Oyster Mushroom Cultivation

Part III. Mushrooms Worldwide

Chapter 11

Mushrooms for the Tropics

GROWING *PLEUROTUS TUBERREGIUM*

Omoanghe (Omon.) S. Isikhuemhen[1] & David S. LeBauer[2]
[1] North Carolina A&T State University, USA.
[2] University of California, USA

Introduction

History and distribution

The King Tuber Oyster Mushroom, *Pleurotus tuberregium* (Fr.) Singer is a tuberous basidiomycete. Consumption is widespread in Nigeria and across many tribes in sub-Saharan Africa. Unlike most other wild and cultivated mushrooms, it has a sclerotia stage in its lifecycle that is resistant to extreme environmental conditions found in some parts of its distribution areas, for example, the hot dry season in West Africa. Figure 1 shows 2kg sclerotium collected in the wild.

Figue 1. Anwar Ibrahim holding a 2kg sclerotium

Sclerotia can be stored for many years before used as food, medicine or inoculum and induced to produce sporophores (fruitbodies) . Fruitbodies (Fig. 2) harvested at a young stage are delicious edibles that may be eaten fresh or dried for future use. The local people who use this fungus for food and medicine usually collect the sclerotia from the wild, but it is getting difficult to find sclerotia due to the depletion of its forest habitat. However, easy growing method of this fungus is established to produce sclerotia using many lignocelullosic agricultural wastes as cultivation substrates.

Figure 2. Ebikare Isikhuemhen holding large mushrooms induced from sclerotium

P. tuberregium is indigenous to tropical Africa and the Australasian-Pacific regions of the world (Pegler, 1983; Isikhuemhen *et al*., 2000a) including sub-Sahara Africa, Madagascar, Malaysia, Papua New Guinea, Northern Australia, New Caledonia, Indonesia, Myanmar and the Yunnan province of China.

Demand for sclerotia all over the world remains high while harvest from the wild is reduced. The natural habitat of this mushroom is declining with deforestation and the conversion of forests into agricultural fields; meanwhile wild sclerotia have become scarce and very expensive, especially during the dry season (October to

April) when it is not easy to collect them in the wild. Africans who have left their home and reside in foreign lands usually, take their eating habits with them, which is why today powdered sclerotia can be found in African food stores across the US, Canada and many European. Powdered sclerotia sold at such stores are always imported from Africa. As the wild source is lost, the demand for cultivation of this mushroom is likely to continue its increase. Wide adoption of cultivation by indigenous people will permit continued use of *P. tuberregium* in the diet and provide income. Various kinds of available agricultural wastes can serve as an ideal substrate for its growth; the cost of production can be quite low and adaptable to low technology methods.

Mushroom collection and usage by the local people

Indigenous peoples of Nigeria incorporate collected mushrooms, including *P. tuberregium*, into their diet and medicine (Oso, 1977, Ogundana, and Fagade 1982; Akpaja *et al*., 2003). Similarly, people in Ghana (Obodai *et al*., 2004), Cameroon (Kuyper *et al*., 2002) and Republic of Benin (Victor Ekun, personal communications) collect wild mushrooms and use as food and medicine. Sclerotia and fruitbodies of *P. tuberregium* are also collected from the wild and then either consumed or preserved for later use. Women and children collect and sell sclerotia at roadside stands for seasonal income and some financial autonomy. The Igbo people are the major consumers of *P. tuberregium* throughout Nigeria, using the fungus in place of meat as well as for its medicinal properties (Akpaja *et al*., 2003).

Life Cycle

The life-cycle described here is based on previously reported observations (Isikhuemhen *et al*., 2000a, Oso 1977, Pegler 1983) in nature and experiments conducted on the cultivation of *P. tuberregium* (Isikhuemhen *et al*., 2000b). In nature, dead wood is colonized either by mycelia in the soil or airborne spores discharged from individual fruitbodies. *P. tuberregium* is a white rot fungus, which derives nutrients from the degradation of lignocelullosic material (usually hardwood). During the rainy season it is possible to see a decaying log filled with fruiting mushrooms during early years of its colonization. Once the substrate is fully colonized and has reached an advanced stage of decay, sclerotia will form at the end of the rainy season. The sclerotia survives the dry and hot season until the rain returns, at which point the sclerotia will either continue to enlarge or form sporophores (mushrooms).

Sclerotia are a compact mass of mycelia tissue that serves to store food during unfavorable conditions and able to fruit when favorable conditions return. Sclerotia are dark brown on the surface, white inside, spherical to ovoid in shape and as large as 30cm in diameter. Sclerotia weighing up to 6kg are commonly harvested and sold between June and September in Nigeria (Oso, 1977). These are harvested from the wild from decaying logs and adjacent soil. These are either used as sclerotia or buried and watered regularly to induce fruit bodies (Okhuoya and Etugo 1993; Oso, 1997).

