2020 BIOAg Project Report

Report Type:
Final Report (no-cost extension requested through June 2021)

Title:
Evaluating Commercial Specialty Mushroom Production Feasibility for Diversified Farms and Small Woodland Owners in Western WA

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Abstract:
Forest-cultivated mushroom (FCM) production systems may be a yet-untapped, economical, low-impact, ecologically-appropriate enterprise for smaller-scale diversified farms and woodland owners in the western Pacific Northwest (PNW). This project represents the first Extension effort in the PNW on this subject as a commercial enterprise. The western PNW environment has 1) markedly milder winter temperatures, 2) more limited choices of native hardwoods, and 3) patterns of markedly drier, lower-humidity summers than regions where these systems are currently used for commercial production. We conducted two years of field research two distinct regions of western WA, where we evaluated 1) multiple species of locally available hardwoods for their potential to sustain mushroom production 2) strategies to sustain critical moisture levels needed to maintain production viability through low-humidity summers, and 3) indicators of potential for FCM systems to be used for commercial production. Mushrooms produced in wood-chip bed systems (*Stropharia*), those produced in "totem" systems with large wood rounds (and *Pleurotus* and *Hericium*), and shiitake (*Lentinula*) strains that are unresponsive forced-fruiting (induced by immersing logs in water for 24-hrs) all exhibited, poor, compromised potential for commercial production. Conversely, systems producing shiitake strains that respond well to forced-fruiting illustrated considerable commercial production potential due to sizeable, reliable, market-quality yields that were on par or greater than yields observed in the Eastern US. Log moisture content was not a reliable predictor of shiitake yield, but was a prominent factor associated with whether yields were delayed or completely absent. Substrate species was a standout driver of moisture retention and shiitake yield, with logs from PNW-sourced feral birch (*B. populifolia*), and native red alder (*A. rubra*) respectively producing the greatest total shiitake yields over 3 harvests in 2020 and 1 harvest in 2021. Feral sweet cherry (*P. avium*) has produced has reliably low to moderate shiitake yields that have increased over time, while native big leaf maple (A. *macrophyllum*) overall has produced poorly with absent, delayed, and highly variable yields. Logs of garry oak (*Q. garryana*) were added into a second 2020 sub-trial in Vancouver, but logs may not be completely colonized with mycelium yet, as yields were remarkable as of the first harvest. Wood density and bark integrity were observed to be apparent factors influencing log moisture retention. Birch and cherry had the greatest log densities and outstanding bark integrity, leading to excellent moisture retention. Alder exhibited moderate to low bark integrity, low wood density, leading to the highest magnitude of moisture loss, but an exceptionally high initial moisture content. Maple had poor bark integrity, moderate wood density, and unexceptional initial moisture content which led to poor retention of critical moisture levels during shiitake colonization in year 1. Oak logs had considerably higher density than birch but lower moisture retention, likely due to moderate bark integrity. Patterns across datasets and proximal evidence are indicating that early log moisture is likely important in assuring complete and rapid colonization of the log with shiitake mycelium, and it's resilience low levels of log moisture after colonization. Shiitake logs that were covered with white spun polyester fabric and soaked once for 24 hours in summer of the first year showed the most notable

potential to guard against excessive moisture loss, although this treatment does not appear to have a reliable, direct relationship with cumulative yields over time, but rather with early yielding. Project-related information has been disseminated to farm and forest owners, researchers and educators via numerous Extension websites, social media, educational events, newsletter articles, popular press and conferences. The project supported compilation of a substantial high-quality dataset suitable for 1-2 peer-reviewed journal publications, along with comprehensive imagery and video for use in Extension guides. It also supported ongoing trials that laid a foundation to substantiate needs for future research and development and has supported two additional applications for funding to date.

Project Description:

Forest cultivated mushroom production systems are common in Japan and China and use freshly-harvested hardwoods logs as a substrate to produce mushrooms at a commercial scale under existing forest canopy. Since the 1980s, Extension researchers in the eastern US (including the eastern-midwest) have refined several systems for diversified farmers and forest owners to produce forest-grown specialty mushrooms on hardwood log substrates. To date though, there is a marked absence of institutional, research-based knowledge about the viability of commercial, forest-grown specialty mushroom production in the western Pacific Northwest (PNW), despite a potentially favorable production climate, proximity to premium markets, and outstanding interest from PNW farm and forest owners. Foreseeable aspects potentially affecting these systems' viability in the PNW are 1) differing and relatively limited species of locally-sourceable hardwood substrates, 2) common dry spells during PNW summers that could compromise critical thresholds of log moisture needed for sustaining mushroom production vitality, and 3) potential effects resulting from milder winters (commonly wet, and with limited periods of freezing weather) and unknown effects of insect pests or competitive native fungal species.

Figure 1. *Trial site illustrating "bolt" systems for shiitake mushroom production (small-diameter logs horizontally stacked in a "crib stack" in the foreground) and totem systems for lion's mane and oyster mushroom production (large diameter rounds stacked vertically in background left). The figure shows a control treatment equipped with a weather data logger and two moisture-managed treatments under breathable, spun polyester fabric covers (background right) within a treatment block at the project's main trial site in Vancouver, WA.*

In light of this, the aim of this project is to:

1) Establish baseline, research-informed estimations of the viability of adapting current forest-grown commercial mushroom production systems to western PNW environments;
2) Investigate economically feasible, regionally appropriate management practices for commercial mushroom operation development in the region;
3) Develop foundational, research-based information for stakeholders and researchers to use in future decisions about the potential for commercial forest-grown mushroom enterprise development in the western PNW;
4) Increase awareness of forest-grown mushrooms as a commercial enterprise, potential pitfalls, and current knowledge gaps for forest owners and diversified farms.

