PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [4] : A01G 1/04

A1

(11) International Publication Number: WO 86/ 06247

(43) International Publication Date: 6 November 1986 (06.11.86)

(21) International Application Number: PCT/US86/00937

(22) International Filing Date: 28 April 1986 (28.04.86)

(31) Priority Application Number: 728,176

(32) Priority Date: 29 April 1985 (29.04.85)

(33) Priority Country: US

(71) Applicant: NEOGEN CORPORATION [US/US]; 620 Lesher Place, Lansing, MI 48912 (US).

(72) Inventors: OWER, Ronald, Dean ; 3320 21st Street, San Francisco, CA 94110 (US). MILLS, Gary, Lynn ; 1670 Southshore Drive, Apartment C-2, East Lansing, MI 48823 (US). MALACHOWSKI, James, Anthony ; 1678 Bliss Street, Haslett, MI 48823 (US).

(74) Agents: WATT, Phillip, H. et al.; Room 900, 135 South LaSalle Street, Chicago, IL 60603 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent).

Published
With international search report.

(54) Title: CULTIVATION OF *MORCHELLA*

(57) Abstract

Culturing ascocarps or fruitbodies of species of the genus *Morchella.* Mycelia are provided nutrients and subsequently produce nutrient-rich mycelia and attendant sclerotia. The mycelia and attendant sclerotia store sufficient nutrient to supply the ascocarps that are developed later. The mycelia and attendant sclerotia are establishd in a nutrient poor substratum. Mycelia and attendant sclerotia are induced to give rise to ascocarp development by initially maintaining the mycelial growth and attendant sclerotia in an environment that is poor is exogenous nutrients and by exposing the mycelia and attendant sclerotia to a high level of water. After induction, primordia appear. The period from primordia appearance until midway to maturation of the fruitbodies represents a critical period during which the fruitbodies are prone to abort. During this critical period, particular attention is directed to maintaining favorable conditions. The fruitbodies, which may be grown to maturation, are harvested.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GA	Gabon	MR	Mauritania
AU	Australia	GB	United Kingdom	MW	Malawi
BB	Barbados	HU	Hungary	NL	Netherlands
BE	Belgium	IT	Italy	NO	Norway
BG	Bulgaria	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	LI	Liechtenstein	SN	Senegal
CH	Switzerland	LK	Sri Lanka	SU	Soviet Union
CM	Cameroon	LU	Luxembourg	TD	Chad
DE	Germany, Federal Republic of	MC	Monaco	TG	Togo
DK	Denmark	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali		
FR	France				

CULTIVATION OF MORCHELLA

The present invention is directed to cultivation of the morel fungi i.e., species of Morchella, including their mature, edible ascocarps.

BACKGROUND OF THE INVENTION

The genus Morchella contains the species of mushrooms known as morels or sponge mushrooms. They belong to the ascomycetous fungi. True morels are edible and delicious. Indeed, some consider them the most delectable of all the fungi. While the taste of these mushrooms is known and loved by those who search the forests in the early spring, morels are unavailable to the general population because heretofore they have defied cultivation such as would be practical for commercial production year round.

To the connoisseur of mushrooms, morels are known by their ascocarp or fruitbody (the visible mushroom). One would suppose that if these fungi grow freely without cultivation in the wild or natural state, cultivation methods would have been developed to maximize their production. This, however, has not been the case. There are reports of growing morels outdoors; however, no one has succeeded in cultivating morels like the common Agaricus species or other edible forms in environmentally controlled rooms for harvesting throughout the year.

Ascocarp or fruitbody production is the mature embodiment of the sexual reproduction cycle of the morel. The mature ascocarp containing ascospores or germ spores represents the culmination of a life cycle highlighted by an internal mating of two haploid nuclei to form a diploid nucleus which undergoes meiosis to form new haploid ascospores. Both autogamous and heterogamous pairing prior to meiosis have been reported for Morchella. An alternative life cycle is an asexual process in which conidia (asexual spores) are produced and from which new mycelium, containing haploid nuclei, can be grown.

Also, as a means of protecting the species under certain conditions, the vegetative mycelia coalesce into hardened bodies known as sclerotia which may lie dormant during periods of unfavorable conditions. Accordingly, fruiting of the morel occurs during select conditions; a situation recognized by mushroom hunters who have experienced "bad years" for morel gathering.

