UNIVERSITY OF WISCONSIN-LA CROSSE

Graduate Studies

ONE MAN GATHERS WHAT ANOTHER MAN SPILLS

CULTIVATING OYSTER MUSHROOMS

ON INVASIVE PLANTS:

AN ALTERNATIVE

SUBSTRATE

A Chapter Style Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

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By Kyle Kaszynski

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ABSTRACT

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Invasive plant species are taking over forests and other ecosystems globally, creating a large reservoir of unused lignocellulolse biomass. In the upper-midwest, buckthorn (*Rhamnus cathartica*) and honeysuckle (*Lonicera maackii*) are two prevalent woody invaders of forest ecosystems. The objective of this study was to determine if these forest exotics could be successfully used as a sustainable alternative substrate for mushroom cultivation. Since *Pleurotus* species produce a wide array of lignocellulolose degrading enzymes and can break down many different substrates and contaminants, members of this genus were chosen for the study. Two strains of the fungus were chosen for this study; one that is used in commercial cultivation practices and one that was isolated from a fruiting body found growing near the invasive plants used in the study. The two strains of oyster mushroom were grown on buckthorn and honeysuckle along with control treatments of oak and straw. Time to colonization, time to first harvest, first yield, total yield, and biological efficiency (BE) were all measured and then compared to the control treatments. Although both strains of *Pleurotus* performed best on the straw substrate, there was no significant difference within a strain of the yield and BE on the buckthorn, honeysuckle, and oak substrates. These results suggest that invasive species can provide an alternative sustainable substrate compared to currently used hardwood woodchips.

TABLE OF CONTENTS

PAGE

LIST OF FIGURES vii

LIST OF TABLES viii

CHAPTER I: INTRODUCTION 1

CHAPTER II: LITERATURE REVIEW 4

Invasive Plants 4

Fungi 12

Oyster Mushrooms 14

Mushroom Cultivation 17

Research Objectives 19

Research Questions: 19

Hypotheses and Research Aims: 20

CHAPTER III: MATERIALS AND METHODS 22

Overview- 22

Culture collection 23

Spawn preparation 24

Invasive species removal 24

Making woodchips 26

Inoculation of woodchips with local and commercial *Pleurotus ostreatus* 26

Inoculation of straw with local and commercial *Pleurotus ostreatus* 27

Primordia Initiation 28

Fruitbody Development 30

Experimental Design: 30
Statistical analysis 31
CHAPTER IV: RESULTS 33
Strain comparison in petri dish 33
Days to colonization 34
Days to First Harvest 35
First Yield 37
Total Yield 40
Biological Efficiency 42
CHAPTER V: DISCUSSION 45
Strain Selection 45
Substrate 46
REFERENCES 52

LIST OF FIGURES

FIGURE PAGE

1. *Lonicera maackii* distribution in the U.S. 6

2. *Lonicera maackii* distribution classification in Wisconsin. 6

3. Distribution of *Rhamnus cathartica* in the U.S. 8

4. *Rhamnus cathartica* distribution in Wi. 9

5. World mushroom production per country. 18

6. Bark and leaves of buckthorn, honeysuckle, and oak. 25

7. Mushroom fruiting chamber. 29

8. Flow chart of experimental design. 32

9. Flow chart of straw design. 32

10. Mean days to colonization. 35

11. Primordia of commercial strain and local strain 36

12. Mean day to first harvest 37

13. Fruiting bodies 38

14. Mean first yield fresh weight of fruiting bodies 39

15. Fruiting bodies 37

16. Mean total yield of fresh weight of fruiting bodies 41

17. Mean biological efficiency 43

LIST OF TABLES

TABLE PAGE

1. Estimated production of edible cultivated mushrooms by species.. 17

2. Mean growth of local and commercial strains of *Pleurotus* 33

3. General growth patterns of commercial and local strains of *Pleurotus* on buckthorn, honeysuckle, oak, and straw substrates. 44

CHAPTER I

INTRODUCTION

The twenty-first century will bring a number of new challenges to humans due to an increase in the world population. The world population was a little over 6 billion people at the end of the 20th century and is currently over 7.3 billion (census.gov April 2016). The rapid increase in population size brings new issues for mankind to face, such as a need to produce more food, an increase in pollution, and an overall decline in human health (Chang and Miles 1989). The demand to supply more food will require sustainable and efficient methods to produce nutritious food products. New methods will focus on maximizing yield and decreasing time to harvest.

In addition to influencing the harvest, sustainable and efficient methods would also reduce the amount of pollution produced by farming practices. Anthropogenic forces are causing a rise in the amount of pollution in the world. Some forces include automobile traffic, fertilizer, and pesticide use. Therefore, the need to produce more food has directly and indirectly increased the amount of pollution being produced. This increase in pollution has consequences such as decreasing the value of natural environments, allowing exotic species to invade new territory, and also has been linked to a decline in human health (Dockery *et al.* 1992).

Living unhealthy lifestyles in polluted environments has led to a decline in human health. Poor eating habits have contributed to heart diseases being one of the leading causes of death in the world (Murray and Lopez 1997). Changes need to be made to human diet to improve the health of the human population. It has been shown that certain species of fungi can provide food to help feed an increasing population, can degrade various pollutants, and may also provide compounds to improve human health (Chang and Miles 2001). The beneficial impact that fungi can have on the welfare of mankind in the 21st century has been termed the 'nongreen revolution' (Chang and Miles 2004). Therefore, it is critical that scientists study these organisms to optimize the potential for utilizing fungi for our benefit. This includes advancing the understanding of fungal ecology, characterizing extracellular enzymes produced by fungi, and enhancing cultivation techniques.

Mushrooms are one group of fungi that provide a good source of nutrients and improve human health (Chang and Buswell 1996). Many mushrooms contain all the essential amino acids in favorable ratios, contain a high amount of protein, and are a good source of vitamins (Chang and Buswell 1996). In addition to providing a functional food for people, some mushrooms can also act as a "nutriceutical," which means that an extract can be made from the mushroom and/or mycelium that can be taken as a dietary supplement to improve health (Chang and Buswell 1996). Therefore, incorporating more mushrooms into our daily lives, both as functional foods and nutriceuticals, may help better human health (Cheung 2010).

It has been shown that some mushroom-producing fungi can help feed a growing population, can degrade certain contaminated sites, and can also improve

human health. However, there are still more environmental issues that mankind must deal with. Another problem facing a number of different ecosystems are invasive species. Invasive plants are colonizing new territory and out-competing native species. This then leads to a change in the insect community, followed by a change in the avian community, and so on up the food chain. Again, fungi may be able to help control this problem. Woody, invasive plant species, which are comprised mainly of cellulose, lignin, and hemicelluloses, can be degraded by various fungi. Therefore, cultivators can utilize these sources of lignocellulolytic material as a substrate to grow edible mushrooms to help feed people, clean polluted areas, improve health, and also increase the health and value of the forest ecosystem.

CHAPTER II

LITERATURE REVIEW

Invasive Plants

Invasive plant species are a critical problem in many ecosystems that has led to numerous consequences (Wang *et al.* 2012). Since invasive plant species are altering the dynamics of many different ecosystems, a restorative techniques are needed that are economically and ecologically effective for removing, or at least controlling, invasive plants. There are various practices in place for the eradication of invasive plants, but none that are sustainably utilizing the biomass of these invasive plants. Sustainable methods would remove the invasive species from the ecosystem, and then use this forest waste product in an economically and ecologically beneficial way. Therefore, there is a need for a technique that will break down this woody biomass and make a usable product such as food, compost, or fertilizer.

Exotic invaders are creating a serious problem for ecosystems and, coupled with habitat loss and climate change, leave less room for endangered animals and plants by degrading natural habitats (Poulette and Arthur 2012). Invasions of exotic plant species allows for less space and water for native plants, and invasives have also been shown to affect nutrient cycling, decomposition, and disturbance regimes (Vitousek *et al.* 1997). Therefore, it is thought that invasive plants can shift

interspecific interactions, thereby reducing biodiversity (Heneghan *et al.* 2006). In addition to invasive plants decreasing biodiversity, they are also decreasing the overall value and productivity of these forest ecosystems, especially in the midwest (Miller and Gorchov 2004, Poulette and Arthur 2012).

Throughout the midwest, there are two common invasive woody shrub species that are altering ecosystem dynamics, European buckthorn (*Rhamnus cathartica)* and bush honeysuckle (*Lonicera maackii*). Each has been shown to change soil properties (such as pH and nutrient ratio), thereby enhancing their ability to invade new areas (Heneghan *et al.* 2006, Poulette and Arthur 2012). Also, invaders may be able to adapt to new or changing surroundings more quickly than native plants, making exotic invaders even more harmful (Evans *et al.* 2013).

Bush honeysuckle is an example of a very successful invasive plant that originates from Asia and is particularly problematic in the midwest. Bush honeysuckle is in the plant family Caprifoliaceae and is a deciduous shrub that can grow up to six meters in height. This plant produces yellow and white fragrant flowers in the summer, which develop red berries that are eventually dispersed by birds. It has not yet invaded much of Wisconsin (Figure 1), but Wisconsin still classifies this species as prohibited or restricted (Figure 2). Not only does this exotic plant demonstrate tremendous plasticity in many morphological traits, but it has also been shown to alter the microhabitat surrounding the plant by changing soil properties, such as pH, carbon to nitrogen ratio and water availability (West *et al.* 2010). These traits coupled with climate change, which may allow for more northern colonization, and anthropogenic disturbances, which create new habitats

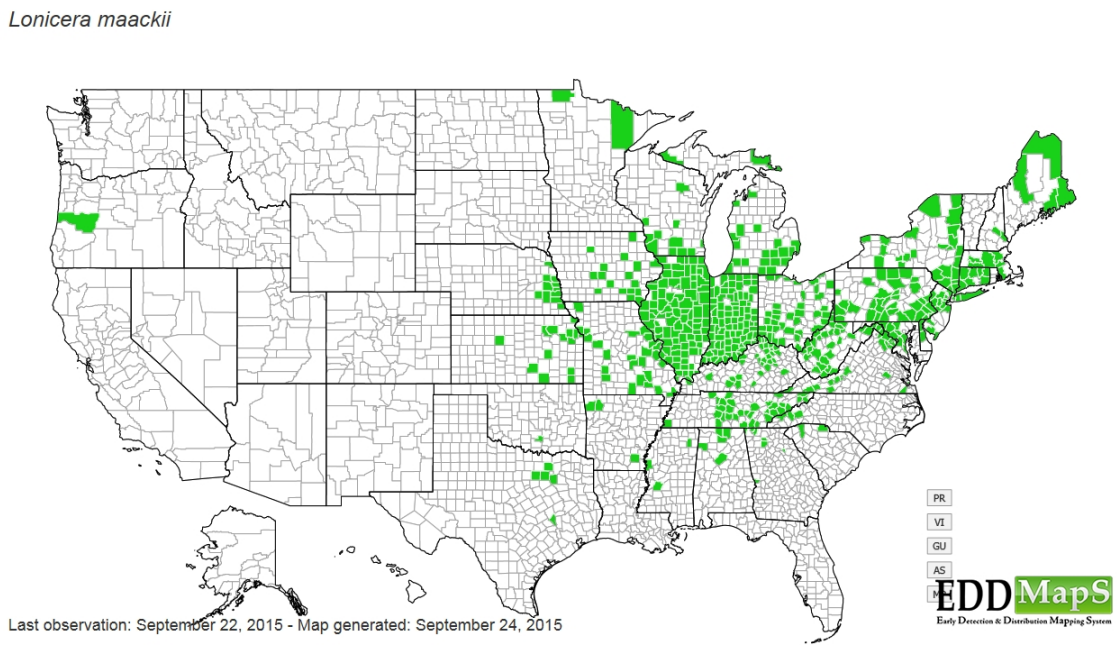

Figure 1. *Lonicera maackii* distribution in the U.S. Image taken from www.invasive.org.

