Pacific Northwest
NATIONAL LABORATORY

PNNL-31970

Molecular level characterization of wildfire aerosol induced changes to plant health and value

September 2021

William Kew
Swarup China
Georg C Jansson

U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
under Contract DE-AC05-76RL01830

DISCLAIMER

PACIFIC NORTHWEST NATIONAL LABORATORY
operated by
BATTELLE
for the
UNITED STATES DEPARTMENT OF ENERGY
under Contract DE-AC05-76RL01830

Printed in the United States of America

Available to DOE and DOE contractors from the Office of Scientific and Technical Information,
P.O. Box 62, Oak Ridge, TN 37831-0062
www.osti.gov
ph: (865) 576-8401
fax: (865) 576-5728
email: reports@osti.gov

Available to the public from the National Technical Information Service
5301 Shawnee Rd., Alexandria, VA 22312
ph: (800) 553-NTIS (6847)
or (703) 605-6000
email: info@ntis.gov
Online ordering: http://www.ntis.gov

PNNL-31970

Molecular level characterization of wildfire aerosol induced changes to plant health and value

September 2021

William Kew
Swarup China
Georg C Jansson

Prepared for
the U.S. Department of Energy
under Contract DE-AC05-76RL01830

Pacific Northwest National Laboratory
Richland, Washington 99354

Abstract

The potential impacts of wildfire smoke on plant health and value are of increasing concern, due both to increasing awareness, and more frequent wildfire events. Wildfires produce substantial amounts of atmospheric pollution in smoke and particulate matter which can travel thousands of miles. These smoke events can blanket entire agricultural regions and cause impacts to plant development. One notable example of this is ‘smoke taint’ in wine, where vines, exposed to smoke, absorb many small volatile phenolic (VP) compounds. These VPs can be metabolized (glycosylated) and transported throughout the plant. Later, during downstream processing (fermentation of the grape musts), these glycosylated VPs can be hydrolyzed to re-release and volatilize the aroma-active phenolic compounds. The final product, thus, smells of smoke. Despite the obvious commercial and general research interests here, the nature of wildfire impacts on plant health are incompletely understood. Using established metabolomics and organic matter (aerosol) characterization methods, the overall aim of this proposal is thus to study wildfire smoke impacts on plant health and value, in the context of a model system (V. vinifera).

This project closed early, and this report reflects some of the limited work conducted prior to conclusions. Specifically, this report discusses some sample preparation and initial MALDI-mass spectrometry analysis.

Acknowledgments

Thanks to; Tom Collins (WSU) for consultation and assistance with sample collection. Steve Wiley and Christer Jansson for proposal support. Dusan Velickovic for MALDI preparation assistance. Yessica Carnely for sample preparation assistance. Finally, Sotiris Xantheas and the LDRD committees for this opportunity.

Acronyms and Abbreviations

- MALDI – Matrix Assisted Laser Desorption Ionization
- FTICR – Fourier transform ion cyclotron resonance
- MS – Mass Spectrometry
- NMR – Nuclear Magnetic Resonance
- NOM – natural organic matter
- DHB – dihydroxybenzoic acid
- NEDC - N-naphthylethylenediamine dihydrochloride

Contents

Figures

1.0 Introduction

This project closed prematurely and so this report reflects the limited work completed. The report will begin with a copy of the original proposal, and then highlight some of the work perform.

1.1 Original Proposal

1.1.1 Aims

The potential impacts of wildfire smoke on plant health and value are of increasing concern, due both to increasing awareness, and more frequent wildfire events. Wildfires produce substantial amounts of atmospheric pollution in smoke and particulate matter which can travel thousands of miles. These smoke events can blanket entire agricultural regions and cause impacts to plant development. One notable example of this is 'smoke taint' in wine, where vines, exposed to smoke, absorb many small volatile phenolic (VP) compounds. These VPs can be metabolized (glycosylated) and transported throughout the plant. Later, during downstream processing (fermentation of the grape musts), these glycosylated VPs can be hydrolyzed to re-release and volatilize the aroma-active phenolic compounds. The final product, thus, smells of smoke. Despite the obvious commercial and general research interests here, the nature of wildfire impacts on plant health are incompletely understood. Using established metabolomics and organic matter (aerosol) characterization methods, the overall aim of this proposal is thus to study wildfire smoke impacts on plant health and value, in the context of a model system (V. vinifera).

