RURAL INDUSTRIES RESEARCH
& DEVELOPMENT CORPORATION

Cropping the French Black Morel

A preliminary investigation

A report for the Rural Industries Research and Development Corporation

by S. Barnes and Ms. A. Wilson

May 1998

RIRDC Publication No 98/44
RIRDC Project No UT-12A

© 1998 Rural Industries Research and Development Corporation.
All rights reserved.

ISBN 0 642 54064 0
ISSN 1440-6845

Cropping the French Black Morel: A Preliminary Investigation
Publication No. 98/44
Project No. Ut-12A

The views expressed and the conclusions reached in this publication are those of the author and not necessarily those of persons consulted. RIRDC shall not be responsible in any way whatsoever to any person who relies in whole or in part on the contents of this report.

This publication is copyright. However, RIRDC encourages wide dissemination of its research, providing the Corporation is clearly acknowledged. For any other enquiries concerning reproduction, contact the Publications Manager on phone 02 6272 3186.

Researcher Contact Details
S. Barnes and A. Wilson
Tasmanian Institute of Agricultural Research
Department of Primary Industry & Fisheries,
Tasmania
New Town Laboratories
NEW TOWN TAS 7008
Telephone: (03) 6233 6864

In submitting this report, the researcher has agreed to RIRDC publishing this material in its edited form.

RIRDC Contact Details
Rural Industries Research and Development Corporation
Level 1, AMA House
42 Macquarie Street
BARTON ACT 2600
PO Box 4776
KINGSTON ACT 2604

Phone: 02 6272 4539
Fax: 02 6272 5877
Email: rirdc@rirdc.gov.au.
Website: http://www.rirdc.gov.au

Published in May 1998
Reprinted May 2003
Printed on environmentally friendly paper by Canprint

Foreword

Morels (*Morchella* species) are one of the most popular group of the wild edible fungi collected in Europe and North America.

Black fleshed fruit bodies are produced by *Morchella angusticeps*, *M. elata* and *M. conica*, while *M. esculenta*, *M. crassipes* and *M. deliciosa* produce yellow or white mushrooms. Black morels demand the greatest market price and *M. angusticeps* is the best known of these species.

Morels grow in some parts of Australia and in Tasmania. The fruiting season in Tasmania extends from October and, depending on the winter weather, until December.

Twelve years ago, a US patent was granted to Ower, Mills and Malachowski to cultivate the white morel (*Morchella esculenta*) under controlled conditions. The patent and following adapted patent are held by the multinational company Neogen. White morels are being commercially cultivated by this company.

The potential exists for developing a significant French black morel industry in Australia based on artificial production technologies.

This publication reviews different techniques for inducing morel fruiting with the aim of identifying factors critical to fruit body initiation and describes experiments to investigate the growth response of both American and Tasmanian morel isolates to a number of different substrates and environmental conditions.

The report is part of RIRDC's New Plant Products program, which aims to facilitate the development of new industries based on plants or plant products with commercial potential for Australia.

Peter Core
Managing Director
Rural Industries Research and Development Corporation

Table of Contents

Executive Summary

Mushroom connoisseurs consider morels (*Morchella* species) among the finest edible fungi, and fresh specimens are much sought after when they come into season in spring in Europe and North America. Black fleshed fruit bodies are produced by *M. angusticeps*, *M. elata* and *M. conica*, while *M. esculenta*, *M. crassipes* and *M. deliciosa* produce yellow or white mushrooms. Black morels demand the greatest market price and *M. angusticeps* is the best known of these species. The potential exists for developing a significant French black morel industry based on artificial production technologies.

Five strains of *M. angusticeps* and two strains of *M. esculenta* were imported from the USA to begin pilot studies in the induction of fruiting. However the eventual use of imported isolates in commercial production could raise problems over either property rights and/or quarantine issues (ie. the possible release into nature of a morel isolate or species not present in Australia). It would be preferable to source Tasmanian isolates for a Tasmanian morel industry. Morels have been introduced into Australia, although the taxonomic identity of introduced species is unclear. In Tasmania fruitbodies were collected in Hobart area, Southern Tasmania. Ecological aspects, e.g. the role of morel mycelium and conditions triggering fruiting, is not yet clear. In order to highlight any consistent pattern to fruiting, full descriptions of the different sites have been collated and exact times of fruit body production noted. Fruit bodies collected have not been formally identified but their morphology suggested *M. elata*, a black morel.

