

ABSTRACT

DURMUS, TARIK. Forest Farming of Shiitake Mushrooms (*Lentinula edodes*) (Under the direction of Dr. Erin O. Sills).

Mushrooms such as shiitake (*Lentinula edodes*) are often grown in forest farming systems. This thesis is comprised of two chapters on forest farming. In the first chapter, I reviewed the literature on the ecological impacts of forest farming, finding that this system can affect the population dynamics where it is implemented. It also can provide waste resource for biofuel/bioenergy and can affect the wild animals in the ecosystem. In the second chapter, I analyze the likelihood and quantity of mushroom production on logs of four different tree species, three years after inoculation. White oak (*Quercus alba*) is most commonly recommended for shiitake production. I compare it to tree-of-heaven (*Ailanthus altissima*), red maple (*Acer rubrum*) and sweetgum (*Liquidambar styraciflua*), testing two different mushroom strains (west wind and naturalized). After controlling for size of the log and strain of mushroom, sweetgum was not significantly different from white oak in terms of the likelihood of a log surviving and producing mushrooms, or the quantity of production per log. Factors other than species that affect production include the length of the logs (positively and significantly related to the probability of mushroom production), the weight of the logs (positively and significantly related to the yield of mushrooms), and the strain of shiitake (higher probability of production on logs inoculated with west wind).

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Forest Farming of Shiitake Mushroom (*Lentinula edodes*)

by
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CHAPTER 1: ECOLOGICAL IMPLICATIONS OF FOREST FARMING

Forest Farming System

Forest farming is an agroforestry¹ practice simply defined as the cultivation of understory crops within an established forest (USDA 2012). The system can be applied in natural forests or plantations. As a more comprehensive definition, "*forest farming is a distinctive approach that combines the management practices of conventional forestry with those of small-scale farming or gardening to attain an environmentally and economically sustainable land use system*" (USDA 2012, Page 1). One of the particular benefits of this system is that forest farming can generate income to supplement timber production from the forest stands. Producing crops under the forest canopy can be one of the main characteristics of the system that differentiates it from wild harvesting (USDA, 2012).

A forest farming system provides a shaded ground for crops that require shade. Medicinal crops and mushrooms are the most common products of this type of system.

In North America, the use of forest farming system has been increasing in recent decades (Chamberlain et al., 2009). Crops like pods of mesquite (*Prosopis spp.*), honey locust (*Gleditsia triacanthos L.*) and mulberry (*Morus spp.*) are grown in the southern region of the US as livestock forage (Chamberlain et al., 2009). Forest managers, farmers, and landowners are starting to choose this new system to maximize the benefit from their lands (Chamberlain, 2009). Forest farming is a concept that provides land for agriculture under a forest canopy (USDA,2012). However, growing agricultural crops in a natural forest stand could bring ecological changes in the environment. Bringing new plant species in an environment might affect the population dynamics and might also increase the competition for nutritional resources.

¹**Agroforestry** is a land management approach that combines forestry and agriculture to use the ecosystem for crop production, livestock, animal herbivory. There are five agroforestry practices: silvopasture, alley cropping, forest farming, riparian forest buffers, windbreaks (USDA, 2012).

Moreover, increasing the number of plant species in an environment, especially agricultural crops, might affect the number of wild animals in the area. Crops can draw attention for animals and high number of herbivores can affect the plants in terms of recovery and reproduction. Another point in this system is that crops can provide below-ground biomass that benefit environment. Similarly, farming operations in the forest might leave waste behind, this waste can be deformed crops, cultivation logs or nut shells. The effect of these waste products in the environment could be an essential part of forest farming.

In this chapter, I search the literature for information on the ecological impacts of forest farming to the ecosystems to answer these objective points, along with the main differences and similarities between wild-harvesting and forest farming, and also retrieved possible impacts of wild-harvest that could be applied to forest farming.

Literature Review

For this chapter, I searched the scientific literature for publications that discuss the ecological implications of forest farming. I used five different databases; CAB Abstracts (Provided by NC State Library), Science Direct (<https://www.sciencedirect.com>), NCSU (North Carolina State University) Library website (<https://www.lib.ncsu.edu>), Google Scholar (<https://scholar.google.com>) and Web of Science (Provided by NC State Library). First, I defined the key phrase likely to appear in relevant literature: "ecological impacts of forest farming". Second, I identified keywords representing the main concepts of interest. In CAB abstracts, I used the advanced search option to search for key concepts by combining keywords with the "AND" command tool. The three concepts that I combined were (1) "forest farming", (2) "impact* OR implication* OR consequence*" and (3) "ecolog*".²

² The "OR" command was used to search for similar words that could be included in the title, and the symbol asterisk (*) was used to search different variations of words.

First concept (1) resulted in 131 results with a book focusing on forest farming. The combination of concept one (1) and two (2) resulted in 15 resources while the combination of concept one (1) and three (3) resulted in 57 resources. The combination of all the concepts resulted in 10 resources in the CAB Abstracts database. In Science Direct, search title "ecological implications of forest farming" did not give results and searching only "forest farming" term resulted in 150 resources. A separate search of "forest farming and "ecological impacts" resulted in only four resources. NCSU website provides a more title-based search, and I used the key phrases that I identified for the title search. Google Scholar search on "forest farming" phrase led to few resources that focus on forest farming. Web of Science search resulted in 83 ecological resources for the key phrase, however, "forest farming" search resulted in 63 resources in total, and 26 of them were in forestry topic.

Through this literature search, I selected resources that could be relevant to the topic that I am searching, and I also obtained articles by analyzing the cited works of literature of resources I obtained. As a result, I used 15 resources including two books written by J. Russel Smith and J. Sholto Douglas. These books reported studies conducted in different parts of the world from Asia to America, and also included definition and explanations about forest farming system and its history.

Even though literature search resulted in a substantial number of resources, only a few of them were relevant to the ecological impacts of forest farming system. Besides these few articles, I identified articles that focus on wild harvesting/collecting and its ecological effects. Also, some of the articles compared wild harvest and cultivation that is explaining the effects of forest farming. These articles also pointed out the differences and similarities between forest cultivation and wild collection, and also their effects to determine the suitable system to nature. The crops that were used in the studies were mainly medicinal and aromatic non-timber plant

species. As a result of web search, I also reached two different website resources that are focusing on explaining/defining forest farming system.

Findings

People have been using forest farming system to obtain resources for centuries however the name "forest farming" is recently formalized and managers and landowners started to use it in forest management practices. (Chamberlain et al., 2009). The main reason for using this system is to generate additional income and also benefit the ecosystem sustainably. That is supported by Schippmann et al. (2006) with the idea of "a sustainable system that works for every part of the ecosystem". In this case, the need is that an approach which takes four interlinked scales into account at the: (1) landscape level; (2) community and ecosystem level; (3) plant population level; and (4) genetic level (no literature identified) (Schippmann et al., 2006). Ecological impacts of forest farming system can be examined on these four scales.

1-Landscape Level

The effects of forest farming on landscapes can result beneficially. A damaged landscape could lead a gap in the ecosystem, on the other hand, using forest farming system could restore a landscape and provide sustainable management. In an example study, in Corsica Island, families supported themselves by gathering nuts, while the presence of the chestnut (*Castanea spp.*) forests has helped that the land is protected from erosion (Smith, 1987). In this circle, people had benefits from the forests, and keeping these forests helped landscape as well as the ecosystem in a sustainable way.