The sclerotia stage is not common among white-rot fungi. Among cultivated mushrooms, only *Morchella* spp. is known to form sclerotia as part of their lifecycle. However, only the sclerotia of *P. tuberregium* are valued as food and medicine independent of its ability to produce mushrooms. Not all strains of *P. tuberregium* form sclerotia; some strains from Australia and Indonesia have been observed to fruit directly without ever forming sclerotia (Isikhuemhen, 2000a). The sclerotia stage provides a unique advantage to the cultivation of this species because it eliminates the need for costly fruiting conditions and because sclerotia may be stored for many years without loss of viability.

Under cultivation, it is common to find 1 and up to 3 sclerotia per 1kg (dry weight) bag of sawdust substrate. Aborted sclerotia primordial less than 10cm diameter may be more numerous on wheat straw substrate, which is incapable of generating new mycelium therefore unsuitable for use in propagation techniques described below (Isikhuemhen *et al*., 2000b). Wild strains from Nigeria have exhibited slower colonization and greater yield on

wheat straw when compared to strains from Australasian-Pacific regions; some hybrid strains were highest yielding (Isikhuemhen *et al*., 2000b). Recently, we have obtained biological efficiency of 73.5% using improved strain grown on supplemented sawdust in laboratory experiments (Isikhuemhen and Vaughans, unpublished).

Generally, *P. tuberregium* as a tropical mushroom grows best around 30°C. Fruiting requires high humidity to provide the large volume of water required for mushroom fruit body development.

Products and Uses

Food

Sclerotium can be soaked in water for 12-24 hours and after the skin is peeled off, the whitish inner tissue is milled into paste. This paste is used to substitute in part or whole for the melon seeds (*Citrulus lanatus*) in the preparation of 'egusi' soup, or mixed with corn flour and fried (Isikhuemhen and Okhuoya 1995; Nwokolo 1987). When sclerotia are induced to form sporophores, the ideal stage for harvest is 5-7 days after emergence, before the cap becomes upturned. These mushrooms are cut into pieces and used as meat in soups (Nwokolo 1987). Analysis of both sclerotia and sporophores shows that they are rich in carbohydrate, protein, vitamins, and minerals, while low in fats (Nwokolo 1987; Okhuoya and Ajerio 1988).

Medicine

The sclerotium of *P. tuberregium* has long been used for food and medicine by various tribes of Nigeria (Akpaja *et al*., 2003; Isikhuemhen and Okhuoya, 1995). The Igbo use it to treat heart problems, while it is used to treat asthma, cough, and obesity among people of the Edo State (Isikhuemhen 1995, Isikhuemhen *et al*., 2000a). Other known uses include treatment of headache, stomach ailments, colds and fever, smallpox and high blood pressure (Oso, 1977). Okhuoya *et al*., (1998) reported that in Ghana they are used in medicine for illness that relates to malnutrition and anemia in children and in the rural areas it is used as one of the ingredients in the embalming of dead bodies.

Materials

In this chapter, we discuss specific materials used as substrates. However, it should be noted that *P. tuberregium* can be cultivated on a variety of lignocellulosic substrates, including many agricultural byproducts, sawmill waste, and even cardboard. The farmer should first assess what substrates are readily obtainable at low cost, and experiment with combinations of substrates to determine which produce greatest yields. Corn, rice, and wheat straw (*Zea mays*, *Oryza sativa*, and *Tritiucm aestivum*), oil palm fruit fiber, cassava (*Manihot escunlenta*), banana leaves, corncobs, cotton waste, hardwood sawdust, paper, and cardboard are all suitable substrates. Where available, oil palm fruit fiber is not matched for yield and low incidence of contamination (Okhuoya and Okogbo 1990).

Bags: Bags made specifically for mushroom cultivation with gas-exchange filters are available from Unicorn (www.unicornbags.com), though grocery bags are a suitable alternative.

Spawn

Starter culture

The method you want to employ in the cultivation of this fungus can determine your starter culture or material. If you want to cultivate this fungus on sterile substrate using a semi-intensive or intensive cultivation process, you can isolate pure culture from mushroom fruit bodies germinated from sclerotia. Obtaining a pure culture from wild fruit bodies, sclerotia, or spores is possible using standard tissue culture techniques outlined by Stamets (1984). A pure culture of the mycelium of this fungus can be obtained by incubating tissue from inside the stipe near the base of

the cap or from inside the sclerotia on standard malt extract agar in a Petri dish incubated at 30℃. We have observed that isolating pure cultures directly from sclerotium can be difficult at times due to contamination problems associated with inherent microbial load in wild sclerotia. Pure cultures of this fungus are available from the author and major culture banks all over the world.

