Mushroom Cultivation Manual for the Small Mushroom Entrepreneur

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CHAPTER 1
INTRODUCTION

WORLD OF MUSHROOMS

Dr Gunter Pauli, Founder ZERI, Author "The Blue Economy"

I discovered the world of mushrooms in 1994 during a meeting organised in Beijing by the Royal Academy of Sciences of Sweden and the Chinese Academy of Sciences. Prof. Dr. Carl-Göran Hedén, former Director of the Biology Department of Karolinska Institute, and Prof. Dr. Li Wenhua, Dean of the School of Environment of the Renmin University had invited a select group to discuss new ways of responding to the urgency to respond to the basic needs for people in terms of water, food, health, housing, energy and jobs. As the head of a think tank that was charged with formulating new ideas for business at the United Nations University in preparation of the Kyoto Protocol, that was to become a reality three years later I was a student in this room filled with scientists. Whereas all presentations inspired me, there was one that surprised me: Prof. Dr. Shu-ting Chang, the Dean of the Faculty of Biological Sciences of the Chinese University of Hong Kong introduced the audience to his latest findings in mycology.

The simple and clear message made a lasting impression. First the fact that biowaste rich in fibres should never be left to rot or landfill, that it should turn into a substrate for mushroom farming. Instead of rotting debris that generates methane gas, mushrooms would produce food only emitting carbon dioxide. That was a breakthrough in the run-up for the global agreement on climate change. Second, mushrooms supply a wealth and breadth of essential amino acids in such abundance that if compared dry-base with meat, it could compete. This offered an insight that was very new to me, since I was hardly acquainted with the white button mushroom and never considered it nutritious. The talk of the day was not about this Agaricus bisporus, rather of the wealth and diversity of Chinese mushrooms which have been farmed over centuries including the shiitake.

Prof. Shuting Chang made us realise that any country with a food processing industry could build up a mushroom business. I immediately invited him to join us for meetings in Windhoek at the University of Namibia, in Zimbabwe at Africa University, and in Colombia at the Federation of Coffee Farmers. His message was loud and clear: the straw of wheat, the water hyacinth from the lakes and the waste of coffee all served as a substrate for mushrooms. When ST, as friends call this guru of mycology, sat down with Dr. Jorge Cardenas, the President of the cooperative that united 650,000 coffee farming families he strongly advised the leadership that the future of coffee is not in producing more coffee, rather the future of coffee is in the transformation of all the coffee waste into mushroom substrates. CENICAFE, the research centre of the Coffee Federation embarked on a seven year program and studied every component from the stalks from the bush that need pruning, the pulp the is fermenting off the beans, the silver film of the roasted coffee, and the grounds after brewing was mapped for its use. It was like finding bonanza in a world that was passing through a harsh crisis.

Since only 0.2% of the coffee harvest is actually ingested, the opportunities are vast. The key is how to harness this opportunity, either on the farm, or at the point of consumption. Fortunately, a network of entrepreneurs emerged around these opportunities. These entrepreneurs were not located in the capital cities and were flush of cash, these were community leaders operating in the periphery of society like Carmenza Jaramillo in the peri-urban zones of Manizales, the Coffee Capital of Colombia, and Margaret Tagwira, the laboratory technician in charge of tissue culture who worked with orphan girls in Zimbabwe. Both realised that mushrooms on coffee is not just a biological process, it is an opportunity for a social transformation. Hundreds of entrepreneurs took notice and started small scale businesses. In the region of El Huila, 90 production centres were started in less than a few years and in Zimbabwe, hundreds of orphans found a new opportunity in life as the mushrooms provided them food security which gave them the self-confidence to fight against abuse.

When Chido Govera, one of the first orphan girls to get trained at the age of eleven in the farming of mushrooms on grass clippings, corn cobs and water hyacinth, something that is within reach of everyone, committed to bring this technique to everyone. She traveled throughout the country (and later throughout Africa and beyond) and when she explained at Chipinge (Zimbabwe) to women working the coffee farm for less than two dollars per day, that on the waste from the farm it is possible to get food for their children within a few weeks time, then these women would get up, sing, dance … and do it. The farming of mushrooms once demonstrated that it works, through the cooking of a local dish, enriched with freshly harvested fruiting bodies, is followed-up by action. There is no need to write a strategic paper, a business plan, a strengths and weaknesses - opportunities and threats analysis, a pilot project or a technology audit. Farming mushrooms starts with an awareness that you have all what is needed available, and that if you put your mind to it, and follow a few basic hygiene rules, then you will be able to harvest … perhaps even for the rest of your life.

Twenty years later, there are an estimated 5,000 mushroom on coffee farms. While a few have attempted to go for large scale production (like Setas de Colombia in Medellin), and one exploited the experiences to create a (failed) network of franchise mushroom farms, the initiatives have been growing rapidly around the world from farms in Harare, to urban initiatives in San Francisco, and innovation hubs in Rotterdam where young entrepreneurs void of any exposure to mushroom farming in the centre of the city now have trained 30 others to start their business. Prof. Chang was keen on insisting that the farming of mushrooms was half science and half art, and indeed when Chido Govera farms mushrooms it seems so easy, whereas others have to struggle to get going but once they master the art, it is a great satisfaction to witness the spreading of something that seems that simple and yet has many hurdles to overcome.

The main obstacle is the clarity that farming mushrooms is not just a potential business, it is also an opportunity to transform society, beyond climate change benefits. Mushrooms empower people, and provide access to healthy food, generating jobs, while transforming available resources (unfortunately considered by many as waste), cascading food and nutrition, addressing fundamental social and ecological issues. While the creation of 5,000 farming operations is by many considered a remarkable result, it is by no means a success. The ZERI Network, this web of thousands of scientists and practitioners from around the world is convinced that the annual production of 10 million tons of coffee waste that continues to be discarded at farms, industries and cafes or restaurants provides enough material for at least one million initiatives. And if we consider coffee, why not consider tea, corn cobs, sawdust and rice straw all varieties of biomass that represent an ideal substrate for mushrooms to grow. We quickly see the creation of another 100 million tons of amino acids and the production of perhaps as much as 50 million tons of feed. These are major shifts in our capacity to produce food and respond to immediate needs alleviating hunger where it is needed most. Every refugee in any camp could learn how to farm mushrooms. And yet, we prefer to supply processed food in aluminium packs.

Mushrooms are not just healthy food, mushrooms hold the potential of transforming our modern day society into an entrepreneurial world, where we succeed in building up a more resilient community first and foremost because we transform biomass into food, and the waste of this food is most of the time a great feed for animals, cascading nutrition, matter and energy. It allows our economies to grow without expecting our Earth to produce more, we learn with the mushrooms how to do more with what the Earth already produces. This is a gift we received from our Chinese mentor, and a practice we learned from our African, Latin American and European mycologists who worked tirelessly in propagating this know-how open source, sharing what we learn, and learning from each other in order to offer society a chance to stamp out hunger, generate more jobs and empower young people to have a purpose in life.

Mushrooms as a conventional food

Mushrooms: Conventional Food and Alternative Medicine.

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Most edible mushrooms have a number of properties in common. The most obvious is their high water content, which varies from 85 to 95 % of their fresh weight and makes mushrooms vulnerable for bruising and loss of storage quality. After harvest, mushrooms must be kept cool to prevent water loss and discoloration. When this is not done properly or when mushrooms are affected by bacteria or parasitic fungi, most mushrooms will have only a very short shelf life, lose their food quality and cannot be sold.

The dry matter of mushrooms consists mainly of fibrous carbohydrates and further of proteins, (unsaturated) fats and a very high number of very diverse compounds: anti-oxidative polyphenols, vitamins, and inorganic elements as phosphorus (P), potassium (K) and magnesium (Mg), The diverse compounds are present at low concentrations but their biological effects may be impressive. Table 1 shows the composition of dry matter of the white button mushroom Agaricus bisporus (from Stojkovic et al. 2014), also known colloquially as the champignon.

	Agaricus bisporus	Agaricus braseilliensis
Ash	6.0±0.2	8.2±0.6
Proteins	3.0±0.3	13.3±0.2
Fat	1.8±0.1	2.8±0.1
Carbohydrates	89.1±0.3	75.8±0.3
Energy	384.8±0.7	381.1±2.2

Table 1. Main composition of cultivated Agaricus bisporus and Agaricus brasiliensis (= Agaricus blazei), expressed in grams per 100 grams dry weight.

In addition to the major components shown in Table 1, mushrooms contain bactericidal and fungicidal components that form a natural protection against offensive microbials, and could also be applied as protective agents in biological foods such as yoghurt (Stojkovic et al. 2014).

The question arises whether mushrooms are a healthy food. Shii-take (Lentinula edodes) is reported to have app. 14 % of its dry weight as protein (Sistani et al. 2007), which makes it comparable to some vegetables, wheat and rice (Chang & Buswell, 1996), but it is less than that of animal meat. Further the protein seems high in glutamic acid and aspartic acid but is low in methionine and cysteine. The answer to the question is that for vegetarians mushrooms are not a very healthy food. Assuming a MDI (minimum daily intake) of 60 grams protein per day, it can be easily calculated that even a daily consumption of 1 kg of fresh mushrooms would not suffice. The advice is then to use a variety of lean meat and various vegetables and or mushrooms to supply the required protein.

Agaricus bisporus is a valuable source of magnesium, phosphorus and vitamin D. Vitamin D
is readily available from Agaricus bisporus and Pleurotus sp. after exposure to sunlight or other sources of UV-B light, the fungal wall component ergosterol being converted to Vitamin D2. Post harvest drying of these mushrooms in sunlight induces high amounts of Vitamin D. Different animal studies have shown that light induced edible mushrooms are safe and that Vitamin D2 is indeed available (Calvo et al. 2013). In a human experiment with 26 patients that were deficient in Vitamin D2, Urbain et al. (2011) have described that UV irradiated Agaricus bisporus could improve their Vitamin D2 status the same way as synthetic Vitamin D2.

The energy content of mushrooms is low which makes them suited for a low calorie diet that is much desired in the affluent western world.

Mushrooms can be considered a high dietary fiber food, which relates to its non-digestible carbohydrates, mainly chitin. Agaricus bisporus contains 41% of its carbohydrates as dietary fiber and Pleurotus sajor caju 44% (Goyal et al. 2015). This high content of dietary fiber makes mushrooms suitable as an anti-constipation food or to be used in a diet designed to prevent this modern times' hindrance of human well being. In patients with functional constipation, fiber supplements derived from Auricularia (ear) mushrooms significantly improved constipation related symptoms without serious side effects (Kim et al. 2004).

Apart from the Vitamin D2 precursor ergosterol, Agaricus bisporus contains significant amounts of the vitamins B2 = riboflavin (24% of recommended daily intake per 100 gram fresh), Vitamin B3 = niacin (18%) and Vitamin B5= panthotenic acid (15%). 9% of the recommended daily intake of Potassium (K) and 9% of phosphorus can be supplied by the same 100 grams of fresh product (from: USDA SR-21 database).

The polyunsaturated fatty acids (PUFA) present in mushrooms are often mentioned as contributing to good health. Up to 80% of the edible mushrooms' fatty acids are of apolyunsaturated nature (Reis et al. 2012), but the amount per serving of 100 grams fresh mushrooms is maximum 0.15 grams of PUFA. It seems not very likely that this small amount can play an important dietary role.

Mushrooms as an alternative medicine

Medicinal mushrooms are mushrooms or extracts from mushrooms that are thought to give treatment for various diseases, yet these effects remain unconfirmed in mainstream science and allopathic medicine. In the Western world, i.e. the USA and the EU, they are not approved as medicines for therapy or prevention. Such use of mushrooms therefore falls into the domain Traditional Chinese Medicine (TCM) or Complementary Medicine.

In spite of the remark above, mushrooms have been employed in Chinese and Japanese medicine for hundreds of years. Ganoderma lucidum also known as Reishi or Ling Zhi was used as a remedy against various cancers for over 500 years and Lentinula edodes, shii-take, was found to enhance "vital energy" and cure colds since the Ming dynasty (Mizuno, 1995). It is only since the 1960's that medicinal mushrooms were introduced in Europe and the USA as possible cure for many diseases and that has started a search for the validity of the statements about medicinal successes, that is still continuing.

Mushroom compounds that are causative in (presumed) medicinal effects are high molecular weight polysaccharides, and a variety of smaller compounds as polyphenols and triterpenes, and many molecules that have possible signaling functions but have not yet been defined.

The effects of mushrooms and mushroom components on human and animal health have been studied in vitro as well as in vivo. Tens of thousands of scientific articles have been published over the past 50 years, but up till now no definite conclusions could be drawn whether mushrooms and their components can cure severe disease in humans.

The compounds

Polysaccharides are building components of the fungal cell wall; they consist of a long chain of the sugars glucose, mannose and galactose connected by 1→3, 1→4 and 1→6 bonds. Depending on their conformation they are called α(alpha) or ß (beta) chains. They can be either water soluble or non-soluble.

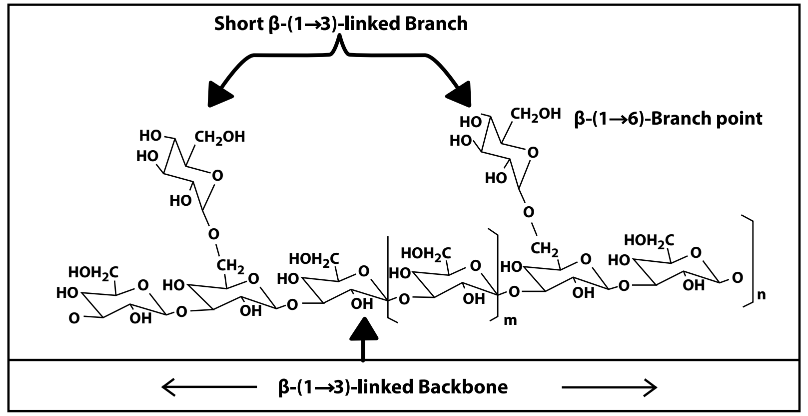

Figure 2. Schematic drawing of the structure of a 1→3, 1→6 ß glucan.

Best known examples of mushroom beta glucans are lentinan and schizophyllan from Lentinula edodes and Schizophyllum commune respectively. Some active beta glucans are bound to peptides, examples are polysaccharide K and polysaccharopeptide from Trametes versicolor. The molecular weights of these materials vary from 10.000 d. to 1.5 x 106 d.

Polyphenolic compounds consist of large multiples of organic rings carrying one or more OH groups. They have many functions in nature. They can determine color and taste, and are also involved in the oxidative status of organisms as they can give off oxygen or bind oxygen, depending on pH and oxygen supply. The polyphenols are the main anti-and pro-oxidants present in mushrooms. They can also specifically combine with cell membrane or nuclear receptors and be responsible for intracellular signaling effects.

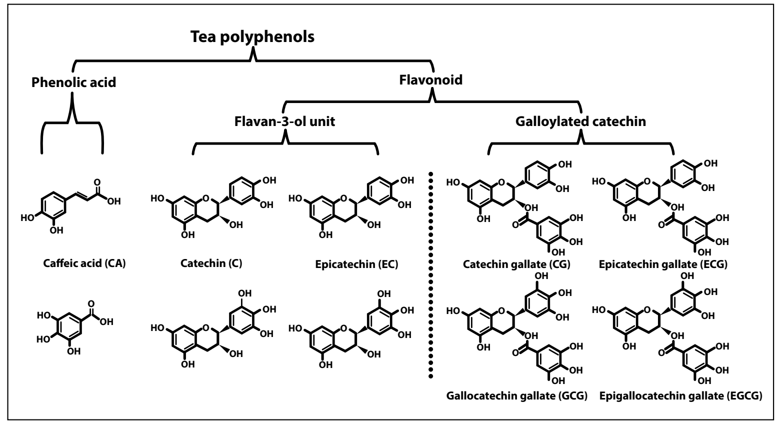

Figure 3. Schematic drawing of tea polyphenols.

Comparable compounds are present in mushrooms.

Triterpenes or triterpenoids are carbohydrates consisting of one or more pentacyclic (5-ring) structure. In animals and fungi their biosynthesis is through lanosterol; they form a structural part of the cell membrane and they could be involved in signaling by binding to cell membrane receptors. Although many have been extracted from medicinal mushrooms, the functions of most are not known. Well known examples of triterpenoids are the ganoderic acids from Ganoderma lucidum.

Effects of mushroom compounds

<u>Effects of Polysaccharides</u>

The biological effect of mushroom polysaccharides is attributed to their recognition by immune cells, leucocytes, and membrane receptors as Dectin-1, the toll like (TLR) receptors and/or the (complement receptor) CR3. Binding affects the proliferation and differentiation of the cells and determines thereby their function in immunity.

(1→3),(1→6) β glucans are the major immunomodulatory polysaccharides; they determine the activity and the direction of the immune system. Mushroom polysaccharides can activate innate immunity and cause the secretion of pro-inflammatory cytokines such as TNF-α (tumor necrosis factor-alpha), IFN-γ (interferon -gamma) IL-1β (interleukin -1 beta) from immune cells like macrophages, natural killer cells (NK Cells) and T-lymphocytes. Dendritic cells (DC) are the phagocytic cells of the innate immune system, and they present the antigens they absorb to the start point of the adaptive immunity, the T-helper cell. When gastric or colorectal cancer patients were supplemented with PSK one month after surgical resection, PSK was reported to shift the T-helper cells balance (Th1/Th2) toward Th1 dominance resulting in increased cytotoxicity for cancer cells. When Th1 is high, the immunity is shifted to inflammatory effects, when Th2 is high the effect is immunosuppressive. Inflammation forms a defensive barrier against infectious disease and growth of abnormal cells such as cancer. Anti-inflammatory (immunosuppression) activity could prevent and possibly soften the overactive immunity in various autoimmune diseases and allergies.

<u>Bactericidal and fungicidal effects</u>

Mushrooms can be easily colonized by bacteria and fungi. During evolution they have adapted to these threats by developing defense mechanisms. Many mushrooms extracts show antimicrobial properties. Agaricus blazei extracts showed MIC's and MBC's that were equal to or better for inactivation of P. aeruginosa than those of ampicillin and streptomycin (Stojkovic et al. 2014).

<u>Anti Quorum sensing</u>

Many pathogens use the formation of biofilms as a defense against their host's immune system and against antibiotic treatment. Biofilms are vast bacterial populations in a host that are protected by a layer of polymeric substances. Biofilms use quorum sensing for their protection, a bacterial coordination system that allows density-dependent cell–cell communication. Considering the rapid spread of multidrug resistance, the development of new antimicrobial or antivirulence agents that act upon newly adapted microbial targets has become a very pressing priority. Agaricus blazei and also Inonotus obliquus (Chaga) were found to have anti quorum sensing compounds next to more common antimicrobials (Sokovic et al. 2014). Application of these findings awaits techniques for large scale production and purification of anti quorum sensing compounds.

Antioxidant effects

Oxidative stress caused by an imbalanced metabolism and an excess of reactive oxygen species (ROS) lead to a range of health disorders in humans. Our endogenous antioxidant defense mechanisms and our dietary intake of antioxidants potentially regulate our oxidative homeostasis. Numerous synthetic antioxidants can effectively improve defense mechanisms, but because of their adverse toxic effects under certain conditions, preference is given to natural compounds, such as from mushrooms. Almost all mushrooms show considerable anti-oxidant activity. Edible mushrooms might be used directly in enhancement of antioxidant defenses through dietary supplementation to reduce the level of oxidative stress. Kozarski et al. (2015) have recently published and extended review of antioxidants of edible mushrooms.

The diseases

Mushrooms and their components have been used during ages as a traditional medicine in the prevention and therapy of a variety of diseases.

Cancer

Although hundreds of studies were published on the curative effects of mushrooms and their extracts on various cancers in experimental animals, no convincing information is available on the effects in humans that justifies a definite conclusion. Instead many studies are biased, too small, non-randomized, and non-conclusive. The Cochrane Institute is an objective observer of medical testing in humans. Their latest report is on the effects of Ganoderma lucidum medication in cancer patients (Jin et al. 2016) and stated:

“Our review did not find sufficient evidence to justify the use of G. lucidum as a first-line treatment for cancer. It remains uncertain whether G. lucidum helps prolong long-term cancer survival. However, G. lucidum could be administered as an alternative adjunct to conventional treatment in consideration of its potential of enhancing tumor response and stimulating host immunity. G. lucidum was generally well tolerated by most participants with only a scattered number of minor adverse events. No major toxicity was observed across the studies.”

For Polysaccharide K (PSK) from Coriolus versicolor the situation is not much different. PSK may improve the immune function, reduce tumor-associated symptoms, and extend survival in lung cancer patients. PSK was reported to enhance docetaxel-induced prostate cancer tumor suppression, apoptosis and antitumor responses. Cochrane has started a new search to estimate the effects of PSK on cancer.

Use of G. lucidum and of PSK, in the fight against cancer are repeatedly mentioned to increase the quality of life of cancer patients. What the cause of this effect is, still needs to be evaluated.

In breast cancer patients quality of life increased after supplementary shii-take extract treatment; the same as for A. blazei extract (Ahn et al. 2004).

These are only a few examples of many anti cancer assays in humans. The results are hopeful, but nothing is definite yet.

A possible interesting feature of Agaricus bisporus is its anti-aromatase activity, that could be deployed in the prevention and treatment of breast cancer in postmenopausal women; but here again this awaits further research. Work of Chen’s group (Grube et al. 1999) in the City of Hope Institute has revealed promising results.

In Japan 30 years of experience with the aforementioned PSK as an adjuvant in cancer therapy led to 2 impressive metastudies demonstrating that immunoactivation with PSK together with surgery and chemotherapy led to an average longer survival in 3000 colorectal cancer patients as well as benefits to patients with gastric carcinoma (Sakamoto et al. 2006, Oba et al. 2007).

Although these are impressive results, the adjuvant effects of medicinal mushroom components in cancer therapy need to be further studied.

Autoimmune diseases and allergies

Mushroom polysaccharides decrease the concentration of pro-inflammatory cytokines as TNF-a and IFN-g in lipopolysaccharide stimulated cell systems in vitro. This proves that mushroom polysaccharides can be immunosuppressive under certain conditions.

Although there are several case stories on curative effects of mushroom polysaccharides on

human rheumatoid arthritis and other autoimmune diseases, reliable and published evidence is very scarce. Some experiments in rats have shown that induced arthritis can be cured by application of Phellinus polysaccharides (Miao et al, 2015), but the overall information consists mostly of interesting theories, rumors and non-published cases.

Hetland et al. (2011) found anti-allergy effects of Agaricus blazei, i.e. AndoSanTM, in mice, explainable by changes in cytokines. Most of the interesting work of this group was however done on ulcerative colitis and Crohn's disease in humans. They (Therkelsen et al, 2016) have carried out a randomized single blinded clinical study on the effects of AndoSanTM on 50 patients with symptomatic ulcerative colitis of which 24 were treated for 21 days and 26 served as controls. Fatigue, quality of life for bodily pain, vitality, social functioning and mental health improved in the AndoSan™ group. There were no alterations in general blood samples and fecal calprotectin. This supports its use as a supplement to conventional medication for patients with mild to moderate symptoms from ulcerative colitis. The patients did not report any harms or unintended effects of AndoSan™ in this study.

<u>Neurodegenerative diseases</u>

In the coming 5 decades average human life expectancy will considerably grow, and as a result an increase of age dependent decline in immunocompetence, and an increase in systemic diseases and in neurodegenerative disease is to be expected. Cancer, atherosclerosis, diabetes and obesity have already increased and will continue to do so.

Neurodegenerative diseases as Alzheimer's disease, Parkinson's disease, dementia and stroke are mostly age dependent. Mushrooms such as Antrodia camphorate, Ganoderma lucidum, Grifola frondosa, Hericium erinaceus, Phellinus linteus and Pleurotus giganteus may improve memory and cognition functions. The mushrooms (either extracts from basidiocarps/mycelia or isolated compounds) reduced beta amyloid-induced neurotoxicity and had anti-acetylcholinesterase, neurite outgrowth stimulation, nerve growth factor (NGF) synthesis, neuroprotective, antioxidant, and anti-(neuro)inflammatory effects (Phan et al 2015). Phellinus linteus ethyl acetate extract containing mostly polyphenols was found neuroprotective in vitro by reducing oxidative stress and preventing apoptosis (Choi et al, 2016). In mice with experimentally induced stroke, intraperitoneal treatment with anti-oxidative P. igniarius polyphenol extract at low concentration caused a reduction of the infarction volume by 62.2% compared to untreated mice (Suabjakyong et al. 2015).

