Swedish substrates suited for circular production of the edible gourmet mushroom maitake

Svenska substrat lämpade för cirkulär produktion av gourmetsvampen maitake

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Swedish University of Agricultural Sciences, SLU
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Abstract

A selection of locally sourced substrates were evaluated for hyphal growth and fruiting body production of the edible gourmet mushroom maitake (*Grifola frondosa*). Hyphal growth was evaluated on a wide range of substrates (30) including different leaves, sawdust and waste from food production and the substrate that performed best was potato peelings, which showed development of a remarkably dense mycelium.

Fruiting body production was studied on eight different substrates including enriched sawdust of alder, birch, oak and fir and the residues apple pomace, oat okara, betfor (a feed product based on residues from sugar beets) and a mixture of betfor and deproteinized rapeseed cake. A literature study was performed to determine commonly used cultivation conditions for *G. frondosa* and these were applied in the fruiting body experiment.

Despite problems with mould infection in the cultivation chamber mushrooms could be harvested from the boxes with enriched fir substrate. Other substrates, such as rapeseed and betfor mix, had primordia but these had not reached full size at the end of the project. It appears *G. frondosa* mycelium thrives on waste products from agriculture and food production, but further work is needed to determine if fruiting body production is possible on these types of substrates. However, fruiting bodies can be produced on enriched fir substrate which is an easily available substrate in Sweden. Also, aspects of circular production, i.e. reusing the spent mushroom substrate in further production, is highlighted in this work.

Keywords: Grifola frondosa, maitake, mushroom production, spent mushroom substrate, circular production

Preface

My long-standing interest in mushrooms led me to my supervisor Malin Hultberg who helped me develop this project. The idea with this project was to spread knowledge regarding the gourmet mushroom maitake and investigate the possibility of using locally sourced materials for its potential production in Sweden. Developing a suitable mushroom substrate that can be easily sourced domestically and be part of a circular production would go a long way in making the cultivation process sustainable and creating a wholly local source; an important part of creating a greener mushroom production. Another part that interested me was the potential to perhaps be part of or at least contribute to a growing market for edible mushrooms and the prospect of using them as a meat substitute.

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Abbreviations

SMS	Spent mushroom substrate
RH	Relative humidity
FW	Fresh weight
DW	Dry weight
BE	Biological efficiency ((FW of harvest/DW of substrate)×100)
MA	Malt agar

1. Introduction

1.1. Mushroom production in Sweden

Mushroom production today in Sweden relies heavily on imported materials (Stridsberg & Tullander 2017; Hansson & Hansson 2014). This includes materials such as grain spawn, mycelium and substrates. Replacing one or more of these with a locally sourced alternative would bring us one step closer to entirely locally produced mushrooms. Using locally available Swedish substrates could also give mushroom production a smaller carbon footprint, making it more environmentally friendly and sustainable. This can be further improved by striving to achieve a circular production system.

1.2. Circular production

In current years, increasing awareness of the negative effects of development based on the concept of "take, make and dispose" has driven a change towards development of a circular economy. From a practical perspective, a circular production system means utilising your resources in such a way that they don't leave the area (e.g., filtering and reusing water) or using waste products that otherwise would have been thrown away (e.g., composting discarded vegetables). Within the confines of this study, this could for instance be done by using agricultural waste as a substrate for mushroom production and, after harvest of the fruiting bodies, reusing the spent mushroom substrate (SMS) as animal feed (Grimm & Wösten 2018). For substrates less suited as feed, e.g. sawdust-based substrates, an option for development of a circular production system is to reuse the SMS for biofuel production (Chen et al. 2020).

2. Background

2.1. The edible mushroom *Grifola frondosa*

Grifola frondosa is an edible polypore mushroom that is commonly known as maitake. Maitake, meaning dancing mushroom (Stamets 1993), is generally light grey at maturity, with white pores under the caps. It is typically found growing at the base of deciduous trees such as oak and beech but has been found on the evergreen Douglas fir (*Pseudotsuga menziesii*) as well. A benefit of cultivating a polypore mushroom is that these do not need neither high humidity nor a lot of water to thrive, keeping the water cost and impact of production down (Stamets 1993).

Grifola frondosa is a basidiomycete fungus that is native throughout North-eastern Japan, parts of North America, temperate forest regions in China and Europe at large (Stamets 1993). The fruiting bodies contain approximately 90.1% moisture, and the dry matter is composed of 70.7% carbohydrates, 6.1% crude ash, 3.8% crude fat and 19.3% crude protein (Wu et al. 2021). Spores are approximately 3-5 x 6-9 µm in size and oval in shape (see fig. 1).

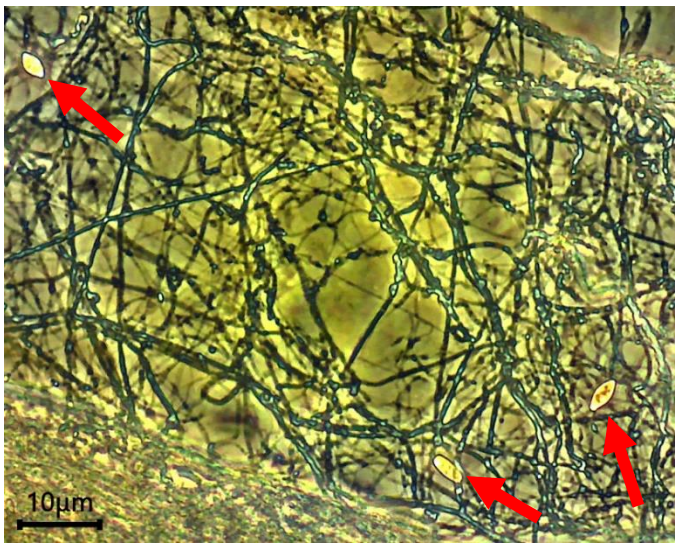

Figure 1: Mycelium of G. frondosa (10µm scale bar). The red arrows are pointing at spores that can be seen in the top left and bottom right corner.

Maitake is a common culinary mushroom in some parts of the world, such as Japan (Stamets 1993). It has been a popular culinary and medicinal mushroom in China for hundreds of years (Pan et al. 2018; Hong et al. 2013), but yet it remains a rather obscure mushroom in Sweden. It's considered a gourmet mushroom and the taste is described as nutty with a meaty texture (Jones 1998). There is a growing demand in Sweden for both meat substitutes and edible mushrooms, and maitake, with its meaty texture, would lend itself well to such use.

Some studies have been done on its effect on various diseases. For instance, in *in vitro* conditions, it's been found to be effective against the HIV virus (Stamets 1993). In other *in vitro* studies, certain maitake derived polysaccharides have been shown to have both antioxidative and immunoregulatory effects (Wang et al. 2016; Inoue et al. 2002). Hong et al. (2013) tested the maitake polysaccharide MT-α-glucan on mice and found that it seemingly delayed aging thanks to these effects by, among other things, improving motor and memory skills. However, the medical aspects of maitake is not in the scope of the present study which instead will focus on cultivation.

Finding a substrate that works well and is easily available in Sweden would be of interest to mushroom enthusiasts and hobby growers alike. Currently, mushroom production in general relies heavily on imported materials and it is mainly white button mushrooms that are produced. Finding a suitable local substrate for maitake would therefore bring consumers a new type of edible mushroom that also has the benefit of being more sustainably produced. However, maitake is considered difficult to cultivate (Malek et al. 2012; Mau et al. 2003) partly due to the limited range of optimal cultivation parameters (Gregori et al. 2016; Švagelj et al. 2008), so a study into which conditions and substrates are suitable is needed, which is part of what will be investigated in this project.

2.2. Mushroom substrates

The most commonly produced mushroom in the western world is the white button mushroom (*Agaricus bisporus*) (Grimm & Wösten 2018). The white button mushroom is a humus-inhabiting fungus which means it grows in soil and is therefore cultivated on different mixes of compost/soil etc. There are also rot mushrooms (categorised as brown-, soft- or white-rot) that live on wood. *G. frondosa* is a white-rot mushroom. This means it grows on wood, both living and dead, and breaks down the lignin in the process (Nordiska ministerrådet 2012b). Maitake is usually cultivated on enriched hardwood sawdust such as oak (Stamets 1993), but there's every opportunity to be creative and try other types of lignocellulosic products as substrate. The agricultural industry produces several different types of lignocellulosic waste products. Commonly available ones include straw (e.g. what's left after harvest of wheat, corn etc.) and oil press cake (e.g.

sunflower and rapeseed seeds etc. after oil production). Spent mushroom substrate (SMS) is quite often composted, but if the cultivated mushroom sufficiently breaks down the lignin present in the substrate it would be possible to use the SMS as animal feed. Using a lignocellulosic waste product and then using the SMS as animal feed would make it possible to keep a circular production system (Grimm & Wösten 2018).

2.3. Global statistics

China's share of the worlds mushroom production has always been significant. In 2014, China stood for just over 73% of all mushrooms produced worldwide (Pandey et al. 2018). Mushroom production has increased rapidly over the last couple of decades. In 1978 about 1 billion kilograms were produced and just 35 years later, in 2013, it had increased to as much as 34 billion. This increase is considerably bigger than worldwide population increase over the same time period, which means that mushroom consumption has gone up and now sits at about 4.7 kg per person and year. About 85% of worldwide production of edible mushrooms are from five genera: *Lentinula* (22%), *Pleurotus* (19%), *Auricularia* (18%), *Agaricus* (15%) and *Flammulina* (11%) (Royse et al. 2017).