To begin to address these objectives, the project included research trials designed to produce foundational information regarding the viability of current eastern-US-developed commercial mushroom production systems in PNW environments. The trials aim to clarify 1) best practices for maximizing production regarding substrate choice, 2) substrate moisture management, and the 3) suitability of the most commonly cultivated forest-grown mushroom species for production in the western PNW. Two distinct western PNW regions were targeted for replicated trial locations with one location serving as the main trial site and the other serving as a satellite site with truncated trials. The trials chiefly focus on “bolt” production systems for producing shiitake (*Lentinula*) mushrooms at all locations (see Figure 1). At the main site location, additional evaluations of 1) “Totem” systems for producing lion’s mane (*Hericium*) and oyster (*Pleurotus*) mushrooms and 2) wood-chip bed production systems for producing wine cap (*Stropharia*) mushrooms are being conducted. The two most common native PNW hardwood species, Red alder (*Alnus rubra*) and bigleaf maple (*Acer macrophyllum*) are the primary mushroom log substrates being evaluated at all sites. Additional evaluations of non-native wild sweet cherry (*Prunus avium*), and gray birch (*Betula populifolia*) substrates are being conducted at the main trial location (see Appendix 1). Cherry was included because it is commonly found as a feral species growing throughout historic farming regions. Birch was chosen because it is a fast-growing, escaped ornamental found growing feral throughout urban and suburban areas, and because the closely related paper birch (*B. papyrifera*) species is a native to northwestern WA, eastern slopes of the Cascades and western slopes of the northern Rockies. Moisture management treatments focused on three methods of modifying humidity and evaporative potential to mitigate potential log moisture loss throughout the summer (see Appendix 1) with a combination of using breathable, spun polyester fabric covers in summer with passive water diffusion or active sprinkler irrigation under these covers, or immersive soaking of freshly inoculated logs followed by covering. In 2020 additional sub-evaluation including garry oak (*Quercus garryana)* and logs harvested before and after winter dormancy were established at the main trial site. Garry oak chosen for evaluation because of its availability as a native species found growing throughout the Willamette Valley, Puget Sound lowlands, and western/central Columbia Gorge regions, and because of traditional observations that oak logs are most the most naturally suitable substrates for shiitake production. Substrate harvest timing was chosen for evaluation because it can affect bark retention, which in-turn, can affect the log’s ability to support mushroom production in the long-term.

Because this is a nascent research area, a peer-reviewed journal publication was targeted as a core project output. Extension outreach products were targeted to be web-based for purposes of conducting ongoing edits/updates as research progress is made, but enough information may likely be available by the end of 2021 to produce an Extension manual. The project team produced multiple workshops on commercial mushroom production, our preliminary trial results, and potential growth of future specialty mushroom markets. Trial sites also dually served as demonstration sites. Multiple presentations at conferences, invited talks to stakeholder groups, and press were targeted as a project output.

Outputs:

Overview of Work Completed and in Progress:

- Two replicated research trials established in 2019 in Vancouver and the south Puget Sound continued throughout 2020 and into 2021(See Figure 1, Table 1, and Appendix 1). Circumstances and emerging perspectives on the commercial production potential of these systems prompted a near complete shift in focus on systems producing certain types of shiitake mushrooms as the trial evolved into its second and third seasons.
- The shift to focus on shiitake included establishing an additional replicated sub-trial at the Vancouver site in 2020 to augment knowledge gaps that arose in 2019. These sub-trials included 1) an evaluation of garry oak as a PNW native substrate for shiitake production, and 2) the effect of substrate harvest timing (cutting logs during vs. after winter dormancy) on production (see Table 2 and Appendix 1). This additional sub-trial allows for an evaluation of garry oak against select species we included in 2019’s trials. Our 2019 trials were also cut later than planned due to logistical circumstances (just as winter dormancy was threatening to break) and this additional sub-trial will allow us to evaluate whether log substrates cut during winter dormancy truly have a longer productive lifespan than those cut afterwards, or whether there are differential effects between species. The sub-trials were completely established by midsummer 2020.
- Shiitake “logs” inoculated in 2019 largely began producing mushrooms in 2020 (see Figure 2) and allowed for the first full year of yield data to be taken, along with the first harvest of 2021. Three there were major shiitake harvests of throughout spring, summer and fall 2020 that were dominated

by a single “wide range” strain of shiitake (oriented to producing within a wide range of ambient temperatures) that was common to all trial sites. Yield data from a second wide-range strain and a warm weather-oriented strain were collected simultaneously from the south Puget Sound trials. Yield data collection on a cool weather-oriented strain also began as intermittent production commenced in late fall and into the winter season at both trial locations.

- Oyster mushrooms began intermittently producing a limited amount of mushrooms in the totem systems in 2020, which allowed for collection of yield data from late fall into winter and spring 2021 (see Appendix 1). Logs inoculated with lion’s mane spawn did not produce any mushrooms in 2020 and precluded the ability to evaluate yield for this species.
- Wine cap mushroom (aka. *Stropharia*) wood-chip bed trials at the main trial site that were not established in 2019 due to capacity constraints, were instead established by late spring of 2020 (see Table 3 and Appendix 1). The wood chip bed systems included all four substrate species included in the 2019 shiitake and oyster/lion’s mane trials, and drip-irrigated vs. non-irrigated sub-treatments. Stropharia began producing limited sporadic yields in fall of 2020 into early summer 2021.
- Second measurements of log moisture content originally planned for Fall 2019 were precluded by capacity constraints and were instead completed in spring of 2020 for the shiitake bolt system. The second moisture measurement on logs in totem systems was abandoned primarily due to risk of damage to the totem structures. In the 2020 Vancouver sub trials, initial moisture log measurements were taken after bolt cutting in spring 2020, and second measurements were taken in late fall 2020.
- Educational and outreach outputs included seven educational events (see Figure 3), and three newsletter articles. Instructional videos are in the editing process, and data analysis has begun for preparing publications for submittal in 2021. Additional Extension events, and how-to videos will be produced after the project end date.
- Two proposals to expand the current project were submitted during the project period; a smaller scope proposal was denied funding in early 2021 ($30K), while a much larger, more comprehensive multi-year proposal submitted in May 2021 is still pending as of 2020 ($175K).