Figure 2. *Lonicera maackii* distribution classification in Wisconsin. This species is prohibited in red counties and restricted in orange counties. Image from dnr.wi.gov.

for exotic species, allow for even more invasion and for an expansion of the range of *Lonicera* species (Wang *et al.* 2012). Interestingly, bush honeysuckle plants from more northern regions have been found to grow larger, produce more seeds, and thus have a greater capacity to invade novel habitats (Evans *et al.* 2013).

Invasion into forest ecosystems by *L. maackii* has also been shown to decrease growth and fecundity of native perennial forest herbs (Miller and Gorchov 2004). In turn, this can affect plant communities and entire ecosystems. Invasive plants in general cost the United States about $34 billion dollars in damage and control each year (Miller and Gorchov 2004). Clearly, invasive plants have a negative impact on ecosystems diversity and even the country's economy.

Similar to bush honeysuckle in many regards is the woodland invader common buckthorn (*Rhamnus cathartica)*. Buckthorn is in the plant family Rhamnaceae and is a deciduous shrub that can grow up to 10 meters tall, producing yellow flowers in late summer that turn into black drupes in early fall. The fruits (and seeds) are bird dispersed. The drupes contain a compound called emodin, which acts as a laxative to the birds, causing the birds to widely disperse the plant seeds as they move throughout the ecosystem. It has been shown that buckthorn can alter nutrient composition, mineralization rates, pH and even soil moisture, leading to altered ecosystems that may persist for many years (Heneghan *et al.* 2007).

Common buckthorn is a shrub from Eurasia that was brought to America as an ornamental in the 19th century, where it has invaded many ecosystems,

especially in the northern Midwest (Figure 3; Knight *et al.* 2007). High fecundity, fruit and seeds that are dispersed by birds, high percentage of seed germination, ability to colonize disturbed areas, and production of secondary compounds (such as allelochemicals) contribute to the successfulness of this invader (Knight *et al.* 2007). When buckthorn invades a new ecosystem, the structure and services of the ecosystem change. This in turn alters carbon storage and carbon cycling (Larkin *et al.* 2014).

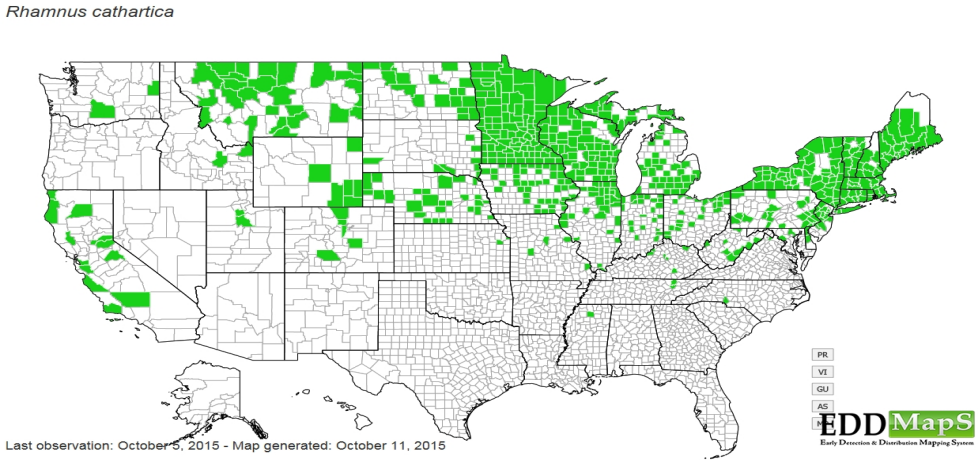

Figure 3. Distribution of *Rhamnus cathartica* in the U.S. Image from www.invasive.org.

Rhamnus cathartica is a particularly aggressive invader in the northern midwest ecosystems (Figure 4). Invasion of *Rhamnus cathartica* co-facilitates the invasion of an exotic earthworm, creating an 'invasional meltdown' (Heneghan *et al.* 2007). This increase in the invasive earthworm population can lead to an increase in the rate of litter decomposition, which facilitates more growth of *R. cathartica.*

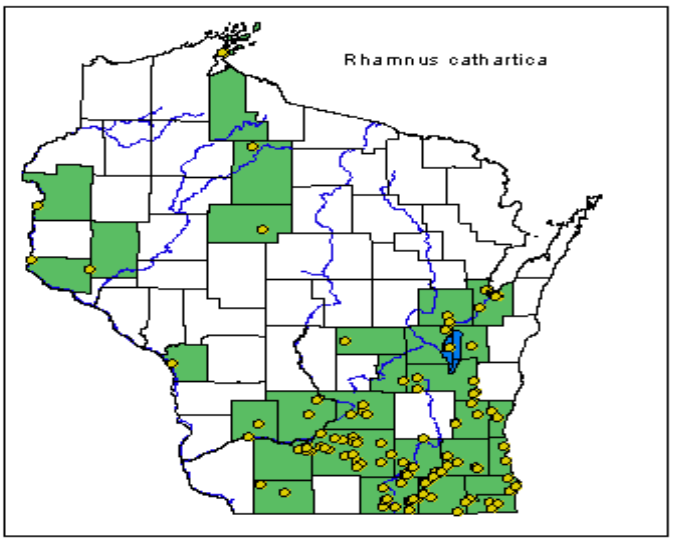

Figure 4. *Rhamnus cathartica* distribution in Wi. Image from www.botany.wisc.edu.

It is worthwhile noting that the anatomy of *Rhamnus* species makes it more resistant to degradation, meaning the dead plant material can stay in the ecosystem for a longer period of time. The reason for this is the large vessel elements that contain high amounts of lignin in the wall, leading to increased lignin content overall in the xylem (De Micco and Aronne 2012). Since lignin is a recalcitrant molecule, this makes buckthorn more resistant to degradation. In forest ecosystems, nitrogen is typically tied up in living organisms, since there is no nitrogen in lignin or cellulose, making it a limiting resource (Barron 2003). This poses a threat to ecosystem health because all organisms require nitrogen to grow and live. Buckthorn is better at assimilating nutrients from the environment than many other native plants, altering nutrient cycles so that many native plants are unable to grow (Knight *et al.* 2007).

Understanding how invasive species change and shift entire ecosystem dynamics is key to developing strategies for the conservation and restoration of natural areas (Heneghan *et al.* 2007, Knight *et al.* 2007, Larkin *et al.* 2014). When buckthorn is found growing along with honeysuckle, both species produce dense thickets that displace native seedlings and adult plants, also reduces sunlight, and decreases the herbaceous species richness (Larkin *et al.* 2014).

Therefore, something must be done to at least slow invasion of exotic plants before the native plant communities have vanished. There have been many attempts to eradicate invasive plants by using various methods, including manual removal and spraying chemical herbicides. When the plants are manually removed, they are typically piled and then burned, wasting many organic nutrients and releasing excess CO_2 into the environment (Das and Mukherjee 2007).

Removing the invasive plant species and using the woody biomass as a substrate to cultivate edible mushrooms to feed growing populations may benefit both forest ecosystems and mankind. In many areas of the world where woody substrates may not be readily available, researchers are experimenting with growing edible mushrooms on weed plant species (Das and Murkherjee 2007, Mintesnot *et al*. 2014, Fernandes *et al.* 2015, Alananbeh *et al.* 2014). The woody substrate from the manual removal of the invasive shrubs is composed mainly of lignocellulolytic biomass. This biomass is made primarily of cellulose, hemicellulose, and lignin (Manavalan *et al*. 2014). Various wood decay fungi are known to degrade these compounds, forming simple compounds from these complex polymers (Lang *et al*. 1996, Manavalan *et al*. 2014). More on this later.

It is important to develop a plan for the management of invasive plant species before they take over more territory. As described earlier, manual removal followed by burning the plant material is one technique in place. However, disposal of plant material in this way leads to increased pollution through CO_2 release, and there is a large waste of organic nutrients that could be used as a fertilizer or a substrate to grow a high protein food source (Das and Mukherjee 2007). The technique tested here is to manually remove the invasive plants, allow them to dry, make woodchips of the material, and then inoculate the plant material with the mycelium of a local oyster mushroom (*Pleurotus* species). Using fungi to breakdown cellulose/lignin based plant materials is currently being researched as a way to rid these waste products and produce a protein, mineral, and vitamin rich food source (Mintesnot *et al.* 2014). After mushrooms have been harvested and the fungus has decomposed the plant biomass, the colonized woodchips that have already produced mushrooms can then be used as spawn to install outdoor mushroom beds that will continue to produce mushrooms. Alternatively, the spent, colonized substrate can be made into mushroom compost that can be used as a fertilizer to grow more edible crops. Therefore, it is important to understand the role of fungi in their ecosystems so we can better use these organisms to benefit humankind.

