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STUDIES ON THE MOLECULAR IDENTIFICATION, BIOLOGICAL CHARACTERISTICS AND INDOOR CULTIVATION OF *MORCHELLA* SPP.

A Thesis in

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ABSTRACT

True morel (*Morchella*) is a genus of edible fungi in the order Pezizales, Ascomycota, well known for its desirable flavor. However, most morels are collected in the wild and the short fruiting season makes it one of the most prized edible fungi in the world. Recently, morels have been cultivated in China to meet the growing demand of edible morels. The purpose of my thesis is to study the biological characteristics of *Morchella* and provide information to improve on the success of future *Morchella* cultivation. The *Morchella* cultural collection in the Spawn Lab at the Pennsylvania State University is reidentified. The isolates in the collection were identified decades ago based on morphology and older species concepts. As a result, many species seem to be misidentified. Here, the *Morchella* isolates were reidentified based on molecular phylogenetic analysis of the nuc rDNA internal transcribed spacer (ITS1-5.8D-ITS4) sequences. Based on the cultivation history, geographic origin and characteristic recorded fruiting patterns, four *Morchella* isolates (WC 833 *M. rufobrunnea*, China 1 *M. importuna*, WC 374 *M. americana* and WC 766 *M. exuberans*) were selected to study the growth characteristics and used for indoor cultivation cropping trials. Mycelial growth is the first stage in the life cycle of *Morchella*, and *Morchella* fruit bodies develop from pseudosclerotia, which are masses of mycelia acting as a resting stage. Satisfactory mycelial growth and pseodosclerotia production are both thought to be required for successful morel cultivation. The effects of pH, light and temperature on mycelial growth and pseudosclerotia formation were described for four selected *Morchella* isolates. PH and light had a significant effect on mycelial growth for three of the *Morchella* isolates, with no affect observed for *M. exuberans*. Light significantly affected the pseudosclerotia formation for *M. rubobrunnea* and *M. importuna*, while pH had no effect. With the exception of *M. exuberans*, which obtained the most mycelial growth at 30°C, mycelial growth and pseudosclerotia formation were maximized at 20°C. *M. americana* and *M. exuberans* did not produce any pseudosclerotium under any of the tested conditions. Conditions resulting in both the most mycelial growth and pseudosclerotia production *in vitro* were pH6, darkness (0 lux), and 20 to 25°C for *M. rufobrunnea*, pH5 to 6, darkness (0 lux), and 20 to 25°C for *M.*

importuna. The condition preference for mycelial growth of *M. americana* was found to be pH5 to 7, darkness (0 lux) to low light intensity (400 lux), and 20°C. Mycelia of *M. exuberans* achieved the fastest mycelial growth in media with a pH value of 5, 25 to 30°C, and light intensity did not influence its mycelial growth. In addition, two indoor morel cultivation experiments were conducted in the Mushroom Research Center (MRC). The results indicated that the outdoor morel cultivation techniques, currently being followed in China, are feasible for indoor cultivation, even though many questions remain. Mature fruit bodies of *M. rufobrunnea* were successfully obtained from two substrates: soil + straw and soil + sawdust in experiment 1 but not in experiment 2, even though many primordia were formed in experiment 2. *M. rufobrunnea* and *M. importuna* appear to be the most promising candidates of morel cultivation based on the results from both biological growth studies and the indoor cultivation experiments. These studies can contribute knowledge to the field of *Morchella* cultivation by providing information on environmental factors that contribute to desirable mycelial growth and pseudosclerotia formation, which are both thought to be required for successful morel cultivation. These results will certainly contribute to our understanding of the required growing parameters needed for successful *Morchella* indoor cultivation.

Key Words: *Morchella*, morel cultivation, environmental factors, identification

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Chapter 1

Literature Review

Economic importance of morels

True morels (*Morchella* spp.) are prized, edible fungi, belonging to Ascomycota, Pezizomycetes, Pezizales, Morchellaceae, and *Morchella*. Due to their short fruiting season, favorable flavor and value in the food and medical industry, mycologists have been attempting to cultivate morels for decades, and it has been proven very difficult. However, recently in China, morel cultivation has expanded and the annual export of dried morels reached 900,000 kg in 2015, averaging $160 US dollars per kilogram (Du et al. 2015). Large quantities of morels have been harvested in China, India, Pakistan, Turkey, and North America (Pilz et al. 2007), though the majority are through the collection of wild mushrooms and not through cultivation.

Taxonomy of *Morchella*

To date, there are 334 names identified under *Morchella*, including species, subspecies and varieties, reported in the latest database of the Index Fungorum (http://www.indexfungorum.org/names/Names.asp). Due to the high diversity and variation in morphology under different environmental and ecological conditions and during different developmental stages, the use of homonyms and synonyms in *Morchella* spp. very often exists, which has caused confusion and difficulties in identifying *Morchella* spp. (Du et al. 2012b). Before the development of molecular techniques, *Morchella* spp. were initially divided into three groups: black morels, yellow morels and semi-free capped morels. In 1998, Guzmán and Tapia (1998) proposed a fourth group, named the blushing species, in the genus *Morchella*.

The application and development of DNA sequencing techniques and phylogenetic analysis has made great progress in fungal identification. Sequencing of ITS loci consisting of the 5.8S rRNA gene along with flanking ITS regions, LSU, SSU, RNA polymerase, and RAPD-PCR analysis have been used in the identification of *Morchella*. Wipf et al., 1996, Wipf et al.,1999 sequenced the ITS locus with the 5.8S rRNA gene of a morel in the Elata Clade (*M. conica*) and a yellow morel in the Esculenta Clade (*M. esculenta*) to elucidate the discrepancy between two morel groups. The length of the ITS locus was confirmed to be 740 - 760 bp for the Elata Clade and 1150 - 1220 bp for the Esculenta Clade. It should be noted that the name *M. conica* was not correct and has been applied to different *Morchella* species- *Morchella deliciosa*, *Morchella purpurascens*, *Morchella tridentina*, and *Morchella vulgaris* (Richard et al. 2015). ITS and RAPD-PCR analysis was used by Pilz et al. (2004) to study the phylogenesis of *Morchella* located in northeastern Oregon.

O' Donnell et al. (2011) used genealogical concordance phylogenetic species recognition (GCPSR) to investigate the species limits within *Morchella*. In their study, three lineages within *Morchella* were identified: a basal monotypic lineage represented by *M. rufobrunnea*, the Elata Clade (black morels) and the Esculenta clade (yellow morels). Even though it was stated in this study that 177 specimens representing the global genetic diversity were finally selected, samples from eastern Asia were underrepresented (Du et al. 2015). The phylogenetic diversity of *Morchella* was studied for 361 specimens, collected from 21 provinces in China between 2003 to 2011, by analyzing ITS rDNA sequences (Du et al. 2012a). Forty Esculenta Clade (yellow morels), 30 Elata Clade (black morels) and 1 in the rufobrunnea Clade were selected.

M. anatolica belongs to the rufobrunnea Clade, along with *M .rufobrunnea* (Taşkın et al. 2012). Three additional *Morchella* species were identified and named as *Mel*-35 , *Mel*-36 (Elliott et al. 2014) and *Mel*-37 (Pildain et al. 2014) in the Elata Clade and Kuo (2012) described 19 phylogenetic *Morchella* species in North America. It is worth noting that 6 species described by Kuo (2012) and Clowez (2010)

were reported to be synonyms by Richard et al. (2015). For example, *M. conica* was determined to be illegitimate and was a synonym of *M. purpurascens*. Four previous unnamed *Morchella* species were described as *M. mediterraneensis* (Mel-27), *M. fekeensis* (*Mel*-28), *M. magnispora* (*Mel*-29), and *M. conifericola* (*Mel*-32) by Taşkın et al. (2016).

Even though ITS rDNA sequencing has been used in many identification studies on *Morchella*, ITS rDNA sequencing can only identify approximately 70% of the known species (Du et al. 2012b).. The error rate is especially high when identifying morels in the species-rich Elata Subclade and two closely related species in the Esculenta Clade. In addition, more than 66% of the named *Morchella* sequences were misidentified in GenBank and the application of synonyms exacerbates the problem (Du et al. 2012b). In order to ascertain the identities and achieve reliable results, additional gene sequences along with dedicated accessible references are needed.

Global distribution pattern of *Morchella*

Sixty-five phylogenetic distinct species have been identified in *Morchella* with 34 species found in East Asia or China, of which 20 species are endemic. Of 27 species present in Europe, and of 21 species present in North America, 12 and 14 are endemic respectively (Du et al. 2015). Many *Morchella* species are found in Turkey with roughly twice as many species found there compared with other regions in Europe (Taşkın et al. 2012). *Mel*-10 (*M. importuna*), *Mel*-19, *Mel*-2 (*M. tridentina*) are found in Western North America, Eastern North America, Europe and Asia. *Mel*-7 (*M. eximia*), which is found in Western North America, Eastern North America, Europe, Asia, Oceania and South America, is the most widely distributed among all the disjunct species (Du et al. 2015).

Trophic mode of *Morchella*

Successful cultivation suggests that at least *M. importuna*, *M. sextelata*, and *M. eximia* are saprophytic. *M. tomentosa, M. sextelata, M. eximia* and Mel-8 were reported as obligate fire-adapted species. *M. exuberans* and *M. importuna* are considered facultative fire adaptive species (Pilz et al. 2007).

Fruiting bodies of some species in the "black morel" clade can be found commonly after fire (Kuo et al. 2012). Stable isotopes have indicated that some *Morchella* species are saprotrophic (Hobbie et al. 2001). Hobbie et al. (2016) accessed the nutritional strategy of post-fire morels using isotope techniques (δ13C and δ15N) and radiocarbon analysis (Δ_{14} C). They determined the isotopes values for ectomycorrhizal or saprotrophic fungi by comparing the stable isotopes and radiocarbon in *Morchella* with values from different fungi found in the literature. High 15N enrichment in *Morchella* indicated that the primary nitrogen source for *Morchella* was not recently burned litter. Similar 13C and 15N enrichments to saprotrophic *Plicaria* suggested that *Morchella* assimilated carbon and nitrogen the same as *Plicaria*, which indicated saprotrophic nutrition in *Morchella spp.*. Unfortunately, the authors failed to provide the species names.

Buscot and Roux (1987) reported that *M. rotunda* were able to penetrate into root tissues, indicating a plant parasitic or symbiotic relationship. An ectomycorrhizae association was described between *Picea abies* (Norway spruce) and *M. rotund*, *M. esculenta*, and *M. elata* by Buscot and Kottke (1990) and Buscot (1992a, b, c). With *M. rotunda*, a compact mycelial sheath was formed around the roots, which may serve as nutrient source for fructification, and the formation of ectomycorrhizal mycelium acting as a survival structure for morels in forest ecosystems (Buscot and Kottke 1990). Subterranean pseudosclerotia of *M. elata* ensheathed mature roots of *Picea abies* (Buscot and Kottke 1990). Buscot (1992a) found that *M. elata* only formed an ectomycorrhizae by replacing a previous ectomycorrhizae formed by other fungi. Seven ectomycorrhizal types were identified from 155

ectomycorrhizal root tips of *Picea abies* by Buscot (1994). Additionally, an endobacterium was found growing within the Hartig net of this ectomycorrhiza (Buscot 1994), though the exact role the endobacterium plays is unknown. However, Dahlstrom et al. (2000) found that *Morchella* isolates formed similar structures as the ones described by Buscot (1992a, c) without the help of bacteria or the presence of previously colonized fungi.

Dahlstrom et al. (2000) reported morels could form an ectomycorrhiza sheath with Pinaceae, but failed to indicate the *Morchella* species. In addition, they pointed out that additional microbes were not required for the formation of ectomycorrizas of morels. Stark et al. (2009) suggested the association between morels and orchids by analyzing the root-extracted DNA. Q. Li et al. (2013) speculated that morels with black pilei were considered saprobes, while those with yellow pilei were mycorrhizas. Miller (2005) was issued a patent (US 6907691) for morel cultivation using the mycorrhizal association between *Morchella* mycelium and a tree seedling, though Miller also did not indicate the species in his patent. Baynes et al. (2012) reported that *M. eximia* could infect cheatgrass roots but it was not clear if *M. eximia* was acting as a pathogen, saprophyte or symbiont in this relationship.

The trophic strategies of *Morchella* remains to be studied and discussed. Based on the existing data and information, it is likely that *Morchella spp.* include saprophytic, mycorrhizal and facultative mycorrhizal species.

Life cycle of *Morchella*

Life cycle

The first life cycle of *Morchella* spp. was proposed by Volk and Leonard (1990). This life cycle is general and does not confine to any specific *Morchella* spp. In this life cycle, the primary mycelium,

secondary mycelium, the formation of pseudosclerotia, germination of pseudosclerotia and the development of a fruiting body is identified.

Pilz et al. (2007) proposed a life cycle of true morels. Their life cycle is based on Volk and Leonard's life cycle, but it includes the cellular stages, showing the nuclei status in the hyphae or mycelium. What is worth mentioning is that this life cycle also includes some ecological conditions under which this life cycle takes place, such as soil necromass remaining after fires and live tree ectomycorrhizae associations.

Alvarado-Castillo et al. (2014) proposed a more complicated and integrated life cycle of *Morchella* spp.. This life cycle integrates all of the previous models and experimental observations and known research related to this genus. This life cycle starts with the fruiting body, ascocarp or ascoma. Each ascus produces eight ascospores which are released for dispersion. Under certain environmental conditions, such as drought and low temperatures, ascospores can grow and germinate to form hyphae. These hyphae can continue to grow and form primary mycelium. There are two subsequent pathways that the primary mycelium may develop; one is that primary mycelium may produce conidia and the other is that the primary mycelium can continue growing, intertwining, and develop into compact masses, which can then give rise to pseudosclerotia formation. Generally, the primary mycelium fuse with other mycelium from the same or another ascocarp through anastomosis and secondary mycelium is then produced. Even though Volk and Leonard (1990) suggested that anastomosis of two compatible primary mycelia occurs with and nuclei pairing in secondary mycelia, other haploid nuclei coexisting in the same septum instead of pairing to form dikaryons often exists. The haploid hyphae might fuse with existing heterokaryotic mycelia to simply contribute its own haploid nuclei, which seems to increase gene diversity and may be a strategy to adapt to environmental changes (Kaul 1997) In addition, it seems that mycelia of some *Morchella* isolates (species not indicated) do not anastomose (Volk and Leonard 1990). There are on average 10 to 15 nuclei in each septum in the secondary mycelium, but numbers can reach

up to 65 (Volk and Leonard 1990). The secondary mycelium can also grow repeatedly and intertwine to form pseudosclerotia. During this process, the secondary mycelium may form chlamydospores (Alvarado-Castillo et al. 2012), which are thick-walled and function as resting fungal cells. Under certain environmental stimuli, such as drought, change of temperature, and exposure to fire and flooding, the pseudosclerotia germinate and give rise to the carpogenic mycelium, which will then develop into a fruiting body, the ascocarp. However, it is not clear whether the pseudosclerotia produced from the primary mycelium can develop into fruiting bodies directly, though its' haploid nature indicates that is unlikely to occur (Pilz et al. 2007). It is also unknown whether the secondary mycelium is able to develop into a fruiting body or not.

W. Liu et al. (2017a) proposed a life cycle of *M. importuna*, the most widely cultivated *Morchella* species in China. There is no intrinsic difference between this life cycle and the one Alvarado-Castillo proposed. Under favorable conditions, the ascospore of *M. importuna* germinates and forms the primary mycelium.

The nuclear behavior during meiosis and ascosporogenesis on *M. importuna* was studied using confocal laser scanning microscopy (P. He et al. 2017). The study suggested that a total of six nuclear divisions typically took place during ascosporogenesis. First, two meiotic divisions and subsequently one mitotic division gives rise to eight nuclei. The nucleus in each ascospore then undergoes three mitotic divisions and finally six to eight nuclei are formed in each ascospore. Two idiomorphs, *MAT1-1* and *MAT1-2* were identified on *M. importuna* and population genetic investigations suggest heterothallic characteristics of three *Morchella* isolates, *M. importuna, M. sextelata* and *Mel-20* (Chai et al. 2017). Du et al. (2017) noted that 14 *Morchella* species in the Elata Clade are all heterothallic. W. Liu et al. (2018b) investigated two monospores with a different mating type isolated from *M. importuna*. Their results indicated independent mating type structures in the two monospores, so they suggested that *M. importuna* might be a heterothallic fungus.

Pseudosclerotia of *Morchella*

Concept of sclerotia

Fungi can use a variety of strategies to survive under unfavorable conditions (Blackwell 2011). Many fungi are able to produce durable structures to facilitate dispersion or survival (Stajich et al. 2009), while some form a kind of multicellular structure, which is a dense aggregation of fungal tissue called sclerotia (Willetts 1971). Generally, sclerotia consist of a peripheral rind, which is a layer of pseudoparenchymatous, melanized cells, and the rind encases a broad medulla. However, sclerotia produced by *Morchella* are actually not true sclerotia in the classic sense, such as those produced by *Sclerofinia sclerotiorum*, which are characterized by the medulla and rind, they are considered pseudosclerotia (Volk and Leonard 1990), even though the name sclerotia was used in many studies.

Role of pseudosclerotia in the life cycle of Morchella

Based on Volk and Leonard's theory, pseudosclerotia formation is an indispensable period in the life cycle of *Morchella* spp. and plays an essential role in the successful development of fruiting bodies of *Morchella* spp.. It is thought that pseudosclerotia can be produced from both primary mycelium and secondary mycelium. However, Volk and Leonard suggested that only secondary mycelium is able to give rise to pseudosclerotia (Volk and Leonard 1990), but additional evidence is needed. Formation of pseudosclerotia is a response to unfavorable environment conditions and also a strategy that fungi use for nutrient storage, which suggests they are the products of a long-term biological evolution (Xiong et al. 2015).

In 1982, Ower was successful in the cultivation of *M. rufobrunnea* for the first time in history. He used a jar method to produce pseudosclerotia and then stimulated the germination of pseudosclerotia to promote fruiting body development. Three patents were issued based on this method, and many mycologists have since been attempting to study the mechanism of pseudosclerotia development. However, pseudosclerotia are not always formed under experimental conditions, and the ability and requirements of pseudosclerotia development varies greatly depending on the species (C. Ding et al. 2008).

Furthermore, types of pseudosclerotia may differ as well. Buscot (1993) studied mycelial differentiation of *M. esculenta* in pure culture. Based on morphogenetic and additional characteristics, two types of pseudosclerotia were identified from freshly germinated ascospores: early, encrusting pseudosclerotia (EES), and late, isolated pseudosclerotia (LIS), which were initiated by growth interruption and aging of the culture respectively. It is likely that EES were associated with imperfectly developed fruiting bodies or some kind of sexual structures, while LIS functioned as storage and resting structures. On the basis of the interaction of *Morchella* with bacteria, two types of pseudosclerotia were produced: pseudosclerotium type 1 (ST1) and pseudosclerotium type 2 (ST2). ST1 initiated first and aggregated near the inoculum. ST2 formed after ST1. On the contrary to ST1, ST2 were dispersed on the medium, matured faster and became pigmented (Stott and Mohammed 2004). Stott and Mohammed (2004) found that *M. hortensis* and *M. rigida* only produced pseudosclerotia on the nutrient-rich medium when inoculated on the nutrient poor medium beforehand. An isolate of *M. esculenta*, originating from Greece, also only produced pseudosclerotia on nutrient-rich media after being grown on nutrient-poor media first (Philippoussis and Zervakis 2000). However, one Tasmanian isolate was able to produce pseudosclerotia on both nutrient-poor and rich media no matter whether it was inoculated on nutrient poor medium or not. Philippoussis and Zervakis agreed with Buscot (1993) and Faris et al. (1996) that a nutrient-poor environment is essential for pseudosclerotia production. But Singh and Vema's (2000) research indicated that nutrient-poor conditions are not necessary for the development of pseudosclerotia.

In their study, six media were used to cultivate *Morchella* spp.. Malt Extract Agar was found to be the best culture medium to get the maximum radial growth and the only one that supported pseudosclerotia development in all of the test isolates.

Recently, P. He et al. (2018) first highlighted the involvement of autophagy and apoptosis and lipid accumulation in pseudosclerotia morphogenesis of *Morchella importuna* (Pseudosclerotia initial [SI], pseudosclerotia development [SD], pseudosclerotial maturation [SM]). They compared the structural features of the undifferentiated mycelial stage and three main pseudosclerotial stages by using transmission electron microscopy. The characteristics of autophagy was observed during the SI phase and apoptotic characteristics were found in some cells during the SD phase. Moreover, they found lipid was the energy-rich substance in both hyphae and pseudosclerotia of *M. importuna*. Pseudosclerotia had a significantly higher content of lipid than that in hyphae, which is consistent with the hypothesis that pseudosclerotia serve as nutrient storage organs.

Factors that affect pseudosclerotia production

External factor

Amir et al. (1992; 1993; 1995) studied the effect of medium composition and water potential on pseudosclerotia formation on *M. esculenta* using the split plate method (two media that differ in nutrient content on one plate separated by a plastic barrier). Their study emphasized the important role of high turgor potential, which is required for the growth of hyphae, in the production of a large quantity of pseudosclerotia and this turgor potential is regulated by metabolizable substrates. Pseudosclerotia production was measured by dry weight as well as calculating their surface area with the aid of a camera and computer program. Water, solute and turgor potential were measured as described by Thompson et al.

(1985). Their study defined six growth stages of *M. esculenta* during hyphal growth and pseudosclerotia formation on a split plate. The first stage included the growth and extension of mycelium on the nutrient-poor (noble agar/NA) side. Hyphae were weak, characterized by large vacuoles and growth toward the nutrient-rich (potato dextrose agar(PDA)) side. Stage II began when hyphae reached the PDA side of the plate, followed by decreasing the size of hyphal vacuoles and declining of the hyphal glucose content. It was worth noting that the direction of the cytoplasm stream, including nutrient substances, reversed when the growth of mycelia reached the end of the plate. The cytoplasm moved from old mycelia (PDA side) to young mycelia (NA side), but no explanation was provided. Carbohydrates, which were assumed to be mannitol or arabitol, were translocated from the younger mycelium to the older mycelium. The initiation of pseudosclerotia on the NA side indicated the beginning of stage III. Stage IV was characterized by the enlarging pseudosclerotia and this enlargement was supported by the rapid transportation of a large amount of nutrients to the young pseudosclerotia. Color of the mycelium changed from white to yellow-brown during stage V and stage VI was characterized by the formation of the peripheries of some pseudosclerotia. They also pointed out that nutrients from mycelium on the PDA side were transported to the other side and the pseudosclerotia from the NA side served as a nutrient sink. Moreover, their study indicated turgor pressure gradients in a split plate, which explained the rapid translocation of nutrients to pseudosclerotia during the morphogenetic process.

Volk (1989) used a jar method (Ower et al. 1986) to study the effect of a variety of conditions on pseudosclerotia formation. Complex medium worked better and a smaller sized container achieved better biological efficiency (BE), calculated as the wet weight of pseudosclerotia divided by the dry weight of the substrate. He also pointed out that illumination was a limiting factor for the development of pseudosclerotia. In addition, carbon was not a limiting factor while nitrogen was. Zhao et al. (1997) reported that glucose, mannitol, mannose and diammonium phosphate promoted pseudosclerotia formation. They also stated that light is not necessary. However, during the process of making spawn, there was a significant difference between the light side and the dark side of the spawn container if it was

not rotated on a regular basis. On the side that was occasionally exposed to light, large amounts of pseudosclerotia were produced. On the contrary, production of pseudosclerotia on the dark side was poor, indicating that light plays a role in pseudosclerotia formation (W. Liu et al. 2017a). Volk and Leonard (1990) reported that low temperatures or nutrient deficiency is required for the formation of pseudosclerotia of *Morchella* spp. Wang (1997) reported that between 15 to 20°C is the best temperature for the formation of pseudosclerotia of *Morchella* spp. Wang also pointed out that the number and size of pseudosclerotia can differ significantly on different cultural media even for the same isolate of *Morchella* spp.. Li et al. (1998) reported that VB1 favors the formation of aerial pseudosclerotia. Kanwal and Reddy (2011) compared the effects of various nitrogen (N) and carbon (C) sources on pseudosclerotia formation and development for *Morchella elata* and *Morchella crassipes.* They concluded that for the formation of larger-sized pseudosclerotia of *Morchella*, ribose, mannitol, and glucose are the best carbon sources and the best nitrogen source is sodium nitrate. They also studied ligninolytic enzyme production on different substrates and during pseudosclerotia formation in *M. crassipes* (Kanwal and Reddy 2014). Besides carbon and nitrogen, ligninolytic enzyme production in *M. crassipes* was influenced by ligninolytic chemical and natural inducers (Kanwal and Reddy 2010). Their study demonstrated that ligninolytic enzymes, such as laccase, Manganese peroxidase, and lignin peroxidase are induced in lignin-rich substrates. These enzymes result in lignin degradation in *M. crassipes* and it is hypothesized that lac enzymes play an important role in pseudosclerotia formation and maturation.

Intrinsic factor

Chen et al. (2014) analyzed the differences between gene expression of pseudosclerotia-producing and non- pseudosclerotia-producing single spore isolates from *M. conica* using RT-PCR. Thirteen different positive gene fragments which were thought to be involved in pseudosclerotia formation of *M. Conica* were identified by comparing the gene expression of two types of isolates to the

housekeeping gene 18S rRNA. Some positive fragments were found similar to genes involved in lipid metabolism, nitrogen metabolism, the *Omp*A family protein coding gene and keratin-associated protein 5, 6 coding genes. Some positive fragments that could not be found in the NCBI are thought to be the specific genes controlling pseudosclerotia formation and differentiation.

Q. Liu et al. (2018) studied the effect of reactive oxygen species on pseudosclerotia formation of *M. importuna*. In their study, a higher hydrogen peroxide concentration was observed in the mycelial growth region compared to the pseudosclerotium-forming region. Moreover, pseudosclerotia formation and gene expression of organisms possess superoxide dismutases (SOD) was correlated to the concentration of hydrogen peroxide. It was hypothesized that the MARK pathway was involved in pseudosclerotia formation in *M. importuna*.

Mitospore

The mitospore stage of *Morchella* spp. was first reported by Molliard (1904a) in his experiment on artificial cultivation of *Morchella*. He identified the white mold on the soil surface as the hyphomycete *Costantinella*. Subsequently, Molliard (1904b) demonstrated that low relative humidity is required for the development of mitospores. However, he failed to observe the germination of mitospores on various media. Paden (1972) also reported the development of mitospores in *M. elata*. Ower found massive conidia blooms during his cultivation experiment on *Morchella*. According to the life cycle provided by Volk and Leonard (1990), mitospores can be produced from primary mycelium but no explanation was given to their role in fruit body formation. Masaphy (2010) closely observed the morphology of mitospores during cultivation of *M. rufobrunnae.* W. Liu et al. (2016a) analyzed the morphology and structure of mitospores on *M. importuna*. The mitospores of *M. importuna* are hyaline, spherical, non-septate, smooth, 3.5 to 5.2μm in diameter. Conidiophores are cambiform, slightly curved like an “s”, and are (2.1 - 6.2) × (14.1 - 18.5) μm in size. Four to six conidioiphores can be found on the specialized

hypha, which is 6.0-8.5um long, hyaline and thinner than the vegetative hypha. Conidiogenous cells contain between 2-7 nuclei. Most of the mitospores have only one nucleus, though two, three and four nuclei are occasionally found. Mitosis can be clearly observed in a single mitospore, indicating the mitospores with two or more nuclei may result from mitospores with a single nucleus. Even though the ability to produce mitospores has been demonstrated with *M. importuna* during artificial cultivation, the specific role of the mitospores is not yet clear. The relationship between the production of *Morchella* fruiting bodies and the number of mitospores, and the role of mitospores in the entire life cycle of *Morchella* spp. needs further investigation.

Etiology of *Morchella*

Carbon source

Zhu et al. (2011) noted that the best carbon source for mycelial growth of *M. esculenta* is soluble starch based on the growth rate of mycelium, and the size and thickness of the colony. Using the dry weight of mycelia, the content of intercellular polysaccharides and the yield of polysaccharides as the indicators, Yang et al. (2007) pointed out that soluble starch as well as sucrose are the best carbon sources for the growth of various *Morchella* spp. (the name of specific species was not provided). This contradicts previous reports that the best carbon source for mycelial growth is glucose for strain LWY-1(Chai et al. 2010) and strain M-yan-5 (Ren et al. 2006). However, the species of these two strains were not indicated. In production, wheat, sawdust, corn stover, cottonseed hulls and compost have been used as carbon sources for *Morchella* spp. Nevertheless, *Morchella* has limited abilities to degrade lignin, hemicellulose and pectin (W. Liu et al. 2017a).

Nitrogen sources

S. Liu et al. (1998) reported that the best nitrogen sources for the mycelial growth of *M. conica* (reported to be an illegitimate name (Richard et al. 2015)) *in vitro* are aspartic acid, cysteine and sodium nitrite, while Dong (2004) reported that potassium nitrate and sodium nitrate are the optimal nitrogen sources for *M. angusticeps*. Yang et al. (2007) noted that soybean meal, peptone and fish gelatin are the best nitrogen sources based on dry weight measurements of mycelium, the content of intercellular polysaccharides and the yield of polysaccharides as the indicators. Zhu et al. (2011) noted that carbamide is the best nitrogen source followed by potassium nitrate and calcium nitrate for *M. escultanta*. Xie et al. (2009) pointed out that the best nitrogen source for five *Morchella* strains - M. angusticeps, M. conica, *Morchella* spp. (50647), *Morchella* spp. (50648) and *M. esculenta* are ammonium sulfate, peptone, yeast extract, sodium nitrate, and sodium nitrate respectively.

The diverse conclusion regarding the best nitrogen source for the growth of *Morchella* is related to the diversity of the stains of *Morchella* and the concentration of nitrogen source. Unfortunately, there is not adequate, consistent and convincing data indicating the best carbon to nitrogen ratio or the best source of carbon or nitrogen for optimum growth and development of *Morchella* (W. Liu et al. 2017a).

Mineral salts

Mineral salts play an important role in maintaining the normal functions of fungi, such as adjusting the osmotic pressure and the concentration of hydrogen ions. They are also components of fungal cells that are involved with maintaining the activity of enzymes. Zhu et al. (2011) reported that $MnSO_4$ (30 mg/L) promotes the growth of *M. esculenta*, while $MgSO_4$, K_2SO_4, NaCl and Na_2MoO_4 neither promote nor inhibit the growth of *M. esculenta*. However, KH_2PO_4, $FeSO_4$, $ZnSO_4$, $NaSeO_3$, $CuSO_4$, $CoCl_2$ and $Ni(NO_3)_2$ potentially inhibit mycelial growth of *M. esculenta*. $CoCl_2$ only slightly

suppressed mycelial growth of *M. esculenta* when its concentration was around 10 mg/L. As the concentration of $CoCl_2$ increased, the effect of inhibition of mycelial growth also increased. $CuSO_4$ with a concentration of 70 mg/L and $Ni(NO_3)_2$ with a concentration of 90 mg/L completely stops mycelial growth of *M. esculenta*.

Temperature

Chen and Guo (2007) reported that 24 to 26°C is optimum temperature for *M. esculenta* mycelial growth. Mycelia start to die when temperatures exceed 34°C. According to the growers' experiences, low temperatures facilitate the differentiation of mycelia (personal communication). Growers believe that a satisfactory yield of mature fruit bodies will be obtained when the air temperature is below 4°C for at least one month before the primodia formation for *M. importuna* and *M. esculenta* (personal communication). It is also believed that temperature fluctuation of more than 10°C in a day facilitates primodia formation for these two species (personal communication). Nevertheless, additional data is required to determine if a low temperature period is required for initiation of fruit bodies.

Humidity

W. Liu et al. (2017a) discussed the moisture and humidity levels required for general morel cultivation (mainly *M. importuna*, *M. sextelata*, *M. septimelata*, and *M. esculenta*). Ideally, during the incubation period, the moisture of spawn should be between 60 to 65%. If it is too high, air movement is inhibited. As a result, anaerobic conditions are created and mycelial growth is suppressed. The moisture of the soil should be between 18 to 28% before spawning, while during the mycelial growth stage, it is better to drop the moisture to between 15 and 25%. When fruiting bodies start to form, a soil moisture level of 20 to 28% is required. Air humidity levels between 65 to 80% is optimal during spawn run and

mycelial growth stage. During fruit body maturation, 85 to 95% air humidity is needed to avoid damage to young fruiting bodies from drought.

pH

Similar to many fungi, *Morchella* spp. are able to grow under a broad pH range. Chen and Guo (2007) noted that the optimal pH is 6.4 to 8.7 based on the condition of mycelial growth and pseudosclerotia formation of *M. esculenta*. Calcium oxide is commonly used to adjust the pH of soil if it's too acidic (W. Liu et al. 2017a).

Light

Volk (1989) noted that light inhibits the pseudosclerotial growth of *M. crassipes*. However, it was reported that mild light can actually stimulate the formation of pseudosclerotia during spawn production of *M. importuna* (W. Liu et al. 2017a). There have been some reports on the effects of light on some other edible fungi. For instance, it was reported that light treatment increases the production of *Flammulina velutipes* (M. Liu and He 1997), and light has an effect on the development and spore production of *Ganoderma* (Hao et al. 2011).

Oxygen and carbon dioxide

Adequate oxygen should be provided since *Morchella* spp. are aerobic fungi. Mycelia of *Morchella* spp. are not reported to be sensitive to carbon dioxide levels (W. Liu et al. 2017a). However, 400 to 600 ppm of carbon dioxide has been reported to be the optimal level for the growth of fruit bodies (W. Liu et al. 2017a).

Morel cultivation

History and progress of morel cultivation

Since 1882, when outdoor cultivation of morels was first reported by Roze (1882) in France, many researchers and mushroom lovers have attempted various methods of cultivating morels. In 1904, Molliard reported successful cultivation of morels in an apple compost. Ower claimed successful indoor artificial cultivation of *M. rufobrunnae* in 1982. Subsequently, Ower applied for three patents (US Patents 4594809, 4757640, and 4866878) for morel cultivation in 1985, 1986 and 1988 respectively. These studies revealed the optimal temperature, humidity and other essential factors during different stages, as well as providing a method of transformation from vegetative growth to sexual reproduction. Moreover, he emphasized the important role of pseudosclerotia in fruit body development in his method (Ower et al. 1985; 1986; Ower 1988). Methodology provided by these patents was adopted by Neogen Corporation, Domino's Pizza Inc., The Terry Companies of Wayzata (Terry Farm) and Diversifield Natural Products Corporation (DNP, currently named Gourmet Mushrooms Inc.). With the help of Mills, the second author of the patents, Terry Farms cultivated morels indoors and it was stated that 1400 fresh morels could be produced per week. However, for unknown reasons, this production line was closed around 1999. Subsequently, Mills worked for DNP and the company started selling fresh morels in 2005. Due to the reduction of output and bacterial contamination, they had to stop growing morels in 2008, and since, there have not been any reports of commercial artificial cultivation of morels in the USA.

In 2005, a patent for the cultivation of *Morchella* was issued for SC Miller. The key process of his method was inoculating a tree root system with *Morchella* mycelium. Masaphy (2010) reported a relatively detailed biotechnology of morel cultivation. She was successful in getting fruiting bodies of *M. rufobrunnae* in a soilless system in laboratory-scale experiments. She also used a multi-layer cultivation

method proposed by Ower to cultivate pseudosclerotia. These pseudosclerotia then served as the "seeds", which were then inoculated into the compost and water treatment was applied to stimulate the germination of the pseudosclerotia (Ower 1986, Volk 1990). Masaphy (2010) also pointed out that the primary reasons for the failure of morel cultivation are the deficiency in knowledge about the development of the ascocarp and difficulties and unknowns regarding species selection.

Chinese scientists have been attempting to unveil the mystery of *Morchella* since the 1980's. The first report of morel cultivation in China was proposed by Ding (1983) in 1983, though he failed to publish detailed records of the cultivation process. In the same year, Gu (1983) cultivated spores on a medium and bean-sized pins were observed after 55 days of cultivation. However, no mature fruiting bodies were obtained. Zhu Douxi, who is honored as the "father of morels in China", was issued the first morel cultivation patent in China in 1994 (D. Zhu 1994). Later, four more patents for morel cultivation were issued in 2001, 2007, 2009 and 2012 (D. Zhu 2001; D. Zhu and He 2007, 2009, 2012), respectively. The 2001 Patent reduced the amount of organic matter needed in the soil to between 5 to 20% compared with 90 to 95% requirements stated in the first patent. Excessive organic components were thought to facilitate bacterial contamination and was more expensive for those considering large-scale commercial production. The Patent in 2012 was the most detailed one among those five issued to D. Zhu. In this patent, D. Zhu provided the formulation of a medium for the mother culture, detailed processes of making spawn, spawning, and management strategies during different stages. According to published papers and patents of D. Zhu, the techniques he reported belong to what is considered "bionic cultivation", since the species of *Morchella* he used were not identified and managements of factors, such as light, humidity and temperature, were difficult to manage.

Apart from Miller, researchers in China also attempted to use the ectomycorrhyzal relationship between *Morchella* mycelium and tree seedlings (Zhao et al. 2009), but they failed to examine the

effectiveness of this ectomycorrhyzal symbiosis. Compared with the cultivation process described by D. Zhu, the production period is much longer and techniques are more complex.

In the early 21st century, some farmers in the Yunnan province, China, created a special method for morel cultivation. They placed a piece of poplar wood on a layer of spawn and then added another layer of spawn on the top of the wood, repeating this sequence until a "pyramid" was obtained. Then they covered the "pyramid" with soil, simulating a "natural environment". This method was reported to be feasible using *M. importuna* (Zhao at al. 2014), but the large consumption of wood is not environmentally friendly.

Intercropping patterns between morels and wheat is currently applied in some regions in China, especially for those who do not count on growing morels to make profits. The most obvious advantage of this pattern is the additional output of wheat when not cultivating morels. Therefore, this pattern is only temporary and a way to cut losses to confront with the immature cultivation technology (W. Liu et al. 2017a). However, the yield of mature morel fruit bodies is generally 10 to 30% higher when intercropping with wheat comparing to greenhouse cultivation (personal communication). But it is not clear whether this intercropping pattern effects the production of wheat or not.

The most significant and important process discovery in morel cultivation in China may be the application of exogenous nutrient bags. Strictly speaking, the concept of additional nutrient sources was first described by Ower et al. in his patent in 1986 (Ower et al. 1986). However, they did not highlight or compare the importance of the additional nutrient sources in the cultivation of *Morchella* and this theory was never used in morel cultivation until D. Zhu's reports.