No reliable data concerning the effects of mushrooms and their extracts on human neurodegenerative disease are available at present. The observation that higher dietary intake of Vitamin D2 was associated with lower risk of developing AD among older women could possibly relate to a higher intake of ergosterol from sunlight exposed dried mushrooms. Further information on the possible use of mushrooms against neurodegenerative disease can be found in the excellent review article of Phan et al. (2015).

<u>Diabetes</u>

Diabetes type 2 is a rising problem in the modern world. Lifestyle, diet and genetics are causal in the development of obesity and diabetes type 2. As many conventional drugs show adverse side effects,

a search was done for potential effects of mushrooms in treatment and prevention of diabetes. So far there is only a single statistically reliable study published on the effects in humans. Hsu et al. (2007) performed a clinical randomized double-blind placebo controlled trial with 72 Chinese subjects who had proven diabetes type 2 for over a year and who had been taking gliclazide and methformin for over 6 months. They showed that treatment with 1.5 grams of Agaricus blazei extract per day for 12 weeks improves the insulin resistance of the patients from 6.6 to 3.6 in the homeostasis model assessment for insulin resistance, blood pressure was lower and fasting triglycerides of the patients were much lower than the controls. Surprisingly, no further results have been published since.

Summary

In the text above a personal vision is given on the role of mushrooms as food and as alternative medicine. Mushrooms are a conventional food of rather average quality. They can be part of a healthy diet when consumed with other sources of protein. Eaten in normal quantity they do not supply sufficient protein for a vegetarian diet. On the other hand they may form a delicacy of unheard quality due to their subtle taste.

As an alternative medicine they cannot replace allopathic medical treatments. In cancer they may yet function as adjuvants, in other diseases they could play a slightly more prominent role. In the future this may change. It seems relatively sure that they can increase quality of life during conventional cancer treatment and may have a positive effect on soemautoimmune diseases, e.g. Crohn's disease.

Most mushrooms carry cell wall polysaccharides that cause immunomodulatory effects; it seems likely that the mechanism is the (re)establishment of equilibrium, i.e. immune homeostasis. This could explain pro- and anti-inflammatory effects exerted by the same compounds in different patients with the same disease.

The role of mushroom anti-oxidants is generally overvalued. Polyphenols can be pro- as well as anti-oxidant depending on the redox situation in situ. The effects of pro-oxidatives and the reactive oxygen species they induce, are insufficiently known in both cause and cure of disease. Receptor/ligand interactions are still between mushroom compounds, e.g. fatty acids and triterpenoids and others, and animal cell membranes should be studied and may rapidly lead to more understanding of the role mushroom compounds may play in human and animal health.

I have not tried to give a complete overview, I have scavenged fragments from Scopus.

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Mushroom substrate preparation (Phase I and Phase II Composting): What is it and what are your options?

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The first, and probably most important, step in producing white button mushrooms (i.e. champignons) is proper substrate preparation, involving a two-phase composting process prior to spawning. Phase I consists of high-temperature composting (~80°C) with the goal of creating a uniform, high-moisture substrate that is selective for the mushroom to grow and feed upon. If you travel around the world, you will see that there are several different methods being utilized to achieve these objectives, several of which are described in this text. After a uniform, high moisture compost is produced via Phase I composting, the substrate must then be pasteurized and conditioned in a process known as Phase II composting. This too can be accomplished using a variety of methods, each with its own benefits.

Before starting, a grower must decide what raw materials they will use to make the compost. The most common – and some say best – raw materials used for mushroom substrate production consist of wheat straw or wheat straw-bedded horse manure, poultry manure and gypsum. These materials are primarily used in Europe, North America, Australia and South Africa, areas where a long history of button mushroom production exists. The basics for compost formulation are to utilize a carbohydrate source (wheat straw), mixed with a protein source (poultry manure) and gypsum (a necessary ingredient used worldwide). Though gypsum is an ingredient that must be included in the formula, the source of carbohydrate and protein varies globally as well as locally, within the same country, depending on availability and costs. Not just any carbohydrate source can be utilized for button mushroom composting. The carbohydrate source should not consist of materials high in lignin, such as woody materials. Unlike white rot fungi that are able to efficiently degrade lignin (e.g. Pleurotus ostreatus: oyster mushroom), the button mushroom does not produce the necessary enzymes needed to break down this complex carbon. Rice straw is an alternative carbon source sometimes utilized in Asia to supplement or replace wheat straw. Though rice straw is quite different from wheat straw physically, it can be efficiently utilized to grow mushrooms if handled properly. In the eastern region of North America, mulch grass-based hay (timothy, orchard, brome grass, etc…) is often used in place of, or to supplement, wheat straw. Grass hays are tougher to compost and take longer to properly prepare; however, compost produced from these grasses can produce equally well as straw-based formulas. The same holds true for other carbon sources, often agricultural waste products.

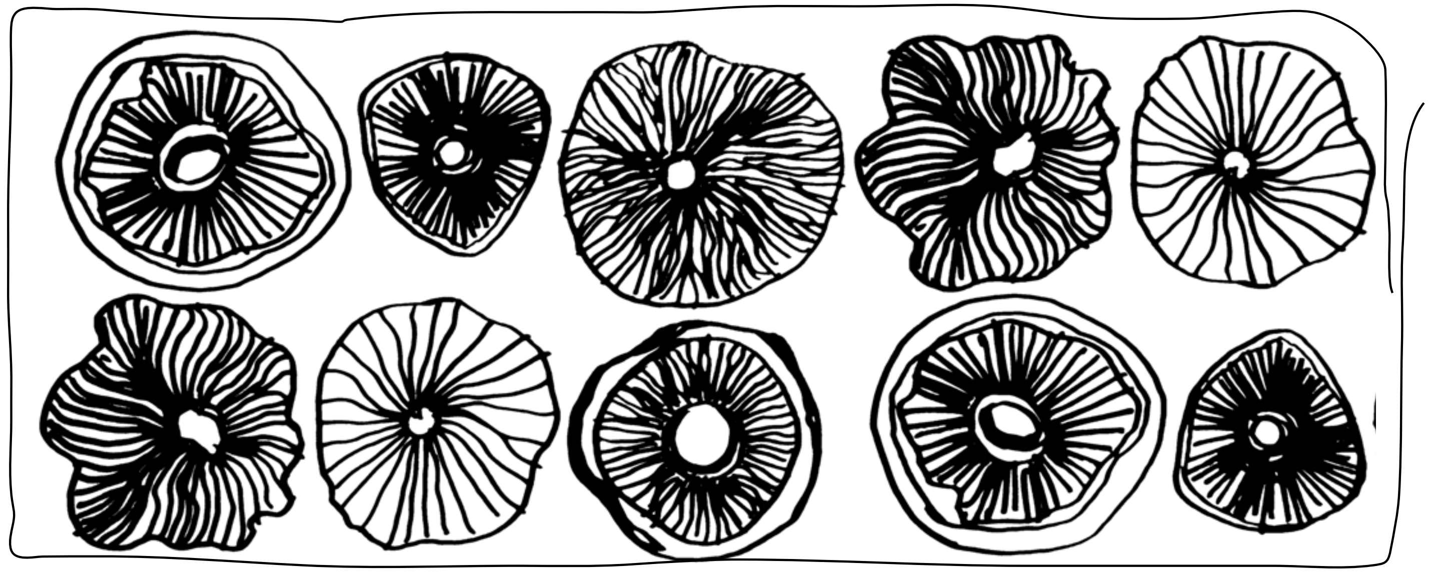
One alternative agricultural "waste product" currently being incorporated into commercial compost formulations is corn stover (the plant material left in the field after harvesting Zea maize). Corn stover is much different in appearance than wheat straw. Typically it may contain small pieces of the cob, the physical differences causing concern amongst many growers to incorporate this product into their formulas. However, the carbohydrate analyses of corn stover is quite similar to wheat straw and preliminary studies have found that it appears to produce as well as wheat straw when handled correctly. As is the case with any alternative raw material, growers should make changes slowly and check yields to ensure that any modifications to their formula or composting process have no negative impacts on yield or quality. Since corn stover is physically larger (lower surface to volume ratio), initial work that we've done at the University has been studied utilizing a longer composting period to allow for adequate breakdown and ensuring adequate moisture holding capacity, compared to a 6 day Phase I composting period with wheat straw. Composters that are already composting for longer periods may not need to extend the length of composting, it will depend on existing materials and composting systems being utilized.

Similarly, alternative nitrogen sources to poultry manure can be used based on availability of raw materials. Poultry manure is typically used because of the readily available supply and low costs. Vegetable meals (soybean and cottonseed) can be used to replace poultry manure as can ammonium sulfate and synthetic fertilizers. However, even if formulated for the same nitrogen content, varying nitrogen sources can impact the amount of ammonia produced during composting (ammonia is beneficial in substrate preparation) and it can have significant impacts to conditioning during Phase II composting. If applied at the wrong time (late Phase I) or if applied at too high a concentration, it may become very

difficult to clear the ammonia during Phase II.

In addition to deciding on which materials to utilize, growers have to determine the best method to mix the raw materials and incorporate water. The cheapest method is to turn piles with a pitch fork. However, this is very labor intensive and, unless turned aggressively, is difficulty to produce uniform piles with high temperatures (often because of the lack of size of a pile turned by hand). This method is typically not used in countries that have large farms; however, there are small farms that currently still use this method in some countries. A better approach is to turn the pile (or windrow) using a mechanical loader, allowing for piles to be made larger and often reach higher temperatures. However, this method still does not allow for adequate mixing and the compost is typically not very uniform, making it more challenging to consistently produce high yields. A slightly more capital intensive system is to turn the pile with a mechanical turner designed to mix compost. This system not only allows for large piles (and thus higher temperatures) but it also helps produce uniformity in the compost, subsequently leading to much more consistent, higher yields. Mechanical turning is still frequently used in North America, though turners are typically not used much anymore in Western Europe. The newest technology, and most advanced system, is to produce compost using a forced aerated system in a structure known as a Phase I bunker. Phase I bunkers minimize the chances of producing anaerobic compost by forcing air into the substrate, typically through a system of pipes built into the floor. These systems allow for more flexibility when adding water to the substrate and may reduce the time needed to compost. This flexibility and quality comes at a substantial cost for the engineering and building of these units.

Phase II composting has two objectives: 1) pasteurization to eliminate unwanted pests and competitor fungi as well as kill any mushroom and human pathogens and 2) condition the compost (removal



of free ammonia which is toxic to the mushroom mycelium). Phase II composting can be achieved in a growing room, whether cropping in trays or beds. The Phase I substrate is filled into the beds or trays inside of the growing room and compost temperatures are raised to approximately 60°C by injecting steam into the room. Temperatures are then lowered to approximately 48°C to allow for conditioning to occur. Conditioning is the process in which beneficial microbes convert free ammonia to protein to be later used as a food source by the mushroom. The second method is to utilize a bulk Phase II composting system in a specialized structure called a tunnel. A tunnel is similar to a bunker in that it provides aeration through floor to the compost, however, the air is recirculated to allow for uniform temperatures to be achieved (in the headspace, air supply and compost). Fresh air entering a tunnel system is also filtered to prevent recontamination of the compost with pathogens and flies. Running Phase II composting in the growing rooms requires an external energy source (boiler) to provide the steam and it also occupies growing space for up to 2 weeks until Phase II composting is complete. The tunnel system allows for greater flexibility of moisture content of the compost and requires little energy to reach and maintain compost temperature set points. It also allows for more crops to be produced in a room per year due to the fact that the growing space is not occupied for the duration of the Phase II. However, the design and construction of a Phase II tunnel, along with the necessary specialized equipment needed to fill and empty can be a very expensive undertaking for a small farmer.

If you ask a composter, a grower, or a consultant what the best formula and system is to make compost for mushroom production you are sure to get different answers based on a person's experience and background. Many often feel that the only way to grow mushrooms is to do it "right", meaning that modern Phase I bunkers and Phase II tunnels are needed and only wheat straw or wheat straw-bedded horse manure based formulations should be utilized to reach optimum yields. Though they may be correct that aerated bunkers and wheat straw produce the highest yields, I don't necessarily always agree that it's the best option for everybody. It's possible that a grower doesn't need to pick 35-40 kg/m2 to make a profit, depending on the scenario; a profit may be made at 20 kg/m2, not to say that much higher yields can't be obtained on different raw materials, they can. Each operation and farm is different regarding labor availability, raw material costs, energy costs and the market for the mushrooms produced. Therefore the design of each farm should be based on the region and the marketplace. Growers do not always need to stick with what the other guy is doing, especially if the other guy is located hundreds or even thousands of kilometers away, just because that farm has years of experience and they are picking high yields. There are a multitude of potential raw materials available for mushroom growers to try in their compost formulation, some may work better than others, but we should not be close minded and accept that there is only one way to do things. Utilizing other materials may just require a different way of thinking and require making adjustments to what is considered "standard" composting procedures. Staying flexible and looking at alternative, more economic materials may be beneficial for smaller growers based on their profit margin and geographic location.

CHAPTER 2. GUIDEBOOK

Mushroom Cultivation Manual for the Small Mushroom Entrepreneur

Ivanka Milenkovic
System Ekofungi

Contents

Introduction

Mushroom cultivation seeks to recreate natural processes in controlled conditions. This applies to a high-tech approach as much as for a simple and small production. The better the growing chamber is able to simulate nature, the better the final result will be, the better the quality and quantity of the edible fruiting bodies – or mushrooms – will be.

This is the secret of a successful production.

But what does this term, successful production, entail? Is it the ability to create fruiting bodies on some substrate under controlled conditions? Or is it rather the opportunity for a sustainable business generated by this product?

Given their biological, physiological and nutritive characteristics, it is not overly- ambitious to expect that mushrooms – especially those from the Pleurotus (oyster mushroom), Lentinus (shii-take) or Auricularia family – can be grown on a variety of substrates made from cellulose-containing material. Even this small step requires some skill, yet it is probable that mushrooms will grow. However, if these fruiting bodies are to be used as the basis of a small business, the producer needs to be knowledgeable of as well as provide and maintain favorable conditions.

The purpose of this manual is to present information that is indispensable for producing cellulose-utilizing mushrooms. Another goal is to provide instructions on how to establish a sustainable mushroom production based on available materials.

To be frank: with some luck a producer will end up with mushrooms regardless whether the substrate is of a poor quality or if the growing conditions he provides the fungi are near impossible. However, an acceptable yield of good quality which grants the grower with an income requires know-how and most importantly an understanding of the process!

This manual will try to explain the processes of mushroom production in the simplest and most effective way, in a way that is easy to understand. It is envisioned for novices as well as for those already active in the business. The author's approach is straightforward: the producer has to understand

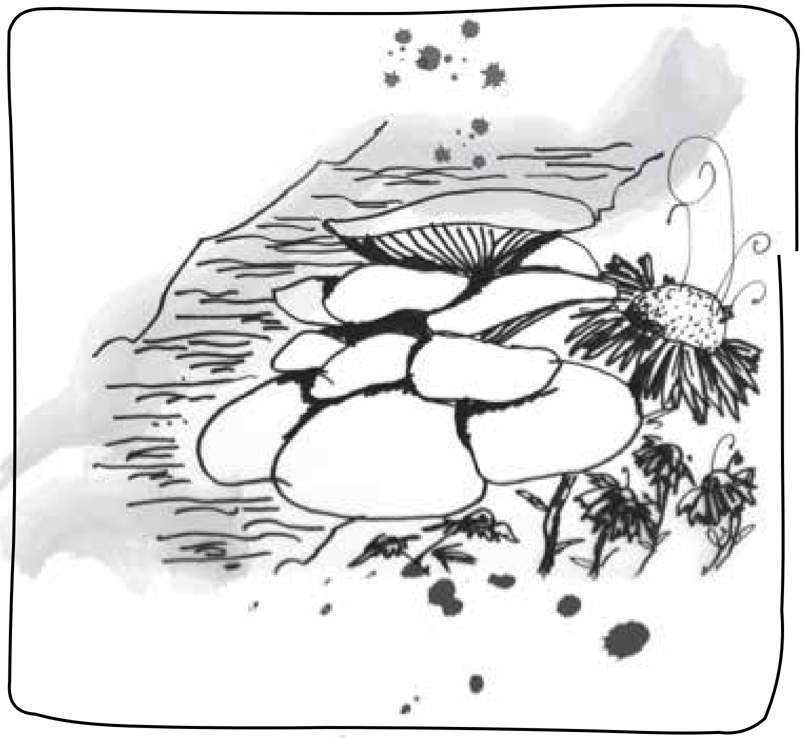
the process. Each step should be clear and logical: the producer needs to know what is important and why. Specific details of the approach taken by each individual practitioner is less important, since different contexts will largely influence these decisions (area available, raw materials available, location, desired production volume, etc.). Regardless of these factors, the production process MUST provide clear results, which are in fact nothing more than fulfillment of the mushroom's biological needs.

To conclude, it is not important how some phase/need of production will be achieved, it is important to fulfill basic needs, and to understand why they are important in the first place.

The Kingdom of Fungi

Fungi include more than 4.000.000 different species, found even in the most hostile and extreme conditions. They are able to survive extremely high or extremely low temperatures. It is little surprise that fungi can be found deep in ice on Earth's poles, and immediately after wildfires or other catastrophes. In most cases, fungi survive due to their ability to form spores. What seeds are to plants is similar to what spores are to fungi, spores result from asexual reproduction and are protected from extreme conditions for extended periods of time.

In natural ecosystems, fungi represent a high-potential cleaning mechanism. They find and degrade complex materials from their surroundings to use as their food.



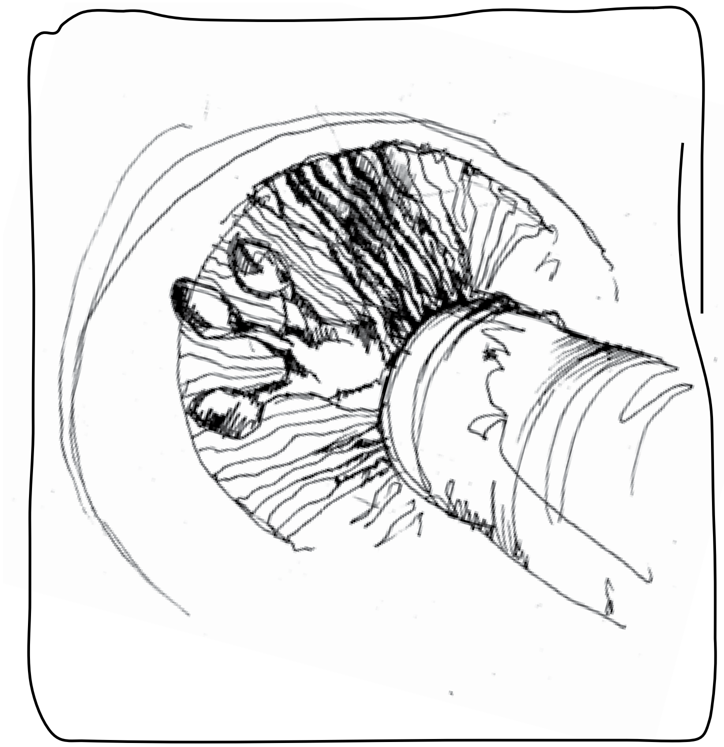
Unlike plants, fungi cannot capture carbon from the air and therefore, like animals, have to digest organic compounds such as carbohydrates, fats and proteins; they are heterotrophic. Unlike animals, fungi digest food by excreting enzymes outside their body and absorb food, or digested organic compounds, into their bodies through osmosis; they are osmotrophic. This feeding patterns requires a large surface area relative to body size, and thus the fungal body is an interconnected net-like mass of mycelium, made from thin thread-like tubular hyphae which branch and extend radially as they grow.

It is difficult to know the exact age of fungi as fossil records are scant, the oldest fossil discovered as of yet being only 400 million years old. Heckman et al. (2001) used DNA sequencing used to estimate that fungi first appeared 1.5 billion years ago. They suggest. Fungi started to colonize land 1 billion years ago, probably in the form of lichens (compound organisms of symbiotic algae/cyanobacteria and fungi), greatly contributing in changing atmospheric composition by increasing oxygen content. In comparison, plants appeared on land around 700 million years ago, and it is believed the very assisted by fungi to adapt to the new conditions (Pirozynski & Malloch, 1975). Animals in turn, did not become proper habitants of land until around 400 million years ago (Munnecke et al., 2010).

Once on land, the evolution of higher fungi was a gradual process. Higher fungi are those that belong to Basidiomycota and Ascomycota phyla. Encyclopedia Britannica provides a good overview of this timeline:

- 500 million years ago: hyphae similar to modern Basidiomycota appear.
- 300 million years ago: fungi with characteristics similar to Ascomycota and Basidiomycota first appear.
- 130-200 million years ago: fungi with easily recognizable mushroom fruiting bodies appear.

The Assembling the Fungal Tree of Life Project identified 558 taxa in 430 genera, 68 orders and five phyla (Lutzoni et al., 2004). However, the field of fungi classification is wide and due to contemporary molecular methods novel findings and reorganisations are to be expected. And, as all mycologists will tell you, there is certainly many more fungi that have not been discovered than those that have.



Which mushroom species are cultivated?

Mushroom producers are interested in those fungi species that can be seen with naked eye, known as macromycete or mushrooms. Systematically speaking, most of the species that are interesting for the cultivator, because they are edible and can be cultivated under artificial conditions, are in the class Agaricomycetes, order Agaricales.

Although cultivated and edible mushrooms can differ morphologically, biological classification clearly points to common characteristics. Without considering cell morphology and sexual reproduction (which is the basis for the biological classification), we would like to emphasize that all species from the class Agaromycetes have the same life cycle.

All mushroom species, that are of interest for producers and gatherers, go through two phases in their life cycle:

1. Mycelium growth, also known as vegetative phase, and
2. Fruit body formation or the germinative phase.

When conditions are right, fungal spores germinate into a single cell which extends, multiplies and branches to make hyphae. A mass of hyphae interconnects into a web to make mycelium. This is the mushroom body and it will keep on developing as long as the conditions for vegetative phase remain favorable.

From the perspective of mushroom production, spore fertility is the most important characteristic of the hyphae. Mycelium generated through spore germination can either be fertile (able to form a fruiting body) or sterile (unable to form a fruiting body). Spore fertility depends on the mushroom species or a biological mechanism called the recombination process that occurs during the moment of spore formation. Sterile mycelium is only able to form a fruiting body in case that it encounters another hyphae of a different sex-type, wherein it become fertile. Only fertile mycelium has the necessary morphological characteristics that will enable efficient and robust growth.

In the vegetative phase, fungi colonizes its substrate through mycelium growth. In the moment of stress (it can be any large deviation from the optimal conditions), the mushroom will switch to an-

other developmental phase: from the vegetative phase to the germinative phase. The final result of this phase is the edible mushroom. The germinative phase has one important purpose and that is spore formation. When you look at the fruiting body, spores could be found on the lower side of the cap, on fine structures that are called lamellae or tubules.

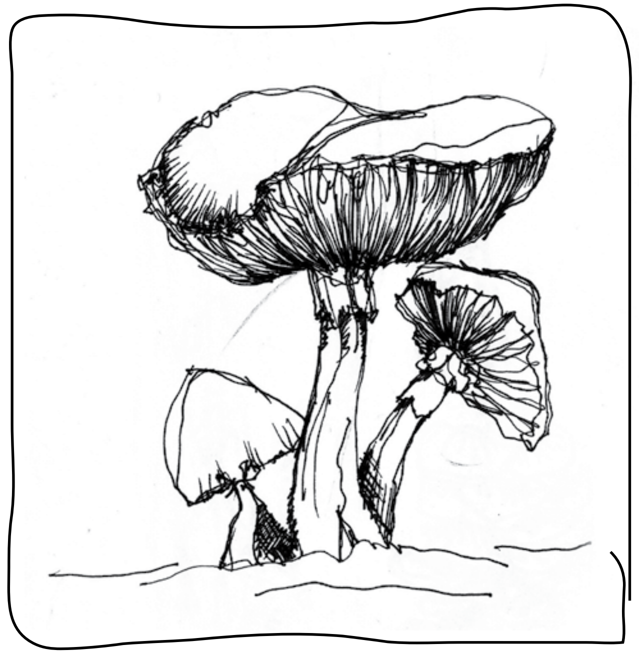
Spore formation is a very important phase during which genetic recombination is occurring in the fruiting body. This genetic mechanism has an effect on the fungi that will later develop from the spore. Special envelopes protect the spore, enabling its prevalence in unfavorable conditions. After all, fruit body formation is a response to the unfavorable conditions. Therefore, we can conclude that the formation of the fruiting body – that is the mushroom – is a response to non-optimal conditions. Wind, water and insects facilitate the spatial scattering of spores and enable the spreading of the mushroom realm.