Fungi

In nature, there are various organisms that degrade recalcitrant molecules, allowing other organisms access to nutrients (Ashton *et al.* 2005). Fungi are organisms that can break down forest waste products into nutrients that plants are then able to assimilate. By cutting down invasive plant species and inoculating the dried plant material with a particular fungus, the fungus would be able to transform the plant material into compost that can be used to fertilize local gardens and also produce more mushrooms for consumption and/or sale by people, benefiting both the ecology and the economy of the local area.

Mushrooms are the epigeous or hypogeous fruiting bodies of macrofungi belonging to the phyla Ascomycota or Basidiomycota (Chang 2001) that are large enough to see with a naked eye and be picked by hand. Filamentous fungi grow via thin, thread-like structures called hyphae. Saprotrophic, mushroom-producing fungi are eukaryotic organisms that break down organic molecules in nature through the use of extracellular enzymes. However, this is only one example of how fungi can get their nutrients.

There are three main nutritional modes of fungi: saprophytic, mutualistic and parasitic. Saprotrophic fungi obtain nutrients by breaking down dead organic material. Mutualistic fungi have some sort of symbiotic relationship with other living organisms where both organisms benefit. Parasitic fungi get nutrients by stealing them from another living host, thereby causing the host some sort of harm. The nutritional mode of interest for this project is saprophytic fungi.

Saprophytic fungi obtain energy by breaking down large organic molecules thereby recycling nutrients back to the ecosystem. By secreting exoenzymes out of the growing tip of the hyphae, and breaking down complex molecules into smaller ones, fungi can then ingest the simpler molecules through active transport. Once the substrate has been partially degraded, the hyphae can then utilize turgor pressure to grow through the weakened substrate. All of the hyphae make up the vegetative body of the fungus, known as the mycelium.

It is important that the fungus secretes these extracellular enzymes with a manner of control, only at the growing hyphal tip, ensuring that competitors do not steal nutrients or invade the degraded substrate (Moore *et al.* 2011). Some fungal species are specific about the type of substrate they can break down, whereas others are more generalists. The particular substrate that is utilized depends on the type of fungus.

Saprotrophic wood decay fungi are classified as either white rot or brown rot. The former break down lignin, leaving the cellulose, while the latter break down cellulose and leave lignin. Most white rot fungi are simultaneous white rot fungi meaning that they degrade lignin as well as cellulose and hemicelluloses. These fungi are primarily responsible for the decomposition of lignocellulosic plant material. One such genus of simultaneous white rot fungi, that has a suite of enzymes capable of degrading a large number of substrates, is *Pleurotus* (Lang *et al.* 1996, Manavalan *et al.* 2014).

Oyster Mushrooms

Cultivation of edible exotic and specialty mushrooms is gaining increased popularity, and currently *Pleurotus* species are the third most commonly cultivated mushroom in the world (Wang *et al.* 2015). Oyster mushrooms (*Pleurotus* spp.) are rich in lignocellulytic enzymes, making them a perfect candidate for breaking down the large organic compounds of these invasive plants into smaller molecules (Mintesnot *et al.* 2014). Members of the genus *Pleurotus* are classified as white rot fungi that possess lignocellulosic-degrading enzymes, including many non-specific peroxidases and laccases that have the ability to degrade various different compounds (Lang *et al.* 1996, Mintesnot *et al.* 2014). Oyster mushrooms are found in tropical and subtropical forests all over the world and are easy to cultivate in artificial settings (Fernandes *et al.* 2015). Their mycelium is able to degrade a wide variety of substrates, from weed plants to paper scraps to coffee grounds to diapers (Fernandes *et al.* 2015, Espinosa *et al.* 2015). Members of *Pleurotus* are primary decomposers, with the ability to degrade different complex phenolic polymers (Mintesnot *et al.* 2014). Therefore, *Pleurotus ostreatus* was chosen in the current study as the edible mushroom-producing fungus to degrade the woodchips of *Rhamnus cathartica* and *Lonicera maackii*.

There has been much research done outside of the United States looking at growing *Pleurotus* species on various "waste" substrates. One such study in India (Das and Mukherjee 2007) experimented with cultivating *Pleurotus* species on local abundant weed plants and also supplemented their typical substrate (rice straw) with various dried weed plants (Das and Mukherjee 2007). They found that

supplementing substrates with some of the weed plants actually decreased the time to fruiting, whereas mushrooms grown on other weedy plants increased the protein content of the mushrooms (Das and Mukherjee 2007).

A group in Saudi Arabia (Alananbeh *et al.* 2014) studied the efficiency of using common agricultural wastes as a way of cultivating oyster mushrooms as part of "the water and environment friendly agriculture strategies" initiative. The group used date palm leaves supplemented with other agricultural wastes as the substrate for fruiting the mushrooms. They found that the mushrooms grown on the substrate supplemented with agricultural wastes (wheat straw) contained higher fiber content (carbohydrates) and also more potassium. These researchers concluded that *Pleurotus* species are able to help manage agricultural waste by converting lignocellulosic material into protein-rich biomass.

Fernandes *et al.* (2015) researched the nutritional constituents of a *Pleurotus* species that was grown with paper scraps as a substrate. They used blank paper and paper with printed ink as substrates with wheat straw as a control. They found that the fungus was able to colonize all substrates and produce mushrooms. Although the biological efficiency and nutritional content of the mushrooms grown on paper were not as high as those on the wheat straw control, the study still offered a novel way to reduce paper waste.

A graduate student in Hawaii researched the possibility of cultivating oyster mushrooms on local fast growing tree species (Tisdale 2004). They selected five different woody species that had abundant plant biomass. The student was able to cultivate oyster mushrooms on all of the substrates and then determined which

plant substrate was most efficient for growing mushrooms to produce a profit. The study also noted that neither cellulose nor lignin content nor nutritional content of the plant material had an effect on the biological efficiency or economic yield of the mushrooms produced.

While the above studies demonstrate the wide variety of substrates *Pleurotus* can successfully colonize, the most commonly used substrate for cultivating oyster mushrooms is enriched sawdust and/or woodchips of hardwood tree species such as oak, maple, and poplar (Stamets 1993). The material from hardwood trees is comprised mainly of lignin and cellulose compounds. However, many species of hardwood trees take a long time to fully mature and have high economic value (lumber, furniture, etc.). Thus, removal of these trees for the sole purpose of cultivating mushrooms is not economically or environmentally sustainable. The woody substrate from the manual removal of the invasive shrubs (buckthorn and honeysuckle) is also composed primarily of cellulose, hemicellulose, pectin and lignin (Manavalan *et al*. 2014). Currently there are no techniques that utilize the woody plant biomass produced by these invasive species to grow food crops to feed a growing population. The technique proposed here removes the invasive plants from forestland and then uses that biomass as a substrate to produce edible specialty mushrooms that are delicious and nutritious. With a growing population and increased development of land, plant habitats are frequently becoming degraded. This leaves less space for native plants and leads to a decrease in overall biodiversity. Therefore it is important that a restoration technique is developed that helps restore habitats to a place that supports native plant growth and rejuvenation, while at the same time being environmentally and

economically viable.

Mushroom Cultivation

People have been cultivating edible mushrooms (*Auricula auricula*) since at least 700 A.D. (Moon and Lo 2014). Then, around 1000 A.D., people began to cultivate *Lentinula edodes* (Shiitake or Shianju) on logs in China (Sánchez 2004). In Paris, around 1650 A.D., the Europeans began cultivating *Agaricus bisporus*, the common button mushroom (Chang and Miles 1984). These two mushrooms (*Lentinula* and *Agaricus*) accounted for the majority of cultivated mushrooms in the 1980's at 14% and 70% of the market, respectively (Chang and Miles 1984). Recently, however, there has been a shift to cultivate more exotic varieties, including an increased production of *Pleurotus* species (Table 1). Although mushrooms are both a delicacy and a staple in some cultures, cultivation of gourmet and exotic species of edible mushrooms only began gaining popularity within the past few decades in the United States (Chang and Miles 1989). These gourmet, exotic species of mushrooms are now considered "specialty mushrooms" in the cultivation industry.

Table 1. Estimated production of edible cultivated mushrooms by species. Adapted from Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products 2014.

Genus	% of World Cultivation
Agaricus	30
Pleurotus	27
Lentinula	17
Auricularia	6
Flammulina	5
Others	15

Mushrooms are an important food source because they have a high protein and mineral content, low fat content and sometimes medicinal value (Chang and Miles 1984). Not only are they important as a food source, but the spent mushroom substrate can be used as an effective fertilizer for crop plants (Chang and Yau 1981). However, only 45% of mushrooms are consumed fresh, whereas the remaining 55% are consumed in a canned or dried form (Moon and Lo 2014). This could be due to the lack of mushroom cultivators to provide fresh mushrooms to certain parts of the world. In most areas of the world, mushrooms are only produced outdoors seasonally, when environmental conditions are favorable. China currently leads the world in mushroom production, producing 47% of the world's mushrooms (Figure 5).

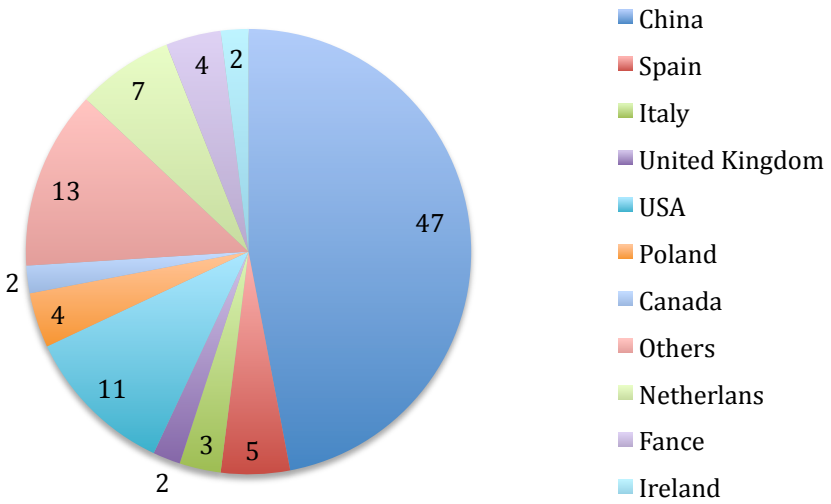

Figure 5. World mushroom production per country by percent. (Adapted from Harsh and Joshi 2008).

Enriched hardwood sawdust and straw are currently the most commonly used substrates for the commercial cultivation of *Pleurotus* mushrooms. These substrates can be expensive for cultivators to obtain in certain parts of the world. In addition, the decline of hardwood forests in the United States suggests that farmers should begin to experiment with alternative substrates for mushroom cultivation.