The theory of using nutrient bags is that the sexual development of *Morchella* requires a comparatively nutrient-poor environment, but the nutrient-poor soil substrate is not able to supply enough nutrients for the growth of newly formed mycelium. Therefore, additional nutrients are required to

support the formation of mycelium. However, the additional nutrients should be removed later in the process in order to facilitate the sexual reproduction, which will develop into fruiting bodies.

Morel cultivation in China

Current status

The cultivated area of morels in China is currently 9470 hectares, compared to just 405 hectares in 2011, and nearly four times greater than the amount in 2014 (Figure 1-1) (W. Liu et al. 2017b). Morel cultivation regions are located in more than twenty Chinese provinces. Sichuan province accounts for 44 percent (Figure 1-2) of China's production area.

Chinese scientists have decided upon the following species to be used for artificial cultivation: *M. importuna*, *M. sextelata*, *M. septimelata*, and *M. esculenta*. *M. importuna* is the most widely cultivated species in China (accounting for more than 95 percent) due to the superior productivity and stability (W. Liu et al. 2017a). To date, farmland cultivation and forest farming are the most common morel cultivation patterns in china. Figure 1-3 shows the widely-applied morel cultivation technique used in China.

Spawn production

The quality of the spawn largely affects the entire morel cultivation process. The mother culture can be obtained from fresh fruiting bodies of *Morchella* using spore isolation, tissue isolation or matrix isolation (W. Liu et al. 2017a). For different purposes, different strain isolation methods may be recommended. Spore isolation is recommended for strain selection when breeding new cultivars, since the spores are genetically different from the mother culture. Spawn produced from tissue isolation has the same genetic information as the mother culture. Thus, for general spawn production, tissue isolation is

recommended to maintain its productive traits (W. Liu et al. 2017a). The most commonly used media for morel mother cultures includes potato dextrose agar (PDA), PDA with humus, and complete yeast medium (CYM) (W. Liu et al. 2017a). Mother spawns are often produced in glass bottles and autoclave-friendly plastic bags are widely used for the final spawn.

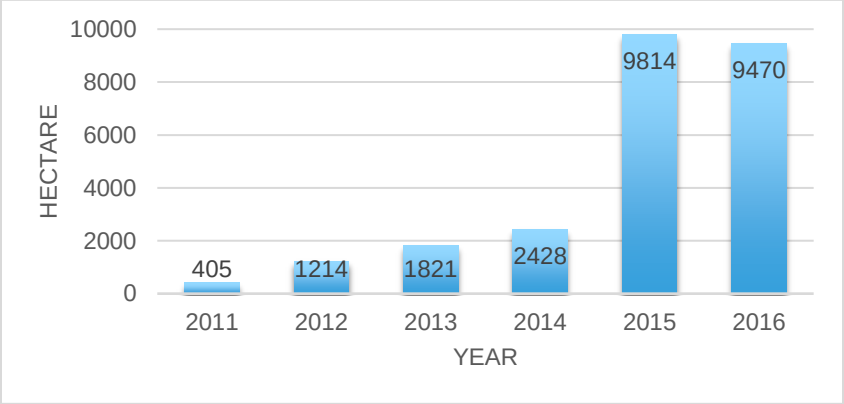


Figure 1-1. Cultivated area of morels in China by province from 2011 to 2016

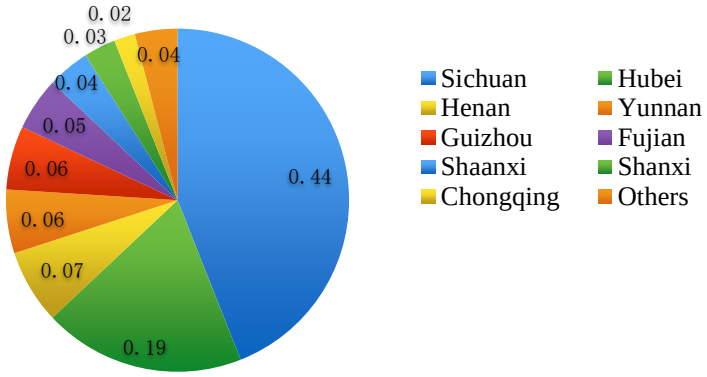


Figure 1-2. The distribution of morel cultivation in China in 2016

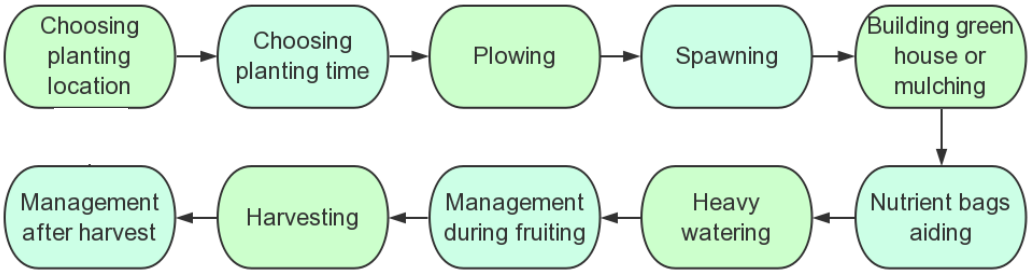


Figure 1-3. Morel cultivation flow chart

Selection of cultivation location

Different areas may choose different cultivating patterns according to the various weather conditions in different regions. In plain and basin areas, locations that are flat, near water sources, of good drainage ability with loose soil, and are suitable for morel cultivation (*M. importuna*, *M. sextelata*, *M. septimelata*, and *M. esculenta*). Generally speaking, fields suitable for plants should work well for morel production (personal communication).

Plowing

Before plowing, weeds and plant debris from last season should be removed. Moreover, calcium oxide or calcium carbonate (250 to 370 kg per hectare) or plant ashes (1000 to 1240 kg per hectare) can be sprayed onto the soil to adjust pH of the soil to 6.4 - 8.7. After adjusting the soil pH, if necessary, the land is typically plowed to build beds (0.8 to 1.5 m wide) and furrows (0.2 to 0.3 m wide and 0.2 to 0.25 m deep) (W. Liu et al. 2017a).

Spawning

In Sichuan, Hubei (personal communication), and Shanxi (Ping 2019) provinces, China, spawning typically occurs during the first ten days of November to the end of November when the outside highest temperature drops to 20°C (personal communication). In plateau regions and areas further south, the spawning is done earlier (personal communication). For different cultivating patterns, the spawning rate varies. Generally, the application amount of spawn is around 500 to 1000 kg per hectare (W. Liu et al. 2017a). Two spawning patterns are widely applied in China (W. Liu et al. 2017a): 1. Spawn is spread directly on the bed and an additional layer of soil is added on top of the spawn; 2. Spawn is placed in V-

shaped furrows on the beds and then are recovered with soil. On one hand, technically, good dispensability makes spaying a more effective way to spawn. On the other hand, spawn in the furrows provides the opportunity for mycelia to grow from nutrient rich side (the site with spawn) to nutrient poor side (the site with no spawn), which is favorable for pseudosclerotia formation.

Film mulching

The application of mulching films is a recent, innovation in the morel cultivation industry and is utilized in some regions in China. This process reduces the costs of morel cultivation, which makes the rapid expansion of morel cultivation more feasible (W. Liu et al. 2017a). Plastic film mulching can be used both in addition to and in place of the shade cloth housing. Black plastic mulch is most commonly used in the artificial cultivation of morels in China. It is suggested to choose mulches with a thickness of 0.006 to 0.008 mm (Zhang et al. 2017). Width of mulches is maintained 10 to 20 cm narrower than the mushroom beds. After spawning, mulches are placed on the beds and fixed by stones or soil with a 50 cm interval (Zhang et al. 2017). On the basis of the stability of the mulch, openings should be made on the mulches to allow air circulation (Zhang et al. 2017).

About one day after spawning and mulching, mycelial growth can be seen on the soil (personal communication). When adding nutrient bags at approximately 7 to 10 days after spawning, the stones or soil on one side of the plastic should be removed so that the plastic mulch can be moved to one side to allow for the application of the nutrient bags (personal communication). After application of the bags, growers then cover the furrows again. W. Liu et al. (2017a) noted that the optimal mulch removing time is 20 to 25 days after adding nutrient bags. Zhang et al. (2017) suggested that it is better to remove the plastic mulch 10 to 20 days before fruiting, which depends a lot on personal experience.

There are several advantages of using a plastic mulch (Zhang et al. 2017). Moisture preservation and waterlogging prevention: On one hand, mulches prevent the moisture from evaporation and on the other hand, raindrops can move along with the mulches into the grooves. Even long periods of rainy days won't cause serious damages to the beds. The plastic also provides protection from light and provides weed control. Strong light will suppress the mycelial growth of morels. Weeds provide habitat for pests and sabotage the effectiveness of air circulation, contributing to a high-temperatures and a high-moisture environment, which is favorable for fungal and bacterial infections. Plastic mulch also provides heat preservation by absorbing heat from the sunlight and warming the soil temperature, which is favorable for mycelial growth during the winter when temperatures are low. Another possible advantage of the plastic mulch is that it may reduce conidia growth, even though the necessity of conidia in the life cycle of *Morchella* is not clear (W. Liu et al. 2016a), it is proposed that excessive conidia will consume nutrients making them unavailable for *Morchella* fruit body initiation. However, it should be noted that the role of conidia in the morel fruiting body initiation is not clear yet. Mulching is also effective in controlling the cost. The consumption of spawn can be reduced to 420 bags per hectare (14 × 28 cm plastic bag, 0.6 - 0.7 kg per bag) to obtain the same yield by using mulch instead of growing in shade cloth housing (Zhang et al. 2017).

Nutrient bags

This is one of the most essential steps throughout cultivating morels. Nutrient bags with openings are added to the soil with the openings facing the soil surface approximately 7 to 20 days after spawning (W. Liu et al. 2017a). It is suggested that 4450 to 4950 bags be used, evenly dispersed per hectare (W. Liu et al. 2017a). Mycelia of *Morchella* will use the nutrients contained in the bag and grow into the bags. Nutrients in the bags are absorbed and transferred by mycelia to the soil mycelia, which supports the fruiting body development. Removal of the bags takes place 20 days before fruiting bodies develop (Wang et al. 2016). Based on limited information, the composition of the nutrient bags does not appear to

be critical. There are many available formulas for nutrient bags listed in Chinese patents (Qin et al. 2015; Shi 2014).

Heavy watering

Apart from the addition and subsequent removal of supplemental nutrients, the other way to stimulate fruit body development is through heavy watering (W. Liu et al. 2017a; Ping 2019). After removing the bags about 20 days before fruiting bodies develop, heavy watering is recommended between 1 to 3 times onto the beds. Heavy watering or complete submersion is also used to initiate fruiting with other cultivated mushrooms (ex. *Lentinula edodes* - Shiitake logs) (Niu 1994).

Management during fruiting

It is worth noting that the fruiting bodies of some *Peziza* spp. have commonly been observed in Chinese production systems about one week before the fruiting of *Morchella* (*M. importuna*, *M. sextelata*, *M. septimelata*, and *M. esculenta*) (W. Liu et al. 2017a). Therefore, the presence of these fungal fruiting bodies is considered a signal for the coming of the fruiting period of Morels.

The pins of morels are fragile and can be damaged easily when temperatures are less than 0°C. One method to minimize frost damage is to add straw or plastic mulch on the beds to keep the soil warm. When small, immature pins develop into small fruiting bodies, air humidity should be maintained between 85 to 95% (W. Liu et al. 2017a; Ping 2019) and soil moisture levels between 28 to 35% is recommended (Zhang et al. 2017). When the fruit bodies grow to 1.5 to 3 cm tall, the air humidity should be maintained while the soil moisture should be reduced to 18 to 25% (W. Liu et al. 2017a). In the later maturation period, when fruiting bodies are growing quickly, it is recommended to maintain the

temperature between 12 to 16°C and increase soil moisture to 20 to 25% (W. Liu et al. 2017a). During the maturation period, the air humidity should be lowered to 70 to 85% (W. Liu et al. 2017a).

Harvest

When the fruiting body grows to 10 to 15 cm tall with a characteristic appearance of morels, then growers harvest using a knife and cut the fruit body at the bottom of the stipe. Mature fruit bodies can be dried at a low temperature and stored for a long period in a dried state (personal communication).

Issues and perspectives

Spawn quality

Quality spawn increases the likelihood of successful mushroom cultivation. *Morchella* species display different morphology on different media (Guler and Ozkaya 2009), which is a major issue in the development of a spawn quality evaluation standard. To date, no morel spawn quality standard is available and the evaluation mostly depends on farmers' experience and pseudosclerotia quality (W. Liu et al. 2017a).

Spawn aging has been one of the major problems in morel cultivation (W. Liu et al. 2017a). The characteristics of spawn aging include fragile mycelia, slow mycelial growth rate, week anti-competitor ability and a delayed harvest, all of which will contribute to a reduced morel production. Senescence (aging process) of *M. elata*, characterized by slow mycelial growth, premature pigment production and death of hyphal tips, was observed by successive subculturing (P. He et al. 2015). Microscopy studies

revealed the involvement of autophagy, apoptosis and necrosis in senescence of *M. elata* (P. He et al. 2015). Nevertheless, the molecular mechanism of these behaviors needs further study.

Moreover, a decrease in spawn vigor is often observed in morel spawn production as well (personal communication). The application of weak spawn characterized by thin, weak mycelia and prolonged cultivation cycles may be detrimental to morel production directly. Since spawn quality is a key component of mushroom cultivation, more attention should be given to the issues on spawn quality of *Morchella*.

Reduction of soil nutrients

A continuous cropping cycle has demonstrated some problems in morel cultivation. The perpetual productivity of morels on the same soil tends to decrease yields after two to three years as reported by farmers. Yield reduction has forced farmers to find new land to cultivate. Unless researchers soon determine the essential nutrients for morel production, the cost of continuously cultivating new land for morel production may become prohibitive due to the need for shaded structures being built for outdoor cultivation.

Discussion

Between 1975 to 1995, researchers from all over the world have done many studies on the morphology, physiology, ecology, genetics and cultivation of *Morchella* spp.. However, research on *Morchella* have primarily been restricted to taxonomy during the past ten years. Before the development of DNA-sequencing techniques and molecular phylogenetic analysis, morphological based taxonomy of *Morchella* was used and is not as accurate since the morphology of *Morchella* varies under different development stages and under different climate and ecological conditions (Volk and Leonard 1989; Kuo

et al. 2012; Richard et al. 2015). Lately, molecular analyses based on the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) were conducted to investigate the phylogeny and biogeography of *Morchella* (O 'Donnell et al. 2011; Taşkın et al. 2012; Du et al. 2012a, 2012b; Kuo et al. 2012; Richard et al. 2015).

Understanding the life cycle and reproductive systems are critical for successful morel cultivation. The general life cycle of *Morchella* species was studied by a few researchers (Volk and Leonard 1990; Pilz et al. 2007; Alvarado-Gastillo et al. 2014) and the importance of pseudosclerotia in the entire life cycle of *Morchella* was emphasized. It has been proposed that *Morchella* fruit bodies are developed from pseudosclerotia produced from primary mycelia or secondary mycelia. However, no evidence can be found to support this theory. Factors affecting mycelial growth and pseudosclerotia formation on some *Morchella* spp. were investigated *in vitro* by several researchers (Volk and Leonard 1990; Amir et al. 1992, 1993, 1995; Zhao et al. 1997; Li et al. 1998; W. Liu et al. 2017a; Kanwal and Reddy 2011; Q. Liu et al. 2018). Nevertheless, the results and conclusions have not been consistent due to the difference strains and experimental designs. For example, light was considered a prohibiting factor on pseudosclerotia formation of *M. esculenta* (Volk 1989), while W. Liu et al. (2017a) noted that light actually promotes pseodosclerotia formation of *M. importuna* during spawn production. Q. Liu et al. (2018) concluded that light does not affect pseudosclerotia formation of *M. importuna*. Thus, it is necessary to study the biological characteristics of specific *Morchella* species to provide biological knowledge required for morel cultivation. Moreover, the exact role of conidia in the life cycle is not clear. Reproductive systems of *Morchella* spp. are not completely understood. Several studies indicated that many species in the Elata Clade are heterothallic (Pagliaccia et al. 2011; W. Liu et al. 2018). Du et al. (2017) proposed a mixed mating system of *Morchella* - heterothallism and haploid fruiting which forms sterile fruit bodies. However, more studies on the determination of mating systems of other *Morchella* spp, such as *M. rufobrunnea*, are needed to facilitate the strain selection for morel cultivation.

The trophic mode of *Morchella* has not been settled yet. The successful cultivation of *M. rufobrunnea*, *M. importuna*, *M. sextelata*, *M. septimelata*, and *M. esculenta* suggests that these species are saprophytic. However, an ectomycorrhizal association between *M. rotunda* and Pinaceae plants was also reported (Buscot and Kottke 1987, 1990). It seems that the trophic mode of *Morchella* varies according to the species. Some *Morchella* spp. may be only saprophytic, while some are mycorrhizal. It was also hypothesized that *Morchella* are facultative mycorrhizal species (Du et al. 2015), but no evidence was provided. For morel cultivation, it is critical to select feasible species that are able to obtain their nutrients saprophytically using the current cultivation procedures. Obligate mycorrhizal *Morchella* species are definitely not good options for morel cultivation.

A comparative complete outdoor morel cultivation process has developed in China, in which the addition of nutrient bags is one of the key steps. However, the necessity of adding the nutrient bags and how the nutrient makeup of the bags affects morel fruit body formation remain to be studied. Thus far, four *Morchella* spp. (*M. importuna*, *M. sextelata*, *M. septimelata*, and *M. esculenta*) have been cultivated commercially in China and *M. importun*a accounts for more than 95% of the morel cultivating area (W. Liu et al. 2017a). Compared to outdoor cultivation, which is largely restricted by the natural conditions, such as nutrients in the soil and weather conditions, indoor cultivation seems to be a better option to cultivate morels commercially on a year-round basis. However, whether the outdoor morel cultivation pattern is feasible for indoor cultivation is unknown. Even though there have been some studies on indoor morel cultivation (Zhu and He, 2007; Y. Liu et al. 2013), the feasibility of these techniques remains skeptical. There is an urgent need to develop an indoor morel cultivation procedure. Additional research is needed to determine the parameters (temperature, light condition, CO_2 level etc.) of indoor cultivation for specific *Morchella* spp.. In addition, strain selection is critical in both indoor and outdoor cultivation of morels. *Morchella* spp. are diverse (Richard et al. 2015) and it is the foundation to select the most suitable species for indoor and outdoor cultivation.

In this thesis, I correctly identified the *Morchella* isolates in the Penn State University culture collection using molecular phylogenetic analysis and studied the biological characteristics of four *Morchella* isolates (WC 833 *M. rufobrunnea*, China 1 *M. importuna*, WC 374 *M. americana* and WC 766 *M. exuberans*) *in vitro* with an emphasize on mycelial growth and pseudosclerotia formation aiming to provide information to improve on the success of future *Morchella* cultivation. Indoor morel cultivation research was also conducted in order to find a feasible procedure and select the potential *Morchella* isolates for commercial morel cultivation.

Chapter 2

Identification of *Morchella* Cultural Collection

Abstract

True morels (*Morchella*) are one of the most highly-prized edible fungi. Due to the high vaiability of apothecium color and shape of morels and the insufficient understanding of phenotypically informative morphological characteristics within the genus *Morchella*, morphological species recognition (MSR) has causing much confusion. The isolates in the *Morchella* cultural collection in the Spawn Lab at Penn State were identified decades ago based on morphology. As a result, many species seem to be misidentified. Here I proposed a revision of the Penn State *Morchella* cultural collection based on molecular phylogenetic analysis of the nuclear rDNA region encompassing the internal transcribed spacers 1 and 4, along with the 5.8S rDNA (ITS). Thirty-three isolates were identified in this study and the identifications of three of the isolates were confirmed by O'Donnell et al. (2011). Finally, the identifications of 36 isolates were determined, and fifteen, twenty, and one isolate in the updated *Morchella* cultural collection belong to the Esculenta Clade, the Elata Clade and the rufobrunnea Clade respectively. Based on the new identification of the *Morchella* cultural collection, four *Morchella* isolates were selected for further study.

Introduction

Morchella have been identified into three main clades, the Esculenta Clade, the Elata Clade and the rufobrunnea Clade (O'Donnell et al. 2011). These clades are comprised of at least 27, 36, and 2 phylogenetically distinct species respectively (O'Donnell et al. 2011; Du et al. 2012a; Votic et al. 2014). To date, there are 334 names identified under *Morchella*, including species, subspecies and varieties, reported in the latest database of the Index Fungorum

(http://www.indexfungorum.org/names/Names.asp). Due to the high diversity and variation in morphology under different environmental and ecological conditions and during different developmental stages, the use of homonyms and synonyms in *Morchella* spp. very often exists, which has caused confusion and difficulties in identifying *Morchella* spp. For instance, one species was identified as *Morchella conica*, which was determined to be illegitimate and was a synonym of *M. purpurascens* (Richard et al. 2015). Thirteen species from the Penn State collection were identified as *M. esculenta*, which was reported as the most commom Esculenta clade morel in North America (O'Donnell et al. 2011). However, a recent study indicated that *M. esculenta* is a European species and not present in North America (Richard et al. 2015). Thus, the identifications of numerous *M. esculenta* in the collection may be incorrect. Moreover, subsequent research relies on the correct identification of *Morchella* species. The objective of this research was to use the internal transcribed spacer (ITS) nuc rDNA (ITS1-5.8S-ITS4) to accurately identify the *Morcella* cultures in the Penn State University cultural collection.

Material and Methods

Morchella isolates

Thirty-eight *Morchella* isolates from the Penn State University Spawn Lab culture collection (Table 2-1).

Culture preparation and DNA extraction

Thirty-eight *Morchella* isolates were removed from liquid nitrogen storage to be recovered and inoculated onto the middle of 100 mm x15 mm Potato Dextrose Yeast Agar (PDYA) (Potato Dextrose Agar (Difco™ 39 g/L) + Yeast Extract (Difco™ 1.5 g/L)) plates at room temperature. When the mycelial

growth reached the edge of the plate, the edge of the colonies were cut using a 5 mm diameter cork-borer. Two plugs of mycelia from each isolate were transferred into 50 ml Potato Dextrose Broth (PDB) Media (Difco™ 24 g/L) in 250 ml flasks and incubated at room temperature. When the diameter of a colony in the PDB was approximately 2 cm, the samples were stored in a 4℃ refrigerator to inhibit growth of the faster growing cultures, until all of the isolates obtained a similar level of growth. Cultures were grown in the PDB for 3 to 7 days at room temperature depending on growth rates of the different isolates. Mycelia were then removed from the flask using a sterilized inoculation needle and placed in an Eppendorf tube (1.8 - 2.0 ml) at room temperature. Sterilized needles were used to squeeze out the liquid from the mycelia in the Eppendorf tube and the liquid was carefully poured out. Mycelia were then lyophilized for 48 hours. Freeze-dry samples were stored at -20℃ until they were used for DNA extraction.

DNA extraction was done following the instructions of the AllPrep® Fungal DNA/RNA/Protein Kit from MoBio (now Qiagen). After the DNA extraction, DNA samples were stored at -20℃ until used for further analyses.

Polymerase chain reaction (PCR) and agarose electrophoresis

PCR was used to amplify the ITS segments of DNA in the samples. The internal transcribed spacer and 5.8s rDNA (ITS) with ITS-1 (forward primer: TCCGTAGGTGAACCTGCGG)/ITS-4 (reverse primer: TCCTCCGCTTATTGATATGC) were used for all 36 samples (two isolates were dead) in this study. .For each PCR reaction, 1 µl of the template DNA was used in a 25 µl reaction mixture containing 0.5 µl of dNTP mix (10 mmol), 0.5 µl of each primer, 5 µl of 10 × PCR reaction buffer, 0.125µl *Taq* DNA polymerase (New England Biolabs, MA, USA) and 17.375 µl of sterile distilled water. The PCR program used included an initial 30 min hot start at 94 °C and 30 cycles of 20 s of denaturation at 94 °C, 30 s of annealing at 60 °C, and 60 s of elongation at 68°C, followed by an additional 5 min extension at 68 °C.

Subsequently, PCR products and a 1 kb DNA ladder (New England Biolabs, MA, USA) were separated on a 1% agarose gel stained by SYBR Green I Nucleic Acid Gel Stain (New England Biolabs, MA, USA) and observed under UV illumination. This step was to determine whether the PCR was successful, whether the resulting product was the correct size and whether the concentration of the resulting product was suitable for cycle sequencing.

Data collection and analysis

DNA samples were sent to the Nucleic Acid Facility at Penn State. The alignment and edition of the partial DNA sequence data was conducted with Geneious 10.2.3 (Biomatters Ltd). For each isolate, the ITS1 forward sequence and the ITS4 reserve sequence were aligned and poor quality bases were trimmed off the ends of the aligned sequence. Each aligned DNA sequence chromatogram file was viewed and analyzed visually to ensure that all ambiguous bases were correctly called and miscalled bases were edited manually in the software. The edited sequences were compared within Genbank and *Morchella* MLST (www.westerdijkinstitute.nl/Morchella/) to identify the species or isolates.

Results and Discussion

. WC 328 and WC 743 were not able to be recovered on either PDYA or malt extract media. The sequence data of WC 198 and WC 757 were too poor to analyze. In this study, PCR results showed that the primer sets ITS1 and ITS4 amplified an approximately 700 bp to 1500 bp fragment from 32 isolates (Figure 2-1). Three *Morchella* clades, the Elata Clade, the Esculenta Clade and the rufobrunnea Clade were included in this *Morchella* collection (Figure 2-1).. Most fragments amplified by the primer set ITS1 and ITS4 were around 1500 bp in the Esculenta Clade, while the sizes were generally smaller in the Elata Clade and the rufobrunnea Clade. The old ID only matched with the new ID in 8 isolates (Table 2-1).

Isolates identified as *M. americana* were the most abundant in the *Morchella* collection, followed by isolates identified as *M. importuna* and *M. esculenta*. *M. americana* was thought to be native to North America and was recently introduced to Europe (Richard et al. 2015). *M. esculenta* was only found in Europe and has been one of the most common and widely distributed yellow morels in Europe (Richard et al. 2015). Two Pennsylvania collected *Morchella* isolates (WC 327 and WC 374) which were previously identified as *M. esculenta* are actually *M. americana* and no Pennsylvania collected isolate was identified as *M. esculenta*. *M. importuna* has been the most widely cultivated *Morchella* spp. in China. It is a widespread and genetically variable species (O'Donnell et al. 2011). *M. exuberans*, *M. tomentosa* and *M. eximia* were reported to be fire-adapted *Morchella* species (O'Donnell et al. 2011).

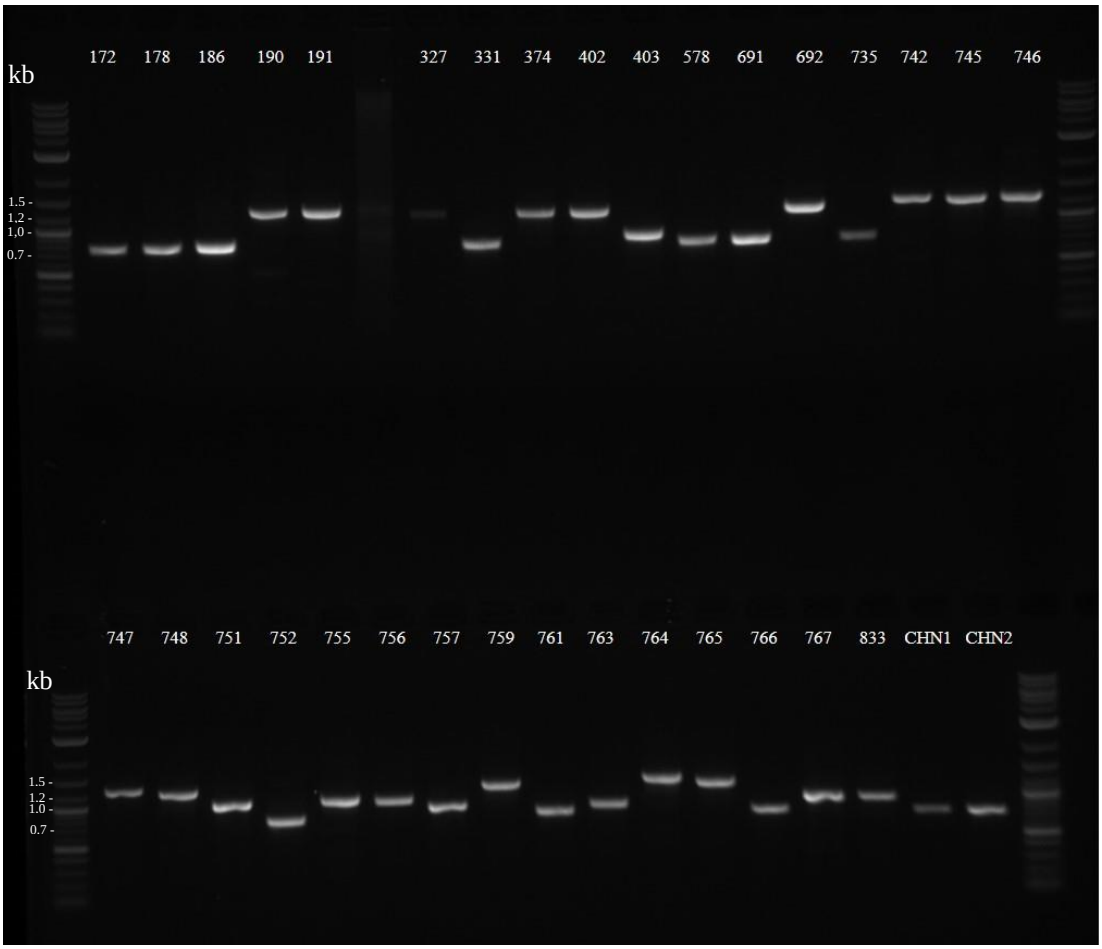


Figure 2-1. PCR amplification of ITS1-5.8S-ITS4 region of *Morchella* isolates

The first and last lane represent the 1 kb New England Biolabs DNA ladder. The numbers above the bands indicate the PCR products were amplified from the corresponding DNA of *Morchella* isolates. For example, number 172 refers to *Morchella* isolate WC 172. CHN1, CHN2 refers to isolate China 1, isolate China 2 respectively.

List of *Morchella* isolates in the cultural collection belonging to the Esculenta Clade (O'Donnell et al. 2011) **based on the results in this study.**

WC 190 (NRRL # 20872) *M. prava*: ITS sequence data was 100% identical (1071/1071 bp) to *M. prava* (GenBank accession JQ723099, Dewsbury et al. 2013). The same isolate was identified as *M. prava* by O'Donnell et al. 2011. The culture was collected in West Kettle River, British Columbia, Canada.

WC 191 *M. prava*: ITS sequence data was 100% identical (1071/1071 bp) to *M. prava* (GenBank accession JQ723099, Dewsbury et al. 2013). The culture was collected in West Kettle River, British Columbia, Canada. It was a duplicated of WC 190.

WC 327 *M. americana*: ITS sequence data was 100% identical (1030/1030 bp) to *M. esculentoides* (GenBank accession JQ723067; Du et al. 2012). The name *M. esculentoides* was corrected to *M. americana* by Richard et al. (2015). The culture was collected in Julian, PA, USA.

WC 374 *M. americana*: ITS sequence data was 99.8% identical (1038/1040 bp) to *M. americana* (GenBank accession KM587949; Richard et al. 2015). The culture was collected in Fisherman's Paradise, PA, USA.

WC 402 *M. americana*: ITS sequence data was 100% identical (1030/1030 bp) to *M. esculentoides* (GenBank accession JQ723067; Du et al. 2012). And the name *M. esculentoides* was corrected to *M. americana* by Richard et al. (2015).

WC 692 (NRRL # 20877) *M. americana*: ITS sequence data was 99.8% identical (1040/1042 bp) to *M. americana* (GenBank accession KM587975; Richard et al. 2015). The same isolate was identified as *M. americana* by O'Donnell et al. (2011). The culture was collected in UT, USA.

WC742 (NRRL # 20853) *M. esculenta:* ITS sequence data of WC742 had 99.8% identity (1131/1133 bp) to *M. esculenta* (GenBank accession KM588023; Richard et al. 2015). According to updated information from USDA, NRRL 20853 was identified as *M. esculenta* (Link) Persoon, and the same as CBS 275.88 in CBS catalog. It was collected in Czechoslovakia in 1993.

WC 745 (NRRL # 20884 or 66866) *Mel*-12: ITS sequence data was 100% identical (1112/1112 bp) to *Mel*-12 (GenBank accession JQ723079.1; Du et al. 2012). The same isolate was identified as Mel-1by O'Donnell et al. (2011). The culture was collected in Japan.

WC 746 (NRRL # 20885 or 66865) *Mes*-9*:* ITS sequence data was 99.9% identical (1141/1142 bp) to *Mes*-9 (GenBank accession KM485936.1; Chen et al. 2014, unpublished) and was 100% identical (1112/1112 bp) to *Mes*-9 (GenBank accession JQ723094; Du et al. 2012). The same isolate was identified as *Mes*-9 by O'Donnell et al. (2011).The culture was collected in Japan.

WC 747 (NRRL # 20886) *Mes*-9: ITS sequence data was 99.9% identical (1141/1142 bp) to *Mes*-9 (GenBank accession KM485936.1; Chen et al. 2014, unpublished) and was100% identical (1126/1126 bp) to *Mes-9* (GenBank accession JQ723094; Du et al. 2012). The same isolate was identified as *Mes-9* by O'Donnell et al. (2011).

WC 748 (NRRL # 20914 or 66862) *M. ulmaria*: ITS sequence data was 100% identical (1058/1058 bp) to *M. ulmaria* (GenBank accession JQ723091; Du et al. 2012). The identification of Du was also confirmed by Richard et al. (2015).

WC 759 (NRRL # 22335) *M. americana*: ITS sequence data was 99.8% identical (1031/1034 bp) to *M. americana* (GenBank accession KM587975; Richard et al. 2015).

WC 765 (NRRL # 22476 or 66864) *M. americana*: ITS sequence data was 99.8% identical (1032/1034 bp) to *M. americana* (GenBank accession KM587975; Richard et al. 2015). The same isolate was identified as *M. americana* by O'Donnell et al. (2011).

WC 764 (NRRL # 22475) *M. esculenta*: ITS sequence data was 99.8% identical (1130/1131 bp) to *M. esculenta* (GenBank accession KM588023; Richard et al. 2015).

List of *Morchella* isolates in the cultural collection belonging to the rufobrunnea Clade (O'Donnell et al. 2011) **based on the results in this study.**

WC 833 *M. rufobrunnea*: ITS sequence data had 99.9% identity (842/843 bp) to *M. rufobrunnea* (GenBank accession KM588017, Richard et al. 2015).

List of *Morchella* isolates in the cultural collection belonging to the Elata Clade (O'Donnell et al. 2011) **based on the results in this study.**

WC 172 *M. importuna*: ITS sequence data was 100% identical 672/672 bp) to *M. importuna* (GenBank accession KM204675; Carris et al. unpublished).

WC 178 *M. importuna*: ITS sequence data was 100% identical (672/672 bp) to *M. importuna* (GenBank accession KM204675; Carris et al. unpublished).

WC 186 (NRRL # 20869) *M. brunnea*: ITS sequence data had 99.6% identity (711/714 bp) to *M. brunnea* (GenBank accession FJ594059, Sulej et al. unpublished) and 100% identity (672/672 bp) to *M. brunnea* (GenBank accession KM204675, Carris et al. unpublished). The same isolate was identified as *M. brunnea* by O'Donnell et al. (2011). The culture was collected in West Kettle River, British Columbia, Canada.

WC 331 (NRRL # 20871) *M. angusticeps*: ITS sequence data was 100% identical (681/681 bp) to *M. angusticeps* (GenBank accession GU551410; O'Donnell et al. 2011). The same isolate was identified as *M. angusticeps* by O'Donnell et al. (2011). The culture was collected in State College, PA, USA.

WC 403 (NRRL # 20826) *M. punctipes*: ITS sequence data was 99.6% identical (825/825 bp) to *M. tomentosa* (GenBank accession KM588008; Richard et al. 2015). The same isolate was identified as *M. punctipes* by O'Donnell et al. (2011).

WC578 *M. eohespera*: ITS sequence data was 100% identical (705/705 bp) to *M. eohespera* (GenBank accession KT819369, O'Donnell et al. 2015).

WC 691 *M. importuna*: ITS sequence data was 99.8% identical (718/720 bp) to *M. importuna* (GenBank accession MG121861.1, Li et al. 2017).

WC735 (NRRL # 25407) *M. angusticeps*: ITS sequence data was 100% identical (681/681 bp) to *M. angusticeps* (GenBank accession JN085151; O'Donnell et al. unpublished) and 99.8% identicle to

M. angusticeps (GenBank accession GU551410; O'Donnell et al. unpublished). The same isolate was identified as *M. angusticeps* by O'Donnell et al. (2011). The culture was collected in Kent, OH, USA.

WC 751 (NRRL # 22207) *M. tridentina*: ITS sequence data was 99.7% identical (878/881 bp) to *M. tridentina* (GenBank accession KM587964; Richard et al. 2015). The same isolate was identified as *M. tridentina* by O'Donnell et al. (2011).

WC 752 (NRRL # 22208) *Mel*-8: ITS sequence data was 99.8% identical (650/651 bp) to *Mel*-8 (GenBank accession MH127532, Rockefeller et al. et al. 2018). The same isolate was identified as *Mel*-8 by O'Donnell et al. (2011).

WC 755 (NRRL # 20851) *M. tridentina*: ITS sequence data was 100% identical (886/886 bp) to *M. tridentina* (GenBank accession KT875083; Garibay-Orijel et al., unpublished) and 99.6% identical (892/896 bp) to *M. tridentina* (GenBank accession KM587964, Richard et al. 2015). The same isolate was identified as *M. tridentina* by O'Donnell et al. (2011).The culture was collected in BC, Canada.

WC 756 (NRRL # 22314) *M. tomentosa*: ITS sequence data was 99.8% identical (823/825 bp) to *M. tomentosa* (GenBank accession KM587989; Richard et al. 2015).

WC761 (NRRL # 22447) *Mel*-23: ITS sequence data had 100% identity (713/713 bp) to *Mel*-23 (GenBank accession JQ618526.1, Du et al. 2012). The same isolate was identified as *Mel*-23 by O'Donnell et al. (2011). This culture was collected in Finland.

WC 763 (NRRL # 20862) *M. Punctipes*: ITS sequence data was 100% identical (726/726 bp) to *M. Punctipes* (GenBank accession JQ723026; Du et al. 2012). ITS sequence data had 99.6% identity (764/767 bp) to *M. Punctipes* (GenBank accession KM588008, Richard et al. 2015).

