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Investigation into Cultivation Parameters for Australian Species of *Lepista*

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ABSTRACT: Wild material of *Lepista nuda* was collected in Australia, identified and pure cultures isolated. Research was undertaken to provide information on environmental and physical parameters required for the cultivation of Australian *Lepista nuda*. Australian isolates were compared with French isolates on agar and composted substrate. The optimum and minimum growth temperatures of Australian isolates were found to be higher than those of French isolates *in vitro*. The growth rate of Australian isolates was more than double the rate of French isolates at all temperatures except 5°C. This suggested that Australian isolates had the capacity to colonise substrate more rapidly than French isolates. The addition of 10% uncomposted cereal straw to composted substrate encouraged hyphal growth but discouraged the initiation of fruiting bodies of Australian isolates. A cold shock encouraged more hyphal aggregations at 12°C than at 15°C, indicating that low temperature may be more beneficial to fruit body initiation.

Australian isolates differed from overseas isolates in morphology. Whether they are new species or varieties or the result of environment and habitat has yet to be determined. This study has provided previously unknown data on the effects of substrate and temperature on hyphal growth and fruit body production of Australian *Lepista* isolates. This information will form the basis for the development of viable commercial cultivation techniques for the exotic/wild mushroom industry.

1 INTRODUCTION

In Australia there are probably 3000 to 5000 species of macroscopic fungi, most of which have not been properly described (Wood 1992).Only a small proportion have been assessed for edibility. The increase in consumption of wild mushrooms in many countries has led to a worldwide demand for new and exotic mushrooms in the market place and given impetus to explore new sources of edible mushrooms. In addition, the harvesting of edible mushrooms from the wild must be curbed to ensure that the environment and ecosystems where these mushrooms grow are not destroyed. Therefore it is important to explore these sources (Purkayastha and Chandra 1985) of new mushrooms and develop techniques for their cultivation.

Worldwide there are 200 species of edible fungi of which only 25 are widely accepted as human food and are cultivated (Pathak 1986, Chang 1981, Hashioka and Arita 1978). Fungi other than *Agaricus bisporus* represent about 30% of world production (Olivier 1991) but all fungi are used at the dinner table for their culinary properties with nutritional value being of secondary importance (Singer 1961). The collection and consumption of mushrooms from the wild has increased significantly in Australia in recent times. The influx of migrants with a European heritage into Australia has created a demand for edible mushrooms produced locally. Amongst the gilled fungi, there are a number of edible species in the families *Agaricaceae, Bolbitiaceae, Lepiotaceae* and *Tricholomataceae*.

Within the *Tricholomataceae, Lepista nuda (syn: Tricholoma nudum; Rhodopaxillus nudus; Clitocybe nuda*), commonly known as Wood Blewitt, has an international reputation as an excellent edible species and is found growing wild in Europe, the Americas and Australia. The combination of its lilac colour, solid fleshy structure, good shelf life, delicious flavour and aroma makes it attractive to the market. Therefore the development of commercial cultivation techniques for *L. nuda* is highly desirable. While research into cultivation techniques on wild material from these regions has been documented, no research has been undertaken on Australian isolates.

Developing techniques for commercial cultivation requires a detailed understanding of the environmental and nutritional parameters which optimise vegetative growth and induce fruiting bodies. An understanding of the effect of temperature and substrate on fruit body formation is necessary to maximise yields for growers. The development of an appropriate substrate requires the chemical, physical and biological conditioning of composted matter. This creates an environment selective for a particular species that is critical for fruit body production. Factors to be considered in the substrate are microbial activity, physical characteristics, pH, chemical components, aeration, water content, substrate composition and extent of composting undergone by the substrate. Some species require the application of a casing layer of peat or soil to enhance yield and quality. The physical and chemical characteristics, optimal depth and number of applications for a given casing material must be determined. The environmental conditions of temperature, light, O_2, CO_2, watering and care must be carefully managed to encourage fruit body formation. Previous studies on substrate requirements of *Lepista* (Vaandrager and Visscher 1981) indicate that fruit body production is enhanced by the addition of 10% uncomposted straw to commercial *Agaricus* compost. Guinberteau *et al.* (1989) and Brian *et al.* (1979) reported that a cold shock is essential for the formation of fruit bodies of *L.nuda* and temperatures of 8-15°C have been found to be effective. However, Australian isolates have been found under more variable environmental and substrate conditions than European isolates (Breitenbach and Kranzlin 1991, Moser 1978).

The objective of the project was to provide the mushroom industry with viable commercial cultivation techniques for Australian isolates of *L. nuda*. At this early stage several areas have been explored. Australian forms of *L. nuda* have been isolated from the wild and identified. These isolates have been found growing under different environmental and nutritional conditions. Investigations into the effect of temperature and substrate on hyphal growth and fructification (essential in any commercial cultivation techniques) have been explored and some comparisons made with French isolates.

2 MATERIALS AND METHODS

2.1 *Morphology and identification*

Under Australian conditions *Lepista* appears in the wild from May to July and during this period isolates of *Lepista* were collected from various sites. Voucher documentation, identification and isolation of strains was undertaken. Comparisons were undertaken with French isolates of *Lepista*, provided by Dr. Guinberteau of Station de Recherches sur les Champignons at Institut National de la Recherche Agronomique, France.