Preparation of substrate for spawn production

Many substrates can be used for spawn preparation. Those reported so far are oil palm fruit fiber (*Elaeis guineensis*) hereafter referred to as OPF, wheat bran (*T. aestivum*) supplemented sawdust and whole grains: untreashed rice (*O. sativa*), millet (*Setaria macrochaeta*), sorghum or Sudan grass (*S. bicolor*) and wheat (*T. aestivum*). Use of the above grains follows standard methods for using grains to prepare spawn (Stamets 1984). Here we provide a variety of spawn production methods that have been used in the past to prepare spawn, starting with our standard wheat grain.

Wheat grain

Unthreashed wheat grain is first soaked in water overnight and then parboiled for 10 min. Excess water is drained. Calcium carbonate ($CaCO_3$) is added at 1% w/w and grains are spread out to air dry for 20 minutes. Spawn bags are made with 500g of these grains and sterilized at 121℃ for 30 minutes. After cooling, bags are inoculated with 8-10 agar blocks (approximately 1cm2) colonized by actively growing mycelium of *P. tuberregium*. This inoculation process is best done under sterile airflow such as under a laminar flow hood to decrease contamination. Inoculated spawn materials are incubated either in incubators or clean rooms at 30℃. Colonization of 500g spawn substrate bags usually takes less than ten days (Isikhuemhen *et al*., 2000b). At lower temperatures, total colonization will require more time. Care should be taken to avoid contamination, and any contaminated bag should be removed and autoclaved before disposal. Colonized spawn can be stored at 5-10℃ without loss of vigor or vitality for up to six months. However, longer storage periods can increase the chances of spawn becoming contaminated. Using pieces of agar from sclerotia collected in the wild is not recommended for inoculation spawn of this nature, because in most cases you will have contamination problems. It is possible to use pieces of sclerotium cultivated on sterile substrate for spawn inoculation, but the rate of contamination is still much higher than when pure cultures on agar blocks are used.

Sawdust

The supplemented sawdust recipes used by commercial mushroom growers (Stamets, 1984) work well for this species. Hardwood sawdust supplemented with 20% w/w wheat or rice bran and wet to 65% moisture content supports healthy spawn growth. Under less sterile conditions, spawn can be made by adding sclerotia pieces to sawdust at 65% moisture, which has been boiled for 3 hours. When sterile conditions are not available, sawdust should not be supplemented.

Oil palm fruit fiber

Oil palm fruit fiber (OPF) can be used for spawn preparation. It is known to have low incidence of contamination, which is attributed to its nutrient content that allows *P. tuberregium* to grow quickly to colonize the substrate (Okhuoya and Okogbo, 1990). OPF is an abundant waste generated from oil palm fruit processing, a major industry in many tropical countries where *P. tuberregium* is found. Fresh OPF is soaked in water for six hours, drained and loaded into spawn bags, sterilized, inoculated and incubated as per grain spawn above. Usually, 2kg bags prepared as such achieve total colonization within seven days at 30℃, forming a compact mass that can be easily broken down into smaller pieces and used for inoculation.

OPF is also a very good material to use for spawn preparation under sub optimal sterile conditions. Fresh OPF

can be soaked in water for six hours, boiled at 100°C for 3 hours and the water drained before inoculation with the inner parts of freshly collected or presoaked sclerotia pieces. In a similar manner, sawdust spawn can be prepared. However such sawdust should not be supplemented to reduce incidence of contamination. In some cases, we have been able to make spawn from fresh sawdust that was wetted to 65% water content and inoculated with pieces of sclerotia. These methods of spawn preparation under sub-optimal or non-sterile conditions can be very useful in developing countries where sterile facilities may be too expensive to obtain.

The direct use of sclerotia as spawn, i.e. inoculation of cultivation substrate with pieces of sclerotia is also common. However, the contaminant load from wild collected sclerotia is very high. To do this we recommend soaking the sclerotia in water over night if they are dried or have been stored for over three months (this is not necessary if collected fresh). The soaked sclerotia are clean washed in boiled water followed by removing the outer skin with a clean knife. The whitish inside core of the sclerotia are cut into pieces (usually not less than $2cm^3$ a piece) and used for inoculation.

It is reported that soaking in bleach solution have been used to achieve surface sterilization, but we do not recommend it due to the fact that compounds or by products resulting from its degradation or breakdown may be toxic to human health This is especially so if the produce is to qualify as organic food or aimed at export to countries where there are stringent control on the chemicals used in food production and processing.

Substrate

Bulk substrate preparation

Many lignocellulosic wastes can support growth of *P. tuberregium* (Fasidi and Olorumaive 1994; Isikhuemhen and Okuoya 1995; Okhuoya and Etugo 1993; Okhuoya and Okogbo 1990). Sawdust and straw are readily available at little or no cost, easy to pre-treat, and are less prone to contamination compared to other agricultural wastes.