Isolations obtained from both mushroom stipe and pileus tissue cultures were often contaminated. It was necessary to develop a technique to isolate single spores cultures from fresh mushrooms collected in the field. The asci of the morels released their ascospores in sterile, covered petri-dishes. If the air is too dry or the morel immature the asci will not release the ascospores. The spores were observed and counted using a haemocytometer and Wild M20 microscope. The ascospores were then diluted to a known concentration with sterile water and pipetted onto filtered water agar. The spores grew within 10-24 hours and were cultured onto a yeast malt extract agar. The rapid growth of the fuzzy rust colour mycelia resulted in microsclerotia forming on the agar plates.

A review of literature was carried out, focusing on any existing techniques for inducing the fruiting of different morel species. Twelve years ago (1986), US patent no. 4594809 was

granted to Ower, Mills and Malachowski for the cultivation of the white morel (*M. esculenta*) under controlled conditions. The patent and following adapted patent (Ower *et al*. 1988; Patent No. 4757640) are held by the multinational company Morel Mountain, based in Mason, Michigan. White morels are currently being commercially cultivated by this company. Limited success has been achieved by others in obtaining fruiting under controlled conditions.

Why are morels so difficult to fruit under controlled conditions? The answers to these questions lie in an understanding of the life cycle of the morel. A stage of the morel life cycle not present in the other cultivated mushrooms is sclerotium formation. The sclerotium of the morel is a relatively large structure (1mm -5 cm diameter) composed of aggregated mycelium - large cells with very thick walls that allow the fungus to survive adverse natural conditions, such as winter. In the spring, the sclerotium has two options for germination; to form a new mycelium or to form a fruiting body. It is very easy to get the sclerotia to form a new mycelium but very difficult to force it to form a fruiting body. Very specific conditions of nutrition, humidity, carbon dioxide levels and temperature must be met for fruiting initials or primordia to form and for these to develop into fully mature fruit bodies.

The patents themselves describe a process for formation of sclerotia that are competent to produce fruiting bodies.

The first experiment in this project involved replicating the environmental conditions stipulated in the patent using an American strain of *M. angusticeps*. Basically morel mycelium is inoculated on a fairly nutrient-poor substrate (soil). It is allowed to use its limited nutrient reserves to reach a nearby nutrient-rich substrate. The nutrients are then translocated back into the old mycelia where the sclerotia are formed as they begin to store the nutrients as lipids. These sclerotia formed can often be quite large. When the sclerotia are mature, the nutrient source is removed, and the sclerotia chilled. After a couple of weeks water is "percolated" between the sclerotia in the soil, perhaps simulating spring rains. After 10-12 days, small primordia should appear, and, if the conditions are correct, the morels mature in 12-15 days. In this first experiment seven different locally available timbers were shredded and each tested as the hardwood chip component in the soil substrate.

Sclerotia were successfully obtained but no fruiting primordia were observed when these sclerotia were subjected to chilling and flooding. The reason for the lack of fruit body initiation may have inadequate drainage of the substrate. Three different hardwoods (eucalypt, oak, elm) were chosen as those stimulating better growth and formation of sclerotia when incorporated into the soil substrate.

In the second experiment local strains of morels were used as well as the American strains. The soil substrate recipe was modified and charcoal was added to the recipe to simulate burnt conditions experienced in nature. The second experiment was also given a longer chilling phase and more drainage holes were added because in the first experiment drainage was not adequate.

The growth of mycelium of the local strains was more prolific than that of the American strain. Sclerotia were formed but once again these sclerotia failed to give rise to fruiting primordia.

However primordia were formed from sclerotia in two petri dishes containing potato dextrose agar in which local strains of morel (DPI96005) had been cultured at approximately 4°C for eleven months. It is possible that certain strains of this fungus will fruit more readily. This isolate has been retained for further experimentation.

The project (although short in duration, Aug. 1996 - Aug. 1997) achieved significant progress:

- gaining an increased background knowledge about *Morchella* species from existing literature
- collecting both foreign and Tasmanian isolates of *Morchella*
- conducting two pilot studies on the artificial production of morels which were successful in producing sclerotia although did not proceed to form fruiting primordia

INTRODUCTION

The fungal genus *Morchella* is widely distributed throughout the world and comprises edible species well known for their adaptability in growing under various ecophysiological conditions, either in association with plant hosts or as independent saprotrophs.

