Project Title: Investigating the Influence of Glycosylated Aroma Precursors and Thiophenols in Smoke-Affected Hops

Project Summary: Wildfire smoke is an emerging threat to hop quality, with smoke-exposed hops posing potential risks to beer aroma and flavor. While volatile phenols (VPs) have been linked to smoke taint in hops through previous research by our workgroup, the role of glycosylated phenols (GPs) — non-volatile compounds that may release VPs during fermentation — remains unexplored in hops. In wine, GPs have been identified as key precursors of smoke characteristics, along with thiophenols (TPs) that are responsible for meaty and ashy aromas. This project will investigate the occurrence, concentration, and sensory impact of GPs and thiophenols (TPs) in smoke-affected hops and beer. Using both commercially sourced and field trial samples, we will quantify GPs, VPs, and TPs in a range of hop varieties with differing smoke exposures. We will screen common brewing yeast strains for their ability to hydrolyze GPs into VPs, conducting small-scale fermentations and sensory analyses to evaluate aroma outcomes. If warranted, pilot scale beers will be brewed with high-GP hops and yeasts with high hydrolytic enzyme potential and will be analyzed chemically and sensorially. Also, depending on outcomes from the TP testing, individual TPs and mixtures may be tested in beer to determine their sensory relevance. By integrating chemical and sensory data, this research will clarify how previously unexplored smoke-derived phenols contribute to off-flavors in beer and assess the potential for fermentation to modulate these impacts. The results will provide critical, actionable information for growers and brewers navigating the increasing risk of smoke exposure, and support the development of strategies to maintain hop and beer quality in wildfire-prone regions.

Proposed Duration: 1 year (single year extension to the current 3-year project on smoke & hops)

Project Leader: Thomas H. Shellhammer

Nor'Wester Professor of Fermentation Science

Professor of Food Science

Department of Food Science & Technology

Oregon State University, 100 Wiegand Hall, Corvallis, OR 97331

Office: 541 737-9308, Fax: 541 737-1877 e-mail: tom.shellhammer@oregonstate.edu

Technical Assistant: Cade Jobe, Graduate Research Assistant

Cooperators: Dr. Cole Cerrato Dept Food Science & Technology, OSU

Jeff Dailey, John I Haas

Tiffany Pitra, Yakima Chief Hops

Amount Requested: \$75,356 (FY 2026).

Other Funding Sources and Support: The proposed work involves significant direct and in-kind contributions of labor and materials from collaborators.

Send funding to: Agricultural Research Foundation

1600 SW Western Blvd., Suite 320

Corvallis, Oregon 97333

Charlene Wilkinson, 541 737-3228, charlene.wilkinson@oregonstate.edu

Project Title: Investigating the Influence of Glycosylated Aroma Precursors and Thiophenols in Smoke-Affected Hops

Statement of Problem:

Wildfire smoke exposure is an increasingly significant challenge for hop growers, potentially compromising hop quality and aroma profiles. While volatile (free) phenolic compounds (VPs) have been established as markers of smoke exposure in hops, the role of glycosylated phenols (GPs)—non-volatile forms in which phenolic compounds are bound to sugar moieties—remains uninvestigated. In wine grapes, exposure to wildfire smoke leads to the uptake of airborne VPs, which are rapidly glycosylated by the plant to form GPs. These bound compounds are odorless but act as latent reservoirs of smoke aroma precursors that can be hydrolyzed during fermentation, releasing free VPs and contributing to undesirable sensory attributes such as ashy and smoky aromas. Elevated concentrations of GPs in wine grapes have been directly linked to smoke-tainted wines. A similar mechanism may be at play in hops, with significant implications for brewing quality. Although GPs have not been studied in hops outside our research group, preliminary data from our lab indicate that GPs are indeed present and can be enzymatically converted to free VPs under fermentation conditions. In bench-top trials using an industrial glucosidase enzyme treatment to release VPs from the glycosidically-bound GP forms, we observed significant increases in VPs after 72 hours of enzyme incubation (Table 1).