2-Community and Ecosystem Level

Forest farming can be applied as an ecosystem support mechanism that could restore the balance between plants and ecosystem. Trauernicht and Ticktin focus on effects of NTFP

cultivation under a forest canopy. The study that they conducted can be evaluated under genetic level. Plantations of the palm *Chamaedorea hooperiana*, on community composition and structure of an old-growth tropical rain forest in southern Mexico was studied. The findings from the experiment showed that forest sites were significantly higher in overall species richness than plantation sites. When broken down into life history groups, richness was more significant in forest sites for all woody species, woody understory species, shrubs and woody individuals ≥ 10 cm dbh.

In contrast, species richness did not significantly differ between forest and plantation sites for the larger woody individuals ≥ 20 cm dbh. Forest sites were also found to have a significantly higher richness of understory trees and fleshy-fruited trees and shrubs than plantation sites. No differences were found in the richness of all palms and cycads or of uncultivated palms (Trauernicht and Ticktin, 2005).

Similar to Chamberlain et al. (2013), Xia et al. (2018) pointed out on a topic that includes forest farming and use of forest farming waste. Chinese chestnut (*Castanea mollissima*) and *Jatropha curcas* are widely used species in forest farming in China for their nut and biodiesel precursor. The seeds of these crops are harvested with their shell; once the product is separated, the shell waste remains in the land. Xia et al. (2018) claim that it will be beneficial to both farmers and the environment if these wastes were converted to bioenergy, biofuels, or biomaterials using modern biomass conversion technologies. As a result, converting shell waste to biomass pyrolytic polygeneration can be defined as an effective method to produce high-value products. However, leaving these shells in the land would create another subject to experiment. Usually, remained bark, branches and sawdust in the land provide biomass resource to the area, it is not known yet if leaving shell waste on the ground would provide a biomass resource (Xia et al., 2018).

3-Plant Populations Level

According to studies, population level can be the most visible ecological effect of forest farming system. "A sustainable system for harvesting medicinal and aromatic plants is one where fruits, seeds or other plant parts can be harvested indefinitely from a set area without detrimental impact on the structure and dynamic on the harvested plant populations" (Schippmann et al., 2006). According to Ticktin (2004), NTFP harvest from an environment can cause a change of the survival rate, growth, and reproduction of harvested individuals. This change on essential rates can affect the structure and dynamics of populations. Because sustainability of resource use requires that harvest rates do not exceed the capacity of populations to replace the individuals extracted (Ticktin, 2004).

About variation in plant part, Ticktin (2004) also clarifies that "when a portion of plant material is harvested from an individual, the nature and quantity of nutrients and photosynthetic capacity removed, and the potential for survival and effective propagation will depend on the kind of material harvested". Even though Ticktin explains the situation as NTFP harvest, this application shows similarities with forest farming system. In both cases, plants or plant parts are removed from the environment or plants are added to environment in forest farming system. It is also stated in the paper that light availability may be a limiting factor for some understory species. Some management practices, such as thinning of dense populations or sowing of seeds, may result in growth rates of harvested populations that exceed those of non-harvested populations (Ticktin, 2004).

Medicinal plants or mushrooms draw attention from wild animals and over-use of these areas by animals could create an ecological issue. Farrington et al. (2008) carried a research study on the effects of deer herbivory and harvest on population dynamics of American ginseng (*Panax quinquefolius*). Six ginseng populations observed for eight years in the Ozark Highlands

of east-central Missouri. The results showed that the effects were not additive; however, deer herbivory and human harvest each negatively affected the population dynamics of American ginseng. Although deer herbivory decreased the population growth rate of American ginseng by decreasing fecundity, growth, and stasis, it also had a positive effect on survival, because it hid plants from human harvesters. The interactive effects of deer browse, and harvesting were especially active in this species because deer preferred to browse the same larger plants sought by harvesters (Farrington et al., 2008).

Results and Discussion

The research studies that were carried on different perspectives of forest cultivation, wild harvesting and ecological impacts give a point of view to make assumptions on ecological implications of forest farming system. However, further research needs to be done on the subject to be able to understand the effects of this system to nature.

Forest farming system is a broad area with many different aspects that need understanding. Different plant species will need different treatments in nature, and it is essential to understand the possible effects of these treatments on the ecosystem. To give an example to explain the effects of this system, forest farming of shiitake mushroom can be a good example to evaluate the research points that need to be conducted. First, to cultivate the mushrooms, trees are cut down and cut into logs for cultivation, and it is a silvicultural question that “what kind of treatment has to be applied in the land?”, and if the shade will be enough for a secondary product to grow.

Another point is that the waste from forest farming system, Xia et al. (2018) conducted their research on this subject with particular tree species, but there is a considerable need about a variety of tree species and secondary product species. For example, shiitake mushroom cultivation usually takes 3-5 years and after that logs might be left in the area or might be used

for other purposes such as firewood. If the logs left on the ground after the cultivation process, “what kind of ecological impact would they have on the field?” is another question that needs answers with further studies. Other parts of the ecosystem are also needing to be evaluated.

Medicinal plants or mushrooms can be preferred by wildlife and producing these might increase the number of wildlife species in the area. To give an example from shiitake cultivation, different kind of insect species can use cultivation logs to live, and this can increase the number of other animal species that use them for the food source. Few studies explain the deer impact on NTFPs, but the effects of other animal species can be an essential angle to understand the ecosystem relationship. For mushroom cultivation, pathologic aspects of forest farming can be another point to be evaluated. In general, the ecological effects of forest farming are a broad area of study, and there are only a few studies have been conducted on it. To be able to determine if this system has positive or negative impacts on nature, we need more detailed studies on different habitats with different species that include wildlife effect, pathologic and chemical relations in nature.

Ecological implications of forest farming system are less studied area. The articles that I found mostly focused on the effects of wild harvesting of NTFPs, which can somewhat be related to the effects of cultivated areas since crop production in forest farming system is an installed application. Main ecological problems in the studies were population dynamics change, forest farming effect on wild animal effects, and waste management. However, there is a big gap to be understood about the ecological effects of this system from all perspectives. Although these effects were explained in a different application, some of them can be applied to forest farming system. They also can be considered as connected results of each other. Over-harvesting could be a danger to the ecosystem, or over-planting the crops would create a population increase of wild animals in the area that they could end up damaging the overstory or slowing down the

reproduction of tree species. It is a process that needs to be observed over the years due to the time of reproduction of tree species.

CHAPTER 2: FOREST FARMING OF SHIITAKE MUSHROOMS (*Lentinula Edodes*)

Introduction

Shiitake mushrooms (*Lentinula edodes*) have been grown on oak (*Quercus* spp.) logs for centuries. In this chapter, I report the results of a test of shiitake mushroom cultivation on logs of sweetgum (*Liquidambar styraciflua*), red maple (*Acer rubrum*) and tree-of-heaven (*Ailanthus altissima*) in comparison to white oak (*Quercus alba*), considering both the probability and quantity of mushroom production per log, three years after they were inoculated with shiitake spawn. There is a high opportunity cost of using white oak to cultivate mushrooms, because it is also a valuable timber species (Robert Bardon, personal communication), as evidenced by the fact that it is one of the few hardwood species listed individually in timber price reports. The question that I address in this chapter is whether shiitake cultivation on logs of species with lower timber value is as likely to result in production, and will produce as much, as cultivation on white oak logs. To explain why the species of the log may matter, I first review what is known about the biology and management of shiitake.

The mushroom species *Lentinula edodes* is known by various names around the world. Even though it was first cultivated in China, perhaps the most common name, shiitake, is derived from the Japanese words "shii," which means *Castanopsis cuspidata*, and "take," which means mushroom. Black mushroom is another name used in the US (Chen, 2005). In addition to being one of the most popular edible mushrooms, shiitake is also known as a medicinal product (Tokimoto, 2005).