It is a general object of the invention to provide a method for culturing morels in a manner suitable for commercial production of ascocarps throughout the year under controlled conditions.

DEFINITIONS

For purposes of clarity, terms used in this application are defined generally as following in C.J. Alexopoulos and C.W. Mims, Introductory Mycology, 3rd Ed., John Wiley & Sons, New York (1979):

Ascocarp - a fruitbody containing asci.

Ascospore - a meiospore borne in an ascus.

Ascus - (pl. asci) a sac-like cell generally containing a definite number of ascospores (typically eight) formed by free cell formation usually after karyogamy and meiosis; characteristic of the class Ascomycetes.

Condiophore - a simple or branched hypha arising from a somatic hypha and capable of bearing at its tip or side one or more conidiogenous cells.

Conidium - (pl. conidia) sometimes called conidiospores, a nonmotile asexual spore usually formed at the tip or side of a cell; in some instances a pre-existing hyphal cell may transform into a conidium.

Hypha - (pl. hyphae) the unit of vegetative structure of most fungi; a tubular, filamentous cell containing asexual nuclei.

Mycelium (pl. mycelia) mass of hyphae constituting the body (thallus) of a fungus.

Primordium (pl. primordia) the beginning stage of any structure.

Sclerotium (pl. sclerotia) for the purpose of this document, sclerotium is defined as hyphae that contain stored nutrient reserves, that may aggregate, that may contain embedded amounts of substratum and that also may form hardened structures (referred to herein as hardened sclerotia).

Substratum (pl. substrata) (for the purpose of this document substratum will be defined as) the soil-like material which serves as the habitat in which the fungus grows and from which the fungus produces fruitbodies.

SUMMARY OF THE INVENTION

The invention provides for the culturing of species of the genus Morchella to produce mature ascocarps or fruitbodies. Vegetative mycelia are fed nutrients for development into sclerotia, which under some forms of cultivation may take the form of hardened sclerotia. The nutrient-rich sclerotia contain sufficient stored nutrients to supply substantially the entire nutrient requirements for subsequent development of fruitbodies. Subsequent to feeding, the environment of the mycelial growth and attendant sclerotia is substantially altered in order to promote the sexual cycle of growth in which ascocarps (visible mushrooms) are produced. Contributing to this process is removal of available exogenous nutrients. Also contributing to this process is exposure of the mycelial growth and attendant sclerotia to high levels of water. The sexual cycle of growth is first evidenced by the appearance of primordia and culminates in mature fruitbodies. The growth period from primordia appearance to about the time of fruitbody maturation is an especially critical time of development, and conditions are carefully controlled to minimize abortion of the developing fruitbody. One important factor in minimizing abortion of the developing fruitbody is to ensure previous storage of sufficient nutrients in the mycelial growth and attendant sclerotia, particularly neutral lipids, to support fruitbody maturation. Other important factors are the maintainance of correct air humidity and substratum moisture during fruitbody development and proper ventilation during fruitbody development. Other factors include optimal air velocity relative to the habitat and the maintenance of a daily water loss from the habitat.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention provides for the culturing of morels to produce ascocarps or fruitbodies. The spawn that are used for morel cultivation are nutrient-rich mycelial growth and attendant sclerotia, including hardened sclerotia which are resting bodies and nutrient reservoirs that are somewhat resistant to unfavorable conditions. The nutrient-rich mycelial growth and attendant sclerotia, depending upon environmental conditions, may either sustain additional vegetative mycelial growth or may be induced to give rise to the sexual cycle and mature ascocarps. Nutrients, particularly neutral lipids in the form of triglycerides, are stored in the mycelial growth and attendant sclerotia, and during the sexual cycle, substantially all of the nutrients for fruitbody development are drawn from these and other stored nutrients.

Accordingly, the invention provides for production or cultivation of mycelial growth and attendant sclerotia, providing the same with nutrients so as to ensure sufficient storage of nutrients in the mycelial growth and attendant sclerotia for subsequent development to ascocarps. Conditions are then adjusted appropriate to induce mycelial growth and attendant sclerotia to the sexual growth cycle. Substantial care is taken during development from primordia appearance to ascocarp maturation to maintain conditions that ensure that the developing ascocarps do not abort. In particular, conditions of soil moisture, humidity and air exchange are adjusted to promote ascocarp development and minimize disease.