Research Objectives

The goal of this experiment was to develop new, inexpensive, sustainable substrates to use for cultivating mushrooms for a food source and a way to dispose of agricultural/forestery waste and plant material. Another goal was to compare the efficiency of a locally growing, wild strain of *Pleurotus* to a commercially available strain of *Pleurotus* that is known to perform well. The two strains were used to determine if the locally growing strain of *Pleurotus* was any more adept at degrading plants that were growing near the mushroom. Not only will this research provide ways to help restore ecosystems and also offer a way to stimulate local economies, but it may also offer insight into the ecology and physiology of *Pleurotus* species of fungi.

Research Questions:

Will a local ecotype of oyster mushroom (*Pleurotus* species) grow on the wood of locally abundant invasive the plant species *Lonicera maakii* (Bush honeysuckle) and/or *Rhamnus cathartica* (Buckthorn)? I want to see if the mycelium of *Pleurotus* species will be able to colonize the woody waste of these

invasive plants. Next, I want to determine if this plant biomass can supply enough nutrients to the fungus to support mushroom production.

How will the yields and biological efficiencies of *Pleurotus* grown on buckthorn and honeysuckle compare to that of the standard practice of growing *Pleurotus* on oak woodchips or straw? If the fungus can colonize the invasive plant material and can produce mushrooms from the substrates, I then compared the yield of mushrooms from the invasive plants to the yield of more traditional techniques for growing oyster mushrooms.

Hypotheses and Research Aims:

H_1-

Pleurotus mycelium will be able to colonize the woodchips of the invasive plant species but at a slower rate than the mycelium colonizes the oak wood chips.

H_{o1}-

There will be no difference in the amount of time that it takes the *Pleurotus* mycelium to colonize the various substrates.

H_2-

There will be different total yields of mushrooms on the various woody substrates.

H_{o2}-

There will be no differences between the total yields on the various substrates.

H_3-

The time to the first harvest will be shorter on the oak substrate than the invasive plant substrates.

H_{o3}-

There will be no difference in the time to first harvest across all substrates.

H_4-

The greastest biological efficiency (BE) will be on the oak substrate.

H_{o4}-

There will be no difference in BE across the different substrates.

H_5-

The commercial strain of oyster mushroom will out preform the local strain of oyster mushroom in days to colonization, first yield, total yield and biological efficiency.

H_{o5}-

There will be no difference between the two strains of oyster mushrooms for days to colonization, first yield, total yield and biological efficiency.

CHAPTER III

MATERIALS AND METHODS

Overview-

Invasive plant species (*Rhamnus cathartica* and *Lonicera maackii*) were cut and allowed to dry. The plant material was then made into woodchips. The woodchips were inoculated with either the spawn of a locally growing oyster

mushroom (*Pleurotus* species) or the spawn of a commercial strain of oyster mushrooms. The commercial strain of the fungus is known to produce well in culture and was compared to the local strain. Yield, biological efficiency, days to colonization and days to fruiting was measured for all treatments.

Culture collection

Samples of *Pleurotus* sp. fruiting bodies were collected in the Fall of 2014 and 2015 in Hixon Forest, La Crosse County, Wisconsin and in the summer of 2015 in Woodstock, McHenry County, Illinois. The samples were fruiting from dead *Quercus* species. Fruiting bodies were taken back to the lab at University of Wisconsin-La Crosse and tissue samples were taken from multiple basidiocarps: the mushroom was torn in half to expose the content, and tissue cultures were taken from the inside of the mushroom, just above the hymenium. The tissue was placed on malt extract agar (MEA) (BD Biosciences) and allowed to fully colonize the petri dish (7-10 days). MEA is the standard agar used to culture wood-decay fungi. Cultures were incubated in the dark, upside down at 26^{o}C. Samples were subsequently sub-cultured until a pure culture with only *Pleurotus* mycelium was achieved. The samples were also observed for speed of growth, susceptibility to contamination and vigor of mycelial growth. The strain that was quickest growing and with the least amount of contamination was chosen for this project.

A commercial culture of *Pleurotus ostreatus* obtained from Fungi Perfecti (Olympia, WA) that is known to show vigorous growth was used to compare an

established, high yielding strain to a strain cultured from a local area. This strain was grown on malt extract agar (MEA) as above.

Growth of each of these strains on MEA will be analyzed at 7, 10, 12, and 14 days. The diameter of mycelial growth will be recorded as a way to determine the speed and vigor of growth of each strain.

Spawn preparation

Spawn was grown on 100% organic rye berries from Mountain High Organics (Milford, Connecticut). The grains were boiled at a rapid boil for one minute and then removed from heat, and allowed to soak for 30 minutes hydrating the grains to a moisture content of ~55 percent. The water was then drained, and the grains were placed under cool running water until they were cool enough to touch. The grain was placed into one-quart glass Mason jars with holes drilled in the lids that utilize a piece of TyVek to act as a filter. The jars with the hydrated grain were then autoclaved at 15 psi (121.1^0C) for 90 minutes and allowed to cool in the autoclave. Once the jars had cooled, they were moved in front of a laminar flow hood. The jars were inoculated with ¼ of a fully colonized Petri dish, with mycelium cut into small pieces. Jars were then kept in a dark room at ~20^0C and allowed to colonize for 14-21 days.

Invasive species removal

The invasive plant species *R. cathartica* (buckthorn) and *L. maackii* (honeysuckle) were identified and removed from a property in Bull Valley, Illinois,

where all the species of interest were growing in close proximity. Plants were identified by leaf and bark morphology (Figure 6). The invasive plants were removed manually by cutting with a hacksaw about 5 cm from the forest floor. The plants that were chosen had a trunk diameter of 4-5 cm. Multiple large branches from a live burr oak (*Quercus macrocarpa)* were cut and allowed to dry to be used as the control substrate. After plants were cut and removed, the remaining stump was covered with a dark plastic bag and secured with wire to prevent sunlight from reaching the plant. This was done to prevent the stump producing new shoots of plant growth and the plant reestablishing. Plant material was dried in the sun for 10 days prior to making the wood chips to prevent binding of the wood chipper blades.

Figure 6. Bark (top) and leaves (bottom) of buckthorn, honeysuckle, and oak (left to right).

Making woodchips

After the plant material had been allowed to dry for 10 days, a Vermeer model BC600XL wood chipper with a 15.24 cm square feed was rented from Burris Rental Company in Volo, Illinois. The dried plant material was fed through the wood chipper separately and wood chips approx. 2-4 cm in length were made. The chips were then stored in separate 28-gallon plastic containers until ready for inoculation.

Inoculation of woodchips with local and commercial *Pleurotus ostreatus*

The bags that were used as a container for the substrate inoculation were 3 mil. polypropylene gusseted bags that were 5"X4"X18" and fitted with a 0.2 micron filter patch (Unicorn Bags, Plano, TX). Dried woodchips (250 g) were placed into separate bags. Tap water was then added to the woodchips to bring the moisture of the woodchips to 50%. Prior to the woodchips being hydrated, 100 g of woodchips were baked at a low heat for three hours. The woodchips were then re-weighed in order to determine the initial moisture content of the chips. Then the appropriate amount of water was added to bring the woodchips up to 50% moisture content. The bags with the hydrated, uninoculated woodchips were then sterilized in a commercial 41.5 quart All American pressure cooker at 15 psi (121.1^{0}C) for 90 minutes. The bags were allowed to cool in the sterilizer. Once cooled, the bags were inoculated in front of a laminar flow hood. Each bag (with the dry weight of 250 grams) was then inoculated with 30 grams of fully colonized rye berries. When the spawn is added to the woodchips the stage is known as the spawn run. The bags were then heat sealed with an impulse sealer and shaken to evenly distribute the

colonized rye berry inoculum. The bags of inoculated substrate were then transferred to a dark room with a temperature of ~20°F and allowed to colonize for 12-21 days. The number of days it took the fungus to fully colonize the substrate was recorded and compared.

Inoculation of straw with local and commercial *Pleurotus ostreatus*

Polypropylene gusseted bags (3 mil.) that are 22.5" x 8.25" x 4.75" and fitted with a 0.2 micron filter patch (Fungi Perfecti Olympia, WA) were used as a container for the straw substrate. The straw was chemically pasteurized prior to inoculation with the fungus as follows: chopped straw was placed in 32 Gallon rubber-made containers and filled with water until all of the straw was completely submerged. Then 10 grams of hydrated lime (calcium hydroxide) (Mississippi Lime, St. Louis, MO) was added to the water to bring the pH above 12. The lime was then thoroughly mixed in and the straw was allowed to soak in the solution for 24 hours. After 24 hours, the water was drained from the straw by placing the straw in a burlap sack for 1 hour. Once the straw was fully drained the straw was spread out on a tarp and allowed to further dry until a moisture content of 50% was achieved. This was done using the same method as was used to measure the moisture content of the woodchips. Hydrated straw (500 g) was placed into each container (bag fitted with a filter patch). The straw was then inoculated with 75 grams of spawn. The spawn was mixed thoroughly into the straw and the bags were heat-sealed. The bags of inoculated substrate were transferred to a dark room with a temperature of ~20°C,

relative humidity of ~30% and allowed to colonize for 12-21 days. The number of days it took the fungus to fully colonize the substrate was recorded and compared.

Primordia Initiation

When the bags of substrate were fully colonized (about 10-14 days after inoculation), the bags were moved to the fruiting chamber (Figure 7). A slit was cut into each bag to release the CO_2 that had built up in the bag and also to expose the mycelium to fresh oxygen. The increase in the O_2 to CO_2 ratio is one trigger to get the fungus to produce mushrooms (Stamets and Chilton 1983). The primordia chamber had 12 hours of light followed by 12 hours of dark, a light intensity of 1500 lumens, a relative humidity of 90-100%, a CO_2 concentration of <1000 ppm and an average temperature of 55^0F. The introduction of light and high humidity along with the drop in temperature are other signals for the fungus to begin producing primordia (Stamets and Chilton 1983). About one to two weeks later the bags were moved into the fruiting chamber, and primordia began to develop.

Figure 7. Mushroom fruiting chamber.