Table 1. Preliminary results of GP analysis in several varieties of hops with varying smoke exposure levels.

| Sample No. | Smoke Exposure | Sum of VPs (no enzyme) | Sum of VPs (with enzyme) | % Increase of VPs | Variety | Comment |
|----------------|-------------------|---------------------------|--------------------------------|-------------------------|------------|--------------------------------------|
| 1 a | None | 23.2 | 30.0 | 29% | Citra | Control (not smoked) (2024 WA) |
| 2 a | None | 20.8 | 30.8 | 48% | Citra | Control (not smoked) (2024 OR) |
| 3 b | Low | 23.0 | 32.7 | 42% | Willamette | Flagged by brewer as smoky (2021 WA) |
| 4 b | Medium | 63.1 | 160.9 | 155% | El Dorado | Flagged by brewer as smoky (2021 WA) |
| 5 ^c | High | 55.1 | 170.2 | 209% | Citra | Smoked in-field (2024 OR) |
| 6 c | High | 86.8 | 424.0 | 389% | Citra | Smoked in-field (2023 OR) |

^a From 2024 harvest grown in Washington or Oregon.

Brewers have reported instances of beers developing smoky characteristics during storage, despite initially passing quality control checks. This delayed onset of smoke-related flavors may be explained by the gradual release of VPs from GPs over time. The results observed in Table 1 offer compelling evidence that GPs may play an important role in the contribution of smokiness from hops to beer. Additionally, brewer's yeast's may vary in hydrolytic potential with respect to VPs, since prior work has shown that brewer's yeast produces glucosidase enzymes capable of releasing monoterpene alcohols from their glycosylated precursors^{3,4}—the same enzymes could work to liberate VPs from GPs.⁵ The results from our preliminary investigation combined with the glucosidase activity of brewer's yeast suggest that further exploration of GPs in hops is necessary. If hop-derived GPs are hydrolyzed by brewer's yeast during fermentation or beer storage, they may contribute to the delayed emergence of smoke-related off-flavors, even in hops that initially appear unaffected. Understanding this potential pathway is essential for hop growers and brewers seeking to manage the risks associated with wildfire smoke exposure.

^b Flagged by brewers as smoky, grown in 2021 in Washington.

^c From 2023 and 2024 in-field smoke exposure trials as part of an HRC funded project.

Separately, recent investigations in wine grapes have identified thiophenols (TPs) as potent contributors to smoke-taint in wine.⁶ In wine, combinations of VPs and TPs in spiked wines most similarly resembled wines made from grapes that were tainted during wildfire smoke events indicating that both VPs and TPs play a role in aromatic characteristics.⁶ Individually, thiophenols are known to contribute meaty or ashy flavors in wine and other food systems.⁷ Since meaty, artificial BBQ, and savory are common descriptors for smoke-affected hops, it is possible that TPs play a similar sensorial role in hops.

We believe that continued exploration is warranted to determine whether GPs and TPs consistently occur in hops and how they may vary across different hop varieties or smoke exposure conditions. Additionally, the capacity of brewer's yeast to hydrolyze GPs during fermentation remains unknown. Finally, the impact of thiophenols on hop and beer sensorial attributes is wholly unexplored. This gap in knowledge presents a critical barrier to understanding how wildfire smoke impacts the sensory quality of hops and, ultimately, beer flavor. Addressing this issue will provide practical information for both hop growers and brewers by identifying potential risks associated with smoke-affected hops and offering insights into how fermentation processes might mitigate or exacerbate smoke-related flavor changes.

Justification and Significance of Research:

Understanding the presence, concentration, and variability of glycosylated phenols (GPs) and thiophenols (TPs) in both clean and smoke-exposed hops is critical because they may contribute to smoke-related off-flavors in beer, potentially at levels higher than what is already contributed by free phenols in smoke-affected hops. This research will advance scientific knowledge by elucidating the chemical nature and behavior of GPs and TPs in hops and beer, including an assessment of the relative contributions of free phenols introduced during the kilning (drying) process versus those derived from the hydrolysis of bound glycosidic precursors. Clarifying the origin and extent of volatile phenol (VP) expression through these different pathways will provide valuable insights for both growers and brewers into the risks associated with wildfire smoke contamination. Ultimately, the findings may support the development of mitigation strategies to maintain hop quality and beer flavor integrity, contributing to the sustainability of the hop industry and the brewing sector in the face of future wildfire smoke events.