According to the USDA (2017), in the 2016-2017 season, commercially grown mushrooms had a value of United States dollars (USD) 40.8 million including 10.5 million pounds of shiitake produced by 182 shiitake growers. The average wholesale price of log-grown shiitake mushroom was USD3.89 per pound (USDA, 2017).

The first reports of cultivation of shiitake are from mountainous areas of China during the Sung Dynasty (960-1127) (Przybylowicz and Donoghue, 1990). In 1920, shiitake mycelium was first grown on a suitable material base. In 1943, the technique of inserting colonized wooden wedges into logs was developed, leading to higher yields (Przybylowicz and Donoghue, 1990).

The life cycle of shiitake begins with basidiospores dropped by grown mushrooms and spread through the air and environment. When basidiospores land in suitable environments, they develop a new hyphal structure called primary mycelium. The primary mycelium stage is short, and this structure cannot produce mushrooms by itself. To be able to form mushrooms, two primary mycelia grow together and form a new structure called secondary mycelium. Secondary mycelium is a productive stage that includes most of the shiitake mushrooms' vegetative stage. Secondary mycelium grows below the surface of the host, typically in the sapwood of trees. The next step is for the primordia to break through the surface and develop mushroom bodies. The mushroom bodies grow larger and develop gills under the cap. Those gills contain basidiospores that begin the cycle again reproduction (Przybylowicz and Donoghue, 1990; Chen, 2005).

Carbon and nitrogen help shiitake to compile proteins, nucleic acids and sugars, and a specific ratio of carbon to nitrogen is vital for vegetative mycelium growth (25:1) and mushroom production (40:1). Shiitake use polysaccharides, cellulose, hemicellulose, and lignin as their energy source, as well as sugars in the mycelial growth phase (Chang and Miles, 2004).

Environmental conditions can affect survival, growth rate, time of fruiting, yield, and the shape of mushrooms (Przybylowicz and Donoghue, 1990). Shiitake can grow in a temperature range between 4 and 35 °C, but the optimal temperature is 24-27 °C. Depending on the strain, the fruiting phase requires a temperature of 15 ±1-2 °C. Another critical factor is moisture content, because that affects how nutrients disintegrate. Disintegrated nutrients are absorbed by mycelium when they have the proper moisture, generally considered to be 50-75 % (Chen,

2005). In addition, 70% shade is considered optimal for growing shiitake (Przybylowicz and Donoghue, 1990). Last, shiitake need an environment in the pH range of 3-7. However, pH 3.5-4.5 is desired for primordia and fruiting body formation (Chen, 2005).

Shiitake mushrooms have been grown on a wide range of hardwood species with variable success (Tokimoto, 2005). Chen (2005) suggests species in the family *Fagaceae*, especially Shii (*Castanopsis* spp.), oaks (*Quercus* spp.), and other Asian oaks and beeches (*Fagus* spp.) It is not easy to determine the exact list of species for shiitake cultivation. The cultivation of shiitake is not only promoted by tree species but also environmental conditions, which makes it harder to rank the species for suitability due to different environmental conditions (Tokimoto, 2005).

The log species has been found to affect the number of mushrooms produced each flush, the number of flushes from a single log, and the taste and size of the mushrooms produced (Przybylowicz and Donoghue, 1990). This has been attributed to differences in nutrient content, wood density, and bark characteristics (Przybylowicz and Donoghue, 1990). Factors other than species include the season when a tree is felled and the age of the tree. The best season to fell trees for shiitake production is when the logs contain the highest content of carbohydrates and other organic substances, and their bark is not easily peeled off. Przybylowicz and Donoghue (1990) suggest that shiitake logs should be cut just before the buds break in the spring. Chang and Miles (2004) recommend using logs from trees that were 15 to 20 years of age. However, standard practice in mushroom cultivation is to use a mix of young and old logs, with variable bark thickness and heartwood fraction.

Factors Influencing Production

"In China, Korea and Japan, shiitake growers choose primarily oak (*Quercus*) trees" (Tokimoto, 2005: Page 48). One of the key reasons for this is that the strong bark of oak supports shiitake fruiting for an extended period (Tokimoto, 2005).

In addition to species, other characteristics of the logs also matter for shiitake production. Usually, logs are cut into 100 cm in length (Tokimoto, 2005), because this makes them easy to manage and carry. In this experiment, logs were cut into lengths varying from 66 to 153 cm. Longer logs are more difficult to submerge in the soaking tanks, which are used to boost mushroom fruiting. Likewise, lighter weight logs may float in the soaking tanks, reducing absorption of water. Finally, deformation of the logs (into anything other than straight pieces) can make them more difficult to stack in the tanks, thus potentially affecting production.

There are various strains of shiitake used under different weather conditions. West wind is commonly used in Virginia (our study site). Naturalized LE strain was found naturally growing on logs in Virginia, encouraging a grower to use this strain for commercial production (Mark Jones, personal communication). Since this strain naturally grows in the area, it could be the most suitable for cultivation of shiitake in Virginia.

In a forest farming setting, logs can be infected by other competitive fungi or pathogens. Competitive fungi could reduce the nutrient amount inside the wood, and this could even cause the death of shiitake mycelium (Mudge, 2010).

Research Questions

In this experiment, logs of tree-of-heaven, red maple, and sweetgum are tested as alternatives to white oak logs, which are generally considered the best for shiitake production. The main concept is to compare the species in terms of productivity and suitability. The value of white oak timber value is reported in the timber market reports for the eastern US, while the

other species are grouped in categories with lower prices. There can actually be a net benefit from harvesting trees of these other species to use for shiitake production, e.g. because sweetgum and tree of heaven can be invasive.

My study takes advantage of an experiment installed in March 2014, when logs of these four species (*A. rubrum*, *L. styraciflua*, *A. altissima*, and *Q. alba*.) were inoculated with two strains of shiitake and placed under the forest canopy on the Randolph Experimental Farm in Virginia. Three years later, I assessed the outcome: Are there differences between tree species and between mushroom strains for survival of the logs, the probability that they yield mushrooms (yes/no), and the quantity of mushrooms produced? To answer these questions, I control for shiitake strain (naturalized vs west wind) and the size (length and weight) of the logs.

Materials and Methods

At the beginning of 2014, an experiment to test production of shiitake on logs of different tree species was installed on Randolph Farm by two Virginia State University scientists, Dr. Greg Frey and Dr. Marcus Comer. In the next section, I describe the study site, experimental design, and materials that were used in this experiment. In the following section, I describe my methods for evaluating the outcomes of this experiment three years later, in 2017. I collected data in two stages: in June of 2017, I counted the remaining logs, soaked them, and measured the fraction of logs still producing and the total quantity of mushrooms produced by species, and in September of 2017, I again soaked the logs and measured the quantity of mushrooms produced on each individual log.

Experimental Site

Randolph Farm is located at 37°13'37" North longitude and 77°26'52" West latitude near Petersburg, VA (Figure 1). The farm is utilized as 416-acre agricultural learning center that focuses on “new and niche crops, alternative cropping methods, horticultural crops, nutrient management, water quality, animal production and aquaculture production”

(<http://www.agriculture.vsu.edu/randolph-farm/index.php>).

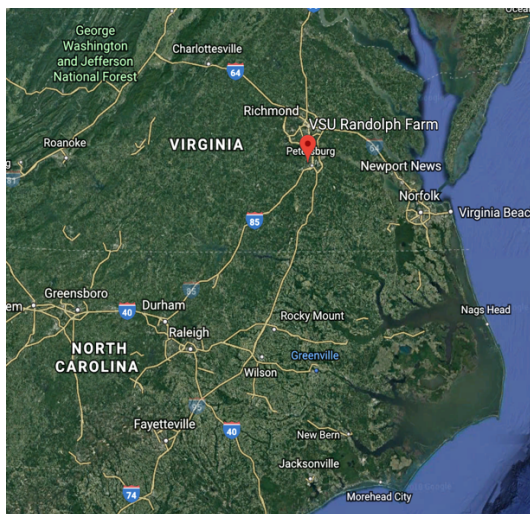


Figure 1 Location of Randolph Farm



Figure 2 Location of experimental site on Randolph Farm.