2.4. Aim

The aim of the present study was to compile knowledge on how *Grifola frondosa* is cultivated and to examine the suitability of different substrates, all easily available in Sweden, for supporting its growth. The aim was also to study the potential for developing a circular production system *of G. frondosa* with the long-term goal of reusing the spent mushroom substrate as feed.

The specific objectives were

- To evaluate hyphal growth of *G. frondosa* on a wide array of potential substrates

- To evaluate fruiting body formation of *G. frondosa* on 4 sawdust-based substrates and 4 substrates based on residues from food industry

- To determine the impact of fungal growth on total protein concentration in the substrates based on residues from food industry

2.5. Limitations

Substrates based on residues from food industry were selected based on their availability when the project started. Undoubtedly, there are other residues that may be interesting to test. Furthermore, in order to perform the project within the time frame of a degree project, certain limitations in the experimental set-up have been necessary:

- Only one strain of *G. frondosa*, strain M9827 from Mycelia BVBA Belgium, has been used.

- Only one set-up of climate conditions have been tested for fruiting body production and hyphal growth.

- The climate chamber was only available for a limited amount of time (one month).

3. Materials and method

3.1. Literature study

The literature used in this study was found by searching for key terms in Web of Science and Google Scholar. The collected literature was used to compile information on cultivation as a reference for the cultivation efforts in the experimental parts of this study as well as for general information on *G. frondosa* and circular production.

3.2. Experimental work performed in the study

3.2.1. Microorganism

The fungal strain *Grifola frondosa* M9827 was obtained from Mycelia BVBA, Belgium, and used in all experiments. Long-term storage of the strain was carried out at room temperature on malt agar (MA). For production of inoculum intended for the hyphal growth tests, the strain was propagated on MA for one month at 21 °C. Circular slants (diameter 15 mm) from the MA plates were then used as fungal inoculum in the hyphal growth experiments (fig.2). For the experiment on fruiting body production fresh grain spawn of the strain was obtained from Mycelia BVBA, Belgium.

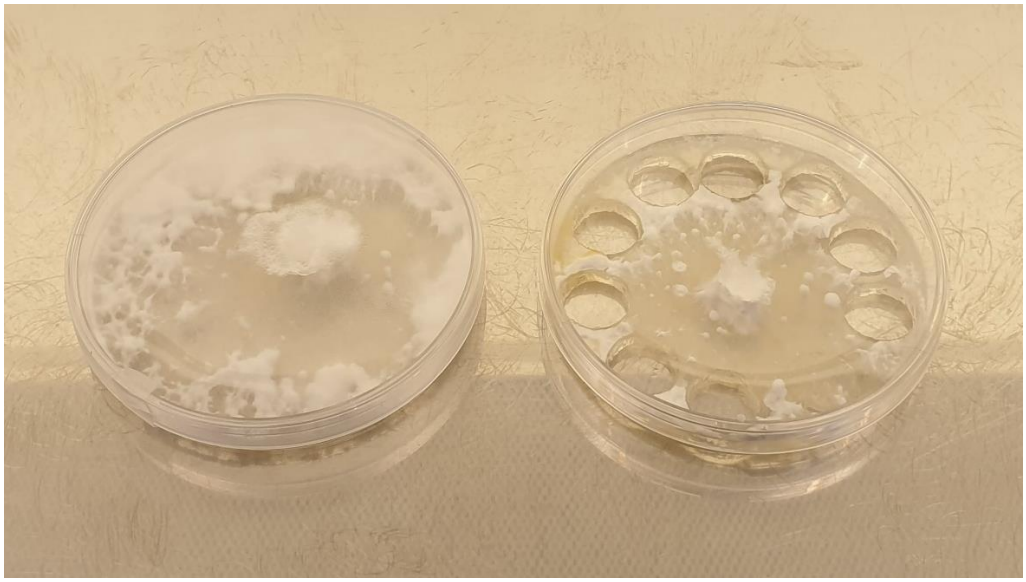

Figure 2: Left: Petri dish with G. frondosa mycelium growing on MA. Right: Petri dish with G. frondosa mycelium growing on MA where 15 mm circular slants have been removed and used for inoculation of substrates in the hyphal growth experiment.

3.2.2. Substrates

Fruiting body production and hyphal growth was studied on the substrates listed in table 1 below. For the hyphal growth experiment, all sawdust was tested both enriched (10% wheat bran, 10% rolled oats and 2% of calcium carbonate) and on its own without any additives, i.e. non-enriched.

Table 1: List of all substrates used in this study. Experiment: F=fruiting body production, H=hyphal growth. All sawdust substrates were of unspecified species.

Substrate	Type	Experiment
Beech (*Fagus sylvatica*)	Leaves	H
Oak (*Quercus robur*)	Leaves	H
Horse chestnut (*Aesculus hippocastanum*)	Leaves	H
Norway spruce (*Picea abies*, needles)	Leaves	H
Sycamore (*Acer pseudoplatanus*)	Leaves	H
Willow (*Salix alba*)	Leaves	H
Alder	Sawdust	F, H
Birch	Sawdust	F, H
Fir	Sawdust	F
Oak	Sawdust	F, H
Apple pomace	Waste	F
Betfor	Waste	F, H
Betfor+ deproteinized rapeseed cake* (50/50 dw)	Waste	F, H
Cardboard	Waste	H

Carrot peelings	Waste	H
Oat okara	Waste	F, H
Potato peelings	Waste	H
Spent coffee grounds	Waste	H
Hay	Plant biomass	H
Malt extract agar	Standard laboratory media	H

* This substrate is further described below

The pH of the substrates was determined after autoclavation. The remaining substrates after the start of the experiment (see section 3.3.1) were mixed with 100 ml of distilled water. The suspensions were stored at 4ºC and the pH was recorded after 48 hours.

Malt agar, a standard laboratory medium, was also inoculated with mycelia and used to serve as a comparison to the other substrates.

Fruiting body production was studied on four sawdust-based substrates (alder, birch, oak and fir of unspecified species). The hardwood sawdust was obtained from Lilla Rökeriet, Kristianstad (https://www.lillarokeriet.se) and the softwood sawdust from Setra Group (https://www.setragroup.com). The size of the sawdust was 2-4 mm for alder, birch and fir and 3-8 mm for oak. The sawdust was enriched according to a standard recipe with 10% of wheat bran, 10% rolled oats and 2% of calcium carbonate (dry weight dw/dw) (Stamets 2000). The sawdust-based substrates were rewetted with distilled water to reach a moisture content of 65% and the initial pH ranged between 7.2-7.5. The pH values were determined according to the standard EN13037.

Also, four substrates based on residues from food production were included in experiments on fruiting body production: betfor (sugar beet pulp), okara of oat, pomace of apple and deproteinized rapeseed cake. The betfor-based substrate was amended with 2% calcium carbonate to compensate for low pH and rewetted to a moisture content of 68%. The pH in this substrate was 6.6 when the experiment started. The substrate based on okara of oat had an initial pH of 6.9 and calcium carbonate was not added to this substrate. The moisture content was 62% when the experiment started. The pomace had an initial pH of 3.5 and 2% calcium carbonate was added. This resulted in a pH of 4.6 in the pomace-based substrate when the experiment started. The moisture content of this substrate was 75%. The deproteinized rapeseed cake had an initial high pH of 9.8 and a very dense structure. It was therefore mixed with betfor (50/50 dw/dw) to create a structure more conducive for gas exchange as this is needed for fungal growth. The pH was 6.9 at the start of the experiment and moisture content was 73% in the mixed substrate.

3.3. Experimental set-up

3.3.1. Hyphal growth

The substrates were autoclaved and then added to sterile petri dishes and spread out in order to cover the entire surface as evenly as possible. An agar slant (diameter 15 mm) of *G. frondosa* mycelium was carefully placed in the middle of the dish (fig. 3). Three replicates were created for each substrate, resulting in 63 in total (fig. 4). The inoculated petri dishes were incubated at 21 °C for 14 days and then photographed and analysed to calculate hyphal surface coverage in percentage.

Figure 4: Petri dish with leaves of Acer pseudo-platanus inoculated with an agar slant of G. frondosa mycelium.

Figure 3: The 63 petri dishes marked and ready for substrates and inoculation.

3.3.2. Fruiting body production

All substrates were autoclaved and when the substrate had cooled down, spawn of *G. frondosa* was added in a concentration of 10% (dw/dw). Inoculated substrate was packed in boxes suitable for mushroom production (Sac O2, Nevele, Belgium) and a total weight of 0.6 kg of substrate (wet weight) was packed in each box. The closed boxes were incubated at 21ºC for 15 days and then at 19ºC for 35 days. After this the substrates were densely colonized by the mycelium and the boxes were moved to a climate chamber to induce fructification. The boxes were spread out and placed in such a way that replicates of the same substrates were not placed next to each other, in order to ensure results were not affected by possible microclimates present.

Based on the results obtained in the literature study, the conditions used in the climate chamber should have been 90% RH, 17°C and 200 lux (~3-4 µmol/m^2/s) in a 12h cycle. However, the limitations of the chamber were such that the light could not go low enough and was instead set at 50 µmol/m2/s. This was lowered to 7-11

µmol/m2/s with cloth coverings. CO_2 concentration was monitored and not allowed to rise above 600 ppm, as Chi et al. (2009) recommends levels of between 500 to 800 ppm which they found increased maitake fruiting body initiation speed and resulted in higher yields. Levels of 1000 and above resulted in misshapen and deformed fruiting bodies of lower quality (Chi et al. 2009).