Methods, Results, and Discussion:

Methods:
Two replicated research trials were established in 2019 in two differing western PNW ecosystem regions- 1) the greater Willamette Valley (at the main trial location in Vancouver) and 2) the South Puget Sound (the satellite location, in using two sites in Lacey and McCleary). The experimental design used at each trial site is a spatially-balanced complete block design with split-plots and four replications (see Table 1). At the main trial site, each replication contains a shiitake bolt production system and lion’s mane & oyster totem production system, with 1) four moisture management treatments, 2) split-plots with four substrate species, and 3) split-split-plots with two different shiitake strains in the shiitake bolt system (see Table 1). The satellite sites each contain two of four total replications of an abbreviated trial containing only shiitake bolt systems, two moisture management treatments, split-plots of two species of substrates and split-split-plots with three different strains of shiitake (two wide range, one cold weather, and one warm weather strain). All replications were placed in shaded, protected locations prioritizing dominant evergreen canopy, and/or north facing aspects, and were also individually sited to capture a stratified range of microclimates that may be encountered in the western PNW. Two treatment replications of the total four at each trial location were placed in two distinctly different locations where relatively dry, windy conditions could be captured along with relatively humid, stagnant microclimate conditions.

Table 1*. Trial treatment layout established at each research site in 2019.*

Trial Location *(Site)*	**Replications** *per site*	**Mushroom Species** *(System)*	**Sample Units** *per replication*	**Main Treatment Plot** *Moisture management*	**Sample Units** *per treatment*	**Split-Plot** *Substrate*	**Sample Units** *per substrate*	**Split-Split Plot** *Strain*	**Sample Units** *per strain*
Main (Vancouver)	4	Shiitake (Bolt)	64	Control (unmanaged moisture)	16	Red alder	4	Wide-range shiitake strain	2
				Covered + passive irrigation		Bigleaf Maple			
				Covered + active irrigation		Wild Cherry		Cool-weather shiitake strain	2
				24-hr immersive soak > covered*		Paper Birch			
		Lion's Mane (Totem)**	16	Control (unmanaged moisture)	4	Red alder	1	NA	NA
				Covered + passive irrigation		Bigleaf Maple			
				Covered + active irrigation		Wild Cherry			
				24-hr immersive soak > covered*		Paper Birch			
		Oyster (Totem)**	16	Control (unmanaged moisture)	4	Red alder	1	NA	NA
				Covered + passive irrigation		Bigleaf Maple			
				Covered + active irrigation		Wild Cherry			
				24-hr immersive soak > covered*		Paper Birch			
Satellite (Lacey & McCleary)	4 (2 per site)	Shiitake (Bolt)	48	Control (unmanaged moisture)	24	Red Alder	12	Wide-range shiitake strain 1	3
								Wide-range shiitake strain 2	
				Tarped + passive irrigation		Bigleaf maple		Warm weather shiitake strain	
								Cool weather shiitake strain	

**Triple layer of 85% light transmission breathable white spun polyester cover (aka "floating row cover", "Reemay®"*

*** Production systems for lion's mane and oyster mushrooms are identical but are not intended to be compared to each other in statistical analyses.*

Moisture management treatments at all sites include 1) un-managed controls (i.e. no moisture management) vs. 2) covered + "passive irrigation" treatments that use static water containers under breathable, spun-polyester fabric covers to modify relative humidity. Two additional moisture management treatments are being trialed at the main trial site, including 1) a covered + "active irrigation" treatment using mist emitters on irrigation timers, and 2) a treatment where substrates were soaked for 24 hrs. post-inoculation, and then placed under covers (see Appendix 1). A weather station was installed on each control treatment to capture baseline temperature, relative humidity, windspeed, and light intensity conditions within a given trial block. Temperature and humidity loggers were installed under. Core response variables being evaluated are 1) temperature and relative humidity in controls and within each moisture management treatment, 2) log substrate moisture changes over time, and 3) total mushroom yield over time. Observations of factors potentially affecting the system's prospects to support a viable commercial mushroom production enterprise are additionally being documented to inform project results. Response variables will primarily be used to estimate effects of substrate species and moisture management treatments on mushroom yield, and to produce estimates of yield dynamics that can be used to inform enterprise budgets.

Trees used for substrates at the main Vancouver Site in 2019 were cut by the end of March. Shiitake logs were inoculated beginning in late April, and finished by June, and placed in the final trial site and under the influence of moisture management treatments by July. Totem systems were inoculated and placed under the influence of treatments by the beginning of September. Trees used for the Lacey and McLeary satellite trial sites were cut by mid-April, inoculated by mid-May, and placed in each trial site and under the influence of moisture management treatments by June. Log moisture content measurements were taken when logs were initially cut, one year later in late spring 2020, and again in fall 2020 following the summer season. Moisture measurements were determined from a 3”-deep log round cut 3” in from the end of the log; cut rounds were then weighed immediately, and then again after ~1 week of forced air drying at 220° F to estimate log moisture content. Harvests of wide-range and warm-weather shiitake strains began at both sites began in June 2020 following a 24-hr immersive soaking of the logs in water to initiate a flush of fruiting (termed “forced-fruiting”). This harvest process was repeated two more times at an interval of ~7-8 weeks, with the last harvest of 2020 occurring in October, and the first harvest of 2021 occurring in June. Logs inoculated with cool-weather shiitake strains were soaked in October after the third log moisture content measurements had been taken in an effort to try and initiate fruiting and assure that sufficient log moisture was maintained; small harvests of sporadic production began the same month and have continued steadily into winter 2020-2021 every two weeks. Fresh mushroom yield weight and mushroom quantity of was recorded for each log at each harvest timing. All mushrooms in force-fruited treatments were harvested when the majority of mushrooms were at a market-mature stage. Yields focused on single day harvests, although immature mushrooms (closed gills) were occasionally harvested ~1-2 days later, as needed and/or to develop an estimate of single-day harvest yield vs. full yield potential.