WC 766 (NRRL # 22216) *M. exuberans*: ITS sequence data had 100% identity (718/718 bp) to *M. exuberans* (GenBank accession KM587918, Richard et al. 2015). The same isolate was identified as *M. exuberans* by O'Donnell et al. (2011).

WC 767 (NRRL # 22218) *M. tomentosa*; ITS sequence data was 100% identical (825/825 bp) to *M. tomentosa* (GenBank accession KM587989; Richard et al. 2015). The same isolate was identified as *M. tomentosa* by O'Donnell et al. (2011).

China 1 *M. importuna*: ITS sequence data was 100% identical (710/710 bp) to *M. importuna* (GenBank accession JQ618780; Du et al. 2012). The culture was collected in China.

China 2 *M. importuna*: ITS sequence data was 99.7% identical (707/709 bp) to *M. importuna* (GenBank accession JQ618776; Du et al. 2012). The culture was collected in China.

In summary, 33 *Morchella* isolates were identified based on molecular phylogenetic analysis of ITS region from the original 38 isolates recovered from the Penn State University *Morchella* cultural collection. WC 328 was dead and China 3 was contaminated, so no new identification was made for these two isolates. WC 198 and WC 757 were identified as *M. brunnea* and *M. popliphila* by O'Donnell et al. (2011). WC 743 was identified as *M. semilibera* by O'Donnell (unpublished). These three identifications were adopted in this study. Finally, the identifications of 36 *Morchella* isolates were determined. Fifteen, twenty and one isolate in the updated *Morchella* cultural collection belong to the Esculenta Clade, the Elata Clade and the rufobrunnea Clade respectively (O'Donnell et al. 2011). The origination area of these isolates include: Asia, Europe and North America. Results of this study should help inform *Morchella* isolates selection for further research. For morel cultivation. Saprophytic *Morchella* species with high

potential for commercial cultivation are the ones that should be focused on. Based on this updated list of *Morchella* cultural collection (Table 2-1), four *Morchella* isolates were selected for the subsequent biological characteristic studies and morel indoor cropping experiment: WC 833 *M. rufobrunnea*, China 1 *M. importuna*, WC 374 *M. americana* and WC 766 *M. exuberans*. WC 833 *M. rufobrunnea* originated from a commercial isolate cultivated in North America. A successful fruit body initiation of *M. rufobrunnea* in laboratory-scale experiments was reported in 2010 (Masaphy 2010). The mature fruit bodies of *M. rufobrunnea* have been successfully initiated in the Mushroom Research Center (MRC) at Penn State. China 1 was originated from China and *M. importuna* is the most widely cultivated *Morchella* species in China (W. Liu et al. 2017a). *M. americana* is native to North America and was thought to be recently introduced to Europe (Richard et al. 2015). WC 374 was collected in PA, USA, so it might be a potential *Morchella* isolate that can be cultivated outdoors in Pennsylvania. *M. exuberans* was reported to be fire-associated species (Richard et al. 2015). Previous cultivation of *M. rufobrunnea* (Ower 1982) and *M. importuna* (Du et al. 2015; W. Liu et al. 2017a) indicates these two species are saprophytic. *M. americana* is often found under ashes dead and dying elm trees, but occasionally it can be found associated with conifers (information provided by Midwest American Mycological Information (MAMI)), which indicates this species is mostly likely saprophytic but can be mycorrhizal. *M. exuberans* often fruits on conifer burning sites (Miller et al. 2017) suggesting that *M. exuberans* might be mychorrhizal, but it fruit once the tree is killed. Lastly, other phylogenetic informative loci are needed in order to better understand the important facets of morel cultivation, such as nutrient mode and mating systems of different *Morchella* species.

Table 2-1. Identification of *Morchella* cultural collection

WC #[a]	NRRL #	Old ID in collection	New ID in this study	Clade/Lineage	Collected Area	Month/year collected
833	-[b]	*M. esculenta*	*M. rufobrunnea*	rufobrunnea	Terry Farms, Auburn, AL-USA	8/25/1998
190	20872	*M. deliciosa*	*M. prava*	Esculenta	West Kettle Creek, British Columbia, Canada	-
191	-	*M. deliciosa*	*M. prava*	Esculenta	West Kettle Creek-duplicate, British Columbia, Canada	-
327	-	*M. esculenta*	*M. americana*	Esculenta	Julian, PA-USA	5/20/1981
374	-	*M. esculenta*	*M. americana*	Esculenta	LCS; Fisherman's Paradise,PA-USA	6/4/1905
402	-	*M. crassipes*	*M. americana*	Esculenta	-	5/9/1983
691	-	*M. elata*	*M. esculenta*	Esculenta	-	7/18/1986
692	20877	*M. esculenta*	*M. americana*	Esculenta	UT-USA	7/18/1986
742	20853	*M. esculenta*	*M. esculenta*	Esculenta	-	9/1/1993
745	20884/66866	*M. esculenta*	*Mes*-12	Esculenta	Japan	9/1/1993
746	20885/66865	*M. esculenta*	*Mes*-9	Esculenta	Japan	9/1/1993
747	20886	*M. esculenta*	*Mes*-9	Esculenta	-	9/1/1993
748	20914/66862	*M. esculenta*	*M. ulmaria*	Esculenta	-	9/1/1993
759	22335	*M. esculenta*	*M. americana*	Esculenta	-	9/1/1993
764	22475	*M. esculenta*	*M. esculenta*	Esculenta	Oland, Sweden	9/1/1993
765	66864	*M. esculenta*	*M. americana*	Esculenta	Cove Junction, OR-USA	9/1/1993
172	-	*Morchella angusticeps*	*M. importuna*	Elata	Wrede isolate	5/13/1975
178	-	*M. angusticeps*	*M. importuna*	Elata	Merritt's #2	5/24/1975

186	20869	*M. angusticeps*	*M. brunnea*	Elata	West Kettle River, British Columbia, Canada	5/30/1975
198	20870	*M. angusticeps*	*M. brunnea*	Elata	Juliet Creek, British Columbia, Canada	6/22/1975
331	20871	*M. angusticeps*	*M. angusticeps*	Elata	St. College, PA-USA	5/5/1981
403	20826	*M. semilibera*	*M. punctipes*	Elata	PA-USA	5/1/1983
578	-	*M. elata*	*M. eohespera*	Elata	-	2/4/1986
735	25407	*M. conica*	*M. angusticeps*	Elata	B. Bunyard; Kent, OH-USA	4/30/1993
743	20859	*M. semilibera*	*M. semilibera*	Elata	-	9/1/1993
751	22207	*Morchella sp.*	*M. tridentina*	Elata	-	9/1/1993
752	22208	*Morchella sp.*	*Mel*-8	Elata	-	9/1/1993
755	20851	*Morchella sp.*	*M. tridentina*	Elata	-	9/1/1993
756	22314	*M. esculenta var. atrotomentosa*	*M. tomentosa*	Elata	-	9/1/1993
757	22315	*M. semilibera*	*M. populiphila*	Elata	-	9/1/1993
761	22447	*M. elata*	*Mel*-23	Elata	Finland	9/1/1993
763	20862	*M. semilibera*	*M. punctipes*	Elata	-	9/1/1993
766	22216	*M. elata*	*M. exuberans*	Elata	OR-USA	9/1/1993
767	22218	*M. esculenta var. atrotomentosa*	*M. tomentosa*	Elata	OR-USA	9/1/1993
China 1	-	*M. importuna*	*M. importuna*	Elata	China	2017
China2	-	*M. importuna*	*M. importuna*	Elata	China	2017
328	-	*M. esculenta*	-	-	Julian, PA-USA	5/20/1981
China3	-	*M. importuna*	-	-	China	2017

a. The reference number of *Morchella* isolates from the Penn State University Spawn Lab culture collection.

b. (-): Not known.

Chapter 3

Studies of Biological Characteristics of Four *Morchella* isolates

Abstract

Morels are one of the most prized edible fungi and morel cultivation has been catching researchers' interests. Mycelial growth is the first stage in the morel life cycle and it is believed that pseudosclerotia formation is one of the most important phases during the morel life cycle. Good mycelial growth and satisfactory pseudosclerotia formation are thought to be required for successful morel cultivation. However, how environmental factors effect mycelial growth and pseudosclerotia formation of many *Morchella* species is unknown. Even though there have been some reports accessing the effects of environmental parameters on mycelial growth or pseudosclerotia formation of *Morchella*, there is little known about the specific requirements due to the ambiguity of species in many of these studies. In this study, the effects of pH, light intensity and temperature on mycelial growth and pseudosclerotia formation of four *Morchella* isolates selected from Penn State *Morchella* cultural collection（*Morchella rufobrunne*, *M. importuna*, *M. americana* and *M. exuberans*) were investigated. Conditions resulting in both the fastest mycelial growth and most pseudosclerotia production *in vitro* were pH 6, darkness (0 lux), and 20 to 25°C for *M. rufobrunnea*, pH 5 to 6, darkness (0 lux), and 20 to 25°C for *M. importuna*. An Initial pH of the medium from 5 to 8 did not significantly affect pseudosclerotia formation of *M. rufobrunnea* and *M. importuna*. *M. americana*, and *M. exuberans* did not produce any pseudosclerotium under any of the test conditions in this study. The optimum environmental conditions for mycelial growth of *M. americana* was found to be pH 5 to 7, darkness (0 lux) to low light intensity (400 lux), and 20°C. Mycelia of *M. exuberans* achieved the fastest mycelial growth in media with a pH value of 5, 25 to 30°C, and light intensity had no influence on mycelial growth. This work will promote a better understanding of

the effect of environmental factors on mycelial growth and pseudosclerotia formation of *Morchella*, which are necessary for the improvement of morel cultivation.

Introduction

Morchella species are highly prized, edible fungi belonging to Ascomycota, Pezizomycetes, Pezizales, Morchellaceae, and *Morchella*. Due to their short fruiting season, favorable flavor and value in the food and medical industry, mycologists have been attempting to cultivate morels for decades, and it has proven very difficult. Ower et al. (1986) claimed successful indoor cultivation of *M. rufobrunnae* in 1982 and subsequently applied for three patents (US Patents 4594809, 4757640, and 4866878) for morel cultivation. These studies revealed the optimal temperature, humidity and other essential factors during different stages, as well as leading to detailed studies on the life cycle of morels. Mycelial growth is the first stage in the life cycle of *Morchella* species. Moreover, the essential role of establishment of pseudosclerotium was emphasized and has become the central focal point in morel fruiting. Many fungi are able to produce durable structures, such as pseudosclerotium to facilitate dispersion or survival. The pseudosclerotium of morels is a compact structure composed of large cells with thick walls which allows the fungus to survive under unfavorable environmental conditions (Volk and Leonard 1990). Formation of pseudosclerotium of morels is also a strategy the fungus uses for nutrient storage (Buscot, 1989). Amir et al. (1992, 1993, 1995) studied the effect of medium composition and water potential on pseudosclerotia formation on *M. esculenta* using the split plate method. Volk and Leonard (1989) used a jar method to study the effect of a variety of conditions on pseudosclerotia formation. They pointed out that complex medium worked better for pseudosclerotia formation and a smaller sized container achieved better biological efficiency. He also stated that illumination was a limiting factor for the development of pseudosclerotia. However, during the process of making spawn, there is a significant difference between the light side and the dark side of the spawn container if it is not rotated on a regular basis (W. Liu et al. 2017a). On the side that was occasionally exposed to light, large amounts of pseudosclerotia were

produced. On the contrary, production of pseudosclerotia on the dark side was poor, indicating that light plays a role in pseudosclerotia formation.

Good mycelia growth and satisfactory pseudosclerotia formation is required for successful morel cultivation. The first step to successfully cultivate morels is to be able to culture them under conditions that maximum their mycelial growth and pseudosclerotia formation. Environmental factors may have an effect on the mycelial growth and sclerotia formation of *Morchella.* Even though there have been some reports accessing the effects of environmental parameters on the mycelial growth or pseudosclerotia formation on *Morchella* (S. poplarLi et al. 1998; Zhao et al. 1998; X. Liu et al. 2004; Kalyoncu et al. 2009), many of them did not provide the name of the species. Besides, the identification of *Morchella* in previous studies may be inaccurate due to the use of homonyms and synonyms (Richard et al. 2015) and poorly annotated sequences in GenBank (Du et al. 2012b). Therefore, previous studies provide little reference value for culture and morel cultivation. The aim of this study was to quantify the effect of pH, light intensity and temperature on the mycelial growth and pseudosclerotia formation of four *Morchella* isolates: *Morchella importuna, M. rufobrunnea, M. americana and M. exuberans*. Here we hypothesized that: 1) For each *Morchella* isolate, there is a pH range and a temperature range that it can grow, but there is an optimal pH and temperature that results in the fastest mycelial growth and most pseudosclerotia production. 2) The effect of light on *Morchella* varies by species and 3) Not all tested *Morchella* isolates can produce pesudosclerotia under tested conditions.

Material and Methods

Morchella isolates

Four *Morchella* isolates; *Morchella importuna, M. rufobrunnea, M. americana* and *M. exuberans* were selected from the Penn State culture collection to be utilized for this study.

Table 3-1. Four isolates used in this study.

WC #[a]	NRRL #	Species	Clade/Lineage	Collected Area	Month/year collected
833	-[b]	*M. rufobrunnea*	rufobrunnea	Terry Farms, Auburn, AL-USA	8/25/1998
China 1	-	*M. importuna*	Elata	China	2017
374	-	*M. americana*	Esculenta	LCS; Fisherman's Paradise,PA-USA	6/4/1905
766	22216	*M. exuberans*	Elata	OR-USA	9/1/1993

a. The reference number of *Morchella* isolates from the Penn State University Spawn Lab culture collection.

b. (-): Not known.

Effect of pH on the mycelial growth and pseudosclerotia formation

Potato Dextrose yeast Agar (PDYA) medium was prepared in four flasks and the pH was adjusted to 5, 6, 7 and 8 respectively using 1 N NaOH or HCl and measured using a pH meter (Sesion MM374, Hatch) before sterilization (Farooq et al. 2014). Agar plugs (5 mm diameter) containing actively growing *Morchella* mycelia were taken from seven-day old cultures using a cork borer, and were placed in the center of the 100 mm × 15 mm PDYA (PDA(Difco™ 39 g/L) + Yeast Extract (Difco™ 1.5 g/L)) plate and sealed with parafilm. Only one plug was inoculated to one plate. Inoculated dishes were incubated at room temperature (20 ± 1°C). Five replicates were maintained for each isolate growing under each pH condition. The experiments were repeated three times.

Dishes were observed daily for characteristic mycelia and pseudosclerotia formation. Two perpendicular straight lines were drawn on the bottom of the petri dishes with the crossing point coinciding with the center of the 5 mm disc. Radial growth measurements (mm) were recorded every day for each isolate until the mycelial growth reach the petri dish edge in any dish. Pseudosclerotia can be identified by the following characteristics: 1) Pseudosclerotia are convex, round or irregular, condensed masses surrounding by mycelia. There is a clear boundary between pseudosclerotia and mycelia in the

plate. 2) Pseudosclerotia usually form near the inoculation cite and the plate edges. 3) Pseudosclerotia are white at first and then turn yellow to brown. 4) The sites of the agar where pseudosclerotia form are usually brown pigmented. Mycelial color, colony shape, distribution of mycelia and, if present, the color, shape, area and distribution of pseudosclerotia in each dish was recorded for 20 days post inoculation (dpi). The area of pseudosclerotia in each dish was measured using Image J software (https://imagej.nih.gov/ij/), an open source image processing system.

Effect of light on the mycelial growth and pseudosclerotia formation

PDYA medium was prepared in flasks and the pH of the media was adjusted to 6 by using 1 N NaOH and measured with a pH meter (Sesion MM374, Hatch) before sterilization. The culture preparation was made following the previously described method. The effect of light on mycelial growth and pseudosclerotia formation of four *Morchella* isolates were studied by growing the fungal cultures under the following light conditions: 0 lux, 400 lux continuous light and 2000 lux continuous light provided by LED lights (Lightning Ever®, 12V, 6000K). Light intensity was measured by a Dr. Meter® digital light meter. All plates were incubated at 25°C. The temperature of the chambers was monitored using a thermometer and recorded every 2 days to confirm that the light source did not increase the temperature in the chambers. Five replicates were maintained for each isolate growing under each light condition. The experiments were repeated three times. The measurement of mycelial growth and pseudosclerotia formation was made following the previously described method.

Effect of temperature on the mycelial growth and pseudosclerotia formation

PDYA medium was prepared in flasks and the pH of the media was adjusted to 6 by using 1 N NaOH. The culture preparation was made following the previously described method. The effect of

temperature on mycelial growth and pseudosclerotia formation was studied by growing the fungal cultures at 20°C, 25°C and 30°C in incubators without light. The temperatures of the chambers were checked using a hand-held thermometer and recorded every 2 days. The experiments were repeated three times. The measurement of mycelial growth and pseudosclerotia formation was made following the previously described method.

Morphological characteristics of pseudosclerotia development

Agar plugs (about 5 mm diameter) containing actively growing mycelia of *M. rufobrunnea* or *M. importuna* (these were the only 2 species that formed pseudosclerotia under the conditions being tested) were transferred from seven-day old cultures using a cork borer and were placed in the center of the Petri dishes containing PDYA media with initial pH of 6. Colonies of *M. rufobrunnea* and *M. importuna* were observed using a digital microscope (Celestron 44308, US) (× 40). The pseudosclerotia were taken out from the media with a sterilized hood and chopped into small strips on a slide, covered with a glass coverslip and observed with a Nikon Eclipse E400 microscope (Nikon, Minatoku, Tokyo, Japan) (× 400 and × 1000) to characterize the pseudosclerotia.

Data analysis

The experimental design for the growth characteristics were completely randomized factorial experiments (four isolates and four pH media, four isolates and three light conditions, four isolates and three temperature), with 15 replicates (5 plates × 3 replicates) per treatment. The non-parametric analysis of variance (Kruskal-Wallis test, $p<0.05$) was carried out on the data for mycelial radial growth and pseudosclerotia formation. A multiple comparison test (Pairwise Wilcox test, $p<0.05$) was carried out on data using R software (R.app GUI 1.70).

Results and Discussion

Effect of pH on mycelial growth and pseudosclerotia formation

In this study, all *Morchella* species grew over the pH range of 5 to 8. For *M. rufobrunnea*, the complete colonization of at least one dish was observed at 4 dpi. At 4 dpi, significant differences were only observed between treatments pH5 and pH6 for mycelium radial growth ($p<0.05$) and the fastest growth was observed on pH6 (Figure 3-1Aa). There was no significant difference in pseudosclerotia area among all the pH levels tested (Table 3-2) ($p>0.05$).

M. importuna generally colonized the entire dish in 3 to 4 days. PDYA medium with an initial pH of 5 and 6 resulted in the fastest mycelial growth among all the pH levels tested. A pH of 7 and 8 suppressed the mycelial growth of *M. importuna* compared to a pH of 5 or 6. At 4 dpi, the growth differences between pH5, pH6 and pH7, pH8 were statistically significant ($p<0.0001$) (Figure 3-1Ab). The colony morphological characteristics for the different pH levels were similar (Figure 3-2b). There was no significant difference in pseudosclerotia area among all the pH levels tested (Table 3-2) ($p>0.05$).

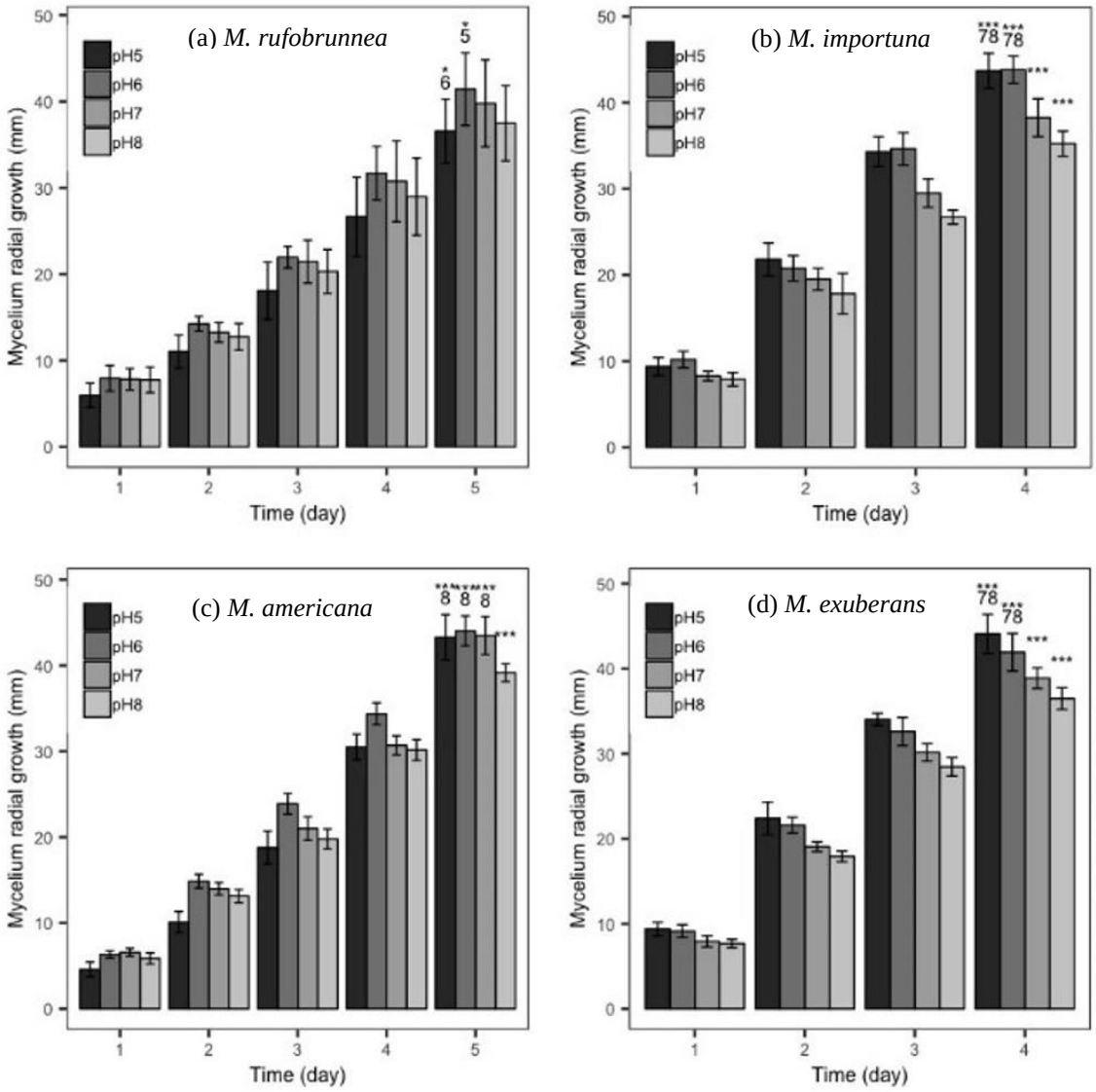
M. americana grew well on PDYA with a pH of 5, 6 or 7, but it grew significantly slower at pH 8 ($p<0.0001$) (Figure 3-1Ac). The colony morphological characteristics were not different at any of the pH levels tested (Figure 3-2c). Mycelia was first white to light yellow and turned to orange around 3 dpi. No pseudosclerotium was observed at 20 dpi (Table 3-2).

Table 3-2. Effect of pH, light and temperature on pseudosclerotia formation of four *Morchella* isolates

Isolate	Treatment	Pseudosclerotia area (mm^2)[a]	Isolate	Treatment	Pseudosclerotia area (mm^2)
M. rufobrunnea	pH5	550.05 ± 183.97 abcde	*M. americana*	pH5	-[b]
	pH6	651.91 ± 385.87 abcde		pH6	-
	pH7	442.52 ± 150.13 de		pH7	-
	pH8	424.64 ± 212.84 e		pH8	-
	Dark	544.61 ± 232.21 abcde		Dark	-
	Low light	160.95 ± 153.02 f		Low light	-
	High light	13.15 ± 45.47 g		High light	-
	20°C	508.93 ± 167.6 bde		20°C	-
	25°C	627.38 ± 206.19 bde		25°C	-
	30°C	0 ± 0 g		30°C	-
M. importuna	pH5	538.98 ± 162.34 abcde	*M. exuberans*	pH5	-
	pH6	715.78 ± 247.38 acd		pH6	-
	pH7	671.93 ± 186.03 acde		pH7	-
	pH8	626.93 ± 144.50 ac		pH8	-
	Dark	727.66 ± 288.86 ab		Dark	-
	Low light	690.38 ± 314.58 ab		Low light	-
	High light	113.26 ± 164.37 f		High light	-
	20°C	543.5 ± 220.83 abde		20°C	-
	25°C	509.67 ± 192.45 abde		25°C	-
	30°C	0 ± 0 g		30°C	-

a. Data shown are the means of 15 replicates from three independent experiments ± SD. Values followed by the same letter are not significantly different according by the Pairwise Wilcox test ($p = 0.05$). Pseudosclerotia area was measured at 20 dpi.
b. (-): no pseudosclerotia produced

A


(a) M. rufobrunnea
(b) M. importuna
(c) M. americana
(d) M. exuberans
pH5
pH6
pH7
pH8
Mycelium radial growth (mm)
Time (day)

B

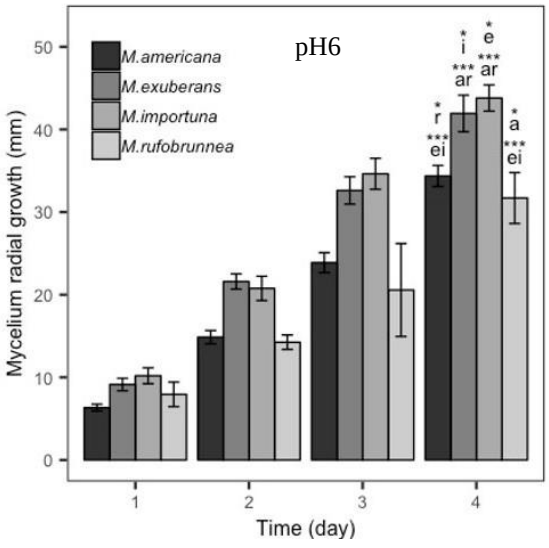

pH6
M.americana
M.exuberans
M.importuna
M.rufobrunnea
Mycelium radial growth (mm)
Time (day)

Figure 3-1. Effect of pH on mycelium radial growth of four *Morchella* isolates

A. Effect of pH on mycelium radial growth of (a) *M. rufobrunnea*, (b) *M. importuna*, (c) *M. americana* and (d) *M. exuberans*. Data shown are the means of 15 replicates from three independent experiments ± standard deviation (SD). Statistical analysis was conducted on the data of the last measuring day for each isolate. Bars with "*", "**", "***" at the top without numbers represent values that are significantly different from all other pH values with a p value less than 0.05, 0.001, 0.0001 respectively. Bars with "*", "**", "***" at the top with numbers represent values that are only significantly different from pH values listed below the asterisk(s) with a p value less than 0.05, 0.001, 0.0001 respectively. Numbers "5", "6", "7" and "8" represent "pH5", "pH6", "pH7", and "pH8" respectively.

B. Mycelium radial growth of *M. rufobrunnea*, *M. importuna*, *M. americana* and *M. exuberans* at pH6. Data shown are the means of 15 replicates from three independent experiments ± standard deviation (SD). Bars with "*", "***" at the top with letter(s) represent values that are only significantly different from species listed below the asterisk(s) with a p value less than 0.05, 0.0001 respectively. Letters "r", "I", "a", and "e" represent "*M. rufobrunnea*", "*M. importuna*", "*M. americana*" and "*M. exuberans*" respectively.

M. exuberans generally completely colonized the dish at 4 dpi. Significant differences were observed among growth rates for all pH conditions at 4 dpi (pH5 and pH6, $p<0.001$; others, $P<0.0001$). The optimal pH for mycelial growth was pH 5 and the growth decreased as the pH of the medium increased. Mycelia was white and aggregated, condensed at the edge of growth, but was relatively sparse in the center. Aged growth rings were formed as the development continued. The mycelia under pH 5 and 6 appeared more condensed than mycelia under pH 7 and 8 (Figure 3-2d). No pseudosclerotium was observed at 20 dpi (Table 3-2).

Species comparisons

At 4 dpi, all four isolates were significantly different from each other when grown on medium with pH 6 in terms of mycelial growth. *M. importuna* had the fastest growth rate, followed by *M. exuberans*, *M. americana* and *M. rufobrunnea* (Figure 3-1B). There was a significant difference between *M. importuna* and *M. exuberans* in terms of mycelial growth at 4 dpi ($P<0.05$). Mycelial radial growth of *M. importuna* and *M. exuberans* was significant faster than *M. americana* and *M. rufobrunnea* ($P<0.0001$).

X. Liu et al. (2004) reported the optimal pH for mycelial growth and pseudosclerotia formation for two *Morchella* spp. (species names were not indicated in the paper) was between 5 and 7, indicating the tested *Morchella* isolates favors slightly acid to neutral environment. J. He et al. (2004) concluded that the optimal pH for mycelial growth of five *Morchella* isolates (species names were not indicated in the paper) was 6.5. However, Chen and Guo (2015) reported that *M. esculenta* grew well at pH values between 6.4 to 8.7, which was neutral to slightly alkaline, and the optimal pH was 7.7. Q. Liu et al. (2018) reported mycelia of *M. importuna* grew well at pH values between 4 to 9, and pseudosclerotia weight was not significant different for pH 5, 6, 7, and 8, which is consistent with our results. It seems that most *Morchella* species can grow at pH values between 5 to 8. However, the optimal pH differs

according to specific *Morchella* isolates. Chen and Guo (2007) pointed out that wild morels are likely to be found in the location where there is a lot of limestone and plant ashes. Nevertheless, many morels are collected from the soil with a pH value less than 7.0 in North America (Information provided by The Ohio Mushroom Society), implying that a neutral to alkaline environment is not necessary for the growth of some *Morchella* species. Our results demonstrate that all tested *Morchella* isolates could grow between pH 5 to 8 and the optimal pH for mycelial growth was pH 6, 5 to 6, 5 to 7, and 5 for *M. rufobrunnea*, *M. importuna*, *M. americana*, and *M. exuberans* respectively, suggesting these four tested *Morchella* isolates prefer slightly acidity to neutral media. However, pseudosclerotia growth was not significantly different for pH values from 5 to 8 for *M. rufobrunnea* and *M. importuna*. This may be because even though mycelial growth was slower at higher pH values, mycelia were still able to colonize the entire plate 1 to 3 days later and nutrient in the media was depleted triggering the formation of pseudosclerotia The timing of sclerotia formation was delayed, but the total area of sclertoia were not affected. For morel cultivation, the optimal soil pH differs depending on cultivated isolates. Slightly acidity to neutral soil seems to be a good option for all four tested *Morchella* isolates in this study. Even though our results indicated that the optimal pH for the mycelial growth of *M.exuberans* was 5, *M. exuberans* still grew well at pH 6, 7 and even 8. If multiple *Morchella* isolates in this study are considered for future cultivation, it will be convenient to adjust the soil pH to 6 to 7. pH can strongly influencenutrient availability (Abubakar et al. 2013). However, it is more complicated in soil since soil pH may affect the biotic factors, such as biomass composition of fungi and bacteria (Rousk et al. 2009), the effect of which on *Morchella* mycelial growth is unknown. Thus, the results obtained *in vitro* may not accurately predict similar results in morel cultivation. More studies are needed to investigate the effect of pH on mycelial growth of *Morchella* in soil.

(a) *M. rufobrunnea* (b) *M. importuna*

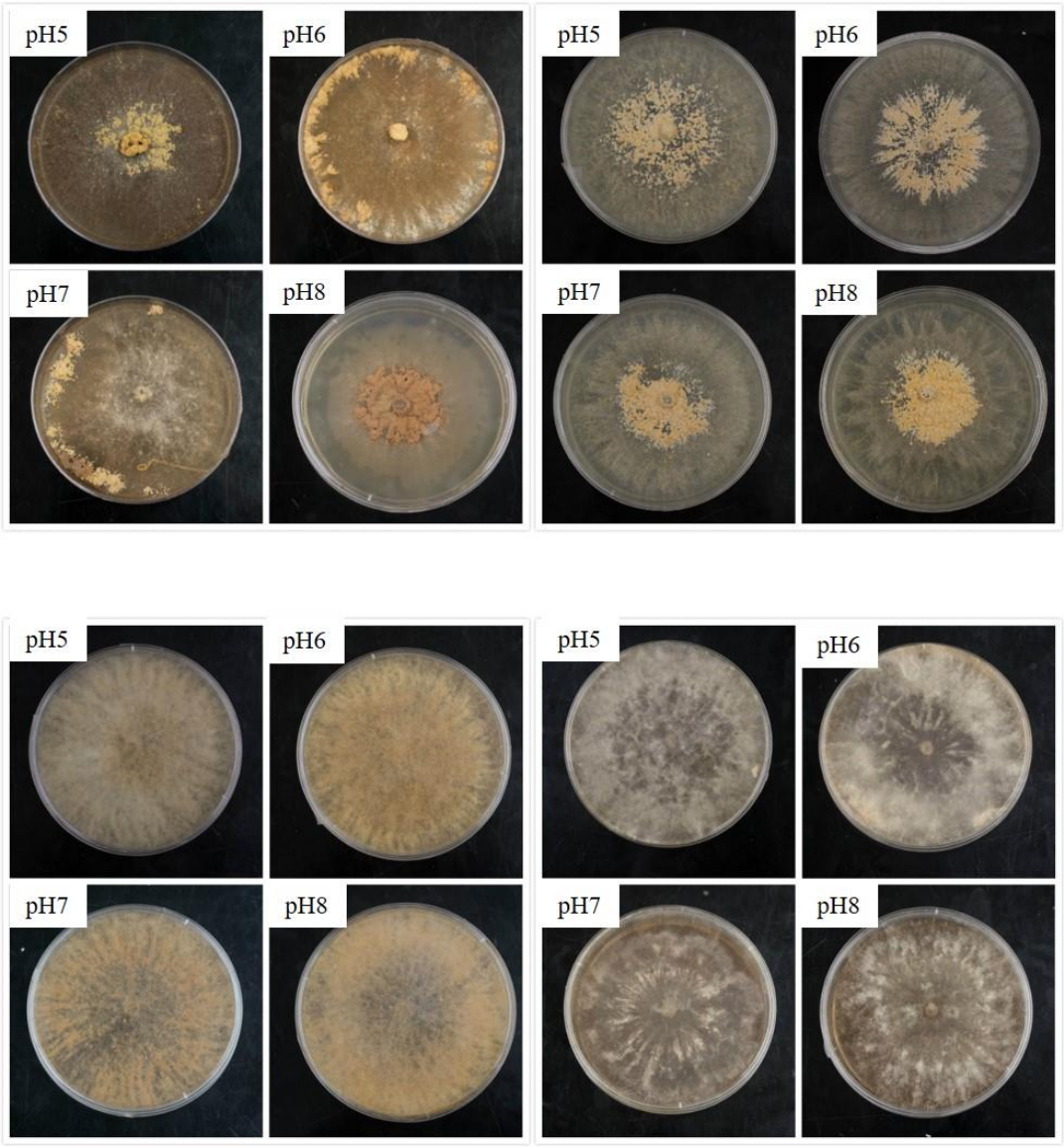


Figure 3-2. Effect of pH on pseudosclerotia formation of (a) *M. rufobrunnea*, (b) *M. importuna*, (c) *M. americana* and (d) *M. exuberans*. All isolates were cultured on PDYA medium with different pH values at 20°C for 20 days. All pictures were taken at 20 dpi.

Effect of light intensity on mycelial growth and pseudosclerotia formation

Light had a significant effect on mycelial growth for all tested isolates, except for *M.exuberans*. For *M. rufobrunnea*, the optimal light condition for mycelial growth was found under darkness (Figure 3-3a). *M. americana* and *M. importuna* showed no significant difference in growth under dark and 400 lux continuous light (low light) ($p>0.05$) (Figure 3-3b and c). However, a significant decrease in mycelial growth rate was observed under 2000 lux continuous light (high light) compared to two other conditions for *M.exuberans*, *M. americana*, and *M. importuna* (Figure 3-3a, b, and c). In particular, the mycleia color of *M. rufobrunnea* was light brown between 400 and 2000 lux continuous light and light yellow when grown in darkness (Figure 3-4a and b). The mycelia of *M. exuberans* when exposed to the 400 and 2000 lux continuous light treatments was less dense than under darkness, and the mycelial growth was the least under 2000 lux continuous light conditions (Figure 3-4d).

For *M. americana and M.exuberans*, no pseudosclerotium was observed under the three light conditions tested (Figure 3-3c and d, Table 3-2). However, light had a significant effect on pseudosclerotia formation for both *M. rufobrunnea* and *M. importuna* (Figure 3-3a and b, Table 3-2). The pseudosclerotia area under darkness was significantly larger than growth at 400 and 2000 lux continuous light conditions for *M. rufobrunnea* ($p<0.0001$), while it was not significantly different between 0 lux and 400 lux continuous light for *M. importuna* ($p>0.05$) (Table 3-2).

Light can make considerable alterations on fungi in many metabolic systems, such as carotenoid metabolism, polysaccharide and carbohydrate metabolism, fatty acid metabolism, nucleotide and nucleoside metabolism (Tisch et al. 2009). Besides, secondary metabolite production was found to be regulated by light as well (Tisch et al. 2009). A significant decrease in mycelial growth was observed when exposed to 24 h light in a cabinet containing 40 W, warm, white fluorescent tubes compared to under 24 h dark for four bioluminescence fungi (Weitz et al. 2001), which is similar to the results in this

study. Volk (1989) noted that light inhibited the pseudosclerotial growth of *M. crassipes*. Zhao et al. (1997) stated that light was not necessary for pseudosclerotia production. Q. Liu et al. (2018) also reported that lightning conditions did not affect the pseudosclerotia formation on *M. importuna*. However, it was noted that during the process of making spawn, the side that was occasionally exposed to light produced large amounts of pseudosclerotia. On the contrary, production of pseudosclerotia on the dark side was poor, indicating that light plays a role in pseudosclerotia formation (W. Liu et al. 2017a). One reason to account for the different results observed in this study compared to previous studies could be related to the difference in light intensity, light wavelength, and light stability tested. Prevoius studies have documented the effects of light on some other edible fungi. For instance, it was reported that light treatment increases the production of *Flammulina velutipes* (M. Liu and He 1997), and light has a positive effect on the development and spore production of *Ganoderma* (Hao et al. 2011). Our results indicated that light does not promote mycelial growth for all four tested *Morchella* isolates. Strong light (2000 lux) should be avoided in both culture preparation and morel cultivation except for *M. exuberans*. However, it was noted that scattering light (200 to 1100 lux) triggers primordial formation according to farmers' experiences (W. Liu et al. 2017a). Sexual development may be stimulated by light, but the mechanisms involved are unknown. Combined with the results in this study and farmers' practical experiences, we provided some suggestions on morel cultivation here: For outdoor cultivation, using of shading nets and black mulching films are suggested in morel cultivation to adjust the light intensity. For indoor cultivation, the light could be adjusted manually according to the development stage of *Morchella*. Completely darkness is suggested during the mycelial growth stage and then some light (200 to 1000 lux) can be provided to facilitate the sexual development.