Oregon State University is particularly well-poised to conduct this research because of the world-class hop chemistry lab led by Dr. Tom Shellhammer, aided by the Smoke, Wine, and Grape Analytical Lab funded in part by the state of Oregon and in part by the USDA Specialty Crop Research Initiative to study smoke taint in wine grapes. The lab, located within the Department of Food Science & Technology, includes equipment for studying smoky compounds in hops, including a gas chromatograph with a triple quadrupole mass spectrometer (GC-MS/MS), a gas chromatograph with a quadrupole time of flight detector (GC-QToF), a liquid chromatograph (LC) with a QToF detector and two LCs with triple quadrupole mass spectrometers. This equipment is needed for GP analysis because of the unique nature of the precursor, which is soluble and measurable by liquid chromatography, and the resultant VP, which is volatile and measurable by gas chromatography. Additionally, the OSU mass-spectrometry center has supplementary advanced instrumentation that may be utilized to look at smoke-related analytes, if needed.

Another major advantage for OSU is the close relationship with Drs. Elizabeth Tomasino, James Osborne, and Cole Cerrato (collaborator on this project) within the Department of Food Science & Technology, who have collectively published several peer-reviewed articles on the topic of smoke taint in wine grapes, including on the impact of GPs and TPs in wine. Dr. Tomasino specializes in wine flavor chemistry, specifically in correlating smoky compounds with sensory characteristics, while Dr. Osborne focuses on

how microbial factors contribute to the formation of smoky compounds during fermentation and aging of wine, and Dr. Cerrato focuses on analytical chemical analyses for compounds that impact flavor and aroma. These collaborative relationships are invaluable resources for helping to understand how smoke compounds might interact with hops. They can also provide access to flavor standards and methodology for analyzing targeted compounds.

Research Objectives:

The primary objective of this research is to investigate the role of glycosylated phenols (GPs) and thiophenols (TPs) in hops exposed to smoke and their potential impact on hop and beer quality. To achieve this, we will address the following specific objectives:

<u>Objective 1</u>: Characterize the presence, concentration, and variability of glycosylated phenols (GPs) in both clean and smoke-exposed hops across multiple cultivars.

<u>Objective 2</u>: Evaluate the ability of brewer's yeast to enzymatically hydrolyze GPs during fermentation, possibly resulting in the release of volatile phenols (VPs) into beer.

<u>Objective 3</u>: Investigate the occurrence and sensory impact of thiophenols in smoke-affected hops and their contribution to undesirable aroma compounds in beer.

Project Description/Procedure/Methods:

Objective 1: Characterize the presence and concentration of glycosylated phenols in hops

To quantify the occurrence and concentration of glycosylated phenols (GPs) and volatile phenols (VPs), we will analyze both commercially sourced and field trial smoke-exposed hop samples. We will collect lots from commercial suppliers, encompassing a range of cultivars (e.g., Cascade, Citra®, Mosaic®, etc.) and varying smoke exposure histories (clean, moderate, and heavily smoke-impacted). In parallel, we will analyze hops from our controlled in-field smoke exposure trials (Citra®) conducted in 2023 and 2024, where exposure parameters (e.g., duration, AQI, particulate matter, developmental stage, VP concentration) have been measured and are well documented.

Volatile phenols (e.g., guaiacol, 4-methylguaiacol, cresols) will be extracted using headspace solid-phase microextraction (HS-SPME) and quantified using gas chromatography-tandem mass spectrometry (GC-MS/MS). Glycosylated phenols will be analyzed by enzymatic hydrolysis followed by HS-SPME extraction and GC-MS/MS, where commercial β -glucosidase preparations will be used to release VPs from glycoside conjugates. Where possible, internal standards will be used to ensure accurate quantification. We will evaluate differences in GP concentrations across hop varieties, regions, and smoke exposure levels, and calculate the relative proportion of bound (GPs) to free (VPs) phenols to estimate potential aroma release upon fermentation.

Objective 2: Yeast screening to evaluate hydrolytic potential

From Objective 1 results, we will select one or more hop varieties with high GP concentrations for yeast screening trials. We will select a mix of ale and lager yeast strains prioritized based on known β -glucosidase activity (from literature or commercial databases) and usage in the brewing industry. Benchtop fermentations will be conducted with un-hopped wort supplemented with the hops identified in Objective 1. Fermentations will be performed in triplicate, and samples will be taken at key time points

(e.g., day 0, 3, 7, post-fermentation) for chemical analysis. GPs and released VPs will be quantified using the same HS-SPME GC-MS/MS workflows from Objective 1.