As nature commands: mushroom cultivation technology

Mushroom production is the only economically-feasible biotechnology that converts complex organic molecules into more simple ones that can be used as food by humans. Fungi possess an aggressive enzyme complex: exogenous cellulolytic enzymes with the capacity to degrade cellulose molecules in a fast and efficient way. A profitable production must provide those conditions that promote fungal enzymatic function. In effect, all that we want and need to do is to efficiently simulate the natural processes typical for cellulose-degrading species from the order of Agaricales. Our job is to eliminate all obstacles and provide those conditions that will enable the fungi to complete its life cycle.

Cultivation technology can be divided in three segments, each of them equally important and indispensable.

1. Spawn production
2. Substrate preparation
3. Fruit body production



Spawn production

Spawn is comparable to a stem cutting of a higher plant: similarly, spawn vegetatively propagates fungi of the same genetic material.

From a genetics perspective, macromycetes are unpredictable and unstable organisms. The genetics and breeding of these fungi as well as subsequent preparation of spawn for commercial purposes is a very sensitive and delicate process. Laboratories with specialized equipment tackle this issue through molecular genetic methods, selecting strains to meet the needs of commercial producers.

This is the origin of strains used in commercial cultivation. Once created, a strain is the protected property of its author. It is guarded in hibernation under strict laboratory conditions, necessary in order to keep the genetic stability of the strain.

Commercially, a strain's mycelium is cultivated on sterile grains to create spawn. However, innovative approaches have enabled the use of artificial substrates as a growing medium for the mycelium of macromycetes.

At the beginning of spawn production, the mycelium culture needs to be revitalized from hibernation. The mycelium is then multiplied on liquid or solid media in the laboratory. Mycelium of a good quality is then transferred onto sterile grains —typically rye (Secale cereale), millet (Panicum miliaceum) or sometimes wheat (Triticum durum) – and then grown, under stable conditions free from microorganisms, until the mycelium envelops each grain. Each grain then becomes a potential inoculation point when it is applied to the substrate.

Attempting to produce mycelium on your own will most probably yield some result. Macromycetes mycelium can be propagated vegetatively from parts of the fruiting body (bought in a store for example) because of their biological and physiological characteristics. Another option is to multiply the spawn bought from commercial producer on grains.

However, amateur spawn resulting from these activities is extremely risky and often has damaging consequences for production. Beside that, there is the issue of the right to propagate biological material that is the intellectual property of its authors without their permission. Even when the culture is derived directly from a wild mushroom variety, the problems with amateur mycelium production are serious and place the success of the whole production into question.

The preservation of genetic stability is critical – vitality, growth ability and growth speed, robustness in non-ideal nutritive conditions are all important characteristics. As mentioned before, macromycetes' genetics are unpredictable and unstable. Repetitive multiplying by non-professionals generally leads to loss of all these important characteristics, and leads to blunders and losses in production.

These details are important background information, but also lead to the conclusion that in-house spawn production is not recommended for small mushroom entrepreneurs regardless of experience. In addition, it should be noted that the pure culture of a selected macromycete is difficult to obtain.

As a result of our experience as both producers and in mushroom projects around the world, we strongly recommend mycelium produced by Sylvan Inc., the world leader in this industry.

Substrate

As we have seen, all cultivated species are closely related.

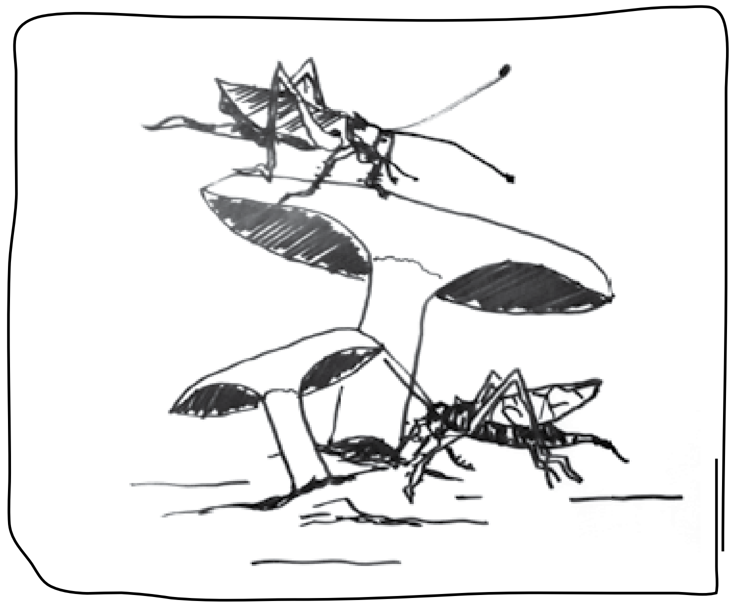
However, as in life, some family members are more hard-working and capable than others. In the mushroom world, this means being able to prepare their own food from complex cellulose molecules that are the main building blocks of plants. The hard-workers include shiitake, oyster mushrooms, poplar mushrooms; they can be grouped under the name of cellulose-degrading mushrooms or white rot fungi. On the other hand there are family members not able to prepare their own food: the white button mushroom (commonly called champignon), CoprInus (shaggy cap), etc.   The main difference between these two groups is their physiology and the differing way that they digest their food.

During the vegetative growth phase, during which a fungi's mycelium is expanding, the white button mushroom feeds off of the so called ligno-humus complex. During this phase, the quantity of simple sugars present in the compost remains almost unchanged. However, during the germination phase, these simple sugars are degraded to power the formation of the fruiting body. To generate degradation, the mushroom secretes an arsenal of exo-enzymes (i.e. enzymes outside of the fungal body). Some scientists believe that the mechanism behind the absorption of degraded molecules from compost is unique in the mushroom world. It is very interesting to note that the white button mushroom has been shown to grow up to two times slower in sterile substrate – clearly, the mushroom has forged a special relationship with specific microorganisms present in the compost.

White button mushrooms fulfill all their nutritive requirements from the growing medium, known as substrate or compost, which is prepared in a process called composting. It is important to note that mushroom compost cannot be made from composting any organic material (that is, material of plant origin). Furthermore, the mushroom composting process differs significantly from the composting process that turns organic materials into compost for use in the cultivation of plants.

Straw and chicken/horse manure are the raw materials most often used for preparation of compost for white button mushroom production. The process can be successfully realized in specific conditions and with specific hardware, and presently with the help of automation/software.

Compost for the white button mushroom (and those similar to it) is a complex system/environment with specific physical, chemical and biological characteristics, which together create the conditions needed for the mushroom to complete its life cycle. One of the most important characteristics of compost is its high selectivity. This means that its conditions favor a narrow range of organisms, one of which is the white button mushroom.



Beside the inherent biological complexity of cultivating the white button mushroom, their high vulnerability contributes to the trend of intensifying cultivation. Industrial production is characterized by high efficacy, fast cycles and high yield. In order to achieve these results, dedicated R&D addresses all aspects of production, from technology to breeding and genetics. White button mushroom strains developed for these production systems are very demanding, or we can say spoiled, regarding their growing conditions. As a consequence, small scale producers with the ambition to supply the local market best look past the production of white

button mushrooms.

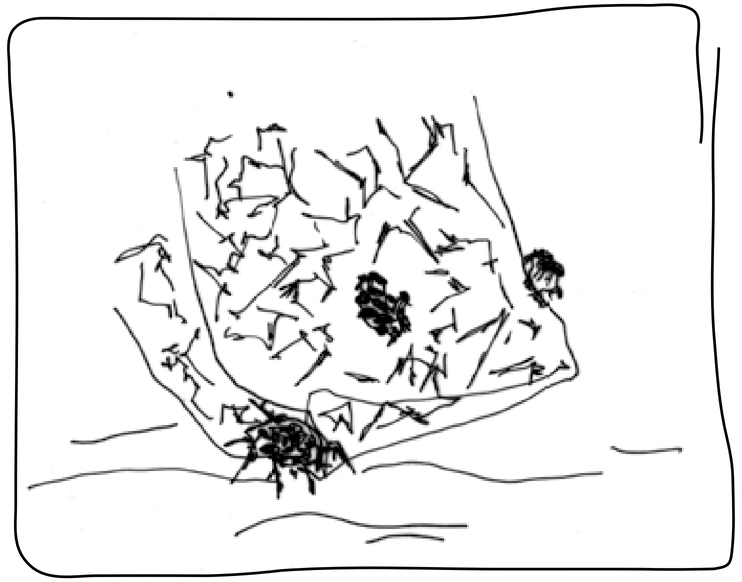
Luckily there is a second group of hardworking mushrooms with significant physiological differences – the cellulose-degrading or white rot fungi. The oyster mushroom belongs to this group, and in nature grows on the residues of dead higher plants. Their vegetative growth is driven exclusively by their own enzyme complex: given sufficient moisture in the material, enzymes degrade complex lignocellulose molecules into simpler molecules that the mushroom can make use of. The fructification phase does not have specific nutritive requirements. These characteristics are exploited for production processes of white rot fungi species.

In a controlled environment such as that of a growing chamber, the oyster mushroom requires these very elements: cellulose containing material to provide energy, and sufficient moisture content of the material to enable enzymatic activity. Another important point that requires attention of the producer is eliminating the enemies of the oyster mushroom. In most cases these are other fungi that feed off of similar components like the oyster mushroom. They are biologically more robust and resistant and easily outcompete cultivated mushrooms.

How to choose raw materials?

White rot fungi are cultivated on cellulose-containing materials. The lignocellulose complex, which contains cellulose, is a structural material. Materials rich in lignocellulose include:

- woody plants, from which sawdust, wood chips, or even logs are used for mushroom production;
- grains, from which straw is used for mushroom production;
- other plants with solid stem, from which hard stem pieces, branches, etc. are used for mushroom production;
- and husks from coffee beans as well as brewery spent grain, coffee waste, coffee are used for mushroom production.



People often ask if they can use greenish plants or greenish parts of plants (for example leaves) for mushroom production. The answer is no! A short reminder: oyster mushroom and other white rot fungi grow exclusively on the lignocellulose complex. The greenish part of plants (those which would stain your hand green upon being squeezed) cannot be a raw material for the production of substrate since they do not contain the lignocellulose complex.

The first criterion to identify a suitable material for mushroom substrate is the amount of cellulose it contains. The material needs to be available to the producer: located nearby and there must be a way to transport it.

Simply put, sustainable production of oyster mushroom (and cellulose-degrading species) can only be achieved if suitable cellulosic material is available up to 30 km away from the production site. The reason for this is simple: raw materials for mushroom substrate are lightweight and bulky, and long distance transportation is not economically justified. **Thus, the choice of raw material for substrate is dictated by its availability and how easy it is for the producer to collect it.**

The second criterion, no less important, is its cleanliness. This term as it is used here means that the material should be free from dust, dirt and other impurities that do not contain cellulose; examples include an orange peel in coffee waste, green leaves among branches, cellophane amongst straw. The term also refers to:

- Lack of microorganisms like molds that can be seen with the naked eye;
- And that the material was not intensively treated with chemicals during its production or handling.

The presence of molds can be easily observed, but the situation is quite different for the presence of chemicals or a high heavy metal content. Some protective agents used in contemporary plant production have the effect of preventing fungal growth on their byproducts after harvest. In these cases, it is possible for the fungi to successfully grow on a contaminated substrate, but the chances of absorbing pesticide residues along with its food is high. In the end, such negatively enriched mushrooms will end up on our plates.

The only way to address these issues is to develop a relationship with the supplier, or to find out how the material was produced or handled.

In the urban context, mushroom production on coffee waste is very popular and attractive. Given the variation of type and quantity of coffee consumed across cultures and possible challenge to efficiency of collection, this material can be complemented by or replaced with: brewery spent grain from the local mini-breweries, good quality cardboard without plastic, fine-cut branches from parks, etc.

In rural or peri-urban areas where straw, sawdust, or corn cobs are available, these materials should be prioritized since mushroom production with them is more secure and easy to handle.

The substrate preparation process

Substrate preparation for the oyster mushroom and similar species needs to fulfill several conditions:

- Competitors that use the same food source as our mushroom must be eliminated,
- Access to cellulose in the selected material,
- And sufficient moisture in the material/substrate.

Based on experimental knowledge, long-term experience, and understanding of the biology and physiology of white rot fungi, the above listed goals can be achieved with a large variety of processes in different conditions and circumstances.

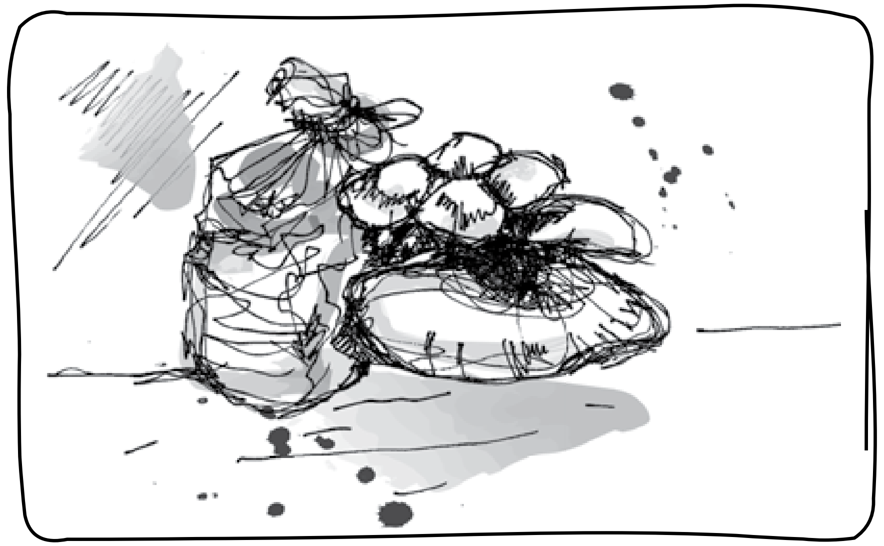
Common sterilization and pasteurization processes will not be emphasized as the basic, primary and only possible ones.

Elimination of potential competitors

Most problems that occur during the production of mushroom substrate relate to this aspect. Producers design their processes to achieve pasteurization or even sterilization of the material designated for substrate. Of course, there is no doubt that these approaches can contribute to a successful production. However both of these processes intensively consume energy and thus entail a high cost. Also, the effects of these treatments on other key production parameters has to be taken into account. For example, pasteurization with steam dries out the material. Finally, substrate after it is subjugated to pasteurization/sterilization will most likely be exposed to open air at some point, after which the intent behind these expensive processes is lost.

The recommended approach to eliminate potential competitors is to expose the material to ample and intensive washing, using clean water and letting it flow through the material. The water used for this process can be reused for other activities on the farm or in the household, but it cannot be reused more than once for substrate washing. The water will contain impurities that were washed from the material. The water can, however, still be used to maintain the level of moisture in the air in the cultivation chambers.

Washing can take anywhere from 1 to 24



hours, depending on the type of material and quantity involved. Simply put, one can tell the process is finished when water coming out from the material is clean.

Ensuring cellulose availability and sufficient moisture in the substrate

The most important part of the substrate preparation phase is to provide easily accessible food – that is, cellulose – for fungal mycelia. This entails loosening and/or breaking bonds between cellulose and other parts of the lignocellulose molecule.

The material has to be fragmented. Fracturing, splintering and/or cutting the substrate's raw material damages the protective layer whose purpose in nature is to protect plants by repelling water. On the molecular level, the lignocellulose structure is cracked. Clearly, materials such as sawdust or coffee waste do not have to be fragmented; all other materials have to be cut into particles that are 2-5 cm long and wide.

After being cut up and washed, the material is then immersed in water, which should submerge it completely. Prolonged soaking leads to relaxing/loosening of the links between cellulose and lignin, even for the hardest cellulose-containing material. The result is that cellulose in the substrate is more available for mushroom mycelium. Soaking length depends on the type of raw material (Table 2).

Providing conditions for the successful activity of fungal enzymes

Cellulose degrading mushrooms secrete enzymes outside of their mycelium and into their immediate environment, where the enzymatic activity occurs. The efficiency of this activity enables rapid growth of mycelium through the substrate, simply because it is able to provide plenty of food for itself. It is important that the fungi has a strong start after the substrate has been inoculated for it to compete with microorganisms that feed on the same food. The main condition to encourage this is to have sufficient moisture in the substrate. The water has to be absorbed by the substrate, as the substrate cannot be submerged during production. Why? Excess of water means a shortage of oxygen, and without oxygen there is no growth or survival of mycelium. We can conclude that we need to maximize the amount of water absorbed by the substrate.

How do we achieve this?

There are many technical solutions available – the producer needs to choose the one that suits his conditions the best. One thing should always be kept in mind: the most adequate approach is the one with which the final product will have a competitive price on the market. Only in this way will production will be sustainable.

1. Cutting procedure

In the case of coffee waste, coffee husk, spent brewery grain or sawdust, cutting can be skipped. However, in the case that material with larger particle is used including straw, branches, cardboard, etc., the material has to be cut into pieces from 2-5 cm length. The easiest way to do this is to use a hammer mill, typically available in an agricultural setting, but other solutions can be found that are more appropriate to specific conditions of the producer. No matter which process you choose, the outcome has to be the same: fragmented material.

2. Washing procedure

All raw materials (straw, corn cobs, sawdust, wood chips, cardboard, etc.) except coffee waste has to be exposed to a large quantity of running clean water. The washing lasts until the water coming out of the material is clean. If the material was stored and transported under conditions of low hygiene (for example: parks near the streets, bulk corn cobs in trailers) longer washing is necessary. Similarly, in these cases the producer will see clean water leaving the material when it is clean.

There is one specific situation that requires washing even when the material is clean: the use of brewery spent grain. This material is very clean, even sterile, yet has a high content of simple sugars and proteins. The oyster mushroom and other white rot fungi do not use them. On the other hand, these molecules are a great source of food for other species that compete with the oyster mushroom or can cause it harm. In order to eliminate this threat, brewery spent grain must be exposed to running water for an extended period, and then left submerged to soak for 24 hours. During the soaking period, the water needs to be changed from time to time as it will become saturated with proteins and sugars from the material.

Even after intensive washing it is recommended to mix brewery spent grain with some other material less rich in sugars and proteins, in order to make the substrate less attractive for the growth of competitor fungi.

Coffee waste does not need washing if it is used recently after it has been collected. However, use of this material does require well-tuned logistics, including an agreement with the coffee waste supplier to use clean collecting containers. We recommend that a mushroom producer provides the clean dishes and delivers them in return for one full of coffee waste. In addition, collected coffee waste needs to be used for mushroom production in a period no longer than 4 days since it was used for brewing.

It must be underlined: washing raw materials for substrate is the most important step in the mushroom production process.

3. Adoption of adequate amount of water

Celluloise containing weeds	24 hours
Straws	36 hours
Sawdust from deciduous trees	96 hours

Table 2. Soaking time for various materials to prepare substrate.

If the material is to be soaked for more than 24 hours, water needs to move around from time to time. This can be achieved with a simple submersible pump or just by change of water. This procedure has to ensure that the availability of oxygen is refreshed to prevent anaerobic processes from occurring.

The water can be heated, but that depends on the conditions in which the producer operates and her/his possibilities. It is generally recommended to heat up the material during the soaking phase. However, it must be noted that the temperature of the water should not exceed 55°C. The reason for this limit has been repeated several times in this text and must always stay prominent in the mind of the producer – financial feasibility.

Heated water is better absorbed by the material. Thus, its lignocellulose bonds are loosened more efficiently, resulting in a shorter substrate preparation process. However, heating water does not achieve pasteurization. That is a completely different approach. The goal of the mushroom producer that is in tune with biological rhythms of the process is to provide food for the white rot fungi, not to kill the microorganisms present on the food source. Even if he would like to do this, it is very difficult to achieve in a small-scale production.

After soaking, excess water has to be eliminated from the substrate because it diminishes oxygen availability, which is crucial for the growth of mycelium.

If the producer decides to use coffee waste, special attention has to be paid to the amount of moisture. S/he must ensure that this material did not lose the moisture that it acquired during the brewing of coffee. If indeed the material is too dry, then it has to be carefully soaked using fine filter bags. We will remind the reader that a crucial goal of substrate preparation is to provide adequate moisture in the substrate, which is absolutely necessary for the function of cellulose-degrading enzymes that provide the fungi its food.

4. Inoculation

Hygiene is the basic and most important condition that has to be fulfilled during inoculation. It is also important to check if the substrate is cold; the substrate's temperature must not exceed 20°C during inoculation.

This process must be conducted in a clean space, sheltered from the wind and from insects.

Spawn bought from specialized producers has to be broken into small pieces with clean hands. The next step is to mix spawn with the substrate: 2.1 kg of spawn (3L) to 100 kg of substrate. It should be noted that spawn is typically measured by its volume (L).

Spawn needs to be mixed into the substrate slowly and evenly, by hand or some other convenient tool. A uniform spread will ensure that mycelial growth from the inoculation points will interconnect as fast as possible into a single organism, leaving little resources for competitors to grow.

No matter which material is used to prepare the substrate, the mushroom producer must pay attention to its pH. Optimal pH is between neutral to slightly alkaline, a range more suitable for cellulose-degrading mushrooms than for competitors. We recommend the addition of about 0.5 % $CaCO3$ in tiny grumps during the step of substrate and mycelium mixing.

The substrate is then packed into the polyethylene (plastic) bags. They must not be biodegradable because the mushroom will degrade them during its growth. The bags' dimensions are not a critical issue for production, but the general recommendation is 60-70 cm high and about 40 cm width. It is important to note that small bags holding 1-2 kg of substrate are not recommended for production, except in the case that inoculation is only for fun. Substrate in bags that have a smaller volume easily loses moisture and this complicates fructification. The thickness of the bags is more important: it should allow the substrate to be firmly packed into it without ripping.

Before the bags are packed they need to be perforated. The cuttings should be made in the shape of cross, with the dimensions 2x2 cm. The distance between the perforations should be about 25 cm.

Mushroom cultivation

As was already mentioned, mushroom cultivation is the simulation of natural processes in a controlled environment.

The greater the degree of quality with which natural conditions are replicated in the growing chambers, the better the result that can be expected.

In order to understand the cultivation process and the parameters involved, the producer should think about the natural environment in which edible, medicinal and all other kinds of mushrooms can be found:

First of all, they cannot be found in places where water runs or lies in puddles. Mushrooms inhabit humid places, away from direct sunlight. The same principals must be applied in the production chamber: one must not focus on high humidity or produce puddles in growing unit.

Secondly, mushrooms cannot be found in places where air currents can be felt or are intense. Similarly, artificial conditions must avoid intense or uncontrolled flowing of air.

Thirdly: when is it that we find mushrooms in nature? It is always after a sudden change in the environment, like that following rain or a storm, when the air is refreshed. These conditions have to be replicated in cultivation chambers to ensure that mushrooms mark undisturbed and good growth.

Fourthly, mushrooms that are convenient for cultivation do not inhabit extremely hot or extremely cold places. This means that the temperature in the growing unit should be between 15 and 25°C.