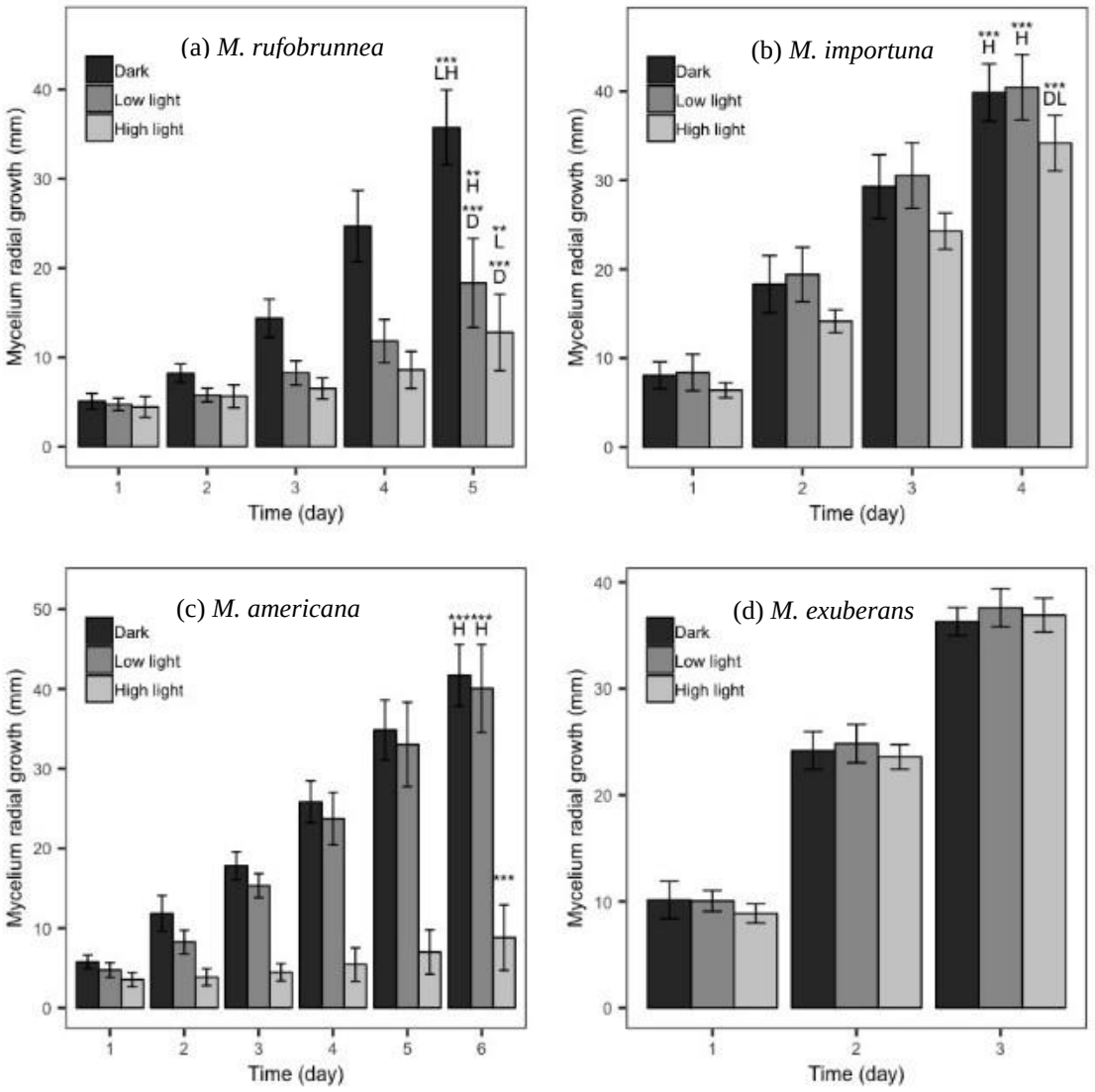


Figure 3-3. Effect of light intensity on mycelium radial growth of (a) *M. rufobrunnea*, (b) *M. importuna*, (c) *M. americana* and (d) *M. exuberans*. Data shown are the means of 15 replicates from three independent experiments ± SD. Statistical analysis was conducted on the data of the last measuring day for each isolate. Bars with "*", "**", "***" at the top without numbers represent values that are significantly different from all other light conditions with a p value less than 0.05, 0.001, 0.0001 respectively. Bars without asterisk at the top represent values that are not significantly different from all other light conditions. Bars with "*", "**", "***" at the top with letter(s) represent values that are only significantly different from light conditions listed below the asterisk(s) with a p value less than 0.05, 0.001, 0.0001 respectively. Letters "D", "L" and "H" represent "dark" (0 lux), "low light" (400 lux), and "high light" (2000 lux) conditions respectively.

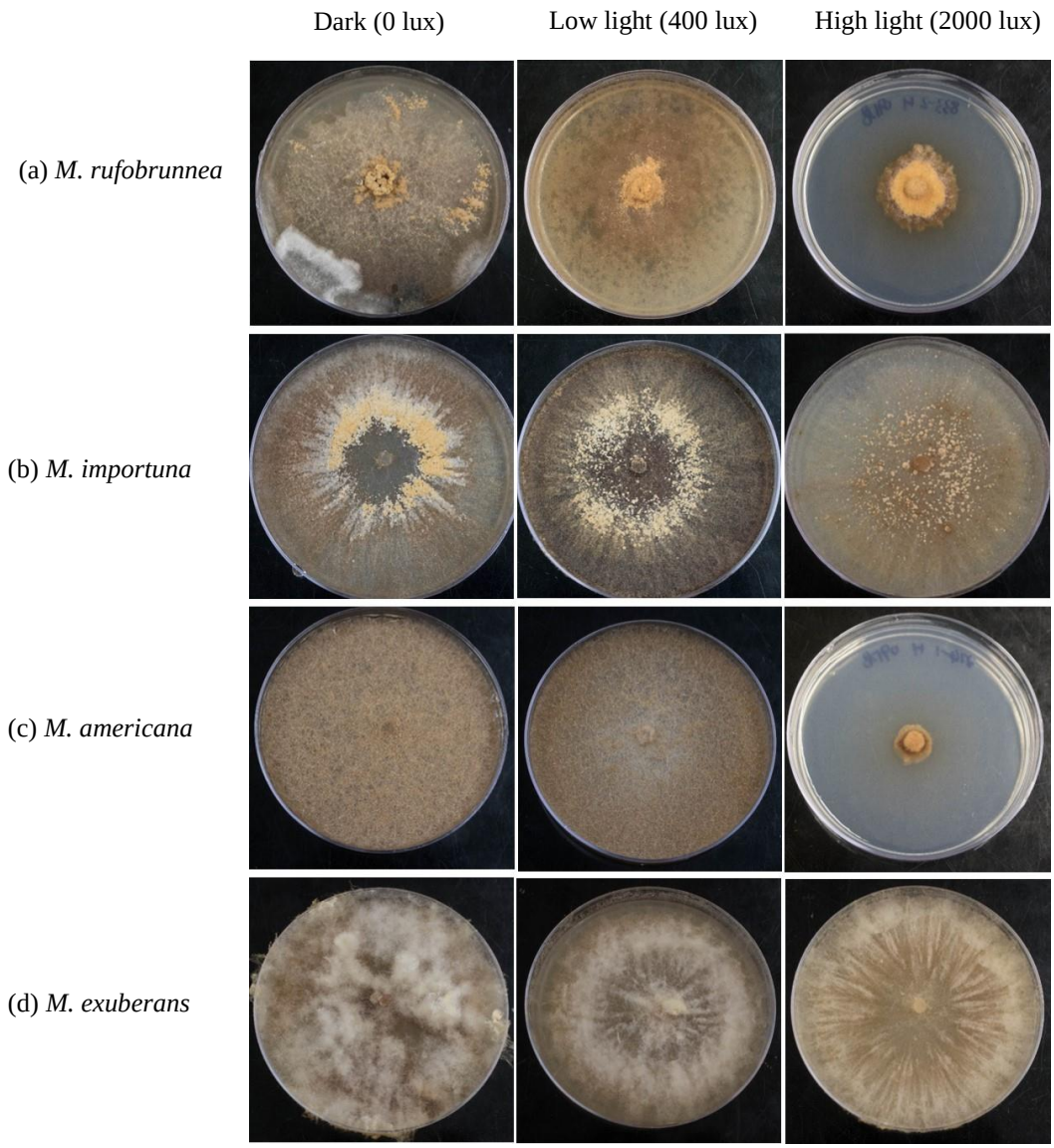


Figure 3-3. Effect of light intensity on pseudosclerotia formation of (a) *M. rufobrunnea*, (b) *M. importuna*, (c) *M. americana* and (d) *M. exuberans.* All isolates were cultured on PDYA medium under different light conditions at 25°C for 20 days. All pictures were taken at 20 dpi.

Effect of temperature on mycelial growth and pseudosclerotia formation

Results demonstrated that temperature had a significant effect on mycelial growth for all four isolates and on pseudosclerotia formation for *M. rufobrunnea* and *M. importuna*. *M. rufobrunnea* and *M. importuna*, demonstrated significantly faster mycelium radial growth at 20°C and 25°C compared to

growth at 30°C at 5 dpi ($p<0.0001$) (Figure 3-4a and b). Growth was significantly different among all three temperatures tested for *M. americana* at 5dpi, with the fastest at 20°C, followed by 25°C, and slowest at 30°C ($p<0.0001$) (Figure 3-4c). Interestingly, mycelial growth was found to be faster at 25°C and 30°C compared to 20°C for *M. exuberans* at 3 dpi ($p<0.0001$) (Figure 3-4d). One hypothesis to explain the observed differences between *M. exuberans* and the other three *Morchella* isolates is the difference in the ecological niches of the isolates. *M. exuberans* was reported to be post-fire *Morchella* species, which may suggest better adaptation of *M. exuberans* to higher temperatures. The colony color of *M. rufobrunnea* and *M. importuna* at 25°C and 30°C was dark brown compared to light brown at 20°C (Figure 3-4a and b).

Pseudosclerotia formation was significantly affected by temperature for *M. rufobrunnea* and *M. importuna*. No significant differences were observed for pseudosclerotia growth between 20°C and 25°C for *M. rufobrunnea* and *M. importuna* (Table 3-2). At 30°C, no pseudosclerotia was observed for *M. rufobrunnea* and pseudosclerotia area was considerably smaller for *M. importuna* (Figure 3-5c and d, Table 3-2). No pseudosclerotium was observed under any of the three light conditions tested for *M. americana* and *M.exuberans* (Figure 3-5c and d, Table 3-2).

X. Liu et al. (2004) noted the optimal temperature for pesudosclerotia formation was from 15 to 20°C and 20 to 30°C for two different strains, but the species names were not provided. They also stimulated the outdoor temperature fluctuation and less pseudosclerotia were formed compared to growing at a stable optimal temperature. Chen and Guo (2007) reported mycelia of *M. esculenta* could grow between 2 to 34 °C and the optimal temperature was 24 to 26°C. Q. Liu et al. (2018) found the most favorable temperature for *M. importuna* was 20°C with mycelium being unhealthy and no pseudosclerotium formed at 30°C, which is consistent with the results obtained in this study. Our results indicated that the optimal temperature was 20 to 25°C for all four tested isolates in terms of mycelial

growth except for *M. exuberans*, which grew better between 25 to 30°C. Even though the optimal temperature for mycelial growth of those previously mentioned *Morchella* species was generally between 20 to 26°C, in morel production, the optimal spawning temperature is when the maximum temperature is 20°C when other microorganisms in the soil are in a state of semi-dormancy (W. Liu et al. 2017a). According to farmers' experiences, a satisfactory yield of mature fruit bodies will be more likely be obtained when the air temperature is below 4°C for at least one month before the primordial formation period and the temperature difference is at least 10°C during the day, thereby facilitating primordial formations in morel cultivation (personal communication). Nevertheless, additional data is required to determine if a low temperature period is required for fruit body initiation. For culture and spawn preparation, conditions with optimal temperatures was recommended by growers. For outdoor cultivation, growers suggest spawning when the air temperature starts to drop and the highest air temperature is below 20°C. For indoor cultivation, air temperature can be adjusted manually to below 20°C. However, more studies are needed to investigate how air temperature fluctuation affects mycelial growth, pseudosclerotia formation and primordial formation both in lab and in the field.

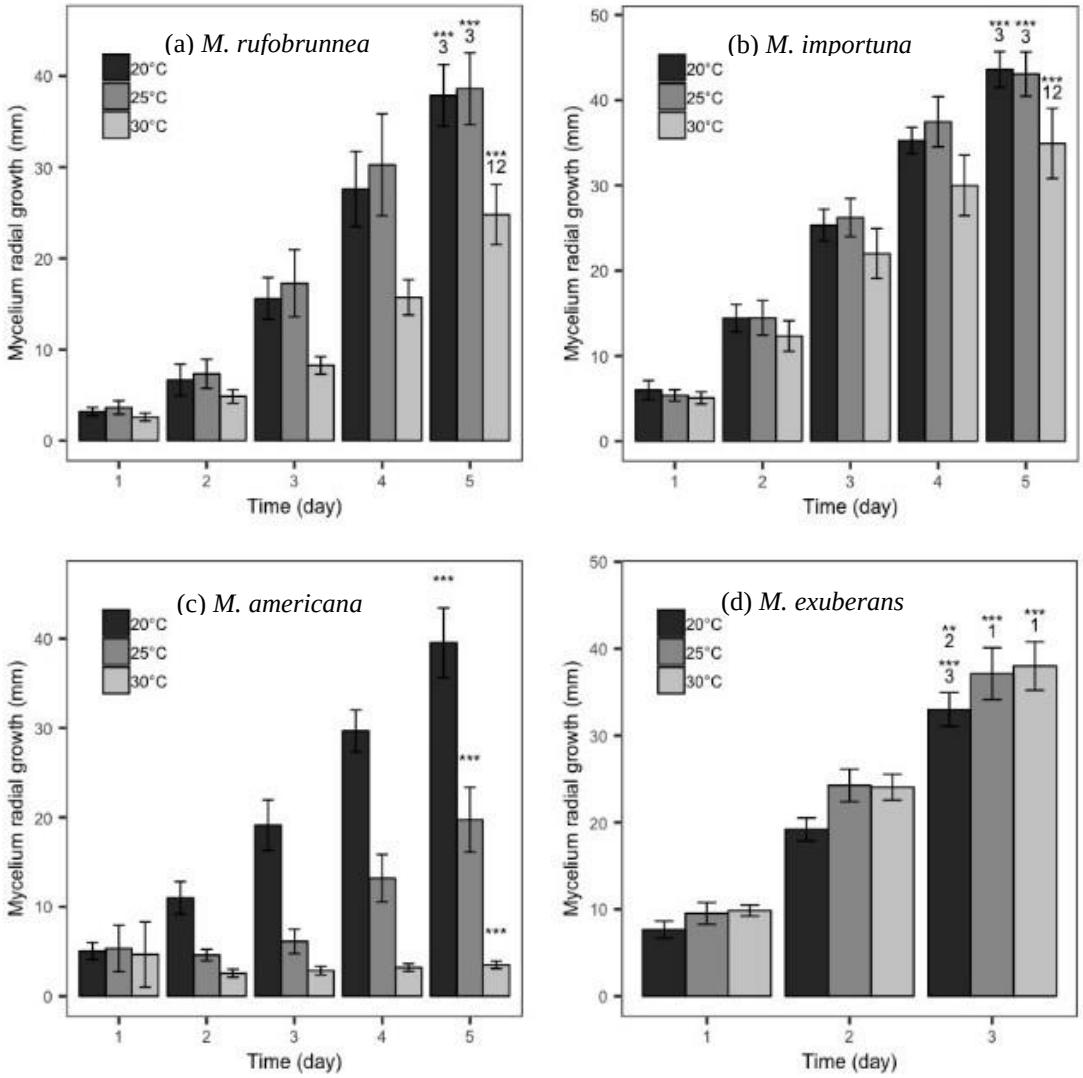


Figure 3-4. Effect of temperature on mycelium radial growth of (a) *M. rufobrunnea*, (b) *M. importuna*, (c) *M. americana* and (d) *M. exuberans*. Data shown are the means of 15 replicates from three independent experiments ± SD. Statistical analysis was conducted on the data of the last measuring day for each isolate. Bars with "*", "**", "***" at the top without numbers represent values that are significantly different from all other temperature treatments with a p value less than 0.05, 0.001, 0.0001 respectively. Bars with "*", "**", "***" at the top with letter(s) represent values that are only significantly different from temperature treatments listed below the asterisk(s) with a p value less than 0.05, 0.001, 0.0001 respectively. Numbers "1", "2" and "3" represent "20°C", "25°C", and "30°C" treatments respectively.

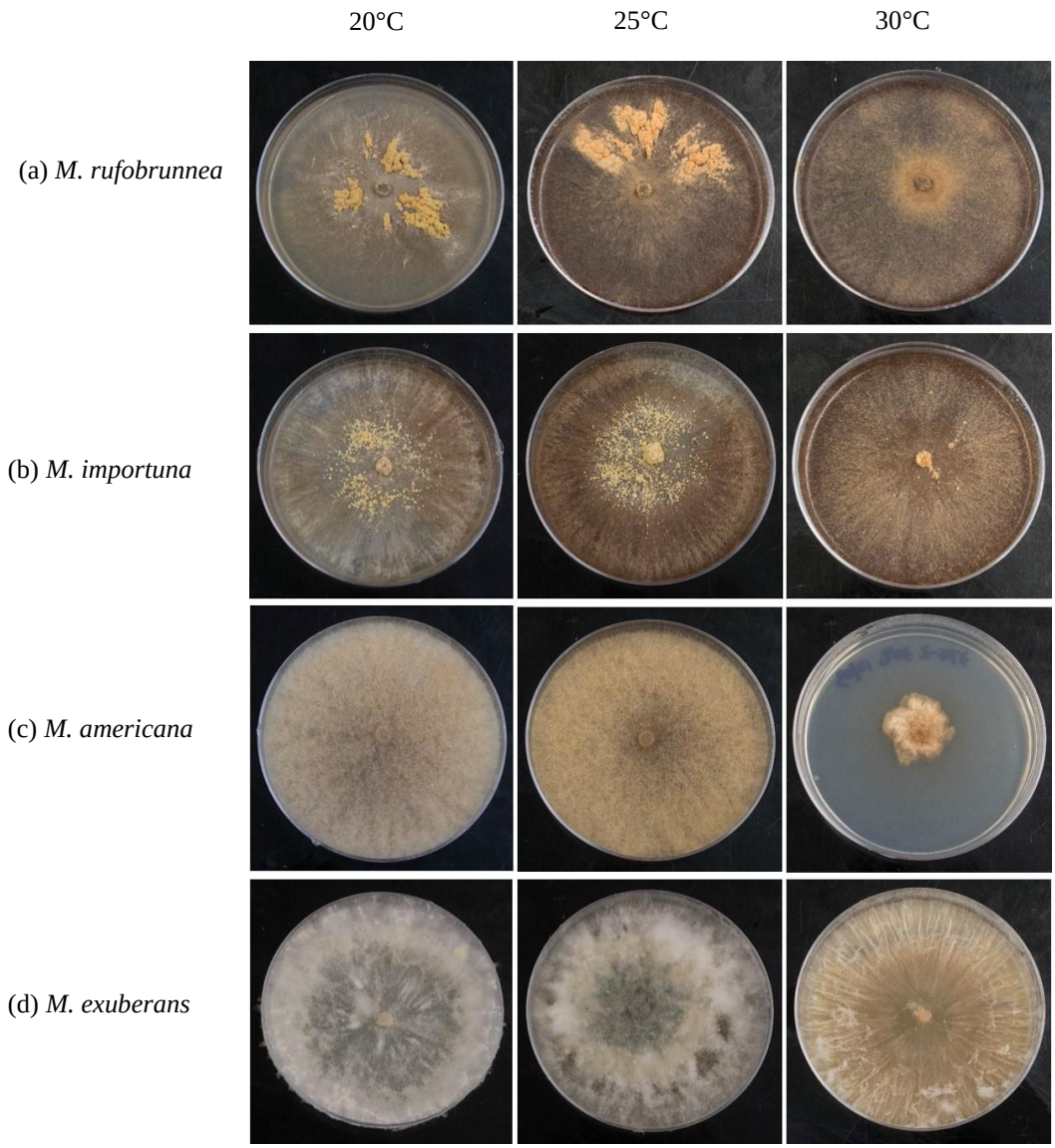


Figure 3-5. Effect of temperature on pseudosclerotia formation of (a) *M. rufobrunnea*, (b) *M. importuna*, (c) *M. americana* and (d) *M. exuberans*. All isolates were cultured on PDYA medium with an initial pH value of 6 at 20, 25 and 30°C for 20 days. All pictures were taken at 20 dpi.

Morphological characteristics of pseudosclerotia development

The *M. rufobrunnea* and *M. importuna* were cultured on PDYA media with an original pH of 6 under darkness at room temperature (20 ± 1°C). Pseudosclerotia formation did not initiate until mycelia colonized the enitre plate. Mycelia of *M. rufobrunnea* were white at first and then turned yellow and brown. White pseudosclerotia initials were found at 5 dpi on the inoculation site. Pseudosclerotia area expanded on the inoculation site and the turned to yellow around 8 dpi (Figure 3-5C). Concentric pseudosclerotia distribution patterns were also observed in some dishes (Figure 3-2a). At 15 dpi, pseudosclerotia became dark brown and clear boundaries between the pseudosclerotia and the surrounding mycelia could be observed (Figure 3-5E). Cell walls appeared to become thicker as pseudosclerotia morphogenesis progressed (Figure 3-5E and F).

M. importuna mycelia were white to light grey at first and then turned light yellow to brown. At 5 dpi, white to light yellow pseudosclerotia initials were formed (Figure 3-5G and H). The initials developed into yellow to brown pseudosclerotia at 8 dpi (Figure 3-5I and J) and eventually developed into mature pseudosclerotia masses (Figure 3-5K and L). The plasma membrane appeared to be shrinking during SD to SM phase in both isolates (Figure 3-5D, F, J and L).

P. He et al. (2015) compared the structural features of the undifferentiated mycelial stage and three main pseudosclerotial stages on *M. importuna* by using transmission electron microscopy and first highlighted the involvement of autophagy and apoptosis and lipid accumulation in pseudosclerotia morphogenesis of *M. importuna*. They indicated that the pseudosclerotia of *M. importuna* can be formed from the repeated branching as well as enlargement of either terminal hyphae or subordinate hyphal branches. Q. Liu et al. (2018) observed the pseudosclerotia and hyphal (outside the pseudosclerotia region) formation process in *M. importuna*. It was found that swollen cells were observed at 4 to 5 dpi in the center, while the outside region remained as mycelia. As the pseudosclerotia matured, characterized

by cell aggregation and dark brown pigmentation, senescent and aging cells were found outside the pseudosclerotia region. In this study, *M. rufobrunnea* and *M. importuna* displayed similar patterns. Pseudosclerotia initials usually developed at the center of the plate and then expanded cells aggregated with a white color then turned yellow to brown. For *M. importuna*, small white to yellow aggregates were formed first and aggregated to a larger pseudosclerotium in the center later. However, for *M. rufobrunnea*, pseudosclerotia were generally formed in the center most of the time, but occasionally some also formed near the plate edges. The detailed pseudosclerotia mophogenesis of *M. rufobrunnea* and how environmental conditions effect pseudosclerotia morphogenesis of both *M. importuna* and *M. rufobrunnea* require further study.

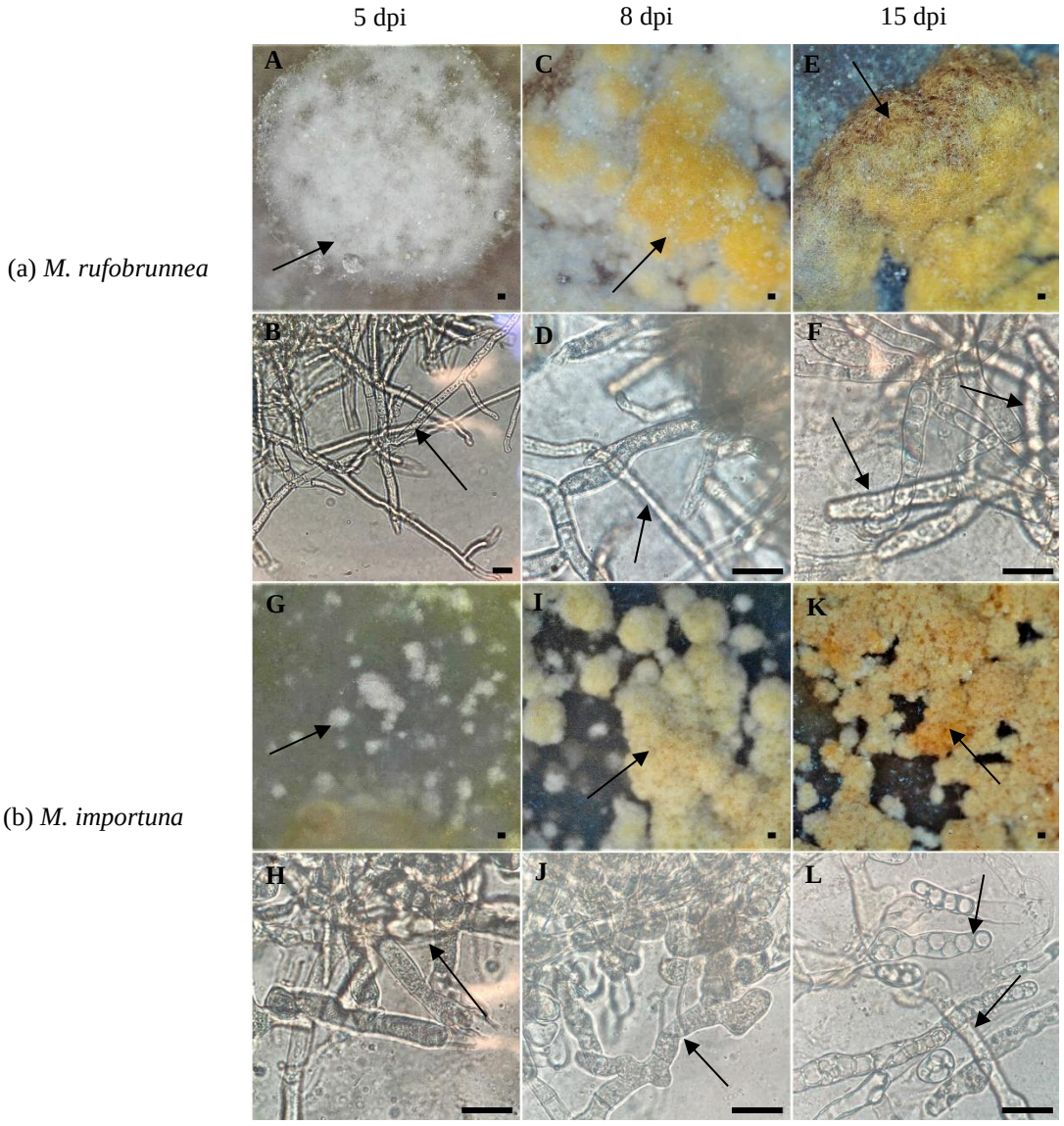


Figure 3-5. Partial enlarged detail of pseudosclerotia (row 1 and 3) and the microscopic characteristic of pseudosclerotia (row 2 and 4) of (a) *M. rufobrunnea* and (b) *M. importuna* at 5 dpi, 8 dpi, and 15 dpi. At 5 dpi, mycelia were interwoven (Figure 3-5A, B, G, H, arrow). At 8 dpi, the mycelial masses turned yellow (Figure 3-5C and I, arrow) and cell walls were thickened (Figure 3-5D and J, arrow). Orange to brown pseudosclerotia with obvious boundaries with surrounding mycelia (Figure 3-5E and K, arrow) were at 15 dpi. Note the thickened well wall and shrunken plasma membrane (Figure 3-5 F and L, arrow). Scale bars = 20 μm.

Conclusion

This study suggested that environmental factors, including pH, light and temperature, play an important role in mycelial growth and pseudosclerotia formation on *M. rufobrunnea*, *M. importuna*, *M. americana*, and *M. exuberans*. All four isolates tested in this study were able to grow in the initial media with a pH of 5 to 8. Slightly acidic to neutral initial agar media is most favorable for mycelial growth, while alkaline conditions of the medium suppressed the mycelial growth. However, initial pH of the medium did not significantly affect pseudosclerotia formation of *M. rufobrunnea* and *M. importuna*. *M. americana*, and *M. exuberans* did not produce any pseudosclerotium under any of the test conditions in this study. Light intensity had different effects on different *Morchella* isolates. High light intensity (2000 lux) significantly suppressed mycelial growth, except for *M. exuberans*, and differences in light intensity also affected pseudosclerotia production for *M. rufobrunnea* and *M. importuna*. Darkness (0 lux) and low light intensity (400 lux) were found to be the most favorable conditions for mycelial growth for *M. rufobrunnea*, but pseudosclerotia formation was significantly suppressed under low light conditions (400 lux) compared to darkness (0 lux). *M. importuna* grew equally well and formed pseudosclerotia with no significant difference under low light intensity (400 lux) and darkness (0 lux). The temperature preference for mycelial growth of *M. rufobrunnea*, *M. importuna*. *M. americana*, and *M. exuberans* was found to be 20 to 25°C, 20 to 25°C, 25°C, and 25 to 30°C respectively. It is worth noting that *M. exuberans* grew well

at 30°C, at which the mycelial growth of three other *Morchella* isolates was highly suppressed. One possible explanation could be due to the special ecological niches of *M. exuberans*, which was reported to be a post-fire *Morchella* species. Moreover, the pseudosclerotia morphogenesis of *M. rufobrunnea* and *M. importuna* was investigated and light microscopic observations represented complex pseudosclerotia formation patterns. Good mycelia growth and satisfactory pseudosclerotia formation is required for successful morel cultivation. The results of this research will provide some guidance for future culture preparation, spawn production and indoor and outdoor morel cultivation.

Chapter 4

Indoor cultivation of four *Morchella* isolates – Cropping Experiment

Abstract

Morels (*Morchella*) are commercially important edible mushrooms well known for their desirable flavor and medicinal value. The outdoor cultivation of *Morchella* species has been achieved in China in recent years. Even though *Morchella* species are diverse in the wild, species that can be cultivated are limited. In this study, we discuss two morel indoor croppping experiments at the Mushroom Research Center (MRC) at The Pennylvania State University. In experiment 1, we selected five substrates and two nutrient bags for use in indoor *M. rufobrunnea* cultivation. *M. rufobrunnea* was the first reported cultivated *Morchella* species in North America. In experiment 2, we selected four *Morchella* species: *Morchella rufobrunnea, M. importuna, M. americana* and *M. exuberans,* based on previous research and relevant information for indoor cultivation experiments. Nutrient sources are essential for mycelial growth and fruiting body development. We wanted to test available and effective carbon sources that contribute to more efficient fruiting body development and higher yields, and compare results between different treatments and between different species. These results can provide data that will be used in the design of future research. In experiment 2, two substrate carbon sources (sawdust and straw) were used as soil amendments for use in indoor *Morchella* cultivation. Difference in fruiting body initiation and yield were assessed based on the two different substrate carbon treatments and for four different *Morchella* species in an indoor cultivation system at the MRC. Mature fruit bodies were successfully initiated in experiment 1 but not in experiment 2, even though many primordia were formed on one *Morchella* isolate in experiment 2. Factors that may contribute to the different results in two experiments are discussed.

Introduction

Morels (*Morchella*) are well known for their savory flavor and multiple bioactive compounds. Morels are often collected in the wild and their fruiting season is very short (Pilz. et al. 2007). Thus, the cultivation of morels has been attracting many mushroom enthusiasts' attention. The first morel fruit body initiation was reported by Ower (1982). He studied morel indoor cultivation on *M. rufobrunnea* and applied for several patents. However, due to the lack of basic biological knowledge of morels and some unknown reasons, indoor commercial production of morels was not successful (Ower et al. 1986, 1988, 1989; Pilz. et al. 2007). Recently, outdoor cultivation of morels has been developing rapidly in China. The application of exogenous nutrient bags has made a great promotion on commercialized morel outdoor cultivation (W. Liu et al. 2017a). Thus far, four *Morchella* spp. (*M. importuna*, *M. sextelata*, *M. septimelata*, and *M. esculenta*) have been cultivated commercially in China and *M. importun*a accounts for more than 95% of the morel cultivating area (W. Liu et al. 2017a). Nevertheless, morel cultivation has been proven to be a challenging task due to the lack of knowledge on basic biology about morels, such as their complex life cycle, strain polarity and ploidy, mating systems, trophic modes and aging, which has caused notable economic losses (W. Liu et al. 2018). Compared to outdoor cultivation, which is largely restricted by natural conditions, such as nutrient content of the soil and weather conditions, indoor cultivation seems to be a better option to consistently cultivate morels commercially. Even though there have been studies on morel indoor cultivation since Ower (D. Zhu and He, 2007; Y. Liu et al. 2013), the feasibility of these indoor cultivation techniques is skeptical. There is an urgent need to develop a successful morel indoor cultivation process. Whether the outdoor morel cultivation process is feasible for indoor cultivation is unknown. Even though *Morchella* species are diverse in the wild, species that can be cultivated may be limited based on nutritional requirements. Nutrient sources are essential for mycelial growth and fruiting body development. In this study, two morel indoor cultivation experiments were

conducted in the MRC. In experiment 1, five substrates and two nutrient bags were selected for use in indoor *M. rufobrunnea* cultivation. In experiment 2, four *Morchella* species: *Morchella rufobrunnea*, *M. importuna*, *M. americana* and *M. exuberans*, two substrates and one nutrient bag were selected based on previous research and relevant information for indoor cultivation experiments. Both experiments stimulated the curently used ourdoor cultivation techniques. The effect of substrates with different carbon sources and nutrient bags with different sources on conidia production, primordial formation and mature fruit body initiation of differernt *Morchella* species were examined in two experiments. Factors that may have contributed to the different results observed in the two experiments are also discussed.

Experiment 1

Material and Methods

Morchella isolates

WC 833 *M. rufobrunnea* obtained from the Penn State Mushroom Spawn Lab was utilized for this study.

Substrate and nutrient bags preparation

Substrates with five different treatments were used in this study: (1) soil + sawdust (wet, pH buffered with limestone) (8% by weight); (2) soil + wheat straw (chopped and wet); (3) soil + wheat straw (chopped and wet) more straws; (4) soil + fresh mushroom compost (8% by weight); (5) soil + aged mushroom compost (8% by weight). Soil pH was adjusted to 7 with limes and then was mixed with straws or wood shavings. Each tub was filled to 50 lb. Each treatment consisted of six replicates. There were totally 30 tubs (1 isolates × 5 treatment × 6 replicates).

Two different nutrients bag formulas were used for each isolate in this study: (1) wheat and sawdust at a ratio of 3:2 (m/m); (2) corn and sawdust at a ratio of 3:2 (m/m). The moisture was adjusted to 40% using tap water. Each plastic autoclavable bag (12 cm × 24 cm × 5 cm) was filled with 350 g of the mixture and sealed with a twist tie. The bags were autoclaved for 35 minutes at 121°C before using them. Two nutrient bags with the same formula were used for each tub. For each substrate treatment, six replicates were divided to two group corresponding to nutrient bags with two formulas.

Spawn production

Morchella mycelia grown on PDYA medium were used to inoculate rye grains. Two plugs of agar containing active growing *Morchella* mycelia were inoculated onto sterilized rye grains in a 250 ml flask incubated at room temperature for 7 days. Rye grains were then transferred from the 250 ml flasks to sterilized rye grain spawn in 1000 ml flasks and incubated at room temperature for 35 days. Flasks were shaken every two days. Spawn was ready to use after the *Morchella* mycelia completely colonized the grains.

Rye grains used for spawn production were produced using the following formulas: For 250 ml flasks, 50 ml beaker level full of rye grain, 1/2 tsp. Calcium Carbonate, powder (lime), 1/4 tsp. Calcium Sulfate (gypsum) and 60 ml warm water were mixed together and then autoclaved for 35 minutes at 121°C. For 1000 ml flasks, 250 ml beaker level full of rye grain, 2 tsp. Calcium Carbonate, powder (lime), 1 tsp. Calcium Sulfate (gypsum) and 220 ml warm water were mixed together and then autoclaved for 35 minutes at 121°C.

***Morchella* indoor cultivation experiment set up**

Thirty tubs were randomly assigned to four three-level shelves in one room in MRC (Table 4-1). For each tub, 77g spawn were mixed with the soil substrate. A sterilized knife was used to cut a 3 to 4 cm opening on the bags and two sterilized nutrient bags were placed on the soil surface for each tab with the opening attached to the soil surface at 45 days post inoculation (dpi). The tubs were watered heavily at 55 dpi using tap water and then nutrient bags were removed at 90 dpi. The used bags were steamed to kill the microbes before disposal.

The procedure of morel indoor cultivation and relative parameters are listed in Table 4-2. When the size of fruit body stops increasing, the stem of fruit body was cut and harvested. Each fruit body was measured for length and weight. Harvested fruit bodies were dried at room temperature.

Table 4-1. The room map of *M. rufobrunnea* indoor cultivation (experiment 1) in MRC

Higher level	Middle level	Lower level		Lower level	Middle level	Higher level
	2[a] W[b] 30[c]			1 W 18	1 C 17	1 C 16
	4 C 5			1 W 15	1 C 14	5 C 13
	4 C 8			1 W 12	5 C 11	5 C 10
3 W 25	3 C 26	2 C 17	Asile	5 W 9	4 C 8	4 W 7
3 W 22	3 C 23	2 C 24		5 W 6	4 C 5	4 W 4
3 W 19	3 C 20	2 C 21	Door	5 W 3	4 C 2	4 W 1

a. The treatment number of the substrate: “1” represents treatment soil + peat; “2” represents soil + wheat straw; “3” represents soil + wheat straw (less straw); “4” represents soil + fresh mushroom compost; “5” represents soil + aged mushroom compost.
b. The treament of nutrinet bags: “W” represents wheat + sawdust; “C” represents corn + sawdust.
c. Randomly assigned tub number.