The first step of morel production is the development of sclerotial spawn. The use of sclerotia as spawn represents a preferred aspect of the invention with regards to efficient production of morels. Although cultivation of morels can be effected starting each cycle with spores, production is much slower and, thus, beginning each growth cycle with spores is impractical for commercial cultivation. In addition, the traditional use of grain spawn as substrate inoculum is generally inappropriate because inoculation with grain spawn tends to lead to cultures that are highly contaminated with other fungi and bacteria.

One method of culturing hardened sclerotia for use as inoculum spawn is to fill a container with wheat or other vegetative material to between about 40 to about 80 percent of its volume. The wheat is then covered with a perforated liner, typically plastic film or metal foil, although other materials can be used, and the remaining 20 to 60 percent of the container volume is then nearly filled with moist soil. The volume of the container may range from about 50 ml to multiple liters, but is typically about 500 ml. The wheat berries or other vegetative material may be supplemented with additional nutrients consisting of both organic and inorganic nitrogen sources, other minerals, vitamins and carbohydrates which help to promote storage of the nutrients that are required during subsequent ascocarp development. The container is covered and autoclaved to kill possible contaminating organisms. The soil layer of the sterilized container is inoculated with ascospores, with vegetative hyphae or with small pieces of sclerotia, and the jar is again sealed. The container is maintained at a temperature of between about 10°C and about 30°C and preferably between about 18°C and about 22°C.

Hyphae from the inoculum grow through the soil layer and colonize the grain. After about one week, a loosely compacted mass of hyphae appear in the soil layer. Microscopically viewed, the hyphal cells become highly branched, septate and swell to a barrel shape. This is then followed by the adhesion of adjacent cells to form a solid mass that is visible to the naked eye. It is the hyphal cells of the sclerotia which store the materials obtained from the colonized grain. The sclerotia cultivated in this manner are, at maturity, hard structures which can become quite large. Virtually all of the total soil layer can become enmeshed in the the hardened sclerotia.

At this point, the hardened sclerotia are harvested for use as spawn. Some of the developed hardened sclerotia may be reserved as "jar inoculum" for producing additional sclerotia, or for other uses.

The use of sclerotia as spawn has several advantages with respect to the efficient production of morels. In addition to growing at a rate commensurate with serving as a steady source of inoculum, sclerotia may be preserved for extended periods of time. It is possible that in nature sclerotia remain dormant for extended periods of time, such as over the winter months, until conditions are favorable for initiation of growth. Storage at about 5°C is found to be satisfactory for long-term preservation.

Mature sclerotia are used as spawn to inoculate the substratum. Two variations on the method of the present invention may be followed. In a first of the variations, mature sclerotia is divided into pieces which are used to inoculate a substratum; these sclerotial pieces produce hyphae, which upon addition of nutrients produce additional mycelial growth and attendant sclerotial mass within the substratum before induction to the sexual cycle. In the second variation, sclerotia, i.e., hardened sclerotia that have been developed in the jars, are directly inoculated into a substratum, and the additional mycelia which grow therefrom are induced to the sexual cycle, without adding nutrients.

An important aspect of the invention is induction or triggering of the fungus to the sexual growth cycle in which ascocarps are produced. One important contributing factor in induction is deprivation of available exogenous nutrients to the fungus so that assimilation and storage of nutrients by the fungus ceases or significantly slows. Accordingly, the environment of the fungus is altered from a nutrient-rich environment to a nutrient poor environment. For purposes of this invention, a "nutrient poor" environment is an environment lacking readily available nutrients for supplying developing ascocarps, whereby the nutrients for such developing ascocarps are the nutrients which have been stored in the mycelial and attendant sclerotia prior to induction.

Another important factor which appears to contribute to induction is exposure of the fungus to high quantities of water in the substratum in which the fungus is growing. Typically, the substratum is hydrated substantially to saturation for the purpose of promoting induction of nutrient-rich mycelia and attendant sclerotia to the sexual cycle. By substantially saturated is meant at least about 90% of the capacity of the substratum, but preferably approaching 100% capacity. Preferably, during exposure to high quantities of water, there is a continuous exchange of water. This may be accomplished, for example, by percolating water through the substratum in which the fungus is growing. Although Applicants are not bound to any theory as to why the high level of water seems to promote induction, the water may provide a triggering "shock" to the system, e.g., by change in osmotic pressure.