The morel (5-13cm tall) and resembles a natural sponge on a stalk (Fig. 1). The hollow, dome-shaped cap is various colours, depending on the species. The taxonomy of *Morchella* species is confusing and subjective. The much prized black "French" morel is *Morchella angusticeps*. The morel taxa, which include, white, yellow and black forms, are numerous. The black morels are a naturally definable cluster, including *Morchella angusticeps*, *M. conica*, and *M. elata*. The yellow or white morels include *M. esculenta*, *M. deliciosa*, and *M. crassipes*.

Buscot (1989) suggests that the ecological status of morel fruiting remains unresolved for primarily two reasons. The first is that there may be many described species, varieties, ecotypes that require different conditions for fruiting. The second reason is that the mushrooms (ascoma) are produced under two different ecological conditions.

The first condition is the occurrence of morels as pioneers of recently disturbed sites with the production of ascoma rapidly declining after the disturbance. Under the second condition, in stabilised forest soils with high competition for nutrients, the morel mycelium contracts complex (pseudomycorrhizal) associations with tree roots (Buscot 1992). Buscot and Roux (1987) observed that voluminous, subterranean morel sclerotia ensheathing sectors of mature tree roots were connected with the ascomata.

The reliance on collecting the wild morel and consequent seasonal variation in quantity and quality limits the development of any industry based on the supply of this product. There are valuable export markets in Europe and North America.

The total mushroom market has been estimated (Agtrans Research) 37,000 tonnes/year by 2003. If a maximum of 10% of the total market comprises specialty mushrooms this gives their total production of about 4000 tonnes/year. If morels constituted 5% of the specialty mushroom production (200 tonnes/year) at current Australian prices (A$40-A$100/kg) this product would be worth A$8-20M /year.

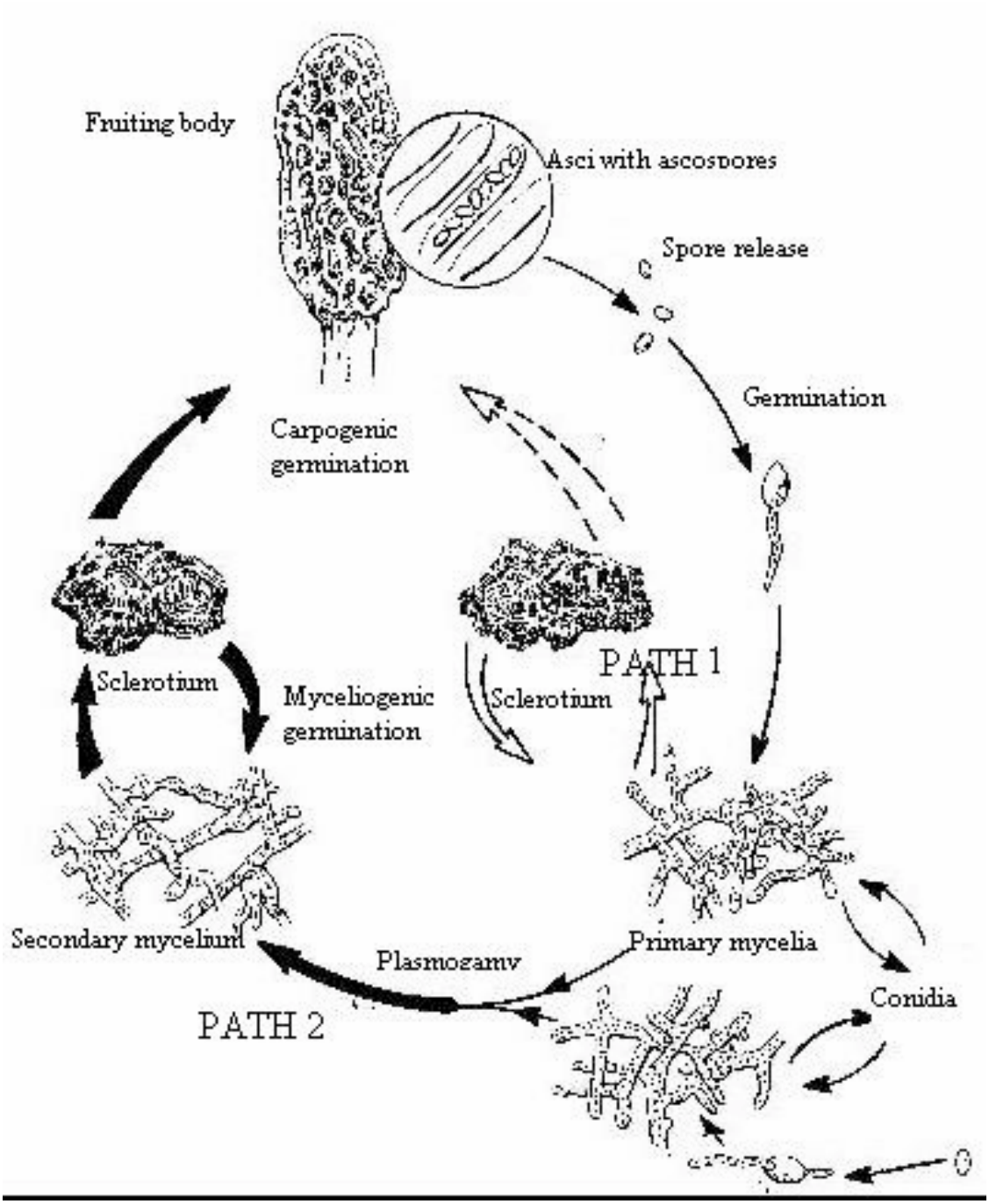
There are scientific reports on success with growing morels from as early as 1883 (using substrates consisting of Jerusalem artichokes, apples, pumpkin and various other vegetable matter). Recent attempts to control fruiting both outdoors and indoors have only met with sporadic and limited success. In 1982 Ronald Ower of San Francisco succeeded in regularly cultivating morels. At this point the Neogen company of East Lansing, Michigan became interested in the process. Neogen is affiliated with Michigan State University and at that time was already the holder of a number of biotechnology patents. Neogen was able to convince Ower to come to East Lansing to develop a commercial process for growing morels. In April of 1986, U.S. Patent no. 4,594,809 was issued. Ron Ower did not live to see the patent granted since he had been murdered a few weeks before in San Francisco. The patent and following adapted patent (Ower et al., 1988; Patent No. 4757640) are held by Neogen, a multinational company. White morels are currently being commercially cultivated by Morel Mountain, based in Mason, Michigan.