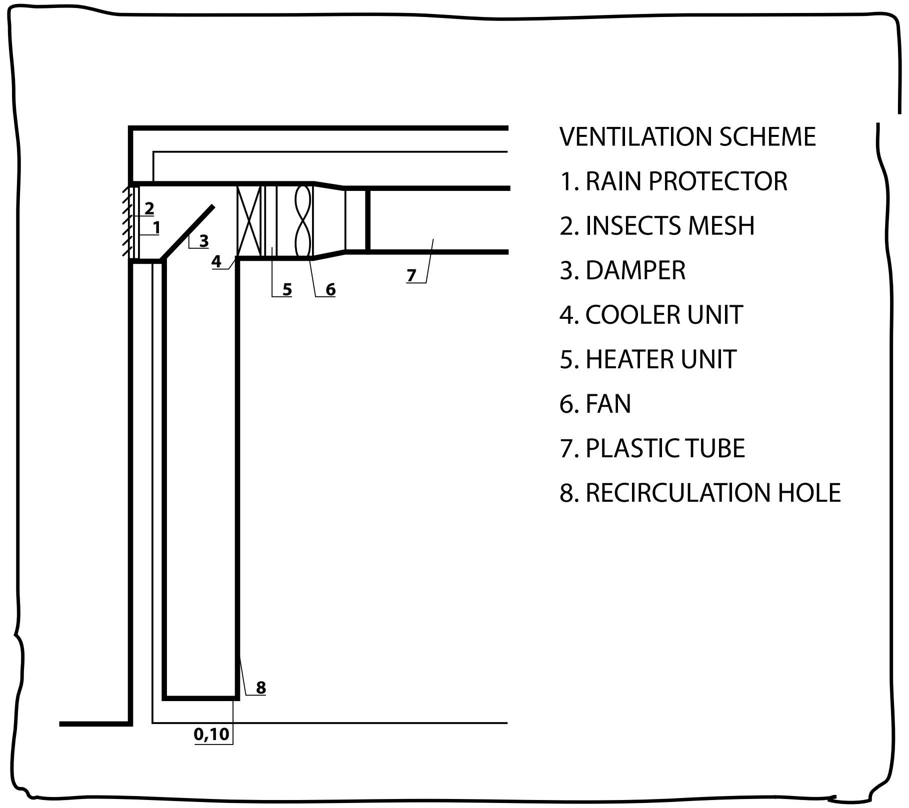
Ventilation system

The following conditions need to be provided in all cases, regardless of whether we are referring to a micro, small or hi-tech production. The process is in essence the same in all these cases.

The growing chamber must have no more than one fan. The capacity of the fan should be 10 exchanges of the total volume of air in the chamber per hour. If the room, for example, has an area of 25 m2 and is 3 m high (volume of 75 m3), the fan's capacity should be able to exchange 750 m3 of air per hour.

The fan is placed within a mixing box whose bottom is movable (it can be lifted up and down). There is a vertical duct coming out from the bottom of the box. The end of duct away from the mixing box has

no closing on its end, opening to the growing chamber and located near the floor. At the same time, the mixing box is located up against the wall of the growing unit, with an opening to allow the fan to freely draw in air from outside. The bottom of the mixing box has a damper, which when lifted allows the fan to pull in air from the bottom of growing unit (i.e. above the ground) into the mixing box. On the other hand, when the damper is closed, the fan will draw in only the outside air into the mixing box with its full capacity. The mixing box may be improvised or constructed in a sophisticated manner; however the main structural design that enables its basic function has to be maintained. The figure below shows a mixing box.



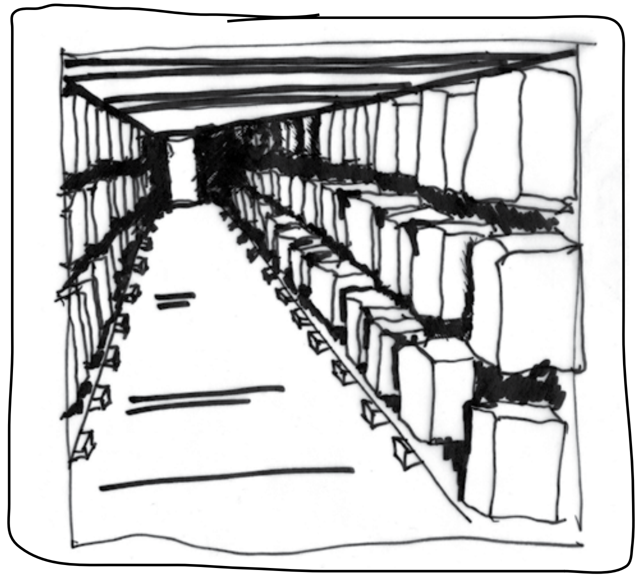
Heating or cooling air for the growing unit is achieved by using heat exchangers installed in the mixing box. It is not allowed to use air-conditioning units. Why? As has already been stated, moderate air flow is a must for successful mushroom production. Another fan will increase the speed of airflow, and result in no mushrooms!

The fan should be set up to blow air into a polyethylene tube whose diameter is equal to the fan's diameter, placed across the ceiling of the growing unit. The tube should be perforated with the holes in two rows, positioned like clock hands pointing to half past one and half past ten if one is facing the opening of the tube. The end of the tube should reach the other end of the growing chamber, and be tied in a knot, forcing all air to flow through the holes.

In this way we assure even air flow in the chamber and guarantee that each cutting on the bag of substrates receives an equal amount of good quality air.

Air humidity

Humidifying a growing unit can be achieved simply by pouring water on the floor. Using sprinklers or nozzles for this purpose is not recommended. However, the need for additional air humidity may arise, as during hot periods when cooling is necessary – remember how much water is drained from air-conditioned rooms in the summer. This can be addressed by a so called water curtain. In other words, air that enters the growing chamber is forced to pass through a curtain of pouring water or a wet barrier, thus absorbing moisture as it enters the growing chamber. At the same time, the water curtain lowers the temperature of the incoming air, achieving a double effect of humidification and cooling on hot days.



Organization of Space

Besides providing micro-climatic conditions, the organization of space is vital. The positioning of racks and shelves has to be carefully planned. Cellulose degrading mushrooms do not require shelves, which can entail high expenses. Instead, hooks and racks can be used.

It is important to always have in mind that mushroom cultivation is nothing more than the simulation of natural processes. Rapid air flow should be avoided, and thus the amount of substrate in growing unit must be optimized. One cubic meter of space must not contain more than 20 kg of substrate. How the substrate is arranged is not important.

The cultivation process

Mushroom cultivation begins at the moment of substrate inoculation with spawn, and can be split into two phases.

Incubation phase

The incubation period is when all the small pieces of vegetative mushroom body, scattered in the substrate as spawn during inoculation continue to grow in order to interconnect with each other. At the same time, surely other microorganisms like molds are also present in the same substrate and grow as well. Some of the microorganisms do not harm our mushroom while others compete with it for food and space. In order to encourage efficient growth of our mushroom's mycelium and to give it an advantage over competitors, key parameters are addressed and optimized during the substrate preparation step. However, once in the cultivation chambers, additional steps must be taken during the incubation period to support the mushroom.

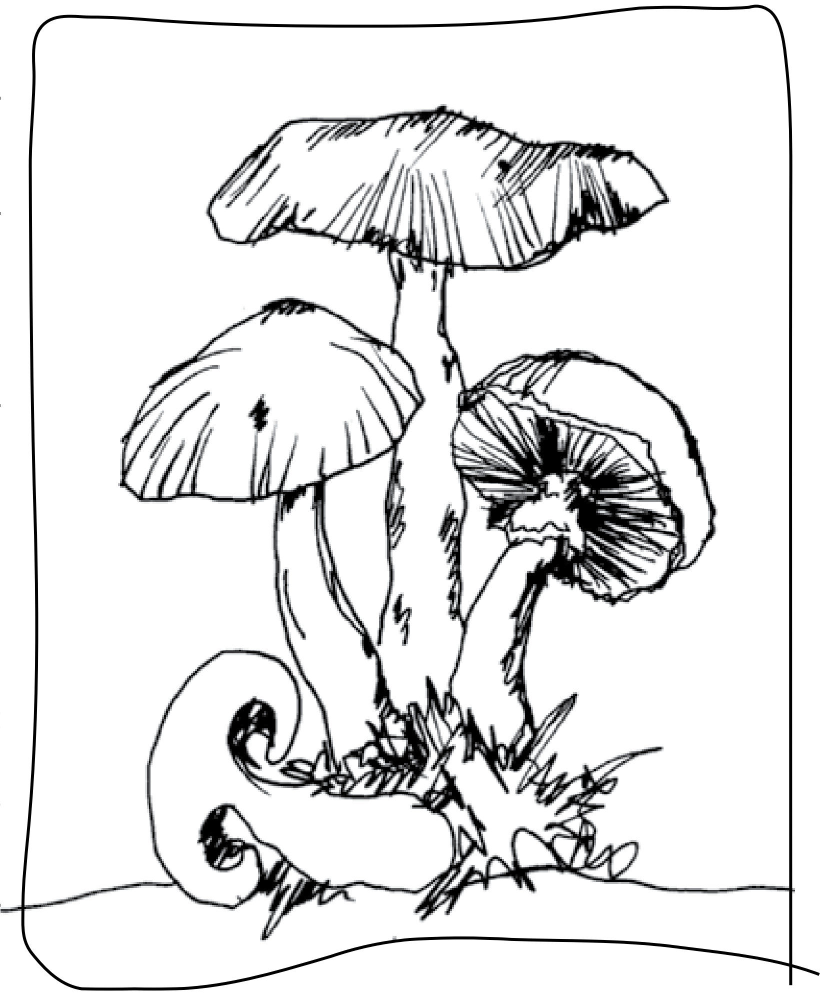
Firstly, temperature in the chamber must be maintained between 20 to 22°C. The inflow of fresh air is completely eliminated by closing the damper regulating inflow of fresh air from outside the cultivation chamber. The floor needs to occasionally be covered in water to maintain air moisture.

In such conditions, mycelium will grow rapidly and after three weeks the whole substrate will be covered by mycelium. The smell in the growing unit should be pleasant, of mushrooms and forest litter, and certainly not of dankness or mold. **Smell is the best indicator of a good situation in growing chamber and is the first to signal that something has gone wrong.**

Fructification phase

After about three weeks, around each cross-shaped hole cut into the bag there will be a thickening of mycelium tracing the shape of the hole.

This is the moment for a dramatic change in micro-climatic conditions in the growing unit, to simulate a storm. In effect we mimic conditions after the rain: inflow of fresh air. This means that the fan should work at full tilt. With these changes, a switch is stimulated in the mycelium from a vegetative growth phase to a phase in which fructification bodies are formed.



In the initial moments of fruit body formation, temperature in the growing chamber should be decreased to between 16-18°C. The fan should work constantly and the dampers should be open so that 70% of air comes from the outside the growing chamber and 30% is drawn from the growing unit.

The floor should be periodically dampened. It is important for the mushroom producer to simulate day and night for cellulose-degrading mushrooms. Artificial lights – specifically which kind is not critical – should be switched on and off routinely.

Three to four days after fructification was initiated, mushrooms will start to appear in bunches from the holes in the bags

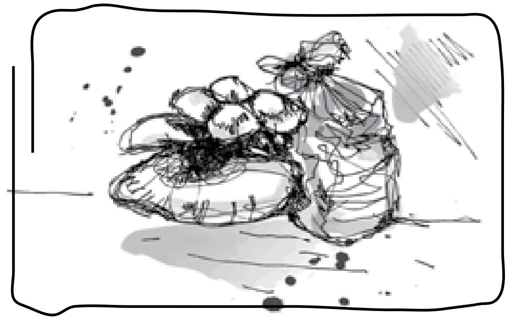
Mushrooms grow from substrate in so called waves or flushes. During the first wave it can be expected that every cutting on the substrate bag is decorated with a bouquet of oyster mushroom.

Bunches should be carefully picked from the bag, at the moment when the oyster mushroom's cap starts to bend up.

The size of the fruiting body does not indicate maturity of the mushroom – its shape does!

The right moment for harvest.

The spot from which the bunch was removed needs to be cleaned from remaining fragments of mycelium, and be airy and ready for the next flush.


The conditions at the beginning of fructification need to be maintained during whole period. It is desirable that after the first wave is over, to cutoff inflow of fresh air and turn off the light for two to three days. Opinions are divided on this practice, but it might be that it intensifies the second wave. The second flush will come after 7 days and lasts for 5 days.

The whole fructification period should result in a yield of about 18 kg of fresh oyster mushrooms per 100 kg of inoculated substrate. This proportion (18%) is estimated for conditions prevalent in intensive small scale production, where one cycle takes about 7 weeks. It is important to note that conditions in real production and the laboratory are not the same. Theoretical yield can be much higher but the length of growing cycles and repeatability of results should be taken into account. A small mushroom business should not be planned on the basis of high expectations, but on realistic and objective ones.

Preparation of growing unit/hygiene

Before dwelling into this subject, it is important to remind the reader that the approach to mushroom production presented in this handbook is rooted in the physiology and biology of cellulose degrading mushrooms, and in a preference to optimize conditions that favor mycelium growth rather than trying to eliminate competitors. In this way, the mushroom has an edge to win in its fight against competitors without problems. With this in mind, we can see that hygiene of the level present in sterile spaces of hospitals and surgery rooms are difficult to be replicated in a small mushroom business. Moreover, it is very expensive to do so. The question also remains: is it really necessary?

The spaces used during the production process include those where: substrate is prepared, substrate is inoculated, the growing chambers and the space where harvested mushrooms are stored and packed. Simply put, all these spaces must at all times be clean and neat.

The space is which substrate is prepared needs to be washed after this step is completed, making sure to remove all fragments of substrate. All containers and accessories that were used have to be

cleaned. After washing, these materials should be dried and put back into a space designated for their keeping.

The space for inoculation must also be cleaned every time after this step has been completed. The producer must take care that little bits of mycelium do not remain hidden out of sight.

Before the next inoculation step starts, the working surface should be disinfected, for example with 96% alcohol. Of course, household products for disinfection are also acceptable.

If the working space for substrate preparation as well as for the inoculation is too large and it proves difficult to maintain as in a household, then the best alternative is to use copper-sulfate (i.e. $CuSO_4$). Of course, this substance is used in addition to intense cleaning of the work space before its application. Instructions provided on the package for its use should be followed.

Hygiene of a growing unit

The growing unit which houses the inoculated substrate must be as clean as a kitchen! At the end of the previous production cycle after the bags of spent substrate have been ejected, the growing chamber must be carefully cleaned of all fragments and sediment, large and small. This includes all that can be seen with the naked eye and remains from the recently completed production cycle. After this, the chamber is to be washed. For this purpose, it is important that the growing unit was initially located where this is possible. Washing is conducted only with water from a municipal water supply or another source which provides clean/filtered water. Clean growing units must be occasionally painted before new substrate can be put in. Painting is needed only when dark spots can be seen in the corners of the chamber implicating the emergence of molds.

Regardless whether a chamber is painted or not, after cleaning it is necessary to heat the chamber to 18°C for the 24 hours and then apply a mild copper sulfate treatment. This is a classical approach in the battle against molds and it is best to follow instructions available on the packaging.

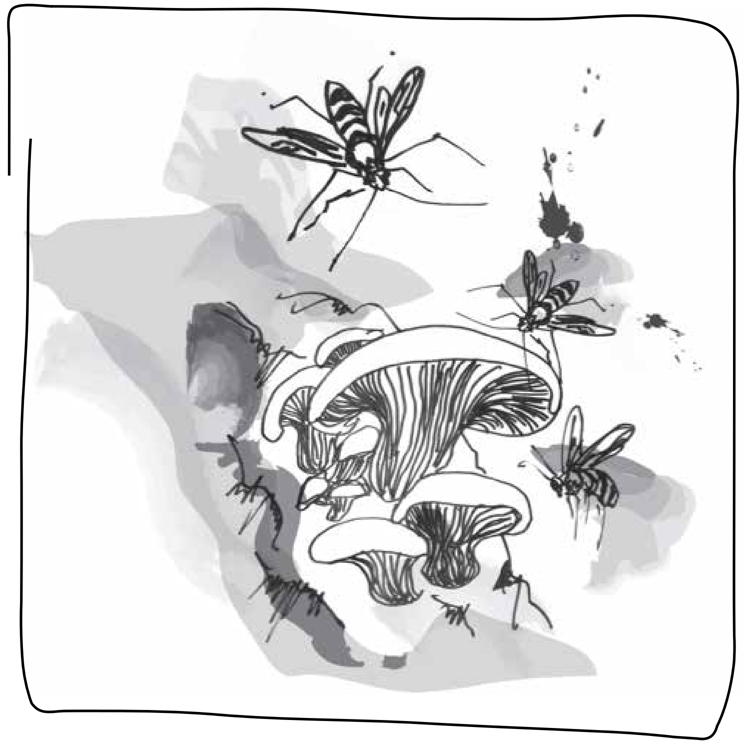
During cultivation, the growing unit should also be treated like a clean kitchen: do not leave waste, do not enter with dirty footwear and maintain everything clean and tidy. We must not forget that we are producing food, and doing so by emulating nature. With the knowledge that nature does not have anything sterilized, similarly we can conclude that we also do not need sterile conditions.

Problems in the production (in situ)

There are always problems that arise during production, and there always will be. Cultivation of oyster mushroom is specific in the way that few remedies exist once the substrate has been inoculated. Therefore, the small mushroom entrepreneur should be aware of the most common problems they will face, provided in the following list. It is also important to understand the problem and its cause to avoid wrong remedies, which unfortunately often make a situation worse than it is.

1. Poor mycelium growth emanating from spawn (in the substrate bag).

● Mycelium starts to grow from spawn fragments and suddenly stops. The substrate does not change color or show signs of contamination.



The cause: poor chemical composition of raw materials – full of pesticides and/or additives.

● Mycelium fails to grow from the spawn, and it turns green in color.

The cause: old/poor quality spawn, spawn was not properly stored, moisture is too high in the substrate or spawn was inoculated while substrate temperature was above 30°C.

● Sporadic growth, wherein mycelium grows well from some spawn fragments and not at all from other fragments.

The cause: unequal substrate quality, often due to unequal moisture content but also possibly due to raw materials used for the substrate; pay attention to equally mix spawn equally when inoculating substrate.

If the substrate begins to change color before it is incubated in the growing unit, this implies heavy presence of pathogens or competitors. It is likely a problem with the quality of raw materials used for the substrate.

This is a good occasion to accentuate that if mycelium has already covered the substrate up to 50%, as visible from the outside of the bag, it does not have to be ejected from the growing unit. Our mushroom is a strong fighter and it is likely to best its competition at this point. However, the substrate bag must should not be opened to avoid the spreading of competitor/pathogen spores.

2. Poor fructification

● Long stem of fruiting bodies, from slightly elongated to a fruiting body characterized by a long stem and a small cap; yet with no change in color.

The cause: insufficient air in the growing unit at the moment of primordia formation (i.e. when a white ring of mycelium forms around the cuts in the bag).

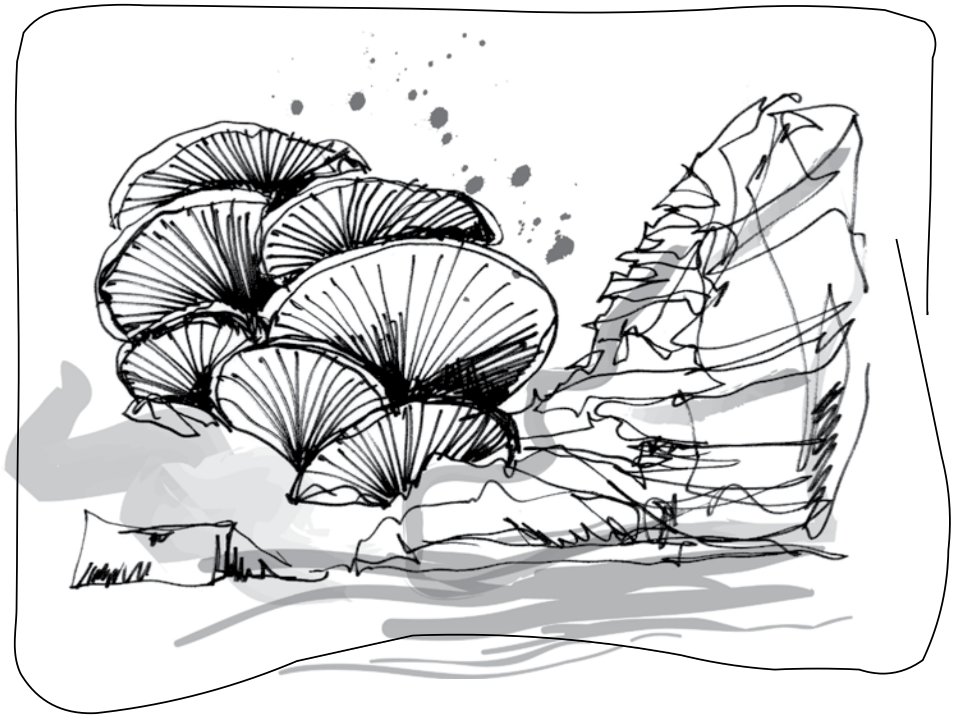
● Elongation of the stem of the fruiting body with a change in the color.

The cause: unsuccessful emulation of day/night rhythm, or insufficient lighting (often due to insufficient duration of lighting).

● Cauliflower shapes of mycelium on the cross-shaped cuts in the substrate bag.

The cause: besides the possibility that the spawn carries a low genetic potential, most likely a lack of moisture in the substrate.

It is possible to partly address this issue by pulling back the bag from the substrate and moistening with a gentle shower. However, this can only be done if there is no trace of other fungi/molds (e.g. Trichoderma spp., Penicillium spp., etc.) and the mycelium has mostly covered the substrate. The showering should be conducted very carefully to avoid damaging the mycelium and to avoid adding too much moisture – fungi are neither aquatic organisms nor frogs.



Conclusion

Mushrooms are the product of an organism that is very much alive. An organism, which like all life, has its own biological needs along its life cycle that must be

met for it to yield a healthy and beautiful bouquet of mushrooms at its natural crowning glory. For this, the mushroom cultivator must develop a profound familiarity with his fungi. Problems will arise, as they do for all growers: doubtlessly, knowledge will prove the most effective remedy.

In this book, we provide the fundamentals of mushroom cultivation in the form of a manual, based off of years of experience in various contexts: from highly automatized almost laboratory conditions to simple straw-roofed production in tropical mountains, and everything in between! However, like a spore awaiting the right conditions, this inoculated knowledge has to come to life through practice. We guarantee one thing: success does not come without many failures.

For the mushroom cultivator that wants to take this art to the next level, to become a small mushroom entrepreneur, he must balance costs of his operations – including his own time – with revenues from mushroom yields. He must design his sustainable business to survive and thrive in the untamed reality of running a private business.

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Use of straw-based substrate from the production of Pleurotus ostreatus mushroom as animal feed

Dr Milan Adamović
System Ekofungi

Introduction

After the production of oyster mushroom (Pleurotus ostreatus), the substrate on which the mushrooms were cultivated that remains is called spent mushroom substrate (SMS), spent mushroom compost or, simply, spent substrate. Different materials are used to prepare the compost on which oyster mushrooms are cultivated: most commonly straw or stover from crops (wheat, oat, barley, rye, soybeans, rice) are used, but sawdust, wood chips, bark and branches, sugar cane, husks of soya, cotton, peanuts, grape seeds, byproducts from the brewing industry (brewery spent grain), byproducts from the coffee industry (chaff, pulp and coffee waste) and other materials rich in lignin and cellulose are used. Thus, oyster mushroom production is based on using cheap waste materials from other industries, that are ballast and may cause damage to the environment. Global oyster mushroom production from intensive industrial production systems amounts to over a million tons annually, resulting in over three tons of spent substrate. The issue of how to manage this waste stream is important to address from the perspective of environmental protection and renewable energy. So, how can it be managed? Many approaches have been tried resulting in varied experiences including: biogas production, in the paper industry, cellulose-based sugar, single cell proteins, white button mushrooms (i.e. champignons), pellet production for heating, etc. It has also been demonstrated that SMS can be used as animal feed. In this way, industrial scale mushroom production can be incorporated into a fast organic matter cycle, resulting in high-value animal products and a lower carbon footprint of the production systems involved.

1. Chemical composition of biomass and straw based substrate from Pleurotus ostreatus mushroom cultivation

Lignocellulosic materials that may be used for the cultivation of mushroom Pleurotus ostreatus are showed in Table 1.

Table 1. Lignocellulosic materials (straw and hay) that may be used for the cultivation of the Pleurotus ostreatus mushroom.