In the first variation, sclerotia are divided into pieces between about 0.5 and about 4 cubic centimeter in size and inoculated into a thin layer of substratum which is typically between about 1 to about 4 cm deep. Good results occur when there are about 6 to about 30 cc. of divided sclerotia per square meter of substratum surface. If hardened sclerotia are used, mycelial growth from the inoculum is enhanced by soaking the sclerotial pieces in water just prior to inoculating them into the substrate.

Preferred support substratum is nutrient poor, permitting the availability of nutrients to be controlled through application and subsequent removal of an external nutrient source to the substratum. Suitable substratum includes any standard bark, soil or sawdust compost or potter's soil with or without added minerals known to those skilled in the art. For example, Supersoil[R] (R.McL. Co., San Francisco) has been used successfully either directly from the commercially sold bag or leached two times with two equal (v/v) volumes of water. The substratum should allow adequate drainage, should provide buffering capacity, should have good water-retaining capabilities, and should provide adequate aeration to allow proper gasous exchange. The substratum that is now being used is about 25% sand and about 75% organic material. A small portion of lime is also added. The organic portion of the soil is primarily ground fir bark (85%) and also contains 10% sphagnum and 5% redwood bark. The soil mixture has an available water content of 55% and an air capacity of 25%. It is expected, however, that a more optimal substratum may be developed.

The substratum is steam-pasteurized or hot water-pasteurized or autoclaved. Pasteurized substratum is then typically mixed with water to produce a workable slurry. The slurry is poured into a tray that has holes in its bottom for drainage. After the slurry is added to the desired depth in the tray, it is allowed to drain until the soil is void of gravitational water; i.e., is below field capacity, allowing for maximum air spaces. This is advantageous in at least two ways. First, it allows for increased production of mycelia and attendant sclerotia, and more specifically, mycelia and attendant sclerotia are formed throughout the substratum. Secondly, removal of standing water helps to minimize later microbial contamination problems. Also, as an alternate approach for tray preparation, trays may first be filled with the substratum as above, then pasteurized.

After the poured substratum is inoculated with pieces of sclerotia, the temperature around the tray is maintained between about 10°C and about 22°C, the relative humidity is maintained between about 75 and about 95 percent, and the water content of the substratum is maintained between about 50% and about 75%. Soon after inoculation, hyphae grow from the sclerotia and completely colonize the tray in about one week. As the mycelia and attendant sclerotia develop, no further water is added, thereby allowing the substratum to dry, preferably to a substratum moisture content of below about 75%. Drying of the substratum prior to feeding is considered to be an important factor in inhibiting growth of bacteria and other fungi which would harm or compete with the developing morels.

Morels, being fungi, do not produce their own food as do photosynthesizing plants, but rather obtain their total nutrient supply from external sources. As a nutrient-poor substratum is deliberately provided, the morel tissue must at some time be provided the requisite nutrients, and in this variation, nutrients are fed to the mycelia growing from the inoculum. The additional mycelia and other at sclerotia that develop from the vegetative growth after nutrient addition should contain, in stored form, substantially all of the nutrients that are needed for efficient fruitbody development.

Nutrients are provided to the mycelia growth in a manner so that the nutrients may be later withdrawn to leave the substratum again nutrient poor. Removal of nutrients promotes differentiation into the sexual cycle and decreases the incidence of contamination.

As a convenient means of providing a removable source of nutrients, a nutrient-rich medium is placed onto the substratum, into which source hyphae can grow and from which source the hyphae can distribute nutrients throughout the mycelial colony. As one means of providing such a source, jars are prepared similar to those used to culture hardened sclerotia. Typically, jars are nearly filled with organic material; a perforated heat resistant liner (usually metal foil) is placed over the organic material; and the liner is covered with soil to the top of the jar. The jar is again covered with another layer of perforated foil, further sealed with a sheet of metal foil and then sterilized.

The nutrient source with which the jar is filled provides the organic material. The organic material is metabolized and eventually is stored in the mycelia and attendant sclerotia as carbohydrates and lipids. The stored material is eventually utilized for ascocarp formation. The nutrient source most commonly used in the development of this cultivation method is wheat berries; however, other vegetative material, including mixed compost, is suitable. If wheat berries are the nutrient source, they should be provided at a ratio of about 1000 grams to about 8000 grams (dry wt.) per square meter of substratum. However, this ratio could vary significantly and can be considered only as a general approximation.