Table 4-2. The timetable and relative parameters of morel indoor cultivation (experiment 1)

Days after spawning	Activity	Parameters		
		Soil Temperature（°C)	Air Humidity (%)	Soil Moisture(%)
0	Spawn	17	80-90	15-25
45	Add Nutrient bags	17	80-90	15-25
90	Remove nutrient bags	17	85-95	20-30
	Fruiting period management	17	85-95	20-30
	Harvest	17	85-95	20-30

Experiment 2

Material and Methods

Morchella isolates

Four *Morchella* isolates, *Morchella importuna* (China 1), *M. rufobrunnea* (WC 833), *M. americana* (WC 374), *M. exuberans* (WC 766) obtained from the Penn State Mushroom Spawn Lab were utilized for this study.

Substrate and nutrient bags preparation

Two soil substrates with different carbon sources, oak wood shavings (sawdust) and straw, were used for all isolates. Soil was supplemented with chopped wheat straw (4% by dry weight) or supplemented with oak wood shavings (8% by weight). Soil pH was adjusted to 7 with crushed limestone (calcium carbonate) and then the soil was mixed with straws or wood shavings and 35 lb of each mix was filled into a tub to a 4 inch layer of perlite (to allow for soil drainage and raise the soil level to near the surface of the tubs). Each treatment consisted of six replicates with a total of 48 tubs (4 isolates × 2 carbon sources × 6 replicates). The soil and substrate samples were sent to the Penn State University Agricultural Analytical Services Laboratory to analyze for chemical composition (Table A-3, A-4, A-5, A-6, A-7).

Wheat grain and oak sawdust were mixed together at a ratio of wheat to sawdust of 3:2 (m/m). The moisture was adjusted to 40% using tap water. Each plastic autoclavable bag (12 cm × 24 cm × 5 cm) was filled with 350 g of the wheat/sawdust mixture and sealed with a twist tie. The bags were autoclaved for 35 minutes at 121°C before using them. Two nutrient bags were used for each tub. The nutrient bag sample was sent to the Agricultural Analytical Services Laboratory to analyze for chemical composition (Table A-8).

Spawn production

Morchella mycelia grown on PDYA medium were used to inoculate rye grains. Two plugs of agar containing active growing *Morchella* mycelia were inoculated onto sterilized rye grains in a 250 ml flask incubated at room temperature for 7 days. Rye grains were then transferred from the 250 ml flasks to sterilized rye grain spawn in 1000 ml flasks and incubated at room temperature for 35 days. Flasks

were shaken every two days. Spawn were ready to use after the *Morchella* mycelia completely colonized the grains.

Rye grains used for spawn production were produced using the following recipes: For 250 ml flasks, 50 ml beaker level full of rye grain, 1/2 tsp. Calcium Carbonate, powder (lime), 1/4 tsp. Calcium Sulfate (gypsum) and 60 ml warm water were mixed together and then autoclaved for 35 minutes at 121°C. For 1000 ml flasks, 250 ml beaker level full of rye grain, 2 tsp. Calcium Carbonate, powder (lime), 1 tsp. Calcium Sulfate (gypsum) and 220 ml warm water were mixed together and then autoclaved for 35 minutes at 121°C.

Morchella **indoor cultivation experiment set up**

Fourty-eight tubs were randomly assigned to four three-level shelves in one room in MRC (Table 4-3). For each tub, 77g spawn were mixed with the soil substrate. Two sterilized nutrient bags were placed on the soil surface for each tab at 15 days post inoculation (dpi). A sterilized knife was used to cut a 3 to 4 cm opening on the bags and two sterilized nutrient bags were placed on the soil surface for each tab with the opening attached to the soil surface at 15 dpi. The tubs were watered heavily at 55 dpi using tap water and then nutrient bags were removed at 65 dpi. The used bags were steamed sterilized before disposal.

The procedure of morel indoor cultivation and relative environmental growth parameters are listed in Table 4-4. When the size of the fruiting bodies stopped increasing, the stem of fruiting bodies will be cut and harvested. Each fruit body will be measured for length and weight.

Table 4-3. The room map of *Morchella* indoor cultivation in MRC

Higher level	Middle level	Lower level		Higher level	Middle level	Lower level
833[a] sa[b] 33[c]	CHN st 6	374 st 45		766 st 11	CHN st 1	833 sa 25
CHN st 2	374 sa 27	833 sa 34		CHN sa 22	CHN st 10	374 sa 35
CHN sa 14	766 sa 23	833 st 40	Asile	374 st 48	CHN sa 21	374 sa 28
374 st 41	374 st 46	833 st 38		833 st 43	374 sa 32	766 st 8
CHN sa 13	766 st 3	374 sa 36		766 sa 19	766 sa 15	833 st 44
766 sa 24	CHN sa 18	CHN sa 5		374 sa 31	766 st 4	374 st 47
833 st 37	766 sa 16	833 sa 26	Door	374 st 42	766 st 7	766 st 12
CHN st 9	833 sa 39	833 sa 29		766 sa 20	833 sa 30	CHN sa 17

a. The isolate number in the Mushroom Spawn Lab, Buckhout Lab, Penn State. 833: *M. importuna*; CHN: *M. importuna*; 374: *M. americana*; 766: *M. exuberans*.

b. Carbon source. sa: sawdust; st: straw.

c. Randomly assigned tub number.

Table 4-4. The timetable and relative parameters of morel indoor cultivation

Days after Spawning	Activity	Parameters		
		Soil Temperature (°C)	Air Humidity (%)	Soil Moisture (%)
0	Spawn	17	80-90	15-25
10-15	Add Nutrient bags	17	80-90	15-25
50-60	Heavy watering	17	85-95	20-30
65-75	Remove nutrient bags	17	85-95	20-30
	Fruiting period management	17	85-95	20-30
	Harvest	17	85-95	20-30

Air humidity and temperature, and soil moisture and temperature measurement

Air humidity was adjusted using a hydrofogger humidifier connected to a 60 minute Tork® timer, measured by a digital hygrometers daily. Soil humidity was adjusted by watering and measured using a tensiometer. Air temperature was measured with a thermometer placed in the center of the room. Soil temperature was measured using a thermometer probe placed into the soil.

Mycelial growth and conidia growth in soil and nutrient bags rating

Mycelial growth and conidia growth on the soil surface were recorder every two days post inoculation. Mycelial growth and conidia growth in nutrient bags were recorded every two days after placing on the soil. Mycelial and conidia growth were ranked from 1 to 5 following the ranking standard (Figures 4-1 and 4-2).

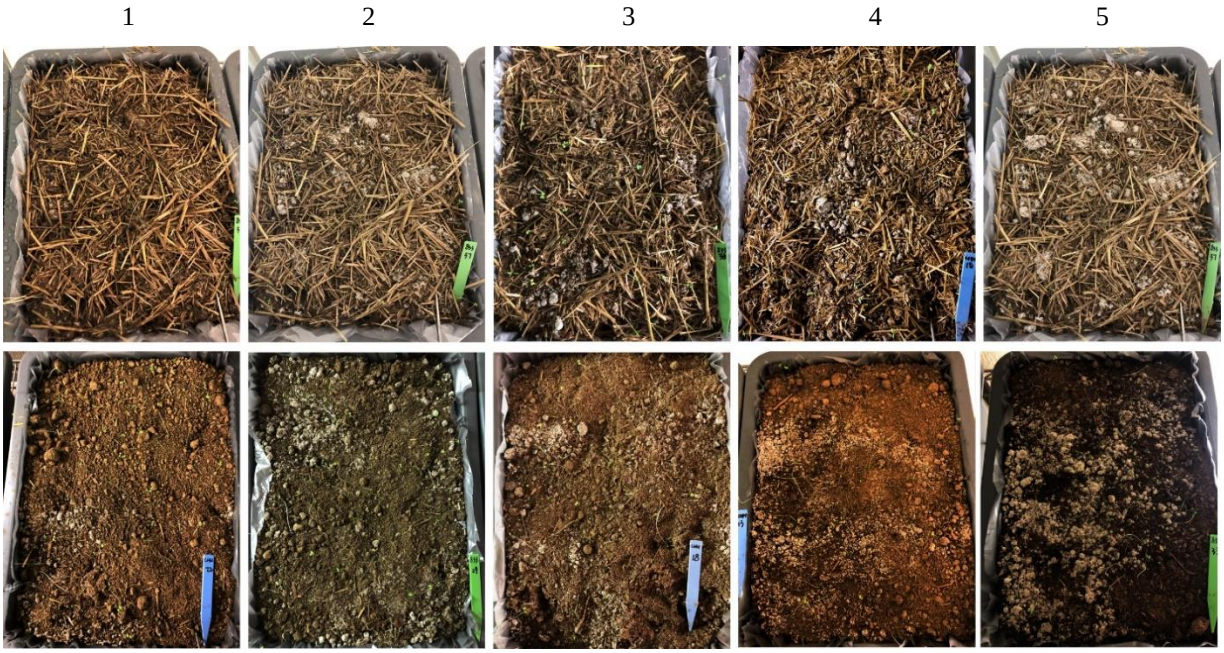


Figure 4-1. Ranking standard for conidia growth in soil. Upper row: soil + straw; Lower row: soil + sawdust.

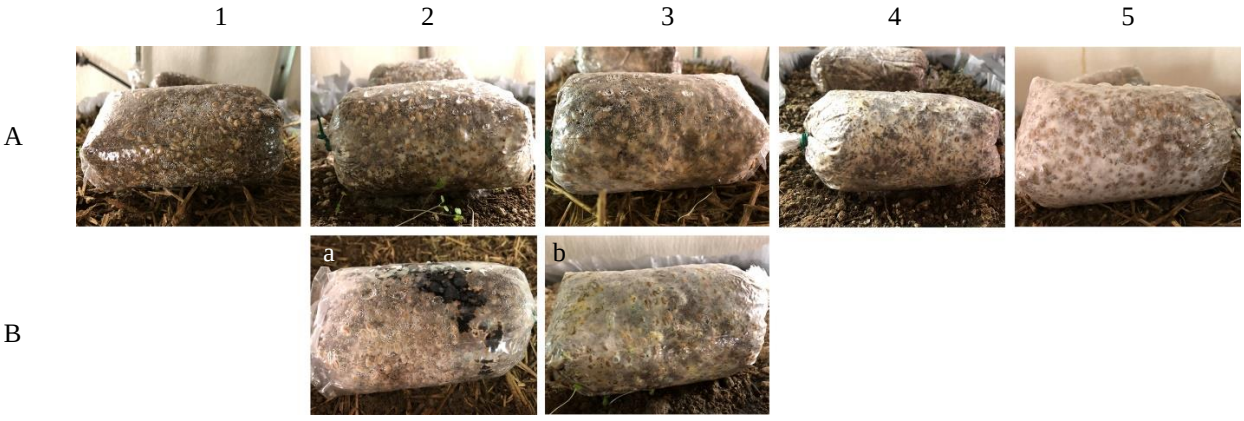


Figure 4-2. Mycelial growth in nutrient bags.

A. Ranking standard for mycelial growth in nutrient bags.

B. Contamination in nutrient bags. Black (a), yellow and green (b) contaminations.

Result and Discussion

Experiment 1

The *M. rufobrunnea* isolate used in this study produced rich pseudosclerotia on the PDYA media. Agars with active growing mycelia were transferred to grains to produce spawn. At 2 to 3 dpi, white thin mycelia could be found on the surface of the substrates. At 14 dpi, mycelia of all isolates turned yellow to brown. At 15 dpi, white layer of asexual condia were produced on the surface of both substrates in the tub 6, 9, and 12. No obvious conidial formation was found in other tubs. Nutrients bags were added at 45 dpi. Room temperature was kept at 68°F (20°C) during the whole experiment. After decline of the conidial layer, the white conical knots on the surface of the substrate was first found in tub 17 and 18 at 56 dpi, which was filled with soil and peat. The knots continued to grow and developed into the fruit body primordia. The characteristic curved head with white to light beige ridge and brown pits was formed from the primordial fruit body. The fruit body grew very fast and doubled the size in 5 days. It took approximately 16 days to mature from the primordial period (Figure 4-3). Unexpectedly, even though many small primordia were formed in tub 17 and 18, all of them died after one to two weeks and failed to develop into mature fruit body. Finally, 9 mature fruiting bodies were obtained in this study. The length of the mature fruit bodies ranged from 12 to 18 cm and the fresh weight ranged from 18.05 g to 55.8 g. According to the results in this experiment, the formulas of nutrient bags did not make a difference on the fruit body initiation. Dai (2018) reported the optimal application density of nutrient bags for outdoor cultivation of WC 833 *M. rufobrunnea* (the same isolate as we used in this study) was to add nutients bags at a rate of 2400 g/m^2 on the beds at 15 dpi. The optimal formula of spawn substrates and nutrient bags was 80% wheat berry, 9% wheat bran, 9% wood chips, 1% lime, and 1% gypsum (by weight) and

the moisture was adjusted to 60% (by weight). Wheat and corn worked equally well in terms of sclerotia production in nutirient bags. However, the moisture in the bags was easily lost because the density of the filled bags was too low (too much air space). Moreover, *Morchella* cound not consume the nutrients in the bags very fast, so more contamination was found in the bags filled with corn. Such problems were not observed in our study, possibly because the growing room used in our experiment was cleaner than the greenhouse where Dai conducted his study. In morel production, growers generally use what is available and cheap when preparing nutrient bags (personal communication). *Morchella* fruit bodies were successfully initiated under substrate treatment 1 (soil + sawdust), 2 and 3 (soil + wheat straw). Three, five and two were harvested from treatment 1, 2 and 3 respectively. No mature fruit body was harvested from treatment 4 and 5. The color of the fruit bodies varied from light beige to dark brown due to different environmental conditions, such as light intensity, humidity and temperature. Even though the variation in the environmental factors were controlled to minimize varibaility, due to the location of the humidifier and the position of the tubs on the shelves, there was some difference in the light condition, soil moisture and temperature. The tubs located at the lower level of the shelves were more likely to be flooded, while those placed at the higher level were drier. Tub 10, 7 and 4 were became saturated due to excessive free water from the humidifier. As a result, no primordial or mature fruit bodies were formed in these tubs. Tubs 15, 18, 19 and 22 were drier compared to other tubs and no fruit bodies were formed from these tubs as well.

Figure 4-3. Fruit body initiation in *M. rufobrunnea*. (a) A 5 days old fruiting body. Note the white base, stem and slightly pigmented conical shaped head; (b) A 10 days old fruiting body: Note the changing in size and color of the head. The ridge turned light grey and pits turned brown to grey; (c) A 14 days old fruiting body. Note the changes in size and color. The stalk turned light beige; (d) A 16 days old mature fruiting body. The mature fruit body reached 12 to 13 cm in length.

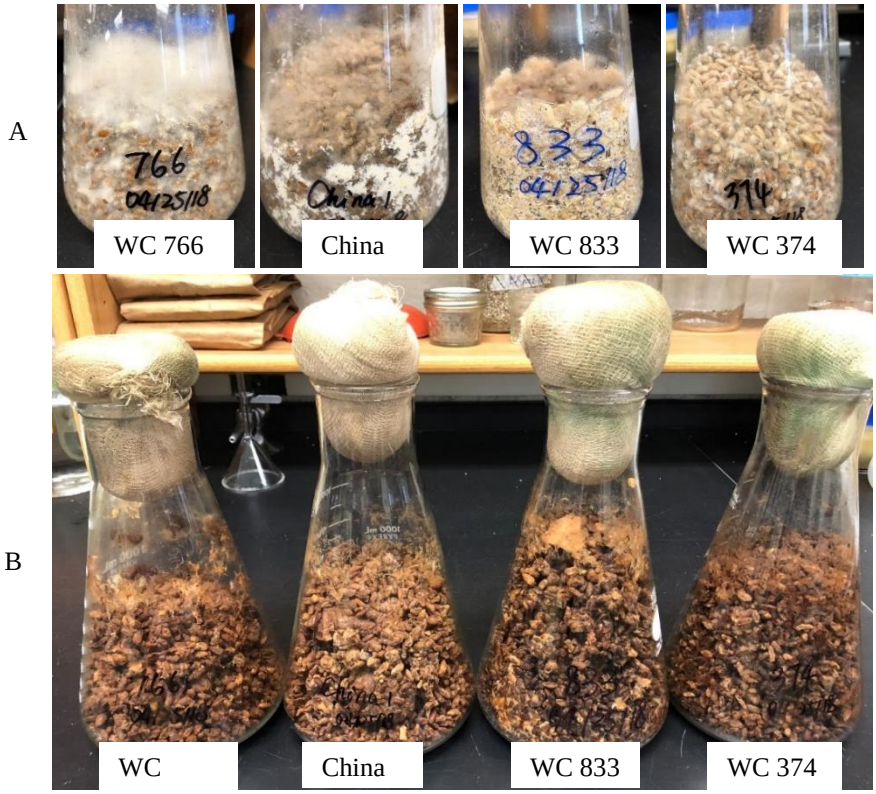


Figure 4-4. Spawn production.

A. The grain mixture at 6 dpi.

B. The spawn in the flasks were ready to use at 35 dpi.

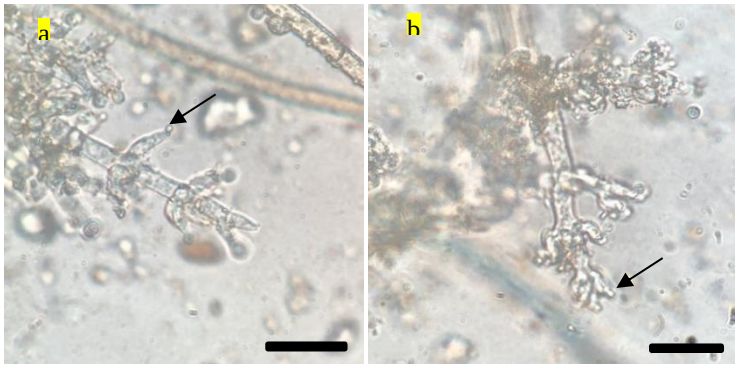


Figure 4-5. Microscopic image of asexual spores produced on the surface of the substrate.

Conidiophore with conidia (black arrow) of (a) *M. rufobrunnea* and (b) *M. importuna*. Pictures were taken at 7 dpi.

Scale bars = 20 μm

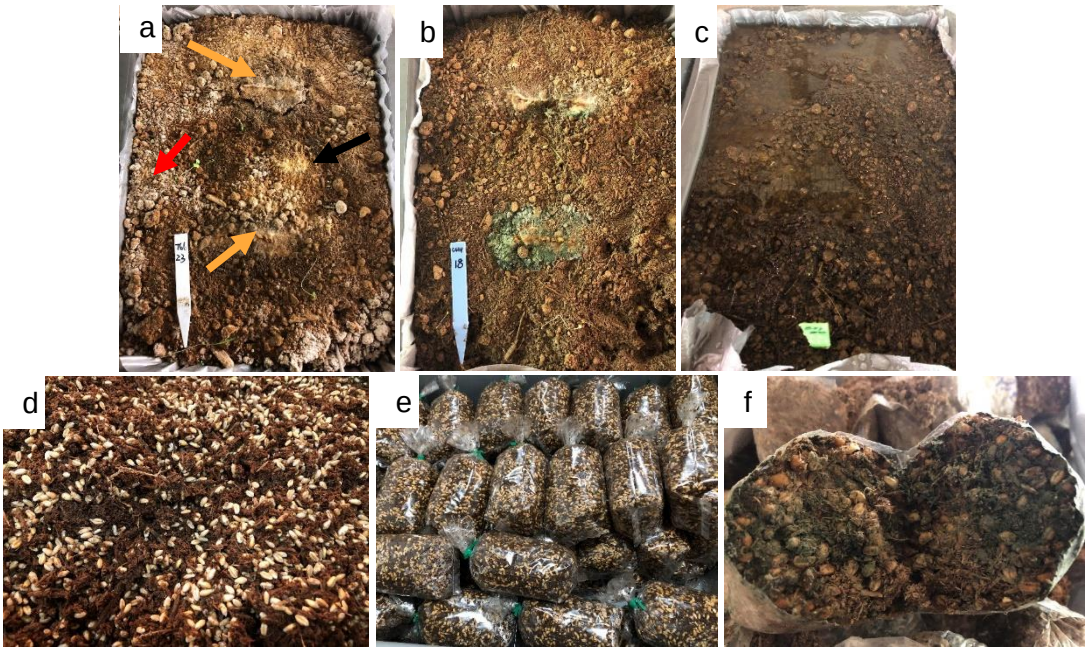


Figure 4-6. a. The surface of the substrate after removing nutrients bags. Note the white layer of conidia (red arrow), pseudosclerotia formation on the nutrient bags attaching site (yellow arrow) and yellow contamination on the soil surface (black arrow); b. Green contamination on the surface after removing nutrient bags.; c. Heavy watering on the tubs after removing nutrient bags; d. The mixture of sawdust and corn used in nutrient bags; e. Nutrient bags filled with sawdust and corn mixture; f. Green contamination in nutrient bags.

Experiment 2

The *M. rufobrunnea* isolate and *M. importuna* isolate used in this study produced rich pseudosclerotia on the PDYA media. Agars with active growing were transferred to grains to produce spawn. White mycelia were produced inside and outside the grains for all four tested isolates. WC 766 produced characteristic large amount white fluffy mycelia. WC 833 *M. rufobrunnea* and China 1 *M. importuna* produced white to yellowish brown condensed masses on the wall of the flasks within one week (Figure 4-4, A). At 35 dpi, the grains were soft, moist and colonized by brown mycelia. Brown masses were found in the spawn of all four isolates (Figure 4-4, B), but the structure of the masses has yet to be defined.

At 2 to 3 dpi, white thin mycelia could be found on the surface of the substrates, which was especially obvious on the straw substrates. At 14 dpi, mycelia of all isolates turned yellow to brown. Nutrients bags were added at 20 dpi. At 15 dpi, white layer of asexual condia were produced on the surface of both substrates for *M. rufobrunnea* and *M. importuna*. The conidia were produced by vegetative mycelium, which may be produced from pseudosclerotia. The amount of conidia and covering area of condia on the surface of the substrates were increased for the first month, while at about 35 to 40 dpi, the amount of observable conidia on the surface of the substrates started to decrease. At 48 dpi, only a small amount of conidia could be observed on the surface of the substrates. For *M. exuberans*, conidia were found on the surface of the straw substrate at 42 dpi in only one tub. However, condia were observed in all replicates for the sawdust substrate at 30 dpi. It is worth mentioning that no conidia were found in vitro experiments for these four tested isolates in this study. The exact role and the importance of conidia in the *Morchella* cultivation is not clear yet. There is a hypothesis that excessive conidia will consume nutrients making them unavailable for *Morchella* fruit body initiation. Microscopy observation showed that conidia were produced on phialides located on conidiophores (Figure 4-5).

In this study, pseudosclerotia were not observed on the surface except the area attached to the nutrient bags. However, whether pseudosclerotia were formed inside the substrates was unknown. One reason is that I did not observe into the substrates in order to keep the substrate intact. The other reason is it was difficult to distinguish the mycelia and pseudosclerotia from the substrates under dark conditions.

For *M. rufobrunnea*, small white initial knots were formed around 90 dpi. The knots continued to grow and developed into primordial stems. Then the stalk and the head with pre-apothecia was formed. Up to 200 small observable knots were observed at 130 dpi, but more than 50% were died at 140 dpi. Some white knots disappeared without any noticing changes. However, those with a bigger size (2 to 3 mm in height) turned dark brown and died eventually. No primordial fruit body successfully developed into mature fruit body. For *M. importuna*, white masses were found on the surface, however no pins and

primordial stage fruiting body was found in this study. For *M. exuberans* and *M. americana*, no obvious formation of pseudosclerotia and primordial fruit body was found.

Similar to experiment 1, some tubs located at the higher level were drier than others. But according to the conidial growth rating, soil moisture did not affect the production of conidia on *M. rufobrunnea* and *M. importuna*.

What caused the difference between Experiment 1 and 2?

Even though the basic techniques and procedures used in two experiments were similar, the results were different: *Morchella* fruit body formation and maturation was successfully initiated in Experiment 1, while not a single mature fruit body was obtained in experiment 2. Here several possible factors that may have influenced the differences in fruiting body maturation are discussed:

1. **Temperature**. The room temperature in experiment 1 was 20°C (68°F) during the whole procedure, while in experiment 2, the temperature was 19.4°C (67°F) for the first two days and maintained 14.4 to 15°C (58 to 59°F) for the most time. In order to trigger the fruit body initiation, the temperature was downregulated to 12.2°C (54°F) at 50 dpi and upregulated to 20°C (68°F) at 67 dpi, but this adjustment did not make noticeable effect on the fruit boy initiation. According to the growers' experiences, low temperatures facilitate the differentiation of mycelia (personal communication). However, in this study, lower temperature did not contribute to desirable mycelial differentiation in experiment 2 when compared to experiment 1. The effect of temperature may be covered up by other factors. Additional data is required to verify the effect of temperature on fruit body initiation on *Morchella* indoor cultivation.

2. **Spawn quality**. Even though the spawn for both experiments were produced following the same protocol, the quality of the spawn may be different. Aging of the cultures was a potential problem in spawn production. We observed weak mycelial growth and diminished pseudosclerotia production on some aged Morchella cultures, especially for *M. rufobrunnea* and *M. americana*. *Morchella* species represent different morphology on different media, which is a major issue in the development of a spawn quality evaluation standard. Due to the fact that there is no available standard for the evaluation of morel spawn quality, the evaluation mostly based on personal experience. Pseudosclerotia production is considered an essential factor in spawn quality evaluation. However, the pseudosclerotia production in the spawn from two experiment is difficult to compare. Unfortunately, I did not take photos of the spawn used in experiment 1, so it is impossible to simply compare the pseudosclerotia production via pictures. Moreover, whether a desirable amount of pseudosclerotia guarantee a good quality of spawn is unclear.

3. **Contamination and flies**. Contamination of nutrient bags was appeared in both experiments, but more frequent and severer contamination was observed in experiment 2. Contamination were observed in both nutrient bags and on soil surface. The nutrient in the nutrient bags were consumed by the contaminators, which resulted in the shortage of nutrient for *Morchella*. Mycelial growth was suppressed in the contaminated area, characterized by lighter color and dryer texture of the substrate. Weak mycelial growth might be a reason for the failure of fruit body initiation. The contaminations were probably originated from the soil or raw materials. Moreover, more flies were found in experiment 2. The eggs may survive in the soil and then developed into flies under warm and moist environment.

4. **Conidial formation**. Conidia were formed in all *M. rufobrunnea* inoculated tubs in experiment 2, while conidial formation was only observed in 3 tubs in experiment 1. Conidia formation is very common in *Morchella* outdoor cultivation (personal communication). As fast as 5 dpi, a white layer

of conidia (2-3 mm thick) could be seen on the soil surface. Environmental conditions, such as light intensity, air humidity, soil humidity and oxygen level, are the key factors that affect the conidial formation. However, how these factors that may affect the conidial formation needs further study. According to farmers' personal experience, complete darkness suppresses the formation of conidia and high oxygen level and lower temperature stimulates the conidial formation. Dai (2018) reported there was a weak, but not significant negative correlation, between conidial production and numbers of primordia, numbers of mature fruit bodies and yield of *M. rofubrunnea*. Unfortunately, it is not clear that what is the light intensity threshold of conidial formation is, and no available data and analysis indicated how air and soil humidity influenced the conidial formation. Nevertheless, conidial production varies largely on *Morchella* species. For instance, the conidial production of *M. sextelata* is generally more abundant than conidial production of *M. importuna* in the outdoor cultivation. In experiment 2, *M. americana* did not produce any conidium in either substrates. In contrast, *M. rufobrunnea, M. importuna* and *M. exuberans* produced conidia in both substrates. In experiment 1, all fruit bodies were collected from the tubs without conidial formation. It seems that conidial formation may not be necessary for *Morchella* fruit body initiation. The relationship between conidial production and yield of morels needs further investigation.

5. **Heavy watering**. Heavy watering is commonly employed to stimulate the fruit body initiation in *Morchella* outdoor cultivation. All the tubs were watered heavily at 65 dpi as soon as the nutrient bags were removed in experiment 2, but this treatment was not applied to experiment 1. Due to the complicated interacting factors, it is difficult to conclude the effectiveness of heavy watering on *Morchella* fruit body initiation. Moreover, heavy watering could be a reason that caused more contamination in experiment 2.

6. **The timing of adding nutrient bags.** Nutrients bags were added to the surface of substrate at 45 dpi and at 20 dpi in experiment 1 and 2 respectively. It is proposed that nutrient bags provide additional nutrients to facilitate mycelial growth and pseudosclerotia formation, which supports the fruit body development. In the outdoor cultivation, nutrient bags with openings are added to the soil with the openings facing the soil surface approximately 7 to 20 days after spawning (W. Liu et al. 2017a). The timing of adding nutrient bags may have an effect on *Morchella* cultivation. Dai (2018) found that the timing of adding nutrient bags (5, 10, and 15 dpi) only effected the fruiting time of *M. rufobrunnea*. The earlier nutrient bags were added, the earlier fruit bodies were formed. However, the yield was not affected by the timing of adding nutrient bags. . Two possible disadvantages may result from adding nutrients bags too early: 1) *Morchella* mycelia may not have completely colonized the surface of the substrate, which can result in the delay of mycelial growth into the nutrient bags. Other competitive microbses, such as *Trichoderma*, can take advantage of this to colonize the nutrient bags due to a lack of competition; 2) Untimely mycelial growth toward nutrient bags may result in inadequate extension of mycelial growth into the soil, which may reduce the fruit body initiation area. This could possibly explain why more contaminated bags were found in experiment 2. Adding nutrient bags too late may delay the nutrient supplies to mycelial growth.

Conclusion

In experiment 1, our results found that the morel outdoor cultivation process was feasible indoors, even though many questions remained. The formula of the nutrient bags was found to have no difference based on the results of fruit body initiation in this study. Many primordia were formed, while only 9 mature fruit bodies of *M. rufobrunnea* were obtained from three tested substrates, and subsequently two substrates (soil + sawdust and soil + wheat straw) were selected for future research. In experiment 2, no mature fruit bodies were formed, however, up to 200 primordia were found on *M. rufobrunnea* in each tub on both tested substrates. The observed differences between the two experiments might be related to

the difference in temperature, spawn quality, contamination of soil, or the timing of adding nutrient bags. More attention should be focused on investigating the effects of environmental growth conditions on primordia maturation to determine why the small fruit bodies never matured in the 2nd experiment and why only a few matured in the first.

Chapter 5

Conclusion and Future Work

The objective of this thesis was to shed light on the biological characteristics of *Morchella* and eventually lead to the successful cultivation of *Morchella*. The first hypothesis was that many isolates in the *Morchella* cultural collection in the Spawn Lab at Penn State were misidentified based on older species concept and outdated techniques. The ITS sequences of the isolates of *Morchella* in the collection were determined and molecular identifications were made comparing these sequences to curated databases. In consideration of the cultivation history, geographical origin and characteristic recorded fruiting patterns, four *Morchella* isolates (WC 833 *M. rufobrunnea*, China 1 *M. importuna*, WC 374 *M. americana* and WC 766 *M. exuberans*) were selected for subsequent studies.

Environmental factors such as pH, light intensity and temperature, may have an effect on the mycelial growth and pseudosclerotia formation of *Morchella*. We hypothesized that: 1) For each of the *Morchella* isolates, there is a pH range and a temperature range that it can grow, but there is an optimal pH and temperature that results in the fastest mycelial growth and most pseudosclerotia production. 2) The effect of light on *Morchella* varies based on light intensity and *Morchella* species. 3) Not all tested *Morchella* isolates can produce pesudosclerotia under tested conditions. The hypothesises were tested by culturing four *Morchella* isolates on PDYA media at four pH levels (5, 6, 7, and 8), under three light intensity conditions (complete darkness (0 lux), low intensity (400 lux), and high intensity (2000 lux)), and under three temperature conditions (20°C, 25°C, and 30°C). For *M. rufobrunnea*, the optimal pH, light condition and temperature resulting in a high level of mycelial growth was at a pH of 6, darkness (0 lux), and at a temperature of 20 and 25°C. For *M. importuna*, the optimal pH, light condition and temperature resulting in a high level of mycelial growth was at a pH of 5 to 6, under darkness (0 lux) and at a low light intensity (400 lux), and at a temperature of 20 to 25°C. For *M. exuberans*, the optimal pH

and temperature was at a pH of 5, and a temperature of 25 to 30°C. Light did not have a significant effect on the mycelial growth of *M. exuberans.* For *M. americana*, the optimal pH, light condition and temperature for mycelial growth was at a pH of 5 to 7, under darkness (0 lux) and low light intensity (400 lux), and at a temperature of 20°C. *M. exuberans* and *M. americana* did not produce pseudosclerotium under any test conditions. The media pH did not affect pseudosclerotia formation for either *M. rufobrunnea* or *M. importuna.* Mycelial growth is the first stage in the life cycle of *Morchella* and pseudosclerotia are essential in the entire *Morchella* life cycle (Volk and Leonard 1990). Based on the results in this study, *M. rufobrunnea* and *M. importuna* demonstrated satisfactory mycelial growth and pseudosclerotia production, so these two isolates are suggested for future cultivation trials. Since high temperatures (30°C) significantly suppressed mycelial growth and pseudosclerotia formation of *M. rufobrunnea* and *M. importuna*, it is suggested that the highest temperature to cultivate these two isolates outdoors should be less than 30°C. It is also suggested to move up the the spawn date in the southern area where the temperature is generally higher to avoid the unfavorable temperatures during the mycelial growth phases when growing *Morchella* outdoors. Light is a suppressing factor of the pseudosclerotia formation of *M. rufobrunnea* and *M. importuna*. The application of mulching films or shade cloths can not only benefit the outdoor cultivation by reserving moisture, but also by protecting *Morchella* mycelia from light. The determination of these optimal environmental conditions resulting in desirable mycelial growth and pseudosclerotia formation will provide guidance on the *Morchella* culture preparation, spawn production and outdoor cultivation.

For the future direction, the following experiments can be pursued:

Study 1

Objective: To test heat and low temperature treatment on pseudosclerotia formation on *Morchella exuberans*.

M. exuberans did not produce any pseudosclerotium under tested conditions. However, it was reported to be a post-fire specie. Heat treatment mimicking wildfires may help triggering pseudosclerotium formation. Interestingly, I found white to orange pseudosclerotia on two old *M. exuberans* cultures growing on PDYA media. These two cultures grew at room temperature (~20°C for the first month and were then stored at 4°C for more than two months. However, pseudosclerotium was not observed in the cultures stored at room temperature. Thus, low temperature treatment may be a potential factor that triggering the pseudosclerotium formation for this species. The experiment can be designed as followed: Three groups of *M. exuberans* will be cultured on PDYA plates at 25°C for 4 to 5 days until the plates are fully colonized. Three cultures will be grown at 4°C, 25 °C and 40 °C for 1 day respectively, and then all the plates will be cultured at 25°C again. Temperature and culturing duration at each temperature may need some adjustments. Plates will be observed every day to determine if any pseudosclerotium form and if any differences are observed based on the different temperature exposure. This study will improve our understanding of the factors that lead to pseudosclerotium development.

Study 2

Objective: To test the effects of environmental factors on *Morchella* spawn production.

The quality of spawn is largely determined by the amount of mycelia and pseudosclerotia formation. Even though the effects of pH, light and temperature on mycelial growth and pseudosclerotia production in vitro were investigated for four *Morchella* isolates, it is not really known how these factors affect the spawn quality and subsequent fruiting potential. Therefore, it is necessary to test how environmental factors affect the amount the mycelia and pseudosclerotia in the spawn and relate how

these factors ultimately affect fruit body production. However, how to effectively and scientifically measure the mycelial growth and pseudosclerotia production in the spawn is a question that needs to be investigated.

Study 3

Objective: To study the effect of successive subculturing on the morphology, mycelial growth and pseudosclerotia formation of four *Morchella* isolates.

The aging of spawn cultures and repeated subculturing are concerning problems in morel cultivation. The use of older spawn may result in reduce yield and profits to farmers depending on how spawn quality impacts fruiting. Microscopy studies revealed the involvement of autophagy, apoptosis and necrosis with the senescence of *M. elata* (P. He et al. 2015). Nevertheless, the molecular mechanism of these behaviors need further study. Delayed mycelial growth, thin mycelia, and the reduction of pseudosclerotia production has been observed in WC 833 and WC 374 after successive subculturing. This study would aim to determine the threshold of subculturing and culture age on *Morchella* spp. viability. It will also be interesting to study if subculturing an isolate in particular subtrates can revive its vigor.

Study 4

Objective: To select species for indoor and outdoor cultivation with high potential for commercial adoption.

Though *Morchella* species are diverse in the wild, species that can be cultivated are limited. In the second cropping trial, I tested four *Morchella* isolates for indoor cultivation, unfortunately no mature

fruiting bodies were formed though many primordia formed for two of the species. Nevertheless, the fruiting body of *M. rufobrunnea* was successfully obtained in the first trail in MRC in 2017 and *M. importuna* is the most widely cultivated morels in China. Based on the successful fruiting of *M. rufobrunnea* in the 1st experiment and the many primoridia observed with *M. rufobrunnea* and *M. importuna* in the 2nd experiment, it is still worth trying to cultivate these two Morchella isolates both indoors as well as outdoors. More work needs done investigating the effects of environmental growth conditions on primordia development to determine why the small fruit bodies never matured in the 2nd experiment and why only a few matured in the first. The first factors that I suggest for testing would be to determine what role humidity, air temperature and light exposure play on fruit body maturation.

Appendix A

Records of *Morchella* Indoor cultivation from Chapter 4

Table A-1. The growth in nutrient bags at 70 dpi.