The patents themselves describe a process for formation of sclerotia (see Fig. 1) that are competent to produce fruiting bodies. The sclerotium of the morel is a relatively large structure (1mm -5 cm diameter) composed of aggregated mycelium - large cells with very thick walls that allow the fungus to survive adverse natural conditions, such as winter. The stages of sclerotium development can be distinguished: initiation (ie. the aggregation of hyphae to form a small sclerotial primordium); increase in size (ie.) branching, growth and compaction of hyphae so that the sclerotium attains its maximum size); and maturation (ie. final differentiation of the sclerotium involving the formation, in most instances, of a rind changes in the composition and distribution of materials within the sclerotial hyphae and its isolation from the parent colony and its surroundings).

In the spring, the sclerotium has two options for germination; to form a new mycelium or to form a fruiting body. It is very easy to get the sclerotia to form a new mycelium but very difficult to force it to form a fruiting body. Very specific conditions of nutrition, humidity, carbon dioxide levels and temperature must be met for fruiting initials or primordia to form and for these to develop into fully mature fruit bodies. Morel sclerotia do not normally form until the nutrients of a substrate have almost run out. One technique to forming large sclerotia is to inoculate morel mycelium on a nutrient-poor substrate (such as soil) and allow it to use its limited nutrient reserves to reach a nearby nutrient-rich substrate. The nutrients are then translocated back into the old mycelia where the sclerotia are formed as they begin to store the nutrients as lipids. These sclerotia can often be quite large and have very much the same

consistency and slippery feel as walnuts. When the sclerotia are mature, the nutrient source is removed, and water is "percolated" between the sclerotia in the soil, perhaps simulating spring rains. After 10-12 days, small primordia appear, and, if the conditions are correct, the morels mature in 12-15 days. On the surface it seems like a very simple process, but as with all mushrooms, there are many points at which the grower can make mistakes and lose the entire crop.

Figure 1. Life cycle of the morel



OBJECTIVES

To investigate the potential for development of a French black morel industry in Australia based on artificial production technologies:

- reviewing world literature
- collecting local and overseas isolates of *Morchella*
- documenting and describing local sites with *Morchella* fruit bodies
- familiarisation with growing *Morchella* isolates in culture and conducting pilot studies to induce sclerotia and fruiting in culture of North American and local isolates.

