

COMMONWEALTH OF AUSTRALIA

THE PATENTS ACT 1952

DECLARATION IN SUPPORT OF A
CONVENTION APPLICATION FOR A PATENTIn support of the Convention Application made for a
patent for an invention entitled:

Title of Invention

" CULTIVATION OF MORCHELLA "

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address(es) of
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do solemnly and sincerely declare as follows:-

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Applicant(s)~~1. I am/We are the applicant(s) for the patent~~*(or, in the case of an application by a body corporate)*

1. I am/We are authorised by
-
- NEOGEN CORPORATION

the applicant(s) for the patent to make this declaration on
its/their behalf.

2. The basic application(s) as defined by Section 141 of the
-
- Act was/were made

Basic Country(ies)

in the United States of America

Priority Date(s)

on April 29, 1985

Basic Applicant(s)

by RONALD DEAN OWER, GARY LYNN MILLS, and
JAMES ANTHONY MALACHOWSKIFull name(s) and
address(es) of
inventor(s)~~2. I am/We are the actual inventor(s) of the invention referred
to in the basic application(s)~~*(or where a person other than the inventor is the applicant)*

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which the applicant(s) is/are entitled to make the application are
as follows: a Deed of Assignment dated April 24, 1985 from RONALD DEAN OWER
to NEOGEN CORPORATION; and a Deed of Assignment dated April 15, 1985 from
GARY LYNN MILLS and JAMES ANTHONY MALACHOWSKI to NEOGEN CORPORATIONSet out how Applicant(s)
derive title from actual
inventor(s) e.g. The
Applicant(s) is/are the
assignee(s) of the
invention from the
inventor(s)

4. The basic application(s) referred to in paragraph 2 of this
-
- Declaration was/were the first application(s) made in a Convention
-
- country in respect of the invention(s) the subject of the application.

Declared at Lansing, MI, USA this 19th day of December 1986

NEOGEN CORPORATION

Brinton M. Miller, Ph.D.
Signature of Declarant(s)

To: The Commissioner of Patents

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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 established 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 in 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.

CLAIM

1. A method for culturing ascocarps of a species of the genus *Morchella* 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, inducing said mycelia and attendant sclerotia into the sexual growth cycle of the species, by depriving said mycelia and attendant sclerotia of substantially all exogenous nutrients and by

(11) AU-B-58671/86
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-2-

exposing said mycelia and attendant sclerotia to high concentrations of water, and maintaining conditions appropriate for development and maturation of ascocarps of the species with the proviso that the conditions are artificially manipulated by man.

8. A method according to Claim 6 or Claim 7, wherein said nutrient source is wheat berries supplied at between 1000 and 8000 gm dry weight per m^2 of substratum surface area.

12. A method according to Claim 11, wherein said water is percolated through said substratum at a rate of between 250 and 1000 ml per m^2 of substratum surface per hour.

13. A method according to Claim 11 or Claim 12, wherein said water is percolated for a period of between about 12 and about 36 hours.

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<p>(54) Title: CULTIVATION OF <i>MORCHELLA</i></p> <p>(57) Abstract</p> <p>Culturing ascocarps or fruitbodies of species of the genus <i>Morchella</i>. Mycelia are provided nutrient 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 established 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 in 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.</p>		

- 1 -

CULTIVATION OF MORCHELLA

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

5

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.

- 2 -

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.

Conidiophore - 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
5 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
10 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

15 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
20 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
25 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
30 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
35 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 maintenance 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.

According to a first embodiment of this invention there is provided a method for culturing ascocarps of a species of the genus Morchella 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, inducing said mycelia and attendant sclerotia into the sexual growth cycle of the species, by depriving said mycelia and attendant sclerotia of substantially all exogenous nutrients and by exposing said mycelia and attendant sclerotia to high concentrations of water, and maintaining conditions appropriate for development and maturation of ascocarps of the species with the proviso that the conditions are artificially manipulated by man.