Straw

Dried straw is shredded with a wood chipper or lawn mower and placed in a pile. As the pile grows, periodic watering combined with frequent turning over of the substrate can help to bring the water content to optimum (ratio of 1:3, straw: water) within a 24hour period. Alternatively, proper moisture content can be achieved by soaking (complete immersion of substrate in water) over night and allowing the soaked substrate to drain for at least 3 hours before heat treatment. After achieving proper water content, substrate can be loaded into cultivation bags before pasteurization or bulk heat-treated. Cultivation bags that can contain up to 5kg wet substrate can be heat sterilized at 100°C for 1 hour on 2-3 consecutive days. Steam sterilization can be done in 55gallon drums filled to 20cm with water and heated over a fire or propane stove, making sure to maintain water in the drum during steaming. Bricks or wire should be used to keep bags above water. Bulk pasteurized substrate can be achieved by steaming the bulk substrate at 100°C in a suitable chamber, but bulk substrate of more than 5kg should be given longer hours of exposure to steam to ensure all substrate reaches 100°C during heat treatment on 2-3 consecutive days.

Heat-treated substrate should be allowed to cool down to at least 45°C before inoculation with spawn. In the case of bags, spawn should be added (5-10% w/w) and carefully shaken down to allow even spawn distribution throughout the bag. Afterward, the bag is either heat-sealed or the mouth of the bag is folded to a tight knob and closed with a rubber ban. For the bulk substrate, spawn materials should be added at similar rate and carefully mixed into the substrate before or during loading into cultivation bags. This process is more efficient, but is not as effective at controlling contamination, which may be introduced during spawning. Usually substrate inoculation with spawn is done under sterile airflow in a laminar flow hood. However, a clean room can be used for this process with low incidence of contamination, provided strict personal hygiene rules are observed.

Composted sawdust and OPF

Figure 3. cultivation of *P. tuberregium* in column bags

Fresh hardwood sawdust and OPF is collected and sun-dried. On a cement platform, sawdust and OPF are mixed in a ratio of 1 : 1 (w/w). Water is added at a substrate: water ratio of 1 : 2. Substrate is piled into 2m diameters and 1.5m tall heaps and covered with a black plastic sheet. Solar and metabolic heating will occur. The pile is turned and mixed once per week for four weeks. Substrate becomes partially fermented and low in contaminants. Substrate can then be loaded into bags and steamed sterilized as above before spawning (Fig. 3).

Substrate not used immediately after fermentation can be dried in the sun and stored for future use, but will require bring water content to optimum capacity (1 : 2 to 1 : 3, substrate: water ration) and sterilized immediately prior to use. In more rural areas where sterilization equipment is not available, pasteurized substrate can be inoculated with sclerotia pieces. In this case, sclerotia pieces should be larger (5-10cm^3) and rate of spawning at least 10% per weight of substrate. When sclerotial is used to inoculate semi or unsterilized substrate, it has high power to overcome contaminants like blue mold (*Trichoderm* sp.). The mycelia growing out of the sclerotial inoculums aggregates into rhizomorphic strands that is used to penetrate and colonize contaminated areas of the substrate (Fig. 4).

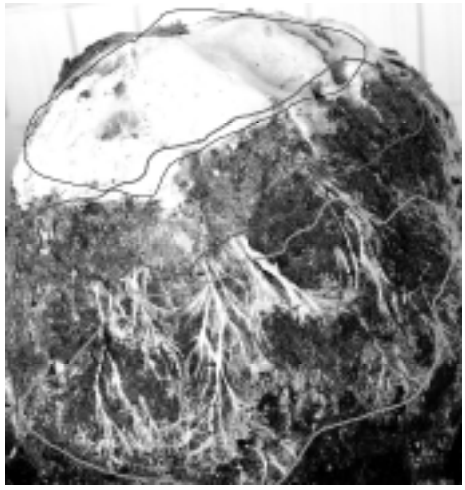

Figure 4. Rhizomorphic mycelia from sclerotia inoculated sawdust colonizing unsterile substrate

Supplemented sawdust

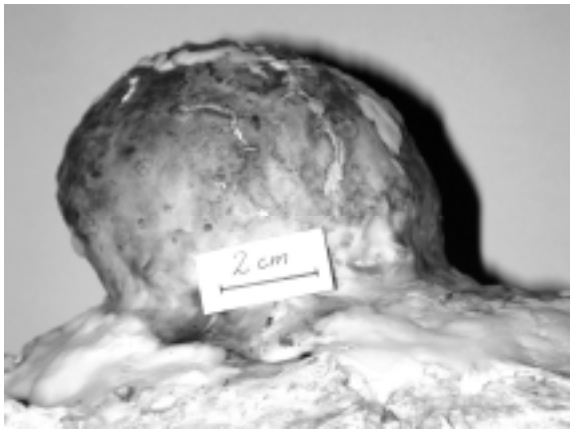

Figure 5. Sclerotia growing on supplemented sawdust substrate.