It is desirable that as much nutrient-rich mycelia and attendant sclerotia be produced within the substratum as is possible during this stage because there appears to be a direct relationship between the amount of mycelia and attendant sclerotia in the substratum and the total weight of ascocarps that develop per unit area of the substratum. Growth of mycelia and attendant sclerotia in substratum parallels growth of hardened sclerotia in jars, and the same nutrient factors which enhance growth in the jars enhance growth in the substratum. Accordingly, the organic material may be supplemented with vitamins, minerals, additional protein and other substances.

In this first variation of the method, the top layer of foil is removed from the cooled sterilized jars, and the jars are inverted onto the surface of the substratum. Hyphae grow upward through the holes in the second layer of foil, gather nutrients and distribute the nutrients to the mycelial colonies. During feeding, the soil moisture is maintained at a level of between about 45% and about 70%, the relative humidity is maintained at between about 85% and about 95% and the temperature is maintained between about 10°C and about 22°C. Feeding continues for a period of between about 7 and about 40 days, typically about 16 days. At the end of the feeding period, both conidia and sclerotia may be observed in substantial numbers on the surface of the substratum.

Having provided the mycelial colonies and newly formed attendant sclerotia with substantially all of the nutrients needed for subsequent ascocarp formation, the nutrient source is removed. Removal of the nutrients is a necessary step for cultivation because the sexual cycle will not commence to any appreciable extent in the presence of excess nutrients that are external to the mycelia. The use of an inverted jar or the like containing nutrient material permits the immediate removal of most of the available nutrients, leaving the mycelia in a nutrient-poor substratum.

Subsequent to removal of the nutrient source, a small amount of additional moisture is added to the substratum, e.g., about 1 liter per square meter of substratum surface, and vegetative growth is allowed to continue for a period of about ten days. During this period, the substratum moisture content is maintained at between about 45% and about 70%, the relative humidity is maintained at between about 85% and about 95% percent, and the temperature is maintained at between about 10°C and about 22°C. After this period the sclerotia are mature.

The mature mycelia and attendant sclerotia, rich in stored nutrients but deprived of exogenous nutrients, are now ready for exposure to high amounts of water, which contribute to induction to the sexual cycle. Preferably the substratum and morel mycelia are hydrated by a slow percolation of water through the substratum for a period of between about 12 and about 36 hours. Water is added to the substratum at a rate of between about 250 and about 1000 ml per hour per square meter of substratum surface area. The substratum and the percolating water are maintained at a temperature of between about 10°C and about 22°C.

In the second variation, mature sclerotia, such as the hardened sclerotia which are produced in the jars, are inoculated into a wetted, nutrient-poor substratum at a much higher rate, e.g., typically between about 1500 and 4000 cc per m^2 of substratum surface. These sclerotia contain the stored nutrients that are necessary for hyphal proliferation and subsequent fruitbody development. The sclerotia may be inoculated into the substratum whole or divided; they may also be inoculated directly from the jars or wetted with water first, e.g., typically an 18 to 24 hour immersion. Inoculation into the nutrient-poor substrate represents deprivation of exogenous nutrients to the sclerotia, one of the factors found to contribute to induction to the sexual cycle of growth.

The other factor found to contribute importantly to induction, i.e., exposure to high amounts of water, may commence contemporaneously with inoculation into the substrate or a relatively short period of time thereafter. The substratum may be thoroughly wetted at about the time of inoculation to provide the high amount of water which promotes induction. Better results, however, are obtained if the sclerotia are maintained in the substratum and their mycelia allowed to colonize the substratum for about seven days under conditions similar to conditions during that period in the first variation when the sclerotia are maintained in the nutrient-poor substratum but before water is percolated through the substratum. Next, in a similar manner to the first variation, water is percolated through the substratum, promoting initiation of primordia from the mycelia.

There are several advantages to the second variation of the method relative to the first variation. One of the more notable advantages to the second variation is the permissible depth of the substratum. For this method, the substratum can be considerably deeper, typically between about 6 and about 16 cm. Cultures with a thicker substratum can contain more sclerotia and thus eventually support more ascocarps per unit area of substratum surface than can a thinner substratum layer.

However, the first variation may be preferred because it is more closely analogous to processes used to cultivate other types of fungi, and therefore, may be more adaptable to cultivation in existing facilities or with available apparatus.