Figure 5: The screen showing the real-time conditions inside the climate chamber.

3.4. Analysis

3.4.1. Fungal growth

Hyphal surface coverage was analysed in percentages. This was done by digitally painting the entire substrate area in white and the entire hyphal-covered area in black (fig. 6). The amount of pixels in the black area was divided by the amount of pixels in the white area and the sum of that was then multiplied by 100. Formula as follows:

$$Hyphal\ coverage, \% = \frac{Pixel\ amount\ of\ hyphal\ area}{Pixel\ amount\ of\ substrate\ area} x\ 100$$

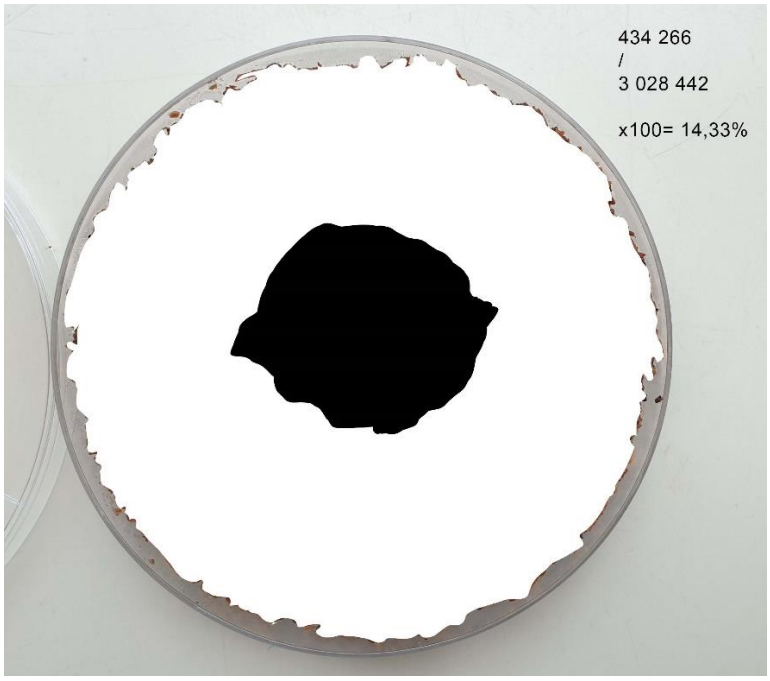

Figure 6: Picture of a petri dish edited for analysis of surface coverage percentage.

In the experiments focusing on fruiting body production the amount of mushrooms (fresh and dry weight) produced in the first flush was determined. The dry weight was recorded after lyophilization. Mushroom production (fresh weight) was related to the amount of substrate (dry weight), in order to determine the biological efficiency (BE) of the substrate, according to the formula:

$$BE = \frac{Fresh\ weight\ of\ mushrooms\ in\ grams}{Dry\ weight\ of\ substrate\ in\ grams}\ x\ 100$$

Total protein content in the fruiting bodies was analysed by the Dumas method (Bellomonte et al. 1987), using a Thermo Scientific™ FLASH 2000 CHNS/O Analyzer and a conversion factor of 4.38 for total nitrogen (Barros et al. 2008).

3.4.2. Impact of fungal growth on protein content in selected substrate

For this analysis, the substrates based on residues from food production, before and after mushroom harvest, was dried at 65 ºC for 48 hours. The dried biomass was milled and total nitrogen (TN) was determined using a Thermo Scientific™ FLASH 2000 CHNS/O Analyzer. A factor of TN x 6.25 was applied to determine crude protein content.

3.5. Statistics

All experiments were set up with three replicates in each treatment. Statistical analyses were carried out using Excel and data were tested for significant differences ($p<0.05$) using T-test.

4. Results

4.1. Literature study

Ways of cultivating *G. frondosa* varies greatly, both in regard to substrates used and growth parameters such as humidity and temperature. *Grifola frondosa* is cultivated on many different types of substrate mixes, but from the studied literature, some ingredients in those stand out as more prevalent than others. The most common is to use some type of sawdust as a base, with wheat bran as an additive. The sawdust types that were the most prevalent were oak (*Quercus* sp.) and beech (*Fagus* sp.).

Waste products from food production was included in several studies in varying amounts. Waste products used in the studied literature include rice straw, soy okara, spent coffee grounds, olive press cake and more (Song et al. 2018; Tanaka et al. 2019; Montoya et al. 2012; Gregori et al. 2009). One study substituted sawdust entirely with corn cob (Song et al. 2018). For a full list of used substrates, see table 2. The growing conditions used are compiled in Table 3.

4.1.1. Substrates

By far, the most common substrate was oak sawdust. Common supplements for enriching the substrate were bran of either wheat, corn or rice. Waste products included in the studies were okara, straw of soybean, rice and corn as well as cottonseed skins, corn cob, spent coffee grounds and olive press cake. Song et al. (2018) found a significant positive correlation between increased yield and addition of corn cob (up to 100% of substrate) and concluded that corncobs could potentially completely replace sawdust as the main substrate. Montoya et al. (2012) supplemented oak sawdust with spent coffee grounds and found that it supported mycelial growth well but did not lead to production of fruiting bodies. Gregori et al. (2009) found that addition of olive press cake decreased yields while addition of crushed corn seeds increased yields.

Table 2: Substrates used in studied representative literature.

Source	Substrates
Tanaka et al. 2019	Oak sawdust, dried okara, hominy feed
Song et al. 2018	Oak sawdust, corn cob, corn straw, soybean straw, rice straw, wheat bran
Li et al. 2017	Cottonseed skins, unspecified sawdust, unspecified bran, red mud, brown sugar
Sato et al. 2017	Birch sawdust, 70/30% birch/larch sawdust, wheat bran
Tabata et al. 2004	Oak sawdust, rice bran
Kirchhoff 1996	Beech sawdust, wheat bran, corn meal
Shen & Royse 2002a	Oak sawdust, white millet, wheat bran, rye and corn meal
Mayuzumi & Mizuno 1997	Unspecified broadleaf wood sawdust, rice bran, wheat bran
Montoya et al. 2012	Oak sawdust, spent coffee grounds, corn bran
Hu et al. 2004	Sawdust and wood meal of oak (*Q. serrata, Q. glauca, Q. acutissima*), Japanese maple, *Castanopsis cuspidata*, Douglas fir, Japanese black pine, Japanese cypress, *Carpinus tschonoskii*, Japanese cedar, Empress tree, rice bran
Gregori et al. 2009	Beech sawdust, crushed corn seed, olive press cake
Stamets 1993	Sawdust of oak, poplar, cottonwood, elm, willow or alder, corn waste, rice bran
Shen & Royse 2002b	Oak sawdust
Shimoda et al. 2012	Beech sawdust, corn bran

4.1.2. Growing conditions

Ways of presenting cultivation parameters varied and occasionally parameters were left out. What was found in the representative literature can be seen in table 3. RH at fruiting was consistently presented, with an average of 86,6%. Temperatures were similar in range across the board, with an average of 23°C at spawn run and 17°C at fruiting. Average substrate wet weight was 2111 g, with some outliers such as the study that was only conducted on petri dishes (25 g). Many different strains were tested. Cultivation time for spawn run and fruiting varied greatly. Average spawn run, fruiting and total time was 62, 19 and 78 days respectively.

Table 3: Complied cultivation parameters from a representative selection of studied sources. N/D: not determined.

Source	RH %	Temp. °C	Added light	Moisture, %	Wet weight, g	Strain	Days
Tanaka et al. 2019	-	Spawn: 23 Fruiting: 18 ±1	No	63	2400	MA52	Spawn: 35 Fruiting: 12
Song et al. 2018	Spawn: - Fruiting: 93-97	Spawn: 23–26 Fruiting: 19–21	Yes (200 lux)	62.5	800	H21	Total: 63-100
Li et al. 2017	Spawn: 80 Fruiting: 70-80	Spawn: 24 Fruiting: 18-23	Yes (N/D)	-	-	Qing gray 151	Total: ~90
Sato et al. 2017	Spawn: 70±5 Fruiting: 90±5	Spawn: 22 ±1 Fruiting: 18±1	Yes (350 lux)	65	-	Gf433 & Mori52	Spawn: 52 Fruiting: - Total: 66.1±1.2–69±1.1
Tabata et al. 2004	Spawn: - Fruiting: 75-80	Spawn: 23 Fruiting: 15	No	70	1000 (DW)	-	-
Kirchhoff 1996	Spawn: 80 Fruiting: 90-95	Spawn: 25 Fruiting: 16	No	63	2000	Gf1 & Gf2	Spawn: 77-84 Fruiting: -
Shen & Royse 2002a	Spawn: - Fruiting: 90-95	Spawn: 20±1 Fruiting: 17±2	Yes (N/D)	55-58	2650	WC828	Total: 63-105
Mayuzumi & Mizuno 1997	Spawn: 70 Fruiting: 85	Spawn: 23 Fruiting: 18	Yes (N/D)	65	2500	-	Spawn: 70 Fruiting: 15
Montoya et al. 2012	Spawn: 60 Fruiting: 70-80	Spawn: 20±1 Fruiting: 16-18	Yes (50–100 lux)	58	1000	PSUMC-C 922	Spawn: 70-75 Fruiting: 10-15 Total: 81-96
Hu et al. 2004	Spawn: 60 Fruiting: No	Spawn: 25 Fruiting: No	No	65	25	G-125	-
Gregori et al. 2009	Spawn: - Fruiting: 85-90	Spawn: 24±1 Fruiting: 17±2	Yes (N/D)	65	3000	GF3	-
Stamets 1993	Spawn: 95-100 Fruiting: 85-90	Spawn: 21-24 Fruiting: 13-18	Yes (500-1000 lux)	-	2270-3175	-	Spawn: 52-60 Fruiting: 30-45 Total:~89
Shen & Royse 2002b	Spawn: - Fruiting: 90-95	Spawn: 20±1 Fruiting:17±2	Yes (N/D)	55-58	2650	Multiple	Total: 56-84
Shimoda et al. 2012	Spawn: - Fruiting: >90	Spawn: 25 Fruiting: 16		65	2500	M51	Spawn: 70 Fruiting: -
Averages:	**Spawn: 74 Fruiting: 86.6**	**Spawn: 23 Fruiting: 17**	**Varies**	**63**	**2111**	**Varies**	**Spawn: 62 Fruiting: 19 Total: 78**

4.1.3. Circular production

The SMS remaining after mushroom production is composed of mycelium and partly degraded plant material and may have several applications. With certain substrates, considerable protein levels can be expected in the SMS arising partly from the substrates and partly from the mycelium colonizing the substrate. Therefore, its use as a feed ingredient could be of interest and may lead to decreased competition between human food and animal feed and lower dependence on imported protein feeds (Grimm & Wösten 2018).