Figure 6. cultivation of *P. tuberregium* in sawdust bed cased with garden soil. Marked spots are points where sclerotia are forming and breaking through the casing material.

Different hardwood sawdust has been tested in the cultivation of *P. tuberregium*. Okhuoya *et al*., (1998) reported various levels of sclerotial yield on different tropical hard wood sawdust. Even for the best sawdust substrates, supplementation with different source of organic nitrogen can improve sclerotia yield significantly, for example using one of our selected strains, we have obtained 735g fresh sclerotia from 1kg sawdust substrate with 5% wheat bran supplementation (Fig. 5). Contaminant loads may be high without suitably sterile facilities. A 20% w/w supplementation is recommended, and cultivation on this substrate should be done in bags to minimize contamination. However, fresh un-supplemented sawdust can be laid in beds and used for cultivation either on

floors of unused rooms, forest floors or under plantation crops like rubber (*Hevea brasilenses*) Cocoa (*Theobroma cacao*) or OPF. In a study of this type of cultivation method, sawdust beds of dimension 1m2 laid in rooms gave yield of 4.0 ± 0.57kg (fesh weight) per bed (Isikhuemhen, 1993). Similarly, Akpaja and Begho (1999) have produced sclerotia from cultivation beds laid under a rubber plantation. Sawdust of suitable hardwood is collected, adjusted for water content (1 : 3 subsrate: water ratio) and lay out in beds on concrete floors or on plastic sheets lay on forest or plantation floors. In this case no heat treatment may be done and inoculation is best by direct sclerotia pieces as described above.

Bed cultivation of *P. tuberregium* requires casing, usually done soon after complete colonization on the bed is observed. This is because sawdust beds can dry up very easily under tropical weather conditions. Generally, when sawdust substrate colonized by mycelia it is difficult to water as applied water will just flow away or stay on top the bed till it evaporates. However, by casing, water content of the substrate is conserved and the casing layer helps to hold the water adder to maintain water levels in the bed until it is slowly absorbed into the bed. Normal casing material used in *Agaricus* production can be used: peat moss supplemented with lime. However there are reports of the use of top garden soil (Fig. 6). Sclerotia formation will be visible within two weeks of casing. It takes longer for colonized and uncased bed to begin sclerotia formation. However, the mechanism by which casing hastens sclerotia formation on beds is not understood.

Logs

Hardwood logs approximately 10-20cm diameter and 1m long can be used to produce both sclerotia and fruit bodies. Okhuoya and Okogbo (unpublished data) tested different logs from different hardwood species and found that almost all tested logs were able to produce sclerotia. However relatively less dense hard wood was earliest to produce sclerotia and in some case, like oil palm log, both sclerotia and non-sclerotia associated fruit bodies were produced. Logs of less economic values, like leftover branches from logging operations and stumps or storm knocked down logs can be used for the cultivation of *P. tuberregium* to obtain sclerotia. The time it takes to produce sclerotia on logs depends on the tree species, log diameter, and strain of *P. tuberregium*.

Standard methods for log inoculation used in shiitake cultivation are applicable to this process. On suitable logs, holes (0.5 × 2cm) are drilled into a diamond pattern with 15cm spacing and spawn injected into such holes with a plunger, followed by waxing with cheese or bee wax. Logs are stacked for one year in shade and prevented from drying out. Any local material of organic origin can be used to cover the inoculated holes, since its function is mainly to prevent the injected spawn from drying up before the mycelium grow into the logs. In the Esan tribe of Nigeria, pomade from palm kernel, Ori-eyo (pronounced ori-ehyor) could be for this purpose. Inoculated logs can be laid on forest floors for colonization and production of sclerotia. Large fallen trees can be inoculated *in situ* and allowed to colonize and produce sclerotia. In such cases, the log can be source of sclerotia year after year till the logs are completely rotted.

We have observed that sclerotia produced on logs under natural conditions are more condensed, heavier and generally store longer than those from on straw or hardwood sawdust substrates.

Cultivation Methods

Inoculation of bulk substrate

When spawn material is fully colonized, it will be a compact mass of mycelia and substrate. In front of a laminar flow hood, spawn is cut into bits 2-4cm^3, which are then ready to inoculate the final substrate. In unsterile or semi-sterile environments, where for example, OPF is used to generate spawn from sclerotia, the spawn can as well be

cut into pieces and used for bulk substrate inoculation.