Tree Species

Logs were obtained from four different hardwood tree species: white oak (*Q. alba*), sweet gum (*L. styraciflua*), red maple (*A. rubrum*) and tree-of-heaven (*A. altissima*) in winter of 2014. The logs were obtained from live and healthy trees. Red maple, sweet gum and white oak logs were harvested from a site near Petersburg where logs were removed for construction purposes. The tree-of-heaven logs were harvested from a cultivated area on Randolph farm. When the experiment was installed, the logs were tagged, and their length and weight were recorded. Length of the logs ranged from 66 cm to 153 cm, and weights ranged from 2.73 kg. to 25.4 kg.

In general, there are two kinds of spawn for *Lentinula* cultivation. One is made by inoculating the fungal mycelium into sawdust medium and is called sawdust spawn. The second kind of *Lentinula* spawn is called wood plug or peg spawn (Chang and Miles, 2004). Most sawdust used for shiitake production originates from sawmills that use band saws (Royse, 2000). Sawdust spawn was selected in this experiment because it is more economical for large quantities of logs. Two wide range strains were tested: west wind (WW) and naturalized *Lentinula edodes* “Cismont” (LE). WW strain is characterized by short rests between fruiting periods of the logs. LE strain was found growing on uninoculated logs near a shiitake production facility in Cismont, Virginia, suggesting that it had become naturalized to that environment (Mark Jones, personal communication). The grower that found this strain has used it commercially and found that it produces a high yield on oak logs.

Inoculation

Inserting mycelium into logs is called “inoculation.” Sixty logs of each species were inoculated, 30 with each mushroom strain. The logs were drilled with 12-mm diameter and 1-inch long drill bit attached to an angle grinder, which spins much faster and more easily drills into non-treated wood than traditional drills. Holes were drilled in a diamond pattern, with 5

inches between holes within rows along the length of log, and 3 inches between rows around the circumference of the logs. After the logs were drilled, they were inoculated using a palm-style inoculator to insert a uniform quantity of spawn into each hole. Pre-heated cheese wax was applied over the spawn and other damaged portions of the bark to seal them using a wax dauber. “When hot wax is applied to holes at about 260 °F (127 °C), the wax forms a thin, tough, translucent coating over the spawn, while log sap and water from the spawn vaporize and boil out” (Przybylowicz and Donoghue, 1990; Page 64). The logs were moved to a wooded site (Figure 2) and stacked after inoculation.

Testing Long-term Productivity

In March of 2017, number of logs of each species remained were determined, and moved the logs to a site where it was possible to install tanks for soaking.

All of the logs were soaked in June and September of 2017, following the same procedure each time. Three cattle tanks were used to soak the logs. The tanks were filled two-thirds with cold water (on regular water temperature). On the first day, approximately half of the logs were put into the water and left to soak for 24 hours; after taking the soaked logs out, they were stacked with a low-ground A-stacking method: logs were arranged on the ground with one side supported by a long, non-inoculated log (Figure 3).



Figure 3 Stacking method used for logs after soaking. One side of the logs are placed on a support log and one side on the ground.

On the second day, the other half of the logs were placed into the tanks and left to soak for 24 hours. While the second batch of logs soaked, the stacked logs were sprinkled with cold water to keep them moisturized. At the end of the second day of soaking, all logs were stacked, sprinkled with water, and left for a week. After eight days, the logs had grown mushrooms that were ready to harvest (Figure 4).



Figure 4 Logs with grown mushroom that are ready to harvest.

To harvest the mushrooms, a knife was used to cut the fruiting bodies off the logs (Figure 5). In the first harvest, collected mushroom from the four tree species were separated into four stacks. After cleaning any outside material from the mushrooms, they were placed into boxes (Figure 6) that were labeled by tree species. In the second harvest, mushrooms from each log were put into a paper bag for weighing. The tag number from the log was recorded on the bag. After collecting and bagging all mushrooms, a scale was used (Figure 7) to weigh the mushrooms produced on each log, and I recorded the weight in kilograms, along with the tag number and characteristics of the log.



Figure 5 Harvest: mushroom bodies are cut close to the bark and taken out by slightly twisting them.



Figure 6 Boxes that were used for storing collected mushrooms in the first harvest. Open boxes were selected to provide air circulation.



Figure 7 Scale used to weigh mushrooms in the field. The scale was set on a flat surface to ensure that it remained in balance.

Shape Abnormality and Disease

During the second harvest, two other characteristics of the logs were also recorded: shape abnormality and disease. I noted whether each log exhibited any shape abnormality or any evidence of disease. Logs that were not reasonably straight were coded as having a shape abnormality. By observation, the health of the logs was evaluated and recorded as either diseased or not. Unusual colors (black, white, brown) on the bark were taken to indicate the existence of disease (or competing fungi) on the logs, but I did not identify the specific diseases. In a forest environment, it is difficult to grow shiitake without having a few other fungi occupy some space on the logs, but commercial cultivators seek to prevent large disease outbreaks (Przybylowicz and Donoghue, 1990).

Data collection

Two different data sets were analyzed. The first data set includes mushroom production in kilograms by tree species at first harvest and second harvest, i.e. 8 observations in total (4 species x two harvests). The second data set includes the mushroom production of each log in kilograms, along with 10 characteristics of each log, including species, mushroom strain, log weight, log length, shape abnormality and disease, i.e. 146 observations in total (146 logs x one harvest). All variables are defined in Table 1 and described in the following section. The distribution of the quantity of mushrooms produced per log in the second harvest is presented in Table 2, along with descriptive statistics for log weight and length.

Table 1 Variables characterizing the logs and production.

Variable Name	Explanation
Tree Species	Indicator of tree species; red maple (RM), sweet gum (SG), white oak (WO), tree-of-heaven (AL).
Mushroom Strain	Indicator of mushroom strain; west wind (WW) and naturalized shiitake strain (LE), where LE refers to <i>Lentinula edodes</i> . WW strain is the reference point.
Log Length	Length of the log in centimeters.
Log Weights	Weight of the log in kilograms (converted from original measurement in pounds)
Shape Abnormality	Indicator of shape abnormality of the log, =1 if some deformation is evident, =0 otherwise
Disease	Indicator of disease existence on logs. "Yes" and "No" answers explain if the log is infected by fungi other than shiitake mycelium.
Mushroom Production	Mushroom production in kilograms.
Production Binary	Binary indicator of production = 1 if production equal to or bigger than 0.1 kilogram, = 0 otherwise

Table 2 Summary statistics for numeric variables: log length (cm), log weight (kg) and production (kg). N = 146.

Statistic	Production (kg)	Log Length (cm)	Log Weight (kg)
Minimum	0	66	2.73
1st Quantile	0	105.2	6.36
Median	0	116	9.54
Mean	0.08	114.7	11
3rd Quantile	0.1	125	14.9
Maximum	0.8	153	25.4

Tree Species

Tree species is a categorical variable, with WO (White oak) as the reference category for the other three species AL, RM, and SG. The experiment started with 60 logs of each species. Over three years, some of the logs disintegrated and/or were stolen. Thus, in 2017, I found 37 logs each of AL and RM, 32 logs of SG, and 40 logs of WO.