Furthermore, lignin is undesirable in animal feed as it is not fully digested by the gut bacteria. This means that all the energy in lignin is not accessible to the animal and that it reduces the amount of useful feed intake (Frei 2013). Usually, pre-cultured mushroom substrate for white-rot fungi is heavy in lignin due to the high percentages of sawdust it contains. Even in cases where sawdust is substituted with some other substance, lignin may still be a big part of it. This is for instance the case if substituting with corn cob or straw, which, although lower that sawdust, also contains lignin in large quantities (Song et al. 2018). The feed suitability of the lignin-heavy parts of mushroom substrate such as waste products of food production may be greatly increased if culturing mushroom on it prior to feeding, as white-rot mushrooms such as *G. frondosa* are known to be efficient in degrading lignin (Montoya et al. 2012).

Also for sawdust-based substrates, which are not suitable as feed, the lignin degradation performed by white-rot fungi is of interest as the potential for extraction of ethanol and thereby use of the SMS for production of biofuel may improve (Chen et al. 2020). Other potential uses for SMS are as filter products (Liu et al. 2017) or simply composting and reusing it for soil improvement due to high content of organic matter and nutrients.

4.2. Experimental study

4.2.1. Hyphal growth

The hyphal growth was assessed in percentages after 14 days of incubation at 21ºC. Due to varying thickness of mycelial mat, results have been further categorised as sparse, moderate or dense. The pH of hyphal growth experiment substrates (excluding malt agar) and complete list of substrates as well as the percentual hyphal surface coverage can be seen in table 4 below.

Table 4: Hyphal growth of G. frondosa on different substrates. The pH of the substrates after autoclaving, at the start of the experiment, is presented (n=1). Hyphal coverage is presented as mean ± standard deviation (n=3). The thickness of mycelium was evaluated visually (see fig. 7).

Substrate	pH	Hyphal coverage, %	Thickness of mycelium
Alder sawdust	4.46	63.4±2.7	Sparse
Alder sawdust E	7.03	14.4±1.7	Moderate
Beech leaves	4.56	46.0±6.3	Moderate
Betfor	3.9	14.3±1.4	Dense
Birch sawdust	4.49	74.3±2.6	Sparse
Birch sawdust E	6.73	42.9±2.3	Moderate
Cardboard	7.3	22.0±9.2	Sparse
Carrot peelings	5.5	23.3±2.2	Dense
Hay	5.28	66.2±9.7	Moderate
Horse chestnut leaves	5.69	73.0±9.3	Sparse
Malt agar	N/A	68.0±3.1	Sparse
Norway spruce needles	4.51	No growth	N/A
Oak leaves	4.15	59.6±2.4	Sparse
Oak sawdust	3.67	9.0±0.9	Moderate
Oak sawdust E	6.46	4.5±0.1	Sparse
Oat okara	6.43	No growth	N/A
Potato peelings	5.42	21.2±0.7	Dense
Rapeseed+betfor	5.36	11.0±6.1	Dense
Spent coffee grounds	4.66	44.0±2.2	Sparse
Sycamore leaves	4.15	38.1±0.4	Moderate
Willow leaves	5.44	31.9±1.9	Dense

Surface coverage of individual replicates varied greatly, from 4 to 83%. The majority of the substrates in the Sparse category covered a rather large area, with most covering between 40-80%. In contrast, the majority of the substrates in the Dense category covered a rather small area, with most of them covering between 10-30%. No hyphal growth was present on any of the replicates of oat okara and Norway spruce needles.

There were some anomalies. Replicate 2 of potato peelings had some type of unidentified infection (likely yeast) that greatly limited hyphal growth and was therefore not included further in the study, such as in the percentage calculations. Replicate 1 and 3 of enriched oak woodchips both had very sparse hyphal growth that was concentrated to the agar slant and a few millimetres surrounding it. Replicate 2 of enriched oak woodchips had no hyphal growth and the mycelium appeared entirely dead. Replicate 1 of betfor/rapeseed had limited hyphal growth similar to that of replicate 1 and 3 of enriched oak woodchips. In spite of this, no sign of infection could be seen in either of these four replicates but given the results it is possible there was infection present.

On several substrates in the sparse category, the mycelium was so sparse that it was barely visible and is therefore rather difficult to see in pictures. Two substrates from each category are presented in fig. 7. Pictures of all hyphal growth results can be seen in appendix 2.

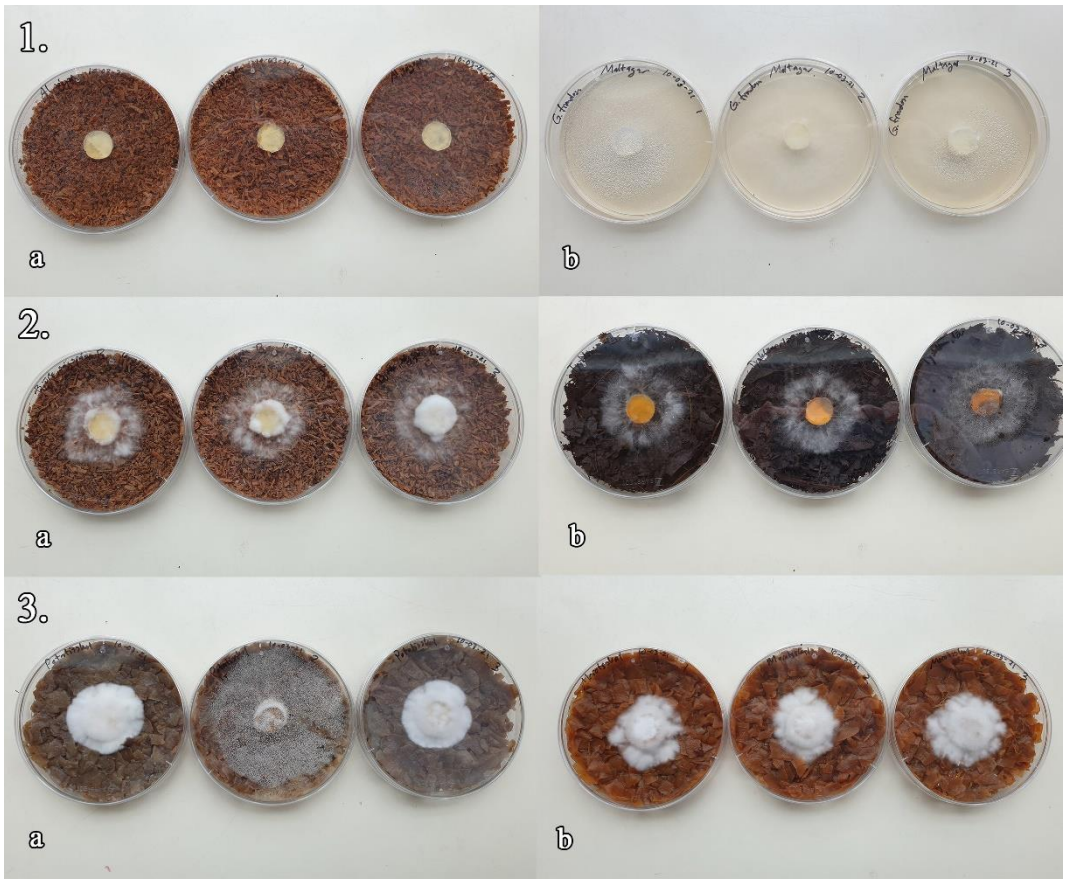

Figure 7: Representative pictures of results of hyphal growth experiment. 1: Sparse category. 1.a: Alder sawdust. 1.b: Malt agar. 2: Moderate category. 2.a: Birch sawdust E. 2.b: Sycamore leaves. 3: Dense category. 3.a: Potato peelings. 3.b: Carrot peelings.

4.2.2. Fruiting body production

After 15 days of incubation at 21ºC, the boxes were moved to a chamber keeping 19ºC. Prior to moving, they were photographed to track progress (fig.8).

Figure 8: Boxes after 15 days incubation at 21ºC. a: Fir. b: Birch. c: Oat okara. d: Oak. e: Betfor. f: Apple pomace. g: Rapeseed+betfor. h: Alder. Boxes were relabelled after the pictures were taken. The replicates are stacked as 2,3,1 starting from the top.