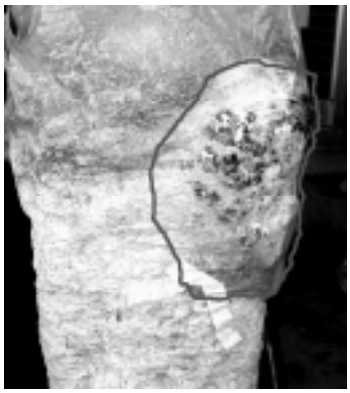

Figure 7. Sclerotium under formation in a cultivation bag filled with sawdust substrate.

Spawn run and sclerotia formation

Spawn run is fastest at 30℃ and > 80% relative humidity. Spawn run will take 20-30 days depending on substrate. Spawn run and sclerotia production can be done in beds if an appropriate growing facility is available or in bags similar to those used for the production of spawn. For large-scale production, growing houses will maximize productivity and efficiency. Alternatively, using bags allows production to be adapted to a variety of scales and eliminates the need to maintain humidity. Cultivation in bags can also decrease contamination. Light is not required for sclerotia formation (Fig. 7).

Cased beds of bulk substrate

Substrate is laid into 1m^2 beds to a depth of 20cm. Bamboo (*Bambusa* sp.) houses with cement floors and palm leaf roofs are suitable. Racks can be used to stack beds and maximize the use of space. Substrate beds are inoculated at a rate of 5-15% (w/w) depending on contaminant load and sterilization method used.

In tropical climates, water loss from beds can be high. Water should be added at a rate of 5L/m^2 every other day for the first three weeks before colonization is complete. After three weeks, bowls containing water placed on available spaces will maintain humidity. Until colonization is complete, windows are kept closed except for one hour during the night.

Casing is done when substrate is completely colonized, usually six-eight weeks after spawning. Topsoil from the garden, sand, or peat is used to cover the colonized beds to a depth of 4-5cm. Casing moisture is maintained by occasional watering. 2-4 weeks after casing, sclerotia will emerge through the casing material. Casing has been shown to increase yields in a number of mushroom species (Stamets 1984) including *P. tuberregium* fruit bodies (Okhuoya and Okoobo, 1990) though the effect of casing on sclerotia yield has not yet been reported. At this stage, watering is increased to prevent sclerotia from drying out. Windows can be opened for six hours during the day to allow ventilation and lower the ambient temperature. Young sclerotia are white, turning dark brown with time. Using this method with proper management can yield 2-4kg sclerotia/m^2.

Bag method

Substrate may also be inoculated in bags up to 8kg each. A rate of 5% (w/w) spawn is added. Substrate may be inoculated from spawn or directly from sclerotia pieces. However where sterile facilities are not available and the possibility of even pasteurization of substrate is remote, pieces of sclerotia can be used to inoculate sawdust substrates loaded into bags after mixing with water. Under such condition, inoculation at the rate of 10% spawn can be successful. When unsterilized substrate is used, supplementation with organic nitrogen sources such as grain should never be used, because contaminants will out-compete the fungus and reduce yields. Sclerotia can be cultivated in the bags with filters similar to those used for spawn production, with the same variety of substrates discussed and not requiring casing. Cultivation in bags requires only that temperature be maintained around 30℃, as moisture will be retained. Sclerotia will be ready for harvest within 12 weeks, when fast growing and high yielding strains are used.

Harvest and storage

At maturity, the sclerotia are harvested, washed clean with water, and air dried (or sun dried) for one week. Fresh sclerotia are approximately 50% water. Dried sclerotia will maintain viability for at least two years if stored at 5-

10℃, or for one year at 28℃. Dr Roy Watling was able to fruit a sclerotium collected in Cameroon after seven years in the green house in Edinburgh (personal communication). Yields of 10% fresh sclerotia from wet substrate (approximately 5% dry mass from dry substrate) can be expected.

Induction of sporophores from sclerotia

To induce mushroom fruiting from sclerotia, first soak the sclerotia in water for 6-12 hours (depending on size). It is necessary to bury the sclerotia in peat, sand, or soil. Sclerotia may be placed in trays or into shallow holes dug in the ground and covered with a casing layer of peat and/or soil. Ambient humidity should be greater than 85%, and it may be necessary to water the casing layer daily with approximately 40mL/ sclerotia. Fruit body formation should occur within 5-15 days.

Mushrooms intended for eating should be harvested before the cap expands, approximately 3-5 days after emergence under temperature conditions of 25-30℃. Two and possibly more flushes can be expected depending on the size of the sclerotium. Sclerotia will form if substrate is colonized at a high temperature, usually in less than 90 days. However, fruiting may be inhibited at this temperature, and will not occur while inside the bag. We have found that 25℃ is ideal for fruiting and Okhuoya and Okogbo (1990) reported that the use of casing material increases yield on a variety of substrates. Mushroom yield will be approximately 25% of sclerotia weight, and 90% moisture. Mushroom yield from sclerotia and mushroom quality is not comparable to other Pleurotus spp. on similar substrates.