The specific aims of the proposal are as follows. **Aim 1) Determine mechanisms of smoke uptake into plant tissues.** Current hypotheses for smoke uptake include transdermal absorption through leaves (e.g., through stomata), or absorption through fruit cuticles. Smoke deposition in soils could also result in root-uptake of these compounds. This proposal will use ultra-high-resolution MS, including MALDI imaging MS,(Hamm et al. 2010) to identify the locations and mechanisms by which smoke organic compounds are incorporated into plant tissues.

Aim 2) Identify the influence of specific aerosol chemistry and particle structure on plant smoke uptake. Organic aerosols are known to undergo structural and chemical changes during transport, depending on the distances traveled, altitudes reached, and meteorological conditions. It is also known that not all smoke events 'taint' wine. However, it is unclear if this variation is induced by intrinsic differences in aerosols (different fire sources produce different aerosols), weathering of the aerosols (e.g. oxidation or hydration), or some other cause (e.g. seasonal susceptibility based on plant development cycle). Deliberate variations in smoke chemistry (e.g. different biomass burning, artificial weathering) will allow investigation of these impacts.

Aim 3) Establish the metabolism and transport pattern of smoke-derived chemicals within plant tissues. Knowing where and how organic compounds are metabolized and transported will inform remediation strategies. For example, if the volatile phenols (VP) are absorbed into the leaves, glycosylated and transported primarily into the fruit skins, this could inform vinification, i.e. to minimize skin contact and extraction during fermentation. Likewise, if the glycosylated VPs are primarily retained within the leaves and stems, fermenting the fruit only

(rather than whole-cluster) will be beneficial. If the smoke event is late in the season, and the glycosylated VPs are resident in the leaves, early pruning of the leaves may also be beneficial.

Aim 4) Establish the impacts on fermentation of smoke-derived compounds. Building on the previous aims, the specific impacts on downstream processing – in this case, vinification and fermentation – will be studied in the context of the different chemistries of smoke the plants were exposed to. This will help to further inform why not all smoke-events yield smoke taint in the wine, as well as to inform what other undesirable effects are occurring – such as reduced ethanol yield, formation of other byproducts, or inhibition of the yeasts.

1.1.2 Mission Relevance

Characterization of aerosol organic matter, especially wildfire derived, and plant metabolomics are important ongoing areas of research within EMSL and are thus important to DOE BER programs. Ensuring agricultural quality and yield, and protecting commercially significant crops, is of keen importance to the USDA. The Washington State Wine Commission has interest in the specific model system here, funding some existing efforts to study “smoke taint”. The potential impacts of smoke on crop yield and quality is important to DOE with regards biofuel production and thus domestic energy protection. Many of the technologies and approaches developed and improved in this project will benefit wider DOE-BER research interests (e.g. plant biology, atmospheric chemistry). Long-term, this research may inform whether if it is possible to use plant material to measure pollution, current and historic, i.e. trees in cities as biosensors for pollution.

1.1.3 Background

Wildfires are an increasing problem around the world due to climate change, drought events, and forestry mismanagement. Especially in North America (e.g. Washington) and Australia, these fires often occur proximal to important agricultural regions. Beyond the immediate devastation, wildfires produce organic aerosols (smoke) which can spread across vast distances (thousands of miles).(Zheng et al. 2020) This smoke is a complex mixture of thousands of different chemicals. It undergoes many poorly understood transformations during its transport from fire source to crop.(Zaveri et al. 2020; Girotto et al. 2018; China et al. 2013) These aerosols comprise many potentially phytotoxic compounds, including volatile phenols (VP). These can be absorbed into the plant tissue, notably through stomata, by mechanisms which remain unclear, where they undergo metabolic transformations, such as glycosylation as a plant-defense strategy against xenobiotic molecules.(Kelly et al. 2014; Härtl et al. 2017) This glycosylation affects the bioactivity, bioavailability, and sequestration of these volatile phenols, but their phytotoxic

