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Occurrence and Initial Observations of *Morchella* in Australia

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ABSTRACT: *Morchella* species grow in the wild in Australia, but no research has previously been undertaken into their biology or cultivation. Isolates of *Morchella* were collected from two sites in Victoria and one in New South Wales and axenic cultures obtained. Soil samples collected from underneath ascocarps at each site were chemically analysed. Mycelial growth of the isolates was measured as a function of temperature. Formation of sclerotia was studied *in vitro* on various solid media, split-plates, and over a range of temperatures.

1 INTRODUCTION

The genus *Morchella* contains the species of edible fungi commonly known as morels and is widely distributed throughout the world. The only previous study on morels in Australia is limited to a description of species (Rifai 1968). Physiology associated with vegetative growth on both solid and liquid media has received attention in the past (Brock 1951, Robbins and Hervey 1965, Kaul 1977a, 1977b). The importance of sclerotia in the life cycle of *Morchella* has been demonstrated by Ower (1982) and Ower *et al.* (1986). They found that sclerotia can be cultured and used to form ascocarps under controlled conditions. Volk and Leonard (1989) used the jar method (Ower *et al.* 1986) to investigate the effects of substrates, growth container size, and light on the formation of sclerotia by *Morchella crassipes*.

Sclerotial formation and development were further described by Volk and Leonard (1990) and Amir *et al.* (1992, 1993) with contrasting results as to whether the sclerotia produced are of the terminal or lateral type. Amir *et al.* (1992, 1993) used a split-plate method to investigate the

morphology and physiology of Morchella during sclerotial formation, as an alternative to the jar method (Ower *et al.* 1986), and described six major stages in growth up to sclerotial maturation. Philippoussis and Balis (1995) recently supported the findings of Amir *et al.* (1993) with their reports that sclerotia had a lateral pattern of induction.

The present work is aimed at increasing the knowledge of the biology of *Morchella* isolated from Australia. In addition, the environmental and nutritional conditions that promote mycelial growth and sclerotial formation by these isolates were investigated on solid media and by the split-plate method.

2 MATERIALS AND METHODS

2.1 *Ascocarp collection and tissue isolation*

Morchella ascocarps were collected from two sites in Victoria (Grampians and Mount Pilot) and one site in New South Wales (Cowra) from late September to early October during 1994 and 1995. Ascocarps were collected along with adjacent soil to the depth of 15 cm, and the site vegetation was recorded. The attached soil maintained the ascocarps in good condition for tissue isolations and spore prints. To obtain tissue cultures, ascocarps were cut longitudinally and tissues (2 to 3 mm) were aseptically removed from the inner pileus and placed on malt extract agar (MEA) (Gibco). After tissue isolation the ascocarps were placed in Petri dishes to obtain spore prints. The lids of the Petri dishes were closed tightly to prevent the loss of moisture.

2.2 *Chemical analysis of soil samples*

Soil collected from under ascocarps was chemically analysed at New South Wales Agriculture. The soil samples were air-dried, ground and sieved through a 2 mm mesh. The pH and electrical conductivity (EC) of the samples were determined in a 1:5 w/v soil suspension in 0.01M $CaCl_2$ at 25°C. Extractable phosphate was measured by the Bray No.1 method (Bray and Kurtz 1945). Total carbon and total nitrogen were determined by the Leco combustion method. Exchangeable cations (Al, Mg, Ca, K, and Na) were measured by leaching the soil with a 0.0025N $BaCl_2$ solution and concentrations of the five cations in the resulting leachate were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES).

2.3 *Mycelial growth on MEA at different temperatures*

Three isolates from each site were selected for use in the growth trials and sclerotial experiments: Isolates G1-G3 from the Grampians site; C1-C3 from the Cowra site; and M1-M3 from the site at Mount Pilot. MEA was chosen as the media for mycelial growth trials after preliminary experiments involving several agar media. Mycelium was grown in 9 cm Petri dishes containing 25 ml MEA. Inoculation was performed by placing a 7 mm diameter plug, cut from the margins of colonies grown for five days on MEA, in the centre of each plate. The inoculated plates were incubated for 5 days at 5, 10, 15, 20, 25, and 30°C in the dark. Growth was evaluated by measuring two perpendicular diameters of the colony.