Results were varied. The ones that fared the best at this point were oak (fig. 8d) and the rapeseed+betfor mixture (fig. 8g). Fir (fig. 8a) showed promising growth. Alder (fig. 8h), apple pomace (fig. 8f) and oat okara (fig. 8c) had the least amount of hyphal spread. Alder had the least of all with only a handful of small hyphal spots. These boxes with alder were opened and an unpleasant smell suggesting bacterial or yeast infections was noted in all boxes and the alder treatment was therefore was discarded. Birch and betfor had an intermediate amount of hyphal spread.

After 35 days incubation at 19ºC, the boxes were moved to a different climate chamber to initiate fruiting body formation (fig. 9).

Figure 9: Boxes in climate chamber, with fibre cloth above to reduce light.

Before they were moved they were photographed once again to track progress (fig. 10). Oat okara had almost no hyphal growth with only a few small spots present at the surface. A few mould infections were detected. Apple pomace replicate 1 was heavily infected and was discarded. Betfor replicate 2 had a small infection at the bottom of the box. It was left as it was since the infection was closed in within the substrate. Betfor/rapeseed replicate 3 had a small infection at the top that was cut out.

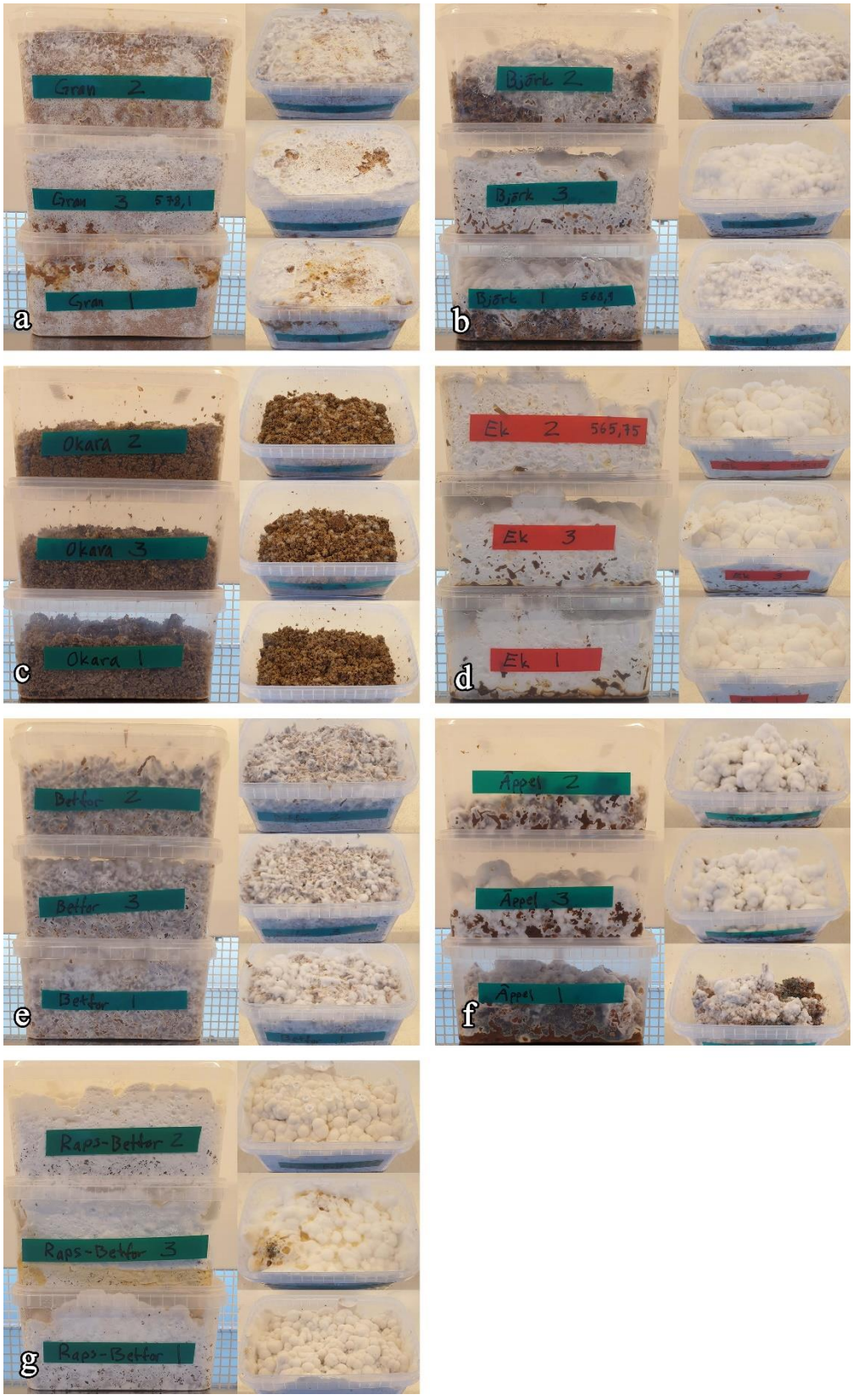

Figure 10: Boxes before relocation to climate chamber. Boxes are stacked in order rep. 2, 3, 1, just as in fig. 8 for easy comparison. a: Fir. b: Birch. c: Oat okara. d: Oak. e: Betfor. f: Apple pomace. g: Rapeseed+betfor.

Fruiting bodies were first observed on day 57. The first fruiting body was on replicate 3 of fir. On day 65, fruiting bodies (primordia) were seen on the two other replicates of fir as well (fig. 11). At this point, mould was present on the substrate of most boxes.

Figure 11: Small fruiting bodies on replicate 1 and 2 of fir.

On day 72, all boxes except for the fir ones were discarded due to large amounts of mould. No signs of fruiting bodies could be seen in any of these boxes, with the exception of rapeseed+betfor replicate 1 that had primordia at the edge of the box (fig. 12). Severity of infection was greatly varied, with severe infection in rapeseed+betfor and almost no infection in any of the fir or betfor boxes.

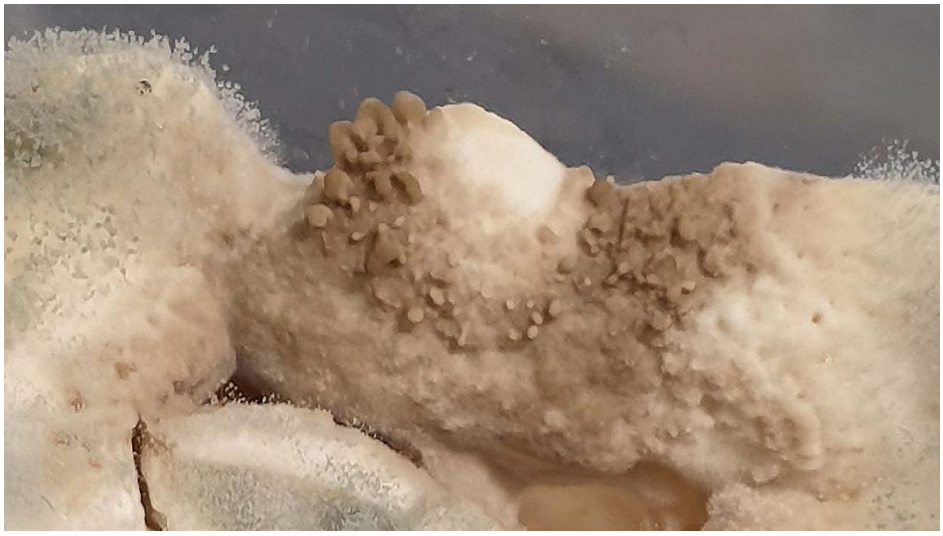

Figure 12: Primordia on replicate 1 of rapeseed+betfor. Mould can be seen surrounding it.

On day 80, all fruiting bodies were harvested and weighed (fig. 13). The fruiting body in rep. 3 was infected with mould at this point. Fresh weight of the fruiting bodies was 42.8±8.3g. The BE of this treatment was 20.3±3.9%. Dry weight of the fruiting bodies was 12.4±1.1% of fresh weight. Total protein content in the fruiting bodies (% of DW) obtained in this treatment was 26.1±1.8%.

Figure 13: Fruiting bodies on replicate 1 of fir at harvest.

Spawn run time was 50 days and fruiting time was 30 days, adding up to a total cultivation time of 80 days.

4.2.3. Spent mushroom substrate

Protein levels increased in all substrates after growth of *G. frondosa*. All results obtained were statistically significant ($p<0.05$).

Table 5: Protein levels (% of DW) in substrate before (fresh substrate) and after (used substrate) cultivation of G. frondosa.

Substrate	Fresh substrate	Used substrate	T-test ($p<0.05$)
Apple pomace	2.3±0.1	4.7±0.1	0.00
Betfor	8.4±0.1	12.7±1.7	0.02
Oat okara	50.3±4.2	No growth	N/A
Rapeseed+betfor	12.9±1.0	16.0±0.7	0.04

5. Discussion

5.1. Literature study

It's quite common, in the studied literature, that some cultivation parameters are left out. It may seem like they are leaving out something important, but it's likely because these parameters not considered important by the authors. It could be due to the production itself not being the focus of that particular study, that the authors themselves were not responsible for the production (purchased mushroom/substrate), that the parameters are assumed to be obvious since it's seen as common knowledge or that they do in fact not affect the production in any meaningful way.