Organic matter	Dry matter %	NEL MJ/kg	Crude protein %	Crude fiber %	NDF	ADF	Cellulose %	Lingin %
Wheat, straw	89	4.0	3.6	41.6	85	54	39	14
Barley, straw	91	4.5	4.3	42.0	80	59	37	11
Oats, straw	92	4,6	4.4	40.5	70	47	40	14
Alfalfa, hay	90	5.1	15	29.0	50	37	28	10
Bahiagrass, hay	91	4.0	9.5	33.0	73	38	31	6
Fescue, hay	92	4.4	9.2	32.6	70	42	34	7
Bermudagrass, hay	93	3.9	8.0	36.0	78	43	33	7
Bluegrass ,hay	90	5.2	9.5	32.2	69	40	34	6
Brome, hay	89	2.9	10.0	37.0	68	43	36	3
Timothy, hay	88	5.0	7.8	32.5	70	40	34	7
Peanut, hay	91	5.1	10.8	33.2	70	41	35	8
Orchardgrass, hay	91	5.0	8.4	37.1	72	45	39	9
Clover, hay	89	5.1	16.0	28.8	46	36	28	8
Pangolagras, hay	91	3.6	5.5	38.0	77	46	37	7
Ryegrass, hay	83	5.0	5.5	36.3	69	45	36	9
Napiergras, hay	90	4.9	7.8	39.0	75	47	35	14
Sorghum, hay	91	5.2	8.0	36.0	68	42	35	6

The lignocellulosic materials (industrial byproducts) that may be used for the cultivation of Pleurotus ostreatus mushroom are showed in Table 2.

Table 2. Lignocellulosic materials (industrial byproducts) that may be used for the cultivation of Pleurotus ostreatus mushroom.

Organic matter	Dry matter %	NEL MJ/kg	Crude protein %	Crude fiber %	NDF %	ADF %	Cellulose %	Lingin %
Cotton, hulls	91	3.9	4.1	47.8	90	73	59	24
Peanut, hulls	91	1.7	7.8	62.9	74	65	40	23
Rice, hulls	92	0.7	3.3	42.9	82	72	33	16
Soybean, hulls	91	7.4	12.1	40.1	67	50	46	2
Oats, hulls	92	3.1	3.9	33.4	78	42	30	8
Safflower, mech. ext.	91	5.6	22.1	32.5	58	41	27	14
Cobs, ground	90	4.6	3.2	36.2	89	35	28	7
Grape marc, pomace	91	2.9	13	31.9	55	54	48	35
Sawdust, salt cedar	90		3.8	40.5	78	61	44	17
Coffee pulp, dehydrated	92		11.3	18.5	49	46		9
Coffee, hulls	88		9.4	36.0	62	51		16
Instant coff. byproducts	91		10.9	44.0	71	66		23

The physical appearance of a bag filled with wheat straw based substrate and the fruiting bodies of the oyster mushroom are shown in Image 1.

Image 1. Oyster mushroom fruit bodies on substrate.

Image 2 shows bags of SMS after cultivation.

Image 2. Landfill of mushroom spent substrate

The physical appearance of high-quality wheat straw based substrate for oyster mushroom cultivation after polyethylene foil has been removed is presented in Image 3.

Image 3. Substrate free from polyethylene foil

From the aspect of animal nutrition, SMS from grain straw-based substrates are the most interesting (wheat, oat, barley, rye). The oyster mushroom uses an arsenal of different enzymes – like cellulase, β-glucosidase, laccase, ligninase – to degrade complex lignocellulose molecules into simpler compounds that the organism uses for its growth. Through degradation, mushroom enzymes change the structure and composition of the original material used in the substrate, that is straw. At the end of the production cycle, SMS contains parent material partly degraded at the molecular level, mycelium and a dense web of fungal hyphae.

The main building material of Pleurotus ostreatus hyphae is chitin, which can hardly be used by animals. Chitin contains proteins and essential amino-acids as well as macro and micro elements. Healthy substrate does not contain mycotoxins (aflatoxin, ochratoxin, citrinin, patulin, zearalenone, sterigmatocystin and penicillin acid). Results show that the amounts of pathogenic bacteria or molds are under the legal limits defined for concentrations of harmful substances in animal feed of plant origin. The chemical composition of wheat straw and wheat straw based substrate for oyster mushroom cultivation is shown side by side in Table 3.

Table 3. Chemical composition of wheat straw and substrate for Pleurotus ostreatus (on 100% dry matter basis)

Indicator	Wheat straw %	Substrate for oyster mushroom %
Crude ash	6,47	7,95
Crude fat	2,00	3,25
Crude fiber	40,57	30,01
Crude protein	3,47	4,58
BEM	47,49	54,21
Ca, g/kg	2,07	5,53
P, g/kg	0,61	0,52

It is hard to preserve SMS from spoilage because it contains a high amount of moisture. Special cold-storage conditions are necessary in the case of prolonged keeping. If the substrate is intended for animal feeding, it has to be used in a short amount of time: a week in summertime and three to four weeks in winter. To prolong shelf-life beyond this timespan, the substrate has to be preserved – the best and the cheapest way is to ensilage.

Ensiling of the wheat straw based substrate left after the production of Pleurotus ostreatus mushroom

Due to it specific smell and taste, SMS is not favored by animals. They prefer to consume it when it is ensilaged and mixed with other feed materials in a form of total mixture ratio (TMR), for example with silage, concentrate, molasses and others.

Ensiling is a biotechnological procedure for preserving wet, bulky and concentrated food on the basis of complex and intense chemical, biochemical and microbiological processes. The most important end product is lactic acid. It is formed by lactic acid bacteria fermenting the soluble sugars present in the compost under anaerobic conditions (without oxygen). Lactic acid is a natural preservative. It has bacteriostatic and bactericidal effect, prevents spoilage and the loss of nutrients. Other bacterial groups like acetic and propionic acid bacteria are also present in material that goes through ensiling process. Silage hosts undesired bacteria like butyric acid bacteria, proteolytic bacteria group, as well as molds and yeasts. If the ensiling conditions are optimal, these microorganisms do not have a large impact on silage quality.

The best way to perform ensiling is to combine the substrate with other materials rich in soluble sugars, like the whole corn plant (while it is immature and still green in color), wet or dry corn based wholemeal. In this case the final product-silage will have better organoleptic characteristics. In other words, silage would be more appealing for animals; consumption improves as well as the net-energy value. Substrate that has been processed in a silage form can be kept for a longer period of time without the risk of spoilage.

Silage quality factors

The best quality silage contains 30-35% of dry matter. The amount of water-soluble sugars should be 6-9% on a dry weight basis.

Ensiling is an anaerobic process: air needs to be pressed out of the material and the possibility of its return should be blocked. Compression is best achieved by using tractors to drive over the material for 10 to 12 hours after the silo has been filled.

Material that is well cut is easier to compress and results in more appealing silage for animals as well as a denser material easier to transport. The ideal length of the whole corn plant is 0.7-1.5 cm. In

case of grasses, legumes and mushroom spent substrate the length should be from 2-5 cm.

Dry and semi-hot weather conditions are ideal for ensiling. Optimal temperature is 20-25°C. If the weather is too dry the material will dry out fast, while rainy weather introduces mud into the silo which results in contamination with pathogenic microorganisms, for example Clostridium bacteria. The best outcome results if the silo is filled in 2-3 days, in which time the material is successively compressed by driving over.

Silage storage facilities

Silage storage facilities should insulate feed material from air flow, and prevent access to underground waters as well as surface runoff.

Silos can take various forms: piles on a concrete surface, in-ground silo trench, horizontal above-ground silo trench, concrete silo tower and air-tight silo (of the Harvestor type). For silo-piles and silo-trench, forage loss can be 15-20%. Horizontal above-ground trench silo better preserves forage quality, especially if it has a roof. Even with those silo losses can be from 10- 15% if they are not properly protected from air flow and rain.

Silos should be covered with plastic foil, which prevents access for air and rain. Foil can be loaded with green mass or other less valuable materials. Usually foils are 0.15-0.20 mm thick. Another solution, which is also the best, is to cover the silo with roof construction.

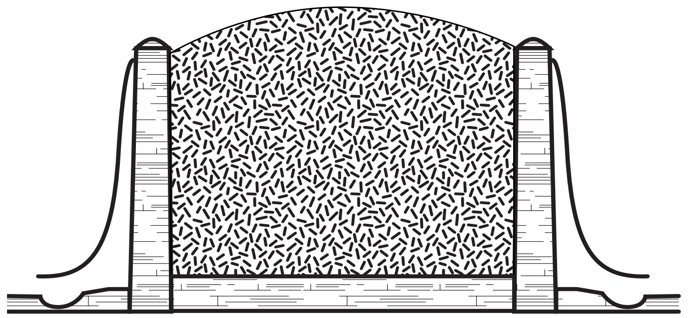


Image 4. Cross-section of silo before covering

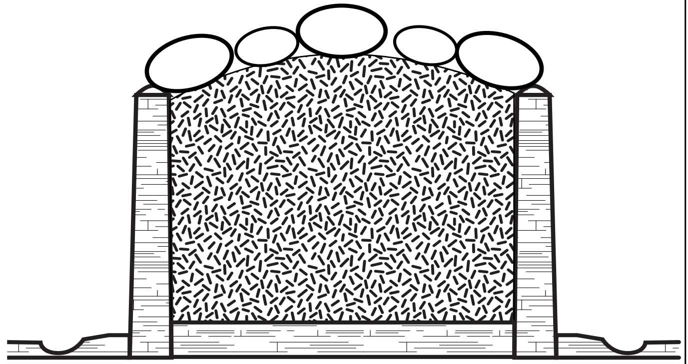


Image 5. Cross-section of silo after covering

A concrete silo tower provides better conditions for keeping silage, and losses amount to 5-10%. The highest quality silage can be obtained from an air-tight silo made from plastic or metal; losses in this case amount to less than 5%.

Polyethylene tunnel-shape foils are another possible solution for silage storage that is very intersting. They are practical, cheap and environmental friendly. The use of these foils yields high quality ensilage, and can be placed anywhere in small farmyard. A special press under 4-5 bars of pressure is used in order to compress the biomass into the foil (Image 6).

Image 6. Press for compress of biomass into the polyethylene tube for ensilaging.

Ensiling with green corn

Straw based substrate does not contain enough soluble sugars for silaging requiring that it be mixed with a material that compensates. SMS mixed with whole corn plant should have 30-35% of dry matter in the moment of ensiling. Considering that fresh SMS has around 25% dry matter, it should be exposed to fresh air for 1-2 days to dry before ensiling. Table 4 presents chemical composition and the quality of silage based on a mixture of green corn plant and oyster mushroom spent substrate (seen in Image 7).

Image 7. Green corn plant and Pleurotus ostreatus mushroom spent substrate based silage (80:20%).

Dry matter	31,00
Crude ash,%	6,30
Crude protein,%	1,71
ADF,%[1]	8,70
NDF,%[2]	13,40
Ca,%	1,38
P,%	0,56
pH	3,68
Lactic acid,%	72,77
Acetic acid,%	27,23

[1] Acid detergent fiber; [2] Neutral detergent fiber
Table 4. Chemical composition and quality of green corn plant and oyster mushroom based substrate.

Silage with SMS and green corn plant achieved a high quality two months into the process. It did not contain butyric acid, and was grade I quality (according to methodology by Flig). Results demonstrate that mushroom spent substrate can be used as silage when it is mixed with whole corn plant while its still immature and green in color, 80:20% (by weight) in the favor of corn.

To what amount can SMS be mixed with green corn plant so that its specific taste does not ruin the attractiveness of the silage as a feed?

The proportion of SMS mixed with corn plant can be up 20% when quality aspects alone are taken into account. However, this proportion does not soften the taste of the resulting silage nor of the ration in which it is mixed – normally including hay and concentrate – significantly. If there is a need to improve the flavor of silage and the meal of which it forms, smaller quantities of molasses can be added.

The major drawback of using the SMS-green corn plant mixture for silaging is that green corn plants are only available seasonally, in the final development stage of the plant.

Ensiling with corn wholemeal

Ensiling of SMS can be accomplished by mixing the substrate with corn wholemeal in proprotion of 80:20, in the favor of substrate. The addition of higher amount of corn wholemeal will increase silage quality and its energetic value. Corn wholemeal should be moist, entailng that the corn should be picked at an earlier stage when dry matter content is 70-75%. In the temperate regions of the Northern Hemisphere, this relates to early autumn: the second half of September or the begining of October. In Table 5 the chemical composition and quality of silage based on substrate and corn wholemeal is shown.

Dry matter	33,00
Crude ash,%	6,80
Crude protein,%	2,4
ADF,%[1]	8,50
NDF,%[2]	11,6
Ca,%	0,56
P,%	0,72
pH	3,72
Lactic acid,%	77,27
Acetic acid,%	27,73

[1] Acid detergent fiber;
[2] Neutral detergent fiber
Table 5. Cheical composition and quality of silage based on oyster mushroom spent substrate and corn wholemea l (80:20%).

At other times of the year, SMS can be ensiled with dry corn wholemeal (80:20%). Silage based on oyster mushroom spent substrate and corn wholemeal is presented in Image 8.

Image 8. Silage based on Pleurotus ostreatus mushroom spent substrate and dry corn wholemeal (80:20%).

Ensiling with other materials

SMS can be used for silage as a mixture with perenninal hay crop (graminea and leguminose) as well as with waste products of plant production (corn stalks, sugar beet with its leaves and other) and agro-industrial byproducts (raw sugar beet pulp, brewery spent grain, apple mash). The ratio of mushroom spent substrate in those mixtures ranges from 10 to 20%. For these mixtures, mushroom spent substrate should be dried for 1 to 2 days in order to regulate the moisture. Dry matter content should be 30-35%. Dry corn stalks, straw, perrenial hay crops or alfalfa (particle size 2-5 cm) could be added in order to absorb the excess of moisture.

Silage additives

Different additives can be used in order to promote a desirable fermentation. At the same time, additives can increase the resulting nutritive value of the silage, improve taste and prevent loss of nutrients. Beside moist or dry corn wholemeal, other materials with a high carbohydrate content like dry sugar beet pulp, whey (powder) and molasses can be used as additives. Dry sugar beet pulp is added in amount of 10-20%, while the ratio for molasses and whey is 5-10%.

Additives are added continuously as layers in the silo as it is being loaded with SMS. Molasses should be diluted because of their high density with semi-hot water (3:1), and sprayed from the bucket or sprinklers. Nozzles to be used should have a larger diameter.

The silage may be used already after 6 weeks, but the best time for opening is 2-3 months after the ensiling process has finished.

Oyster mushroom spent substrate as cattle feed

Various straws are used as a component of feed rations for cattle (wheat, oat, rye, beans, peas, soybeans, lentils); they contain 86-88% of dry matter, 3-4% of proteins and 30-40% of raw cellulose. Thier energy level is relatively low, 2.5-4.0 NEL, MJ/kg and 2.0-3.8 NEM, MJ/kg. They fulfill the role of roughage, important for efficient functioning of a ruminant digestive system like that of cattle; the higher volume fills the complex stomachs of ruminant cattle and encourages digestive motor mechanisms. At the same time, straw contains a large degree of cellulose, which is hard to digest, in effect limiting the extent of its consumption. In contrast to non-ruminant animals, ruminants are able to digest a portion of cellulose from straw into more simple carbohydrate compounds. In turn, acetic acid is formed during fermentation and serves as intermediate for the synthesis of milk and milk fat. It is not rare for straw to be used when there is insufficient feed available. In order to improve straw's nutritional value for cattle, especially regarding the digestion of cellulose, different processing approaches have been tested. This includes physical, chemical and biological treatments.

Straw treatments-in order to improve its utilization efficacy

Physical treatment: Straw is cut into particles 2-5 cm long, milled and cooked with or without pressure. The result is a breakdown of the lignocellulose complex and an increase of utilization efficiency through better consumption and digestion by cattle.

Chemical treatment: Straw is exposed to acids, alkali (NaOH), ammonia, ammonium-hydroxide, urea, alkali hydrogen-peroxide, potassium-xydroxide, sulfur-dioxide, ozone etc. The aim of those treatments is the degradation of the lignocellulose complex, breaking strong bonds in lignin molecule (which is the hardest to be degraded) and production of fodder which is easier to be digested; resulting in higher efficacy of utilization for production of meat and milk.

Biological treatment: Enzymes are used to degrade polysaccharides like cellulose and hemicellulose, as well as lignin. This method ensures that no harmful products are formed and the final result is similar to a chemical treatment. Biological treatment can be combined with chemical treatments, through the use of NaOH and hydrogen-peroxide. One biological approach includes the exposure of straw to fungal enzymes. Typical example for this is the oyster mushroom that has strong enzymes which degrade the lignocellulose complex.

Some of the methods (physical and biological) are present in the preparation of substrate for the oyster mushroom production.

Oyster mushroom spent substrate, as a roughage, can be used as a feed component for ruminant cattle due to the specificity of their digestive system and microorganisms (bacteria, fungi and protozoa) that live in their ante-stomachs. Rumen microorganisms have enzymes that degrade cellulose and hemicellulose to less complex molecules involved in fermentation. Volatile fatty acids (acetic, propionic and butyric acid) are the final products of the fermentation. 30-50% of cellulose and hemicellulose are digested in rumen. Lignin is digested poorly, as it is practically indigestible.

Which SMS can be used as a feed component?

Only the spent substrate that has yielded good fructification is suitable for further use as fodder, fresh or ensilaged. In other words, SMS has to be of a good quality and that the mushroom's enzymes have contributed to the degradation of the straw's lignocellulose complex. Anyhow, substrate that resulted in poor fructification is not good. Why is that? Substrate that showed it was unsuitable for mushrooms is likewise unsuitable for animals.

All the recommendations herein for feed components based on wheat straw SMS are based on scientific experiments with different categories of cattle.

Animal rations for cows

One of the experiments focused on lactating cows. They were fed with corn silage where one part was replaced with the straw based SMS in a ratio of 2:1, in the favor of substrate. Two treatments included groups that were fed with 2 and 3.2 kg/cow/day. Undesired consequences were not observed in regards to production, milk composition and the health of the animals. SMS was mixed with other materials by hand, and it was delivered twice a day (morning and evening). Fodder also contained

alfalfa, spent brewery grain, soybean meal, and complete feed mixture with 18% of total protein. The feed rations reflect standard characteristic for lactating cows.

The treatment groups produced less milk than the control group, 3.73% and 7.28%, respectively. No statistical difference was observed between these two groups.

Treatment groups had milk fat contents of 3.67% and 3.71%, while the control group had a milk fat content of 3.56%. A possible explanation might lie in the fact that oyster mushrooms secrete enzymes that degrade lignin molecules. The outcome is an increase in the bioavailability of cellulose and thus the production of acetic acid, which is a precursor in milk fat synthesis.

The inclusion of SMS based on wheat straw in lactating cow's feed might be justified economically. The possibility of using such meals could be even larger if mixing of the feed was more thorough (total mixture ratio-TMR). The observed results in milk production could be less marked in the case of cows with lower genetic production potential, for example 10-15 kg of milk/cow/day.

On the basis of these results it is suggested to use silage prepared from green corn plants and substrate (80:20) or substrate and corn wholemeal (80:20), in the amount of 4-5 kg/cow/day. Addition of molasses (0.5 kg/cow/day) might improve the meal's taste and consumption.

Image 9. Cow feed with TMR with the substrate

Fresh or ensilaged SMS may be used as a component of feed for cows in the last 60 days of pregnancy in the amount of 1-2 kg if it is of good quality; if it is not it should not be used.

Heifer feed rations

Experiments showed that wheat straw based SMS can be used as feed component for young heifers (4-24 months old) when lower weight gain is desired (0.6-1.0 kg/day). In this case the meal can contain 2-3 kg/animal/day of fresh spent substrate. If the SMS has been silaged it can be administered in amounts of 4-5 kg/animal/day, with the addition of other materials (hay, corn based silage, or total feed mix with 16% of total protein). Addition of molasses (0.5 kg/animal/day) will make this meal tastier for the heifer.

Animal rations for growers

One of the experiments included beef cattle in the second phase of fattening (10-12 months old). The treatment groups were fed with 2.5 kg of SMS (10% of dry matter in meal) and 4.2 kg of SMS (17% of dry matter in meal). The rest of the meal consisted of alfalfa, corn based silage and total feed mixture (12% of total protein) in amounts that are standard for this category of cattle.

Daily growth of experimental groups were lower compared to control (1.149 kg/animal/day): 13 g lower (1.13%) and 158 g (13.75%), respectively.

The experiment showed that wheat straw based SMS can be used as a part of meal for beef cattle. The growth marked was lower but satisfactory, about 1 kg/animal/day, entailing lower expenses.

The amount of fresh SMS in this case can be 2-3 kg/animal/day, and 4-5 kg/animal/day if the substrate was silaged and contains other materials (hay, corn based silage and total feed mix with 13-14% of total protein). Molasses are also welcome to correct taste in the amount of 0.5 kg/animal/day.

Oyster mushroom spent substrate as feed for other animal species

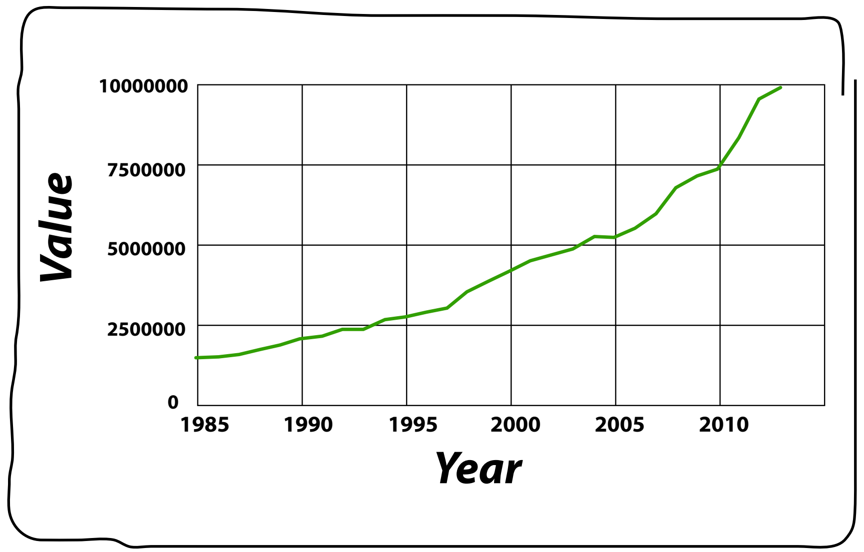
In addition to the use of SMS for cattle feed, other experiments and scientific papers provide information about its suitability as a feed for other animal species. Most of them are obtained in a small scale experiments conducted using ruminants (sheep and buffalo), but there are to papers concerning pigs (sows and growers) too. Some experiments were even conducted on laboratory animals – rats. Evidently, the scientific community, as well as farmers, have demonstrated interest in this topic. Further research is necessary to make precise recommendations for other domestic and game animal species.

Business development for the urban and rural small mushroom entrepreneur

Igor Milosavljevic
System Ekofungi

Who is the small mushroom entrepreneur?

In short, anybody ready to make the effort. It is essential that she/he builds a comparative advantage of their business based on: the knowledge of fundamental underlying biological processes, how to translate biological needs into technical best-practice and, ideally, collective experience from others who are doing something similar. In addition, the entrepreneur should know how to design and build a business. There is much good literature on these subjects to instruct the seeker, both printed and online, and to which this work in its whole aims to contribute. This theoretical knowledge must also be paralleled by practical experience. Covering all these aspects, the EkofungiSchool provides an accelerated initiation for the prospective small mushroom entrepreneur.



Good news: it's boom time in the mushroom business.

The global market for mushrooms has grown an average of 6.4% each year for the past 15 years. Market growth is continuously accelerating since the late 1990s, as can be seen in Figure above. In this period, consumption per global inhabitant grew from 1 kg/year to over 4 kg/year[1].

The market is expected to continue growing at an annual rate of 9.2% between 2016 and 2021[2]. The highest growth is projected in Europe, at 10.8% annually . The global market for shiitake is expected to expand the most, from €6.7 billion to €11.0 billion; with oyster mushrooms in a close second, predicted to expand from €4.6 billion to €7.3 billion[3].

What is driving accelerating growth?

For all consumers, health is a vital and growing concern. Interest in the link between food and health has changed mainstream consumption patterns by valorizing quality aspects related to good health. Consumer perception of mushrooms as a high-quality food, a good component of a healthy diet, is the primary driver of mushroom market growth.

The most eager consumer groups are those that feel this concern to the largest degree. In our experience, this has included:

- Young families: particularly first-time pregnant women and new mothers;
- Pensioners: who are urged by doctors to pay more attention to their diet;
- People with special dietary needs/preferences: vegetarians, vegans, individuals following gluten-free diets;
- Higher income families: are more likely to spend on mushrooms than lower income families[4];
- Young educated professionals: millennials, 24% of EU's population , and are the demographic group most likely to pay premiums for wellness purchases .