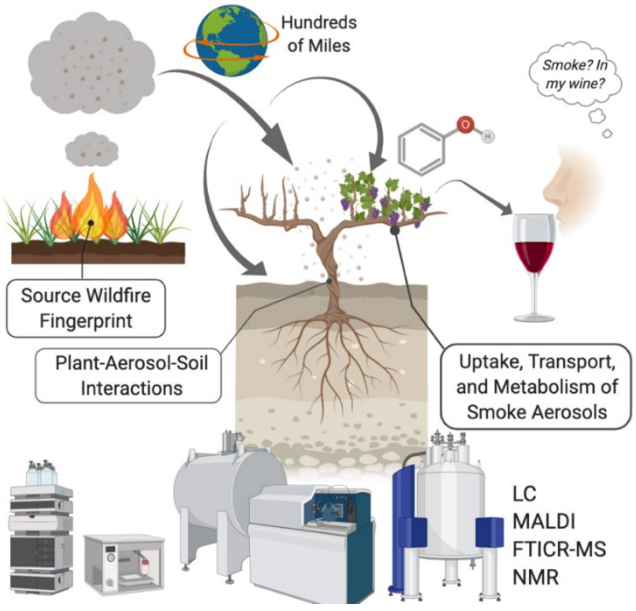

Figure 1 – Graphical Overview of Research Proposed

properties may still impact plant development or fruit ripening, in the case of angiosperms. Potentially more importantly, these xenobiotic compounds may negatively impact downstream processing or commercialization of agricultural crops, such as fermentation for bioethanol production. The proposed research will study *Vitis vinifera* as a model system, wherein wildfires can induce a fault in wine called “smoke taint”(Krstic, Johnson, and Herderich 2015; Noestheden et al. 2017; Noestheden, Dennis, and Zandberg 2018) where the glycosylated phenols are hydrolyzed during fermentation, and as a result, the wine smells smoky. *V. vinifera* represents an ideal model as it grows locally and quickly, and the faults induced have readily detected impacts, making it a sensitive model crop. The research will investigate the mechanisms by which smoke is absorbed into the plant, elucidate the metabolic processes undertaken and their location within the plant tissues, investigate the metabolic impacts on downstream processing, and use these efforts to inform future remediation strategies. It is hypothesized that smoke taint will be induced differentially by various smoke chemistries. Successful efforts to elucidate the nature and mechanisms of wildfire smoke impact on this crop, along with identifying potential remediation strategies, will yield transferable, valuable, and high-impact results to other plants, including bioenergy crops for lignocellulosic or saccharine biofuel production. Further, analytical techniques developed in this project may benefit wider plant biology and atmospheric chemistry research.

1.1.4 Approach/Work Plan

The project will focus on a model system, *V. vinifera,* due to its sensitivity to wildfire smoke, proximal nature to fires and the laboratory, and its commercial and public interest, globally, nationally, and locally in WA. The proposed research will use EMSL and PNNLs world-leading analytical expertise and instrumentation, in collaboration with the Wine Science Center (WSC) at WSU. WSU has both samples and experiments ongoing which will allow us to study this topic and has controlled plant smoking chambers. The PI has already had positive discussions with WSU WSC regarding collaboration. EMSL’s high resolution Fourier transform mass spectrometry (FTMS) and nuclear magnetic resonance (NMR) capabilities will provide molecular characterization of aerosols, plant and fruit extracts, and wine products, allowing us to trace the complex smoke chemistry throughout the entire process. Imaging analysis, including MALDI, will allow spatially resolved characterization of the biochemical processes occurring in the plant tissues. EMSL’s metabolomics platforms, including GC and LC-MS, will allow us to identify previously unknown smoke-related compounds in vines and wine. Many of the techniques required are already well established at EMSL for organic matter characterization or metabolomics, here we will be applying them to address the specific aims above and inform new understanding of this complex problem across scales.

To address **Aim 1,** fruiting vines will be exposed to wildfire smoke in a controlled environment (WSU). The initial study will perform liquid extractions (MPLEx(Nakayasu et al. 2016)) on soil and washed leaf, root, and fruit to identify and quantify low and high molecular weight smoke related compounds within the plant and soil samples (NMR, GC-MS, LC-MS). Smoke will be sampled via bubbling through organic solvent or trapping on aerosol filters, dried and extracted. The advanced instrumentation will yield structural information on more complex and higher molecular weight compounds than usually studied in this area of research. Subsequent experiments will control for each of these pathways by a limited exposure protocol of smoking only specific areas (leaf or soil or fruit). It is expected that significant uptake (indicated by high concentrations of smoke compounds) will occur through the leaf epidermis or fruit cuticle. It is unclear if soil smoke exposure will result in smoke compounds in the main plant. If so, the continuing scope will include root and soil. If not, the focus will be on fruit and leaf. Multi-modal imaging, including environmental scanning and transmission electron microscopy

(ESEM/ETEM), of the leaves and fruits will examine for aerosol particles and subsequent MALDI mass spectrometry imaging will determine if these particles are colocalized with smoke compounds within the tissue, supporting transdermal infusion. MALDI imaging may also reveal localized uptake of the smoke within the leaf (e.g. adaxial or abaxial, edges, tips, or center) based on the leaf structure, rather than specific aerosol particle locations. This will be further controlled by repeated experiments where the leaf tissue is partially protected by a removable wax or otherwise smoke-impermeable barrier, such that localized uptake is induced.