No.[a]	Mycelial growth[b]	Contamination[c]	Texture, Color, Smell
10B	2	0	white to brown, loose, dry
10F	3	0	white to brown, moist, smelly
11B	5	0	white to orange, soft, condense, moist
11F	5	0	white, condense
12B	4	0	white to brown, yellow pseudosclerotia on wall
12F	5	0	brown, condense, soft, yellow pseudosclerotia on wall
13B	2	2	green contamination, smelly
13F	2	2	green contamination, smelly
14B	4	1	some green contamination inside, moist, fermented smell

14F	2	4	green contamination both inside and outsdide, soft, loose, dry
15B	3	2	green contamination only on the surface, white to yellow pseudosclerotia on surface
15F	4	1	black contamination only on surface
16B	5	1	white to brown, loose, moist, soft
16F	3	2	white to brown, loose, moist, soft
17B	3	2	green contamination, soft, loose
17F	4	1	green contamination, soft, loose, dry in the center
18B	0	0	many larvae
18F	0	0	many larvae
19B	4	1	green contamination only on the surface
19F	4	1	green contamination only on the surface
1B	2	1	green contamination on surface, soft, condense
1F	4	0	white, soft, dry
20B	3	2	green contamination, smelly
20F	4	0	yellow pseudosclerotia on wall, condense
21B	4	1	green contamination on surface, soft, white to yellow pseudosclerotia
21F	3	2	green contamination on surface, soft, white to yellow pseudosclerotia
22B	4	1	green contamination on surface, smelly
22F	3	2	green contamination on surface, soft
23B	2	3	green contamination, smelly, soft
23F	4	0	white, pseudosclerotia on wall
24B	5	0	yellow to orange, condense, pseudosclerotia on wall
24F	5	0	white to orange, condense, moist

25B	3	2	green contamination, soft, smelly
25F	4	1	slightly green contamination, white pseudosclerotia on surface
26B	4	2	green contamination, little loose
26F	4	2	green contamination, little loose
27B	4	0	white to brown, soft
27F	5	0	white to brown, condesnse and moist
28B	4	1	brown, black contamination on surface
28F	2	3	green contamination on surface, smelly
29B	2	4	green on surface, soft, moist
29F	4	2	green contamination, loose
2B	5	0	heavy white mycelia, condense
2F	5	0	heavy white mycelia, condense
30B	4	1	green contamination, dry in the center
30F	4	1	green contamination only on surface
31B	2	3	green contamination, soft, dry loose
31F	4	1	green contamination on surface
32B	5	0	brown, white to orange pseudosclerotia
32F	5	0	brown, white to orange pseudosclerotia
33B	2	3	green contamination both inside and outside
33F	0	0	larvae
34B	3	1	green contamination on surface, moist, smelly
34F	0	0	larvae
35B	3	1	green contamination on surface, loose
35F	5	0	white, yellow pseudosclerotia on surface, condense
36B	5	0	white

36F	3	1	black dots, condense
37B	5	0	green, loose
37F	3	3	white to orange, pseudosclerotia on wall
38B	4	0	white to brown, fermented smell, moist
38F	3	1	green contamination on surface, loose
39B	3	0	loose, dry, hard
39F	2	0	loose, dry, hard
3B	5	0	consense, a little dry, orange mycelia
3F	5	0	consense, a little dry, orange mycelia
41B	4	1	soft, smell alcohol
41F	4	2	soft, smell alcohol
42B	3	2	green contamination only on the surface
42F	5	0	white to yellow, condense
43B	4	0	white, condense, moist, white pseudosclerotia on surface
43F	4	0	white to brown, soft
44B	1	3	black contamination on surface
44F	2	2	green contamination on surface, loose
45B	5	0	white to brown, condense, soft
45F	5	0	white to brown, fermented smell, soft
46B	5	0	heavy, condense
46F	5	0	condense
47B	4	1	black contamination, fermented smell
47F	5	0	condense, white to yellow pseudosclerotia
48B	5	0	white to brown, condensed
48F	3	2	black contamination both inside and outside

4B	5	0	white to brown, soft, condense
4F	5	0	white, heavy mycelia on surface
5B	5	0	white to brown, condense
5F	5	0	white to brown, condense
61B	4	0	white, orange pseudosclerotia, condense
61F	3	1	black contamination on surface, loose
6B	3	1	soft, white center, dark outside
6F	3	2	soft, moist, white center, darker outside
7B	4	1	black contamination only on surface, condense
7F	3	1	black contamination only on surface, condense
8B	3	0	white to brown, soft
8F	4	0	white to brown, soft
9B	4	0	soft, moist, white center, darker outside
9F	4	0	soft, moist, white center, darker outside

a. Randomly assigned tub number according to Table 4-1. Each tub had two bags. F: the front bag; B: the back bag.
b. Mycelial growth ranking from 0 to 5.
c. Contamination ranking from 1 to 5.

Table A-2. Morchella indoor cultivation record sheet.

Isolate: 833 *M. rufobrunnea* Straw Spawn Date: 6/1/2018

Date	Air Temperature (F)	Soil Temperature(F)	Conidium[a]	Mycelium	Growth in nutrient bags[b]	Other
6/4/2018	66	63	-	White on surface		Water
6/6/2018	62	60	-	White		Water
6/9/2018	57	56	-	White		Water
6/11/2018	58	56	+	White		Water
6/13/2018	58	56	+	White		Water
6/15/2018	58	58	37-2; 38-2; 39-2; 40-1; 43-1; 44-0	White		Water
6/17/2018	59	57	37-2; 38-2; 39-2; 40-1; 43-1; 44-1	White		Water
6/20/2018	58	56	37-2; 38-2; 39-3; 40-1; 43-1; 44-1	Brown		**Add nutrient bags**
6/23/2018	58	55	37-4; 38-2; 39-3; 40-2; 43-2; 44-3	Orange to brown mycelia		Water
6/25/2018	58	56				Water
6/27/2018	59	57	37-5; 38-3; 39-3; 40-3; 43-3; 44-4	Heavy white mycelia grew around bags		Water
6/29/2018	58	56	37-4; 38-3; 39-3; 40-3; 43-3; 44-4	Brown		Water
7/1/2018	59	57	37-4; 38-4; 39-3; 40-3; 43-3; 44-4	Brown	0	Water

7/3/2018	60	59	37-4; 38-4; 39-3; 40-3; 43-2; 44-4	Brown	0	Water
7/5/2018	63	60	37-3; 38-3; 39-3; 40-2; 43-2; 44-4	Brown	0	Water
7/9/2018	59	57	37-3; 38-2; 39-3; 40-2; 43-2; 44-4	Brown	37-0-1; 38-0-0; 39-0-0; 40-1-0; 43-0-0; 44-0-0	Water
7/12/2018	60	58	37-2; 38-1; 39-2; 40-2; 43-1; 44-3	Brown	37-1-2; 38-1-1; 39-0-0; 40-1-1; 43-2-0; 44-0-0	Water
7/15/2018	60	59	37-2; 38-1; 39-2; 40-2; 43-1; 44-2	Brown	37-1-3; 38-1-1; 39-0-0; 40-1-1; 43-2-0; 44-0-0	Water
7/18/2018	60	58	37-1; 38-1; 39-1; 40-1; 43-1; 44-2	Brown	37-1-3; 38-1-2; 39-2-1; 40-2-2; 43-2-2; 44-0-2	Water
7/21/2018	60	58		Brown		Water
7/25/2018	61	59	37-1; 38-1; 39-1; 40-1; 43-1; 44-1	Brown	37-2-4; 28-2-3; 39-3-2; 40-3-2; 43-2-2; 44-1-4	Water
7/29/2018	60	58	-	Brown		Water

Isolate: 833 *M. rufobrunnea* Sawdust Spawn Date: 6/1/2018

Date	Air Temperature (F)	Soil Temperature	Conidium	Mycelium	Growth in nutrient bags	Other
6/4/2018	66	63	-	White on surface		Water
6/6/2018	62	60	-	White		Water

6/9/2018	57	56	-	White		Water
6/11/2018	58	56	+	White		Water
6/13/2018	58	56	+	White		Water
6/15/2018	58	58	25-1; 26-1; 29-2; 30-1; 33-3; 34-1	White		Water
6/17/2018	59	57	25-2; 26-2; 29-2; 30-1; 33-4; 34-2	White		Water
6/20/2018	58	56	25-2; 26-2; 29-3; 30-2; 33-4; 34-2	Brown		**Add nutrient bags**
6/23/2018	58	55	25-2; 26-3; 29-3; 30-2; 33-4; 34-2	Brown		Water
6/25/2018	58	56	25-3; 26-3; 29-3; 30-2; 33-5; 34-2	Brown		Water
6/27/2018	59	57	25-3; 26-3; 29-3; 30-3; 33-5; 34-2	Brown		Water
6/29/2018	58	56	25-3; 26-3; 29-4; 30-3; 33-5; 34-3	Brown		Water
7/1/2018	59	57	25-3; 26-3; 29-4; 30-3; 33-5; 34-3	Brown	25- green spots; 26-1-1; 29-1-1; 30-1-1; 33-1-1; 34-1-green	Water
7/3/2018	60	59	25-3; 26-3; 29-4; 30-3; 33-5; 34-4	Brown	25-1-2; 26-black-3; 29-2-3; 30-3-4; 33-1-1; 34-2-2	Water
7/5/2018	63	60	25-4; 26-4; 29-5; 30-3; 33-5; 34-4	Brown	25-2-3; 26-3-4; 29-2-4; 30-3-4; 33-1-1; 34-2-2	Water

7/9/2018	59	57	25-4; 26-4; 29-5; 30-3; 33-5; 34-4	Brown	25-3-4; 26-4-3; 29-4-4; 30-3-4; 33-2-1; 34-3-3	Water
7/12/2018	60	58	25-3; 26-3; 29-3; 30-2; 33-4; 34-3	Brown	25-3-4; 26-4-4; 29-5-5; 30-3-4; 33-3-1; 34-4-4	Water
7/15/2018	60	59	25-3; 26-2; 29-2; 30-2; 33-3; 34-3	Brown	25-3-4; 26-4-4; 29-5-5; 30-3-4; 33-3-2; 34-5-4	Water
7/18/2018	60	58	25-2; 26-1; 29-2; 30-1; 33-2; 34-2	Brown	25-4-5; 26-5-5; 29-5-5; 30-5-5; 33-4-2; 34-5-5	Water
7/21/2018	60	58		Brown		Water
7/25/2018	61	59	25-2; 26-1; 29-1; 30-1; 33-2; 34-1	Brown	25-5-5; 26-5-5; 29-5-5; 30-5-5; 33-5-4; 34-5-5	Water
7/29/2018	60	58	-	Brown		Water

Isolate: CHN *M. importuna* Straw Spawn Date: 6/1/2018

Date	Air Temperature (F)	Soil Temperature	Conidium	Mycelium	Growth in nutrient bags	Other
6/4/2018	66	63	-	White on surface		Water
6/6/2018	62	60	-	White		Water
6/9/2018	57	56	-	More white, white mycelial clusters		Water
6/11/2018	58	56	+	White		Water

6/13/2018	58	56	+	White		Water
6/15/2018	58	58	1-1; 5-2; 6-2; 9-2; 10-2	White		Water
6/17/2018	59	57	1-2; 2-2; 5-2; 6-2; 9-2; 10-2	White		Water
6/20/2018	58	56	1-2; 2-2; 5-2; 6-2; 9-2; 10-2	White mycelia turned yellow to brown		**Add nutrient bags**
6/23/2018	58	55	1-3; 2-3; 5-2; 6-2; 9-3; 10-2	Orange to brown mycelia		Water
6/25/2018	58	56	1-3; 2-3; 5-2; 6-2; 9-3; 10-3			Water
6/27/2018	59	57	1-3; 2-3; 5-2; 6-2; 9-3; 10-3	White mycelia grew around bags	More growth	Water
6/29/2018	58	56	1-3; 2-3; 6-2; 9-4; 10-3	Brown		Water
7/1/2018	59	57	1-3; 2-3; 5-3; 6-2; 9-3; 10-4	Brown		Water
7/3/2018	60	59	1-4; 2-3; 5-3; 6-2; 9-3; 10-4	Brown	1-0; 2-0; 5-0; 6-0; 9-2-2; 10-1-1	Water
7/5/2018	63	60	1-2; 2-3; 5-2; 6-2; 9-3; 10-3	Brown	1-0; 2-0; 5-0; 6-0; 9-2-3; 10-1-1	Water
7/9/2018	59	57	1-2; 2-2; 5-2; 6-2; 9-3; 10-3	Brown	1-0; 2-0-2; 5-0; 6-1-1; 9-3-4; 10-0-4	Water
7/12/2018	60	58	1-2; 2-1; 5-1; 6-1; 9-2; 10-2	Brown	1-0-1; 2-0-3; 5-1-1; 6-2-2; 9-3-4; 10-1-4	Water
7/15/2018	60	59	1-2; 2-1; 5-1; 6-1; 9-2; 10-2	Brown	1-0-2; 2-0-3; 5-1-2; 6-2-2; 9-3-4; 10-1-4	Water

7/18/2018	60	58	1-1; 2-1; 5-1; 6-1; 9-1; 10-1	Brown	1-1-2; 2-1-4; 5-2-3; 6-3-3; 9-3-5; 10-1-4	Water
7/21/2018	60	58	+	Brown		Water
7/25/2018	61	59	1-1; 2-1; 5-1; 6-1; 9-1; 10-1	Brown	1-1-3; 2-1-5; 5-3-4; 6-4-4; 9-4-5; 10-1-4	Water
7/29/2018	60	58	+	Brown		Water

Isolate: CHN *M. importuna* Sawdust Spawn Date: 6/1/2018

Date	Air Temperature (F)	Soil Temperature	Conidium	Mycelium	Growth in nutrient bags	Other
6/4/2018	66	63	-	White on surface		Water
6/6/2018	62	60	-	White		Water
6/9/2018	57	56	-	White		Water
6/11/2018	58	56	+	White		Water
6/13/2018	58	56	+	White		Water
6/15/2018	58	58	13-3; 14-3; 17-3; 18-2; 21-2; 22-1	White		Water
6/17/2018	59	57	13-3; 14-3; 17-3; 18-2; 21-2; 22-1	White		**Add nutrient bags**
6/20/2018	58	56	13-3; 14-4; 17-4; 18-2; 21-3; 22-1	White mycelia turned yellow to brown		Water
6/23/2018	58	55	13-3; 14-4; 17-4; 18-3; 21-3; 22-2	Orange to brown mycelia		Water
6/25/2018	58	56	13-4; 14-4; 17-4; 18-3; 21-3; 22-1			Water
6/27/2018	59	57	13-4; 14-4; 17-4; 18-3; 21-3; 22-3	White mycelia grew around bags	More growth	Water

6/29/2018	58	56	13-4; 14-3; 17-4; 18-4; 21-4- 22-4	Brown		Water
7/1/2018	59	57	13-4; 14-3; 18-4; 21-4; 17-4; 22-4	Brown		Water
7/3/2018	60	59	13-4; 14-3; 18-4; 21-4; 17-4; 22-4	Brown	1-0; 2-0; 5-0; 6-0; 9-2-2; 10-1-1	Water
7/5/2018	63	60	13-4; 14-3; 18-3; 17-3; 21-5; 22-5	Brown	1-0; 2-0; 5-0; 6-0; 9-2-3; 10-1-1	Water
7/9/2018	59	57	13-3; 14-3; 17-3; 18-3; 21-4; 22-4	Brown	1-0; 2-0-2; 5-0; 6-1-1; 9-3-4; 10-0-4	Water
7/12/2018	60	58	13-3; 14-2; 17-2; 18-2; 21-2; 22-2	Brown	1-0-1; 2-0-3; 5-1-1; 6-2-2; 9-3-4; 10-1-4	Water
7/15/2018	60	59	13-2; 14-2; 17-2; 18-2; 21-2; 22-2	Brown	1-0-2; 2-0-3; 5-1-2; 6-2-2; 9-3-4; 10-1-4	Water
7/18/2018	60	58	13-1; 14-1; 17-1; 18-1; 21-1; 22-1	Brown	1-1-2; 2-1-4; 5-2-3; 6-3-3; 9-3-5; 10-1-4	Water
7/21/2018	60	58		Brown		Water
7/25/2018	61	59	13-1; 14-1; 18-1; 17-1; 21-1; 22-1	Brown	1-1-3; 2-1-5; 5-3-4; 6-4-4; 9-4-5; 10-1-4	Water
7/29/2018	60	58	+	Brown		

Isolate: 374 *M.americana* Straw Spawn Date: 6/1/2018

Date	Air Temperature (F)	Soil Temperature	Conidium	Mycelium	Growth in nutrient bags	Other
6/4/2018	66	63	-	White on surface		Water
6/6/2018	62	60	-	White		Water
6/9/2018	62	60	-	More white		Water
6/11/2018	61	60	-	White		Water
6/13/2018	60	58	-	White		Water

6/15/2018	61	58	-	White		Water
6/17/2018	59	57	-	White		**Add nutrient bags**
6/20/2018	58	56	-	White mycelia turned orange to brown		Water
6/23/2018	58	55	-	Orange to brown mycelia		Water
6/25/2018	58	56	-	White mycelia grew around bags	some white mycelial growth inside bags	Water
6/27/2018	59	57	-	White mycelia grew around bags	more growth	Water
6/29/2018	58	56	-	Brown		Water
7/1/2018	59	57	-	Brown	White under bags	Water
7/3/2018	60	59	-	Brown	41-2-2; 42-1-1; 45-1-0; 47-2-2; 48-1-1	Water
7/5/2018	63	60	-	Brown	41-2-3; 42-2-2; 45-2-2; 46-0-0; 47-2-2; 48-1-1	Water
7/9/2018	59	57	-	Brown	41-3-4; 42-black-4; 45-2-3; 46-1-1; 47-2-3; 48-1-2	Water
7/12/2018	60	58	-	Brown	41-4-5; 42-black-4; 45-2-3; 46-2-1; 47-2-5; 48-1-2	Water

7/15/2018	60	59	-	Brown	41-4-5; 42-black-4; 45-2-3; 46-2-1; 47-2-5; 48-1-2	Water
7/18/2018	60	58	-	Brown	41-5-5; 42-black-5; 45-3-3; 46-3-2; 47-3-5; 48-2-2	Water
7/21/2018	60	58	-	Brown		Water
7/25/2018	61	59	-	Brown	41-5-5; 42-3-5; 45-3-3; 46-3-2; 47-3-5; 48-2-3	Water
7/29/2018	60	58	-	Brown		Water

Isolate: 374 *M.americana* Sawdust Spawn Date: 6/1/2018

Date	Air Temperature (F)	Soil Temperature	Conidium	Mycelium	Growth in nutrient bags	Other
6/4/2018	66	63	-	White on surface		Water
6/6/2018	62	60	-	White		Water
6/9/2018	62	60	-	More white		Water
6/11/2018	61	60	-	White		Water
6/13/2018	60	58	-	White		Water
6/15/2018	61	58	-	White		Water
6/17/2018	59	57	-	White		**Add nutrient bags**
6/20/2018	58	56	-	White mycelia turned orange to brown		Water
6/23/2018	58	55	-	Orange to brown mycelia		Water

6/25/2018	58	56	-	35 and 28: some contamination	Growth less than straw	Water
6/27/2018	59	57	-	White mycelia grew around bags	More growth	Water
6/29/2018	58	56	-	Brown		Water
7/1/2018	59	57	-	Brown		Water
7/3/2018	60	59	-	Brown	27-1-2; 28-1-2; 31-2-3; 32-2-1; 35-1-0; 36-1-2	Water
7/5/2018	63	60	-	Brown	27-2-3; 28-2-2; 31-2-3; 32-2-2; 35-1-2; 36-3-3	Water
7/9/2018	59	57	-	Brown	27-3-3; 28-3-2; 31-3-4; 332-3-3; 35-3-3; 36-2-3	Water
7/12/2018	60	58	-	Brown	27-3-3; 28-3-2; 31-3-4; 32-3-3; 35-3-4; 36-3-4	Water
7/15/2018	60	59	-	Brown	27-3-3; 28-3-3; 31-3-4; 32-3-3; 35-4-5; 36-4-5	Water
7/18/2018	60	58	-	Brown	27-4-4; 28-4-4; 31-4-4; 32-5-4; 35-4-5; 36-4-5	Water
7/21/2018	60	58	-	Brown		Water

7/25/2018	61	59	-	Brown	27-5-5; 28-5-4; 31-5-5; 32-5-5; 35-5-5; 36-4-5	Water
7/29/2018	60	58	-	Brown		Water

Isolate: 766 *M. exuberans* Straw Spawn Date: 6/1/2018

Date	Air Temperature (F)	Soil Temperature	Conidium	Mycelium	Growth in nutrient bags	Other
6/4/2018	66	63	-	White on surface		Water
6/6/2018	62	60	-	White		Water
6/9/2018	57	56	-	White		Water
6/11/2018	58	56	-	White		Water
6/13/2018	58	56	-	White		Water
6/15/2018	58	58	-	White		Water
6/17/2018	59	57	-	White		Water
6/20/2018	58	56	-	White mycelia turned yellow to brown		**Add nutrient bags**
6/23/2018	58	55	-	Orange to brown mycelia		Water
6/25/2018	58	56	-			Water
6/27/2018	59	57	-	White mycelia grew around bags		Water
6/29/2018	58	56	-	Brown		Water
7/1/2018	59	57	-	Brown	8-1-1; 11-1-1; 12-1-1	Water
7/3/2018	60	59	-	Brown	3-1-1; 4-1-1; 8-1-1; 11-1-1; 12-2-0	Water

7/5/2018	63	60	-	Brown	3-2-2; 4-1-1; 7-7-7; 8-1-1; 12-2-2	Water
7/9/2018	59	57	-	Brown	4-1-2; 7-1-2; 8-1-2; 11-2-3; 12-2-4	Water
7/12/2018	60	58	3-3	Brown	4-1-3; 3-4-4; 7-2-3; 8-2-3; 11-3-3; 12-2-3	Water
7/15/2018	60	59	3-3	Brown	3-4-4; 4-1-3; 7-2-3; 8-3-3; 11-3-3; 12-2-3	Water
7/18/2018	60	58	3-3; 11-2; 8-2	Brown	3-5-4; 4-2-3; 7-3-4; 8-3-3; 11-5-4; 12-2-5	Water
7/21/2018	60	58	-	Brown		Water
7/25/2018	61	59	3-4; 8-2; 11-3	Brown	3-5-5; 4-2-3; 7-3-5; 8-4-4; 11-5-5; 12-2-5	Water
7/29/2018	60	58	-	Brown		Water

Isolate: 766 *M. exuberans* Sawdust Spawn Date: 6/1/2018

Date	Air Temperature (F)	Soil Temperature	Conidium	Mycelium	Growth in nutrient bags	Other
6/4/2018	66	63	-	White on surface		Water
6/6/2018	62	60	-	White		Water
6/9/2018	57	56	-	White		Water
6/11/2018	58	56	-	White		Water
6/13/2018	58	56	-	White		Water
6/15/2018	58	58	-	White		Water
6/17/2018	59	57	-	White		Water

6/20/2018	58	56	24-1	White mycelia turned yellow to brown		**Add nutrient bags**
6/23/2018	58	55	24-1	Orange to brown mycelia		Water
6/25/2018	58	56	20-1; 23-1; 24-1			Water
6/27/2018	59	57	20-1; 23-1; 24-1	White mycelia grew around bags		Water
6/29/2018	58	56	16-1; 20-1; 23-1; 24-1	Brown		Water
7/1/2018	59	57	15-1; 16-1; 19-2; 20-1; 23-2; 24-2	Brown	15-1-1; 16-1-1; 19-1-1; 20-1-1; 24-3-3	Water
7/3/2018	60	59	16-1; 15-2; 19-2; 20-2; 23-3; 24-2	Brown	15-2-2; 16-3-3; 20-2-2; 19-2-3; 23-2-2, 24-3-3	Water
7/5/2018	63	60	15-3; 16-2; 19-2; 20-2; 23-4; 24-3	Brown	15-2-2; 16-2-3; 19-2-2; 20-2-3; 23-3-4; 24-3-4	Water
7/9/2018	59	57	16-3; 15-3; 19-4; 20-3; 23-4; 24-3	Brown	15-2-3; 16-3-3; 20-2-4; 23-4-3; 24-3-5	Water
7/12/2018	60	58	15-4; 16-5; 19-4; 20-3; 23-5; 24-4	Brown	15-2-3; 16-3-3; 19-3-4; 20-2-4; 23-4-3; 24-3-5	Water
7/15/2018	60	59	15-4; 16-5; 19-4; 20-3; 23-5; 24-4	Brown	15-2-3; 16-3-3; 19-3-4; 20-3-4; 23-4-3; 24-3-5	Water

7/18/2018	60	58	15-5; 16-5; 19-4; 20-3; 23-5; 24-5	Brown	15-4-4; 16-4-5; 19-3-4; 20-4-5; 23-5-4; 24-4-5	Water
7/21/2018	60	58	-	Brown		Water
7/25/2018	61	59	15-5; 16-5; 19-4; 20-3; 23-5; 24-5	Brown	15-5-5; 16-5-5; 19-4-4; 20-4-5; 23-5-5; 24-5-5	Water
7/29/2018	60	58	-	Brown		Water

a. Tub number-condidium ranking (from 1 to 5); "-" represents no conidial growth observed in all tubs; "+" represents there was conidial growth at least in one tub.

b. Tub number-front bag ranking (from 1 to 5) - back bag ranking (from 1 to 5)

Table A-3. Soil test report (particle size)

PennState Extension

Agricultural Analytical Services Laboratory
The Pennsylvania State University
111 Ag Analytical Svcs Lab
University Park, PA 16802
(814) 863-0841 aaslab@psu.edu www.aasl.psu.edu

SOIL TEST REPORT FOR:		ADDITIONAL COPY TO:
JOHN PECCHIA MUSHROOM RESEARCH CENTER BIG HOLLOW RD UNIVERSITY PARK PA 16802		
DATE RECEIVED	**DATE COMPLETE**	**COUNTY**
05/16/2019	**5/21/2019**	**Centre**

Particle Size Analysis

Customer ID	Serial #	Lab ID	Sand %	Silt %	Clay %	Soil Textural Class
		S19-25599	35.6	40.1	24.3	Loam

Table A-4. Soil test report (nitrogen and carbon)

PennState Extension

Agricultural Analytical Services Laboratory
The Pennsylvania State University
111 Ag Analytical Svcs Lab
University Park, PA 16802
(814) 863-0841 aaslab@psu.edu www.aasl.psu.edu

SOIL TEST REPORT FOR:	ADDITIONAL COPY TO:
JOHN PECCHIA MUSHROOM RESEARCH CENTER BIG HOLLOW RD UNIVERSITY PARK, PA 16802	

DATE	COUNTY	
05/30/2019	Centre	

Soil Test Report

Lab ID	Field ID	Nitrogen* %	Carbon* %
S19-25599		0.15	1.40

*Combustion Method

Table A-5. Soil test report (soluble salts, organic matter and pH))

PennState Extension

Agricultural Analytical Services Laboratory
The Pennsylvania State University
111 Ag Analytical Svcs Lab
University Park, PA 16802
(814) 863-0841 aaslab@psu.edu www.aasl.psu.edu

Analytical Report

5/20/2019

SOIL TEST REPORT FOR:	ADDITIONAL COPY TO:
JOHN PECCHIA MUSHROOM RESEARCH CENTER BIG HOLLOW RD UNIVERSITY PARK PA 16802	

LAB #	Customer ID	Parameter	Result	Units	Method	Analyst
S19-25599		Soluble salts-Soils	0.24	mmhos/cm	1:2 (soil:water)	SAT
		Organic Matter	2.47	%	Loss on Ignition	SAT
		pH-soil	7.03		1:1 (soil:water)	ACB

Table A-6. Compost analysis report of morel sawdust compost

PENNSTATE

(814) 863-0841 Fax: (814) 863-4540
Agricultural Analytical Services Laboratory
The Pennsylvania State University
University Park, PA 16802
www.aasl.psu.edu

Analysis Report For:	Copy To:
John Pecchia Mushroom Research Center Big Hollow Rd University Park PA 16802	

LAB ID:	SAMPLE ID:	REPORT DATE:	SAMPLE TYPE:	FEEDSTOCKS	COMPOSTING METHOD	COUNTY
C10618	Morel Sawdust @ Spawning	06/07/2018				Centre

COMPOST ANALYSIS REPORT

Compost Test 1C

Analyte	Results (As is basis)	Results (Dry weight basis)
pH	6.8	—
Soluble Salts (1:5 w:w)	0.13 mmhos/cm	—
Solids	81.0 %	—
Moisture	19.0 %	—
Organic Matter	10.9 %	13.4 %
Total Nitrogen (N)	0.11 %	0.14 %
Organic Nitrogen[1]	0.11 %	0.14 %
Ammonium N (NH_4-N)	5.8 mg/kg *or* 0.0006 %	7.2 mg/kg *or* 0.0007 %
Carbon (C)	5.5 %	6.8 %
Carbon:Nitrogen (C:N) Ratio	48.30	48.30
Phosphorus (as P_2O_5)[2]	0.105 %	0.130 %
Potassium (as K_2O)[2]	0.12 %	0.14 %
Calcium (Ca)	0.13 %	0.17 %
Magnesium (Mg)	0.10 %	0.13 %
Sulfur (S)	0.02 %	0.02 %
Sodium (Na)	17 mg/kg	21 mg/kg
Aluminum (Al)	9210.21 mg/kg	11368.96 mg/kg
Iron (Fe)	14980.56 mg/kg	18491.81 mg/kg
Manganese (Mn)	739.58 mg/kg	912.93 mg/kg
Copper (Cu)	28.90 mg/kg	35.68 mg/kg
Zinc (Zn)	32.07 mg/kg	39.59 mg/kg

[1] See comments on back of report .

[2] To convert phosphorus as (P_2O_5) into elemental phosphorus (P), divide by 2.29. To convert potassium (as K_2O) into elemental potassium (K), divide by 1.20.

Table A-7. Compost analysis report of morel straw compost

PENNSTATE

(814) 863-0841 Fax: (814) 863-4540
Agricultural Analytical Services Laboratory
The Pennsylvania State University
University Park, PA 16802
www.aasl.psu.edu

Analysis Report For:	Copy To:
John Pecchia Mushroom Research Center Big Hollow Rd University Park PA 16802	

LAB ID:	SAMPLE ID:	REPORT DATE:	SAMPLE TYPE:	FEEDSTOCKS	COMPOSTING METHOD	COUNTY
C10617	Morel Straw @ Spawning	06/07/2018				Centre

COMPOST ANALYSIS REPORT

Compost Test 1C

Analyte	Results (As is basis)	Results (Dry weight basis)
pH	7.1	—
Soluble Salts (1:5 w:w)	0.18 mmhos/cm	—
Solids	82.9 %	—
Moisture	17.1 %	—
Organic Matter	7.6 %	9.2 %
Total Nitrogen (N)	0.13 %	0.16 %
Organic Nitrogen[1]	0.13 %	0.16 %
Ammonium N (NH_4-N)	17.5 mg/kg *or* 0.0018 %	21.2 mg/kg *or* 0.0021 %
Carbon (C)	2.8 %	3.3 %
Carbon:Nitrogen (C:N) Ratio	21.00	21.00
Phosphorus (as P_2O_5)[2]	0.126 %	0.152 %
Potassium (as K_2O)[2]	0.15 %	0.18 %
Calcium (Ca)	0.14 %	0.17 %
Magnesium (Mg)	0.11 %	0.13 %
Sulfur (S)	0.02 %	0.03 %
Sodium (Na)	24 mg/kg	29 mg/kg
Aluminum (Al)	11549.10 mg/kg	13933.33 mg/kg
Iron (Fe)	18313.51 mg/kg	22094.21 mg/kg
Manganese (Mn)	828.19 mg/kg	999.17 mg/kg
Copper (Cu)	35.96 mg/kg	43.39 mg/kg
Zinc (Zn)	39.54 mg/kg	47.71 mg/kg

[1] See comments on back of report .

[2] To convert phosphorus as (P_2O_5) into elemental phosphorus (P), divide by 2.29. To convert potassium (as K_2O) into elemental potassium (K), divide by 1.20.

Table A-8. Compost analysis report of nutrient bag

PENNSTATE

(814) 863-0841 Fax: (814) 863-4540
Agricultural Analytical Services Laboratory
The Pennsylvania State University
University Park, PA 16802
www.aasl.psu.edu

Analysis Report For:	Copy To:
John Pecchia Mushroom Research Center Big Hollow Rd University Park PA 16802	

LAB ID	SAMPLE ID	REPORT DATE	SAMPLE TYPE	FEEDSTOCKS	COMPOSTING METHOD	COUNTY
C10646	Nutrient Bag 6/12/18	06/22/2018				Centre

COMPOST ANALYSIS REPORT

Compost Test 1A

Analyte	Results (As is basis)	Results (Dry weight basis)
pH	6.0	—
Soluble Salts (1:5 w:w)	0.27 mmhos/cm	—
Solids	54.2 %	—
Moisture	45.8 %	—
Organic Matter	53.3 %	98.4 %
Total Nitrogen	0.66 %	1.22 %
Carbon	24.0 %	44.2 %
Carbon:Nitrogen Ratio	36.1	36.1

Appendix B

The ITS sequences data from Chapter 2

Consensus of 2 sequences: 761-ITS1 extraction, 761-ITS4 (reversed)

CTGCGGAAGGATCATTACCAAGAACCATACAGAAAAGGGAGGCATTAGGG
GACCGACAGGGCTAGTAGCTTATACGTTGTTGAACGTCCAGTATGGACCC
GAAGCCTCCCCCATCTAAACCCTCTGCGTACCCGTCCCTTCTTGCTTCCC
CCGGCATCTCGTCGGGGGGAGGTAACAACCAAAACTCTATGTGAATCAAA
CAGCCGTCAGAATTATAAAACAAACAAAAAGTTAAAACTTTCAACAACGG
ATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG
CCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATAAAAACCTCC
TCCCCCTTCGGGTTTTGTTACTATCGTTGGGGGGTTTTGGCCTAATGGGA
TAGCGATTGGCAATTCGTTTCCCAATGTCCTAAATAGACGTAGACCCGCC
TCCAGATGCGACAGCACCGAGGCCATCAACCGTGGAGTTATGGGATATAA
TAGGCTTGCAGTAAAATGCTCACCTCTCTCCACACGCCGATGGCACGACA
GTTGCAGTTGCGGGCGTAAATTGGAGCCCTTTTCAGGACCCTTGTGGCCT
AGCATCCACCATACATAATTTGACCTCGGATCAGGTAGGGATACCCGCTG
AACTTAAGCATATCAATAA

Consensus of 2 sequences: 751-ITS1 extraction 2, 751-ITS4 (reversed)

CCAAGAACCAAAACACATGAGCGAGGGATCGCGCTCGCGCTGGGCTCGAT
AGACGCGCTAGCACCCGTCAGGCCCGGAGGGCGAACCAGGCCCCTGGGGC
CGGCAGGTTAAACCCAAGGTACCCATCCAAGGTGGACAGTCCTCCCAGAG
TTCGCCCTTCGGCGTCTGCGAGCCATCGGGGCATGGAACCTTGGGGGCTG
TCGGTTTCCCGCTGGGCATACGTCCGATGCCGCCTCGGGGAGCGAAACGT
GGCTCGCGCAGCGTCGCAGCGTTGGTCCCCCACTCACTCACCAAAACCCT
CTGCGTACCTTGCCCACTTGCTTCCCCCGGCCCTCCCGGCCGGGGGGCGA
CACCAAAAACCAAACTCTTTGCGATGAACCGACGTCTGAATGCCAAAAGC
AAAACAAAAACAGTTAAAACTTTCAACAACGGATCTCTTGGCTCCCACAT
CGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCA
GTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGG
GGCATGCCTGTCCGAGCGTCATAAAACCCCCTCCCCCTCGGATTAAATGT
TCCTTGGGGGGTATTGGCCAATGGGATTGCGAGATAGCAATTGTTACCCA
GGCGCCCAAATGCATCAGTACCCCGCCATTTACAGATTTACCAGCACCGA
GGCCGTCAACCGTGGAGTTATGGGAATTATAGGCTTGCAGTAATATGCTC
ACCTCTCTCCATACGCAGGCGGCACACCGGGTAGAGTCGCGGGCGTAAAG

CGGAATCCGAAACGGCCCTCACGGGTAGAGGAGGATCCTTGCGGGCTAGT
CACCCCTCATCACAATTGACCTCGGATCAGG

Consensus of 2 sequences: 748-ITS1 extraction, 748-ITS4 (reversed)

GACAGTGGGGAGAGAGAGAGCCTACCACGATGGATGACGCCCTCAAATTG
GCGGGTTCCACACCACGGTGTCGGTCCGGGGCGGGCCGGCCAGCGCAGCC
TCATCCGAGAAACTCCCGAGAAAGCCCCTTTCAGTGGGGCGGGGGCCGGA
TCATGCAATCAATGCTCTGCATCCACCCGCACGGTTGGGTGAGTGTGCAG
AGTAGGCTATTGCGGCTAAAGGAGGCTGTCTGGCAGAGGGCGGACGATGC
ATGCAGGCTCCGAAGGGAGCCAACCATGTCTTGATTCCCCTGGGGCCGGG
GGGGTTAAAATATCCCCGGCAACACGCATGAACGGACGGCGTGGACCATC
GATGGGGCTGGCATCCTCTCGCCATTGTCCCAACCAAAACCCTCTGTGTA
CCCTTTCCCTGTTGCTTCCCCCGGGGGACTGGCTCCGGCCAGCCGGGGGG
AGAAACCAAGCAAAAACCCTTTTCGCAAAAACAGACGTCTGAATGTTAAA
AACAAAAAAAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCCACATCG
ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT
GAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGG
CATGCCTGTTCGAGCGTCATAAATACCGCTCCTCCTCGGATTGCTTGCGA
TCCCTGGGGGGTTTTGGCAATGTGGTCTCCCCGTGCTTTGAGGGCATGCG
AACGGGCTCCCAGTGCTGAAAGACATAACGTTCCCGGCCGAAACCGGTGG
TTAATTTCACCGGCAGGATTCGAGGCAGGCAGACTGGAGGGCGTCATTAC
GTGGAGTCATGAGGATAGAAACCTCCCCCTTTGCAAGTTATGTTATTGAA
CATTGTTGCTCTGGCAGTTAGATCTGCAGGCCTGCCGGCCTGGGGATGGA
CCTCCCACTCGCAGGCGTCACGGCCACGATAGCGGGCGTTAAACGGAATC
CGATCCGCCTTCACGGGTGGTTGAAGATCCTTGTGGGCTAGCAACCCCTA
CACATATTTGACCTCGGATCAGG

Consensus of 2 sequences: 178-ITS1 extraction, 178-ITS4 (reversed)

CTTCCGTAGGGTGAACCTGCGGAAGGATCATTACCAAGAACCACACAGAA
AAGGGCAGCCGAGGGGCCACCAGGGCTAGTAGCTTTACGTTGTTGAACGT
CCTGGCCGGACCCGGAGCCGCCCCCATCTAAACCCTCTGCGTACCTGTCC
CGCCTTGCTTCCCCTGGCTACCAGCTGGGGGGGAGGAACAACAACCAAAAC
TCTTTGTGAACAAACCGACGTCAGAATCATAACAAAACAAAAAAGTTAAA
ACTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGA
AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
GAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCG
TCATAAAAACCTCCTCCCCCTTCGGGTTTGATTACTATCGTTGGGGGGTT
TTGGCCTAATGGGATAGCGATTGGCAATTAGTTTCCCAATGTCCTAAATA
GACGTAGACCCGCCTCCAGATGCGACAGCACCGAGGCCATCAACCGTGGA
GTTATGGGATATATAGGCTTGCAGTAAAATGCTCACCTTTCTCCATACGC
CGATGGCACACCGGTCGCAGTTGCGGGCGTAAATTGGAGCCCTTTTCAGG
ACCCTCGTGGCCTAGCATCCACCATACACAATTTGACCTCGGATCAGGTA
GGGATACCCGCTGAA