Each local market is different in its composition, and the degree to which such groups (and other groups that value mushrooms) are represented.

How does the small mushroom entrepreneur fit in the world market?

The best opportunity for the small mushroom entrepreneur in her/his local niche market is to offer mushrooms differentiated by high-quality aspects, catering to customer groups that value this trait. To understand how to translate this statement into drafting a business plan, it is useful to examine the structure of the world market.

Champignons are the world's most popular mushroom, accounting for 38% of global sales. Shiitake and oyster mushrooms together make up 40% of the global mushroom market: 24% and 16% respectively[2]. The rest of the market is made-up of other cultivated edible and medicinal mushrooms, as well as mushrooms picked from the wild. Cultivated and wild non-champignon mushrooms are sometimes collectively referred to as specialty mushrooms.

Production of champignons is intensive, based on production systems develop in Western Europe

and North America and replicated in emerging markets around the world. The production systems are characterized by their large scale, achieving low production costs per kg. To establish small-scale champignon production is a challenge, both technically and in regards to establishing a price-competitive position in the market. In addition, consumers perceive champignons to have lower benefits for health than other mushroom types; this consumer perception can be seen in the fact that specialty mushroom market segments are realizing larger growth rates than the market for champignons.

China accounts for 90% of the world's shiitake, and 85% of the world's oyster mushroom production1. There are a large amount of producers, including companies and households, employing low tech production systems. These extensive production systems reliant on low labor costs allow China to realize very high competitiveness in regards to price. However, Chinese mushrooms are scrutinized by experts for their high heavy metal content (e.g. lead and mercury) resulting from high levels of soil pollution in the country, and a high nicotine content. A growing number of large industry buyers refuse Chinese products from traders . This represents a direct opportunity for mushroom entrepreneurs seeking to set-up a medium-sized production, i.e. >10 tons. For all mushrooms entrepreneurs, it signals that they cannot compete on the bulk market and need to in the least differentiate themselves by demonstrating higher-quality and safety of their mushrooms through their composition; this primarily entails attentiveness regarding raw material sourcing, and, preferably, regular quality validation through laboratory chemical composition analyses.

It is also important to note that mushrooms have a very short shelf-life. Quality and aesthetic aspects quickly deteriorate within a week following harvest. Distribution requires constant refrigeration and quick logistics. The consequence is that fresh mushrooms are generally produced within a relative geographic proximity: only 7.4% of mushroom exports are fresh mushrooms . International trade in mushrooms is mostly of preserved types – canned, dried, frozen, etc – mostly originating from China: e.g. 88% of dried mushrooms on the global market are Chinese exports .

In summary, the small mushroom entrepreneur faces low-hanging fruit to position her/himself as a fresh local producer with rigid traceability in the production chain, guaranteeing high-quality that delivers perceived healthy aspects to a large degree. Besides freshness, consumers perceive that local food is beneficial for them: a point of view held by nine out of ten Europeans .

However, this approach should not be taken as a direct prescription but rather as a blueprint for a strong foundation. We have seen a wide range of small mushroom business concepts that deliver added-value well beyond this.

Where to start?

Inherent logistics issues are not the only reason that the small mushroom entrepreneur should focus on the local market. The small mushroom entrepreneur must know his customers very well to recognize important underserved needs in one or several segments of the market and develop a business model that can deliver value in meeting these needs. This requires channels for communication and feedback with end-users, which is not practical or resource-efficient beyond a given distance. Target customers must be identified and characterized, and their problems understood to a large extent. As indicated so far in this text, a major consumer issue is trust in food safety and quality. However, producing a mushroom that is high quality is not sufficient, the product has to reach customers in a manner that fits into their lifestyles. Millenials for example face a tough job market, where competition in on-job performance is high and demanding hours are the norm. For this customer group, providing convenience is a definite must: selling at a green market and fixed time home-delivery might not be optimal solutions to reach them effectively.

The crafting of a good business model is guided by the customer. The mushroom entrepreneur is not psychic, s/he can only make guesses about certain key aspects of the customer groups s/he wishes to target. These guesses should be informed, i.e. hypotheses, and they must be tested. This means continuously communicating with a sample of the customer group to confirming/reject the entrepreneur's assumptions in his business model. The mushroom entrepreneur as an interviewer must ask open-questions: e.g. We have found that problem A is important for other young mothers like yourself, does this sound like it makes sense to you? S/he has to test the following:

- Understand the problem: Who are your customers? What is their top problem relating to what you are trying to solve? How do they solve it today?
- Define the solution: Will it work? Who is the early adopter? Are your adopters willing to pay the price you offer?
- Validate: Do customers understand what is the unique value you are offering? How do you reach more customers? Do you have a financially viable business?

This approach draws entirely from lean startup methodology , developed in Silicon Valley to optimize the product development cycle for startups with limited resources. The basic concept is to closely integrate customers into the product development process, testing all business assumptions before making business decisions. It is important to understand the approach as one of developing and validating a business model. This effort should be charted on a constantly revised lean canvas (available at https://leanstack.com) or business canvas (available at https://canvanizer.com). In its entirety, the canvases cover key aspects of the business model.

What else is important?

The small mushroom entrepreneur will in general have a small production volume. This means that s/he has to seek to generate a large degree of added-value of her/his product. This can be either in regards to the product itself (e.g. organic certification, unique story), through its processing (e.g. cut mushroom, pasta mixes), packaging (e.g. premium packaging), delivery to the customer (e.g. cooked as a meal, delivery). This is particularly true for the urban farmer whose production volume is highly limited by space availability: they must become a true boutique production.

Secondly, the mushroom business entails a long chain of tasks: collecting raw inputs, creating substrate, cultivating fungi on the substrate, distribution and retail. Overall, the entrepreneurs are the major source of labor, and thus labor availability is often a limited resource for the new small mushroom business. This is a good place to accentuate that it is important to maximize the efficiency of business development, for which lean methodology is a great asset. Also, it means that the entrepreneur must seek efficiencies along each chink of the chain. A good way to accomplish this is through close partnerships, often with people with which the business concept and its value resonate. For the mushrooms-on-coffee entrepreneur, this includes café owners that will ensure that hygienic procedures are respected in the collecting of coffee. For some entrepreneurs it may mean selling to restaurants instead of directly to consumers. The small mushroom entrepreneur must develop a business network as one of her/his major assets.

Another important point to consider is if it is feasible for mushroom sales to be the only income stream for the business – or even the main one. Many successful small mushroom entrepreneurs have created a multifunctional farm, where revenue is generated from several products/services. This can include funds from projects designed to deliver social value, or offering classes on home mushroom production (as done by RotterZwam). As time is a highly limited resource for the small mushroom entrepreneur, it is particularly useful to pursue activities that create synergy with mushroom production, for example opening a store in a trendy street to promote the concept to interested consumers. The fact remains that the large majority of classical urban farms (i.e. plant producers) face large challenges to achieve financial feasibility, a large degree of them rely on financing from local government or other donors to survive. Although as of yet less numerous, it seems that most urban mushroom farms will face a similar inherent challenge and will have to seek multiple revenue streams. Classical urban farms have shown great creativity to address this issue, for example creating a beautiful atmosphere which can be rented out for events, like weddings, or recreational activities, like yoga classes. Mushroom farms are typically humid and less picturesque places than rooftop gardens, and thus less suitable for these specific activities, but there is a novelty value which urban mushroom production can offer outside the food value chain that has as of yet to be seized upon fully by many small mushroom entrepreneurs. Creativity is the only limitation for what other business activities can be built around a small mushroom production.

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System Dynamics Analysis: Introduction and an Example for Mushrooms on Coffee for the city of Torino

Ina Matijević, Dipl. ing.

Introduction

The Blue Economy is inspired by non-linear models that prevail in Nature. Environmental systems are highly nonlinear and in combination with socio-economic dynamics create highly complex, interrelated dynamic systems with time delays. The mathematical modeling can capture the key interrelationships through feedback loops and nonlinearities. Computer simulations present dynamic patterns of the business model over a period of time. The nonlinear behaviour of complex systems over time is explained with stocks, flows, internal feedback loops and time delays.

Participatory System Dynamics Model is a tool to design, support and implement the innovative business models that characterize The Blue Economy wisdom. It allows to accelerate and broaden the opportunities for implementation while determining natural limitations. This modeling also permits the quantification of feedback loops and multiplier effects, facilitating a clear understanding of the synergies through cluster approach.

''Prof. Jay Forrester at MIT (Massachusetts Institute of Technology, Boston, USA) built the origins of this computer tool in 1964 as an urban development model. Inspired by the description of the world problems by Aurelio Peccei, a former top executive of FIAT and Olivetti, it was later adjusted into a global model that served as the basis for the Club of Rome report "Limits to Growth". A simplified version of this model is now widely available permitting its use even amongst beginners in math but committed to understand and operate the dynamics of the Blue Economy.

"Mathematics and modeling are the heart and soul of the projects the Blue Economy implements. We could consider it the reversal of the modern day obsession of business plans and strategy developments which have such a linear approach, neglecting the portfolios of opportunities and putting the maximum profit and market share as the ultimate goal.'' Prof. Gunter Pauli

To learn how to grasp the essence of Participatory System Dynamics Modeling anyone can refer to the online course of MIT. Most commonly used SD softwares are Stella and Vensim. They can be downloaded for a free trial period.

This report is made in iThink software of iseesystems.

PARTICIPATORY SYSTEM DYNAMIC MODEL OF TORINO

This simple Participatory System Dynamic model (later in text PSD) of coffee waste in Torino city explains the potential for the local community if they adopt The Blue Economy business model inspired by one of the greatest nutrient transformers - mushrooms.

Torino city has 920,255 habitants with mushroom consumption of 4,800,000 kg/year. It also produces 218.582 kg of coffee waste each month. At the moment this waste goes to a landfill, representing 15 garbage trucks every month that take away this precious resource from the local community.

The simulation is made to calculate the flow of resources per month in city of Torino in kg of coffee waste, mushrooms and leftover substrate in time frame of 12 months. The data is collected using what's available on the internet. There are 2,661 restaurant with average 1.6 kg of waste daily, 281 hotels with average 3.5 kg of waste daily, 90 state/corporate institutions with average 0.5 kg of waste daily and households with cca 2,000 kg daily.

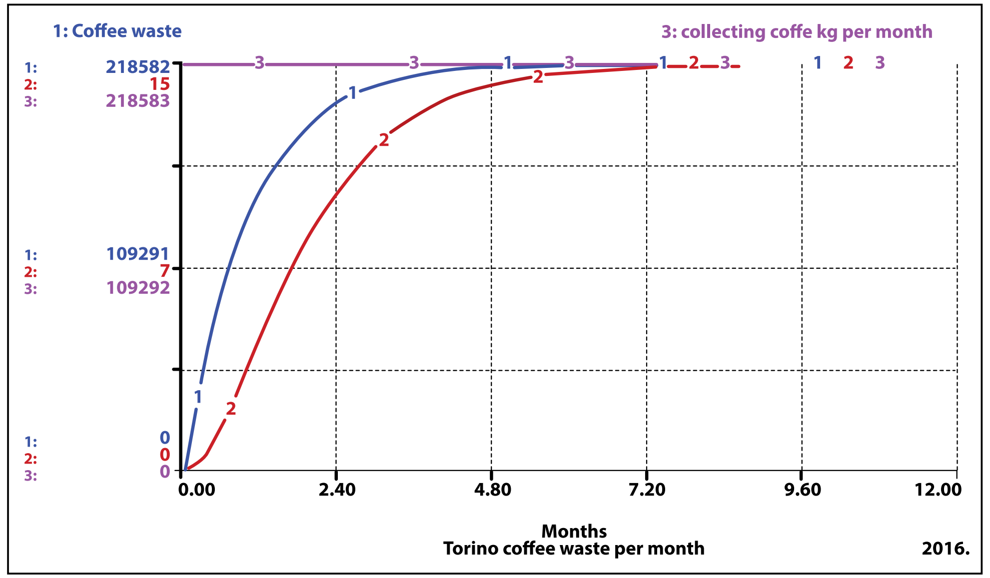


Diagram 1.
Coffee waste produced in Torino per month.

If the local community decides to cascade coffee waste into Cluster on coffee waste starting with mushroom production, we can see the following results:

With 218,583 kg of coffee waste per month, Torino city can produce 37,156 kg of mushrooms per month. For the spent substrate leftover after mushroom production, 26,000 kg goes to the zoo as animal feed and 39,000 kg to city parks as fertilizer. It takes approximately one month to grow mushrooms for the market and two months to collect leftovers for zoo animal feed and city parks.

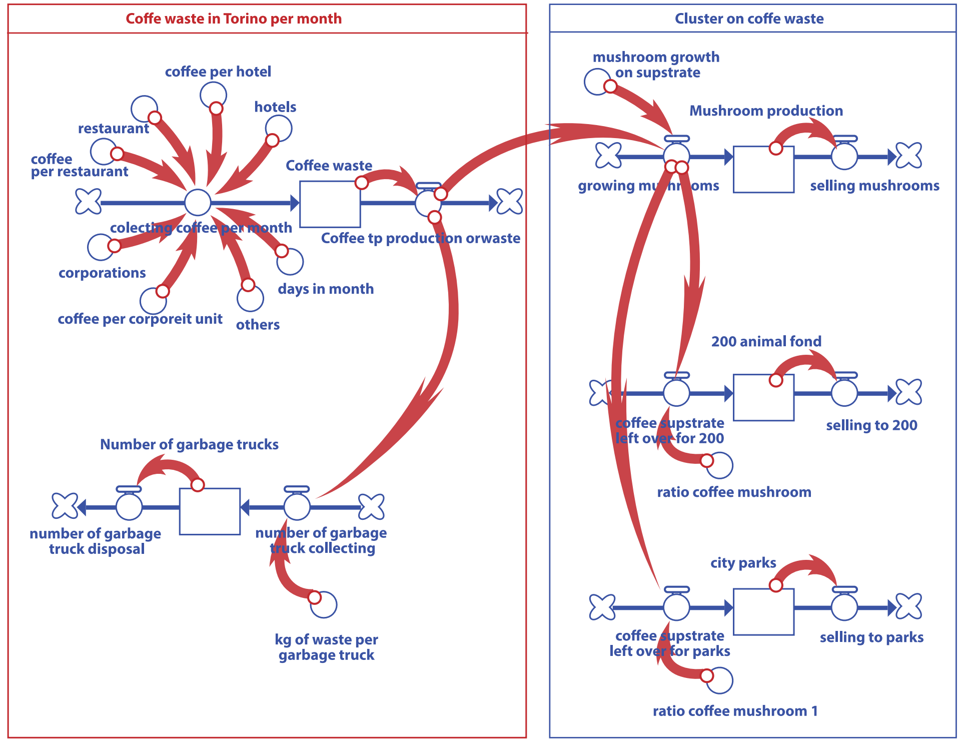
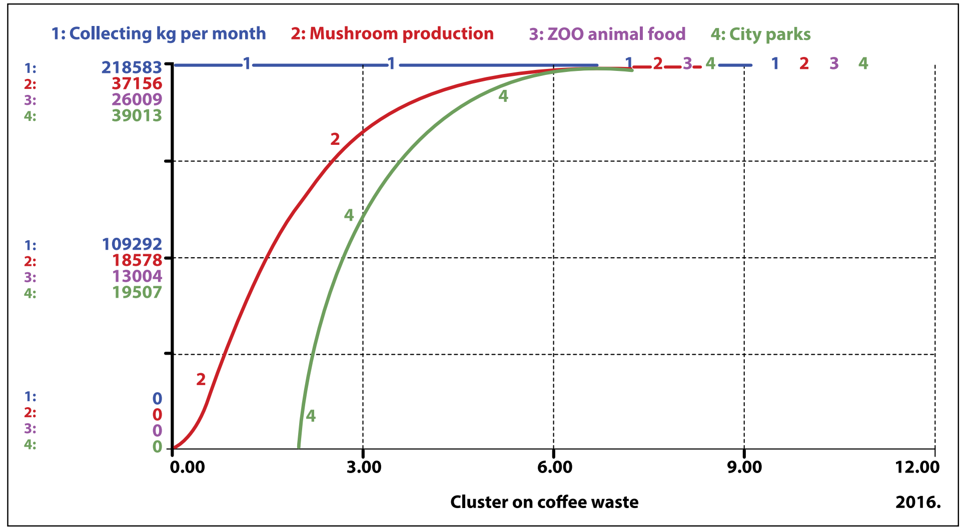
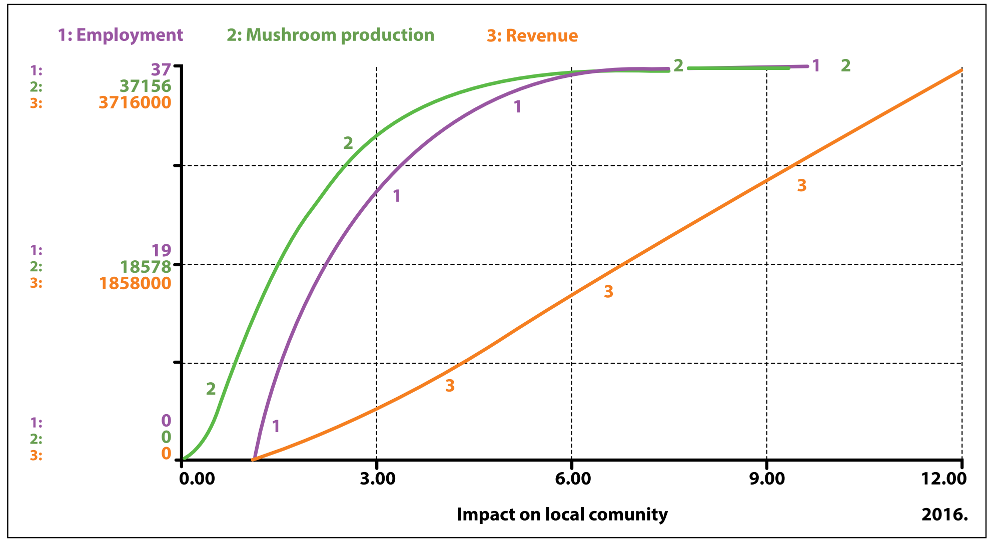


Diagram 2.
Cluster on coffee waste.



The impact on the local community is measured through employment possibilities created and the flow of money that goes back into local community. Almost 10% of Torino's market demand per year for mushrooms can be satisfied with many local, small, entrepreneurial initiatives. This could create up to 37 workplace and circulate almost €4 million in the city of Torino in one year if all the coffee waste is utilized through [The Blue Economy business model](#).

Diagram 3. Impact on local community



For more explanations how to use iThink software and make your own PSD model for local community, please visit [Ekofungi System school video channel.](#)

CHAPTER 3. DESIGN OF SUSTAINABILITY

When passion grows as mushroom

Silvia Barbero, Ph.D. Assistant Professor
Politecnico di Torino (Italy)

My passion for Systemic Design grew on me when its name, as we know it now, was not yet defined. During my bachelor's degree, I had the honour to have Carla Lanzavecchia as one of my professors. Her course, Environmental Requirements of Industrial Products, instilled in me a devotedness towards topics related to environmental sustainability. She was so passionate during her lectures and for her research that she organised an extra workshop for students to delve deeper into sustainability and to consider it in a different way. This was at the beginning of 2000: the mainstream mindset towards sustainability centred on life cycle assessments, and the general perception was that ecodesign products were expensive and ugly. However, her approach was broader. She considered sustainability as a key requirement for products at the same level as others (functionality, ergonomics, industrial feasibility…), and she considered inherent processes behind products in their whole. For those reasons, she organised, together with the coordinator of the Design Degree at Politecnico di Torino, Prof. Luigi Bistagnino, a one-week workshop on Sustainability of Processes, to which she invited prof. Gunter Pauli. I attended this workshop. What I learnt was much more than what I had expected.

The event produced an explosion of passion for the Earth and of trust for human beings. After this workshop, the relation between Carla Lanzavecchia, Luigi Bistagnino and Gunter Pauli grew closer and

closer. Discussions on different projects and research ensued, and an increased awareness crystalized that something should change in the approach. From my side, I strongly believed in a new approach and I decided to write my bachelor's thesis on this topic. I agreed with Carla Lanzavecchia and Luigi Bistagnino that we should examine the possibilities to transform the approach to dealing with the flows of material and energy in our territory, the Piedmont Region, in line with what Gunter Pauli showed us. During this challenging path, when the name Systemic Design started to take shape, Prof. Carla Lanzavecchia passed away leaving behind a large legacy and a responsibility for the research team at Politecnico di Torino.

Prof. Bistagnino carried on with all the activities and by 2002 created a bright new Master of Science in Ecodesign, with one entire semester dedicated to Systemic Design. The programme was not only new for the Politecnico di Torino, but for the entire world. During the first five years, he invited Gunter Pauli to hold a one-week workshop at the beginning of the semester and a one-week workshop at the end. The forward-looking programme started with 12 Italian students, and now counts 100 international students and requires an entrance exam. The lectures have also evolved, as has the name: it is the Master of Science "Aurelio Peccei" in Systemic Design. Experts from different disciplines were involved as teachers from the very beginning (i.e. chemistry, physics, mechanics, history, economy and management), in order to create a multidisciplinary environment for the development of projects.

At the end of the master's programme, I completed another thesis in Systemic Design, working on a coffee plantation for one of Gunter Pauli's projects in Colombia. This was the first time I came across the fact that it is possible to produce mushrooms from the branches of coffee plants (fig.1), and I had the opportunity to study it. At this time, I read that there was an expert in Europe that is able to grow mushrooms on different substrates: Ivanka Milenkovic. The work I did with my master's thesis was well received, and Gunter Pauli invited me to present the project during the ZERI International Forum in Tokyo, held to mark the 10th year since the organization's foundation.

fig.1: coffee plantation in Manizales (Colombia).

At that time, my interest in this topic and the effort I had dedicated towards it were already so strong that the path towards my future job was clear. I continued to feed my hunger for knowledge, enrolling into the second-level international master's of two years in Systems Design at the Politecnico di Torino. The programme included collaboration with and activities in Kyushu Institute of Technology and Kyoto City University of Arts (Japan), University of Hawaii in Manoa (USA), Technical University of Hamburg Harburg (Germany) and Universidad de la Sabana in Chia (Colombia). I had the fortune to have teachers with global fame, like Fritjof Capra, Janine Benjus, Anders Wjikman, Anders Niquist, as well as Gunter Pauli and Luigi Bistagnino. I also had the opportunity to visit many virtuous projects and to meet amazing people around the world with the same attitude to tend to others as well as our planet. I decided to turn this passion into a career, enrolling into a PhD, followed by a research fellowship and my current position as an assistant professor. My present research activities are based on Systemic Design, and I apply this methodology in different sectors and parts of the world to foster local sustainable development. Actually, I'm the coordinator of an Interreg European project: A systemic approach for REgions TRAnsitioning towards a Circular Economy (RETRACE – www.interregeurope.eu/retrace and https://goo.gl/H0LsLz). Furthermore, I'm very honoured to now teach in the Environmental Requirements of Industrial Products course of the bachelor's degree in Design and Visual Communication at Politecnico di Torino. I am doing my best to maintain the high level of work set by Carla Lanzavecchia.

In recent years I have run international projects, like the one in Ahuacuotzingo town (Mexico) with Red Mexicana de Mujeres, Cavideco and Sudemur; in Lea-Artibai (Spain) with Azaro Fundazioa; in Bucharest (Romania) with the National Centre for Sustainable Development of Bucharest (NCSD) and the Romanian Association for the Club of Rome (ARCoR); in Montauban, Tarn et Garonne (France)

with Poult; and local ones, like Energia e Fagioli in Sistema (EN.FA.SI – https://issuu.com/politodesign-stories/docs/enfasi_web and https://vimeo.com/enfasi) cofunded by Regione Piemonte and with the collaboration of Agroinnova, Arese Franco, Molino Borgo San Dalmazzo; agro-industry local development with Agrindustria Tecco srl; and Fondo Noir with Lavazza spa.

