**Emerging Research Issues for Washington Agriculture**

**Proposal for 2021**

1. **Projective narrative**

**I. Title:** From waste to food: conversion of organic waste substrates into gourmet edible and medicinal mushrooms in Washington

* **Lead PI:** David Linnard Wheeler
* **Department**: Plant Pathology
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* **Cooperating PI names:**
  + - Mark E. Swanson, Associate Professor/Forestry Program Leader, School of the Environment, Washington State University. markswanson@wsu.edu

**II. Requested duration:** 2 years

**III.**  **Amount requested for:** 7/1/2021 - 6/30/2022: $50,000 **&** 7/1/2022 - 6/30/2023: $30,000

**IV. Project Abstract, including brief description of Emerging Issue(s) being addressed and the research strategy that is proposed (250 words) :**

Waste streams represent underutilized resources that merit increased emphasis in research in Washington. Stakeholders from agricultural, forestry, brewing, coffee, and restaurant industries have expressed the need for alternative waste disposal outlets. Fortunately, stakeholders from the gourmet mushroom cultivation industry have expressed the complementary need for such substrates in their operations. The goal of this project is to address this emerging issue of waste stream utilization with a biological solution: mushroom cultivation. To produce food from these wastes our objectives are to (i) investigate the cultivation of local mushrooms from regional wastes and (2) teach communities how to cultivate mushrooms. For objective 1, waste residues from the industries mentioned above will be obtained and prepared for mushroom cultivation. Five species of gourmet fungi will then be collected from around Washington. Cultures of these fungi will be maintained by the PIs and a graduate student. Substrates will then be inoculated with each of the five fungal species. To identify which combinations of fungal and waste residues generate the most potential profits, mushroom sporocarp biomass, quality, and production costs will be quantified and compared. Each treatment and trial will be replicated. For objective 2, the PIs will conduct workshops throughout Washington communities, share research results and demonstrate mushroom cultivation techniques. Surveys will be issued to stakeholders before and after workshops gauge the localized interest and adoption of gourmet mushroom cultivation. Ultimately, this research will help ameliorate the emerging issue of waste stream utilization while increasing the production of gourmet edible and medicinal mushrooms.

**V. Rationale & Significance of the problem from the perspective of Washington stakeholders. It should be made clear how the research conducted in this project will close existing gaps in our ability to conduct research that addresses an Emerging Issue for Washington’s stakeholders:** The rationale and significance of this project are twofold. From the perspective of waste stream utilization the rationale is to provide an outlet for organic wastes generated within Washington’s forestry, agricultural, brewing, coffee and restaurant industries. This is at once both a pragmatic endeavor- to reduce waste - and a transformative one, since rigorous research in food production from waste streams is neglected compared to traditional agricultural research.

Further, from the perspective of gourmet mushroom cultivation the rationale is to generate the resources needed to successfully grow, sell, and conduct research on these fungi. The significance of this point should not be underestimated. Agriculture has traditionally focused efforts on plant and animal production. Mushroom cultivation is an age-old but under-researched frontier. Barriers to entry are largely informational and technical. Even with the development of cultivation kits for hobbyists, small to large scale mushroom cultivation is far from its potential. Thus, to provide the resources to stakeholders is to open the doors to an industry well positioned to flourish and prosper in Washington.

As such, stakeholders from disparate industries in Washington are well positioned to profit from the partnership of waste streams with gourmet edible and medicinal mushroom cultivation. The rationale of this project is to satisfy needs from both groups of stakeholders. Stakeholders from agricultural, forestry, brewing, coffee and restaurant industries need a home for their waste products. Fortunately, stakeholders from the gourmet mushroom industry need substrates for cultivation. By connecting these disparate industries we can solve problems for each.

Lastly, the significance of this project is not only in problems but also in opening doors to fruitful research. Once reproducible methods for cultivating local species of gourmet mushrooms on local substrates are available, transformative research can proceed in many directions.