METHODOLOGY

1. **Review of literature**. Standard searches were made of databases
2. **Collection of isolates**

Isolates were imported from North America and France (AQIS permits were obtained).

Tasmanian isolates were obtained from fruit bodies collected from sites in the Hobart area. Fruit bodies were photographed.

Isolations from the stipe and pileus tissue were often contaminated. More success was obtained with isolations from single spores (by collecting fruit bodies (ascoma) of fresh morels and inducing their spore containing structures (asci) to release spores under moist conditions in sterile, covered petri-dishes. If the air is too dry or the morel immature the asci will not release the ascospores. The spores were observed and counted using a haemocytometer and Wild M20 microscope. Once the number of ascospores were known they were diluted with sterile water and pipetted onto filtered water agar. The spores grew within 10-24 hours and were cultured onto a yeast malt extract agar. The rapid growth of the fuzzy rust colour mycelia resulted in microsclerotia forming on the agar plates.

Antibiotics in the media inhibited the growth of the *Morchella* mycelium. It was omitted and the morel mycelia grew rapidly on the agar.

3. Familiarisation with growing *Morchella* isolates in culture: conducting pilot studies to induce sclerotia and fruiting in culture of North American and local isolates

A. Using American strain (M18) of *Morchella angusticeps* and protocol described by the American patent (Ower et al., 1988; Patent No. 4757640). Seven different timbers were trialed as the hardwood component in the soil substrate.

Step 1 - The spawn media is produced in 2000ml flasks containing rye grass seed and a potting soil (sand and peat).

- The seed was covered and soaked with water for twenty-four hours and mixed with one part soil to five parts grain (1litre rye grass seeds : 200ml soil)
- The flasks were fitted with cloth and cottonwool bungs and sterilised in a Smith DSL Steriliser model CSE/ME for one hour at 15 psi.
- Discs of agar cultured with mycelium were aseptically transferred into the cool spawn flasks, using a laminar flow hood and stainless steel work area, wiped down with 95% ethanol, between each inoculation. The flasks' neck and the scalpel were flamed between each inoculation.
- The mycelium was thoroughly mixed in the flasks by shaking for a few days.
- The flasks were placed in a cool, (20 - 21.7 °C) dark place for approximately 4 - 6 weeks.

Step 2 - Preparation and inoculation of the soil substrate used for the development of sclerotia and fruit bodies

- This mixture consisted of 20% sand, 30% potting soil, 50% organic material (of which 80% was small hardwood chips) 10% rice hulls, 5% soybean, 5% sphagnum, lime. Seven different hardwoods were trialed: lime, beech, ash, elm, oak, maple and eucalyptus with 4 replicates of each hardwood.
- Hardwood chips were covered with water and soaked overnight, then drained. The sphagnum was also soaked and drained well before mixing in the other ingredients. All the ingredients were mixed and the pH adjusted to 7.1 - 7.3 with the lime.
- Autoclavable aluminum trays (20.0 x 13.5 x 4.8 cm) were filled with the substrate (leaving 1 cm at the top). The bottom of the tray was punched with drainage holes.

- The substrate was saturated thoroughly with water and allowed to drain completely. A second identical tray was filled to a depth of 1 - 2.5 cm with rye grass seed which was soaked for twenty-four hours. The soil substrate tray was placed on top of the rye grass tray, so that it's bottom rested on the rye seed.
- Each set of two trays was placed inside an autoclave bag fitted with a breather patch and sterilised for one hour at 15 psi.
- 125g of the spawn was inoculated into each of the cooled top trays. The autoclave bags were heat sealed. The inoculated substrate was put in a dark room at 18 - 21 °C for 4 - 6 weeks to form sclerotia. During this time (the spawn run) the relative humidity was kept at 90 - 100 %, CO_2 at 6000-9000 ppm and there were no fresh air exchanges. If pest molds were seen at this stage of the experiment the infected trays were eliminated to prevent the spread into other clean trays.

Step 3 - Chilling of sclerotia

- The rye seed tray was removed from the bag which was reclosed. The soil substrate tray left in the bag was put into refrigeration at 3 - 4 °C for two weeks.

Step 4 - Flooding of sclerotia and soil substrate

- The bagged tray was removed from the coolroom and placed in a fruiting chamber or room. the substrate was slowly saturated with sterile water (18 - 21 °C) for 12- 16 hours and then drained completely for about 24 hours.