Establishment of the 2020 sub-trials in Vancouver followed the same general protocols and experimental design as those used in the 2019 trials but with modifications to accommodate a substrate harvest timing comparison (see Table 2). Trees used for early-cut logs treatments were harvested in February into the first week of March, and trees for late-cut treatments were downed approximately month later (See Appendix 1). Shiitake inoculation began approximately one month after cutting and was completed by late May. Logs were stored in shaded location until they were set into crib stacks in July 2020 adjacent to the crib stacks within each replication of the 2019 trials. Only one moisture management treatment was applied to the 2020 trials, with one of two crib stacks per replication being immersed for 24-hrs before being placed under a triple-layer of the spun polyester fabric covers along with water buckets (passive irrigation). This moisture management treatment was chosen based on promising preliminary observations from the 2019 trials, and because of its technical simplicity. Initial log moisture content measurements were taken at log cutting (Mar/Apr), second measurements were taken after the summer season in October 2020 before winter rains began, and third measurements were taken one year after log cutting (corresponding with the initial-to-second measurement interval 2019 trial), in May 2021.

Table 2. *Additional shiitake sub-trial treatment layout established in Vancouver in 2020.*

Trial Location (Site)	Replications (Per site)	Mushroom Species (System)	Sample Units (Per replication)	Main Treatment Plot (Moisture management)	Sample Units (Per treatment)	Split-Plot (Substrate)	Sample Units (Per substrate)	Split-Split Plot (Strain)	Sample Units (Per strain)
Vancouver	4	Shiitake (Bolt)	16	Control (unmanaged moisture)	8	Red alder	2	Early-cut (during winter dormancy)	1
						Bigleaf Maple			
				24-hr immersive soak > covered + passive irrigation**		Oregon oak***		Late-cut (after spring bud break)	1
						Paper Birch			
		Wine cap (Wood-chip bed)	8	Control (unmanaged moisture)	4	Red alder	2	NA	NA
						Bigleaf Maple			
				Drip-irrigated		Wild Cherry		NA	NA
						Paper Birch			

**All shiitake bolts were inoculated with a single wide-range strain ("West Wind") for consistency with the 2019 trials.*
***Shiitake bolts were placed in crib stacks in July 2020 within several hours after the 24-hr soaking period and covered with a triple layer of 85% light transmission spun polyester cover (aka "floating row cover", "Reemay®") until late October 2020. Water filled buckets were placed underneath the spun polyester covers adjacent to crib stacks as a static source of humidity.*
****Wild cherry (Prunus avinus) substrates (previously included in the 2019 trials) were eliminated from the 2020 sub-trials due to supply and labor capacity constraints. Oregon oak used in the sub-trial was sourced from a privately owned oak restoration planting in western OR due to its protected status in WA.*

Leftover log substrates of red alder, bigleaf maple, wild sweet cherry and birch from the 2019 trials were chipped and used for the wine cap mushroom bed trials. The trial used a spatially-balanced complete block design with split-plots and four replications that includes a 1) two beds of each of the aforementioned substrate species and 2) drip-irrigation treatment in one of each of the two beds (see Table 3). All replications were sited under deciduous forest canopy, with each replication having distinct combinations of microclimate, degrees of shade, and dominant overstory species. Individual beds were 16 ft^2 with a 4"-deep layer of wood chips and ~2 lbs of sawdust spawn added to each bed. Individual beds were separated by 1' wide strips of landscape fabric. All trial beds were established by July 2020. Due to dry conditions immediately following establishment, all beds were soaked with ~25 gallons of water per bed in early August to assure that the wine cap spawn did not become non-viable. The drip-irrigated beds were initially watered for 4 hours every 4 days, but the frequency was increased to 4 hours every other day in August in accordance with observations of moisture retention in the bed as summer weather became drier. All bed irrigation was turned off in mid-October and restarted in July 2021.

***Table 3**. Wine cap mushroom trial treatment layout established in Vancouver in 2020.*

Trial Location ***(Site)***	**Replications** ***per site***	**Mushroom Species** ***(System)***	**Sample Units** ***per replication***	**Main Treatment Plot** ***Substrate***	**Sample Units** ***per substrate***	**Split-Plot** ***Moisture Management***	**Sample Units** ***per moisture management type***
Vancouver	4	Wine-cap (Wood-chip bed)	8	Red alder	2	Control (non-irrigated)	4
				Bigleaf maple			
				Wild cherry		Drip-irrigated	
				Paper birch			

All data analyses conducted to date were performed with JMP Pro 15 (SAS Institute). Logs included across treatments were randomized with respect to logs form the same tree, harvest date, harvest site, inoculation date etc. Potential correlations between samples within the log population regarding factors such as this were vetted before analyses; none were found. Individual logs were therefore considered independent observations and intentional correlation structures within the experimental treatment design (duplicate logs of the same species) were defined within the analysis. Non-categorical analyses exploring generalized associations and correlations between numerical variables were conducted across all logs. In all comparisons of categorical fixed-effect treatments applied to groups of logs within the split-plot experimental design (moisture management and substrate species), treatment replication was defined as a random effect within a mixed-effects model. Any statistics presented in this report are currently preliminary and subject to future refinement. Analyses presented in this report focused on systems and mushroom strains that were observed to have potential for commercial production contexts.