**VI. Description of ability of work/findings to apply across commodities and/or industries:** Mushroom can be cultivated on a wide range of substrates from a diverse array of industries and commodities (Stamets, 2000). For example, we can combine wood residues from sawmill waste with spent grain from breweries and spent coffee grounds from cafes and restaurants to produce nutritious mushroom cultivation substrates. Even in cases of extreme substrate characteristics (for example, with waste from oil spills or plastics), some species of fungi may thrive. In short, gourmet mushrooms can be cultivated on a variety of substrates from across commodities and industries. The species and substrates described below, however, have not been explored in depth with rigorous experimentation.

Cyclical changes in prices in the forest products industry mean that there will be periodic availability of cheap substrate, especially sawmill waste products that might otherwise be diverted to the pulp and paper industry or employed for facility co-generation of electricity. The use of such material for producing marketable edible or medicinal fungi would offer the wood products industry another outlet for byproducts of processing.

**VII. Objectives:** The objectives of this project are to (1) investigate the cultivation of local mushrooms from regional wastes and (2) teach communities how to cultivate mushrooms**.**

**VIII. Research Methodology. Intended Outputs must be clearly stated as well as Outreach/ Extension/Stakeholder involvement activities:** Objective 1 is focused on research efforts while objective two focuses on outreach with stakeholders. Both require stakeholder involvement. For objective 1, we will work with stakeholders to secure consistent sources of organic wastes. Wood residues from mills, spent grain from breweries and spent coffee grounds from cafes and restaurants around Washington will be collected. Each waste source will be homogenized before being systematically mixed in different ratios with other waste sources, as described below. The resultant substrates will be sterilized and used for mushroom cultivation.

A total of five species of gourmet edible or medicinal mushrooms will be used for this project. Three of which, including our local lion’s mane, oyster, and reishi species (*Hericium abietis, Pleurotus pulmonarius,* and *Ganoderma oregonense*), will be collected locally in Washington. Two additional species, the wine-cap mushroom (*Stropharia rugosoannulata*) and shiitake (*Lentinula edodes*) will be purchased from retailers. All of these species are edible and possess medicinal properties (Arora 1986; Trudell and Ammarati, 2009).

Once mushrooms are obtained locally or through retailers they will be cultured and stored under standard laboratory conditions. Next, inoculum or “spawn” of each species will be generated by inoculating sterilized spent grain waste from breweries with cultures of each species. This inoculum will in turn be used to inoculate larger substrates on which each species will produce mushrooms (Stamets, 2000).

Homogenized waste substrates will next be mixed together into three ratios. Each ratio will constitute a treatment: (1) 100% wood, (2) 100% grain (3) 100% coffee, (4) 80% wood : 10% grain : 10% coffee (5) 80% wood : 15% grain : 5% coffee, (6) 80% wood : 5% grain : 15% coffee, and (7) a positive control.

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| --- | --- | --- | --- |
| Treatment | wood | grain | coffee |
| 1 | 100% | 0% | 0% |
| 2 | 0% | 100% | 0% |
| 3 | 0% | 0% | 100% |
| 4 | 80% | 10% | 10% |
| 5 | 80% | 15% | 5% |
| 6 | 80% | 5% | 15% |
| 7 |  |  |  |

A positive control, comprised of commercial cultivation kits for each species, will be included to capture differences in performance between treatments and industry standards. Each treatment will be replicated 5 times and the trial will be repeated once. All substrates will then be sterilized, packed into growth bags and inoculated with standard industry protocols. Inoculated substrates will be arranged in a randomized complete block design (RCBD) and incubated under controlled laboratory conditions. Laboratory conditions will be monitored for reproducibility and to be used as covariates in statistical models. Mushrooms will be harvested as they are produced.