Consensus of 2 sequences: 833-ITS1 extraction, 833-ITS4 (reversed)

GCGGAAGGATCATTACAAAAATCCAACCAAAAAGAGGCGAGGACCGCGGC
CGCCTGGTCTCCCCGGTTAAGAGGCACCCACCCTCAGCCCCCATCTGCGC
GCGGCGGGGGATGCGGGGCCTCCCGTACTGGCCGGGCCGCGGGCCCCTCC
GTCTTCACACAAACCCTCTGTGTACCTTGTCCCCATTGCTTCCCTCGGAA
CGAGAGCTTCTCCGGGCTGGTCCGGGTGGCGGCGCCCCGTGGAACGGGCG
GCGCAACCCGATTGGCCACGCCTGGCTCGCTCCTCGCCCGGGGGGAGGAA
AACCCAAACAAACCCTTTGCGCGAAAAACGACGTCTGAACAGAAAAGAAA
ACAAACTAAAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCCACATC
GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAG
TGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGGGG
GCATGCCTGTTCGAGCGTCATAAAAACCTCTCCACCCCGGGTTCATGAGA
TCCGGGGCGGGTATTGGCGGTGCGGATCCCTCCCCCCTATACAACGGGAC
GGAGGCGCCCACCGCTCAAAGCCATGAAGATAACCCAGCCGAAAGCGCCA
GGGCGTCAACCGTGGAGTCATGAGGAACCACGCCCCGCGAGGGGTGCCTC
CCACTCTGGGCGACCGGTGTATGGAAATGCGGGCGTAAAAGCACGGGAAA
TCCGCGGGAACCGGCGCCGTCTCTGGCTGCGGCCGTGGATGATCCCCGTG
GGGGGCTAGATACCCAATCACATTCAAGTTTGACCTCGGATC

Consensus of 2 sequences: 767-ITS1 extraction, 767-ITS4 (reversed)

AACGAAGCAACGGAACGGTGCATCTGCGCCAGTGACGGGCCAGTCCTGGG
GTGCACGGTAAGACGTCCCGGGCGCTGTTTGACGGTGGAAGGGAGCGTTT
CCAGGCACTCTTGTGCTTGGGCGTATTGACCCCCCACCGTCCTTACCTGA
CACGCACCGGGTCGCACAGAGCGGCCAGGCCCCCCCACAGTGCACATGTT
CCCCTTCCGTTGCGGAGTGGAGTCGAACCAAGACCCTCTGCGTACCTTGC
CTTCCTTGCTTCCCCCCGACCACCTGGGCGGGGGGGGAGAAAACAAACCAC
AAACTCTTTGCGAATAACCGACGTCAGAAAACAAAACAAAAAAAGTTAAA
ACTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGA
AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTT
GAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCG
TCACAAACACCTCTCCCCCTCGGGTTTAGCTACCCTTGGGGGGTATTGGC
CAATGGCATTGCGAGATAGCAATTGTTAGCCAGGAGCCCAAATGCATTAG
TTTCCCGCCATTTTTAAGATGCAACAGCACCGAGGGGTATCCAAATCGGG
GTTCTAATGGTTTGAGGGAGGCCCGCTGCGGTAGTACGCTCGTGGCCTTC
TCCCGCCCCACCGCCGGACCCACATTAGTTGCAGTCGCGGGCGTAAAGCG
ACATCCGATCATCCCTCGCGGGGTGTTGACGATCATCGGGGTTAATAGCC
CCTACACAAAGTTTGACCTCGGATC

Consensus of 2 sequences: 766-ITS1 extraction, 766-ITS4 (reversed)

ACCTGCGGAAGGATCATTACCAAGAACCAAACAGAAAAGGGCAGCCGAGG

GGCCAACAGGGCTAGTAGCTTTACGTTGTTGAACGTCCTGGAACGGACCC
GGAGCCGCCCCCATCTAAACCCTCTGTGTACCTGTCCCACCTTGCTTCCC
CCGGCTACCCGTCGGGGGGAGGAACAACAACCAAAACTCTTTGTGAACAA
ACAGACGTCAGAATCACAACAACAAAAAAAAGTTAAAACTTTCAACAACG
GATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAATGCGATAAGTA
ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC
GCCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATAAAAACCTC
CTCCCCCTTCGGGTTTGATTACTATCGTTGGGGGGTTTTGGCCTAATGGG
ATAGCGATTGGCAATCAGTTTCCCAATGTCCTAAATAGACGTAGACCCGC
CTCCAGATGCGACAGCACCGAGGCCATCAACCGTGGAGTTATGGGATATA
TAGGCTTGCAGTAAAATGCTCACCTTTCTCCATACGCCGATGGCACACCG
GTCGCAGTTGCGGGCGTAAATTGGAGCCCTTTTCAGGACCCTCGTGGCCT
AGCATCCACCTCTCACAATTTGACCTCGGATCAGGTAGGGATACCCGCTG
AACTTAAGCATATCAATA

Consensus of 2 sequences: 765-ITS1 extraction, 765-ITS4 (reversed)

AAGACAGTGGGGAGAGAGGCACCAGCGATGGAGGACGCCGCCACATTGGC
GGGTTCCGTACCCCAGTGTTGGTCCAGGGCGGGCCAGCTGCGACGGTCTC
ATCCGCATTCCCTGCGTTTTGGCAGCAAGCCCCGAATCGGGGTGGGAACG
GATCGTGCAATCAATGCTCTGCAGCCATCCATACGGGTTGGGTGAGCGCG
CAGAGTAGACTATTGCTGCGCTAAAGGAGACCCGTCGCGGCAGGTGGCGG
ACGGCACATGCAGGACTGATCCCAACCATGTGTTGATTCCCCTGGGGCCG
GGGTTGTAGAATACCCCGGCGACTCGCAGACGGCGTGTACCCTCGTTTGG
CCCCCTCTCGCCATTGTCCCAACCAAAACCCTCTGTGTACCCCTTCCCTG
TTGCTTCCCCCGGGGCACTGGCTCCGGCCAGCCGGGGGGAGAAACCAAGC
AAAAACCCTTTTTCGCAAAAACAGACGTCTGAACATCAAAAACAAAAAAG
TTAAAACTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGC
AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTC
GAGCGTCATAAATACCCCTCCTCCTCGGATTGCTTTTGCGGTCCCTGGGG
GGTTCTGGCAATGTGGATTCCCCGTGCTTTGAGGGCATGCGGACGGGCTC
CCAGTGCTGAAAGACATAATGTTCCCGGCCGAAACCGGTGATTAATTTCA
CTGGCAGGATTCGTGGCAGGTAGACTGAGGGCGTCAACCGTGGAGTCATG
AGGGTAGAAACCTCCCCCTTTGCAAGTAACATTGCTCTGGCAGTTAGATC
TGCAGGCCCGACGGTCTGGGGATGGACCTCCCACTCGCAGGCGTCACGGC
CACGATAGCGGGCGTTAAACGGAATCCGATCCGCCTTCACGGGTGGTTGA
AGATCCTTGTGGGCTAGCAACCCCTAAACATATT

Consensus of 2 sequences: 764-ITS1 extraction, 764-ITS4 (reversed)

GACAGTGGCGGTCGAGAGAGAGTCAGCTACGATGGGGCGCGCCGGCAACC
AGACCGCTGGCACACTGGTGGCGGGTACTGTCACCCGCACTTTGTTGCAA
CGTCCAGGGCGAGAGCCTTAGCCGGGTGTGGCCCCATCCGTTAAGCTAGA
CAATTCCATGCTGCCTGCTGCGCTCCCTCCCCACGCCCTGGGCAGCAGCT

CCCGCAATCGGGGGTGGAAGCCGGATCAAGGAATCAATGCTCTGCATACA
TTCATTCAATTGAATGTGGTGCAGAGTAGGTTATTTCTGCTGAAGGGGGA
TCCCCCGGCAGAGGTGGACGGCACATGCAGGCTCCGAACGACGGAGGCCA
ACCATGTCGCTGAGTCCCCTGGGGCTAGGGGCGGTAAAAGCCCCTAGCCA
CTCCCCGCCGACGGCGTTGACCCCATCGTTCTGGCACCCTCTCGCCACTG
TCCCAACCAAAACCCTCTGTGTACCCTTCCCTGTTGCTTCCCCCGGGCAA
CTGGCTCCGGCCAGCCGGGGGGAGAAACCAAGCAAAAACCCTTTTCGCAA
AACAGACGTCTGAACGCAAAAAACAAAACAAAAAAAAGTTAAAACTTTCA
ACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAATGCGA
TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA
CATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATAAA
TACCGCTCCCCCTCGGATTGCTTGCGGTCCCTGGGGGGTTCTGGCAATGT
GGATTCCCCGTGCTTTGAGGGCATGCGAACGGGCTCCCAGTGCTGAAAGA
CATAATGTTCCCAGCCGAAACCGGTGATTAATTTCATCGGCAGGATTCGT
GGCAGGCAGACTGAGGGCGTCAACCGTGGAGTCATGAGGGTAGAAACCTC
CCCCTTTGCAAGTAACATTGCTCTGGCAGTTAGATCTGCAGGCCTGCCGG
TCTGGGGATGGACCTCCCACTCGCAGGCGTCACGGCCACGATAGCGGGCG
TTAAACGGAATCCGATCCGCCCTCACGGGTGGTTGAAGATCCTTGTGGGC
TAGCAACCCCTAAACACATTTTGACCTCGGA

Consensus of 2 sequences: 763-ITS1 extraction, 763-ITS4 (reversed)

GGAAGGATCATTACAAAAAAAACGTTCGGACGGGGGGCACAAGTGGTGGG
AAGCCAAGGCGATGCTAGCTGGACGCCGTGTGGCCTGTGGTTGCCGGGGG
TTAAACGTTCACACCCCCCCTCGCGCAGCACACATCAGTGGCCGAACCCA
GGCAAAGCACCGGCCCCCATCGACTGCCCCCAGTCCCAAAACAAAAACCC
TCTGCGTACTCTCCCCTCCTTGCTTCCCCCGGCACACGTCGGGGGGAGGA
ACACAAACCAAAACTCTTAGCGTGTAACCGCCGTCAGAAATACAGATACA
AAAGAAAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCCACATCGAT
GAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGTACTCCGGGGGGCA
TGCCTGTTCGAGCGTCATAAAACCATCTCCCCCAAGCGTGTTCTACGCTT
GGGGGGTCTTGGCCTGCGGGAAAGCGACTGGCAATCCGCTCCCGAGAGCC
CAAATACAGGAGACCCGCCATCTGATGCGACAGCACCGAGGCCCTCAACC
GTGGAGTCATGGGACTTTTGGATAGCAGTAATATGCTCACCTTTCTTCAC
ACGCCGTTGGCACACCAGTCGCAGTTGCGGGCGTAATATGGAGTCCCCCA
GGGGGGGACCCTTGTGGATTAGCACCCACCTCACGAACTTTGACCTCGGA
TCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAT

Consensus of 2 sequences: 759-ITS1 extraction 2, 759-ITS4 (reversed)

AAAGACAGTGGGGAGAGAGGCACCAGCGATGGAGGACGCCGCCACATTGG
CGGGTTCCGTACCCCAGTGTTGGTCCAGGGCGGGCCAGCTGCGACGGTCT
CATCCGCATTCCCTGCGTTTTGGCAGCAAGCCCCGAATCGGGGTGGGAAC
GGATCGTGCAATCAATGCTCTGCAGCCATCCATACGGGTTGGGTGAGCGC

GCAGAGTAGACTATTGCTGCGCTAAAGGAGACCCGTCGCGGCAGGTGGCG
GACGGCACATGCAGGACTGATCCCAACCATGTGTTGATTCCCCTGGGGCC
GGGGTTGTAGAATACCCCGGCGACTCGCAGACGGCGTGTACCCTCGTTTG
GCCCCCTCTCGCCATTGTCCCAACCAAAACCCTCTGTGTACCCCTTCCCT
GTTGCTTCCCCCGGGGCACTGGCTCCGGCCAGCCGGGGGGAGAAACCAAG
CAAAAACCCTTTTTCGCAAAAACAGACGTCTGAACATCAAAAACAAAAAA
GTTAAAACTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACG
CAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGA
ATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTT
CGAGCGTCATAAATACCCCTCCTCCTCGGATTGCTTTTGCGGTCCCTGGG
GGGGTTCTGGCAATGTGGATTCCCCGTGCTTTGAGGGCATGCGGACGGGC
TCCCAGTGCTGAAAGACATAATGTTCCCGGCCGAAACCGGTGATTAATTT
CACTGGCAGGATTCGTGGCAGGTAGACTGAGGGCGTCAACCGTGGAGTCA
TGAGGGTAGAAACCTCCCCCTTTGCAAGTAACATTGCTCTGGCAGTTAGA
TCTGCAGGCCCGACGGTCTGGGGATGGACCTCCCACTCGCAGGCGTCACG
GCCACGATAGCGGGCGTTAAACGGAATCCGATCCGCCTTCACGGGTGGTT
GAAGATCCTTGTGGGCTAGCAACCCCTAAACATA

Consensus of 2 sequences: 756-ITS1 extraction, 756-ITS4 (reversed)

ACAAACGAAGCAACGGAACGGTGCATCTGCGCCAGTGACGGGCCAGTCCT
GGGGTGCACGGTAAGACGTCCCGGGCGCTGTTTGACGGTGGAAGGGAGCG
TTTCCAGGCACTCTTGTGCTTGGGCGTATTGACCCCCCACCGTCCTTACC
TGACACGCACCGGGTCGCACAGAGCGGCCAGGCCCCCCCACAGTGCACAT
GTTCCCCTTCCGTTGCGGAGTGGAGTCGAACCAAGACCCTCTGCGTACCT
TGCCTTCCTTGCTTCCCCCCGACCGCCTGGGCGGGGGGGAGAAAACAAAC
CACAAACTCTTTGCGAATAACCGACGTCAGAAAACAAAACAAAAAAAGTT
AAAACTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAG
CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATC
TTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTTCGA
GCGTCACAAACACCTCTCCCCCTCGGGTTTAGCTACCCTTGGGGGGTATT
GGCCAATGGCATTGCGAGATAGCAATTGTTAGCCAGGAGCCCAAATGCAT
TAGTTTCCCGCCATTTTTAAGATGCAACAGCACCGAGGGGTATCCAAATC
GGGGTTCTAATGGTTTGAGGGAGGCCCGCTGCGGTAGTACGCTCGTGGCC
TTCTCCCGCCCCACCGCCGGACCCACATTAGTTGCAGTCGCGGGCGTAAA
GCGACATCCGATCATCCCTCGCGGGGTGTTGACGATCATCGGGGTTAATA
GCCCCTACACAAAGTTGACCTCGGAT

Consensus of 2 sequences: 755-ITS1 extraction 2, 755-ITS4 (reversed)

GCGGAAGGATCATTACCAAGAACCAAAACACATGAGCGAGGGATCGCGCT
CGCGCTGGGCTCGATAGACGCGCTAGCACCCGTCAGGCCCGGAGGGCGAA
CCAGGCCCCTGGGGCCGGCAGGTTAAACCCAAGGTACCCATCCAAGGTGG
ACAGTCCTCCCAGAGTTCGCCCTTCGGCGTCTGCGAGCCATCGGGGCATG
GAACCTTGGGGGCTGTCGGTTTCCCGCTGGGCATACGTCCGATGCCGCCT

CGGGGAGCGAAACGTGGCTCGCGCAGCGTCGCAGCGTTGGTCCCCCACTC
ACTCACCAAAACCCTCTGCGTACCTTGCCCACTTGCTTCCCCCGGCCCTC
CCGGCCGGGGGGCGACACCAAAAACCAAACTCTTTGCGATGAACCGACGT
CTGAATGCCAAAAGCAAAACAAAAACAGTTAAAACTTTCAACAACGGATC
TCTTGGCTCCCACATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGT
GAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
CCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATAAAACCCCCTCCC
CCTCGGATTAAATGTTCCTTGGGGGGTATTGGCCAATGGGATTGCGAGAT
AGCAATTGTTACCCAGGCGCCCAAATGCATCAGTACCCCGCCATTTACAG
ATTTACCAGCACCGAGGCCGTCAACCGTGGAGTTATGGGAATTATAGGCT
TGCAGTAATATGCTCACCTCTCTCCATACGCAGGCGGCACACCGGGTAGA
GTCGCGGGCGTAAAGCGGAATCCGAAACGGCCCTCACGGGTAGAGGAGGA
TCCTTGCGGGCTAGTCACCCCTCATCACAATTTGACCTCGGATCAG

Consensus of 2 sequences: 752-ITS1 extraction, 752-ITS4 (reversed)

TGCGGAAGGATCATTACCAAGAACCACACAGAAAAGGGCAGCCGAGGGGC
CACCAGGGCTAGTAGCTTTACGTTGTTGAACGTCCTGGAACGGACCCGGA
GCCGCCCCCATCTAAACCCTCTGCGTACCTGTCCCACCTTGCTTCCCCCG
GCTTACCCGCTGGGGGGAGGAACAACAACCAAAACTCTTTGTGAAGAAAC
AGACGTCAGAATCATAACAAAAAAAAGTTAAAACTTTCAACAACGGATCT
CTTGGTTCCCACATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCT
CTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATAAAAACCTCCTCCC
CCTTCGGGTTTGATTACTATCGTCGGGGGGTTTTGGCCTAATGGGAAAGC
GATTGGCAATTGTCTTCCCAATGTCCTAAATACACTTAGACCCGCCTCCA
GATGCGACAGCACCGAGGCCATCAACCGTGGAGTTATGGGACACCGTTCT
CCATACGCCGATGGCAAACCGGTCGCAGTTGCGGGCGTAAATTGGAGTCC
TCTTCAGGACCCTCGTGGCCTAGCATCCACCATACATAATTNGACCTCGG
ATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGC

Consensus of 2 sequences: 747-ITS1 extraction, 747-ITS4 (reversed)

ACAGTGGCGGTCGAGAGAGAGTCAGCTATACACGATGGGGCGCGCCGGCA
AACCAGACCGCTGGCACACTGGTGGCGGGTACTGTCACCCGCGCCGTTTG
TCGCAACGTCCAGGGCGAGAGCCTTAGCCGGGTGTGGCCCCATCCGTTAT
GCTAGCCAATTCCATGCTGCCTGCTGCGCTCCCTCCCCACGCCCTGGGCA
GCAGCTCCCGCAATCGGGGGTGGAAGCCGGATCAAGGAATCAATGCTCTG
CACCCATTCAGTCAATTGAATGTGGTGCAGTTTAGGTTATTTCTGCTGAA
GGGGGATCCCCCGGCAGAGGTGGACGGCACATGCAGGCTCCGAACGACGG
AAGCCAACCATGATGCTGAGTCCCCTGGGGCTAGGGGTGGTAAAAGCCCC
TAGCCACTCCCCACCGACGGCGTTGACCCCATCGTTCTGGCACCCTCTCG
CCACTGTCCCAACCAAAACCCTCTGTGTACCCTTCCCTGTTGCTTCCCCC
GGGAAACTGGCTCCGGCCAGCCGGGGGGAGAAACCAAGCAAAAACCCTTT
TCGCAAAACAGACGTCTGAACGCAAAAAACAAAACAAAAAAAAGTTAAAA

CTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAA
ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
AACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTCGAGCGT
CATAAATACCGCTCCCCCTCGGATTGCTTGCGGTCCCTGGGGGGTTCTGG
CAATGTGGATTCCCCGTGCTTTGAGGGCATGCGAACGGGCTCCCAGTGCT
GAAAGACATAATGTTCCCAGCCGAAACCGGTGATTAATTTCATCGGCAGG
ATTCGTGGCAGGCAGACTGAGGGCGTCAACCGTGGAGTCATGAGGGTAGA
AACCTCCCCCTTTGCAAGTAACATTGCTCTGGCAGTTAGATCTGCAGGCC
TGCCGGTCTGGGGATGGACCTCCCACTCGCAGGCGTCACGGCCACGATAG
CGGGCGTTAAACGGAATCCGATCCGCCCTCACGGGTGGTTGAAGATCCTT
GTGGGCTAGCAACCCCTAAACACATTTGACCTCGGATCAGG

Consensus of 2 sequences: 746-ITS1 extraction, 746-ITS4 (reversed)

AAGACAGTGGCGGTCGAGAGAGAGTCAGCTATACACGATGGGGCGCGCCG
GCAAACCAGACCGCTGGCACACTGGTGGCGGGTACTGTCACCCGCGCCGT
TTGTCGCAACGTCCAGGGCGAGAGCCTTAGCCGGGTGTGGCCCCATCCGT
TATGCTAGCCAATTCCATGCTGCCTGCTGCGCTCCCTCCCCACGCCCTGG
GCAGCAGCTCCCGCAATCGGGGGTGGAAGCCGGATCAAGGAATCAATGCT
CTGCACCCATTCAGTCAATTGAATGTGGTGCAGTTTAGGTTATTTCTGCT
GAAGGGGGATCCCCCGGCAGAGGTGGACGGCACATGCAGGCTCCGAACGA
CGGAAGCCAACCATGATGCTGAGTCCCCTGGGGCTAGGGGTGGTAAAAGC
CCCTAGCCACTCCCCACCGACGGCGTTGACCCCATCGTTCTGGCACCCTC
TCGCCACTGTCCCAACCAAAACCCTCTGTGTACCCTTCCCTGTTGCTTCC
CCCGGGAAACTGGCTCCGGCCAGCCGGGGGGAGAAACCAAGCAAAAACCC
TTTTCGCAAAACAGACGTCTGAACGCAAAAAACAAAACAAAAAAAAGTTA
AAACTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCT
TTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTCGAG
CGTCATAAATACCGCTCCCCCTCGGATTGCTTGCGGTCCCTGGGGGGTTC
TGGCAATGTGGATTCCCCGTGCTTTGAGGGCATGCGAACGGGCTCCCAGT
GCTGAAAGACATAATGTTCCCAGCCGAAACCGGTGATTAATTTCATCGGC
AGGATTCGTGGCAGGCAGACTGAGGGCGTCAACCGTGGAGTCATGAGGGT
AGAAACCTCCCCCTTTGCAAGTAACATTGCTCTGGCAGTTAGATCTGCAG
GCCTGCCGGTCTGGGGATGGACCTCCCACTCGCAGGCGTCACGGCCACGA
TAGCGGGCGTTAAACGGAATCCGATCCGCCCTCACGGGTGGTTGAAGATC
CTTGTGGGCTAGCAACCCCTAAACACATTTGACCTCGGATC

Consensus of 2 sequences: 745-ITS1 extraction, 745-ITS4 (reversed)

ATGACAGTGGAGAGAGAGAGAGCCAACTGCGGTGGGGGACGCCGTCACTT
CGGCGGGTTCCGTGTACCACTGTGTTGGTCCAGGGCGGGCCTGCCAGGGA
GACCTCATCCGTGTAATCAAATCCTGCTGACCCTAATGCGCTCCCTCCCC
ACGCCTTAGGCAGCAACCCCCCGCATTGGGGGGTGGAGCCGGATCTTAGC
AATCAATGCTCCGCATCCATCCACACAGTTGGGTGCCGGTGCGGAGTAGA

CTATTGCAGCTAAAGGAGGATTCCCTGGCAGGTGGCGGACAACACATGCC
CGGCTCCTTACGCCTTCCGCTCGGTTGGATCCGTCTGCGTAGCCGCTCAT
TGCTGTTGATTCCCCTGGGGCTGGAGGTGTAACGACCTCCAGCGACCCCC
ATCGACGGCGTGGAAACCCATCGTTTGGCACCCTCTCGCCATTGCCCCAA
CCAAAACCCTCTGTGTACCCTTCCCTGTTGCTTCCCCCGGGCACCTGGCT
CCGGCCAGTCGGGGGGAGAAACCAAGCAAAAACCCTTTTCGCAAAACAGA
CGTCTGAATGTAAAAAAACAAAACAAAAAGTTAAAACTTTCAACAACGGA
TCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGC
CCTCTGGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATAAATACCGCTC
CCCCTCGGATTGCTTGCGGTCCCTGGGGGGTTCTGGCAATGTGGATTCCC
CGTGCTTTGAGGGCATGCGAACGGGCTCCCAGTGCTGAAAGACATAATGT
TCCCAGCCGAAACCGGTGATTAATTTCATCGGCAGGATTCGTGGCAGGCA
GACTGAGGGCGTCAACCGTGGAGTCATGAGGATAGAAACCTCCCCCTTTG
CAAGTAACATTGCTCTGGCAGTTAGATCTGCAGGCCTGCCGGTCTGGGGA
TGGACCTCCCACTCGCAGGCGTCACGGCCACGATAGCGGGCGTTAAACGG
AATCCGATCCGCCTTCATTGGTGGTTGAAGATCCTTGTGGGCTAGCAACC
CCTAAACACATTT

Consensus of 2 sequences: 742-ITS1 extraction, 742-ITS4 (reversed)

GACAGTGGCGGTCGAGAGAGAGTCAGCTACGATGGGGCGCGCCGGCAACC
AGACCGCTGGCACACTGGTGGCGGGTACTGTCACCCGCACTTTGTTGCAA
CGTCCAGGGCGAGAGCCTTAGCCGGGTGTGGCCCCATCCGTTAAGCTAGA
CAATTCCATGCTGCCTGCTGCGCTCCCTCCCCACGCCCTGGGCAGCAGCT
CCCGCAATCGGGGGTGGAAGCCGGATCAAGGAATCAATGCTCTGCATACA
TTCATTCAATTGAATGTGGTGCAGAGTAGGTTATTTCTGCTGAAGGGGGA
TCCCCCGGCAGAGGTGGACGGCACATGCAGGCTCCGAACGACGGAAGCCA
ACCATGTCGCTGAGTCCCCTGGGGCTAGGGGCGGTAAAAGCCCCTAGCCA
CTCCCCGCCGACGGCGTTGACCCCATCGTTCTGGCACCCTCTCGCCACTG
TCCCAACCAAAACCCTCTGTGTACCCTTCCCTGTTGCTTCCCCCGGGCAA
CTGGCTCCGGCCAGCCGGGGGGAGAAACCAAGCAAAAACCCTTTTCGCAA
AACAGACGTCTGAACGCAAAAAACAAAACAAAAAAAAGTTAAAACTTTCA
ACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAATGCGA
TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA
CATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATAAA
TACTGCTCCCCCTCGGATTGCTTGCGGTCCCTGGGGGGTTCTGGCAATGT
GGATTCCCCGTGCTTTGAGGGCATGCGAACGGGCTCCCAGTGCTGAAAGA
CATAATGTTCCCAGCCGAAACCGGTGATTAATTTCATCGGCAGGATTCGT
GGCAGGCAGACTGAGGGCGTCAACCGTGGAGTCATGAGGGTAGAAACCTC
CCCCTTTGCAAGTAACATTGCTCTGGCAGTTAGATCTGCAGGCCTGCCGG
TCTGGGGATGGACCTCCCACTCGCAGGCGTCACGGCCACGATAGCGGGCG
TTAAACGGAATCCGATCCGCCCTCACGGGTGGTTGAAGATCCTTGTGGGC
TAGCAACCCCTAAACACATTTGACCTCGGATC

Consensus of 2 sequences: 735-ITS1 extraction, 735-ITS4 (reversed)

GGGGGCGAAGGGGCCGACAGGATTAGTAGCTTATACGTTGTTGAACGTCC
TGGCTGGACCCGGAGCCTCCCCCATCTAAACCCTCTGCGTACCTGTCCCT
TCTTGCTTCCCCCGGCATCTCTCGTCGGGGGGAGGGAACAACCAAAACTC
TTTGTGAATCAAACAGCCGTCAGAATTACGAAACAAACAAAAAGTTAAAA
CTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAA
ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
AACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGT
CATAAAAACCTCCTCCCCCTTCGGGTTTTGTTACTATCGTTGGGGGGTTT
TGGCCTAATGGGATAGCGTTTGGCAATTCGTTTCCCAATGTCCTAAATAA
ACGTAGACCCGCCTCCAGATGCGACAGCACTGAGGCCATCAACCGTGGAG
TTATGGGATATATAGGCTTGCAGTAAAATGCTCACCTCTCTTCACACGCA
GATGGCACGACAGTTGCAGTTGCGGGCGTAAATTGGAGCCCTTTTCAGGA
CCCTCGTGGCCTAGCATCCACCATACATAATTTGACCTCGGATCAGGTAG
GGATACCCGCTGAACTTAAGCATATCAATAA

Consensus of 2 sequences: 692-ITS1 extraction, 692-ITS4 (reversed)

ACAGTGGGGAGAGAGGCACCAGCGATGGAGGACGCCGCCACATTGGCGGG
TTCCGTACCCCAGTGTTGGTCCAGGGCGGGCCAGCTGCGACGGTCTCATC
CGCATTCCCTGCGTTTTGGCAGCAAGCCCCGAATCGGGGTGGGAACGGAT
CGTGCAATCAATGCTCTGCAGCCATCCATACGGGTTGGGTGGGCGTGCAG
AGTAGACTATTGCTGCGCTAAAGGAGACCCGTCGCGGCAGGTGGCGGACG
GCACATGCAGGACTGATCCCAACCATGTGTTGATTCCCCTGGGGCCGGGG
TTGTAGAATACCCCGGCGACTCGCAGACGGCGTGTACCCTCGTTTGGCCC
CCTCTCGCCATTGTCCCAACCAAAACCCTCTGTGTACCCCTTCCCTGTTG
CTTCCCCCGGGGCACTGGCTCCGGCCAGCCGGGGGGAGAAACCAAGCAAA
AACCCTTTTTCGCAAAAACAGACGTCTGAACATCAAAAACAAAAAAGTTA
AAACTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCT
TTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTCGAG
CGTCATAAATACCCCTCCCCCTCGGATTGCTTTTGCGGTCCCTGGGGGGT
TCTGGCAATGTGGATTCCCCGTGCTTTGAGGGCATGCGGACGGGCTCCCA
GTGCTGAAAGACATAATGTTCCCGGCCGAAACCGGCGATTAATTTCACTG
GCAGGATTCGTGGCAGGTAGACTGAGGGCGTCAACCGTGGAGTCATGAGG
GTAGAAACCTCCCCCTTTGCAAGTAACATTGCTCTGGCAGTTAGATCTGC
AGGCCCGACGGTCTGGGGATGGACCTCCCACTCGCAGGCGTCACGGCCAC
GATAGCGGGCGTTAAACGGAATCCGATCCGCCTTCACGGGTGGTTGAAGA
TCCTTGTGGGCTAGCAACCCCTAAACATATTTGACCTCGGAT

Consensus of 2 sequences: 691-ITS1 extraction, 691-ITS4 (reversed)

GACCTGCGGAAGGATCATTACCAAGAACCACACAGAAAAGGGCAGCCGAG
GGGCCACCAGGGCTAGTAGCTTTACGTTGTTGAACGTCCTGGCCGGACCC

GGAGCCGCCCCCATCTAAACCCTCTGCGTACCTGTCCCGCCTTGCTTCCC
CCGGCTACCCGCTGGGGGGGAGGAACAACAACCAAAACTCTTTGTGAACAA
GCCGACGTCAGAATCATAACAAAACAAAAAAGTTAAAACTTTCAACAACG
GATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAATGCGATAAGTA
ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC
GCCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATAAAAACCTC
CTCCCCCATCGGGTTTGATTACTATCGTTGGGGGGTTTTGGCCTAATGGG
ATAGCGATTGGCAATTAGTTTCCCAATGTCCTAAATAGACGTAGACCCGC
CTCCAGATGCGACAGCACCGAGGCCATCAACCGTGGAGTTATGGGATATA
TAGGCTTGCAGTAAAATGCTCACCTTTCTCCATACGCCGATGGCACACCG
GTCGCAGTTGCGGGCGTAAATTGGAGCCCTTTTCAGGACCCTCGTGGCCT
AGCATCCACCATACACAATTTGACCTCGGATCAGGTAGGGATACCCGCTG
AACTTAAGCATATCAATA

Consensus of 2 sequences: 578-ITS1 extraction, 578-ITS4 (reversed)

ATCATTACCAAGAACCACACAGAAAAGGGAGGCAAAGGGGCCTACAGGGC
TAGTAGCTTATACGTTGTTGAACGTCCTGCCTGGACCCGGAGCCGCCCCC
ATCTAAACCCTCTGCGTACCTGTCCCTTCTTGCTTCCCCCGGCATCTCGT
CGGGGGGAGGTAACAACCAAAACTCTCTGTGAATCAAACAGCCGTCAGAA
TTATAAAACAAACAAAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCC
CACATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
ATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGTATTC
CGGGGGGCATGCCTGTTCGAGCGTCATAAAAACCTCCTCCCCCTTCGGGT
TTTGTTACTATCGTTGGGGGGTTTTGGCCTAATGGGATAGCGATTGGCAA
TTCGTTTCCCAATGTCCTAAATAGACGTAGACCCGCCTCCAGATGCGACA
GCACCGAGGCCATCAACCGTGGAGTTATGGGATATAATAGGCTTGCAGTA
AAATGCTCACCTCTCTCCACACGCCGATGGCACGACAGTTGCAGTTGCGG
GCGTAAATTGGAGCCCTTTTCAGGACCCTTGTGGCCTAGCATCCACCATA
CATAATTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAT
CAATA

Consensus of 2 sequences: 403-ITS1 extraction, 403-ITS4 (reversed)

TGCGGAAGGATCATTACAAAAAAAACGTTCGGACGGGGGGCACAAGTGGT
GGGAAGCCAAGGCGATGCTAGCTGGACGCCGTGTGGCCTGTGGTTGCCGG
GGGTTAAACGTTCACACCCCCCCTCGCGCAGCACACATCAGTGGCCGAAC
CCAGGCAAAGCACCGGCCCCCATCGACTGCCCCCAGTCCCAAAACAAAAA
CCCTCTGCGTACTCTCCCCTCCTTGCTTCCCCCGGCACACGTCGGGGGGA
GGAACACAAACCAAAACTCTTAGCGTGTAACCGCCGTCAGAAATACAGAT
ACAAAAGAAAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCCACATC
GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAG
TGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGTACTCCGGGGG
GCATGCCTGTTCGAGCGTCATAAAACCATCTCCCCCAAGCGTGTTCTACG
CTTGGGGGGTCTTGGCCTGCGGGAAAGCGACTGGCAATCCGCTCCCGAGA

GCCCAAATACAGGAGACCCGCCATCTGATGCGACAGCACCGAGGCCCTCA
ACCGTGGAGTCATGGGACTTTTGGATAGCAGTAATATGCTCACCTTTCTT
CACACGCCGTTGGCACACCAGTCGCAGTTGCGGGCGTAATATGGAGTCCC
CCAGGGGGGGACCCTTGTGGATTAGCACCCACCTCACGAACTTTGACCTC
GGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAA

Consensus of 2 sequences: 402-ITS1 extraction, 402-ITS4 (reversed)

AAGACAGTGGGGAGAGAGGCACCAGCGATGGAGGACGCCGCCACATTGGC
GGGTTCCGTACCCCAGTGTTGGTCCAGGGCGGGCCAGCTGCGACGGTCTC
ATCCGCAGTCCCTGCGTTTTGGCAGCAAGCCCCGAATCGGGGTGGGAGCG
GATCGTGCAATCAATGCTCTGCAGCCACCCATACGGGTTGGGTGAGCGCG
CAGAGTAGACTATTGCTGCGCTAAAGGAGACCCGTCGCGGCAGGTGGCGG
ACGGCACATGCAGGACTGATCCCAACCATGTGTTGATTCCCCTGGGGCCG
GGGTTGTAGAATACCCCGGCGACTCGCAGACGGCGTGTACCCTCGTCTGG
CCCCCTCTCGCCATTGTCCCAACCAAAACCCTCTGCGTACCCCTTCCCTG
TTGCTTCCCCCGGGGCACTGGCTCCGGCCAGCCGGGGGGAGAAACCAAGC
AAAAACCCTTTTTCGCAAAAACAGACGTCTGAACATCAAAAACAAAAAAG
TTAAAACTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGC
AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTC
GAGCGTCATAAATACCCCTCCCCCTCGGATTGCTTTTGCGGTCCCTGGGG
GGTTCTGGCAATGTGGATTCCCCGTGCTTTGAGGGCATGCGGACGGGCTC
CCAGTGCTGAAAGACATAATGTTCCCGGCCGAAACCGGTGATTAGTTTCA
CTGGCAGGATTCGTGGCAGGTAGACTGAGGGCGTCAACCGTGGAGTCATG
AGGGTAGAAACCTCCCCCTTTGCAAGTAACATTGCTCTGGCAGTTAGATC
TGCAGGCCCGACGGTCTGGGGATGGACCTCCCACTCGCAGGCGTCACGGC
CACGATAGCGGGCGTTAAACGGAATCCGATCCGCCTTCACGGGTGGTTGA
AGATCCTTGTGGGCTAGCAACCCCTAAACATAT

Consensus of 2 sequences: 374-ITS1 extraction, 374-ITS4 (reversed)

AAGACAGTGGGGAGAGAGGCACCAGCGATGGAGGACGCCGCCACATTGGC
GGGTTCCGTACCCCAGTGTTGGTCCAGGGCGGGCCAGCTGCGACGGTCTC
ATCCGCAGTCCCTGCGTTTTGGCAGCAAGCCCCGAATCGGGGTGGGAGCG
GATCGTGCAATCAATGCTCTGCAGCCACCCATACGGGTTGGGTGAGCGCG
CAGAGTAGACTATTGCTGCGCTAAAGGAGACCCGTCGCGGCAGGTGGCGG
ACGGCACATGCAGGACTGATCCCAACCATGTGTTGATTCCCCTGGGGCCG
GGGTTGTAGAATACCCCGGCGACTCGCAGACGGCGTGTACCCTCGTCTGG
CCCCCTCTCGCCATTGTCCCAACCAAAACCCTCTGCGTACCCCTTCCCTG
TTGCTTCCCCCGGGGCACTGGCTCCGGCCAGCCGGGGGGAGAAACCAAGC
AAAAACCCTTTTTCGCAAAAACAGACGTCTGAACATGAAAAACAAAAAAG
TTAAAACTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGC
AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTC

GAGCGTCATAAATACCCCTCCCCCTCGGATTGCTTTTGCGGTCCCTGGGG
GGTTCTGGCAATGTGGATTCCCCGTGCTTTGAGGGCATGCGGACGGGCTC
CCAGTGCTGAAAGACATAATGTTCCCGGCCGAAACCGGTGATTAGTTTCA
CTGGCAGGATTCGTGGCAGGTAGACTGAGGGCGTCAACCGTGGAGTCATG
AGGGTAGAAACCTCCCCCTTTGCAAGTAACATTGCTCTGGCAGTTAGATC
TGCAGGCCCGACGGTCTGGGGATGGACCTCCCACTCGCAGGCGTCACGGC
CACGATAGCGGGCGTTAAACGGAATCCGATCCGCCTTCACGGGTGGTTGA
AGATCCTTGTGGGCTAGCAACCCCTAAACATATTTGACCT