Mushroom Strain

This variable has two categories: WW (west wind strain) and LE (*Lentinula edodes* naturalized strain). Of the remaining logs, 74 had been inoculated with WW and 67 with LE. LE is used as the reference category. Of the total of 146 logs, there were 5 with damaged tags, which are recorded as NA for strain.

Log Length

This variable ranged from 66 cm to 153 cm, and two box plots were created to show the distribution of logs in the experiment. Figures 8 and 9 present box plots of the length of the logs used in the experiment, by tree species and by mushroom strain. RM and SG have a tighter distribution, while the logs of AL and WO are of more variable length. AL logs tend to be shorter.

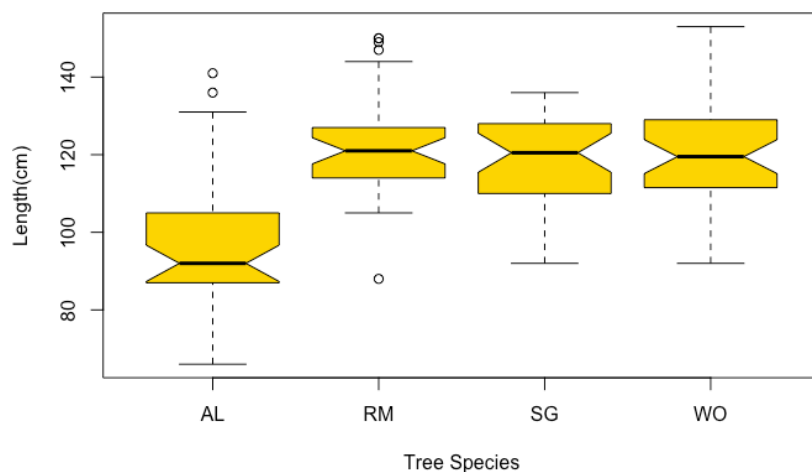


Figure 8 The distribution of log length by tree species. AL had shorter log lengths.

Each box represents one tree species (AL, RM, SG, and WO). The vertical axis represents the length (cm). The middle line shows the median for each species, and outliers around the AL and RM boxes show the logs that are not within inner-quartile range for the log length. For the figure 9, boxes represent the mushroom strains LE and WW. Logs with LE strain is shown as shorter than logs with WW strain.

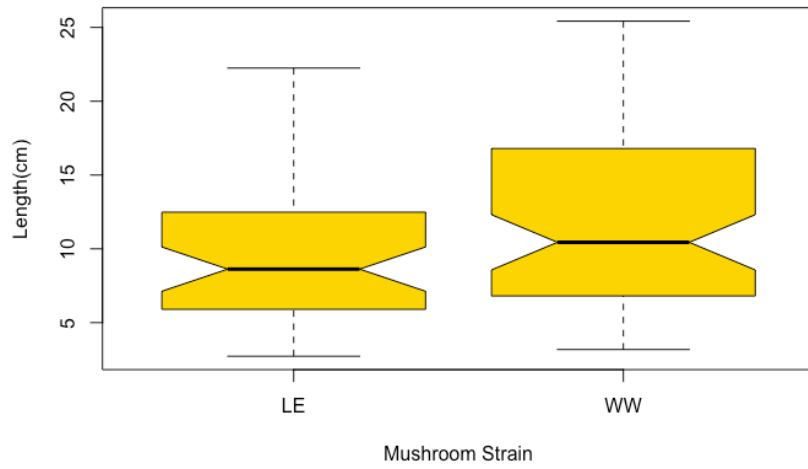


Figure 9 The length of each log by mushroom strain. The median (middle line of boxes) of the two strains are similar.

Log Weight

Weight variable is another numerical variable that ranged from 2.73 kg to 25.4 kg. To demonstrate how tree species and mushroom strain data spread across two box plots were created. Figure 10 shows that AL and RM logs tend to be lighter, while SG and WO logs tend to be heavier. Figure 11 presents the mushroom strain distribution in weight variable. Logs with LE strain tend to be lighter in weight compare to logs with WW strain.

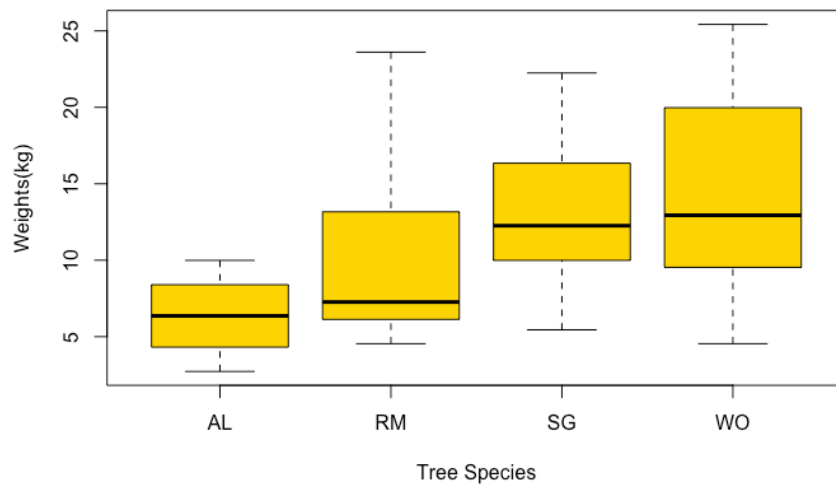


Figure 10 Box plots of weight by species. Bold middle lines show the medians.



Figure 11 Boxplots of weight by mushroom strain. Bold middle lines show the medians.

Shape Abnormality

Table 3 shows the frequency of abnormal log shapes by tree species and mushroom strain. There were relatively more straight logs of SG and WO.

Table 3 The number of logs that have shape deformation for each species and strain.

Strain	Deformation	Species			
		AL	RM	SG	WO
LE	No	14	16	12	20
	Yes	3	3	0	0
WW	No	17	12	18	20
	Yes	3	2	2	0

Production

The second data set includes both the kilograms of mushrooms produced on each log (production_kg) and an indicator for whether each log had substantial (measurable) production

(prod_binary). Of the 146 logs, 79 logs produced no shiitake mushrooms. Another 18 logs produced a very small amount (recorded as 0.01 kg), and were lumped together with zero production, because such tiny amounts could easily have been knocked off or simply hidden under the bark on the logs. Logs that produced more than 0.01 kg were considered productive. I measured the weight of shiitake harvested from each of these logs to the nearest tenth of a kilogram, as shown in Figure 12. If a log produced a weight of shiitake equal to or greater than 0.1 kg ($=0.1, >0.1$), prod_binary is coded as “1”; otherwise, it is coded as “0”.

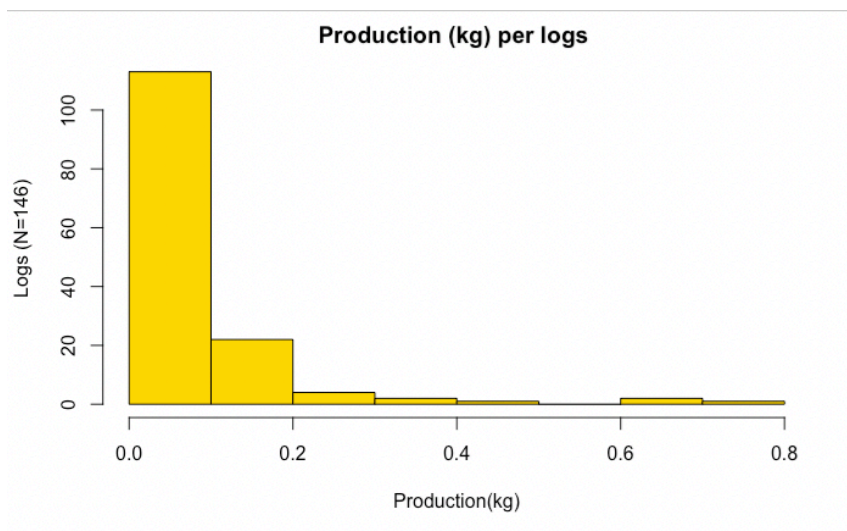


Figure 12 Mushroom production in kilograms.