Simple sensory evaluations (e.g., bench top evaluations and possibly triangle tests) will be conducted on the bench-top-fermented beers to evaluate perceptible differences in aroma. If the results from chemical and sensory tests indicate that GP-derived VPs contribute significantly to beer aroma, pilot-scale fermentations (~150L) may be conducted using high-GP hops and select yeast strains showing strong hydrolytic potential. These beers would be evaluated by a trained sensory panel using discrimination and descriptive analysis techniques to characterize the aroma contributions of GP-derived VPs.

Objective 3: Investigate the occurrence and sensory impact of thiophenols (TPs) in hops and beer

To better understand the role of thiophenols (TPs) in smoke-affected hops, we will analyze all hop samples collected in Objective 1 for the presence of key TPs including thioguaiacol, o-thiocresol, m-thiocresol, and p-thiocresol. These will be quantified via extraction, derivatization, and analysis using liquid chromatography with an MS/MS detector. Concentrations will be compared across smoke exposure conditions and hop varieties to evaluate correlations between TP levels and smoke impact severity.

If chemical analyses suggest that TPs are present in hops at concentrations that might influence aromatic characteristics, we will consider performing model beer studies in which base beers will be spiked with individual TPs and mixtures. Depending on outcomes, sensory threshold studies may be performed to determine detection limits of TPs in beer using ascending forced-choice triangle tests. Additionally, descriptive analysis by trained panels could evaluate qualitative aroma attributes and potential masking or synergistic effects when TPs are combined with VPs. This approach will determine whether TPs contribute to the smoky characteristics observed in beer made from smoke-affected hops.

Outcomes:

These integrated chemical and sensory methods will provide a comprehensive understanding of how glycosylated phenols and thiophenols contribute to the aroma profile of smoke-affected hops and beer. The study leverages field and commercial samples, advanced analytical chemistry, yeast biotechnology, and sensory science to deliver practical insights for hop growers and brewers confronting wildfire-related quality challenges.

Specific outcomes for the project include:

- Quantitative data on glycosylated and volatile phenol concentrations in smoke-affected and unaffected hop samples across a range of varieties, with comparative analysis by smoke exposure level.
- 2. **Identification of brewing yeast strains' capable of hydrolyzing glycosylated phenols** during fermentation, based on both chemical analysis and simple sensory screening.
- 3. **Quantification of thiophenol levels** in smoke-affected and unaffected hop samples across a range of varieties, with preliminary assessment of correlations between TP levels, smoke exposure severity, and sensory impact.

Extension and Outreach Activities:

The outcomes of this project will be shared through industry presentations and publications. Research updates for the project will be presented at annual and biannual brewing and hop industry conferences.

For the hop growing industry, we will share our findings at the Hop Growers of America (HGA) conference, and at the Hop Research Council (HRC) summer update. For the brewing industry, we plan to share project results annually at the Craft Brewers Conference (CBC), the Brewing Summit, and, in 2028, at the World Brewing Congress. We intend to publish our work in peer-reviewed journals such as the American Society of Brewing Chemists, the Journal of Agricultural and Food Chemistry, and other appropriate journals and in non-peer-reviewed, but large audience, trade journals such as the New Brewer.

ATTACHMENTS

Project Timeline:

| | | 2026 | ; | |
|---|--------|--------|--------|------|
| TASK | Winter | Spring | Summer | Fall |
| Analytical analyses | | | | |
| Method development for glycosylated phenols (GP) and thiophenol (TP) analysis | | | | |
| Survey of GPs in commercial hops of varying cultivars and smoke exposure histories | | | | |
| Brewer's yeast screening to evaluate hydrolytic potential of GPs | | | | |
| Survey of TPs in commercial hops of varying cultivars and smoke exposure histories | | | | |
| Sensory analysis of hops and beers with GPs and TPs | | | | |
| (Possibly) Brewing trials to explore effects of transfer of GPs and TPs from hops to beer | | | | |
| Data Analysis, Reporting, and Presentation | | | | |