Fruitbody Development

After primordia formed, the environment was altered to support mushroom formation. In the new environment, lights were on a 12 h on then 12 h off timer, the light intensity was approximately 1500 lumens, the humidity was kept between 85-95% and the temperature was maintained from 12-18^{o}C . The CO_2 concentration was kept under 1000 ppm, and there were about 10 fresh air exchanges per hour. Once the mushrooms reached full maturity, the mushrooms were harvested by cutting the stipe as close to the mycelial block as possible. Biological efficiency (BE) was then determined by dividing the fresh weight of mushrooms (in grams) per block over the dry weight of substrate (in grams). BE is the standard method used to measure the productivity of different stains of mushrooms that are used in cultivation (Tisdale 2004).

Experimental Design:

This experiment was conducted in a randomized block design. There were three blocks in this experiment. Each block represented a time when five replicates from each treatment were inoculated. There were eight treatments with 15 replicates, for a total of 120 independent observations. The treatments were straw inoculated with wild *Pleurotus* spawn (control), straw inoculated with commercial *Pleurotus* spawn (control), oak inoculated with wild *Pleurotus* spawn (control), oak inoculated with commercial *Pleurotus* spawn (control), buckthorn inoculated with wild *Pleurotus* spawn, buckthorn inoculated with commercial *Pleurotus* spawn, honeysuckle inoculated with wild *Pleurotus* spawn, and honeysuckle inoculated

with commercial *Pleurotus* spawn (Figure 8 and Figure 9). All other variables such as environment, date inoculated, and inoculation ratio were held constant to reduce the chance of confounding effects.

Statistical analysis

The data were analyzed using SPSS v. 23.0 (SPSS Inc., Chicago, IL). A one-way repeated measures ANOVA was used to analyze the growth of the strains on petri dishes. A two-way ANOVA was used to analyze the days to colonization, days to first harvest, yields, and biological efficiencies of the three treatments. The main factors using the 2 way ANOVA were the strain and the substrate. Tukey's *post hoc* test was then used to determine differences between treatments at the 5% level of significance.

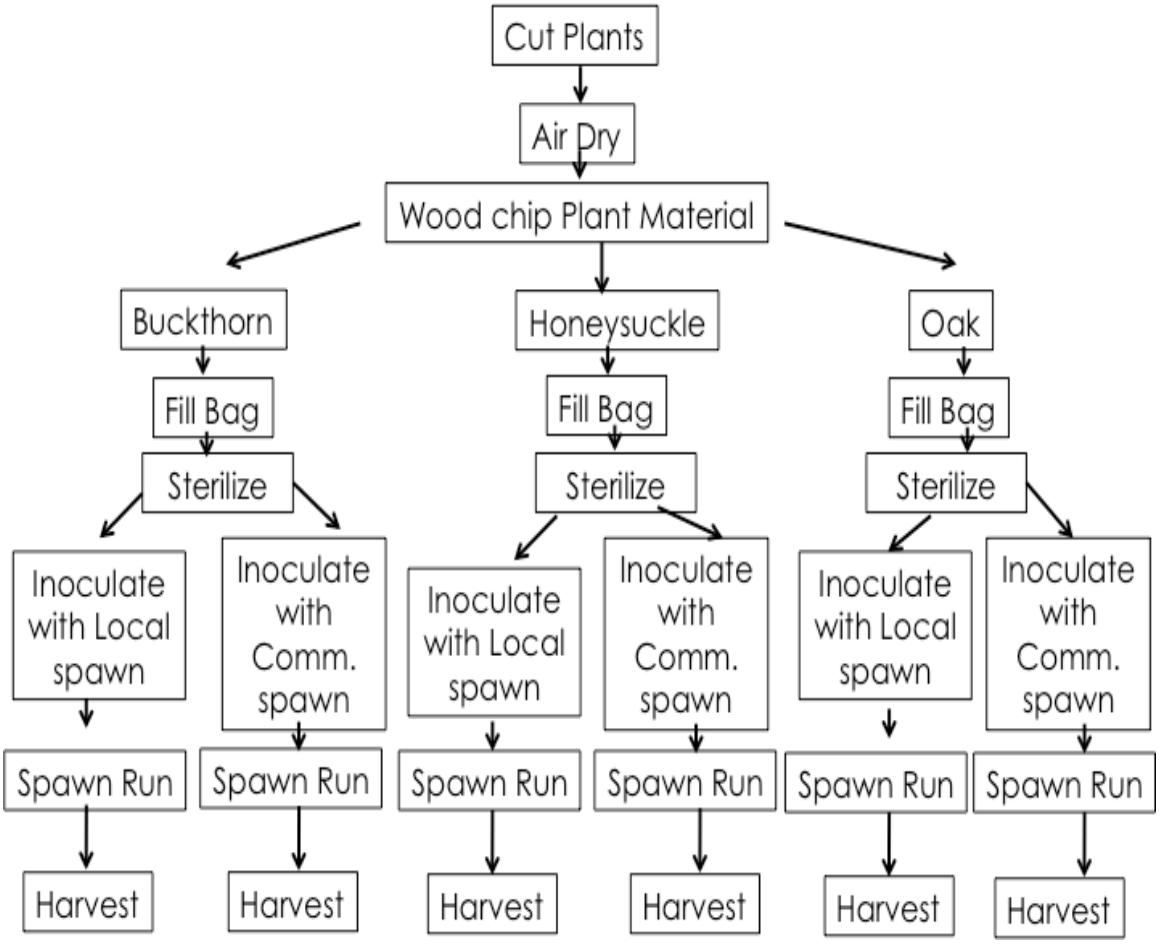

Figure 8. Flow chart of experimental design.

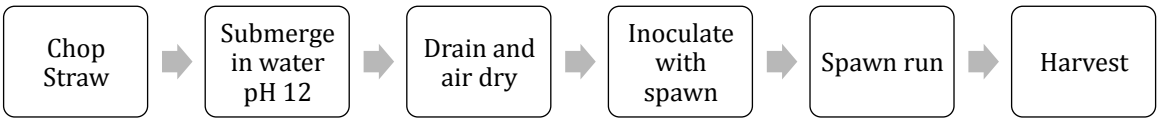

Figure 9. Flow chart of straw design.

CHAPTER IV

RESULTS

Strain comparison in petri dish

Both the local and commercial strains were grown on MEA and examined for radial growth. The first observable growth came after three days for both strains. Although the local strain of *Pleurotus* had a larger radial growth after 14 days than the commercial strain (Table 2), the two were not statistically significant different in growth diameter ($F(1,4)= 0.504$, $p=0.517$). Therefore, the hypothesis that the commercial strain of oyster mushroom would outperform the local strain was rejected. The commercial strain had more aerial hyphae and a more robust mycelium formed (Figure 9). The fastest growing culture of the local strain grew 7.2 cm in 14 days, whereas the fastest culture of the commercial strain grew 6.6 cm in 14 days.

Table 2. Mean growth (diameter in cm ± SD) of local and commercial strains of *Pleurotus* on MEA for 14 days. N=3.

Strain	7 Days	10 Days	12 Days	14 Days
Local	1.63±0.25	4.17±0.31	5.00±0.17	6.63±0.60
Commercial	1.57±0.21	3.87+0.64	4.77+0.38	6.50+0.17

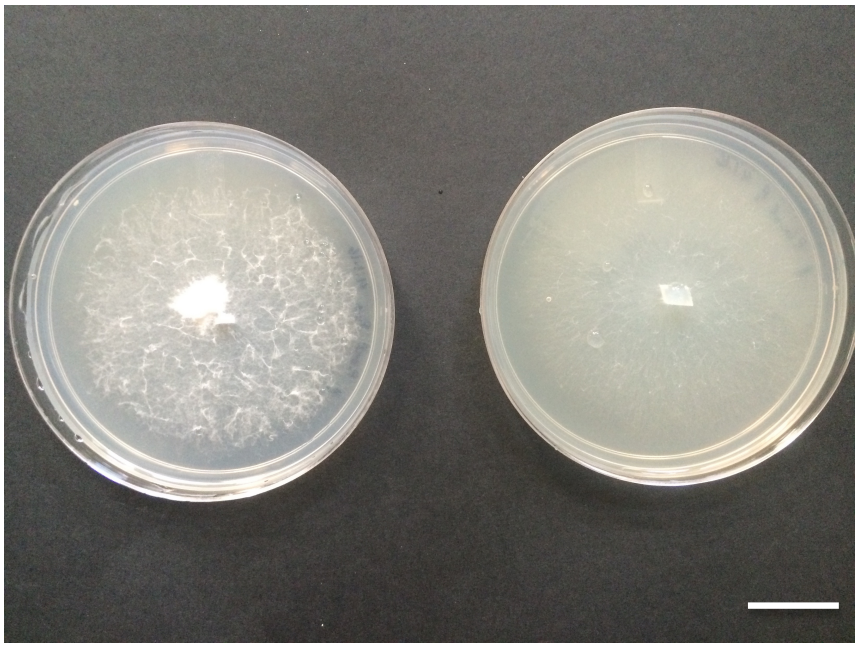

Figure 9. Commercial (left) and local strain of *Pleurotus* grown on malt extract agar. (14 days). Scale bar is 2cm.

Days to colonization

The number of days that it took the fungus to completely colonize the wood or straw substrate was measured (days to colonization). The commercial strain of *Pleurotus* took less time to colonize all of the substrates compared to the local strain ($F(1, 96)=47.72$, $p<0.05$). On average, the local strain took 2.4 more days to colonize the substrate. Straw was the substrate that took the longest to be colonized for both the commercial and local strains of fungus (an average of 12.5 and 15 days, respectively). For the commercial strain, the oak substrate was colonized quickest, an average of 10.7 days, whereas the local strain colonized the honeysuckle substrate the fastest, at an average of just under 12 days (Figure 10). The blocks colonized by the commercial strain again clearly had more robust, denser mycelium than the local strain.