Statistical Analysis

Using the first dataset, the number of logs that produced, and the total amount of mushrooms produced in the first and second harvests across species were compared. One motivation for this analysis was to test for differences between the first and second harvest in terms of the number of productive logs and their productivity. The primary motivation was to test for differences across species. The differences in the number of productive logs and the

amount of mushroom produced in the first and second harvest are presented in Table 4 and visualized in Figure 12.

Using the second data set (N=141), the probability of mushroom production was modeled as a function of log characteristics. The amount of shiitake mushroom production (in kilograms) was modelled using the ordinary least squares regression model. In both the logistics and the ordinary least squares regressions, the effect of species, controlling for all other log characteristics were investigated. Tree species were compared to white oak (control).

The probability of mushroom production was modelled using the following logistic regression model (Ott and Longnecker, 2010):

$$\ln\left(\frac{p}{1-p}\right) = \mathbf{X}\boldsymbol{\beta} + \mathbf{e} \quad (\text{Eq. 1})$$

Where:

$\ln\left(\frac{p}{1-p}\right)$ is the log of odds ratio of mushroom production, transforming the observed data into logit scale,

p is the probability of production,

$\boldsymbol{\beta}$ is the vector of coefficients or effects to be estimated from the model,

\mathbf{X} is the vector of log characteristics, including tree species and log length and,

\mathbf{e} is the vector of the residual errors.

The probability of mushroom production (yes or no) would be a function of tree species, mushroom strain, interaction term between tree species and mushroom strain, size of the log (length and weight) and quality of the log (shape abnormality and disease). The logistic model was run using the SAS software Logistic procedure (SAS Institute Inc. 2018). The regression parameters were obtained on the logit scale. SAS output included the coefficient estimates for

all variables included in the specification. To better interpret the results, the odds ratio, or the ratio of the probability that an event happens over the probability that it does not happen were estimated and visualized (Ott and Longnecker, 2010). The full logistic model including all of these variables had an AIC information criterion of 125.8. After removing the variables without statistically significant coefficients at the 10% level (log weight, shape abnormality, disease and interaction terms), the coefficient estimates for the remaining variables were all significant at the 5% level and the AIC value increased to 153.38.

The quantity of mushrooms produced (kg) on each log as a function of log characteristics including species (S) was modeled using the ordinary least squares regression. The mushroom strain (M), log weight (kg), and log height (cm) at eight months and species (S) were used as independent variables. The OLS model for this:

$$y = \beta_0 + \beta_1 W + \beta_2 H + \beta_3 S + \beta_4 M + \varepsilon \quad (\text{Eq. 2})$$

Where y is the response variable as Production, β_0 is the intercept for the model, $\beta_{01}, \dots, \beta_{04}$ are the coefficient estimates for variables tree species (AL, RM, SG), mushroom strain (LE), log length, log weight, shape abnormality and disease existence, x_1, \dots, x_6 are the predictor values for the variables, and ε is the error term.

Specifically, I modeled the quantity of mushrooms produced on each log (production_kg) as a function of mushroom strain and log characteristics including species, weight, height, and shape deformation. In this smaller sample, it was not possible to estimate the effect of shape deformation in the same model as species.

The initial OLS estimation had one statistically significant variable (log weight, $\text{Pr}(> |t|) = 0.022835$). After dropping variables without coefficients statistically different from zero at the 10% level, I obtained a higher adjusted R^2 value (0.1467) than the initial model with all variables

(adjusted $R^2 = 0.0856$). For the initial model, the F-statistic was 1.535 with a p-value of 0.19, showing that this model was not a good fit. The final model has an F-statistic of 2.719 with a statistically significant p-value of 0.045. The final model specification includes just tree species and log weight. All analysis was carried out in R (R Core Team,2013)) and SAS (SAS Institute Inc.,2013).

Results

Of the 60 logs of each species that were inoculated and stacked in 2014, I was able to locate 40 logs of white oak (WO), 37 logs of tree-of-heaven (AL), 37 logs of red maple (RM), and 32 logs of sweet gum (SG) in 2017. In consultation with Drs. Comer and Frey, I concluded that the rest of the logs had probably degraded and were no longer recognizable as logs. Even among the logs that were identifiable, not all produced shiitake mushrooms. Table 4 presents the number of logs that produced shiitake (defined as producing at least 0.1 kilograms) after the first and second soakings in 2017. For all species except white oak, fewer than half of the logs that were inoculated in 2014 were still productive in 2017. In the second harvest, many fewer oak logs produced, while nearly all of the sweetgum logs that produced in the first harvest also produced in the second harvest.

Table 4 Number of logs that produced mushrooms in the first and second harvest, by species.

Tree Species	Total # of Survived Logs	First Harvest	Second Harvest
white oak WO	40	38	25
tree-of-heaven AL	37	1	2
Red maple RM	37	19	13
Sweetgum SG	32	29	27

The rank order of species in terms of total shiitake production was consistent across the first and second harvest: white oak, sweetgum, red maple, and tree-of-heaven (Figure 13). Production on red maple and tree-of-heaven slightly increased between the first and second harvest, while production on white oak and sweetgum fell.

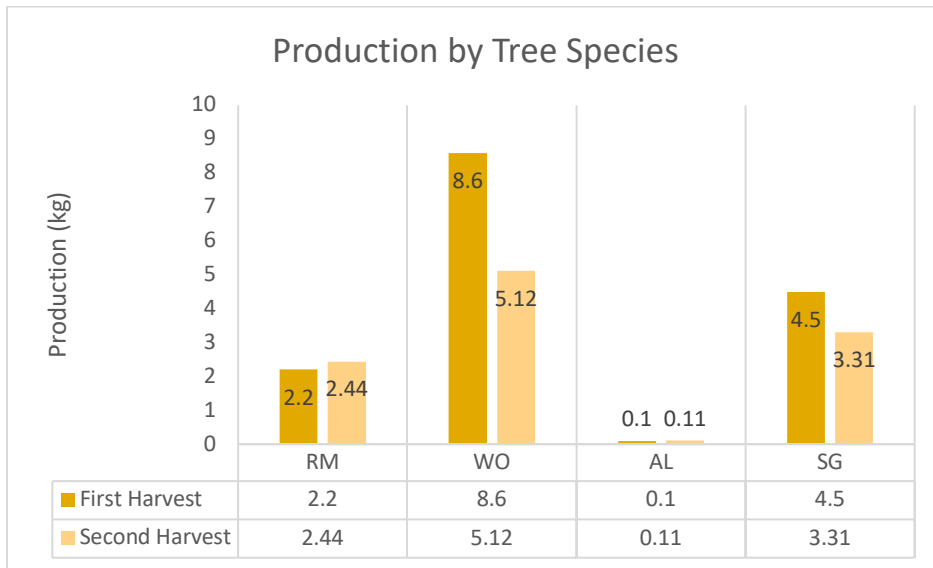


Figure 13 Production by tree species in first and second harvest. Dark yellow columns represent the total yields in the first harvest by tree species and light-yellow columns represent the total yields in the second harvest by tree species.