Step 5 - Optional casing layer

- In the procedure described by the patented recipe the casing layer is optional. The material used in this experiment was the casing material used commercially for Agaric species although not treated with any fungicide which may have adversely affected the growth of the *Morchella* species. It was layered to a depth of 2 - 3 cm.

Step 6 - Induction of primordia and fruit body maturation from sclerotia

- After laying the casing the trays were left for 7-10 days in the fruiting room in the dark for the mycelium to run through. Average air temperature was 16.5 °C, with a minimum of 15.4 °C and maximum of 17.3 °C (prescribed temperatures are higher;

18 - 21 °C). Relative humidity in the fruiting room was kept at 85 - 95 % RH by a humidifier, Defensor AG 505 and automatic sensor set at 85 - 90 % RH. Filtered fresh air exchanges occurred every 1-2 hour with a CO_2 level of 6000-9000 ppm.

- Primordia were supposed to form in three to seven days but the sclerotia in our experiment gave rise to new mycelium not primordia. However if primordia are formed then the substrate moisture is kept at 60 %, relative humidity increased to between 95-100%, air temperature raised slightly to 21 - 22 °C. A light cycle of 12 hours on/ 12 off is established and CO_2 kept at less that 900 ppm with filtered fresh air exchanges every 6-8 hours. During fruit body maturation conditions are altered slightly; temperature is raised slightly to 23 - 25 °C, relative humidity decreased to between 85 - 95 % and substrate moisture kept at 50%.

B. Using American strains (M18 and WC198) of *Morchella angusticeps* and local Tasmanian strain (DPI 96035). The protocol as described above was employed with certain modifications to the soil substrate and environmental stimuli applied.

- Modifications to substrate: Only three timbers were trialed based on results with experiment described above: eucalyptus, oak and elm. The soya bean flour was replaced by a coarser meal (50% flour and 50% grits). Charcoal (to simulate the production of fruit bodies in a fire burnt area) was added. Experimental design was 3 timbers x 2 isolates x 2 charcoal levels (presence or absence). Each treatment had 5 replicates.

- Modifications to environmental stimuli: More drainage holes were punctured in substrate trays to allow for better drainage. The inoculated trays were kept longer in the stage after inoculation during which sclerotia are formed (for 6 - 8 weeks instead of 4 - 6 weeks). The chilling stage was more harsh with one week at 4 °C, then 4 days at 1-2. °C and finally 3 days at 4.0°C.

DETAILED RESULTS

1. Review of literature

Relevant references selected are given in bibliographic section

2. Collection of isolates

Five strains of *Morchella angusticeps* and two strains of *M.esculenta* have been imported from the from USA. French strains were also imported. These isolates are held at the DPIF laboratories in Newtown as well as the precise locations of the morel sites in Tasmania. Detailed information about isolates and morel sites is deemed confidential. Fruitbodies collected were tentatively identified as belonging to *M. elata*.

Description of local morel sites

From August until November 1996 morels were collected from field sites on Mount Wellington. Seven sites were located and soil analysis carried out for five of the sites, because prolific fruitbodies were present at these sites:

Site 1: Morels fruiting: 03/10/96

Morel growing on leaf litter and under rocks

Vegetation: dogwoods, eucalyptus and sassafras.

Temperature	10°C
Humidity	75%
Light	10 lux
pH	6.0
Carbon	11%
Nitrogen	0.49%
Exch Ca	23 ME/100g
Phosphorus	<5.0ppm

Site 2: Morels fruiting: 20/10/96

Morel growing in an old campfire site

Vegetation: eucalyptus, dogwoods and grass

Temperature	12°C
Humidity	78%
Light	250 lux

Site 3: Morels fruiting: 20/10/96

Morels growing under fallen burnt logs, besides rocks and in leaf litter

Vegetation: dogwoods, eucalyptus and ferns

The area was mossy and dark

Temperature	10°C
Humidity	80%
Light	74 lux
pH	5.6
Carbon	9.6%
Nitrogen	0.5%
Exch Ca	13.3 ME/100g
Phosphorus	<5.0 ppm

Site 4: Morels fruiting: 22/10/96

Morels growing along riverbank and some under the riverbank in loose gravely soil.

Vegetation: ferns, moss and dogwoods very close to the river.

Temperature	9°C
Humidity	84%
Light	25 lux
pH	6.0
Carbon	2.2%
Nitrogen	0.11%
Exch Ca	7.8ME/100g
Phosphorus	<5.00 ppm

Site 5: Morels fruiting: 28/10/96

Morels growing in leaf litter and soil near rocks.