Aim 2 will be addressed by repeating the general protocol for Aim 1, controlled smoking of plant and soil, but by altering the smoke itself. This will include burning of different source materials (e.g. grasses or trees), different fuel provenances (species, origin, age and moisture), and smoke conditions (concentration, temperature of burn, temperature of smoke at crop, humidity, time-in-air). In all cases, smoke and plant material will be sampled, extracted and analyzed by ESI and APPI-FTMS using standard organic matter characterization workflows. It is hypothesized specific smokes will leave unique fingerprints in the plant. This will support efforts to understand why not all smoke-events result in smoke taint. If possible, WSU will vinify the differentially smoked fruit to identify which developed smoke taint. The affected wines (and controls) will also be analyzed by FTMS to identify if the unique smoke fingerprints remain detectable and identifiable even post-fermentation. Additionally, pre-existing early detection protocols (i.e. GC-MS of fruit juice) will inform which smoke events may result in smoke taint. Smoke particle structure and elemental compositions will be studied (using SEM/EDX) to determine if there is a correlation between particle structure, smoke source, and induced plant chemical changes.

Aim 3 will utilize temporally resolved sampling of the plant materials after a smoke event. Sampling will be performed hourly (6 hours), then daily (1 week), and then weekly (5 weeks) after smoking. The samples will be stored at -80 °C until all are ready for extraction and analysis. Leaves, stems and fruit will be extracted and analyzed independently. The metabolomics platforms (NMR, LC and GC-MS) will provide quantitative identifications of changing species during this process to understand the metabolic processes occurring and the transportation of both smoke compounds, and smoke-derived compounds, through the plant tissues. Controls (for plant life cycle) will consist of samples of otherwise identically treated, but unsmoked, plants. The primary search targets will be glycosylated small phenols, as demonstrated in the literature. However, it is expected there will be a diversity of glycosylation sugars (not just glucose as observed so far), linkage, and substrate. Structural elucidation of novel metabolites will be performed by high resolution NMR, GC-MS and LC-MS/MS experiments. Two-dimensional $^{1}H,^{13}C$ HSQC, HSQC-TOCSY, and HMBC experiments are ideal for de novo structural elucidation of glycosylated small molecules.

Aim 4 will investigate the impact on downstream processing of smoke leading to smoke taint. To this end, controlled yeast (*S. cerevisae*) fermentation of smoke exposed fruit will be performed. This will inform general fermentation metrics – ethanol yield, residual sugar – as well as formation of other desirable or undesirable compounds (e.g. esters, other alcohols). Relationships between the chemistry of smoke metabolites (Aim 1-3) and the impacts on fermentation will be modeled to understand if the smoke-compounds inhibit yeast metabolism or otherwise affect fermentation processes.

A.1.1 Project Activity

This project terminated prematurely, and so most of the above proposed work was not completed. However, this project did perform some test experiments and explored some sample preparation methodologies.

2.0 Tests and Experiments

2.1 Sample Collection

Sample collection occurred several days after an extremely high atmospheric smoke period in October 2020 from the WSU vineyard off George Washington Way in N. Richland, Washington.

Samples:

1. Varieties – Chardonnay and Syrah
2. Plant Material – Grapes (ripe), grapes (over-ripe), grapes (dried), grape-stems, leaf-stems, and leaves.

Samples were tagged and placed into zip-loc bags and stored at -80°C until required.

2.2 Sample Preparation – Liquid State

Sample extraction procedures were investigated due to the challenges in the heterogeneity of sample types. For example, the soft ripe grape flesh is readily extracted due to its high-water content, however the woody stems or the hard seeds within the grapes were more challenging. Further consideration is paid to the rapid oxidation (as observed by visual browning of tissues) of the samples, necessitating rapid, cold, or anoxic conditions.