Examples of often excluded parameters include light and humidity during spawn run. Light is most likely left out since addition of extra light has little effect on the fruiting body production, with other ambient light usually being enough although fruiting bodies of some species may develop undesirable characteristics such as reduced growth and altered colour (Kuforiji & Fasidi 2009; Kim et al. 2020). Mushrooms have no chloroplasts, which means they do not photosynthesize. It would however have been interesting to know about the light conditions in these studies and how much light is already in the room. Are there windows in the room? Are there lamps, and if so, how strong are they? When are they turned on, is it only when there are people working there, for how many hours is that? Although a specific light level is not always strictly needed for fruiting body development, light is useful at the right stage. Light tells the mycelium that is has reached the surface and that it is time to reproduce (Evert et al. 2013). The same goes for CO_2, which, at low levels, also acts as a signal to initiate primordia formation for the same reason (Evert et al. 2013). This is further corroborated by Chi et al. (2009) who examined *G. frondosa*'s response to varying concentrations of CO_2 and found that lower levels of below 800 ppm were preferable for fruiting body production. When humidity levels up until fruiting body initiation are not mentioned, it is likely due to the substrate containers being sealed at that point. That means no moisture can escape, thus making RH irrelevant.

The great variation in results is partly due to the use of different strains, as can be seen in table 3 (p. 27). Different strains have different qualities and limitations. Some, like Gf433, is able to produce fruiting bodies on sawdust mixes containing woodchips derived from larch despite their presence of the mycelium inhibiting compound taxifolin (Sato et al. 2017). Stamets (1993) also comments on this curiosity, mentioning that maitake has been found growing on larch as well as Douglas fir; an evergreen that also contains taxifolin. Some strains are more vigorous than others, creating bigger fruiting bodies and therefore a higher yield and BE. The strain best suited for one's production needs to be decided on the available controllable parameters such as substrate used and size of substrate containers as well as preferences for fruiting body size and appearance.

Oak sawdust appears to be the favoured choice when picking a main substrate for *G. frondosa* cultivation. Maitake does occur naturally on oak and many studies show good results with oak as a substrate, so it makes sense. However, compared to other more popular mushrooms, there's relatively little information available and few studies done on substrates suitable for cultivation of *G. frondosa* so it may in part be due to tradition.

Although the discussed type of indoor production certainly is possible, there are sources suggesting it may not be the best one for *Grifola frondosa*. Stamets (1993) recommends the buried log method over indoor production. Tabata et al. (2004) examined the content differences between maitake cultivated on sawdust and maitake cultivated on logs and found that the mushrooms grown on logs were higher in sweet tasting amino acids like alanine, while at the same time being lower in bitter tasting ones like tyrosine. From this they concluded that log-grown maitake is likely to have a better and more natural taste than sawdust-grown ones, but also that taste tests will be needed to determine whether the difference really is noticeable outside of lab tests (Tabata et al. 2004). Other reasons for going with log over bag culture may be that it can save on resources. Using log culture means that costs such as air-conditioned rooms, temperature control, lighting, and to some extent watering, can be avoided.

5.2. Cultural aspects

It may seem strange to advocate for increased mushroom production in a country like Sweden where wild mushroom picking is, for many, a cherished pastime and where mushrooms spots are carefully guarded as well-kept secrets. However, this cannot be the case of maitake. Since it is red listed (SLU Artdatabanken 2020), meaning you should not pick them, there are no maitake foraging spots to guard. In the case of wood-living mushrooms such as this, it's especially crucial to leave fruiting bodies alone. The mycelium of soil-living ones is long-lived, so those are not too affected by the removal of fruiting bodies. Wood-living ones, on the other

hand, have a more short-lived mycelium due to their nature of breaking down their substrate which means they have a greater need to reach maturity and release spores in order to spread (Nordiska ministerrådet 2012b). Sale of maitake in the Nordic countries is only allowed if it originates from cultivation or from countries where it is not red listed (Nordiska ministerrådet 2012a). This opens the door for cultivation and production of maitake and gives people the opportunity to sample this gourmet fungi despite its rarity in local nature. For various reasons, such as physical disabilities or long distances to forests, there are of course some people who are not able to forage for mushrooms themselves. Therefore, the production and sale of mushrooms is still important in order to make mushrooms available to everyone.

Interest in mushrooms and fungi as culinary ingredients is growing and there is room for introduction of additional species. Currently in Sweden, white button mushrooms are produced most widely, are commonly available in supermarkets and likely used most frequently. Oyster mushrooms are somewhat common. Chantarelles are also seasonally commonly available in supermarkets. Although prices fluctuate over the season, chantarelles generally remain extremely pricy compared to others. As of writing, prices of white button in local supermarkets range from 20 SEK/kg to 149,75 SEK/kg (see fig. 14), which is cheap in comparison to chantarelles. As of writing, chantarelles are not in season and can therefore not be found in most stores. Online, however, prices range from 177 SEK/kg to 274 SEK/kg for either conserved or frozen chantarelles (Coop.se 2021; Mat.se 2021).

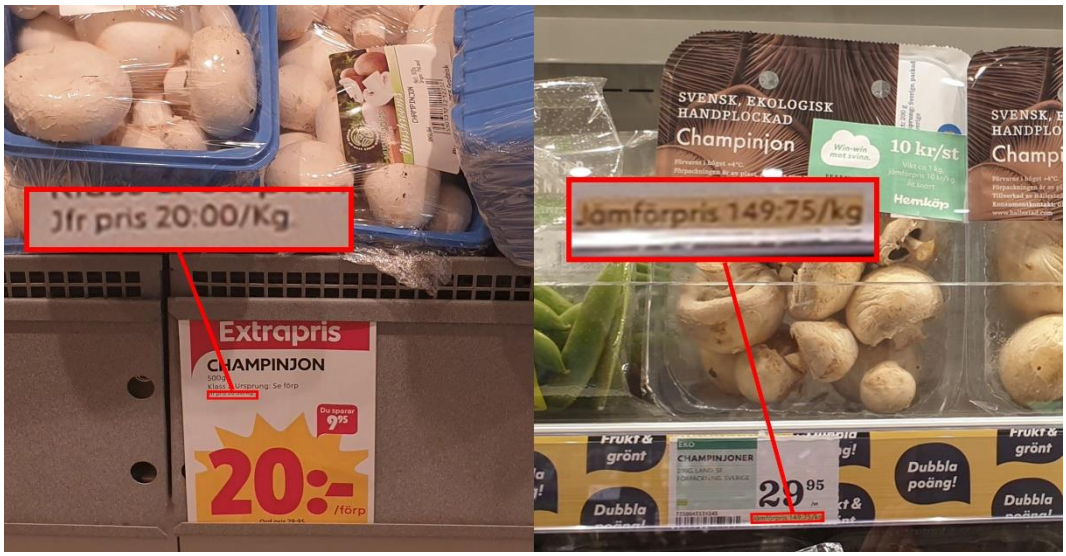

Figure 14: Prices of white button mushrooms in two different local supermarkets. The cheaper ones (left) are on sale and the pricier ones (right) are organic.

There is no Swedish precedent for what the price of fresh maitake mushrooms could be. However, dried and powdered maitake is available at prices of 150-200

SEK/kg (DCG 2021; Rawfoodshop 2021). One could perhaps speculate that the price of fresh maitake mushrooms might be similar to that of other speciality mushrooms, such as enoki (*Flammulina velutipes*) and king oyster (*Pleurotus eryngii*) and therefore range from about 250-350 SEK/kg (Sörboden 2021; Lokalt från Sjuhärad 2020). Prices may reach even higher given the rarity of maitake here and if one can produce them organically.

Different countries have different relationships to mushroom cultivation and consumption. In some parts of the world, such as in China and Japan, it's more common to cultivate mushrooms at home for personal use and at-home cultivation kits are easy to find (Moore & Chiu 2001; Cui et al. 2020). There's no reason as to why this couldn't be the case in Sweden and spreading knowledge about mushroom production is one way to make it more accessible and likely to happen.

5.3. Experimental work

5.3.1. Substrates tested

The substrates tested in this study were chosen by availability and ease of purchase. The potato and carrot peelings were collected personally from normal household use, the hay was purchased at a local pet store and the leaves were collected from the ground when the opportunity presented itself such as while on walks in the local area. There are likely many other substrates that would be possible to use as G. frondosa substrate and would be interesting to try. From the results of this study, the most promising ones seem to be fir sawdust and waste products of food production such as the potato and carrot peelings as well as the betfor and rapeseed press cake combination. Although the fir substrate of course isn't suitable for animal feed it can be part of a circular production system focusing on biofuel production (Chen et al. 2020). Potato peelings or similar substrates might however be well suited in a circular productions system focusing on feed production and should be further evaluated, including tests for fruiting body production. The possibilities are vast and with more time and resources other suitable substrates would undoubtedly be found.

5.3.2. Hyphal growth

Most of the substrates in the Sparse category (tab. 4) covered a large area of the petri dish. This is likely due to *G. frondosa* not favouring the substrate and spreading out in search of something better. The opposite could be true for the substrates in the Dense category, where most covered a comparably small area. This is likely due to *G. frondosa* favouring these substrates and having what it needs for

vigorous growth nearby, thus spreading slowly but growing densely. The reason for the enriched oak substrate being such a poor host for the mycelium is unknown. There was almost no spread on either of the three replicates and the substrate appeared wetter than others. The fact that it seemed wetter might indicate an infection was present, but it could not be seen at the time. The same amount of enriching substances was added to all sawdust-based substrates so an imbalance of that should not have been the issue.