After harvest data on mushroom biomass, substrate biomass, substrate colonization, and production costs will be collected. To detect and estimate differences in response variables as a function of the treatments and covariates, linear models will be used (Kutner et al. 2005). For example, mushroom sporocarp biomass will be modeled as a function of the treatment \* block \* environmental variables \* trial. If treatment effects are detected then post-hoc tests (e.g. Tukey’s honest significant difference) will be used to identify the sources of the differences. To estimate the size of the potential differences, effect sizes will also be estimated. Assumptions necessary for statistical inferences will be investigated visually and empirically with formal tests, including Bartlett’s test for homogeneity of variances and the Shapiro-Wilk test of normality for residual distributions (Zar 1999).

From the experiment described above, the team will identify the ratio of waste substrates that produce the most mushroom biomass for each species. Thus, the expected outcomes for this objective include (1) biomass of five gourmet mushrooms from three different substrates; (2) costs of productions; (3) step-by-step instructions for cultivation.

Results from this project will be published in a peer-reviewed journal and an extension bulletin for Washington State University. Moreover, results will be shared with and demonstrated for stakeholders for objective 2.

For objective 2, the expected outcomes from objective 1 will be extended to communities across Washington. For the proposed waste stream utilization model to function communities must contain receptive markets and stakeholders who produce and utilize waste products. Thus, communities with stakeholders and growing business sectors will be selected.

During year 1 of the project, the PIs and graduate student will establish collaborative working relations with stakeholders in each community (e.g. Pullman, Spokane, etc.) across Washington. Surveys will be issued to gauge localized interest in waste stream utilization with gourmet edible mushrooms. Plans to provide workshops on mushroom cultivation from waste streams will be coordinated with representative stakeholders from each community.

Workshops will be provided in selected communities across Washington state. In the spirit of the land grant mission we will provide cultivation demonstrations and share extension bulletins with participants. Surveys will be issued to participants several weeks after the workshop to gauge interest and adoption of gourmet mushroom cultivation.

Intended outputs for objective 2 include (1) extension of the results from objective 1 and demonstration of mushroom cultivation techniques to communities of stakeholders and (2) community level interest and adoption rates for gourmet mushroom cultivation from waste streams

**IX. A description of how these results will position the group to successfully obtain competitive extramural funding, including information about the intended source of those funds (agency, program, etc.):** The seed money from the project will enable reproducible cultivation of highly valuable gourmet edible and medicinal mushrooms from waste streams. Successful cultivation of highly desirable fungi in genera like *Hericium*, *Pleurotus, Ganoderma, Lentinula,* and *Stropharia* with the described methods will attract interest both from the standpoint of waste disposal as well as production of high-value food items.

From the waste disposal perspective, we will use the results generated from this project to pursue federal funds fromthe Environmental Research and Education Foundation (EREF), the Environmental Protection Association (EPA), and Department of Ecology. These funds will enable us to scale up waste management projects with fungi to a commercial scale.

From the high-value food item perspective, we will pursue funds from the Washington Specialty Crop Block Grant, Agriculture and Food Research Initiative (AFRI), and Sustainable Agriculture Research and Education (SARE) of the U.S. Department of Agriculture. These federal funding sources, especially the Agriculture Systems and Technology or Agriculture Economics and Rural Communities priority areas, would be an excellent programmatic fit for continuing and expanding this research.These funds will enable us to fine-tune mushroom cultivation methods (e.g substrate composition, inoculation methods, etc) with different substrates, species, and environments for the diverse array of stakeholders within Washington State.

**X. Description of the Team, along with individual roles and responsibilities:** PI- Dr. David Linnard Wheeler is an Assistant Professor in the Department of Plant Pathology at WSU. David’s expertise is in mycology, epidemiology of plant diseases, data science and statistical inference. For this project David will oversee and coordinate all research activities. For both objectives David and Mark will work with the graduate student and stakeholders to accomplish tasks in a reproducible and timely manner. Finally, David will be responsible for coordinating the publication of the resultant research and extension efforts that aim to share these findings with stakeholders.