According to a second embodiment of this invention there is provided a method of culturing ascocarps of a species of a genus Morchella comprising cultivating mycelia and attendant sclerotia, providing a nutrient-poor substratum and inoculating said nutrient-poor substratum with said mycelia and attendant sclerotia, promoting mycelial growth in said substratum from said inoculum, feeding said mycelia with a nutrient source which supplies 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 mycelia and attendant sclerotia, hydrating said substratum containing said mature mycelia and attendant sclerotia to expose said mycelia and attendant sclerotia to high concentrations of water, said deprivation of nutrients and said exposure to high concentrations of water contributing to induction of the mycelia and attendant sclerotia to the sexual cycle of the species, and maintaining conditions appropriate for development and maturation of ascocarps with the proviso that the conditions are



artificially manipulated by man.

According to a third embodiment of this invention there is provided a method for culturing ascocarps 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, by depriving said sclerotia and additional mycelia of substantially all exogenous nutrients and exposing said mycelia and attendant sclerotia to high concentrations of water, and maintaining conditions appropriate for development of ascocarps of the species with the proviso that the conditions are artificially manipulated by man.

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 "star 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

- 7 -

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
5 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
10 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
15 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
20 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
25 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
30 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
35 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
5 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
10 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,
15 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
20 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,
25 should provide buffering capacity, should have good
water-retaining capabilities, and should provide
adequate aeration to allow proper gaseous exchange. The
substratum that is now being used is about 25% sand and
about 75% organic material. A small portion of lime is
30 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
35 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

- 10 -

the vegetative growth after nutrient addition should contain, in stored form, substantially all of the nutrients that are needed for efficient fruitbody development.

5 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 500 g dry wt.) per square meter of substratum. This ratio could vary significantly and can only be determined only as a general approximation.

- 11 -

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

- 12 -

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. ^{Typically, water} ~~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 m² 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



- 13 -

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
5 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
10 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
15 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
20 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
25 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
30 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
35 with available apparatus.

Following hydration in either the first or second variation, the substratum is allowed to drain,

- 14 -

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

- 15 -

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
5 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
10 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
15 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
20 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
25 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
30 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.

The claims defining the invention are as follows:

1. A method for culturing ascocarps of a species of the genus Morchella 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, inducing said mycelia and attendant sclerotia into the sexual growth cycle of the species, by depriving said mycelia and attendant sclerotia of substantially all exogenous nutrients and by exposing said mycelia and attendant sclerotia to high concentrations of water, and maintaining conditions appropriate for development and maturation of ascocarps of the species with the proviso that the conditions are artificially manipulated by man.

2. A method of culturing ascocarps of a species of a genus Morchella comprising cultivating mycelia and attendant sclerotia, providing a nutrient-poor substratum and inoculating said nutrient-poor substratum with said mycelia and attendant sclerotia, promoting mycelial growth in said substratum from said inoculum, feeding said mycelia with a nutrient source which supplies 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 mycelia and attendant sclerotia, hydrating said substratum containing said mature mycelia and attendant sclerotia to expose said mycelia and attendant sclerotia to high concentrations of water, said deprivation of nutrients and said exposure to high concentrations of water contributing to induction of the mycelia and attendant sclerotia to the sexual cycle of the species, and maintaining conditions appropriate for development and maturation of ascocarps with the proviso that the conditions are artificially manipulated by man.

3. A method according to Claim 2, wherein said mycelia are cultivated by providing a moist vegetative nutrient source, sterilizing the same, inoculating said moist nutrient source with pieces selected from the group consisting of sclerotia, ascospores, vegetative hyphae, and conidia.

4. A method according to Claim 2 or Claim 3, wherein between about 6 and about 30cm³ of sclerotia are inoculated per m² of substratum surface.



5. A method according to any one of Claims 2 to 4, wherein mycelial growth in said inoculated substratum is promoted prior to feeding by maintaining the substratum moisture content at between 50% and 75%, the relative humidity at between 75% and 95% and the temperature at between 10°C and 22°C.

6. A method according to any one of Claims 2 to 5, wherein said nutrient source comprises a vegetative material.

7. A method according to Claim 6, 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.

8. A method according to Claim 6 or Claim 7, wherein said nutrient source is wheat berries supplied at between 1000 and 8000 gm dry weight per m² of substratum surface area.

9. A method according to Claim 2, wherein during feeding the moisture content of said substratum is between 45% and 70%, the relative humidity is maintained at between 85% and 95% and the temperature is maintained at between 10°C and 22°C.

10. A method according to Claim 2, 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 45% and 70%, the relative humidity at between 85% and 95% and the temperature at between 10°C and 22°C.