I want to share some more words on this last project. It was one of my first projects and it led me to realise how much the Systemic Design approach can really change our economic model, with companies that work in our territory. Also, I had the chance to meet in person the brilliant Ivanka Milenkovic. In 2007, Lavazza S.p.A., one of the most famous Italian coffee companies asked Professor Luigi Bistagnino to find a solution for the spent coffee grounds remaining after brewing the beverage. He wanted me to take part in the research team because of my experience in Colombia on the coffee plantations project. We started with a chemical analysis of Italian spent coffee grounds, and research into possible uses of it. The spent coffee ground contains residual caffeine, tannins, polyphenols, minerals, melanoidins, lipids and waxes, lignin, proteins, ashes and polysaccharides (cellulose and hemicellulose are a little less than 50%). The material is still rich material, though we typically throw it away. Having in mind the amazing production of Shiitake mushrooms in the coffee plantation in Manizales (Colombia), I was wondering if we can do the same spent coffee grounds in Italy. So, after a meeting with Gunter Pauli, he recommended that the best person to help me in this challenging project was Ivanka Milenkovic. His recommendation changed the results of this research and allowed me to meet a wonderful person. She is a visionary agronomist and an inspiring woman, who gave me a sensitive picture of inherent complexity.

Her response was totally unexpected: between the lines of her e-mail you can read her passion. She immediately gave me a positive answer to my request, offering an invitation to go to her workplace to learn how to grow mushrooms from spent coffee grounds. After a couple of weeks, I was in Belgrade and I met her in person. It was an amazing experience. She dedicated a lot of her time to me, I was always with her trying to catch every single word of information. With a lot of patience, she explained the basis of mushroom growing to me, in different substrates and with different species. Then, she set up an experiment for me in order to verify the substrate mix. After this experience, there was time for me to come back in Italy and set up the experiments with spent coffee grounds from Lavazza cafeterias and other waste materials from the local area. Even when I had returned to Turin, she followed my effort at every step, with a continuous and tireless support (fig.2).

fig.2: tests with different substrate and the spent coffee ground.

The main goals of this experiment were to verify feasibility of production (ability to create good substrate material, considering local availability of resources and the suitability of spent coffee grounds for growing edible mushrooms) and logistical feasibility (ability to find the necessary raw materials, equipment, and other essentials in the local area). The field experiment was carried out over a period of four months, during which we tested mushroom growth to determine the most suitable set-up. Our experimental mushroom production process was designed to use raw materials available in or around the city of Turin, except for the spawn.

For optimum growth, mushrooms need a proper substrate into which mycelium can be introduced or inoculated. Mushrooms typically require only low-level energy resources during the growth phase. Once we identified the correct substrate for growing Pleurotus ostreatus, our research analysed the local availability of raw materials on a seasonal basis. This allowed us to determine the best substrate composition with regards to materials available in the local area, knowing that the substrate must contain cellulose, hemicellulose, lignin, minerals, and organic salts (exhausted straw, husks, grape seeds, pruning vines, fruit waste, and sawdust). The exact amounts of these additional components would vary somewhat depending on the locality within the region.

Our experiment yielded positive results, confirming all the hypotheses put forth and proving the feasibility of the proposed project. The average production of fresh mushrooms was around 1.5 kg for each 3 kg of substrate used during the growth period. This harvest was double the estimated biological efficiency. We were able to increase the quantity of mushrooms harvested through step-by-step changes implemented during the growth phase, using a feedback loop that improved substrate preparation techniques and enhanced the ambient conditions (ventilation and moisture) in the growth room. The Pleurotus ostreatus mushroom was recommended by Ivanka Milenkovic due to its good resistance to varying environmental conditions (fig.3).

fig.3: Pleurotus ostreatus production from a mixed substrate with spent coffee ground.

I can say that the results of these experiments were successful thanks to her experience and availability. The Lavazza company was so proud of these results that they decided to present them during the International Fair Salone del Gusto in 2008. This fair is one of the most important events in the world in the wine and food sector: every two years, it gathers the food excellences as expression of culture and identity. For this event we set up mushroom production on spent coffee grounds and other agricultural by-products in order to have ten bags every day (for the five days of the fairs) to show in the exhibition. In the stand, substrate bags with the fresh mushrooms were shown. The whole process was described including the possible use of the spent substrate for production of vermicompost and the nutritional properties of Pleurotus ostreatus (fig.4).

fig.4: exhibition during the Salone Internazionale del Gusto in 2008.

This research resulted in scientific articles, two booklets ("Buone previsioni dai fondi di caffè" and "Fondo Noir" – http://areeweb.polito.it/design-stories/), many speeches in international conferences and, especially noteworthy, a decision by Lavazza to further this study direction. In the following years, with the involvement of prof. Paolo Tamborrini, the research evolved with some tests on other materials (paper, ink, oil, brick…) and the business model for the collection of the spent coffee ground in all the cafeterias in the city centre of Turin. The Italian research team that helped me on these experiments consisted of Alessandro Balbo, Cristian Campagnaro, Andrea Di Salvo, Franco Fassio, Andrea Marchiò, Simona Patrono, Katia Pozzato, Alessandra Rasetti, Dario Toso, Riccardo Vicentini, Andrea Virano, Eleonora Fiore, Miram Bicocca, Matilde Argentero and Milena Barbalinardo (in addition to the people already mentioned).

I really enjoyed meeting Ivanka Milenkovic, not only for the success of this research but for the passion that she transferred to me and the friendship that grew from this occasion. We got to know each other from growing mushrooms on spent coffee ground and now the result is much bigger: a network of visionary people that with the modesty of everyday work are changing the approach to the world.

I can say that she was one of the women that has influenced my carrier the most: she is a model for me in her professionalism and her passhion.

CHAPTER 3. DESIGN OF SUSTAINABILITY ***When passion grows as mushrooms***
Silvia Barbero, PhD, *Assistant Professor, Politecnico di Torino (Italy)*

CHAPTER 4 THE RECIPE FOR SUCCESS

ZERI FOUNDATION´S IMPACT ON EDIBLE MUSHROOMS CULTIVATION IN COLOMBIA AND CENTRAL AMERICA SINCE 1997

Jaramillo Lopez, Carmenza

Researcher Special Projects of CENICAFE (National Coffee Research Center). Chinchina, Colombia until 2003.

Current Member of the International Scientific Committee for Medicinal Mushrooms

Rodriguez Valencia, Nelson

Professor S.T. Chang´s observations on his visit in 1997, by initiative of the ZERI Foundation, initiated a series of investigations in Central and South America, specifically in the coffee zones of Costa Rica, Brazil and Colombia: Caldas, Chocó, Córdoba, Huila, Antioquia and Cundinamarca. They involved the cultivation of edible and medicinal mushrooms on waste streams from agribusiness: impact has echoed into recent years through social projects in rural and urban areas. Meanwhile, industrially productive projects are achieving large sizes despite exploiting new markets. Today, after 18 years, the

most promising potential is shown in production of medicinal mushrooms.

Chronologically, the projects have grown over time, and the training of people in the different places that Professor ST Chang visited on his trips to Colombia is reflected in the number of scientists who have dedicated themselves to fungal science.

ZERI Foundation (Gunter Pauli) and Mario Calderon Rivera were the most important promoters of this vision, working together with Carmenza Jaramillo and Nelson Rodriguez to make it a reality.

CHAPTER 4 THE RECIPE FOR SUCCESS *ZERI FOUNDATION´S IMPACT ON EDIBLE MUSHROOMS CULTIVATION IN COLOMBIA AND CENTRAL AMERICA SINCE 1997 Jaramillo Lopez, Carmenza*
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EKOFUNGI – AUTHENTIC STORY

Ivanka Milenkovic, EKOFUNGI DOO

We, in EKOFUNGI, strongly believe that the core of scientific work should be the benefit of humankind. It should never be at the expense of any single creature.

Science and entrepreneurship are intertwined like mushrooms and the roots of trees: they inspire, support and provide benefit for the participants on both sides. Scientific achievements are, and always have been, the most important resources for the EKOFUNGI. We appreciate them, adjust them and use them in our everyday work. We do the research of our own and exchange it with the scientific institutions who find the inspiration in our work as a representation of a real system-problems. This specific symbiosis enable us to become the unique production facility of edible mushrooms, in Europe as well as in the world.

If an entrepreneur wants to go forward there is only one way: INNOVATE! Open your mind for the advices and experiment! Innovation demands us to be creative. Creativity does not allow us to be closed-minded. On the contrary, creative person appreciate the exchange of ideas through the conversation. He/she shares the ideas unselfishly for the benefit of others. Thus, he/she creates the community of interest, making the world a better place. Only in that kind of world the entrepreneurs can have a prospering small part of its own.

EKOFUNGI is a proof that this kind of entrepreneurship approach is possible. Moreover, EKOFUNGI proved that with this policy, this work-philosophy is the only tool to go through the difficult times, to overcome bad moments and external disturbances that are constantly flushing our existence and business.

EKOFUNGI's wish was and still is to be the spot where the science and practice merges. In reality, we went throughout the difficult times more than once. Very soon we figured out that uniqueness is the only way to survive no matter what the circumstances are. Among contemporary high-tech mushroom production facilities that are typical for the high developed countries and low-cost technological solutions which are present in the countries of East, EKOFUNGI established its own way: We used and merged the solutions of a modern science and our own findings; we applied the laws of biology and fulfilled physiological demands of mushrooms, and finally we used a twenty-year-experience in cellulose waste utilization. The last one was realized in the cooperation with the ZERI foundation. And we succeeded!

Today, EKOFUNGI production takes place in seven tunnels that have been adapted according to the climate zone in which we are situated. The tunnels do not require a huge investment or complicated bureaucratic procedures. We have developed a specific technology for the substrate production, and we produce it for our own needs. Our capacity of oyster mushroom production is 20 t per year. Used substrate is not a waste; we deliver it to the nearby cow-farm. Thus, we create a closed cycle.

On the other side, a local stable provide the horse manure which we use in the white button mushroom compost production. Annually, we produce around 100 t of white button mushroom.

Spent mushroom substrate is directed to the local farmers. They use it as a natural fertilizer, and later their cellulose waste serves as the raw material for the oyster mushroom production.

Obviously, this is a never ending circle. Waste, in a classical meaning, does not exist!
All EKOFUNGI products have an organic production certificate.

The Story of RotterZwam: Blue Economy inspired Urban Mushroom Cultivation for Impact

Mark Slegers
Siemen Cox
Rotterzwam, the Netherlands

Two guys, almost equal in age, both working in a corporate environment and fed up with it. For one it was a training in LEAN which opened his eyes for waste – especially the waste of human talent. For the other a documentary called “The Crash Course - by Chris Martenson” opened his eyes.

They wanted to contribute to a sustainable society and not only talk about it any more. It was time for doing! Both where familiar with the Blue Economy (BE) and specifically the business case of growing mushrooms on coffee waste. The fact that one started 2 and a half months before the other, in the same city, doesn’t matter. After a cup of coffee they decided to tackle this challenge together.

Mark Slegers is a former manager at an energy company. He was educated as a biotechnologist; he knows the details of [nature] biology, chemistry and physics. He worked many years in ICT and energy companies were he was confronted with the lack of possibilities to be creative at his work. As a father of 2 kids he quit his well paid job and started to become an entrepreneur in 2010. In the beginning, he

was active in consulting companies in the green economy, helping them to do less bad, but in 2013 he moved on from consulting to Doing according to the framework of the blue economy. In 2013 he joined RotterZwam and in 2015 they initiated BlueCity, a building where there is [almost] no waste and entrepreneurs work in symbiosis with nature and each other.

Siemen Cox worked in financial services as a team- and project-manager. After watching an online documentary called “the crash course - by Dr. Chris Martenson” he got the insight we need to change the way we organise our society. The second thing that changed his perspective was a permaculture design course in Portugal. It was there that a teacher mentioned the Blue Economy and the business case of growing mushrooms on coffee waste. The thought never left his mind, and he started implementing the dream of a local mushroom farm on the coffee waste of the city of Rotterdam in January 2013.

So there we were. With little money, no experience, in an empty swimming pool in the city centre of Rotterdam and we could find no one willing to share their knowledge on how to grow mushrooms on coffee waste.

The BE states to work with what is locally available. That differs from place to place so we could imagine not all the details being present in the book. Because we believed in the principle, we just started.

But we decided to do it on a different way than those before us. We would share all our knowledge: current, present and past.

We wanted to pave the way for other entrepreneurs to walk this path without having to figure everything out everything by themselves. Also, we didn’t want to deliver our mushrooms to Amsterdam. Not because we don’t like Amsterdam (we leave that to the soccer supporters) but because there is plenty of coffee waste in Amsterdam. Let an entrepreneur in Amsterdam rise up and create jobs, food and reduce waste.

In the very beginning, we shared that we grow mushrooms in a swimming pool and the first people to approach us where the police. A group of 24 strong barged into our farm looking for magic mushrooms. After a good laugh and some picture taking from our side, they parted and we wrote a blog about that experience. The next day we were on the front page of Algemeen Dagblad online, one of the biggest newspapers in The Netherlands. The day after, our phones never stopped ringing.

We were contacted by all the major media outlets and had our first TV appearance the same week. But we didn’t have any mushrooms yet, only bags with substrate that turned green. Luckily we had some pictures they could edit into the film so that the difference between a bag of green mold and oyster mushrooms could not be seen.

Then we had restaurants contacting us through our Facebook page. They ordered mushrooms. But we didn't had any – yet. So we reached out, explained the situation and they were happy to wait. The occasional mushrooms we produced, found willing demand.

But we had to learn by doing, methodically find out all the possible ways of mixing the ingredients. Even to find out what the ingredients were. By simple trial and error it took us a little over 12 months to figure out the 'magic mix'. Next step was to make a DIY growing and fruiting room.

Help from the BE network

That was when Charles van der Haegen initiated a discussion in a Linkedin group. He stated that so many small urban farmers in Europe where all inventing the same wheel. Wouldn't it be a good idea to come together and share what we know, without anybody making money on it.

At RotterZwam we believe in sharing, so we suggested to host the first event from which the Mushroom Learning Network was born. That weekend over 45 farmers from all over Europe came and shared their expertise. We also met Ivanka there.

Ivanka is a real authority in the mushroom world. She has over 25 years of experience under her belt. We decided to do a training with her, at her farm in Belgrade. She gave us the priceless information to build proper growing and fruiting rooms. After this training, we went to next level with full confidence to initiate a crowdfunding campaign and raise the money needed.

The campaign was long and it was not easy, but we managed to raise € 20.000. Enough to go and build the mixing machine and the growing and fruiting rooms.

Master Mushroom Program

After we build the growing and fruiting rooms, we had a tenfold increase in production, from 3 kg per week to 30 kg per week. We could finally start to grow our business. Then we got more and more entrepreneurs contacting us: if we could help them set up a business of their own or if we do franchising.

We had already figured out that franchise was not the way to go. It was part of the old economy and we would have to control what the franchisees are doing. We think that the entrepreneurial spirit works best on the basis of freedom of choice. So we created a four day program in which we share all that we know and all the mistakes we made. The goals are to:

- Make sure that other entrepreneurs cut 8 - 12 months of their learning curve,

- Increase the impact of our business case (we are staying in Rotterdam so others need to stand up elsewhere),
- Create a business network that exchanges knowledge and grows together,
- Do business with each other.

So far we have succeeded in training over 30 entrepreneurs and helping them start up a business in their own city.

Growkit

A lot of people from Rotterdam and beyond where starting to contact us to donate their 'private' coffee waste, the waste they created at home. We where very much surprised by this. The offers kept on coming, so much so that we felt like doing something with it.

We already decide not to produce the regular Growkit because, according to us, it didn't match the story. With a traditional Growkit you are transporting waste from the restaurants to the consumer and 70% of the coffee consumption is at home! So why give the consumer more waste then he already has?

But with all the coffee offers (we didn't accept the coffee because of Trichoderma) pouring in, we knew that the consumer wanted to do something with their coffee waste. That is when we took an idea of another collegue, and build on that.

Bruno Van Haudenhuyse from GandaZwam, taught us the basis of making substrate based on coffee waste, he also had an idea called the 'running pot'. Inspired by that, we developed and build the concept of the RotterZwam Growkit. Of course we shared our results with him and presented him with the first example.

Export

Some of our colleagues who completed the Mushroom Master Program with us, showed interest in using our Growkit in their country. But we didn't feel like exporting it abroad. If we would do that, it would be similar as bringing waste from restaurants to the consumer. So we figured out a different way.

We thought of the principle of think global and act local. We exported the knowledge so that our partners could produce the Growkit in their city and could continue to create jobs locally. In exchange for sharing all the knowledge to build and market the Growkit we get a license fee.

This system allowed us to stay close to what we believe in and minimise CO2 emissions in unnecessary transport. We make less money, but the environment is better off.

Future

The interesting part of the whole thing is that there is a cascade of business cases that build off of either the coffee or the mushrooms. And then we haven't even touched upon the things you can do with worms (yes, they are also involved). At the moment we have more than five business cases placed on a shelf waiting to be developed.

As we don't have the same deep pockets as more established businesses, it takes more time to develop all the opportunities. Because of all the challenges our society faces, this is at times very frustrating. We could do so much more, if we would have better funding.

Don't get us wrong, business is thriving. We have doubled our revenue every year so far and funded RotterZwam well through private institutions. But with some donations, we could drastically increase our impact. It's nice to dream and develop a vision of what we can do to even further create a society based on the Blue Economy and inspired by nature.

Beyond Coffee – The Copenhagen mushroom farm

Ebbe Korsgaard Andersen
Beyond Coffee

Since autumn 2015, Copenhagen has been home to an urban mushroom farm that each month converts 1500 kilograms of organic coffee grounds into ~250 kilograms of fresh oyster mushrooms. The farm is built inside two insulated shipping containers which are placed in a temporarily empty building lot.

Beyond Coffee also runs a small store in the hip Jægersborggade street in Copenhagen. This street is known for its many niche shops, which attract both locals and tourists in big numbers. The store is called Beyond as we do not only sell our mushrooms, but also other sustainable products that are made out of waste. The store acts both as an exhibition room for the concept and as a sales-point for mushroom products.

This chapter will briefly take you through the struggles and achievements that we in Beyond Coffee have experienced in the last year – from planning the first steps to setting up a farm and opening up a shop.

Why containers?

We chose to build our farm inside cooling containers, also called reefers, no longer suitable for their primary purpose. Reefers are used to transport goods that need to be kept cold around the world, but

the cooling element eventually wears down to a point where it is not financially feasible to repair it anymore. At this point they become a waste product of the shipping industry, and they can be upcycled for other purposes. They are usually cheap, and their price is negotiable, especially if you ask around and cross-check prices. The containers are already insulated and have an inside designed to be cleaned easily – both attributes are perfect for mushroom cultivation as we want to control temperature and keep it clean inside.

Not only are the containers a cheap building block (it can easily be stacked in height), they are also very easy to move around, as they are designed for this purpose, and the means to do is widely available – almost anywhere in the world you can hire a truck to move your container. These aspects come

in handy in an urban setting when you are looking for empty spaces for your mushroom farm. In Copenhagen we found that there are many small empty spaces available, but mostly for a short timespan like a year or two. Also, another great advantage is you can design the farm to suit your preference only once and then scale it by adding more units.

Getting started
Finding coffee donors

To get started it is essential to find good sources of coffee grounds. In our experience it proved valuable to find donors that are truly interested in the project. It makes it a lot easier for everything work smoothly. Another good idea is to find donors that are not too busy – meaning that they actually have time to separate the coffee and handle it correctly

We now frequently get asked if we are willing to take the coffee grounds from different places. This means that we can be picky about who we choose to take from. We therefore only accept coffee from places that:

- Are brewing certified organic coffee
- Are truly engaged in the project
- Are willing and able to store the grounds in a refrigerated space
- Are willing to provide us with receipts of the bought coffee, which we need for legal matters

Selling your end product

You should not spend too much time on finding people to buy your mushrooms, before you have the product ready. In our experience it is easier to present the product to your customers when you actually have the mushrooms to deliver. Delivering a test-sample to different restaurants is a great way to move forward. Also, keep in mind that many chefs usually have good connections to other restaurants in town – especially those with the same values (e.g. buying local produce, using organic produce, etc.). It is important to make a good impression from the beginning and to make sure to notify your restaurants if you are looking for more customers. Our experience is that medium to high-end restaurants are the most interested, especially restaurants that promote sustainability and local food.

If possible, you deliver mushrooms to a restaurant from which you also pick up coffee grounds. This creates a strong story from which both the restaurant and your business can benefit.

What we have been successful with
Strong partnerships

From the very beginning we established a good relationship with local restaurants who demonstrated a willingness to support the project and a willingness to pay a little extra for high quality fresh produce with a good story. In part, this was achieved by offering exclusive tours of the farm to some of the head chefs at the restaurants. This is a good way of doing it because it gives your customers (in this case the restaurants) an insight into the work the goes on behind the produce, the whole mindset and also to stress why it is important to deliver fresh non-contaminated coffee grounds to the production.

Media attention

It is useful to have a media strategy from the beginning. Media attention is self-amplifying, so most of the work occurs in the beginning – once you get started the attention will continue to roll automatically. It is best to get covered by a big media bureau at the start as this gives the whole project credibility and many smaller bureaus will learn about the story and also want to cover it.

Luckily, mushrooms being grown on coffee grounds is a great story, one which journalist's love to cover. Keep in mind that there is a breaking factor in journalism, which is quite important to the bigger bureaus in particular. What we did was to promise our story to a big Danish newspaper, which is probably the most read in Copenhagen. This resulted in a big article about us in a big newspaper, which lead other journalists to contact us from other types of media. Within a year we have been featured in several newspapers, on radio, on internet blogs, on TV-news and in a documentary film. Every time we appear in the media we can tell the difference in both the sales and the amount of interested partners who contacts us.

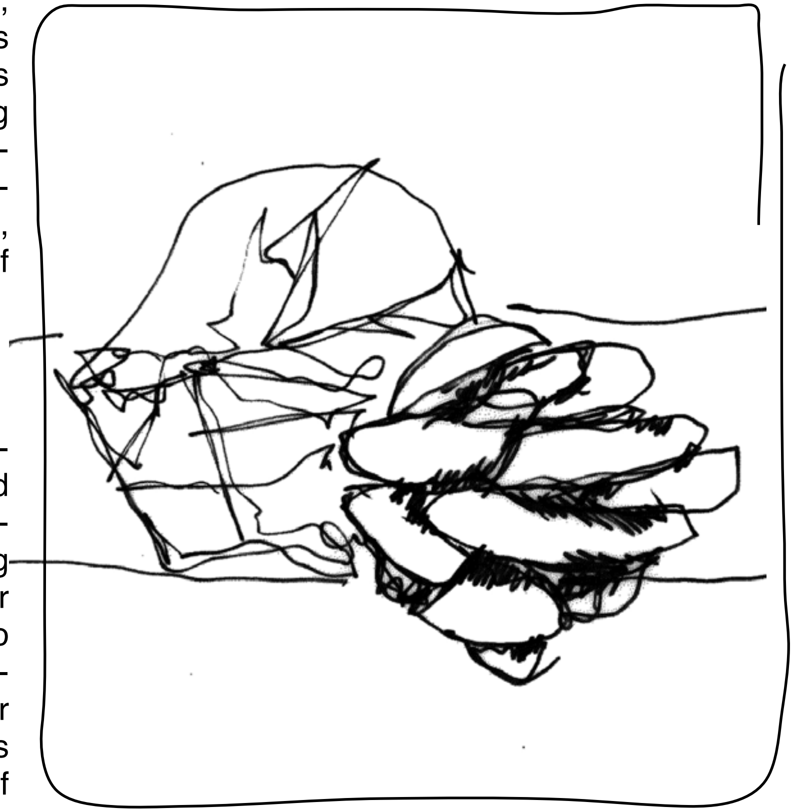
Opening a shop

Opening a physical shop to meet the customers has been a great success for us. In the window facing the street we showcase a bag of substrate with fruiting mushrooms. This is a great way to present the idea to the general public. It is also an eye catcher that makes people stop and triggers their curiosity. Many people enter the shop with a mysterious look on their faces, eager to hear what it is all about. Having your own shop also makes it really easy to test out new products and ideas.

What we could have done differently
Getting good quality grounds

When engaging new coffee ground donors it is super important to make sure that the quality of the coffee grounds is good. If you do not do this, you can end up discarding a great amount of your production due to contamination from molds. We have recently experienced this due to a change in coffee donors. When you take in several new donors and mix all the grounds, it can be very hard to determine where the contamination comes from. We therefore strongly emphasize that coffee grounds from every new donor are tested by themselves, meaning the grounds are not mixed with coffee from any other sources. This gives a really good indication of the quality of this donor. We have made the mistake to mix coffee grounds from new donors with grounds for which we already knew were good quality. This diluted the

amount of bad grounds in a mix of good grounds, which made it hard to see that the new grounds were bad. This means that the new grounds became a part of the production without being suspected as a possible source of contamination. Thus, the lesson learnt is that it is important to be super critical towards new donors, until you are completely sure that the quality of the coffee grounds they provide is good.