Figure 2. *Shiitake production in trials throughout 2020. Birch substrates have dominated yields to date, followed by red alder yields (top left and center). It was not uncommon to observe heavy yields (top left and center) that were followed by modest yields (bottom left) and vice versa. Wide-range shiitake strains produced throughout summer 2020 would commonly last up to a month in refrigerated storage (top right). Cool-weather shiitake strains have thus far produced low, variable, sporadic yields mostly produced from birch substrates (bottom right), which excelled at maintaining log moisture.*

Results and discussion (see Appendix 2):

<u>Yields of force-fruited shiitake:</u> Of the shiitake strains that could be force-fruited, the wide-range shiitake strain,'West wind', was common to both the Vancouver and South Sound trials, and performed well within both trials. A second wide range strain in the south Puget Sound trials performed negligibly, but the warm weather shiitake strain 'Night velvet' produced yields that were comparable with 'West wind'. Evidence from the first four harvests in the 2019 Vancouver trials indicated substantially greater evidence that substrate species is affecting mushroom yield ($p<0.01$) more than our moisture management treatments ($p=0.31$ in Vancouver, $p=0.09$ in south Puget Sound). Birch substrates are illustrating strong yield potential, followed alder whose cumulative yields were only ~18% less than birch after the 2020 harvest season, and the first harvest of 2020. Birch logs overall showed early indications of a strong spawn run and exhibited early fruiting in both the 2019 trials and likewise again in the 2020 sub-trials. By the second harvest of the 2019 trials there were no birch logs left that had not yet yielded a mushroom. This was not the case for any other substrate species, although only 5% of alder and cherry logs were still inactive by the end of the 2020 harvest year. Cherry has been a relatively consistent producer of quality mushrooms with low initial yields that have slowly increased to moderate yields over time; it produced on par with all other substrates at the fourth harvest, but cumulative yields are still 57% less than birch to date. Maple has generally been a poor shiitake producer to date at all locations with absent, delayed, and/or variable yields. A small number of maple logs began to produce large flushes of quality mushrooms by the third and fourth harvests, with the fourth harvest being on par with the other strains for the first time, but with cumulative yields that trail birch by 83%. The 'West wind' strain and 'Night velvet' strain on alder also overtly out-yielded maple in the south Puget Sound satellite trials to date, by a

factor of nearly 8x. The first harvest of the 2020 sub-trials in Vancouver followed similar patterns as the 2019 trials, with birch yielding very well, followed by alder; oak has not produced a notable yield as of yet. There is no apparent effect of early harvested vs. late harvest logs as of yet in the 2020 sub-trials.

General expectations for first-year yields of shiitake in the eastern US are 0.25 lbs./log/harvest and are expected to increase to 0.50 lbs/log in the second year. Yields of shiitake on birch and alder logs in the Vancouver respectively produced an approximate equivalent of 0.57 and 0.34 lbs./log/harvest in the first year of production. First year shiitake yields in the South Puget sound trials on alder were comparatively low, with yields 70% lower than yields on alder in Vancouver. While the Vancouver yields were promising for a commercial production context, it is unclear why the south Puget Sound trials' shiitake yield was much lower than in Vancouver. Several factors that may be possible are: 1) logs were harvested slightly sooner in Vancouver (but inoculated slightly later), 2) slightly higher, more uniform inoculation rates were used in Vancouver, 3) minor differences in pre-season log treatment that could have reduced moisture retention, (including the spun polyester covers being added slightly later than in Vancouver), and 4) that fruiting blankets were only used in Vancouver.

Log moisture retention: A straightforward relationship between log moisture content and shiitake yield appears to be complicated by 1) substrate-specific effects and 2) dynamic relationships between spawn run timing and environmental factors affecting moisture flux from the log. Similar to the yield data, substrate species illustrated greater evidence of an effect on moisture retention than moisture management treatments in the 2019 Vancouver trial data, although both had an effect ($p < 0.01$). In Vancouver wood density was found to be a factor associated with moisture retention (R^2=0.42 in the 2019 trials, and R^2=0.35 in the 2020 sub-trials), and although we could not quantitatively assess bark integrity, this also appeared to be an important factor affecting moisture retention. Birch logs illustrated the most substantial evidence of an ability retain moisture, followed by cherry. These two species had the greatest average wood density in the 2019 Vancouver trials, and bark integrity with robust tensile strength that made it resistant to peeling, cracking, and effectively sheathed the log to increase the wood's resistance to end-splitting. Birch bark also appeared to have a having a notably low porosity which likely inhibits moisture flux. Moisture content change in birch was minimal (2-5%), and it would commonly yield mushrooms earliest and most reliably, even in treatments without moisture mitigation and conditions with high evaporative potential. Oak in the 2020 Vancouver sub-trials had the greatest wood density, but did not retain moisture as well as birch, presumably because its bark is more porous, prone to cracking, and the log more prone to end-splitting. Alder bark was observed to exhibit decent sheathing, but overall was more brittle and porous with low tensile strength compared to birch and cherry. In Vancouver, alder logs had the lost the lowest wood density and greatest magnitude of moisture content in the first year, but with the notable caveat that they also contained the highest average initial moisture content of all substrate species at both sites (46% in Vancouver and 48% in south Puget Sound). Maple bark was observed to be porous, prone to cracking, peeling, and damage from animals. Maple wood density was moderately low, with an average moisture content of 24% after one year in both the South Sound and 2019 Vancouver trials. Maple also constituted the vast majority of non-producing logs at both treatment sites, and in the 2020 sub-trials in Vancouver logs faced a summer with higher evaporative potential than in 2019 trials, maple logs averaged 22% moisture content after the first summer, and only recovered to 26% after the first winter.