These differences in production totals across species partly reflect the numbers of logs of each species that survived and were still producing shiitake, as summarized in Table 4. They also reflect difference in productivity of the remaining logs, as demonstrated by variation across species in the average production per log among the logs that were still producing in each harvest (Figure 14). Average log production is the highest on white oak logs, while tree-of-heaven logs produce the least on average. Average production on tree-of-heaven logs was significantly lower than production on sweetgum or white oak logs. For comparison, Figure 15 shows production

per log by mushroom strain, which is significantly higher for WW than LE.

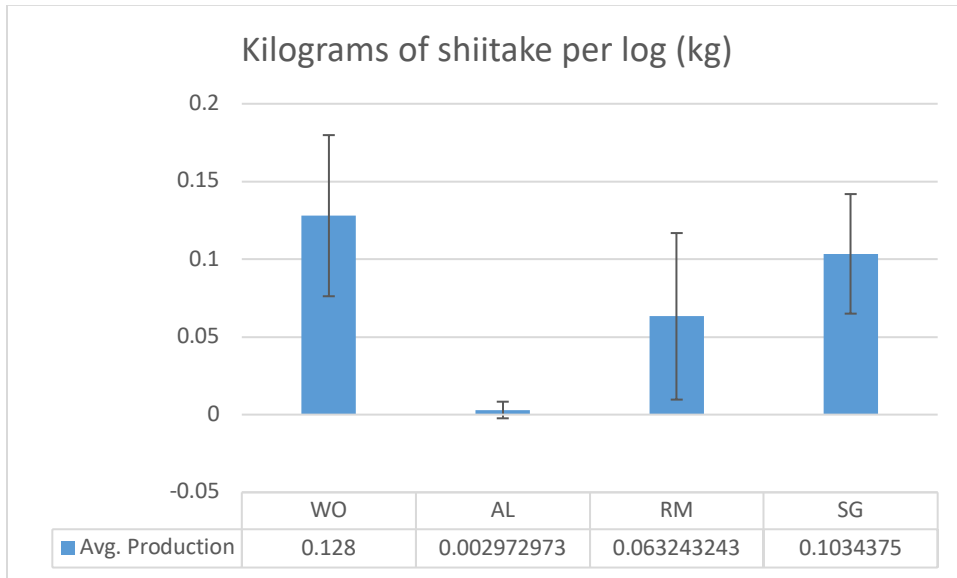


Figure 14 Average production of shiitake per log in the second harvest. The boxes represent the tree species, and their mean values. Black lines in the middle of the bars represent the 95% confidence interval for the tree species.

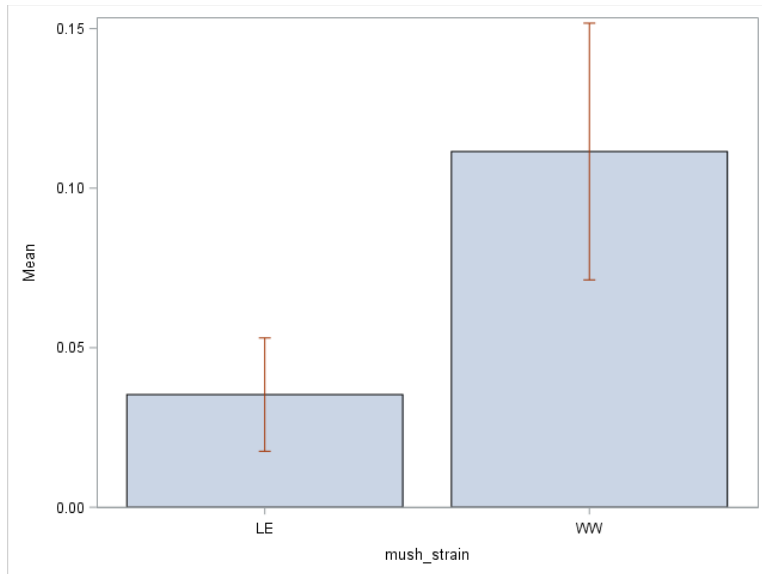


Figure 15 Mushroom production by strain. Red lines in the middle of boxes represent the 95% confidence intervals. Logs with west wind strain had significantly higher mean production.

One possible explanation for variation in the survival, continued production, and productivity of the logs is that some were affected by disease. Table 5 shows the number of logs that appeared to be affected by disease by tree species. The probability of disease may be moderated by mushroom strain. As shown in the table, more WO and RM logs inoculated with the LE strain appeared diseased. Further, two diseased logs of WO with LE strain yielded mushrooms, while diseased logs of the other three species did not produce any mushrooms.

Table 5 The number of logs that appeared diseased for each species and strain.

Strain	Disease	Species			
		AL	RM	SG	WO
LE	No	16	16	11	17
	Yes	1	3	0	3
WW	No	18	14	20	20
	Yes	2	0	0	0

Probability and Quantity of Production by Log

Using the data from the second harvest, I estimate regression models of two dependent variables: production binary and production. “Production binary” is an indicator for whether or not a log produced shiitake in that harvest. “Production” is the quantity of mushrooms in kilograms produced on each log.

Estimation results for a logistic model of the probability of shiitake production are presented in Table 6. The model specification includes all characteristics of the logs, as listed in Table 1, as well as interaction terms between species of log and strain of mushroom as possible. I used an alpha value of 0.05 ($p < 0.05$) to determine statistical significance.

Table 6 also includes the odds ratio. AL, RM, and WG all have odds ratios smaller than 1, showing that using a log species other than WO decreases the probability of production, holding length of the log and strain of mushroom constant.

Table 6 Logistic regression model: columns show variable names, coefficient estimates, standard errors and p-values (N=141, AIC= 125.8).

Effect	Estimate	SE	DF	t-value	Pr> t	Odds Ratio
Intercept	-17.760	750.32	107	-0.02	0.9812	.
AL	-2.5158	1.3335	107	-1.89	0.0619	<0.001
RM	-1.7538	0.8492	107	-2.07	0.0413	0.367
SG	-0.6771	0.7684	107	-0.88	0.3802	0.688
LE	-2.5872	0.9071	107	-2.85	0.0052	9.593e-6
AL-LE	-9.8336	420.61	107	-0.02	0.9814	.
RM-LE	1.5054	1.3339	107	1.13	0.2616	.
SG-LE	0.6062	1.3104	107	0.46	0.6446	.
Log Weight	0.02969	0.04955	107	0.6	0.5503	.
Abnormality-No	1.7308	1.2094	107	1.43	0.1553	.
Disease-No	11.806	750.31	107	0.02	0.9875	.
Log Length	0.04228	0.01987	107	2.13	0.0357	.

For tree species, white oak is the reference category, and the coefficients on the other species show how they influence the likelihood of yielding mushrooms relative to white oak. The probability of production is significantly lower on logs of red maple compared to logs of white oak, while the probability of production on sweetgum was not significantly different from the probability of production on white oak. This is because the largest fraction of white oak logs survived, and of the surviving logs, the largest fraction of sweetgum produce shiitake. The probability of production is also positively and significantly related to the length of the log. The

reference category for mushroom strain is west wind (WW), and logs inoculated with LE strain are less likely to yield mushrooms than logs inoculated with the WW strain.

Table 7 Parsimonious specification for logistic regression (N=141, AIC=134.28)

Effect	Estimate	SE	t-value	Pr > t 	Odds Ratio
Intercept	-4.1607	2.1812	-1.91	0.0586	.
Species -AL	-3.3940	1.1188	-3.03	0.0029	0.03679
Species-RM	-1.8791	0.6039	-3.11	0.0023	0.1674
Species-SG	-0.6094	0.5381	-1.13	0.2594	0.5957
Strain -LE	-1.6500	0.4739	-3.48	0.0007	0.1103
Log Length	0.04449	0.01831	2.43	0.0164	.