I initially suspected that the Norway spruce needles might be too acidic and that they wouldn't support growth for that reason. However, the pH (4.51) turned out to be close to that of beech leaves (pH 4.56) which supported growth just fine, so it appears that the pH was not the reason for the absent growth on the needles. Perhaps taxifolin or another fungal suppressive (Angelis et al. 2016; Hammerbacher et al. 2019) exists in higher concentrations in the needles than in the wood, making growth possible on the fir substrate but not on the fir needles.

Potato peelings were the most densely populated of all substrates with a thick mycelium mat spreading evenly across the surface. Carrot peelings were also densely covered, along with betfor and betfor/rapeseed. This suggests that other residues of food production could potentially be successful mushroom substrates for *G. frondosa* production. Further experiments would need to be conducted to figure out which are the most suitable. The success of these substrates even without the addition of enriching substances is encouraging. The cost of these enrichments is therefor cut and the amount of input to the production is reduced, furthering the impact and value of the circular production concept.

However, it should be pointed out that amount and density of hyphal growth isn’t necessarily a reliable indicator of how suitable the substrate would be for fruiting body production. There simply doesn’t seem to be a strong correlation between the hyphal growth and fruiting body production. In several studies, experiments were started with different substrates at the same time with the ultimate goal of harvesting fruiting bodies. At the end of the experiments, some substrates were densely populated but never produced any fruiting bodies while several others had produced fruiting bodies (Kirchhoff 1996; Shen & Royse 2002a; Shen & Royse 2002b). This indicates that although potato peelings appeared to be a suitable host in the hyphal growth experiment and facilitated impressive growth, there is no guarantee that it will support a satisfactory fruiting body production.

5.3.3. Fruiting body production

The first fruiting body to emerge was on the fir substrate and more specifically, an area at the top of the substrate where some of the dense mycelium mat had been removed due to being stuck to the lid. It’s possible this removal induced the fruiting. Some growers suggest that scratching the surface (called the kinkaki treatment) is

beneficial to induction of fruiting bodies (Kim et al. 2007; Tabata & Ogura 2003; Kinugawa 1993; Lin 2004), although it is not explained why this is beneficial. The success of the fir substrate is interesting considering the presence of fungal inhibitors such as taxifolin (Angelis et al. 2016; Hammerbacher et al. 2019). All boxes expect for the ones with fir substrate were discarded after 62 days due to abundant mould growth. The fir boxes barely had any visible mould on them. It's possible the fungal inhibitors present in fir kept the mould from establishing while at the same time allowing the *G. frondosa* mycelium to establish. I find this discrepancy curious, and it would be interesting to study this further. It's possible it's a specific characteristic of this strain, as with the Gf433 strain mentioned earlier that can handle growing on substrates with taxifolin present.

Fir is abundant in Sweden and forestry involving production and processing of fir for things such as building materials is common. Sawdust is commonly used as a mushroom substrate, however, sawdust of softwoods such as fir is avoided (Stamets 1993). Companies such as Setra, that provided the softwood sawdust used in this project, produces sawdust waste when processing logs into products such as planks and that sawdust could be used for *G. frondosa* production. Acquiring this sawdust waste and using it as substrate while reusing the SMS for biofuel production as described in Chen et al. (2020) would further contribute to the sustainability of mushroom production.

It should also be mentioned that the harvested maitake was compared to white button, shiitake and oyster mushrooms in a simple taste test performed by the author. All mushrooms were first sweated in a pan and then lightly fried in butter. In terms of texture, the maitake was most similar to oyster mushrooms but with even less of the typically spongy mushroom bite and more of the meaty grain type texture like that of cooked chicken and some soy-based meat substitutes, with the textures pleasantly balanced. Even completely unseasoned, the maitake was very flavourful compared to all others tested. The flavour was reminiscent of roasted hazelnuts and barbeque seasoned grilled crispy chicken skin. The flavour was nicely enhanced when pan fired until a nicely seared surface texture. In short, it was very tasty.

5.3.4. Spent mushroom substrate

Changes in the protein levels of the substrates before and after mycelium growth were all statistically significant ($p<0.05$). The increase in protein may have been even greater if the substrates would have produced fruiting bodies. Even so, this does point to substrates indeed being made more suitable as animal feed after cultivation of *G. frondosa*. However, there are other factors to consider such as the levels of chitin present, a compound that is difficult to break down for some animals (Tabata et al. 2018). Regardless, these results are encouraging.

5.4. Conclusions

It is possible to produce *Grifola frondosa* fruiting bodies on a locally available Swedish substrate. In this study it was confirmed to be possible on an enriched fir sawdust media, with fruiting bodies being harvested from all three replicates. It is likely possible on a 50/50 dw combination of rapeseed press cake and betfor as well given the presence of primordia observed. When it comes to hyphal experiments, potato peelings had the densest hyphal growth with carrot peelings coming in at a close second. Agricultural and food production waste products seem like a promising source of substrates. Further studies could investigate additional waste products and test them in larger volumes to attempt fruiting body production.

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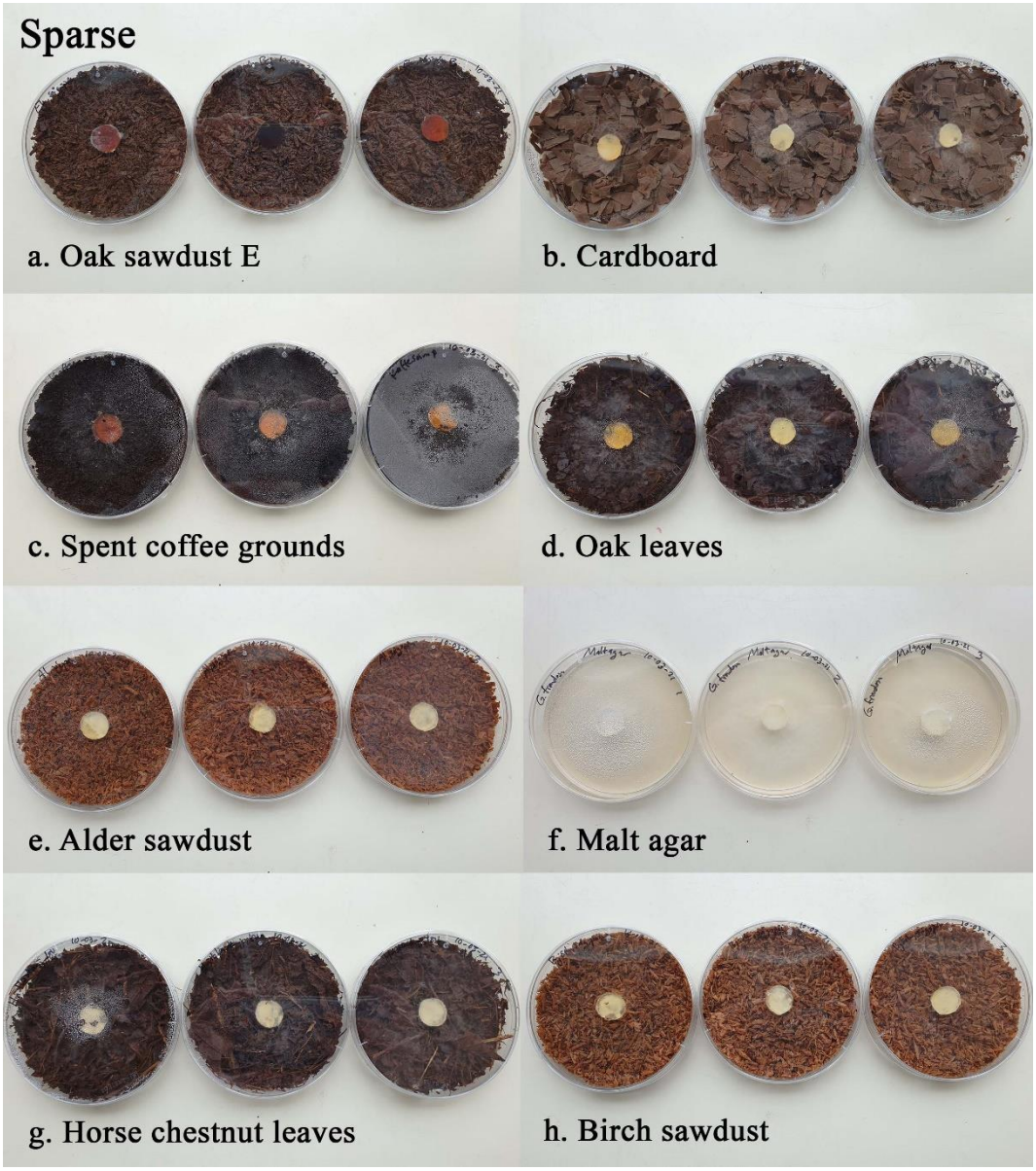
Finally, a thank you to my mother, Maria, and my grandparents, Birgitta and Tore, who kept me going with talk of a (socially distanced, partly vaccinated and corona safe!) celebration when I finished this project.