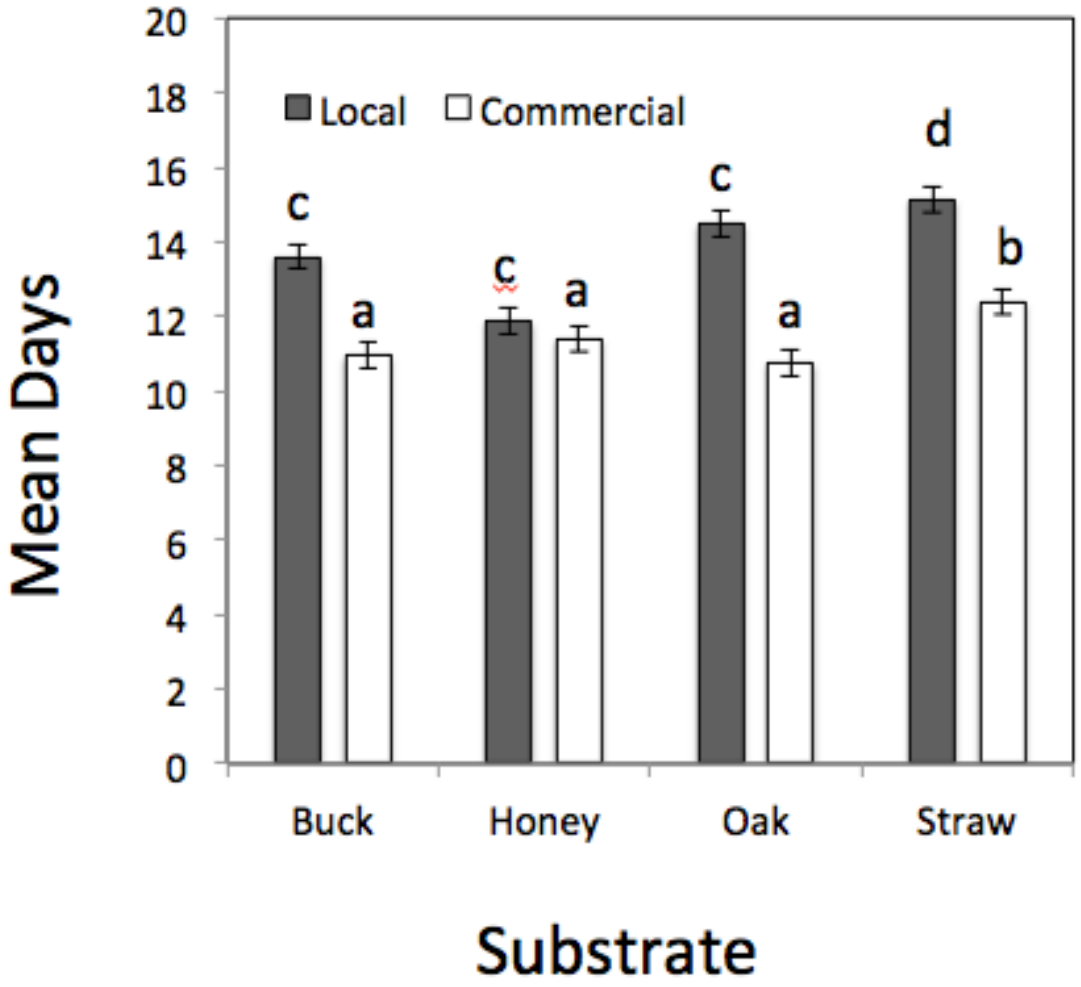

Figure 10. Mean (±SE) days to colonization for commercial and local strains of *Pleurotus* on 4 different substrates. N=15. Bars with different letters represent statistically different results (p<0.05).

Days to First Harvest

Once primordia were formed on each block (Figure 11), the environment was altered to allow the mushrooms to mature. Overall, there was no significant difference in the number of days it took for first harvest between the two strains of fungus (F(1, 2)=4.450, p=0.169). There was also no significant difference between

the substrates (F(3,6)=1.506, p=0.306). However, the commercial strain had the shortest time to the first harvest on the oak substrate, whereas the local strain was quickest on the honeysuckle substrate (Figure 12). The average days to the first harvest for the commercial strain was 32.3 days, and the average for the local strain was 34.5 days. The straw substrate overall took the most number of days to produce the first harvest for both stains of *Pleurotus* (Figure 12).

Figure 11. Primordia of commercial strain (top) and local strain (bottom) fruiting on buckthorn, honeysuckle, oak, and straw (left to right). Scale bar represents 1.8 cm.

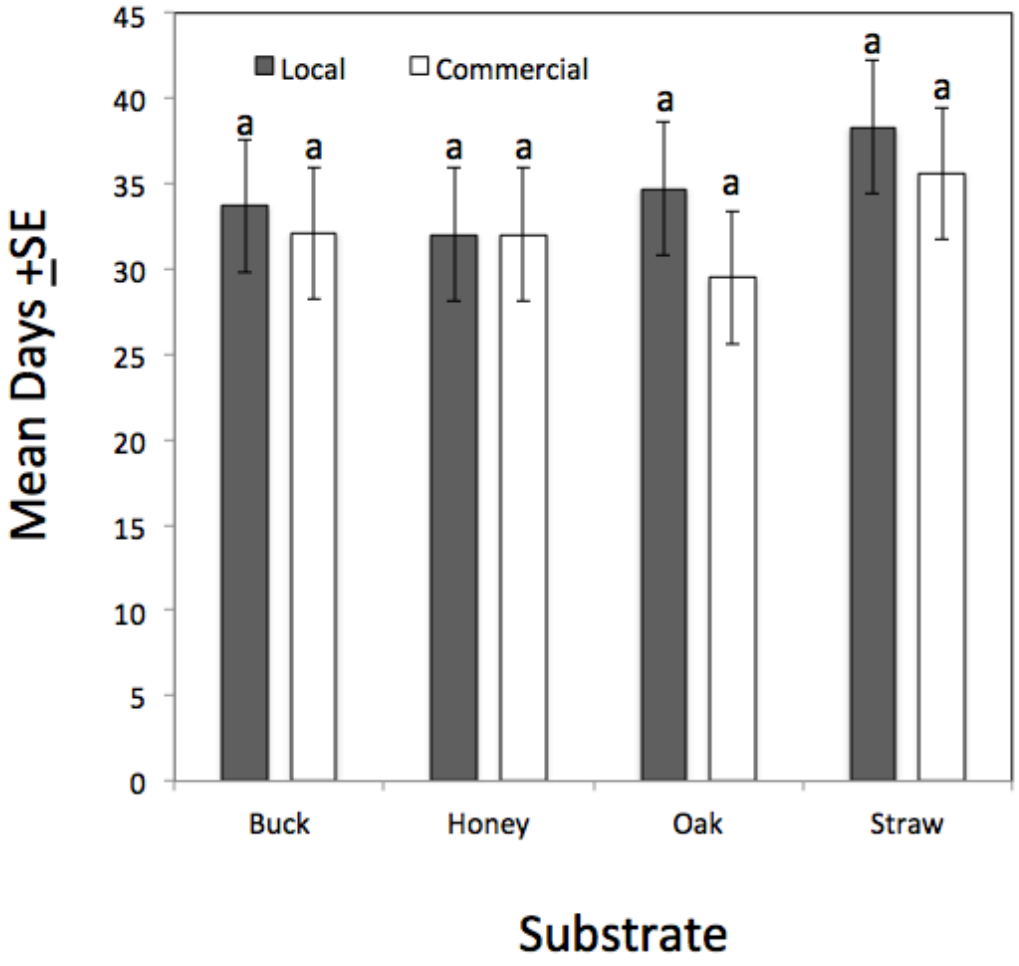

Figure 12. Mean (±SE) day to first harvest for commercial and local strains of *Pleurotus* on four different substrates. n=15. Bars with different letters represent statistically different results (p<0.05).

First Yield

After the primordia formed, the environment in the fruiting chamber was altered so the proper environmental conditions for mushrooms to mature was achieved (Figure 13). The commercial strain of *Pleurotus* produced on average two times the weight of mushrooms than the local strain (F(1,2)=107.08,p=0.09). Straw was the substrate that produced the largest amount of mushrooms for both strains (Figure 14). However, within the strains, there was no significant difference

between the buckthorn, honeysuckle, and oak substrates (Figure 14). Of the woody substrates, both strains produced the most mushrooms on the buckthorn substrate (mean of 22.5 g for the commercial strain and a mean of 10.0 g for the local strain). The lowest first yield was on oak for the commercial strain (17.0 g) and on honeysuckle for the local strain (5.0 g). The local strain did not have any fruiting mushrooms on four of the buckthorn blocks, four of the honeysuckle blocks, and two of the oak blocks. The commercial strain produced at least one flush of mushrooms on all blocks of all substrates. The commercial strain produced darker mushrooms that were typically larger than the local strain (Figure 15, data not shown).

Figure 13. Fruiting bodies of commercial strain (top) and local strain (bottom) fruiting on buckthorn, honeysuckle, oak, and straw (left to right). Scale bar represents 4.75 cm.

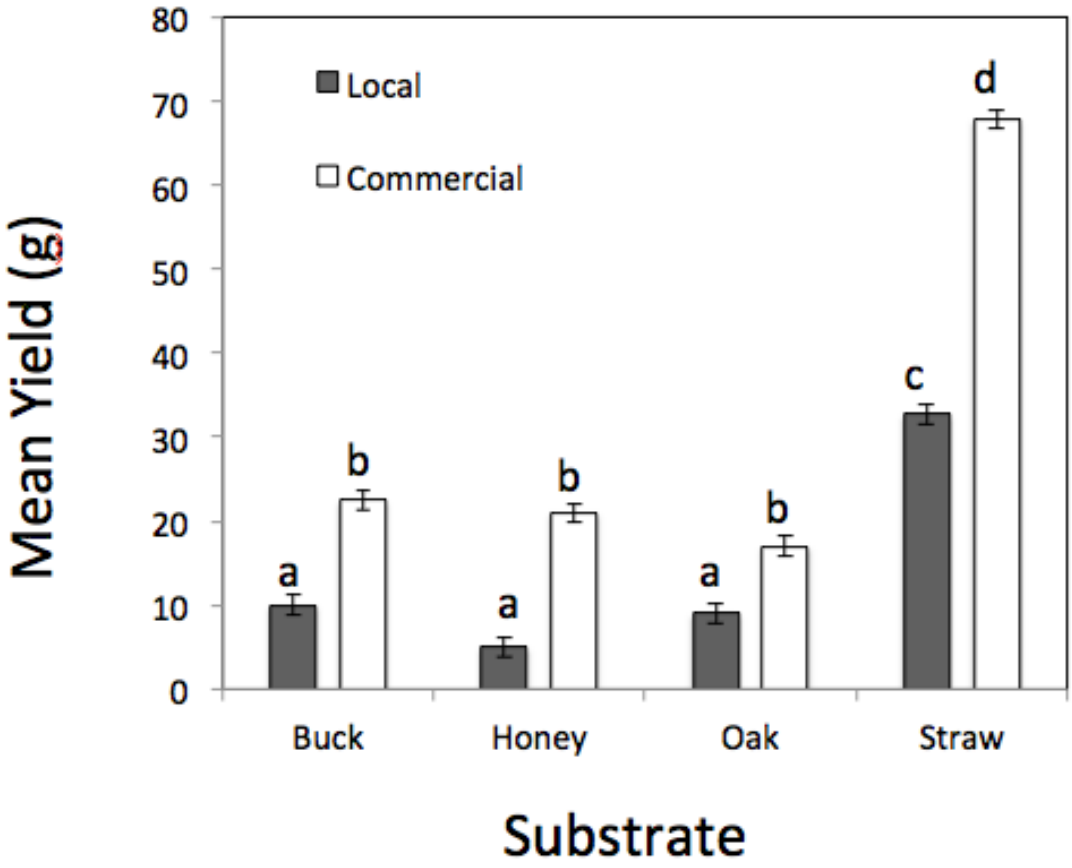

Figure 14. Mean (±SE) first yield of fresh weight of fruiting bodies (g) for commercial and local strains of *Pleurotus* on four different substrates. n=15. Bars with different letters represent statistically different results (p<0.05).