Project Budget

| | Нор | Commission | Other | Total Amount |
|---------------------------------|--------------------|-----------------|--------------|--------------|
| | Research | Request | Sources | Requested |
| | Council Request | (specify state) | | · |
| | | State: | | |
| | | Amount | Amount | |
| | | (cash or in- | (cash or in- | |
| | | kind) | kind) | |
| Salaries ¹ | | | | |
| Graduate Research Assistant | | | | |
| 12 months at 0.49 FTE | \$32,470 | | | \$32,470 |
| Employee Benefits | 8,896 | | | 8,896 |
| Temporary or hourly workers | 8,370 | | | 8,370 |
| Travel ² | 4,120 | | | 4,120 |
| USA Hop Convention Registration | 2,000 | | | 2,000 |
| Equipment | -0- | | | -0- |
| Other (specify) | | | | |
| Smoke compound analyses | 11,500 | | | 11,500 |
| Sensory analyses | 5,000 | | | 5,000 |
| Brewing | 3,000 | | | 3,000 |
| Total | \$75,356 | | | \$75,356 |

¹ Specify the type of position

Budget Justification:

This budget represents a 6% reduction in spending from the 2025 (current) project because of reduced travel costs (no on-site smoking trials).

Personnel - This project will be executed by a doctoral graduate student, 0.49 FTE for 12 months. Temporary or hourly workers consist of one undergraduate student worker to assist with project execution at \$15.50/hour (full time in summer).

Travel

Travel to attend HRC meetings...graduate student (Cade Jobe) and Dr. Shellhammer to attend winter and summer 2026 HRC meetings:

| Item | Winter mtg | Summer mtg | Total |
|-----------------------------------|------------|------------|---------|
| Transportation | 600 | 400 | 1000 |
| Accommodation | 1200 | 800 | 2000 |
| Per diem, 5&2 people x 3 days@ 60 | 700 | 420 | 1120 |
| Total | 2500 | 1620 | \$4,120 |

USA Hop Convention Registration for two attendees at \$1000 each.

Other:

- <u>Smoke compound analyses</u> to quantify free phenols, phenol glycosides (indirect), and thiophenols in hops performed at OSU in collaboration with the <u>OSU Smoke, Wine, and Grape Analytical Lab</u>
 - Free phenols, 60 samples at \$125 per sample = \$7500

² Provide a brief justification for travel funding requested. All travel must be directly related to the project.

- Thiophenols, 20 samples at \$200 per sample = \$4000
- Sensory analyses panelist training and up to 10 descriptive analyses sessions at \$500 each
- Brewing costs involve all materials needed for beer preparation, packaging, and chemical analyses for up to 6 brewing trials.

Other Funding Sources and Support:

The proposed work involves direct and in-kind contributions of labor, materials and potential sensory panel time from industrial collaborators Yakima Chief Hops and John I Haas and materials and transportation costs from hop processors for smoke affected and clean hop material (Yakima Chief Hops, John I Haas, Hopsteiner, and Hollingberry and Son).

Dr. Shellhammer Profile:

Dr. Thomas H. Shellhammer is the Nor'Wester Professor of Fermentation Science in the Department of Food Science and Technology at Oregon State University where he leads the research, teaching, and outreach elements of the brewing-related fermentation science enterprise. Since beginning role at Oregon State University in 2001, he has built an internationally-recognized program in Brewing Science, publishing over 130 peer-reviewed and technical publications and delivering over 300 professional presentations on 5 different continents. During the 2008-2009 academic year he conducted research as an Alexander von Humboldt Senior Research Scholar at the Technical University of Berlin. He is a former President of the American Society of Brewing Chemists, former President of the District NW Master Brewers Association of the Americas, and served on the Board of Examiners (16 years) of the Institute of Brewing and Distilling in London, England. He is a Fellow of the Institute of Food Technologists and the Institute of Brewing and Distilling.

Cade Jobe Profile:

Cade Jobe is a Provost's Distinguished Graduate Fellow and Ph.D. student in Dr. Tom Shellhammer's lab at Oregon State University. In addition to being genuinely enthusiastic about all things brewing science, his research investigates how hop treatment methods impact dextrin-reducing enzymatic activity and how wildfire smoke impacts hop quality. He is a contributor for Brulosophy.com and is the former host of The Brü Lab Podcast, where he recorded over 180 weekly podcast episodes interviewing brewing scientists bringing their research out of the lab and into the ears of the general brewing community. Prior to that, he was a cellarperson and member of the QA/QC team for Blue Owl Brewing Company in Austin, Texas. Cade is also a former lawyer who practiced family law in Austin, Texas before following his dream to study brewing science.