Vegetation: dogwoods and eucalyptus in an excavated area

Temperature	12°C
Humidity	75%
Light	100 lux
pH	5.4
Carbon	9.6%
Nitrogen	0.5%
Exch Ca	10.9ME/100g
Phosphorus	5.0ppm

Site 6: Morels fruiting: 11/11/96

Morels growing in moss and soil on rocks on a waterfall (not flowing)

Vegetation: wattles, eucalyptus and dogwoods

Temperature	13°C
Humidity	80%
Light	30
pH	5.9
Carbon	2.9%
Nitrogen	0.15%
Exch Ca	7.6ME/100g
Phosphorus	10ppm

Site 7: Morels fruiting: 25/11/96

Morels growing by the side of walking track in soil and rock

Vegetation: eucalyptus, manferns, sassafras and dogwoods

Temperature	11°C
Humidity	78%
Light	45 lux

3. Familiarisation with growing *Morchella* isolates in culture: conducting pilot studies to induce sclerotia and fruiting in culture of North American and local isolates

In the first experiment using the patented method and an American isolate mycelial growth was satisfactory and sclerotia formed in the incubation stage. However the sclerotia did not form into fruitbodies after being placed in the fruiting room and flooding with water.

In the second experiment (local strains of morels were used as well as the American strains) the mycelial growth of the local strains was greater than that of American strains. Although there had been modifications to both the media and environmental

stimuli applied, the sclerotia formed in the incubation stage did not form into fruitbodies after being placed in the fruiting room.

However in two petri dishes containing potato dextrose agar and local strain of morel (DPI96005) primordia formed from sclerotia. The PDA agar containing the morel culture had been in a refrigerator at approximately 4°C for eleven months.

DISCUSSION OF RESULTS

Knowledge was gained about morel cultivation. Isolates were collected and local fruiting sites described. This type of preliminary groundwork is necessary to the success of any new venture.

The two experiments have shown that it is relatively easy to produce sclerotia. However it is far more problematic NOT to induce myceliogenic germination of sclerotia but to induce carpogenic germination (to produce a mycelium that forms a primordium). It would seem very specific conditions must be met with to produce carpogenic germination. Perhaps the required environmental conditions for the local strains of morel should more closely mimic the spring conditions where they are found or an inhibitor of the sclerotia needs to be removed before primordium appear.

However this study did serve as a preliminary familiarisation with morel culture. It also provided a strain of morel from Tasmania which did produce primordia in pure culture and this isolate must be further investigated. The capacity to produce primordia and fruit bodies in culture may be a characteristic of a few particular strains and not completely dependent on cultural conditions.

IMPLICATIONS

Preliminary studies have shown the presence of local morel species in a small area of southern Tasmania. The production of morel fruiting bodies from local morel germplasm is possible in the future. DNA identification needs to be completed on the local species and will be done with the University of Tasmania.

The gourmet status of this mushroom and the Australian reputation for clean and green produce would attract international markets for local morel production.

Vital clues have been established for areas of further research. The most important is that sclerotia could be actually harvested after the induction stage and subjected to various treatments, then reseeded back to a substrate. The process is recommended for future research into the commercial production of morels.

RECOMMENDATIONS

1. DNA identification of local morel species.
2. More research into the production of large morel sclerotia.
3. Harvesting of sclerotia and subsequent investigation and manipulation of sclerotia so that primordia are produced from such sclerotia seeded onto a suitable substrate.

INTELLECTUAL PROPERTY

1. Local morel isolates
2. Location of Tasmanian morel fruiting sites

BIBLIOGRAPHY/REFERENCES

Amir, R., Levanon, D., Hadar, Y., Chet, I. 1993. Morphology and physiology of *Morchella esculenta* during sclerotial formation. Mycol. Res. 97: 683-689.

Brock, T. D. (1951) Studies on the nutrition of *Morchella esculenta* Fries. Mycologia, 43, 402-22.

Bunyard, B. A., Nicholson, M. S., Royse, D.J. 1994. A systematic assessment of *Morchella* using RFLP analysis of the 28S ribosomal RNA gene. Mycologia, 86: 762-772.

Buscot, F. 1989. Field observations on the growth and development of *Morchella rotunda* and *Mitrophora semmilibera* in relation to forest soil temperature. Can. J. Bot. 67: 589-593.