Co-PI- Dr. Mark E. Swanson is a forest manager and ecologist with specific expertise in plant community composition and structure. He has worked extensively in forestry consulting contracts involving identification of pathogenic and beneficial fungi and assessment of forest health. He is also a mycophagist, gathering and consuming up to a dozen species of wild edible fungi each year, including from the genera *Hericium*, *Morchella*, *Cantharellus*, *Pleurotus*, *Coprinus*, and *Chlorophyllum*. He will work on sourcing sawmill waste for this research through contacts in the timber industry in northeastern Washington and north Idaho. Other areas of involvement in this research will include statistical analysis (including writing analytical scripts in the R language) and dissemination of findings via extension activity, conference presentations, and field days.

**XI. If applicable, graduate student participation in the project, including identification of the graduate degree program in which the student(s) are or will enroll:** This project is ideal for involvement by a M.S. level student due to tractable field and laboratory procedures, straightforward experimental design, and availability of qualified mentorship on all aspects of the research project. The student will be enrolled in the M.S. Plant Pathology program on the WSU Pullman campus, working with Dr. Wheeler.

**XII. Timeline:** Objective 1 will be completed in year 1 while objective 2 will be completed during year 2.

1. **Appended materials**

**I. ~~If applicable, a Progress Report (for Year-2 applicants – up to 4 pages) and Summary of Results from previous ERI-funded project(s) (1 page)~~**

**II. Reference citations from the Proposal Narrative**

1. Allen, Eric A., Morrison, Duncan J. and Wallis, Gordon W. 1996. Common tree diseases of British Columbia. Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre.
2. Arora, D., 1986. Mushrooms demystified. Berkeley, California: Ten Speed Press, 318 p.
3. Burns, Russell M. and Honkala, Barbara H. 1990. Silvics of North America. Volume 1- Conifers. and Volume 2- Hardwoods. Agriculture Handbook 654 (Washington).
4. Kutner, M.H., Nachtsheim, C.J., Neter, J. and Li, W., 2005. Applied linear statistical models (Vol. 5). New York: McGraw-Hill Irwin.
5. Pilz, David, and Molina, Randy (eds.) 1996. Managing forest ecosystems to conserve fungus diversity and sustain wild mushroom harvests. Gen. Tech. Rep. PNW-GTR-371. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 104.
6. Stamets, Paul. 2000. Growing gourmet and medicinal mushrooms. Ten Speed Press.
7. Trudell, S., and J. Ammirati. 2009. Mushrooms of the Pacific Northwest. A Timber Press Field Guide, Portland, Oregon. 352 p.
8. Zar, Jerrold. 1999. Biostatistical analysis (4th ed.). Prentice Hall, Upper Saddle River, New Jersey. 663 p.

**III. Up to a two‐page CV for each PI or co‐ PI**

**IV. One-page detailed budget including a description of any matching or leveraged dollars (if applicable). Do not include any F&A. All salaries and wages must be included but not benefits. Do not include graduate student tuition. Graduate Students should be paid at the Graduate Research Assistantship levels. *Salary and benefits for permanent faculty and staff cannot be paid from the requested funds*. Resource use efficiency should be clear in the Research Methodology and Approach, and in the Extension Plan.**

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| **Items** | **Year 1** | **Year 2** |
| **Salaries** |  |  |
| 1Graduate Student | 22,128 | 22,128 |
| 2Undergraduate Student | 5,000 |  |
| **Operating Expenses** |  |  |
| Lab Supplies | 12,872 | 4,000 |
| Growth Room | 2,000 |  |
| **Travel** | 5,000 |  |
| **Miscellaneous (extension bulletin,..,)** | 2,000 |  |
| **Extension and Outreach** | 1,000 | 3,872 |
| **Total** | **$50,000** | **$30,000** |

1 Salary for Graduate Student 0.5 FTE of 12 months

2 Salary for Undergraduate Student is $13/hour

**V. Completed Current & Pending Support Form**

**VI. Discussion of overlap of the proposal with currently funded research (250 words)**For PI Wheeler and Co-PI Swanson, there are no overlaps with any currently funded research effort.

**VII. WSU Assurance Statement**