Non-producing logs as a whole tended to have 1) lower average moisture content after one year with a majority having ≤ 24% moisture content, and/or 2) relatively large magnitudes of log moisture loss in that first year. Logs in the south Puget Sound trials overall had a notably greater proportion of logs overall that remained non-producing after the 2020 season than alder and maple logs in Vancouver (58% vs. 32% inactive, respectively). It is notable that despite alder's relatively sharp log moisture decline, it did not appear to preclude alder logs from producing some commendable yields of shiitake in a number of cases at both sites where log moisture content had measured at 24% or less. This proximally suggests that alder's high initial moisture content during the spawn run year may have been an important factor in overall production viability vs. whether log moisture drops below a given "critical" level later on. This observation is supported by maple's similar preponderance to lose log moisture, but compared to alder, maple's lower initial moisture content appeared to predispose it to more readily drop to ≤ 24%, and either delay or preclude a successful colonization of the log and consequent yields.

Compared to controls, relative humidity averaged across the entire two years in moisture-managed treatments in both trials was ~4.4% greater in Vancouver and ~3.7% greater in south Puget Sound, but log moisture measurements from the 2019 trials did not provide clear evidence of an anticipated positive effect on log moisture content. Logs in the 2019 moisture-managed treatments did not maintain greater moisture content than controls, except the soaked + covered treatment In Vancouver and the passive irrigation + covered treatment the south Puget Sound, both of which did not differ from the control. This unanticipated result was likely caused by keeping covers on logs throughout winter 2019-2020, as the fabric was observed to act as an inhibiting barrier to moisture replenishment from winter soaking rains, even if high humidity is maintained under the cover. Covers were subsequently removed for winter in the 2020 sub-trials in Vancouver. This appeared to be effective, as logs in treatments that were soaked and covered throughout summer/fall in the sub-trials had 3% greater moisture content than controls in measurements taken in fall of the first year (6-7 months after logs were harvested, following summer), and likewise maintained a 3.3% moisture content advantage in measurements taken after winter in Vancouver. Moisture measurements from the fall after the first harvest season of the 2019 trials indicated that once periodic soaks for forced-fruiting begins, log moisture is easily replenished to sufficient levels throughout summer.

Despite relatively insufficient individualized evidence of an effect of moisture-managed treatments on shiitake yield, moisture management may have helped safeguard and/or accelerate spawn run. Data trend patterns across datasets and proximal evidence is suggestive that that early moisture during spawn run is an important factor, and that soaked + covered treatments in both the 2019 and 2020 trials in Vancouver exhibited earlier production than in control treatments. In the 2019 trials, pre-season yields were dominated by logs in soaked treatments, and a greater proportion of logs in both the 2019 and 2020 trials soaked treatments were inactive at the first harvest (26% and 54% inactive, respectively) and in contrast to logs in the control treatments (42% and 66% inactive, respectively). This occurred in 2019 despite there being insignificant differences in the average moisture content of logs in each of those treatments measured just before the first harvest. Logs in control treatments maintained a relatively high proportion of inactive logs until the third harvest in 2020, and then returned to a relatively high proportion of inactive logs for the fourth harvest of 2021, even though evidence of a difference in mushroom yield between moisture treatments was low (both for individual harvests and cumulative yield). Data trends are also indicating that overall shiitake production in treatment replications sited in microclimates with higher evaporative potential (hilltop replications in Vancouver and Lacey replications in south Puget Sound) appeared to have a yield lag and/or, lower overall production, and higher incidence of non-producing logs compared to production in locations that were inherently more protected from winds and more consistently humid. These patterns suggest that spawn run may be 1) slowed or accelerated according to log moisture content, and/or 2) that shiitake mycelium may drop into a dormant state when log moisture content drops but is capable of being re-invigorated when log moisture is replenished.

<u>Observations regarding commercial viability:</u> Pest control and mushroom desiccation during dry summer weather were both major concerns regarding quality control and marketability before harvests began. Bolt systems and the ability to force-fruit certain strains of shiitake were observed to have several game-changing advantages for commercial production. In addition to the advantages of log moisture replenishment from soaking logs forced fruiting, the soaking also purges insects from the logs. In addition, we were able to successfully modify the concept of a "fruiting blanket" (spun polyester covers normally used as a moisture mitigator during fruiting) approach to simultaneously control pests during fruiting. After soaking, we used a large piece of spun polyester to completely enclose all of the logs during the fruiting sequence. The complete enclosure helped reduce evaporative potential during fruiting

(which was more critical in drier locations) and was very effective at excluding pests; limited numbers of sow bugs and Portuguese millipedes were the only pests to occasionally would find their way in through small openings. In 2021 we additionally began soaking the fruiting blanket along with the logs to purge insects from the fabric and add a small amount of ambient humidity to the fruiting logs. This method eliminated pests to negligible levels. The majority of mushrooms produced in this manner had marketable quality, and the approach would be easily adapted into a commercial production system. Cool-weather strains were not easily adapted to this method. Unwrapping and re-wrapping the logs is very labor-inefficient for the sporadic low yields that this strain has produced, and the method was consequently abandoned in December 2020. Overall, difficulty controlling pests, very low yields (mostly limited to birch in Vancouver), spontaneous fruiting patterns, non-responsiveness to forced fruiting, propensity to lose log moisture without summer soakings, and frequent winter rain during cool-weather strain fruiting are all formidable factors compromising their viability within a commercial production context.