Marketing and Export

P. tuberregium is not as widely cultivated or well understood as other cultivated mushrooms such as *Agaricus bisporus*(button mushroom) and *Lentinula edodes*(shiitake), which are multi-billion dollar industries in the western world. However, the sclerotia of *P. tuberregium* are very important because of its multiple uses as food and medicine (Akpaja *et al*., 2003, Nwokolo 1987, Oso 1977). The sclerotia are an essential commodity for local consumption, especially in the West African sub-region, has also become a commodity for export to developed countries, since they are sold in specialty food shops across the many continents. The possibility for increased export is predicted on two major facts:

1. People from the regions of the world where the sclerotia is used for food and medicine, who now live in Europe and America actively search for this material in African food shops in those continents. Isikhuemhen has found powdered sclerotia in shops in the UK, US and Canada.

2. The second drive for exports are due to recent scientific studies that is showing sclerotia of this fungus to contain polysaccharides and other compounds with positive medicinal benefits. Publications from Asia have shown that fresh sclerotia of this fungus have high content of useful compounds like β-glucan and lectins that are promising medicinal properties (Zhang *et al*., 2003; Wang and Ng, 2003). In our laboratories we are finding this fungus to have antibiotic properties as well as inhibition of different cancer cell lines *in vitro* (Isikhuemhen and Goktepe, unpublished). At the IMC 7 in Oslo, 2002, Dr. Jae Sung Lee of Department of Biochemistry, Yeungnam University, Korea told us that they were testing incorporating sclerotium powder into bread, as a cheap source of supplement to increase the protein content of bread.

The world is becoming aware of the importance and health benefits of natural foods and supplements. The sclerotia and sporophores of *P. tuberregium* are some of such natural foods. It is very expensive to produce sclerotia in places where temperatures are suboptimal for this fungus because of the overhead cost in environmental control. The production we do to obtain sclerotia for our research is done in incubators set at 30℃ all year round. That will

be expensive for a commercial operation to adopt in a place like central and northern Europe and most part of North America and Asia. However, the same fungus can be produced, even under open or outdoor cultivation, without need for environmental control in places across sub-Saharan Africa (from West to East), Madagascar, southern India, China and Southeast Asia. Therefore, wherever there will be need for the sclerotia of this fungus, it will be more economical to import form the regions where they are easily cultivated. Fortunately, the regions mentioned above are mostly in developing countries.

The method described above for the cultivation of *P. tuberregium* is simple and cheap to operate and it is suitable for use in developing tropical countries. However, this model can be adjusted in both directions with little or no modifications, either for use by rural dwellers (especially women), as small or hobbyist producer, small-scale enterprise or a large commercial producer.

Feasibility of these Methods for Developing Tropical Countries

The methods outlined here were developed specifically to provide methods of cultivating fungi for food and medicine in developing countries with limited technological resources. To this end, we have described the use of agricultural wastes, which are readily available at little or no cost. We have described a variety of methods applicable to various situations. OPF and OPF/sawdust are recommended for greatest yield, low contamination, ease, and applicability to farmers with limited resources. If good sawdust spawn can be produced, log cultivation maximizes substrate inoculation and production from small amounts of spawn, and has a minimal risk of contamination. Another advantage to logs is that they produce for many years. When a sterile lab is available, use of sawdust spawn to inoculate straw, OPF, or other bulk substrates is recommended.

A beginner or untrained mushroom cultivator may have difficulty in the development of pure cultures and pure spawn because this requires some technical skill and is most effective when a laminar flow hood and autoclave are available. This can be overcome by obtaining spawn from commercial spawn manufacturers or creating a cooperative to develop a single spawn production facility to serve many farms. Otherwise, low nutrient substrates such as un-supplemented OPF or sawdust will not host most contaminants.

Contamination by other weed fungi and pathogenic organisms can be minimized by following the instructions given here for spawn and substrate preparation and by maintaining strict hygienic conditions within and around the cultivation premises. A clean room for spawn production and substrate inoculation is recommended. Growing rooms should be thoroughly cleaned between crops. Screened windows and doors can keep the majority of insects away. We have not observed insects attracted to fruit bodies of this fungus as much as we see for other *Pleurotus* species.

Acknowledgements

This chapter is dedicated to Magda and Ebikare Isikhuemhen, and Joseph and Joslin LeBauer. We are grateful to our mentor, Dr. Rytas Vilgalys (Duke University, Durham, USA) for his continuous support and availability for some of our research on Pleurotus species. We wish to acknowledge the support of Unicorn bags (www.unicornbags.com) for the supply of bags we used for cultivation and The Golden Leaf Foundation (www.goldenleaf.org) that is providing financial support for our continuous research on specialty mushrooms including *P. tuberregium*.