Distribute your time

Setting up and running a mushroom business is work intensive. Both mushrooms and molds grow, whether it is a weekend or a workday. This means that time is always a limiting factor for an urban farmer – just like in regular farming. One of the most important things to succeed with this business is to be able to organize the time spent on different projects. Our experience is that the most important thing is always to prioritize the farm and production. If you get behind schedule at the farm, it will take a lot more time to get back on track. A lack of cleaning could for example lead to a contamination outbreak. Once there is an outbreak you have to spend a lot more time on cleaning than you would have originally. It is good advice to always keep a few hours free in your schedule to do extra unforeseen tasks on the farm.

Getting the right coffee

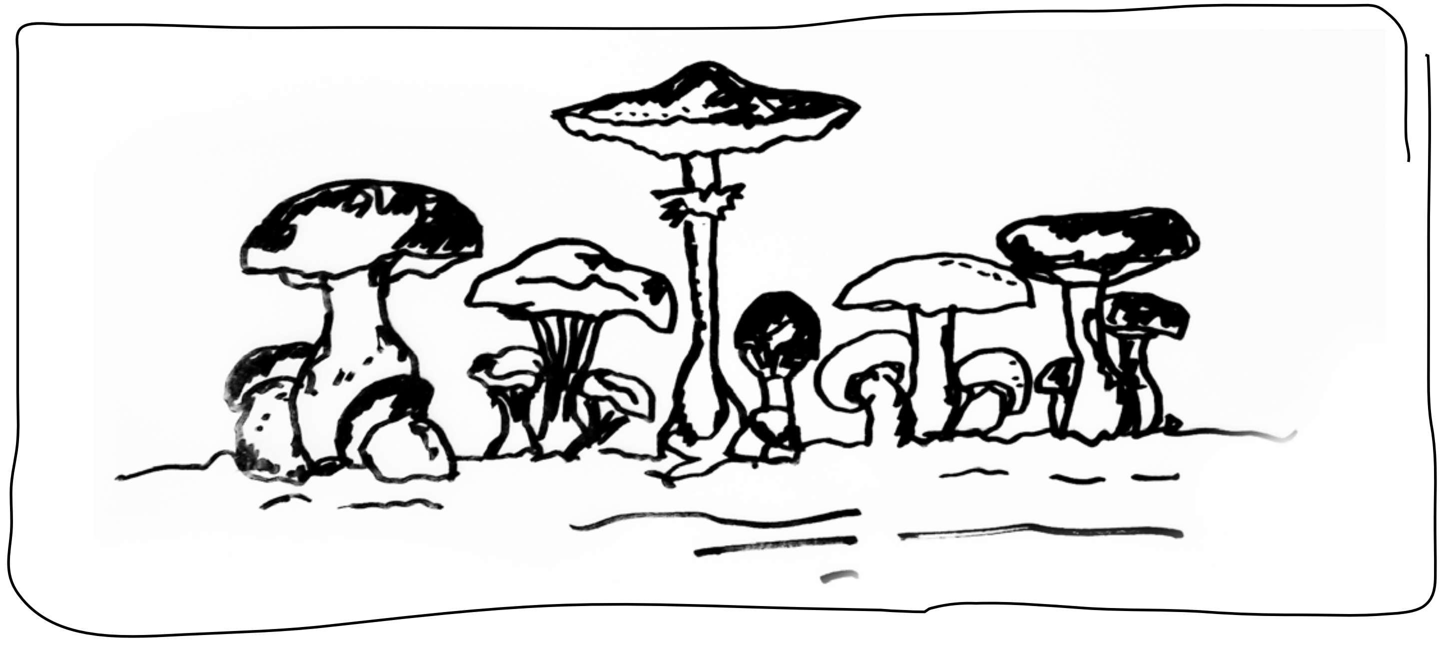
Coffee grounds can have many forms, depending on the method used for coffee brewing. In Denmark it is common to brew big batches of filter coffee in cantinas and big restaurants. When this method is used, the coffee grounds become very wet compared to coffee grounds from brewing espresso. This influences your production and you risk to get substrate that is too wet. This can result in liquid leaking from your growing bags and making a mess in your incubation room. It can also limit mycelium growth in the bottom of your bags, as the water will gather in the bottom and drown the mycelium. We found that mixing a wet substrate with silverskin/chaff collected from coffee roasters is a very efficient way to make the substrate dryer. Chaff is roasted and dry, which means that it can be stored for a long period without it going bad, and thus without causing contamination when it is used in production. When you have made sure that your coffee is of good quality, you can start to mix wet and dry coffee grounds to find the right balance. If you only collect grounds from espresso brewing, then you would probably need to add water to the substrate to get the right moisture level.

Going beyond mushrooms

Our next big project might be beyond mushrooms. We have discovered that the spent substrate from mushroom production is a great source of food for edible insects. We have bred batches of mealworms and crickets, which have only been fed with our spent substrate for their entire lifespan. The crickets are especially interesting as they somewhat taste like what they have been fed. Feeding crickets with spent coffee substrate gives the crickets a pleasant taste of earthiness. Crickets contain all the essential amino acids, which makes it a very good source of sustainable proteins for human consumption.

Unfortunately, Denmark is lacking the legislation to support development of this matter. There are at this moment no rules for how to breed edible insects in Denmark, which means you cannot do it right and legally. Hopefully this situation will soon change.

Our next move is to research on the possibilities to upgrade the facility with an insect farm, also based on disused cooling containers. We believe this has a huge potential – essentially it means that you can produce two healthy and protein rich foods from coffee grounds, using very little resources and space. We encourage everyone engaged in mushrooms production to participate in the development of this field. This way we can use spent substrate for creating yet another healthy food source.



Mushroom Research And Development: The Case Of Namabia

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University of Namibia

Mushrooms have been used for their nutritional value for millennia due to their high possession of qualitative protein content, crude fiber minerals and vitamins (Kosanic, Rankovic, & Dasic, 2013). Besides their nutritional value they produce a wide range of secondary metabolites with high therapeutic value. They are also sources of physiologically beneficial bioactive substances that promote good health (Kosanic et al., 2013).

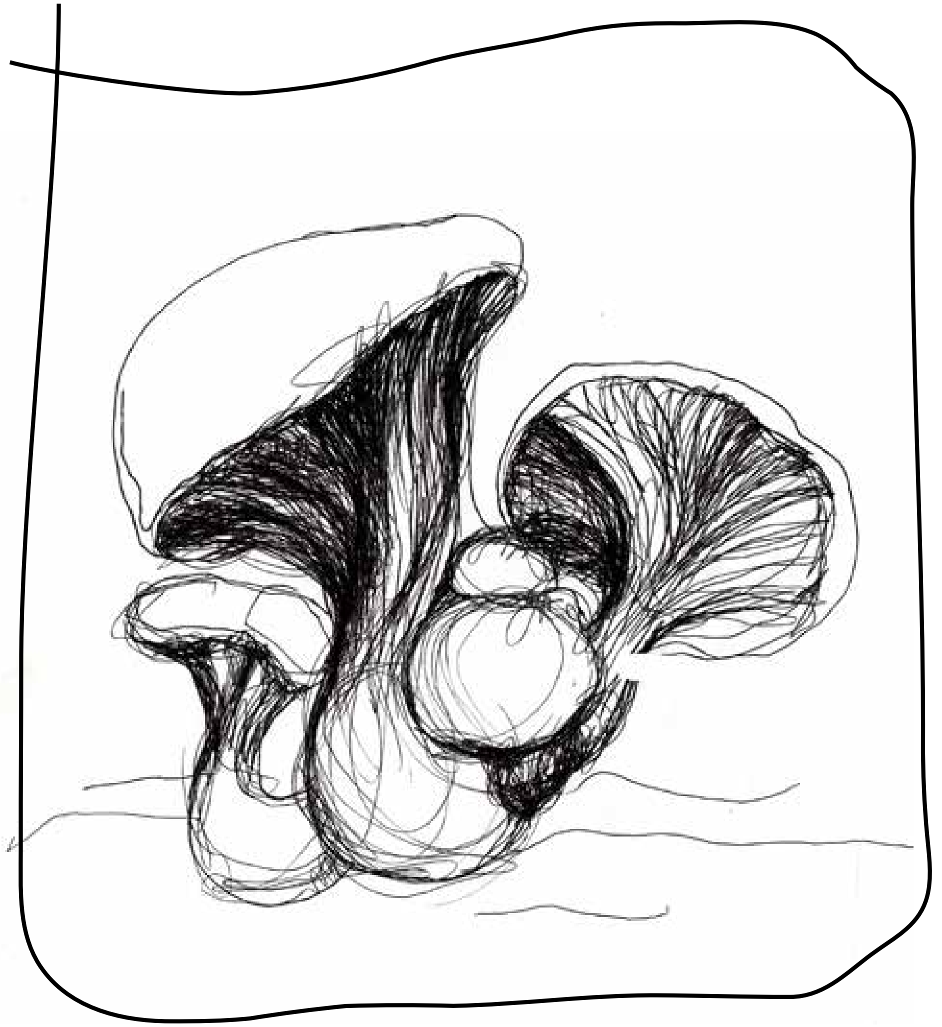
Mushrooms are well known among Namibian communities and are widely consumed in most parts of the country during the rainy season. In Namibia, the utilization of wild mushrooms as food is very common. Wild edible mushrooms provide two main benefits to the people, as a source of food and as an income generation activities. It is very common to see people, particularly young women selling mushrooms by the roadside just after the start of the rainy season. Most mushrooms sold belong to the Termitomyces species especially Termitomyces schimperi, Termitomyces sagittiformis, Termitomyces reticulatus. After the rains, during the months of May and June you will then see farmers selling the most prized mushroom that is known as Omatumbula in Oshiwambo, //nabba by people of Nama or of San heritage, and mafumbula in Rukavango. These are the well-known Kalahari Desert truffles, the Terfezia pfeilii. Truffles are some of the most hunted wild mushrooms that one can also find being sold in local supermarkets.

Mushroom research, cultivation and development were an initiative of Zero Emission Research Initiative (ZERI) Africa Regional Project funded by UNDP and under the leadership of Professor Keto Mshigeni. ZERI, which is a vision, a concept, and a philosophy that aims to catalyze the application of Science and Technology towards food security and the creation of employment opportunities, especially for rural women and the unemployed youth. The concept includes enhancement of people's health and socio-economic welfare; the production of sustainable development and environmental regeneration in rural and peri-urban communities.

ZERI aims at providing affordable options that are viable and suitable to generate income that can contribute to the reduction of poverty for the people in Namibia's rural and peri-urban communities by promoting mushroom farming and mushroom consumption. Thus the project further aims to transfer scientific technology and skills to communities to make productive use of byproducts of agricultural food processing otherwise deemed a waste. Within the University of Namibia, the ZERI Project focuses on mushroom research by domestication of indigenous edible and medicinal mushrooms as well as product development from cultivated indigenous and exotic mushrooms. The Project also ensures that women and the unemployed youth from all communities across the country are trained to take up and practice the scientific technologies and skills of mushroom cultivation for them to become self-reliant. The aim of promoting and increasing public awareness about the benefits of mushrooms as food, medicine and as an income generation activity is important because mushrooms grow relatively fast and does not require complicated post-harvest techniques.

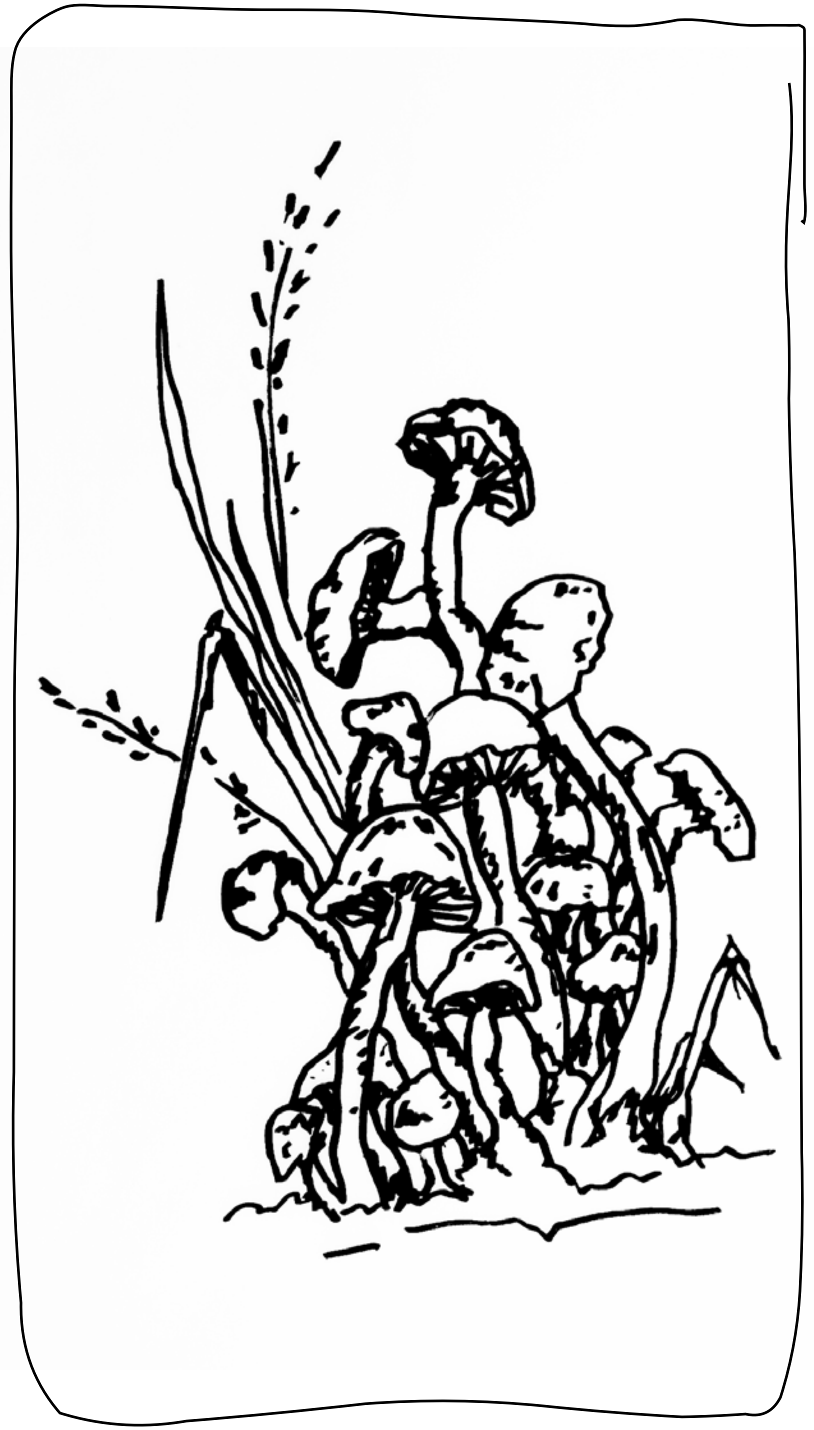
Mushroom farming was introduced in Namibia in the late 90s. Oyster mushroom cultivation was introduced to famers in 2002 by the Ministry of Agriculture, Water and Forestry at Grootfontein in the Otjozondjupa Region. Oyster mushrooms have been cultivated and documented in China, (Mshigeni and Chang, 2000), Pakistan (Sher, Al-Yemeni and Khan, 2011), North America (Cline and Leschen, 2005) and in African countries such as Kenya, Tanzania and South Africa (Kimenju et al., 2009).

In its efforts to promote mushroom farming, the University of Namibia developed and published reader friendly advocacies on mushroom growing in English with some translated into local languages. It is thought that the promotion of oyster mushroom cultivation is best suited to introduce farmers to mushroom cultivation, as these mushrooms can play a major role in poverty reduction, hunger alleviation, promotion of good health and creation of employment for the unemployed. Thus the University of Namibia embarked on research to explore various locally available organic materials to be used as substrates for the production of oyster mushrooms, especially those generated as byproducts from agriculture activities after harvesting. Several experiments were carried out in order to find the best local organic materials to grow the exotic mushrooms that were introduced in the country. Research also focused



on trials to determine the mushroom species that can grow well in Namibian climatic conditions. A comparison of the bioconversion efficiency of mushrooms grown on different locally available substrates was conducted for the few available oyster mushroom species. The likes of Pleurotus sajor-caju, Pleurotus ostreatus and many others oyster mushrooms responded well when tested on some Namibian wild grass and most agricultural wastes. The substrate research went as far as exploring a mixture of grass with seaweeds found along the Namibian costal area: it yielded promising data showing that mushrooms grown on grass supplemented with seaweeds absorbed iodine. However it was suggested that more research was necessary (Kaaya, Kadhila-Muandingi, Lotfy, and Mshigeni, 2012). Currently the recommended oyster mushrooms substrates in Namibia are maize cobs, wheat and rice straw as well as millet husks. Medicinal mushrooms like Ganoderma are grown on woodchips from the local wood carvers.

Currently at the University of Namibia, mushroom research activities are being carried out and coordinated by the ZERI department within the Multidisciplinary Research Centre (MRC) at main campus in Windhoek, the Sam Nuyoma Marine and Coastal Resources Research Centre (SANUMARC) at Sam Nuyoma Campus in Henties Bay at the coast, and at Ogongo Campus in northern Namibia. Apart from the United Nations Development Program (UNDP) which was the main funding agency for mushroom promotion and cultivation in the country, mushroom activities also received funding support from the local bank NEDBANK, the African Union through the Southern African Network for Bio-



sciences (SANBio), Global Environmental Facility (GEF), and the United Nations University (UNU). The university main campus supports mushroom projects in Khomas, Omaheke, Kavango, and Hardap regions. SANUMARC supports mushroom project in Erongo, Otjozondjupa, Karas and Kunene Regions, while Ogongo Campus supports mushroom projects that are in Ohangwena, Omusati, Oshana and Oshikoto regions.

Most of the indigenous Namibian mushrooms such as Ganoderma have been studied on the level of domestication and product development. This followed research carried out in the country which revealed that community members have been and are still utilizing Ganoderma mushrooms for medicinal purposes, both in humans and animals (Ekandjo and Chimwamurombe 2012, Kadhila-Muandingi and Chimwamurombe, 2012, Shikongo, 2012 and Kadhila-Muandingi, Nametso, du Preez, and Mumbengegwi, 2014). The evidence of traditional use of substances from mushrooms by indigenous populations and the abundance of the species in nature highlights the consideration of using natural substances for investigation of medicinal properties. Medicinal mushrooms have shown therapeutic benefits, primarily because they contain a number of biologically active compounds (De Silva et al. 2013).

Research on indigenous mushrooms does not only focus on the ethnomycology by the communities and domestication, but

also on buying and collection of edible undomesticated mushrooms that are sold by the communities. These mushrooms are bought, dried and processed into products such as soups that are promoted as healthy food during the University Research Day; the objective being to pass on the technology and skills to the communities during mushroom cultivation and post-harvest training sessions. Mushroom research and development has now become advanced in Namibia with more communities having been trained in mushroom cultivation and post-harvest processing. During community training, participants are introduced to different types of mushrooms, being taught uses for food and medicine as well as the danger caused by specific poisonous mushrooms, and the basic skills and technology involved in cultivating oyster mushrooms. The facilitators of the training follow a comprehensive program that includes but is not limited to: mushroom culture preparation, spawn preparation, substrate preparations and inoculation, vegetative and reproduction phases, mushrooms post-harvest processing focusing on harvesting and preservation, types of mushroom houses and their construction using different cheap locally available materials and their management as well as topics on mushroom pests and diseases control.

Apart from community training, researchers continue with trials on the domestication of indigenous mushrooms, particularly edible ones. Most research is also focused on mushroom product development with some promising prototypes that can be used in the country and beyond. ZERI plans to develop more products from indigenous mushrooms that can be promoted by passing on the knowledge and skills to the local people. More research is now focusing on mycochemical compounds of interest that are found on indigenous mushrooms for possible product development. Products in the form of nutritional food such as mushroom tea, dietary supplements, soups and chips are of interest because they can contribute to health, food security, income generation and poverty alleviation in the country.

Opportunities and challenges faced by mushroom farmers in Namibia

Even though communities countrywide are trained on mushroom cultivation, most mushroom farmers grow mushrooms for their own consumption and only sell them when they have a surplus. Namibia needs to scale up and grow oyster mushrooms on a large scale to meet demand, which is currently high. Public awareness and mushroom promotion still need to be emphasised in order for the people to become aware of such activities. Famers' field days, agricultural shows, school fun days and University research days can be targeted for awareness creation. Information materials like booklets, brochures and posters on mushroom growing that are translated into local languages can be disseminated at agricultural shows for the promotion of mushrooms as a nutritional and healthy food and as a business opportunity that can be easily taken up by people in order for them to become mushroom business entrepreneurs.

Most of the challenges experienced in the regions in Namibia include lack of cooperation among community members and interpersonal relations, lack of commitment toward project activities, unavailability of substrates in some regions, contamination, and mushroom phobia that sometimes limit the sales of mushrooms to other community members. As a result, more needs to be done to create awareness on the safe consumption of cultivated mushrooms.

In 2009, the African Union NEPAD/SANBio selected the University of Namibia ZERI Project as the Mushroom Node for the Southern African Region. UNAM was recognized as a Centre of Excellence for Mushroom Research and was given the role to coordinate the Mushroom Research, Technology and Innovation (MRTI) activities at regional level. Six southern African countries participated in the Node initiatives, namely: Angola, Malawi, Mozambique, Namibia, Swaziland and Zambia. The University of Namibia is still the Mushroom Node for the Southern African Region due to its outstanding research and development on mushrooms cultivation.

Figure 1. Most wild harvested mushrooms that are sold at local markets. From left Termitomyces schimperi, referred to as "omajova" by the locals and Terfezia pfeilii well known as the Kalahari Desert truffles.

Figure 2. From left, the mushroom house at the University of bNamibia and the inoculation box that is used by the communities as laminar flow hood where tissue cultures and spawn are made.

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FUTURE OF HOPE

Dr Gunter Pauli, Founder ZERI, Author "The Blue Economy"

The work of Chido Govera inspires me most. Imagine this orphan girl who learned tissue culture in the laboratory of Africa University at the age of eleven, who perfected her understanding of abuse and food, and the pressure to get married so that the uncle custodian can collect a few heads of cattle as a dowry, while everyone kind of knows that this is not a gift for marrying an old man who already has two or three wives, but rather a compensation for letting this young girl enter into the sex trade. When Chido grasped the full extend of the opportunity she became determined to share he knowledge with any orphan she could reach. However, first Chido had to perfect her knowledge and first under the wings of Margaret Tagwira, and later exposed to the hands on experiences of Carmenza Jaramillo and Ivanka Milenkovic, Chido went out in the world to put into practice the art of mushroom farming reaching out to coffee communities in Tanzania, villagers around beer breweries in the Congo, Aboriginal families in Sydney, outcasts in Jhansi (India), mining communities in Ghana, technology entrepreneurs in San Francisco, and sophisticated three star chefs in Copenhagen. Chido navigated through frustrating experiences in Germany, realised that her understanding of business made her prone to abuse by those who wanted and needed her name, fame, and ethics to proliferate themselves as social entrepreneurs and yet, after a decade of pioneering in four corners of the world, Chido settled with a solid life experience to create hope.

The Future of Hope is Chido's foundation, located outside Harare, where she welcomes orphans for training, and orphans at risk for a living, taking care of their daily needs, including a warm home, clothing and school fees all funded through the sale of mushrooms to the local Pick 'n Pay supermarket. Philanthropists like Koen Van Mechelen hired her to create art, and the Rotary International Foundation joined in to permit Chido to reach out to more girls at risk scattered through a nation that has difficulty in caring for the most vulnerable in society. However, Chido succeeds in making ends meet and create hope amongst the orphans and is committed to save each and single one she can from the devastating experiences she had to undergo. Soon Chido will not be alone in her extraordinary endeavour, dozens of change agents will emerge from the bottom of society where we expected it the least. That is the real power of mushrooms, modest and perhaps invisible, certainly unexpected but prevailing that is one of the components of the future of farming and food.