OSU Facilities:

Oregon State University is a Tier One research university located in Corvallis, Oregon. It is the land-grant university for the state of Oregon and specializes in Agricultural, Forestry, Engineering, and Oceanic and Atmospheric Sciences. It is the state's largest public research university and holds a top tier research designation from the Carnegie Foundation. The university is located in the middle of the Willamette valley hop-growing region and has a long legacy of hop research dating back to 1932 with the creation of a U.S. Department of Agriculture hop breeding program. The Department of Food Science at OSU is the US's oldest Food Science program and has strengths in food chemistry, food processing, and fermentation science. The department houses a 2 hL pilot brewery and state of the art analytical instrumentation including: several GC-MS units, GC's with pulsed flame ionization detectors for sulfur analysis, HPLC and HPLC-MS. The Shellhammer lab serves as a validated raw hop analysis laboratory and carries out significant hop quality and chemistry research which has been funded in part by prior awards from the Hop Research Council. Laboratory strengths include advanced flavor chemistry, sensory and

statistical analysis, brewing trials, and more as highlighted in past studies: the impact of kilning temperatures on hop quality, the origins of hop aroma, harvest timing effects on hop quality, water soluble hop aroma compounds, investigations into dry-hopping aroma and bitterness, bitterness intensity and quality of isomerized and oxidized hop acids.

References:

- (1) Crews, P.; Dorenbach, P.; Amberchan, G.; Keiffer, R. F.; Lizama-Chamu, I.; Ruthenburg, T. C.; McCauley, E. P.; McGourty, G. Natural Product Phenolic Diglycosides Created from Wildfires, Defining Their Impact on California and Oregon Grapes and Wines. *J. Nat. Prod.* **2022**, *85* (3), 547–561. https://doi.org/10.1021/acs.jnatprod.2c00028.
- (2) Caffrey, A.; Ebeler, S. E. The Occurrence of Glycosylated Aroma Precursors in Vitis Vinifera Fruit and Humulus Lupulus Hop Cones and Their Roles in Wine and Beer Volatile Aroma Production. *Foods* **2021**, *10* (5), 935. https://doi.org/10.3390/FOODS10050935.
- (3) Sharp, D. C.; Vollmer, D. M.; Qian, Y.; Shellhammer, T. H. Examination of Glycoside Hydrolysis Methods for the Determination of Terpenyl Glycoside Contents of Different Hop Cultivars. *J. Am. Soc. Brew. Chem.* **2017**, *75* (2), 101–108. https://doi.org/10.1094/ASBCJ-2017-2071-01.
- (4) Sharp, D. C.; Steensels, J.; Shellhammer, T. H. The Effect of Hopping Regime, Cultivar and β-Glucosidase Activity on Monoterpene Alcohol Concentrations in Wort and Beer. *J. Inst. Brew.* **2017**, *123* (2), 185–191. https://doi.org/10.1002/jib.418.
- (5) Martínez, L.; da Costa, B. S.; Vilanova, M. Comparative Study of Different Commercial Enzymes on Release of Glycosylated Volatile Compounds in White Grapes Using SPE/GC–MS. *Food Chem.* **2025**, 464, 141742. https://doi.org/10.1016/j.foodchem.2024.141742.
- (6) Tomasino, E.; Cerrato, D. C.; Aragon, M.; Fryer, J.; Garcia, L.; Ashmore, P. L.; Collins, T. S. A Combination of Thiophenols and Volatile Phenols Cause the Ashy Flavor of Smoke Taint in Wine. *Food Chem. Adv.* **2023**, *2*, 100256. https://doi.org/10.1016/j.focha.2023.100256.
- (7) Khan, M. I.; Jo, C.; Tariq, M. R. Meat Flavor Precursors and Factors Influencing Flavor Precursors—A Systematic Review. *Meat Sci.* **2015**, *110*, 278–284. https://doi.org/10.1016/j.meatsci.2015.08.002.