REFERENCES

- Akpaja, E.O., O.S. Isikhuemhen, and J.A. Okhuoya. 2003. Ethno mycology and uses of edible and medicinal mushrooms among the Igbo people of Nigeria. *International Journal of medicinal mushrooms* 5: 313-319.
- Akpaja, E.O. and E.R. Begho. 1999. Production of sclerotia of *Pleurotus tuberregium* (Fr.) Sing on wastes under

mature rubber (*Hevea brasiliensis* Muell.Arg.). *Nigerian Journal of Applied Science*, 17: 97-103
- Fasidi, I.O., and K.S. Olorunmaiye 1994. Studies on the requirements for vegetative growth of *Pleurotus tuber-regium* (Fr.) Singer, a Nigerian mushroom. *Food Chemistry* 50: 397-401.
- Isikhuemhen, O.S., 1993. Studies on the cultivation of edible sclerotia of *Pleurotus tuber-regium* (Fr.) Sing. on various farm wastes. M. Sc. thesis. University of Benin, Nigeria.
- Isikhuemhen, O.S., J-M Moncalvo, F Nerud, and R Vilgalys. 2000a. Mating compatibility and phylogeography in *Pleurotus tuberregium*. Mycological Research 104: 732-737.
- Isikhuemhen, O.S., F. Nerud, and R. Vilgalys. 2000b. Cultivation studies on wild and hybrid strains of *Pleurotus tuberregium* (Fr.) Sing. on wheat straw substrate. *World Journal of Microbiology and Biotechnology* 16:431-435.
- Isikhuemhen, O.S., and J.A. Okhuoya. 1995. A low-cost technique for the cultivation of *Pleurotus tuberregium* (Fr.) Singer in developing tropical countries. Mushroom Growers Newsletter 4:2-4.
- Kuyper, T.W., J.F.W. van Dijk and N.A. Onguene. 2002. Knowledge and utilization of edible mushrooms by local populations of the remain forest of South Cameroon. Abstract #365, IMC 7, Oslo, Norway. P.115
- Nwokolo, E. 1987. Composition of nutrients in the Sclerotium of the mushroom *Pleurotus tuber-regium*. *Plant Foods For Human Nutrition*, 37: 133-139.
- Obodai, M, K.A. Vowotor and K. Marfo. 2004. Performance of Various Strains of *Pleurotus* Species under Ghanaian Conditions. *Mushworld publication*, issue 16, 2004-01-12.
- Ogundana, S.K. and O.E. Fagade. 1982. Nutritive value of some Nigerian mushrooms. *Food Chem*. 8: 263-268.
- Okhuoya, J.A., O.S. Isikhuemhen and G.A. Evue. 1998. *Pleurotus tuber-regium* (Fr.) Sing.Sclkerotia and sporophore yield during the cultivation on sawdust of different woody plants. *International Journal of Mushroom Sciences*, 2: 41-46
- Okhuoya, J.A. and J.E. Etugo 1993. Studies on the cultivation of *Pleurotus tuber-regium* (Fr.) Sing. an edible mushroom. *Bioresource Technology*, 44: 1-3
- Okhuoya, J.A. and F.O. Okogbo. 1990. Cultivation of *Pleurotus tuber-regium* (Fr) Sing on various farm wastes. Proceedings of the Oklahoma Academy of Sciences 71:1-3.
  http://digital.library.okstate.edu/oas/oas_pdf/v71/p1_3.pdf
- Okhuoya, J.A. and C. Ajerio.1988. Analysis of Sclerotia and sporophores of *Pleurotus tuber-regium* Fr. an edible mushroom in Nigeria. *Korean Journal of Mycology*, 16: 204-206
- Oso, B.A. 1977. *Pleurotus tuber-regium* from Nigeria. *Mycologia* 69: 271-279
- Pegler, D.N. 1983. *The genus Lentinus, A world monographs. Kew Bulletin Additional Series* 10. London: Her Majesty's Stationary Office ISBN 0-11-242627-1
- Stamets, P. 1984. The Mushroom Cultivator. Agarikon Press. Olympia, WA.
- Zhang, M, L. Zhang, P.C.K. Cheung and J. Dong. 2003. Fractionation and characterization of a Polysaccharide from the sclerotia of *Pleurotus tuber-regium* by preparative size-exclusion chromatography. *Journal of Biochemical and Biophysical Methods*, 56: 281-289.
- Wang, H. and T.B. Ng. 2003. Isolation of a novel N-acetylglucosamine-specific lectin from fresh sclerotia of the edible mushroom *Pleurotus tuber-regium*. *Protein Expression and Purification* 29: 156-160
- Stamets, P. 2000. Growing Gourmet and Medicinal Mushrooms. Ten Speed Press. Berkeley, CA.