Following hydration in either the first or second variation, the substratum is allowed to drain, and the cultures may be aspirated to further remove water. The relative humidity is maintained at between about 85% and about 95%, and the temperature is maintained at between about 10°C and about 22°C. The substratum moisture content is maintained at between about 55% and about 65% during this period.

At the end of this period, i.e., approximately 1-3 days after hydration, morel primordia start to form. Primordia are spherical hyphal aggregates which are about one millimeter in diameter. Within a few days, the primordia form protuberances which represent the first sign of ascocarp fundament formation.

A growth period extending from the initial appearance of primordia until the morel ascocarp reaches a height of about thirty millimeters represents an important period for ascocarp development. During this period, the temperature is maintained at between about 10° and about 22°C and preferably about 18°C, the relative humidity at between about 85 and about 95 percent and the substratum moisture content at between about 50 and about 60 percent. Unless very favorable growth conditions are maintained, immature ascocarps are prone to abort.

It has been found that maximum yields of ascocarps are obtained when the air flow near the substratum is maintained at a substantially steady rate of between about 20 and about 40 cm per minute.

After the morel ascocarp reaches the height of thirty millimeters, conditions are maintained that are favorable to continued development and maturation. The temperature during this part of the maturation may range from about 10°C to about 27°C, the relative humidity may range from about 80% to about 95% percent, and the soil moisture may range from about 30% to about 55%. As the ascocarps continue to develop, they may turn a dark grey, and upon reaching maturity the ascocarp color changes from grey to a golden-brown, at which point the morels are mature. After the first crop of ascocarps are harvested, the cultures may be reinduced to produce a subsequent crop(s).

Using the method as described above with *Morchella esculenta*, yields of 25 to 500 ascocarps per square meter have been obtained.

Although most of the development of the method has concerned isolates of *Morchella esculenta*, the methods of the invention are generally applicable to other species within the genus *Morchella*. For example, success with the species tentatively determined as *Morchella crassipes* and *Morchella costata* have been obtained.

While the invention has been described in terms of a particularly preferred embodiment, modifications obvious to one with ordinary skill in the art may be made without departing from the scope of the present invention. For example, conditions are described hereinabove which are particularly favorable for promoting growth of morels during various stages of their growth, such factors, including substratum moisture, temperature, humidity, air flow, etc. It is to be understood that growth may well proceed, at a less favorable rate at conditions outside of the stated preferred conditions and that short-term excursions from the preferred conditions may not seriously affect the growth rate of morels. Thus, for example, whereas a lower temperature of a favorable temperature range is stated in respect to several stages of growth of the ascocarp, short term temperature excursions to temperatures approaching the freezing point of water are consistent with the continued survival of the ascocarps.

Various features of the invention are set forth in the following claims.

<u>WHAT IS CLAIMED IS</u>:

1. A method for culturing ascocarps of a species of the genus <u>Morchella</u> comprising cultivating mycelia and attendant sclerotia of the species in the presence of a nutrient source that provides both organic and inorganic nutrients for a period of time sufficient for said mycelia and attendant sclerotia to store the nutrient supply needed for subsequent ascocarp development, promoting maturation of said nutrient-rich mycelia and attendant sclerotia and inducing said mycelia and attendant sclerotia into the sexual growth cycle of the species, and maintaining conditions appropriate for development and maturation of ascocarps of the species.

2. A method according to Claim 1 wherein said induction of said mycelia and attendant sclerotia to the sexual growth cycle is promoted by depriving said mycelia and attendant sclerotia of exogenous nutrients.

3. A method according to Claim 1 wherein said induction of said mycelia and attendant sclerotia to the sexual growth cycle is promoted by exposing said mycelia and attendant sclerotia to high amounts of water.

4. A method according to Claim 1 wherein said induction of said mycelia and attendant sclerotia to the sexual growth cycle is promoted by depriving said mycelia and attendant sclerotia of exogenous nutrients and by exposing said mycelia and attendant sclerotia to high concentrations of water.

5. A method for culturing ascocarps of a species of the genus <u>Morchella</u> comprising cultivating mycelia of the species to produce sclerotia, providing a nutrient-poor substratum and inoculating said nutrient-poor substratum with pieces of said sclerotia, promoting mycelial growth in said substratum from said sclerotial inoculum, feeding said mycelial growth for a predetermined period of time with a nutrient source that supplies both organic and inorganic nutrients, then

removing said nutrient source to deprive the mycelial growth and newly formed attendant sclerotia of exogenous nutrients, promoting maturation of said mycelial growth and attendant sclerotia by appropriately regulating the environment thereof, hydrating said substratum containing said mature mycelial growth and attendant sclerotia to expose said mycelial growth and attendant sclerotia to high amounts of water, said deprivation of nutrients and said exposure to high amounts of water contributing to induction of the mycelial growth and attendant sclerotia to the sexual reproductive cycle of the species, and maintaining conditions appropriate for development and maturation of ascocarps.