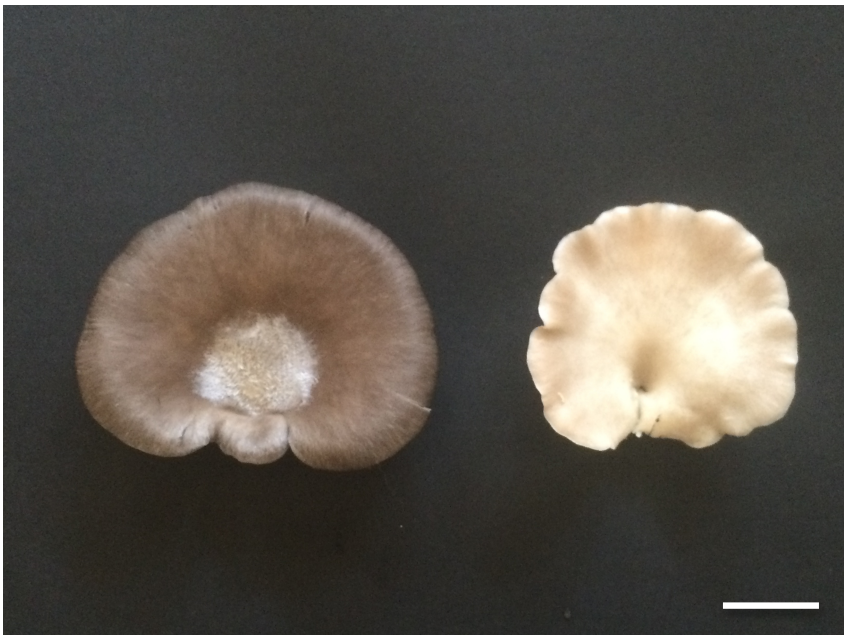

Figure 15. Fruiting bodies of commercial (left) and local (right) strains of *Pleurotus*. Scale bar is 2.0 cm.

Total Yield

The total yield of each block was calculated by combining the first yield and the second yield (if there was one). Not all blocks produced two flushes of mushrooms. The commercial strain had 12 blocks that produced two flushes on buckthorn, nine blocks on honeysuckle, 11 blocks on oak, and 12 blocks produced two flushes on the straw substrate. For the local strain, one block on the buckthorn substrate produced two flushes, five blocks on honeysuckle, seven blocks on oak, and all of the blocks on straw produced two flushes. Again, the commercial strain produced over two times the weight of mushrooms than the local strain (Figure 16). The commercial strain had more blocks that produced a second flush than the local strain (44 versus 28, respectively). Again, using straw as a substrate produced the greatest amount of mushrooms for both commercial and local *Pleurotus* strains, a

mean of 104.0 g and 62.5 g respectively ($F(3,6)=79.69$, $p<0.05$). The lowest mean yield for the commercial strain was found on the oak substrate at 24.0 g, whereas the low mean yield for the local strain was found on the honeysuckle substrate at 8.0 g.

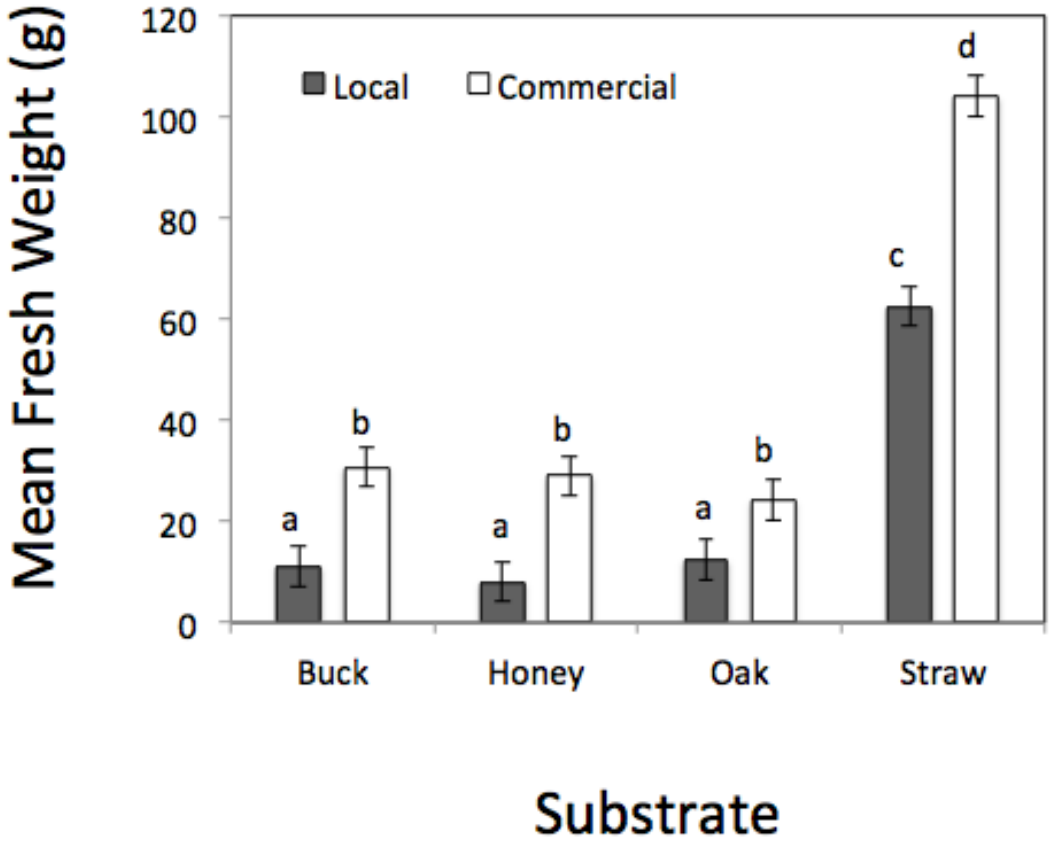

Figure 16. Mean (±SE) total yield of fresh weight of fruiting bodies (g) for commercial and local strains of *Pleurotus* on four different substrates. Total yield may include two flushes of mushrooms (some blocks only produced one flush). n=15. Bars with different letters represent statistically different results ($p<0.05$).

Biological Efficiency

Biological efficiency (BE) is the gold standard for measuring the productivity of each block in mushroom cultivation. BE take into account if the are various starting substrate weights. For this experiment, this is just another way of manipulating the data, since all of the mushroom blocks were the same weight. Therefore, the same pattern that was observed with BE that was seen with total yield. BE was measured by dividing the total fresh weight of mushrooms by the total dry weight of the substrate and then multiplying that number by 100 percent (Tisdale 2004). The commercial strain had a statistically significant greater mean BE than the local strain across all substrates ($F(1,2)=370.75$, $p<0.05$). Within a strain, there were no significant differences between the buckthorn, honeysuckle, and oak substrates, but these three substrates had a significantly lower BE than the straw substrate (Figure 17). However, the lowest mean BE for the commercial strain was found on oak at 9.7%, and the lowest mean BE for the local strain was found the honeysuckle substrate at 3.2%. Both the commercial and local strains produced the highest BE on the straw substrate, producing a mean BE of 41.5% and 25% respectively ($F(3,6)=91.54$, $p<0.05$)(Table 3).

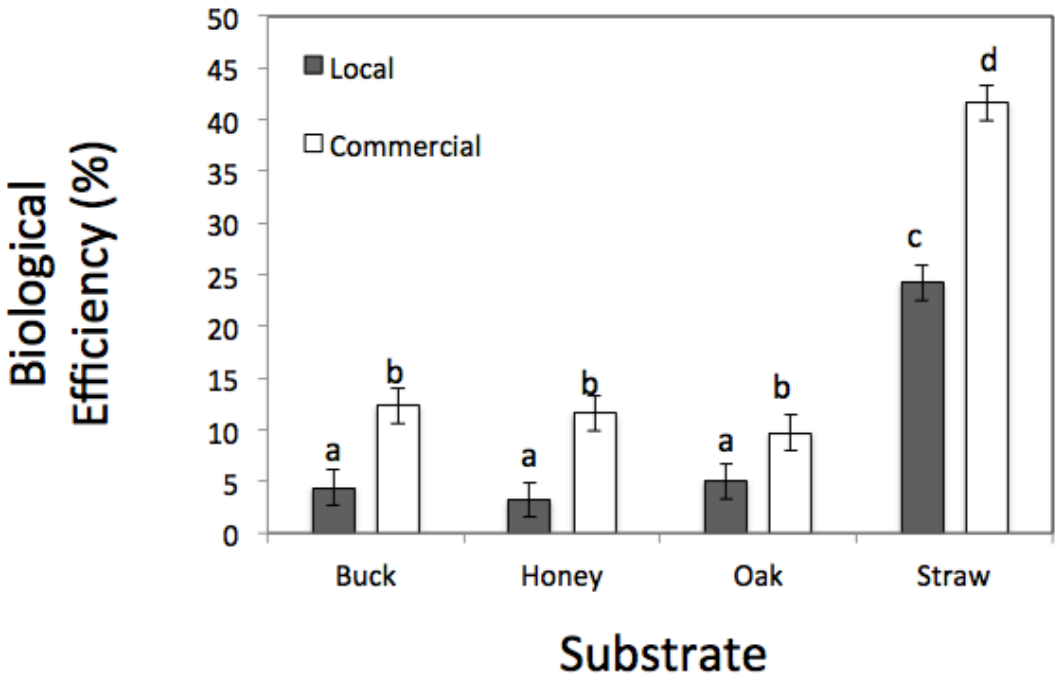

Figure 17. Mean (±SE) biological efficiency (%) for commercial and local strains of *Pleurotus* on four different substrates. BE includes the total yield of mushrooms (some blocks only produced one flush) divided by the dry weight of the substrate (250 g) multiplied by 100%. n=15. Bars with different letters represent statistically different results (p<0.05).