Buscot, F. 1992. Mycorrhizal succession and morel biology. In Myccorrhizas in ecosystems. Edited by D.H. Lewis, A. H. Fitter and I.J. Alexander. C.A.B. International, Wallingford, pp. 220-224.

Buscot, F. 1993. Mycelial differentiation of *Morchella esculenta* in pure culture. Mycol. Res. 97: pg. 136-140.

Buscot, F., and Bernillon, J. 1991. Mycosporins and related compounds in field and cultured mycelial structures of *Morchella esculenta*. Mycol. Res. 95: 752-754.

Buscot, F., and Kottke, I. 1990. The association of *Morchella rotunda* (Pers.) Boudier with roots of *Picea abies* (L.) Karst. New Phytol. 116: 425-430.

Buscot, F., and Roux. J. 1987. Association between living roots and ascocarps of *Morchella rotunda* (Pers.) Boudier. Trans. Br. Mycol. Soc. 89: 249-252.

Carpenter, S. E., Trappe, J. M., Hunt, G. A. 1981. Observations on fungal succession on recent volcanic deposits of Mount St. Helens. In Procceedings, 39th Meeting of the Oregon Academy of Sciences. Edited by J.F. Evett. Oregon Academy of Sciences, Corvallis, Oreg. pp. 36-44.

Deffieux, G., Baute, M. A. 1987. 1,5-D anhydrofructose, the precursor of the pyrone microthecin in *Morchella vulgaris*. Phytochemistry, 26: 1391-1393.

Dennis, R. W. G. 1978 British Ascomycetes. J. Cramer, Vaduz, Lichtenstien.

Gessner, R. V., Romano, M. A., Schultz, R. W. 1987. Allelic variation and segregation in *Morchella deliciosa* and *M. esculenta*. Mycologia , 79: 683-687.

Hervey, A., Bistis, G., Leong, I., 1978. Cultural studies of single ascospore isolates of *Morchella esculenta*. Mycologia, 70: 1268-1275.

Jung, S. W., Gessner, R. V., Keudell, K. C., Romano, M. A., 1993. Systematics of *Morchella esculenta* complex using enzyme-linked immunosorbent assay. Mycologia, 85: 677-684.

Kaul, T. N. 1977. Physiological studies on *Morchella* spp. II. Nitrogen utilization. Mushroom J. 58:2-6.

Leonard, T. J., Volk, T. J. 1992 . Production of specialty mushrooms in North America: shiitake and morels. In Frontiers in industrial mycology. Edited by G.F. Leatham, Chapman and Hall, New York. pp, 1-23.

Levanon, A. R., Hadar, F. Y., Chet, I. 1992. Formation of sclerotia by *Morchella esculenta* : relationship between media composition and turgor potential in the mycelium. Mycologia, 96: 943-948.

Levanon, A. R., Hadar, D. Y., Chet, I. 1993. Morphology and physiology of *Morchella esculenta* during sclerotial formation. Mycologia, 97: 683-689.

Miller, S. L., Torres, P., McClean, T. M. 1994. Persistence of basidiospores and sclerotia of ectomycorrhizal fungi and *Morchella* in soil. Mycologia, 86: 89-95.

Ower, R., 1982. Notes on the development of the morel ascocarp. Mycologia 74: 142-144.

Ower, R., Mills G., Malachowski J. 1986. Cultivation of *Morchella*. U.S. Patent No. 4,594,809.

Ower, R., Mills G., Malachowski J. 1988. Cultivation of *Morchella*. U.S. Patent No. 4,757,640.

Robbins, W. J., Hervey, A. 1959. Wood extract and growth of *Morchella*. Mycologia, 57: 262-275.

Schmidt, E. L. 1983 Spore germination of and carbohydrate colonization by *Morchella esculenta* at different soil temperatures. Mycologia, 75 : 870 -875.

Volk, T. J., Leonard, T. J. 1989a Experimental studies on the morel. I. Heterokaryon formation between monoascosporous strains of *Morchella*. Mycologia. 81:523-531.

Volk, T. J., Leonard T. J. 1989b. Physiological and environmental studies of sclerotium formation and maturation in *Morchella*. Appl. Env. Microbiol. 55: 3095-3100.

Volk, T. J., Leonard, T.J. 1990. Cytology of the life-cycle of *Morchella*. Mycol. Res. 94: 399-406.

Yoon, C. S., Gessner, R. V., Romano, M. A. 1990. Population genetics and systematics of the *Morchella esculenta* complex. Mycologia, 82: 227-235.