2.4 *Sclerotial formation on different solid media and on split-plates*

The following culture media were tested for the production of sclerotia in Petri dishes: MEA (pH 5.4) (Gibco); complete medium + yeast extract (CYM) (pH 6.8) (Leonard and Dick 1973); carnation leaf agar (CLA) (pH 5.5); soil agar (SA) (pH 6); and compost agar (CA) (pH 7.4). CLA was prepared by adding three 1 cm pieces of washed, sterile carnation leaf to Petri dishes containing water agar (WA). SA consisted of 50 g of soil, collected from underneath *Morchella* ascocarps, added to one litre of distilled water. This mixture was stirred by a magnetic stirrer for 90 minutes, sieved using a fine nylon mesh, agar added at 12 g per litre and the medium autoclaved. CA was prepared using dried and milled (1 mm) mushroom compost dispersed in distilled water at 75 g per litre and heated to 100°C. Agar was added at 12 g per litre and 300 ml aliquots of the medium were autoclaved for 30 minutes. Inoculation's were made as in the mycelial growth experiment, except the inocula were taken from WA. The inoculated plates were incubated at 25°C, except CLA cultures incubated at 5, 10, 15, 20, and 30°C.

In split-plate cultures one half of the divided Petri dish was filled with CLA and the other half with MEA. CLA served as the poor medium and MEA as the nutritional medium to investigate mycelium growth and sclerotia production. The effect of inoculation on the CLA side was compared with inoculation on the MEA side of the split plate. Plates were inoculated with a 7 mm diameter plug, cut from the margins of colonies grown for five days on WA, transferred to the periphery of the split-plate and incubated at 25°C.

In all experiments three replicates per isolate were used. Sclerotial formation was assessed after 12 days with respect to number and size. Mycelial growth and sclerotial production on the different media and at

the different temperatures was compared using analysis of variance (COSTAT) and means were separated with the Newman-Keuls test.

Table 1. Composition of soil constituents at *Morchella* sites

Site	pH	EC dS/m	Bray P mg/kg	Tot C %	Tot N %	Al cmol(+)/kg	Mg cmol(+)/kg	Ca cmol(+)/kg	K cmol(+)/kg	Na cmol(+)/kg
Cowra	4.29	0.03	3	1.89	0.13	0.3	0.8	2.2	0.23	<0.1
Grampians	5.61	0.09	<3	5.62	0.31	<0.1	3.1	10.7	0.34	<0.1
Mt. Pilot	4.64	0.03	<3	2.35	0.12	0.1	1.1	3.9	0.18	<0.1

3 RESULTS AND DISCUSSION

3.1 *Observations and analysis of* Morchella *sites*

All three of the sites from which *Morchella* ascocarps were collected were similar in ground vegetation characterised by grasses, mosses and leaf litter. Dominant trees were species of *Eucalyptus* and *Acacia.* Ascocarps were found in the clearings and not close to the base of plants or trees. They did not occur in fairy ring arrangements as reported by Philippoussis and Balis (1995) in a study of *Morchella* species in Greece. *Morchella* ascocarps have been emerging in these established sites for over 20 years with minimal site disturbance and occur over several hectares.

Results of the chemical analysis of the soil collected from underneath the ascocarps are shown in Table 1. Generally, the levels of exchangeable cations and total carbon, nitrogen and phosphorus levels were low, indicating nutrient-poor soils. Soil from the Grampians site had the highest levels of the 10 edaphic variables measured, except for Al. pH was similar at Cowra (4.29) and Mount Pilot (4.64) and higher at Grampians (5.61). Total C was at a higher percentage at the Grampians site (5.62 %) indicating a higher level of organic matter available than at the other two sites. Levels of Ca were highest at the Grampians site (10.7 cmol(+)/kg). Extractable P was similar between the three sites and total N ranged between 0.12 to 0.31 %. These levels of soil constituents contribute to the characterisation of the sites where *Morchella* ascocarps are found in Australia. In comparison with sites in Denmark and Sweden, the soil at the Cowra, Grampians, and Mount Pilot sites in Australia is poorer in soil constituents and lower in pH (Peterson 1985). The pH of the three sites is also lower in comparison with those in India (Kaul 1975).