6. A method according to Claim 5 wherein said mycelia are cultivated by providing a moist vegetive nutrient source, sterilizing the same, inoculating said moist nutrient source with pieces selected from the group consisting of sclerotia, ascospores, vegetative hyphae, and conidia.

7. A method according to Claim 5 wherein between about 6 and about 30 cm^3 of sclerotia are inoculated per m^2 of substratum surface.

8. A method according to Claim 5 wherein mycelial growth in said inoculated substratum is promoted prior to feeding by maintaining the substrate moisture content at between about 50% and about 75%, the relative humidity at between about 75% and about 95% and the temperature at between about 10° and about 22°C.

9. A method according to Claim 5 wherein said nutrient source comprises a vegetive material.

10. A method according to Claim 9 wherein said nutrient source further comprises material selected from the group consisting of organic supplements, inorganic supplements and mixtures thereof that promote storage of neutral lipids in said sclerotia.

11. A method according to Claim 10 wherein said nutrient source is wheat berries supplied at

between about 1000 and about 8000 gm dry weight per m^2 of substratum surface area.

12. A method according to Claim 5 wherein during feeding the moisture content of said substratum is between about 45% and about 70%, the relative humidity is maintained at between about 85% and about 95% and the temperature is maintained at between about 10°C and about 22°C.

13. A method according to Claim 5 wherein maturation of mycelial growth and attendant sclerotia subsequent to nutrient source removal is promoted by maintaining the moisture content of said substratum at between about 45% and about 70%, the relative humidity at between about 85% and about 95% and the temperature at between about 10°C and about 22°C.

14. A method according to Claim 5 wherein said substratum is hydrated by percolating water through said substratum.

15. A method according to Claim 14 wherein said water is percolated through said substratum at a rate of between about 250 and about 1000 ml per m^2 of substratum surface per hour.

16. A method according to Claim 14 wherein said water is percolated for a period of between about 12 and about 36 hours.

17. A method according to Claim 14 wherein said percolating water is maintained at a temperature of between about 10°C and about 22°C.

18. A method according to Claim 5 wherein subsequent to substratum hydration, the water content of said substratum is adjusted to between about 55% and about 65%, the relative humidity is maintained at between about 85% and about 95% and the temperature is maintained at between about 10°C and about 22°C until primordia appear.

19. A method according to Claim 18 wherein from the appearance of primordia until ascocarp

development to a height of about 30 mm, the water content of said substratum is maintained at between about 50% and about 60%, the relative humidity is maintained at between about 85% and about 95% and the temperature is maintained at between about 10°C and about 22°C.

20. A method according to Claim 19 wherein during said period from primordia appearance until ascocarp development to a height of about 30 mm, the air flow near said substratum is maintained at between about 20 and about 40 cm per minute.

21. A method according to Claim 19 wherein from a period from ascocarp development at a height of about 30 mm to ascocarp maturity, the water content of said substrate is maintained at between about 30% and about 55%, the relative humidity is maintained at between about 80% and about 95% and the temperature is maintained at between about 10° and about 27°C.

22. A method for culturing ascocarps of species of the genus Morchella comprising generating mature sclerotia with sufficient stored nutrients for ascocarp development, providing a nutrient-poor substratum and inoculating said sclerotia into said substratum, promoting growth of additional mycelia from said sclerotial inoculum, inducing said sclerotia and additional mycelia into the sexual reproductive cycle of the species, and maintaining conditions appropriate for development of ascocarps of the species.

23. A method according to Claim 22 where the sclerotia used as inoculum are hardened sclerotia and are wetted by immersion in water for about 18 to 24 hours immediately prior to inoculation.

24. A method according to Claim 22 wherein said sclerotia and additional mycelia are induced to the sexual reproductive cycle through the deprivation of exogenous nutrients and by exposure to high amounts of water.

25. A method according to Claim 24 wherein the nutrient-poor substratum into which said sclerotia is inoculated is wetted at about the time of inoculation, whereby deprivation of exogenous nutrients and exposure to high quantities of water is effected contemporaneously.