11. A method according to any one of Claims 2 to 10, wherein said substratum is hydrated by percolating water through said substratum.

12. A method according to Claim 11, wherein said water is percolated through said substratum at a rate of between 250 and 1000 ml per m² of substratum surface per hour.

13. A method according to Claim 11 or Claim 12, wherein said water is percolated for a period of between about 12 and about 36 hours.

14. A method according to any one of Claims 11 to 13, wherein said percolating water is maintained at a temperature of between 10°C and 22°C.

15. A method according to Claim 2, wherein subsequent to substratum hydration, the water content of said substratum is adjusted to between 55% and 65%, the relative humidity is maintained at between 85% and 95% and the temperature is maintained at between 10°C and 22°C until ascocarp primordia appear.



16. A method according to Claim 15, 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 50% and 60%, the relative humidity is maintained at between 85% and 95% and the temperature is maintained at between 10°C and 22°C.

17. A method according to Claim 16, wherein during said period from primordia appearance until ascocarp development to a height of about 30 mm, the air flow surrounding said substratum is maintained at between 20 and 40 cm per minute.

18. A method according to Claim 16, 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 30% and 55%, the relative humidity is maintained at between 80% and 95% and the temperature is maintained at between 10° and 27°C.

19. A method for culturing ascocarps 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, by depriving said sclerotia and additional mycelia of substantially all exogenous nutrients and exposing said mycelia and attendant sclerotia to high concentrations of water, and maintaining conditions appropriate for development of ascocarps of the species with the proviso that the conditions are artificially manipulated by man.

20. A method according to Claim 19, 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.

21. A method according to Claim 19 or Claim 20, wherein the nutrient-poor substratum into which said sclerotia is inoculated is wetted at about the time of inoculation, whereby deprivation of substantially all exogenous nutrients and exposure to high concentrations of water is effected contemporaneously.

22. A method according to any one of Claims 19 to 21, wherein for a period subsequent to inoculation, said sclerotia are maintained in said nutrient-poor substratum at a substratum moisture content of between 45% and 70%, a relative humidity of between 75% and 95% and a temperature of between 10°C and 22°C, and subsequently, said substratum is hydrated to



expose said sclerotia and additional mycelia to high concentrations of water.

23. A method according to Claim 22, wherein said substratum is hydrated by percolating water through said substratum at a rate of between 250 and 1000 ml per m² of substratum surface per hour.

24. A method according to Claim 23, wherein said water is percolated for a period of between 12 and 36 hours.

25. A method according to Claim 23 or Claim 24, wherein said percolating water is maintained at a temperature of between 10°C and 22°C.

26. A method according to any one of Claims 19 to 25, wherein between 1500 and 4000 cc of mature sclerotia are inoculated per m² of substratum surface.

27. A method according to Claim 19, wherein subsequent to induction, the water content of said substratum is adjusted to between 55% and 65%, the relative humidity is maintained at between 85% and 95% and the temperature is maintained at between 10°C and 22°C until primordia appear.

28. A method according to Claim 19, 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 50% and 60%, the relative humidity is maintained at between 85% and 95% and the temperature is maintained at between 10°C and 22°C.

29. 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 20 and 40 cm per minute.

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

DATED this EIGHTEENTH day of JULY 1989
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MTS 11581V

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US86/00937

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *		
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 *		
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 *		
III. DOCUMENTS CONSIDERED TO BE RELEVANT 14		
Category *	Citation of Document, 15 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
<p>* Special categories of cited documents: 14</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"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</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"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.</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 1	Date of Mailing of this International Search Report 1	
28 MAY 1986	19 JUN 1986	
International Searching Authority 1	Signature of Authorized Officer 19	
ISA/US	JAMES R. PEYNER	

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

Category *	Citation of Document, ^{1a} with indication, where appropriate, of the relevant passages ^{1b}	Relevant to Claim No. ^{1c}
A	WO, B, 85/00002 (VALTION TEKNIILLINEN 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 <u>Agaricus bisporus</u> 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, <u>Volvariella volvacea</u> (BULL. ex Fr.) SING." HORTSCIENCE, VOL. 7 NO. 5 OCTOBER 1972 PAGES 461-463 SEE ENTIRE DOCUMENT	1-34