3.2 *Spore release*

Spore prints of the ascocarps indicated a forceful ejection of the ascospores from the asci. The majority of the spores were released outside the area of the ascocarp to a distance of 5 cm. Forceful ejection was described by Schmidt (1979) in a study in which *Morchella* ascocarps rinsed under a trickle of water released a visible cloud of spores and hissed audibly for 2 to 4 s. The simultaneous bursting of asci would help carry spores farther from the hymenium than would occur by individual ascus discharge alone. This method of ascospore release would increase the numbers of spores successfully released into the environment due to the distance spores would be spread.

3.3 *Growth of mycelium at different temperatures on MEA*

Growth of mycelium occurred at all the temperatures. The optimum temperatures for mycelial growth for all three isolates on MEA were 20 and 25°C. There was no significant ($P = 0.05$) difference in growth between these two temperatures. Growth at 5, 10, 15, and 30°C was significantly less. The growth exhibited by G1, C1, and M1 at 30°C was of the unfavourable, sparse mycelial type that is highly branched and slow growing. This type of growth has previously been reported by Willam *et al.* (1956). However, the conditions to cause this growth form to revert to the normal form were not described. In this study it was found that repeated subculturing of mycelium can lead to normal growth, but in the long term this is undesirable and could diminish the ability of the culture to form sclerotia. Heat shock at 50°C over three to seven days has been tested without success in the present study. This unfavourable growth form of *Morchella* is still capable of producing sclerotia, but not to the extent of the normal growth form and this fact could be important in obtaining the optimum yield of ascocarps.

3.4 *Sclerotial formation on solid media and split-plates*

Preliminary screening of the *Morchella* isolates on various media showed that sclerotia ceased expansion and were completely coloured and mature after 12 days. The optimum agar medium for sclerotia production was CYM. Sclerotia produced on this media were formed as an encrusted band in the middle third of the plate or as scattered sclerotia all over the media. CYM produced isolated sclerotia up to 3 mm in diameter. Encrusted bands of up to 1.5 cm width were formed by the coalescence of smaller sclerotia in the size range of <1 to 2 mm. CLA ranked as the sec-

ond best medium and produced isolated sclerotia up to 2 mm in diameter in the centre of plates. SA and CA were poor at inducing sclerotia, only producing one 1 mm sclerotium on each replicate plate for isolates C1 and M1. MEA did not support production of sclerotia by any of the isolates, but has produced sclerotia in earlier trials. There was no significant difference in sclerotial production between the isolates.

Sclerotial production on CLA was statistically shown to be optimum over the temperature range of 10 to 25°C. At these temperatures one to five 1 to 2 mm sclerotia were produced on each plate. G1 was the only isolate to produce sclerotia at 30°C and only in one replicate. None of the isolates produced sclerotia at 5°C. Sclerotial production was not significantly ($P = 0.05$) different between the isolates.

Sclerotial formation by G1, C1, and M1 was investigated under the effect of successive growth on nutrient rich (MEA) and nutrient poor (CLA) media in split plates. In all isolates, sclerotial formation was favoured more when mycelia grew from CLA to MEA than when they grew from MEA to CLA. This result is similar to the occurrence of reverse translocation from mycelia on the nutrient-rich half to the developing sclerotia on the nutrient-poor side of the dish reported in *M. esculenta* by Amir *et al.* (1993), although sclerotia production began before the whole plate had been colonised. In this split-plate experiment sclerotia were only produced on the medium on which the inoculum was made and not on the opposite side of the plate. This contrasts with the findings of Amir *et al.* (1992) and Philippoussis and Balis (1995) who both reported sclerotia production on both the nutrient-rich and nutrient-poor media sides. Isolate G1 produced one to five >3 mm sclerotia when inoculated onto the CLA side, whereas only one to five 1 to 2 mm sclerotia occurred when inoculation was on the MEA side. One to five >4 mm sclerotia resulted when C1 inoculum originated from the CLA side, compared with only one 1 mm sclerotia when coming from the MEA side. M1 also produced one to five >4 mm sclerotia when inoculated on the CLA side, with only one to five 1 to 2 mm sclerotia forming on plates that had been inoculated on the MEA side. There were no significant ($P = 0.05$) differences in the number or size of sclerotia produced by the three isolates. Mycelia grew much faster when inoculation was on the CLA side compared with inoculation on the MEA side. This fast growth on the CLA enabled the mycelia to reach the MEA rapidly and transfer nutrients back to the mycelia and developing sclerotia on the CLA side. Growth of the mycelia on the MEA side was denser across the surface than on plates with growth originating from the CLA side due to the availability of more nutrients.

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