Table 8 OLS model of production, controlling for all log characteristics (except for “disease, because only two white oak logs with disease produced mushrooms). N=53, Adjusted R-squared=0.1503, F=2.011(p<0.08).

Effect	Estimate	SE	t-value	Pr(> t)
Intercept	-0.123825	0.305755	-0.405	0.6881
Species-AL	0.01132	0.180552	0.063	0.9504
Species-RM	0.131426	0.071273	1.844	0.0742
Species-SG	0.004424	0.061495	0.072	0.9431
Strain-LE	-0.06795	0.062444	-1.088	0.2844
Weight	0.01221	0.004824	2.531	0.0163
Length	0.001406	0.002477	0.568	0.5741
Abnormality-Yes	-0.053261	0.164318	-0.324	0.7479

Table 9 presents the estimation results for the parsimonious OLS regression model of the Kg of mushrooms produced per log, including just the logs that produced positive amounts. Production is positively related to log weight.

Table 9 Parsimonious specification for OLS regression. Columns present the variables, coefficient estimates, standard errors, and p-values (N=53, Adjusted R-squared=0.1467, F=2.719(p<0.04472)).

Effect	Estimate	Standard Error	t-value	Pr(> t)
Intercept	4.55974	0.26375	17.288	<2e-16
Species- AL	-0.25543	0.55498	-0.460	0.64810
Species- RM	0.32540	0.223379	1.392	0.17251
Species- SG	0.05644	0.19059	0.296	0.876881
Log Weight	0.04418	0.01552	2.847	0.00725

Among logs that produced a measurable amount of shiitake (>0.1 kg), the production quantity was positively related to the weight of the log (Table 9). The variable for shape abnormality is omitted from the parsimonious specification because it did not have statistically significant coefficients in the initial estimation.

As previously mentioned, data from the second harvest were used to estimate this model. The first harvest serves as a reference for the number of the logs of each species that produce mushrooms. Table 4 illustrates the change of the number of productive logs between harvests. The number of productive logs for WO, SG and RM decreased in the two months between harvests. In the first harvest, they had 38, 29 and 19, respectively. However, in the second harvest these numbers decreased to 25, 27 and 13, respectively. Tree-of-heaven species showed an increase on the number of the productive logs, but only from 1 to 2 logs. In terms of mushroom mass production, only a very tiny quantity of mushrooms was harvested from tree-of-heaven logs.

Summarizing, 146 of the inoculated 240 logs survived for three years after inoculation process. The other 94 logs were missing entirely or were not in a suitable condition to soak for forced fruiting. The number of productive logs among the surviving logs differed by species. In the second harvest, 25 of 40 surviving white oak logs produced mushrooms; 27 of the 32 surviving sweet gum logs gave mushroom yield; 13 of the 37 surviving red maple; and 2 of surviving 37 tree-of-heaven produced mushrooms. Thus, of the 60 logs of each species initially inoculated, there were the most sweetgum logs still producing. However, the kilograms of mushrooms produced per log also varied by species. Even though there were the most productive logs of sweetgum, the highest production was on the white oak logs with 8.6 kg total in the first harvest, and 5.12 kg total in the second, compared to sweetgum with 4.5 kg total in the first harvest and 3.31 kg total in the second harvest.

DISCUSSION

Shiitake cultivation is affected by numerous parameters. Since forest farming is subject to environmental influences, it is hard to perfectly control outside factors. In our experiment, we controlled some of those factors, by soaking logs of different species on the same day and by stacking them in close proximity. We also measured and controlled for factors other than species that could affect the production of mushrooms on tree logs. Tree species (white oak, tree-of-heaven, red maple, sweetgum) and mushroom strains (west wind, naturalized *L. edodes*) were the main variables of interest. In order to test the effects of tree species on shiitake production, we also controlled for log weight, log length, disease existence and shape deformation of the logs.

For both harvests (in June and September), we have information on the total number of productive logs and total amount produced by species. We tested the difference in the weight of mushrooms produced and the number of productive logs across species. We confirmed the conventional wisdom that white oak is more likely to produce shiitake than red maple and tree-of-heaven, and there were also the greatest number of productive logs remaining of white oak. Sweetgum was not significantly different from white oak in terms of likelihood of production and production amount. In the first harvest, the number of productive logs was higher on white oak than sweetgum and in the second harvest, this number decreased for both species but less so among sweetgum logs. It is generally recommended that logs need to rest 4-6 weeks after soaking (Mudge et al., 2010). In our experiment, approximately 13 weeks elapsed between soakings. Nonetheless, total mushroom production and the number of productive logs was lower in the second harvest.

In the second harvest, data on mushroom production were recorded for each log, and two different models were estimated. First, we estimated a model of the probability that a log yielded a measurable amount of mushrooms (equal to or more than 0.1 kg). We find that log length

positively affects the likelihood of production of the logs. Length of the log was unexpectedly significant in the logit model, even after controlling for species. The greater amounts of mushrooms produced in both harvests were from white oak and sweetgum species, and these are the species that have relatively longer logs.

In the logit model, inoculation with the LE strain affects the likelihood of production negatively and significantly. West wind strain is a warm weather strain and adapted to weather conditions in the experimental area, while LE strain was found naturally growing in the area and has not yet been identified as a warm or cold weather strain. In this experiment, mushrooms were harvested in June and September, with summer conditions that are well suited for the west wind strain. Before reaching firm conclusions about strains, they should also be tested in the fall and spring. The LE strain may be more productive in cooler months.

Using OLS, we also modeled the quantity of mushrooms as a function of tree species, mushroom strain, log weight, log length, disease, and shape abnormality, using the sample of logs that had positive production. Log weight has a positive and statistically significant effect on the quantity of mushrooms produced, possibly because those logs also have more surface area that was inoculated, and/or possibly because they were more completely submerged in the soaking tanks. The estimation results suggest no difference among species. However, only two tree-of-heaven logs had positive production, resulting in a large standard deviation for this coefficient.

Other variables were not significant in either the logit or the OLS. Shape abnormality and disease existence were not statistically significant, possibly because these were imprecisely measured. However, multi-collinearity could also have been an issue, as logs of tree-of-heaven had more deformed and diseased logs than logs of other species. There was clearly less

production on tree-of-heaven logs, but that species indicator also did not have a statistically significant coefficient in the models.

In sum, our results confirm that white oak is among the most suitable species for shiitake production, because the logs are durable, likely to continue producing even after three years, and have the highest productivity. Sweetgum appears to be the best alternative. Besides being productive and durable, this species can sometimes show invasive behavior in the eastern US and thus may need to be removed (Adams et al., 2015). Operations to clear this invasive species could be a source of logs for forest farming and cultivation. The OLS regression results also suggest red maple as a possible substitute for white oak.

One weakness of this study is that we only have data on each individual log from the second harvest, making it difficult to explain differences between the first and second harvest. Another statistical challenge is the variable number of logs remaining of each species, including only a very few of the logs of some species actually producing shiitake. Finally, because the logs were left on the ground and unmonitored for years, some of them may have had internal diseases that were not visible.

Future research should examine the characteristics of different species, rather than simply treating species as a categorical variable. These characteristics could include wood density, sapwood to heartwood ratio (cf., Tokimoto 2005), bark structure, and the nutrients and minerals contained in the wood. Identification of the specific diseases on the logs could also potentially lead to insights about the factors driving production.

Conclusion

In conclusion, sweetgum species could provide an alternative log species for the cultivation of shiitake mushrooms, especially when available from operations to clear sweetgum

trees from areas that they have colonized. Further experimentation with more detailed data collection on the logs themselves is recommended.

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