Totem systems allow larger diameter logs to be made use of and are relatively simple to inoculate compared to bolt systems, but overall face all of the aforementioned compromising factors associated with cool-weather shiitake strains. Totems at the Vancouver site began producing oyster mushrooms in fall of 2020 and continued through spring of 2021. Lion's mane has not fruited to date; this species is known to have a longer spawn run than other mushrooms but will be considered to be non-viable if it has not produced by fall 2021. Oyster mushroom production patterns have been overtly variable and sporadic with no observed standout effect from any one treatment (See Appendix 1) or substrate. A notable observation from the totem system in 2020 was pervasive colonization of birch logs by a feral polypore fungus, although a limited yield of oyster mushrooms nonetheless fruited on birch totems in 2020. Another notable observation that may have some limited application oyster mushroom fruiting on totems closest to misters in the active irrigation treatments. Totems have the distinct disadvantage of not being able to be immersed in water for moisture content recharge. A limited number of bolts were inoculated with oyster and lion's mane in 2020 to observe whether there is any potential for them to be alternatively produced with a bolt system.

Wine cap mushrooms began producing very sporadically in late summer of 2020 (See Appendix 1) into late spring. Although the rate of spawn run for wine cap mushrooms offers a rapid return on investment, its commercial viability is substantially compromised by the ability to manage the quality of mushrooms produced. None to date have been marketable quality. Wine cap fruitings occur very quickly (≤2 d) and appear to decline at a likewise rate while also simultaneously succumbing to a myriad of pests. Pest control is formidable due to the mushroom bed's location on the forest floor, where various insects and slugs have ready access to them. The lack of foreseeable market quality control options for these systems is a major concern for applications in a commercial production context.

Publications, Handouts, Other Text & Web Products:
The project was disseminated through articles included in the Association for Temperate Agroforestry's *Temperate Agroforestry* summer newsletter, WSU Extension's *Forest Stewardship Notes* June newsletter, and the Society of American Foresters *The Forestry Source* December newsletter. Formalized Extension guides on these production systems is still somewhat premature at this point in time, but findings to-date will allow for preliminary, basic guides to be produced. Additional photo and video footage was taken 2020, anticipating the need for material in distanced outreach and education during the COVID-19 pandemic, and is currently being edited for posting as an instructional video on YouTube. Datasets produced by this project will be used to produce a manuscript to be submitted for publication to a peer reviewed journal in 2021.

Outreach & Education Activities:
Project information was shared with a minimum of ~330 stakeholders from at least 19 states, Canada, and Portugal, and Mexico throughout 2020 and 2021 via three in-person workshops, two online educational events (see Figure 3), the regional PNW Agroforestry Working Group's annual regional Workshop, and

the North American Agroforestry Conference. Hands-on workshop plans were stymied in 2020 due to COVID-19; instructional video footage was instead created as an alternative approach and used during the two online workshops and presentations in 2020 and 2021. Annotated project photo and video footage was also posted to the WSU Extension SW WA Ag Program Instagram feed which garnered 95 image likes and 140 video views related to the project. A project-culminating workshop is being planned for Winter 2021, along with formulation of an online course using video media collected throughout the project.

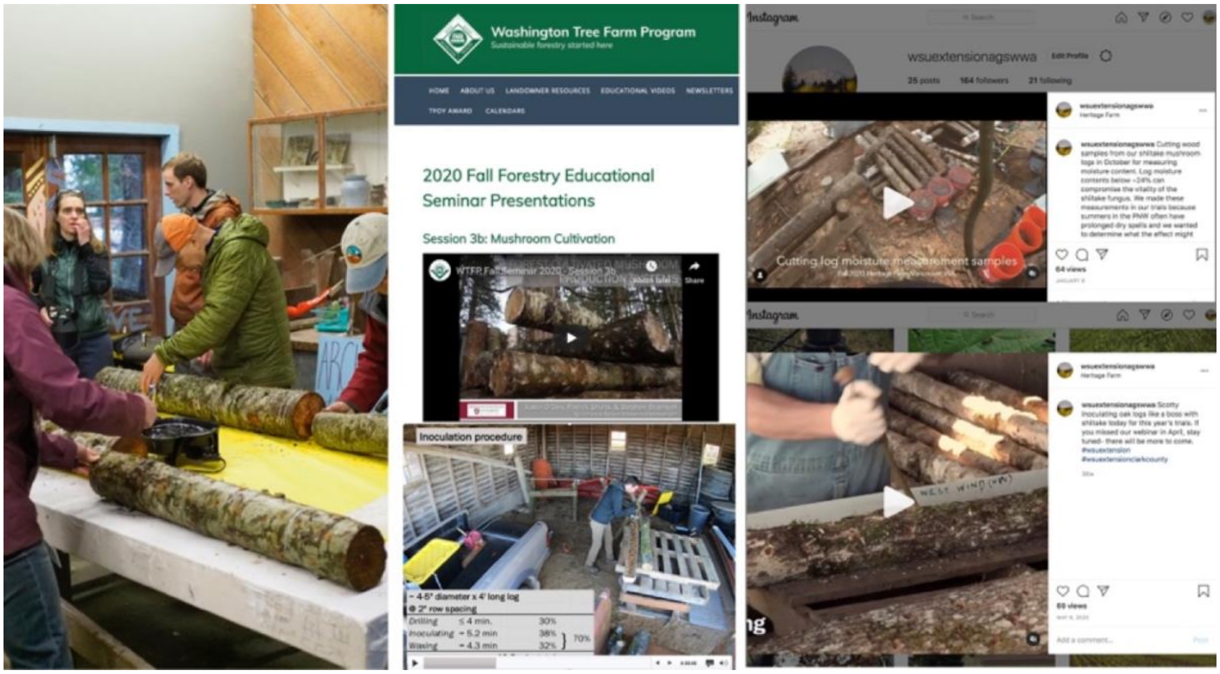

Figure 3. *Various outreach and education outputs from 2020, including a hands-on workshop online workshops featuring enhanced use of video, along with social media posts utilizing video for socially distanced outreach and education.*

Impacts:

Short-Term: A post-program survey from one of our 2020 online events with 120 participants indicated that 62% of respondents reported an intent to implement new management practices based on knowledge gained, 20% of respondents were considering starting a commercial operation after the workshop, 90% indicated more demand for future Extension programming on this subject.