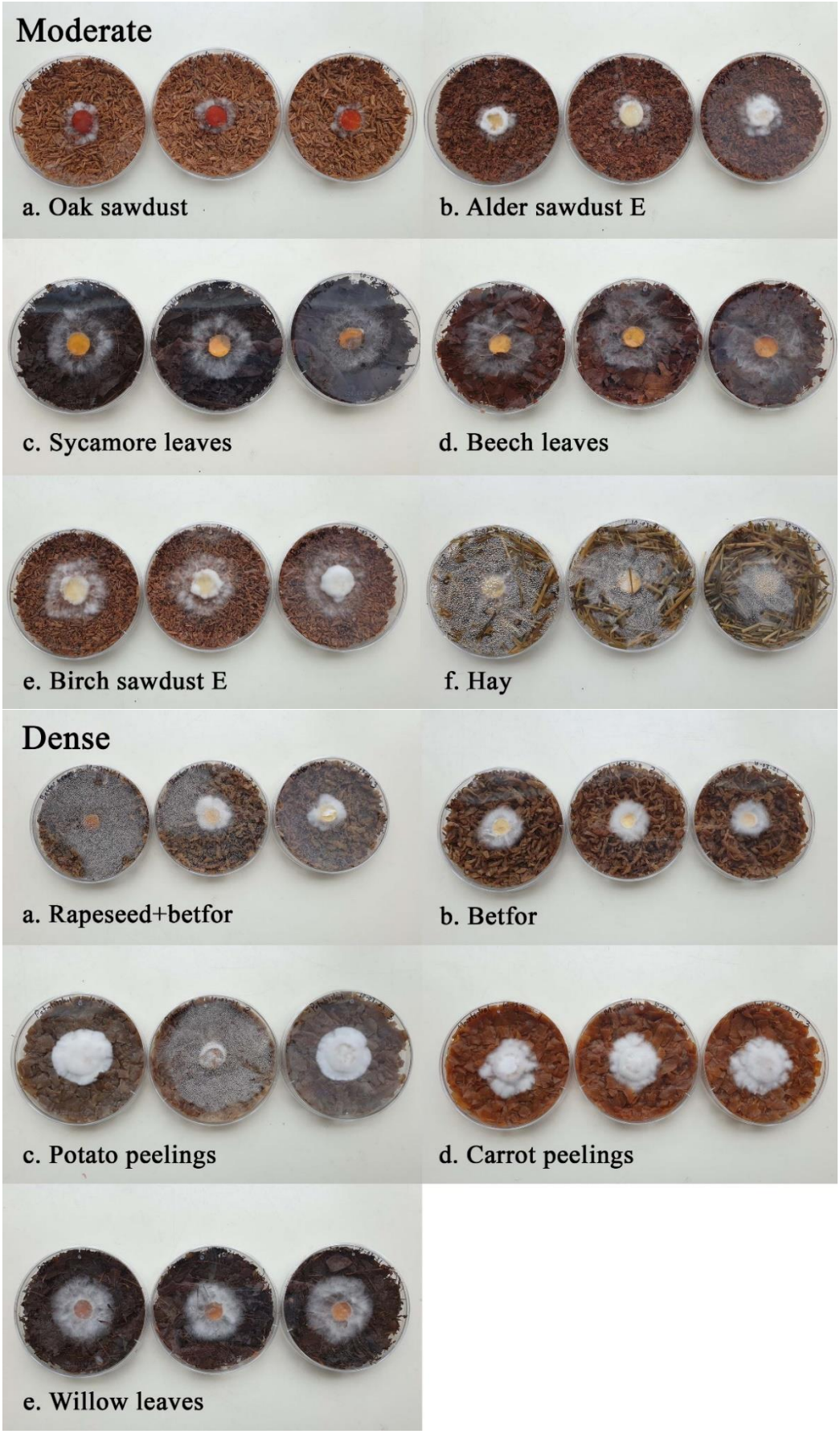
Appendix 1

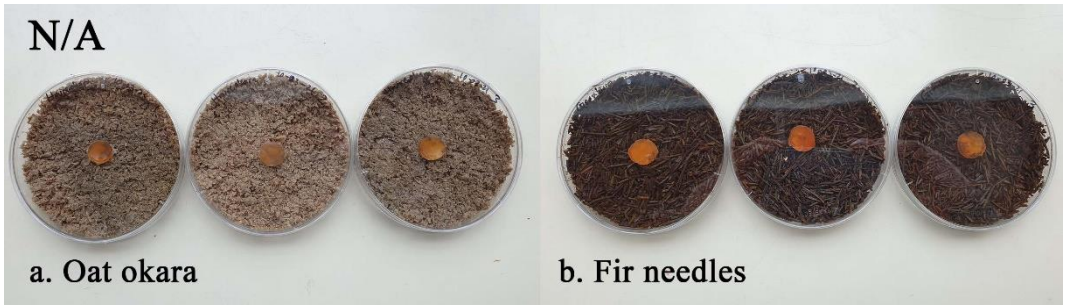
Substrates, alphabetical order	Coverage, %
Alder sawdust 1	66,02
Alder sawdust 2	60,58
Alder sawdust 3	63,75
Alder sawdust E. 1	12,73
Alder sawdust E. 2	14,33
Alder sawdust E. 3	16,14
Beech leaves 1	39,03
Beech leaves 2	47,69
Beech leaves 3	51,52
Betfor 1	15,32
Betfor 2	15,09
Betfor 3	12,74
Birch sawdust 1	77,15
Birch sawdust 2	75,46
Birch sawdust 3	71,89
Birch sawdust E. 1	40,30
Birch sawdust E. 2	44,25
Birch sawdust E. 3	44,38
Cardboard 1	32,43
Cardboard 2	19,05
Cardboard 3	14,68
Carrot peelings 1	22,19
Carrot peelings 2	21,95
Carrot peelings 3	25,92
Hay 1	57,13
Hay 2	65,29
Hay 3	76,46
Horse chestnut leaves 1	65,45
Horse chestnut leaves 2	83,49
Horse chestnut leaves 3	70,14
Malt agar 1	70,44
Malt agar 2	64,93
Malt agar 3	70,44
Norway spruce needles 1	N/A
Norway spruce needles 2	N/A
Norway spruce needles 3	N/A
Oak leaves 1	60,90
Oak leaves 2	61,27
Oak leaves 3	56,83
Oak sawdust 1	9,42
Oak sawdust 2	9,70
Oak sawdust 3	7,91
Oak sawdust E. 1	4,62
Oak sawdust E. 2	N/A
Oak sawdust E. 3	4,47
Oat okara 1	N/A
Oat okara 2	N/A
Oat okara 3	N/A
Potato peelings 1	21,81
Potato peelings 2	N/A
Potato peelings 3	20,75
Rapeseed+betfor 1	4,28
Rapeseed+betfor 2	16,30
Rapeseed+betfor 3	12,56
Spent coffee grounds 1	43,27
Spent coffee grounds 2	46,55
Spent coffee grounds 3	42,35
Sycamore leaves 1	38,59
Sycamore leaves 2	38,23
Sycamore leaves 3	37,65
Willow leaves 1	31,69
Willow leaves 2	30,15
Willow leaves 3	34,00

Substrates	Coverage, % high to low
Horse chestnut leaves 2	83,49
Birch sawdust 1	77,15
Hay 3	76,46
Birch sawdust 2	75,46
Birch sawdust 3	71,89
Malt agar 1	70,44
Malt agar 3	70,44
Horse chestnut leaves 3	70,14
Alder sawdust 1	66,02
Horse chestnut leaves 1	65,45
Hay 2	65,29
Malt agar 2	64,93
Alder sawdust 3	63,75
Oak leaves 2	61,27
Oak leaves 1	60,90
Alder sawdust 2	60,58
Hay 1	57,13
Oak leaves 3	56,83
Beech leaves 3	51,52
Beech leaves 2	47,69
Spent coffee grounds 2	46,55
Birch sawdust E. 3	44,38
Birch sawdust E. 2	44,25
Spent coffee grounds 1	43,27
Spent coffee grounds 3	42,35
Birch sawdust E. 1	40,30
Beech leaves 1	39,03
Sycamore leaves 1	38,59
Sycamore leaves 2	38,23
Sycamore leaves 3	37,65
Willow leaves 3	34,00
Cardboard 1	32,43
Willow leaves 1	31,69
Willow leaves 2	30,15
Carrot peelings 3	25,92
Carrot peelings 1	22,19
Carrot peelings 2	21,95
Potato peelings 1	21,81
Potato peelings 3	20,75
Cardboard 2	19,05
Rapeseed+betfor 2	16,30
Alder sawdust E. 3	16,14
Betfor 1	15,32
Betfor 2	15,09
Cardboard 3	14,68
Alder sawdust E. 2	14,33
Betfor 3	12,74
Alder sawdust E. 1	12,73
Rapeseed+betfor 3	12,56
Oak sawdust 2	9,70
Oak sawdust 1	9,42
Oak sawdust 3	7,91
Oak sawdust E. 1	4,62
Oak sawdust E. 3	4,47
Rapeseed+betfor 1	4,28
Oak sawdust E. 2	N/A
Norway spruce needles 1	N/A
Norway spruce needles 2	N/A
Norway spruce needles 3	N/A
Oat okara 1	N/A
Oat okara 2	N/A
Oat okara 3	N/A
Potato peelings 2	N/A

Appendix 2


Sparse
a. Oak sawdust E
b. Cardboard
c. Spent coffee grounds
d. Oak leaves
e. Alder sawdust
f. Malt agar
g. Horse chestnut leaves
h. Birch sawdust


Moderate
a. Oak sawdust
b. Alder sawdust E
c. Sycamore leaves
d. Beech leaves
e. Birch sawdust E
f. Hay
Dense
a. Rapeseed+betfor
b. Betfor
c. Potato peelings
d. Carrot peelings
e. Willow leaves


N/A
a. Oat okara
b. Fir needles