Table 3. Summary of Data: General growth patterns of commercial and local strains of *Pleurotus* on buckthorn, honeysuckle, oak, and straw substrate. Minimum and maximum values are labeled with the mean in parentheses.

	Spawn Run (Days)	Days to First Harvest (Days)	First Yield (g)	Total Yield (g)	BE (%)
Commercial					
Buckthorn	8-15 (11)	29-34 (32)	15-32 (29.5)	17-42 (31)	6-17 (12.3)
Honeysuckle	7-17 (11)	26-39 (32)	12-30 (21)	12-50 (29)	5-20 (11.5)
Oak	7-16 (10.5)	26-33 (29.5)	9-22 (17)	9-40 (24)	3.5-16 (9.5)
Straw	9-17 (12.5)*	31-43 (35.5)	32-96 (67.5)*	60-129 (104)*	15-70 (41.5)*
Local					
Buckthorn	9-20 (13.5)	38-69 (46)	0-22 (10)	0-27 (11)	0-10 (4.5)
Honeysuckle	8-19 (12)	37-57 (48)	0-13 (5)	0-20 (8)	0-8.5 (3)
Oak	10-20 (14.5)	30-71 (40)	0-15.5	0-30 (12.5)	0-12 (5)
Straw	13-17 (15)*	28-56 (38)	12.5-60 (32.5)*	33-91 (62.5)*	14-36 (25)*

* Indicates significant differences in results (p=0.05)

CHAPTER V

DISCUSSION

Strain Selection

For success in mushroom cultivation, strain selection is one of the most important factors. All other parameters can be set up perfectly, but if the cultivator is using a poor strain, yields will be low and inconsistent. Also, quick growth in a petri dish in the lab does not correlate to a high yield of mushrooms from the substrate. As other reports have noted, e.g. Palikhey (2011), there seemed to be an inverse relationship in the diameter of growth on MEA and the robustness of the mycelium. The dense, more robust mycelium in the petri dish did, however, form dense mycelium on the substrate, whereas the local strain had less vigorous mycelium (Figure 9, pg 31). The first hypothesis stated that both strains would be able to colonize the invasive plant woodchips, but at a slower rate than the oak wood chips. Although the commercial strain in this experiment had much thicker, whiter mycelium after the spawn run across all substrates, the hypothesis that the invasive woodchips would take longer to colonize was not supported for either strain. Cultivators have noted that more dense mats of mycelium are more likely to produce mushroom primordia (Stamets and Chilton 1983). Even though using a productive strain is key for successful mushroom cultivation, it is important for

cultivators to continually be experimenting with new wild strains to guard against genetic stability in the cell line (Stamets 1993).

It was clear in this experiment that the commercial strain outperformed the local strain of *Pleurotus* on all substrates. Not only was the commercial strain able to colonize all of the substrates more quickly than the local, but it also had about twice the yield and biological efficiency across all substrates. This was to be expected as the commercial strain had been selected based on its ability to produce a high yield and have a quicker time to harvest date.

The two strains likely occupy slightly different ecological niches in their environments. Therefore, growing the two strains at the same environmental conditions may have been favoring one strain over the other. For instance, the environmental conditions (temperature, humidity, light) may have been more favorable for the commercial strain, and thus the commercial strain was a better producer. However, sometimes strains that are better for commercial cultivation do not do as well when grown in "wild" conditions, which was not tested in this experiment. In commercial cultivation, it is recommended to experiment with smaller trial batches until the optimum environmental conditions are achieved (Stamets 2000).

Substrate

Both strains had the highest yield and BE on the wheat straw substrate. Although not a waste product, wheat straw has been found to be a more productive

substrate than other forestry and agricultural wastes in time to colonization, yield, and BE (Alananbeh *et al.* 2014). However, the hypothesis that there will be a difference in the yields of mushrooms on the woody substrates was also not supported by the data. Straw, being herbaceous, may have been easier for the hyphae of the fungus to penetrate. Also, straw lacks lignin, a recalcitrant molecule that is difficult to degrade, so the mycelium may have been able to degrade the straw quicker than the woodchips (which contained lignin). All the other substrates were wood chips of various size. These wood chips were denser than the straw, making it more difficult for the fungus to degrade and gain nutrients from them. The anatomy of the chips was likely comprised of primarily cellulose, lignin, and hemicelluloses in varying ratios. Straw also has a higher surface area to volume ratio than the woodchips, creating more surface area for the fungus to colonize.

Since *Pleurotus* fungi are simultaneous white-rot fungi, they possess enzymes to degrade cellulose, lignin, and hemicellulose. These enzymes (cellulase, peroxidase, laccase enzymes) are non-specific. Therefore they can also degrade a number of contaminants, such as polycyclic aromatic hydrocarbons, polychlorinated biphenols, synthetic dyes, and even explosives (Baldrian and Gabriel 2003). Accordingly, both strains of fungi in this experiment were shown to degrade the forest waste products and convert the material into an edible, protein-rich food source.

Of the woody substrates used (buckthorn, honeysuckle, and oak), there were no significant differences within the strains for days to first harvest, first yield, or BE. For this reason, these invasive plant species can provide a sustainable alternative substrate to using oak woodchips. This means that mushroom farmers and forest

restoration practionors or environmental agencies should work together to fully utilize this unused source of plant biomass. Since there were no significant differences in the yields or BE between substrates, cultivators can switch from using the woodchips of valuable hardwood trees to using sustainable resources that were previously being burned. By doing so, people are improving the ecosystems by removing invasive species. Then, the lignocellulosic material from the invasive species would be removed from forests and transported to mushroom farms. Mushroom cultivators can prepare and then inoculate the woodchips with desired mushroom species. Then the mushroom farmers can sell the mushrooms to local markets or restaurants, benefiting the local economy as well. The spent blocks from growing the mushrooms could then be used as "spent block spawn' to further perpetuate outdoor mushroom beds. Alternatively, the spent blocks could be composted and used as a fertilizer to grow crop plants, which can be sold to local markets, further stimulating the local economy. However, other variables need to be considered before a substrate is selected.

Substrates should be selected based primarily on what resources are available and how much they cost. As it has been shown, *Pleurotus* fungi have the ability to degrade a wide variety of materials (from straw to agrowaste to explosives). Thus, experimenting with locally cheap and highly available substrates is recommended. Straw and sawdust may be unavailable in some areas or very expensive, making it difficult to come by (Obodai *et al.* 2003).

The substrates used in this experiment had no additives of any sort. This was done to show that the fungus was only using the plant material as a food source to

grow. However, if these substrates were to be utilized for commercial production it would be recommended to supplement the wood chips with a nitrogen source such as wheat bran or corn meal to help improve yields. This is because lignocellulosic products are typically low in protein and nitrogen (Alananbeh *et al.* 2014). Thus, the low yields and BE obtained in this study were likely due to lack of nutrients in the substrate (particularly nitrogen and protein) and also due to the small amount of substrate used (250 g). Many cultivators can achieve a BE of over 100% growing *Pleurotus* species on various substrate, and to achieve this one needs a very productive strain of *Pleurotus*, a stable controlled environment, and the correct nutritional content in the substrate (Stamets 1993). In nature, some species of *Pleurotus* obtain protein and nitrogen by capturing small invertebrates, such as nematodes (Barron 2003). The fungus sets up traps and secretes sticky enzymes from little "knobs" on the hyphae that lure the nematodes and then trap the invertebrates on the hyphae. The fungus then secretes different extracellular enzymes to degrade the nematode and then ingests the nutrients through active transport. However, in the experiment, any invertebrates that may have been in the woodchips would have been killed through the sterilization process. Therefore, supplementing the blocks with a nitrogen source would likely increase yields and BE.

After the blocks have produced mushrooms, the blocks can then be used as spawn to inoculate mushroom beds. This can be achieved by removing the blocks from the plastic bags they were grown in, breaking up the blocks into smaller pieces and then piling the spent blocks in a pile. Straw is placed down, then a layer of the

"spent block spawn" is added and covered that with fresh woodchips. Water the newly installed mushroom beds periodically in the beginning. Beds can produce mushrooms within one month.

The spent blocks can also be made into compost. The pile should then be watered and turned periodically. This is to ensure homogeneity and maintenance of the microbial communities that do the composting. The partially composted material can then be used as a substrate for secondary decomposing fungi such as *Agaricus bisporus* or *Clitocybe nuda*. Secondary decomposing fungi possess a different suite of extracellular enzymes to degrade substrates than primary decomposing fungi (white-rot and brown-rot fungi). Wang *et al.* (2015) showed that the spent blocks from *Hypsizygus* cultivation could be used as a successful substrate for oyster mushroom cultivation. This is possible due to the fact that *Hypsizygus* is a brown-rot fungus that degrades cellulose and leaves lignin behind. Therefore, when the spent substrate from *Hypsizygus* cultivation is inoculated with *Pleurotus* mycelium, the substrate still has lignin and some protected cellulose available for the *Pleurotus* fungus to degrade (*Pleurotus* degrades both lignin and cellulose).

Alternatively, the spent blocks can be allowed to fully compost and degrade in the compost pile. After a couple of months of turning and watering the pile, the composted material can now be combined with potting soil or peat moss and act as a fertilizer for growing other crop plants (Chang 2001).

Further research needs to be conducted to evaluate other waste products as potential substrates for mushroom cultivation. In addition, different species and strains of fungi should be evaluated for degradation activity and cultivation

potential. In this experiment, wheat straw outperformed the oak substrate. Since straw is herbaceous and oak is comprised of lignocellulolytic compounds, it would be interesting to experiment using herbaceous invasive plants (such as the exotic invasive plant reed canary grass) as an alternative substrate to wheat straw. Before these invasive plants are used in commercial cultivation, additional tests should be conducted, including a nutrient assesments of the mushrooms

In conclusion, wheat straw showed the highest yields and BE. However there were no significant differences in the woody substrates within a strain. Therefore, these invasive plants can provide an alternative sustainable substrate, thereby producing a protein-rich food source, helping restore ecosystems, degrading forestry waste, and producing nutriceuticals that may be used to improve human health.

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