurately describes the relationship between PollenSense and NADP will be crafted by all parties for outreach to network sponsors and the general public.

Schedule: See next page.

Study Schedule

[MOU, memorandum of understanding; NAB, National Allergy Bureau; NADP, National Atmospheric Deposition Program]