Consensus of 2 sequences: 331-ITS1 extraction, 331-ITS4 (reversed)

GGGGGCGAAGGGGCCGACAGGATTAGTAGCTTATACGTTGTTGAACGTCC
TGGCTGGACCCGGAGCCTCCCCCATCTAAACCCTCTGCGTACCTGTCCCT
TCTTGCTTCCCCCGGCATCTCTCGTCGGGGGGAGGGAACAACCAAAACTC
TTTGTGAATCAAACAGCCGTCAGAATTACAAAACAAACAAAAAGTTAAAA
CTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAA
ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
AACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGT
CATAAAAACCTCCTCCCCCTTCGGGTTTTGTTACTATCGTTGGGGGGTTT
TGGCCTAATGGGATAGCGTTTGGCAATTCGTTTCCCAATGTCCTAAATAA
ACGTAGACCCGCCTCCAGATGCGACAGCACTGAGGCCATCAACCGTGGAG
TTATGGGATATATAGGCTTGCAGTAAAATGCTCACCTCTCTTCACACGCA
GATGGCACGACAGTTGCAGTTGCGGGCGTAAATTGGAGCCCTTTTCAGGA
CCCTCGTGGCCTAGCATCCACCATACATAATTTGACCTCGGATCAGGTAG
GGATACCCGCTGAACTTAAGCATATCAATAA

Consensus of 2 sequences: 327-ITS1 extraction, 327-ITS4 (reversed)

ACAGTGGGGAGAGAGGCACCAGCGATGGAGGACGCCGCCACATTGGCGGG
TTCCGTACCCCAGTGTTGGTCCAGGGCGGGCCAGCTGCGACGGTCTCATC
CGCAGTCCCTGCGTTTTGGCAGCAAGCCCCGAATCGGGGTGGGAGCGGAT
CGTGCAATCAATGCTCTGCAGCCACCCATACGGGTTGGGTGAGCGCGCAG
AGTAGACTATTGCTGCGCTAAAGGAGACCCGTCGCGGCAGGTGGCGGACG
GCACATGCAGGACTGATCCCAACCATGTGTTGATTCCCCTGGGGCCGGGG
TTGTAGAATACCCCGGCGACTCGCAGACGGCGTGTACCCTCGTCTGGCCC
CCTCTCGCCATTGTCCCAACCAAAACCCTCTGCGTACCCCTTCCCTGTTG
CTTCCCCCGGGGCACTGGCTCCGGCCAGCCGGGGGGAGAAACCAAGCAAA
AACCCTTTTTCGCAAAAACAGACGTCTGAACATCAAAAACAAAAAAGTTA
AAACTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCT
TTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTCGAG
CGTCATAAATACCCCTCCCCCTCGGATTGCTTTTGCGGTCCCTGGGGGGT
TCTGGCAATGTGGATTCCCCGTGCTTTGAGGGCATGCGGACGGGCTCCCA
GTGCTGAAAGACATAATGTTCCCGGCCGAAACCGGTGATTAGTTTCACTG
GCAGGATTCGTGGCAGGTAGACTGAGGGCGTCAACCGTGGAGTCATGAGG

GTAGAAACCTCCCCCTTTGCAAGTAACATTGCTCTGGCAGTTAGATCTGC
AGGCCCGACGGTCTGGGGATGGACCTCCCACTCGCAGGCGTCACGGCCAC
GATAGCGGGCGTTAAACGGAATCCGATCCGCCTTCACGGGTGGTTGAAGA
TCCTTGTGGGCTAGCAACCCCTAAACATAT

Consensus of 2 sequences: 191-ITS1 extraction, 191-ITS4 (reversed)

AGACAGTGGGGGGAGAGAGAGTGCGTAACCACTTGTGGGGGCCTGCCGTA
CCGTGACTGGCGTGTTCCGGCGCGGCACCTAGGGCGTCCACGGTTCAGGG
CGGGCCAGCCAGGGAACCACATCCGAGTAGTCAAATCCACGCTGCCTGGC
CTGACATCCAGGCAGTACAAGGAGCCGGATCGAGCATTAAACGCTCTGCA
CCCATCCCACACAGTTGGGTGAAGGTGCAGAGCGGATTTCTGTAGCTAAA
GGAGGCACCCCTGGAAAGCGGCGGAGGATGCAAGCAGGGCTGAAACAAAC
CCCTACCACTGCGTCGATTCCCCTGTGGCTGTGGGTGTAAAGACCCACAG
CGACTCGCACCGCGGCAGGACACCACTCTTAGGTTTAGCACGCTCTCGCC
ACTGTCCCAATCAAAACCCTCTGTGTACCCTTCCCAGTTGCTTCCCCCGG
GGAACTGGCTCTGGCCAGCCGGGGGGGAGAAACCAAGCAAAAACCCTTTTT
TCGCAAAACAGACGTCCGAATTTAAAAACAAAACAAAAAGTTAAAACTTT
CAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAATGC
GATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG
CACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATA
AATACCGCTCCCCCTCGGATTGCTTGCGATCCCTGGGGGGTTCTGGCAAT
GTGGTCTCCCCGTGCTTTGAGGGCATGCGAACGGGCTCCCAGTGCTGAAA
GACATAACGTTCCCGGCCGAAACCGGTGATTAATTTCACTGGCAGGATTC
GTGGCAGGCAGACTGAGGGCGTCAACCGTGGAGTCATGAGGATAGAAACC
TCCCCCTTTGCAAGTAACATTGCTCTGGCAGTTAGATCTGCAGGCCTGCC
GGTCTGGGGATGGACCTCCCACTCGCAGGCGTCACGGCCACGATAGCGGG
CGTTAAACGGAATCCGATCCGCCTTCACTGGTGGTTGAAGATCCTTGTGG
GCTAGCAACCCCTAAACATATTTGACCTCGGAT

Consensus of 2 sequences: 190-ITS1 extraction, 190-ITS4 (reversed)

GACAGTGGGGGGAGAGAGAGTGCGTAACCACTTGTGGGGGCCTGCCGTAC
CGTGACTGGCGTGTTCCGGCGCGGCACCTAGGGCGTCCACGGTTCAGGGC
GGGCCAGCCAGGGAACCACATCCGAGTAGTCAAATCCACGCTGCCTGGCC
TGACATCCAGGCAGTACAAGGAGCCGGATCGAGCATTAAACGCTCTGCAC
CCATCCCACACAGTTGGGTGAAGGTGCAGAGCGGATTTCTGTAGCTAAAG
GAGGCACCCCTGGAAAGCGGCGGAGGATGCAAGCAGGGCTGAAACAAACC
CCTACCACTGCGTCGATTCCCCTGTGGCTGTGGGTGTAAAGACCCACAGC
GACTCGCACCGCGGCAGGACACCACTCTTAGGTTTAGCACGCTCTCGCCA
CTGTCCCAATCAAAACCCTCTGTGTACCCTTCCCAGTTGCTTCCCCCGGG
GAACTGGCTCTGGCCAGCCGGGGGGAGAAACCAAGCAAAAACCCTTTTTT
CGCAAAACAGACGTCCGAATTTAAAAACAAAACAAAAAGTTAAAACTTTC
AACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGC

ACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATAA
ATACCGCTCCCCCTCGGATTGCTTGCGATCCCTGGGGGGTTCTGGCAATG
TGGTCTCCCCGTGCTTTGAGGGCATGCGAACGGGCTCCCAGTGCTGAAAG
ACATAACGTTCCCGGCCGAAACCGGTGATTAATTTCACTGGCAGGATTCG
TGGCAGGCAGACTGAGGGCGTCAACCGTGGAGTCATGAGGATAGAAACCT
CCCCCTTTGCAAGTAACATTGCTCTGGCAGTTAGATCTGCAGGCCTGCCG
GTCTGGGGATGGACCTCCCACTCGCAGGCGTCACGGCCACGATAGCGGGC
GTTAAACGGAATCCGATCCGCCTTCACTGGTGGTTGAAGATCCTTGTGGG
CTAGCAACCCCTAAACATATTTGACCTCGGATC

Consensus of 2 sequences: 186-ITS1 extraction, 186-ITS4 (reversed)

TGCGGAAGGATCATTACCAAGAACCATACAGAAAAGGGAGGCATTAGGGG
GCCGACCAGGGCTAGTAGCTTTACGTTGTTGAACGTCCAGTATGGACCCG
AAGCCTCCCCCATCTAAACCCTCTGCGTACCTGTCCCTTCTTGCTTCCCC
CGGCATCTCGTCGGGGGGAGGTAACAACCAAAACTCTCTGTGAATCAAAC
AGCCGTCAGAATTATAAAACAAACAAAAAGTTAAAACTTTCAACAACGGA
TCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGC
CCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATAAAAACCTCCT
CCCCCTTCGGGTTTTGTTACTATCGTTGGGGGGTTTTGGCCTAATGGGAT
AGCGATTGGCAATTCGTTTCCCAATGTCCTAAATAGACGTAGACCCGCCT
CCAGATGCGACAGCACCGAGGCCATCAACCGTGGAGTTATGGGATATAAT
AGGCTTGCAGTAAAATGCTCACCTCTCTCCACACGCCGATGGCACGACAG
TTGCAGTTGCGGGCGTAAATTGGAGCCCTTTTCAGGACCCTCGTGGCCTA
GCATCCACCATACATAATTTGACCTCGGATCAGGTAGGGATACCCGCTGA
ACTTAAGCATATCAATAA

Consensus of 2 sequences: 172-ITS1 extraction, 172-ITS4 (reversed)

CCGTAGGGTGAACCTGCGGAAGGATCATTACCAAGAACCACACAGAAAAG
GGCAGCCGAGGGGCCACCAGGGCTAGTAGCTTTACGTTGTTGAACGTCCT
GGCCGGACCCGGAGCCGCCCCCATCTAAACCCTCTGCGTACCTGTCCCGC
CTTGCTTCCCCTGGCTACCAGCTGGGGGGAGGAACAACAACCAAAACTCT
TTGTGAACAAACCGACGTCAGAATCATAACAAAACAAAAAAGTTAAAACT
TTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAAT
GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCA
TAAAAACCTCCTCCCCCTTCGGGTTTGATTACTATCGTTGGGGGGTTTTG
GCCTAATGGGATAGCGATTGGCAATTAGTTTCCCAATGTCCTAAATAGAC
GTAGACCCGCCTCCAGATGCGACAGCACCGAGGCCATCAACCGTGGAGTT
ATGGGATATATAGGCTTGCAGTAAAATGCTCACCTTTCTCCATACGCCGA
TGGCACACCGGTCGCAGTTGCGGGCGTAAATTGGAGCCCTTTTCAGGACC
CTCGTGGCCTAGCATCCACCATACACAA

Consensus of 2 sequences: China2-ITS1 extraction, China2-ITS4 (reversed)

GGAAGGATCATTACCAAGAACCACACAGAAAAGGGCAGCCGAGGGGCCAC
CAGGGCTAGTAGCTTTACGTTGTTGAACGTCCTGGCCGGACCCGGAGCCG
CCCCCATCTAAACCCTCTGCGTACCTGTCCCGCCTTGCTTCCCCCGGCTA
CCCGCTGGGGGGAGGAACAACAACCAAAACTCTTTGTGAACAAGCCGACG
TCAGAATCATAACAAAACAAAAAAGTTAAAACTTTCAACAACGGATCTCT
TGGTTCCCACATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAA
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCT
GGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATAAAAACCTCCTCCCCC
ATCGGGTTTGATTACTATCGTTGGGGGGTTTTGGCCTAATGGGATAGCGA
TTGGCAATTAGTTTCCCAATGTCCTAAATAGACGTAGACCCGCCTCCAGA
TGCGACAGCACCGAGGCCATCAACCGTGGAGTTATGGGATATATAGGCTT
GCAGTAAAATGCTCACCTTTCTCCATACGCCGATGGCACACCGGTCGCAG
TTGCGGGCGTAAATTGGAGCCCTTTTTTCAGGACCCTCGTGGCCTAGCAT
CCACCATACACAATTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTT
AAGCATATCAATAA

Consensus of 2 sequences: China 1-ITS1 extraction, China 1-ITS4 (reversed)

CTTCCGTAGGGTGACCTGCGGAAGGATCATTACCAAGAACCACACAGAAA
AGGGCAGCCGAGGGGCCACCAGGGCTAGTAGCTTTACGTTGTTGAACGTC
CTGGCCGGACCCGGAGCCGCCCCCATCTAAACCCTCTGCGTACCTGTCCC
GCCTTGCTTCCCCCGGCTACCCGCTGGGGGGAGGAACAACAACCAAAACT
CTTTGTGAACAAGCCGACGTCAGAATCATAACAAAACAAAAAAGTTAAAA
CTTTCAACAACGGATCTCTTGGTTCCCACATCGATGAAGAACGCAGCGAA

ATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG

AACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTTCGAGCGT
CATAAAAACCTCCTCCCCCATCGGGTTTGATTACTATCGTTGGGGGGTTT
TGGCCTAATGGGATAGCGATTGGCAATTAGTTTCCCAATGTCCTAAATAG
ACGTAGACCCGCCTCCAGATGCGACAGCACCGAGGCCATCAACCGTGGAG
TTATGGGATATATAGGCTTGCAGTAAAATGCTCACCTTTCTCCATACGCC
GATGGCACACCGGTCGCAGTTGCGGGCGTAAATTGGAGCCCTTTTCAGGA
CCCTCGTGGCCTAGCATCCACCATACACAATTTGACCTCGGATCAGGTAG
GGATACCCGCTGAACTTAAGCATATCAATAAGCCGGAGGAA

Appendix C

Raw Data of the Mycelial Growth and Pseudosclerotia Formation from Chapter 3

Table C-1. The effect of pH on mycelial growth on WC 833 *M. rufobrunnea*

		Experiment 1					Experiment 2					Experiment 3				
Day	pH	R1[a]	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	pH5	5.25[b]	6	4.75	5.75	4	6.875	7.875	7.5	7.75	8.25	5.25	6	4.75	5.75	4
1	pH6	6.5	6	7	6.25	5.5	8.75	7.875	9.25	7	7.75	9.25	9.25	9	10	9.75
1	pH7	7	6.25	6.5	6.5	6.75	9	7.75	9.25	6.5	7	8.75	9.5	9.5	8.5	8.75
1	pH8	6.5	6	7	5.25	6.5	8	7.5	7	7.75	7.5	10.5	9.5	9.5	8.5	9.5
2	pH5	12.75	13	11.75	12	12.25	12	10.5	11.5	10.5	10.75	11.25	12.75	8	10.75	5.75
2	pH6	15.25	15.5	15.5	15.25	14	13.5	13.75	14	15	14	14	12.75	14.5	13.25	13.5
2	pH7	12.25	10.5	13.5	12.25	12.75	14.5	15	14.5	14	13.5	13.75	12.75	13.75	12.75	13
2	pH8	13.25	10.5	12.25	9.25	10.75	12.875	13.25	14	14.25	14.75	13.75	14	13.5	12.25	12.5
3	pH5	18.375	18.375	18.75	18.25	18.25	19	14.5	19.5	13.5	16	22	24	18	21.5	11
3	pH6	20.75	21.375	21.662	23.5	21.25	20	21	21.75	21.25	21.25	24.25	21.75	24	22.5	23.25
3	pH7	23.5	21.75	24.75	24	24.75	23.25	23.5	23	20	19.25	19.5	18	18.5	19	19
3	pH8	24	21.75	23	19.5	21.5	20	20.25	22.25	23.25	22.5	16	17	18.5	17	18.25
4	pH5	24	24.75	23	23.75	25	27.25	21.75	29	20.25	25.5	35	35	30	32	23.5
4	pH6	26.75	32.25	33	34.5	33	28.75	29.75	28	27	29.5	35.25	34.25	35	35	33.5
4	pH7	34.5	34.25	37	36	36.75	33.5	32.5	33	28.5	29.25	26.25	24	26	25.5	24.5
4	pH8	35.25	34.5	32.5	31	33	28.5	28	31.25	31.25	32	23	22.5	22.5	25.75	23.75
5	pH5	34	33	38	36	39	37	31.5	35	30.5	36.5	45	40	38	40	35.25
5	pH6	38	45	45	45	45	38.5	37.75	35	33.75	38.5	45	45	45	45	40
5	pH7	39	45	45	42	45	45	45	45	37	39	34.5	31.75	34.75	33.75	35
5	pH8	39.5	45	41	40	41	37	38.5	41	38	41	32.75	32	31.25	32.5	31.75

a: replicate. Each experiment had 5 replicates.

b: mycelial growth (mm).

Table C-2. The effect of pH on mycelial growth on China 1 *M. importuna*

		Experiment 1					Experiment 2					Experiment 3				
Day	pH	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	pH5	9	9.5	10	11	10	10	9	11	10	10	8	8	9	8	8
1	pH6	10.25	8.875	11	11.5	10.75	12	9.5	10	11	11	9.5	9.5	9.75	9	9.25
1	pH7	7.5	7.625	8.25	8.25	8.5	8	9	9	9	9	8.25	7.5	7.75	8	8.5
1	pH8	6.875	7.5	8.75	8	7.75	8	8	7	8.5	6	8.5	7.75	8.5	8.5	8.75
2	pH5	23	21	24	24.75	25	22	22	24	21	21	19.75	20	20	19.5	20.25
2	pH6	20.875	19.875	23	23.75	22	23	21	19.5	20	20	19.75	20	20	19.25	19.5
2	pH7	19.625	19.5	21.5	21.25	20.75	19	21	19.5	20	20	18	17.5	18	18.25	18.75
2	pH8	16.125	16.5	18.25	18	17.75	18	17	17	17.5	26	17	16.5	17.75	16.5	17.5
3	pH5	34.75	35.25	36.25	36.25	36	37	34	36	33	32	32	32	32.75	33.5	33.75
3	pH6	34	38.5	36	36.5	37	36	34	34	35	35	32.75	33.5	32.75	32.5	32
3	pH7	28.125	28.125	30.25	29.25	29.75	31	33	31	31	30.5	28	27	27.25	29	29.25
3	pH8	27.375	26	28	27.5	27.75	28	26.5	26	27	26	26	26	26.25	26	26.25
4	pH5	45	45	45	45	45	45	45	45	45	45	39.75	40	40.75	42.25	42.5
4	pH6	41	45	45	45	45	45	45	45	45	45	42.75	43	42	41.5	41.75
4	pH7	36.75	36	39	38	40.5	40	42	41	39	40.5	36	35	35.75	37.25	37
4	pH8	37	36.25	35.5	36.25	37.5	36.5	35	35	36.5	34	35.25	33.5	32.5	33.75	34

Table C-3. The effect of pH on mycelial growth on WC 374 *M. americana*

		Experiment 1					Experiment 2					Experiment 3				
Day	pH	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	pH5	3.75	4.25	4.25	5.25	5	5.5	5	6	5.5	5	3.5	3	4	5	4
1	pH6	6.75	6.75	6.75	6.5	6	6	6	6.5	6.5	5.75	7	5.5	6.5	6.25	6.25
1	pH7	6.75	6.25	7.75	6	6.5	6.25	6.5	6.5	6.5	6	7.25	6.25	7	6.75	6.25
1	pH8	5.5	6.5	6.5	5.5	5.25	5	5	5.5	6	5.5	6	5.75	7	6	7
2	pH5	9.5	9.75	11.25	11	11.5	9	10	12	9	10	8	8.5	10	12	10
2	pH6	15.25	15	15	15.25	14.75	13.5	15.5	15.25	15.5	14.5	16	15	12.75	14.75	15
2	pH7	15.5	14	13.5	13	13.5	14.25	13.75	13.75	14	13	14	13.75	15.25	14.75	13.75
2	pH8	13.5	14.25	14	13.5	13.5	11.75	12.75	12	13	12.75	13.75	12	13.5	13	13.75
3	pH5	18.75	19.25	20.75	17.5	19	19	21	22	20	21	17.5	17	16	17	16
3	pH6	24.75	25	24.75	25	24.5	22.5	23	24.75	24	23.5	24	23	21	25.5	23
3	pH7	21.75	22.5	23	22	23.5	21.25	19.75	20	21	19	21	19	21	20	20.25
3	pH8	21.5	22	21	21	20	19	19	18.25	20	19.5	19	18	19.5	19	20
4	pH5	30	30.5	31.5	28	28.75	29.5	32	33	33	31	29	30	30	31.5	30
4	pH6	35.5	35.5	35.25	35.25	35	33.75	33.75	34.75	34	34	33	33.5	31.75	33.5	37
4	pH7	31	31.75	30.5	30	30.5	30.25	29.25	29.25	30.25	29	31.5	31	33	31.25	32
4	pH8	30	31	31	31	30.5	28.25	28.25	28	30.75	30	31	29.5	30.25	31	32
5	pH5	45	45	45	45	45	45	45	45	45	45	40	38	39	42	40
5	pH6	45	45	45	45	45	45	45	45	45	45	40	45	41	42	42.5
5	pH7	45	45	45	45	45	45	45	45	45	45	41	40	40	41	40.5
5	pH8	38	42	40	40	39	38	39	38	39.25	39	39.75	38	39	39.25	39.25

Table C-4. The effect of pH on mycelial growth on WC 766 *M. exuberans*

		Experiment 1					Experiment 2					Experiment 3				
Day	pH	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	pH5	9.25	9.25	8.5	9.75	9	10	8	11	10	10	9.5	9.25	9.5	8	9.75
1	pH6	8.75	9	9	9.5	8.75	8	9	11	10	10	8.5	9.25	9	8.25	9
1	pH7	7.5	8.5	7.5	7.25	8	8	9	8	9	8	8	7.25	8.75	6.875	7.25
1	pH8	7.5	8	8.25	7.5	7.5	8	8.5	7	8	8	7.5	7	8	6.75	7.5
2	pH5	22	21.5	22	22	21.5	21	17	25	23	23.5	24	24.25	24.25	22	23
2	pH6	21.5	21	21	21.5	21.75	20	20	23	23	22	21.75	22.5	22.5	21	21.5
2	pH7	19	19	18.75	19	19.5	19.5	19	19	20	18.5	19	19	20.25	18	18.5
2	pH8	18.25	18	18.25	17.5	18	17.5	19.5	17	18	18	18.25	17.25	18.25	17	18
3	pH5	34.75	34	33.5	34	33	33.5	32.5	35	34.5	35	34.5	34.25	34.5	33.75	34
3	pH6	33	32.5	33	34	33.5	32	32.5	35	34.5	35	30	32.5	31.25	30	30.5
3	pH7	29.75	29.25	30.5	30.25	30.25	31	31	32	31	30	29	29.25	31.5	28.5	29
3	pH8	28	30	28	28.75	29	28.5	31	28	29	29	27.25	26.5	28	27.75	28
4	pH5	45	42	43	45	45	45	36.5	45	45	45	45	45	45	45	45
4	pH6	45	41.5	41	42	40	39	38	45	41	45	41	45	42	41.5	42
4	pH7	37.5	36.5	40	39.5	38	39	39	40	39	38	37.5	39.5	41	40.25	38.5

Table C-5. The effect of pH on pseudosclerotia formation on WC 833 *M. rufobrunnea*

pH	Pseudosclerotia area (mm^2)	pH	Pseudosclerotia area (mm^2)	pH	Pseudosclerotia area (mm^2)	pH	Pseudosclerotia area (mm^2)
5	268.49	6	287.914	7	200.374	8	327.012
5	284.046	6	302.989	7	203.56	8	358.391
5	397.205	6	328.625	7	339.055	8	687.633
5	502.205	6	348.926	7	349.012	8	824.582
5	404.588	6	375.305	7	372.833	8	775.259
5	509.21	6	400.082	7	395.37	8	429.241
5	509.735	6	515.394	7	395.37	8	489.067
5	533.031	6	569.722	7	421.876	8	386.784
5	550.288	6	629.486	7	471.373	8	281.527
5	570	6	640.757	7	476.292	8	21.875
5	600.597	6	700.843	7	497.356	8	400.007
5	614.839	6	844.878	7	528.237	8	543.2
5	774.487	6	872.959	7	600.719	8	247.506
5	775.891	6	1422.628	7	631.31	8	265.534
5	956.204	6	1538.213	7	755.024	8	331.944

Table C-6. The effect of pH on pseudosclerotia formation on China 1 *M. importuna*

pH	Pseudosclerotia area (mm^2)	pH	Pseudosclerotia area (mm^2)	pH	Pseudosclerotia area (mm^2)	pH	Pseudosclerotia area (mm^2)
5	422.136	6	631.975	7	604.07	8	535.215
5	450.794	6	656.374	7	641.644	8	483.318
5	356.999	6	689.312	7	694.507	8	504.777
5	370.804	6	705.469	7	701.476	8	509.488
5	370.804	6	739.126	7	780.926	8	529.064
5	499.05	6	780.436	7	817.893	8	533.684
5	600.89	6	813.29	7	848.424	8	541.674
5	412.27	6	835.475	7	853.644	8	759.462
5	444.633	6	842.571	7	854.671	8	571.425
5	444.633	6	999.549	7	890.004	8	572.188
5	540.003	6	1019.464	7	897.71	8	615.073
5	589.863	6	1033.812	7	912.634	8	629.437
5	738.45	6	1047.103	7	931.41	8	858.265
5	738.451	6	1192.48	7	962.956	8	920.562
5	905.344	6	1250.222	7	987.015	8	840.289

Table C-7. The effect of light on mycelial growth on WC 833 *M. rufobrunnea*

		Experiment 1					Experiment 2					Experiment 3				
Day	Light Intensity (lux)	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	0	6	5	5	5.5	5.5	6	6	6	5	5	6	4	4	4	3.5
1	400	5	5	5	6	5	4	4	6	5	5	4	5	4	4	4
1	2000	3	4	4	3	3.5	6	5.5	6	5.75	6	5	4	4	4	3
2	0	9.5	8.75	9.75	9.25	9	8	8	9	8	8	7	6	8	8	7
2	400	6	6.25	6	7	6.5	5	5	7	6	6	5	5.5	5	6	4.5
2	2000	7.5	7.25	7	7.5	7	5	5	5.5	4	5	6	4.5	5	4.5	4
3	0	15.25	13.75	15	14.75	13	13	11	12	13	15	18	12	17	15	18
3	400	8	9	8	9	9.5	7	8	11	9	8.5	8	8	7	9	5
3	2000	7.75	7.75	7.75	7.5	8	6	6	7.5	4.5	6	7	5	6	6	5
4	0	23.75	21.75	22.25	23.25	21.5	22	20	21	29	31	22	30	27	31	
4	400	10	10	9	11.25	10.25	10	14	14	11	10	16	11	13	17	11
4	2000	8	8	7.75	8.25	8	7	6.5	9	5.5	8	14	11	11	9	8
5	0	33.75	36.5	31	33.25	28.25	34.5	35	37	35	42	45	32	39	36	38
5	400	13	12	14	18	13	16	23	17	13	17	24	22	22	28	23
5	2000	9.5	9.75	9.5	10.25	10	11	10	12	9	11	21	20	20	16	13

Table C-8. The effect of light on mycelial growth on China 1 *M. importuna*

		Experiment 1					Experiment 2					Experiment 3				
Day	Light Intensity (lux)	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	0	9.25	10	11	9.5	9.25	8	8	9	7	7	6	7	7	6	7
1	400	10	11	10.5	10.5	12	7	9	6	7	9	7	7	7	5	8
1	2000	8	5.5	6.75	7.25	7	6.5	6	7	7	6	5	6	5	6	7
2	0	20.5	22	23.75	22	21	17	18	18.5	19	19	13	16	14	14	17
2	400	22	22	23	24	24	18	20	15	20	19	18	15	16	18	17
2	2000	15.5	12.5	14.5	15.25	15	15.5	15	13	15	14	14	13	11	14	15
3	0	31.75	33	35	32.75	31.75	27.5	30	30	30.5	31	23	28	24	24	27
3	400	33	34	35	35	34	30	33	26	32	33	28	25	25	28	27
3	2000	26.75	23	26	27	27	25	25	25	22.5	23	24	22	20	23	25
4	0	42	43	45	44	43	38	40	38	39	40	35	42	35	36	38
4	400	45	45	45	45	45	38	40	36	40	42	39	37	37	37	36
4	2000	35	35	35	40	35.75	36	37	36	33	32	34	28	29	31	36

Table C-9. The effect of light on mycelial growth on WC 766 *M. exuberans*

		Experiment 1					Experiment 2					Experiment 3				
Day	Light Intensity (lux)	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	0	10	11.25	11	11.25	11.5	10	11	10	11	14	8	7	9	9	8
1	400	11	10	10.25	10	11	11	10	11.5	10	11	8	9	9	10	9
1	2000	10	8.75	8	9	9.25	9	9	9	8.5	10.5	8	8	7	10	9
2	0	25	23.25	25.5	25.5	25.5	23	24.5	23.5	26	28	22	21	24	23	23
2	400	25	24.5	25	24	26	26.5	26	28.5	26	26	21	24	24	23	23
2	2000	25	23	21.75	22	24.5	23.5	23	23	23	25	23	23	24	26	24
3	0	35.25	35.5	35	36.5	37.25	35	36	34.5	36.5	39	36	37	39	36	36
3	400	35	36	37	37	38	41	40	39	38	37	34	39	37	38	38
3	2000	40	35	36	34	39.5	36	37	36	37	36	38	36	38	38	37

Table C-10. The effect of light on mycelial growth on WC 374 *M. americana*

		Experiment 1					Experiment 2					Experiment 3				
Day	Light Intensity (lux)	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	0	5	6	6.5	6.5	6	7	7	6	5	5	5	6	4	5.5	6
1	400	6	5.75	5	6.25	5.25	4	4	3.5	6	5	4.5	4.5	4	4	3.5
1	2000	4	3.75	4	3.5	4.25	3	2.5	4	3	6	2.5	3	2.75	3.25	3.75
2	0	11.75	11	11.25	12.5	12	12	12	11	9	10	10	13	9	16	17
2	400	9	10	9	9	8	5	7	5.5	9.5	8	9	9	10	8	8
2	2000	4	3.75	4	3.5	4.25	4	3	5	3.5	7	3.2	2.6	2.75	3.5	3.75
3	0	18.25	16.5	15.75	18.5	16	17	18	19	17	16.5	17	21	16	20	21
3	400	15	16	15	16	14.5	15.5	17	14	19	17	15	14	15	13	14
3	2000	5	4.5	4.75	4.5	5	4.25	3.25	5.5	3.75	7.25	3.5	3	3.7	4.75	
4	0	25	23	24.75	25.75	25	28	27	28	27	27	22	27	20	28	30
4	400	21.5	21	22	20	20.5	26	27	24	30	29	21	20	23	25	26
4	2000	5	4.75	5	4.75	5.25	6	7	10	4	10	3.5	5	3	3.7	4.75
5	0	32.25	29.5	31.5	33.5	32	40	39	37	38	39	31	35	30	35	40
5	400	26.5	27	28	28.75	26.25	40	39	36	39	40	31	29	36	37	32
5	2000	5.25	6	6.25	5.75	6.25	8	7.5	14	4.5	13	5.75	6.5	5	5.25	6
6	0	37.5	35.5	38.5	37	38	45	45	45	45	45	42	45	37	45	45
6	400	33	32	35	33.5	32.5	45	45	45	45	45	41	37	45	45	42
6	2000	7	7.5	7.75	6.75	7.5	13	8	18	5	18	6.5	7	6.25	6.5	7.5

Table C-11. The effect of temperature on mycelial growth on WC 833 *M. rufobrunnea*

		Experiment 1					Experiment 2					Experiment 3				
Day	Temperature (°C)	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	20	3	3	3	3	3	3	4	3	3.5	3	2.5	4	3	3	4
1	25	3	4	3	3.5	3	3	3	4	5	4.5	3	4	3	3.5	5
1	30	2.5	2	3	3	3	3	2.5	2	3	2.5	3	3	2.5	2	2
2	20	9	5	10	6	6	5	4	5	8	7	7	6	7	9	6
2	25	8	7	10	9	6	5	6	8	10	8	7	8	5	7	6
2	30	3.5	4	5	6	5.5	5	5	4	5	4.5	5	6	5.5	4	5
3	20	20	14	19	16	15	15	10	14	17	16	17	15	15	16	15
3	25	18	20	23	21	15	10	11	17	22	18	16	18	14	18	18
3	30	7	10	9	9	9	9	8	7	8	7	8	9	8	9	7
4	20	30	26	37	31	29	26	18	23	28	30	28	25	28	27	28
4	25	32	36	39	34	29	24	20	33	39	32	28	30	23	29	26
4	30	17	17	17	18	17	12	12	14	15	14	16	18	16	16	17
5	20	40	38	45	40	39	36	29	35	38	38	39	36	40	38	37
5	25	42	40	45	38	42	30	36	39	42	38	39	39	32	41	36
5	30	29	25	27	29	29	18	19	25	25	23	23	25	25	27	23

Table C-12. The effect of temperature on mycelial growth on China 1 *M. importuna*

		Experiment 1					Experiment 2					Experiment 3				
Day	Temperature (°C)	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	20	3	3	3	3	3	3	4	3	3.5	3	2.5	4	3	3	4
1	25	3	4	3	3.5	3	3	3	4	5	4.5	3	4	3	3.5	5
1	30	2.5	2	3	3	3	3	2.5	2	3	2.5	3	3	2.5	2	2
2	20	9	5	10	6	6	5	4	5	8	7	7	6	7	9	6
2	25	8	7	10	9	6	5	6	8	10	8	7	8	5	7	6
2	30	3.5	4	5	6	5.5	5	5	4	5	4.5	5	6	5.5	4	5
3	20	20	14	19	16	15	15	10	14	17	16	17	15	15	16	15
3	25	18	20	23	21	15	10	11	17	22	18	16	18	14	18	18
3	30	7	10	9	9	9	9	8	7	8	7	8	9	8	9	7
4	20	30	26	37	31	29	26	18	23	28	30	28	25	28	27	28
4	25	32	36	39	34	29	24	20	33	39	32	28	30	23	29	26
4	30	17	17	17	18	17	12	12	14	15	14	16	18	16	16	17
5	20	40	38	45	40	39	36	29	35	38	38	39	36	40	38	37
5	25	42	40	45	38	42	30	36	39	42	38	39	39	32	41	36
5	30	29	25	27	29	29	18	19	25	25	23	23	25	25	27	23

Table C-13. The effect of temperature on mycelial growth on WC 374 *M. americana*

		Experiment 1					Experiment 2					Experiment 3				
Day	Temperature (°C)	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	20	6	7	8	8	7	8	9	6	7	8	8	9	9	8	7
1	25	9	9	9	9	8	11	12	9	9	9	10	10	12	9	8
1	30	9	10	10	9	10	10	10	10	11	10	10	10	11	9	9
2	20	18	19	20	20	18	18	21	18	17	21	20	21	20	18	19
2	25	23	25	24	24	24	23	24	22	22	22	25	25	29	26	26
2	30	24	25	25	24	26	22	22	22	23	22	25	25	26	25	25
3	20	30	34	33	34	32	30	32	34	30	37	34	34	34	34	33
3	25	38	38	37	37	38	34	36	36	32	35	40	39	45	37	35
3	30	38	39	39	37	40	36	34	37	33	34	41	41	42	40	39
4	20	29	33	27	29	31	29	33	27	29	31	34	28	30	29	26
4	25	14	18	11	14	16	12	17	10	10	16	10	14	13	11	12
4	30	4	3.75	3	3.5	3.5	2.75	2.75	3.25	2.75	2.75	3.25	2.75	3	3.25	3.8
5	20	39	45	38	39	45	45	40	41	39	35	35	37	43	32	40
5	25	21	27	20	22	22	19	25	14	14	21	17	20	20	18	16
5	30	4.25	4	3.25	4	3.75	3	3.25	3.5	3	3	3.75	3	3.5	3.5	4

Table C-14. The effect of temperature on mycelial growth on WC 766 *M. exuberans*

		Experiment 1					Experiment 2					Experiment 3				
Day	Temperature (°C)	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15
1	20	6	7	8	8	7	8	9	6	7	8	8	9	9	8	7
1	25	9	9	9	9	8	11	12	9	9	9	10	10	12	9	8
1	30	9	10	10	9	10	10	10	10	11	10	10	10	11	9	9
2	20	18	19	20	20	18	18	21	18	17	21	20	21	20	18	19
2	25	23	25	24	24	24	23	24	22	22	22	25	25	29	26	26
2	30	24	25	25	24	26	22	22	22	23	22	25	25	26	25	25
3	20	30	34	33	34	32	30	32	34	30	37	34	34	34	34	33
3	25	38	38	37	37	38	34	36	36	32	35	40	39	45	37	35
3	30	38	39	39	37	40	36	34	37	33	34	41	41	42	40	39

Table C-15. The effect of temperature on pseudosclerotia formation on WC 833 *M. rufobrunnea*

Temperature (°C)	Pseudosclerotia area (mm^2)	Temperature (°C)	Pseudosclerotia area (mm^2)	Temperature (°C)	Pseudosclerotia area (mm^2)
20	857.278	25	739.449	30	0
20	395.187	25	560.44	30	0
20	354.803	25	741.96	30	0
20	483.496	25	431.129	30	0
20	381.015	25	467.593	30	0
20	572.957	25	849.466	30	0
20	407.982	25	960.44	30	0
20	484.231	25	250.295	30	0
20	381.015	25	534.839	30	0
20	867.188	25	387.239	30	0
20	373.552	25	489.972	30	0
20	391.965	25	854.517	30	0
20	668.673	25	585.606	30	0
20	484.231	25	863.035	30	0
20	530.345	25	694.79	30	0

Table C-16. The effect of temperature on pseudosclerotia formation on China 1 *M. importuna*

Temperature (°C)	Pseudosclerotia area (mm^2)	Temperature (°C)	Pseudosclerotia area (mm^2)	Temperature (°C)	Pseudosclerotia area (mm^2)
20	586.076	25	320.018	30	0
20	537.204	25	160.679	30	0
20	289.775	25	426.447	30	0
20	487.922	25	420.018	30	0
20	318.963	25	937.857	30	0
20	265.466	25	616.364	30	0
20	987.62	25	256.032	30	0
20	253.328	25	597.274	30	0
20	819.731	25	501.989	30	0
20	596.079	25	506.761	30	0
20	652.379	25	434.234	30	0
20	444.033	25	727.803	30	0
20	539.036	25	645.129	30	0
20	863.486	25	499.639	30	0
20	511.416	25	594.852	30	0

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