After experimentation with manual actuation of samples, the final protocol involved:

- Dissection of tissues into individual components.
    - Stems were separated from leaves or fruit
    - Skin was removed from fruit
    - Flesh and seeds were separated from each other within fruit.
- Samples were stored on ice when not being directly handled.
- Samples were homogenized with stainless steel beads in a Next Advance Gold homogenizer.
    - Samples were spun up to three times for 15 minutes at max speed to homogenize with miliQ water as a solvent.
    - Dry ice in an adjacent chamber kept samples cool.
    - Some samples were incompletely ground with this process, but longer homogenizations risked heating and oxidation.
- Homogenized samples were then separated from the beads and extracted further with water, methanol, and acetonitrile sequentially or alternatively, during trials.

After extraction of the homogenized sample, the solution was centrifuged, and the supernatant transferred to a fresh Eppendorf for storage.

As NMR and MS are not compatible with particulates suspended in solution, even after centrifugation it was known filtration would be necessary.

Experiments were performed on polypropylene syringes and water-wettable PTFE filters (0.22micron) in polypropylene holders with the solvents used – water, methanol, acetonitrile – to ensure minimal leachate of plasticizers. NMR data – not shown – demonstrated minimal leachate from any of these solvents.

2.3 MALDI Analysis

For MALDI imaging analysis, samples need to be prepared from the intact solid state. For an initial test, a leaf stem was chosen.

The sample was thin sectioned to 20-micron using a cryomicrotome after embedding to hold it's shape.

The samples were sprayed with one of the following matrices - NEDC for negative mode analysis and DHB for positive mode analysis. Samples were analyzed with a Bruker Solarix xR 15T FTICR mass spectrometer at spatial resolutions of 35um or 50um per pixel and a resolution target of 200k at m/z 400.

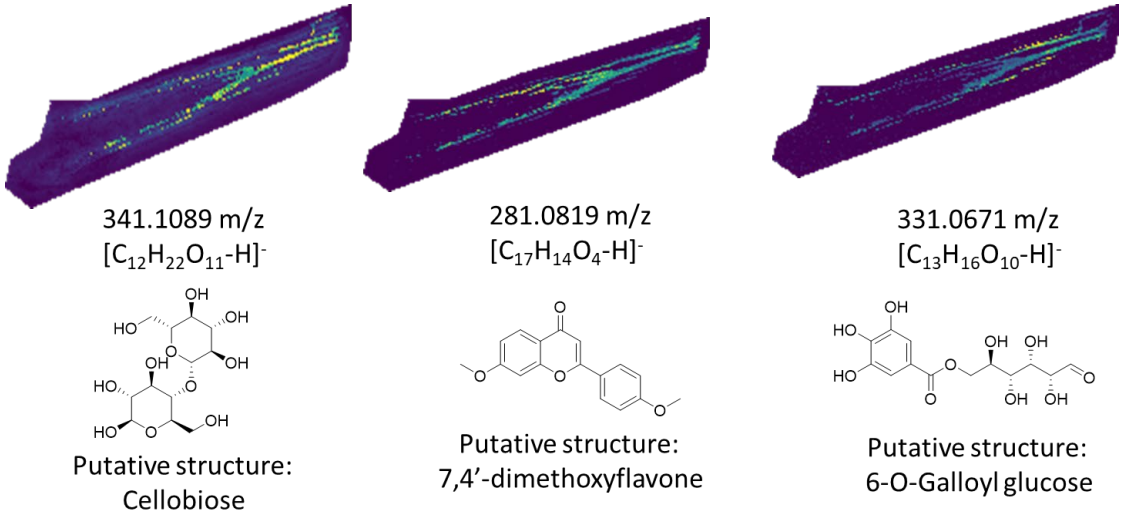

Figure 2 – Example ion images from negative mode MALDI-FTICR on leaf stem showing three different ion images for structurally different compounds. Measured masses and putative assignments are shown – highlighting a carbohydrate, an aromatic molecule, and a glycoside of an aromatic molecule – three key classes under investigation in this project. The images show very similar, but subtly different, localizations of these molecules.

MALDI data analysis was performed with flexImaging and SCILS Lab Pro to convert to imzML format prior to upload to metaspace platform online for annotation against existing libraries.

3.0 Project Termination

Due to staff availability and project commitments, it was decided in early summer 2021 to end this project early. As the scope of work had been laid out for two years, with most of the formal experimental work to be conducted during and after summer (growth, smoke, and harvest events), there is limited completed work in this project.

4.0 References

China, Swarup, Claudio Mazzoleni, Kyle Gorkowski, Allison C. Aiken, and Manvendra K. Dubey. 2013. “Morphology and Mixing State of Individual Freshly Emitted Wildfire Carbonaceous Particles.” *Nature Communications* 4: 1–7. https://doi.org/10.1038/ncomms3122.