26. A method according to Claim 24 wherein for a period subsequent to inoculation, said sclerotia are maintained in said nutrient-poor substratum at a substratum moisture content of between about 45% and about 70%, a relative humidity of between about 85% and about 95% and a temperature of between about 10°C and about 22°C, and subsequently, said substratum is hydrated to expose said sclerotia and additional mycelia to high amounts of water.

27. A method according to Claim 26 wherein said substratum is hydrated by percolating water through said substratum at a rate of between about 250 and about 1000 ml per m^2 of substratum surface per hour.

28. A method according to Claim 27 wherein said water is percolated for a period of between about 12 and about 36 hours.

29. A method according to Claim 27 wherein said percolating water is maintained at a temperature of between about 10°C and about 22°C.

30. A method according to Claim 22 wherein between about 1500 and about 4000 cc of mature sclerotia are inoculated per m^2 of substratum surface.

31. A method according to Claim 22 wherein subsequent to induction, the water content of said substratum is adjusted to between about 55% and about 65%, the relative humidity is maintained at between about 85% and about 95% and the temperature is maintained at between about 10°C and about 22°C until primordia appear.

32. A method according to Claim 22 wherein from the appearance of primordia until ascocarp

development to a height of about 30mm, the water content of said substratum is maintained at between about 50% and about 60%, the relative humidity is maintained at between about 85% and about 95% and the temperature is maintained at between about 10°C and about 22°C.

33. A method according to Claim 22 wherein during said period from primordia appearance until ascocarp development to a height of about 30 mm, the air flow near said substratum is maintained at between about 20 and about 40 cm per minute.

34. A method according to Claim 22 wherein from a period from ascocarp development at a height of about 30 mm to ascocarp maturity the water content of said substratum is maintained at between about 30% and about 55%, the relative humidity is maintained at between about 80% and about 95% and the temperature is maintained at between about 10°C and about 27°C.

.NTERNATIONAL SEARCH REPORT

International Application No PCT/US86/00937

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): A01G 1/04
U.S. Cl. 47/1.1

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	47/1, 1.1, 58; 71/1, 3; 424/415

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	US,A, 3942,969 (CARROLL ET AL) 09 MARCH 1976 SEE ENTIRE DOCUMENT	1-34
A	US,A, 4,164,405 (PINCKARD) 14 AUGUST 1979 SEE ENTIRE DOCUMENT	1-34
A	US,A, 4,229,442 (PINCKARD) 21 OCTOBER 1980 SEE ENTIRE DOCUMENT	1-34
A	FR,B, 2,539,736 (PEBEYRE SA) 27 JULY 1984 SEE ENTIRE DOCUMENT	1-34
A	EP,B, 0,107,911 EVERBLOOM MUSHROOMS) 09 MAY 1984 SEE ENTIRE DOCUMENT	1-34
A	JA,B, 51-1254 (HOHNEN OIL KK) 07 JANUARY 1984 SEE ENTIRE DOCUMENT	1-34
A	JA,B, 53-112156 (KAO SOAP KK) 30 SEPTEMBER 1978	1-34
A	JA,B, 53-124678 (UNITIKA KK) 31 OCTOBER 1978 SEE ENTIRE DOCUMENT	1-34

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
28 MAY 1986	19 JUN 1986
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	JAMES R. FEYRER

International Application No. PCT/US86/00937

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
A	WO,B, 85/00002 (VALTION TEKNILLINEN TUTKIMUSKESKUS) 03 JANUARY 1985 SEE ENTIRE DOCUMENT	1-34
A	MGA BRIGHTON CONFERENCE - OCTOBER 1973, MUSHROOM GROWERS' ASSOCIATION, AGRICULTURE HOUSE, KNIGHTSBRIDGE, LONDON, SWIX 7NJ, "THE CHALLENGE TO Agricus bisporus FROM OTHER FUNGI" DRS. P.J. BELS SEE ENTIRE DOCUMENT	1-34
A	SAN ANTONIO, J.P. ET AL., "CULTIVATION OF THE PADDY STRAW MUSHROOM, Volvariella volvacea (BULL. ex Fr.) SING." HORTSCIENCE, VOL.7 NO. 5 OCTOBER 1972 PAGES 461-463 SEE ENTIRE DOCUMENT	1-34