Intermediate-Term: A follow up survey to the aforementioned online event indicated that 9 of 19 respondents had begun producing forest-cultivated mushrooms at home, and that 2 of these individuals were seriously considering growing them for commercial sale. All of these respondents indicated that either most (7) or some (2) of the information guiding their setup was from the information presented at the event. Two other respondents reported using the information when consulting with landowners. One farmer in Clark County, WA likewise independently reported that they set up a forest-cultivated mushroom system after attending one of our workshops.

Long-Term: Long-term impacts have yet to be noted.

Additional funding applied for/secured:

Project-related funding advancements made in 2020 included 1) inclusion as Co-PD in a USDA-SCRI Planning Grant-funded project focused on developing a commercial specialty mushroom growers network in the western US, and a proposal for a one-year, $25K project to continue and expand the project was applied for but not awarded. A pre-proposal for a three-year $175K project to substantially expand and further this work was applied for in Spring 2021; funding decisions are still TBD.

Graduate students funded:

No graduate students were funded by this project, but it alternatively provided an internship opportunity throughout Summer 2020 for a post-graduate visiting scholar from Kazakhstan studying agroforestry. She reported that she has incorporated the knowledge from that experience into her current work as a freelance environmental consultant in Kazakhstan.

Recommendations for future research:

1) Trialing additional native PNW hardwood substrates – especially vine maple, hazelnut, and native paper birch. With informed management, birch may even be able to produce shiitake on the eastern slopes of the Cascades, and in the western slopes of the Northern Rockies. Oregon ash and pacific dogwood may be considered also.
2) Trials examining existing mushroom strains best suited to the PNW climate, and whether well-suited strains can lengthen the mushroom growing season.
3) Development of novel strains of shiitake adapted to the PNW.
4) Further study of the effects substrate harvest timing on mushroom production longevity.
5) Further exploration of moisture management approaches.
6) Cost/benefit analysis of shiitake inoculation rate as it relates to labor costs vs magnitude of effect on yield.
7) Identifying feral fungal species commonly found on a given substrate species, their degree of competition with the species of mushroom being cultivated, and control methods for any species shown to significantly compromise production.
8) Identifying insect and animal pests of these production systems, and control methods for any species shown to significantly compromise production.
9) Exploration of other specialty mushroom species with potential for commercial forest-cultivated production, especially if they can be force-fruited and produced in a bolt system.
10) Exploration of value-added products and economic assessments of forest cultivated mushroom enterprises within diversified farm operations.
11) Networking with Japanese researchers and producers to connect with contemporary advancements in forest-cultivated mushroom production systems.
12) On-farm mentorship and trialing to facilitate adoption and grower feedback.

Appendix 1:

Two different treatment replications established at the main trial site in Vancouver, WA. Each trial location had two of four total treatment replications that were sited in two contrasting microclimates re: ambient evaporative potential. Photos: Justin O'Dea, WSU.

Figures illustrating moisture management treatments used in trials. From L to R: 1) "Active irrigation" treatment using mist emitters on timers; 2) post-inoculation immersive log soaking before tarping; and 3) "passive irrigation" with static water buckets placed under tarps (foreground, before tarp was placed). The active irrigation treatment used in the totem system can also be seen in the background of the picture on the right. Photos: Justin O'Dea, WSU.

***3**. Aspects of additional sub-trials established at the Vancouver site in spring 2020. An evaluation of substrates including Garry oak (top left and center), and an evaluation of late-cut vs. early-cut substrates (bottom left) were included in the sub-trials. The sub-trials were successfully established with one moisture management treatment in addition to controls (24-hour soaking before stacking and then covering stacks with spun polyester fabric until late fall). Soaked treatments and birch logs were showing strong indications of shiitake colonization by late summer 2020 (bottom and top right). Photos: Justin O'Dea, WSU.*

Wine cap mushroom production trials established at the Vancouver site in 2020. The trials included wood chips of four different substrates (red alder, big leaf maple, wild sweet cherry and paper birch), with half of the plots fitted with drip irrigation and the other half without (top left). The trials produced low, variable, sporadic yields in 2020 that were always unmarketable due to rapid quality decline and pests (right). Coyote damage also plagued the drip irrigation integrity throughout the summer (bottom left). Photos: Olga Romanova (right) and Justin O'Dea (left), WSU.

Modified "fruiting blanket" setup to simultaneously keep conditions humid during shiitake fruiting while also excluding pests from reaching the mushrooms. Photos: Justin O'Dea, WSU.

Highly variable, sporadic yields of oyster mushrooms in the totem systems began in fall of 2020. Totems with lion's mane mushrooms did not produce in 2020. Photos: Justin O'Dea, WSU.

Various production issues encountered throughout the project with feral fungal growth (bottom left), insect pests (top center right and right, bottom center and right), shiitake desiccation from windy conditions during fruiting (top left), and shiitakes that overripened due to harvest timing being asynchronous with unpredictable fruiting patterns a cool-weather shiitake strains (top center right). Photos: Justin O'Dea, WSU.