Girotto, Giulia, Swarup China, Janarjan Bhandari, Kyle Gorkowski, Barbara V. Scarnato, Tyler Capek, Angela Marinoni, et al. 2018. “Fractal-like Tar Ball Aggregates from Wildfire Smoke.” *Environmental Science and Technology Letters* 5 (6): 360–65. https://doi.org/10.1021/acs.estlett.8b00229.

Hamm, Gregory, Vincent Carré, Anne Poutaraud, Benoît Maunit, Gilles Frache, Didier Merdinoglu, and Jean-François Muller. 2010. “Determination and Imaging of Metabolites from Vitis Vinifera Leaves by Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry.” *Rapid Communications in Mass Spectrometry* 24 (3): 335–42. https://doi.org/10.1002/rcm.4395.

Härtl, Katja, Fong Chin Huang, Ashok P. Giri, Katrin Franz-Oberdorf, Johanna Frotscher, Yang Shao, Thomas Hoffmann, and Wilfried Schwab. 2017. “Glucosylation of Smoke-Derived Volatiles in Grapevine (Vitis Vinifera) Is Catalyzed by a Promiscuous Resveratrol/Guaiacol Glucosyltransferase.” *Journal of Agricultural and Food Chemistry* 65 (28): 5681–89. https://doi.org/10.1021/acs.jafc.7b01886.

Kelly, D., Ayalsew Zerihun, Y. Hayasaka, and M. Gibberd. 2014. “Winemaking Practice Affects the Extraction of Smoke-Borne Phenols from Grapes into Wines.” *Australian Journal of Grape and Wine Research* 20 (3): 386–93. https://doi.org/10.1111/ajgw.12089.

Krstic, M. P., D. L. Johnson, and M. J. Herderich. 2015. “Review of Smoke Taint in Wine: Smoke-Derived Volatile Phenols and Their Glycosidic Metabolites in Grapes and Vines as Biomarkers for Smoke Exposure and Their Role in the Sensory Perception of Smoke Taint.” *Australian Journal of Grape and Wine Research* 21: 537–53. https://doi.org/10.1111/ajgw.12183.

Nakayasu, Ernesto S., Carrie D. Nicora, Amy C. Sims, Kristin E. Burnum-Johnson, Young-Mo Kim, Jennifer E. Kyle, Melissa M. Matzke, et al. 2016. “MPLEx: A Robust and Universal Protocol for Single-Sample Integrative Proteomic, Metabolomic, and Lipidomic Analyses.” *MSystems* 1 (3). https://doi.org/10.1128/msystems.00043-16.

Noestheden, Matthew, Eric G. Dennis, and Wesley F. Zandberg. 2018. “Quantitating Volatile Phenols in Cabernet Franc Berries and Wine after On-Vine Exposure to Smoke from a Simulated Forest Fire.” *Journal of Agricultural and Food Chemistry* 66 (3): 695–703. https://doi.org/10.1021/acs.jafc.7b04946.

Noestheden, Matthew, Katelyn Thiessen, Eric G. Dennis, Ben Tiet, and Wesley F. Zandberg. 2017. “Quantitating Organoleptic Volatile Phenols in Smoke-Exposed Vitis Vinifera Berries.” *Journal of Agricultural and Food Chemistry* 65 (38): 8418–25. https://doi.org/10.1021/acs.jafc.7b03225.

Zaveri, Rahul A., John E. Shilling, Alla Zelenyuk, Maria A. Zawadowicz, Kaitlyn Suski, Swarup China, David M. Bell, Daniel Veghte, and Alexander Laskin. 2020. “Particle-Phase Diffusion Modulates Partitioning of Semivolatile Organic Compounds to Aged Secondary

Organic Aerosol." *Environmental Science and Technology* 54 (5): 2595–2605. https://doi.org/10.1021/acs.est.9b05514.

Zheng, Guangjie, Arthur J. Sedlacek, Allison C. Aiken, Yan Feng, Thomas B. Watson, Shira Raveh-Rubin, Janek Uin, Ernie R. Lewis, and Jian Wang. 2020. "Long-Range Transported North American Wildfire Aerosols Observed in Marine Boundary Layer of Eastern North Atlantic." *Environment International* 139 (April): 105680. https://doi.org/10.1016/j.envint.2020.105680.

Pacific Northwest National Laboratory

902 Battelle Boulevard
P.O. Box 999
Richland, WA 99354
1-888-375-PNNL (7665)

www.pnnl.gov