2.2 *Temperature effects on mycelial growth rate*

The mycelial growth response of Australian and French isolates of *L. nuda* from warm and cool climates were compared at different temperatures to determine maximum and minimum temperatures for hyphal growth. Four Australian isolates (A1 and A4 from warm climates, A2 and

A3 from cool climates) and four French isolates (F1 and F2 from warm climates, F7 and F8 from cool climates) were grown on malt extract agar plus 2% yeast (MEAY) at 5, 12, 15, 20, 25 and 30°C. Previous studies in this laboratory had shown that MEAY supported the most favourable hyphal growth. The plates were inoculated with a 5mm plug of mycelium cut from the margins of a 5-day old colony growing on MEAY. Radial growth was evaluated by measuring two perpendicular diameters of the colony and all data analysed by analysis of variance (ANOVA) and least significance difference (LSD).

2.3 *Compost and cold shock*

Commercially-produced *Agaricus* compost (S1) and *Agaricus* compost plus 10% uncomposted cereal straw (w/w) (S2) were placed into plastic growing trays with a surface area of 580cm^2. The trays contained approximately 1.5kg of substrate and were inoculated at 2 to 3% w/w with *Lepista* mycelium grown on rye grain supplemented with 0.5% $CaCO_3$. Trays were sealed with a plastic lid (containing four 1.5cm holes plugged with cotton wool and placed at 25°C in a cabinet with temperature and light control. After 12 days, substrate was cased with a 75% moisture content 50/50 mix

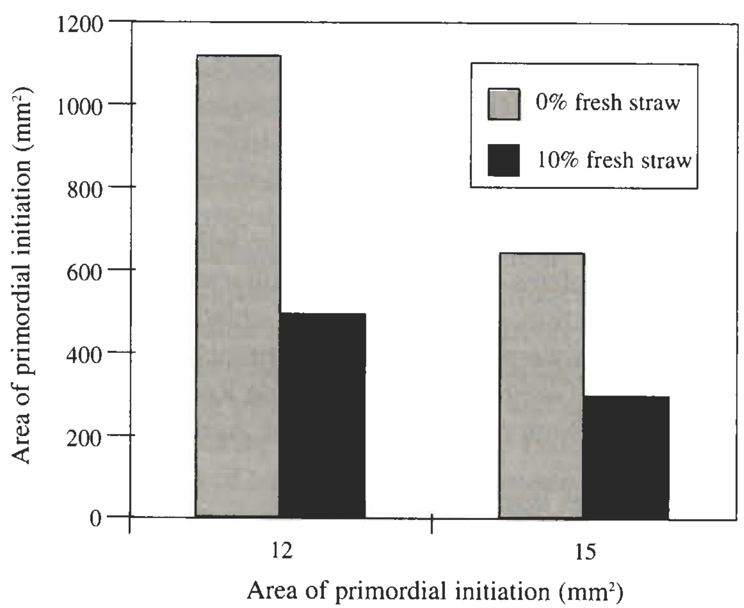

Fig. 1. Effect of substrate and cold shock on primordial initiation.

of blond/dark peat. After hyphae had grown through the casing (approximately 8 days), lids were loosened to allow air movement over the substrate and cold shock of 12°C or 15°C was applied to replicates of both substrates by moving the containers to cooler incubators.

3 RESULTS

The isolates from different temperature and habitat environments had distinctly different forms. Macroscopic and microscopic work confirmed the isolates belong to Tricholomataceae *Lepista* with most isolates being species *nuda*.

In cool climates (5°C to 25°C during the fruiting season) *Lepista* were found in leaf litter under shrubs (*Rhododendron sp., Camellia sp.*), trees (*Cedrus deodara, Quercus suber*) or Kentucky Blue grass (*Poa pratensis*). Basidiocarps were robust and fleshy, lilac to lilaceous brown. The pileus was 75 to 132mm, lilaceous brown, shiny, convex to shallow convex with age. Stipes were 50 to 78mm x 25 to 33mm.

In warm climates (11 to 35°C during the fruiting season) *Lepista* were found in Kikuyu (*Pennisetum*) and Couch grass (*Cynodon dactylon*) in groups or rings. Basidiocarps were strong lilac to lilaceous brown. The pileus was 30 to 75mm, strong lilac, shallow convex with umbo at all stages. Stipes were 65mm, bright lilac to deep lilac or lilaceous brown.

The optimum (25 to 30°C) and minimum (>5,<12°C) growth temperatures of Australian isolates were found to be higher than those of French isolates (22 to 25°C and <5°C, respectively). Australian strains exhibited no growth at 5°C. Most notable was that the growth rate of Australian isolates was more than double the rate of French isolates at all temperatures except 5°C.

Visual assessment and comparison of spawn run trays revealed that the addition of 10% uncomposted cereal straw encouraged hyphal growth through the substrate. Fruiting bodies of Australian strains of *Lepista* did not develop fully on any compost, but primordial initials developed in both types of substrate. These were counted and measured to record an area of pre-primordial coverage of the compost surface in mm^2.

The comparison between composts and the comparison between a 12°C and a 15°C cold shock were combined and the results of the primordia initiation are illustrated in Fig. 1. A cold shock encouraged greater development of hyphal aggregations at 12°C, leading to primordia development, than at 15°C.

4 DISCUSSION

Australian isolates differed from overseas isolates in shape, form and size. Whether they are new species/varieties or the result of environment and habitat has yet to be determined.

This study has provided previously unknown data on the effects of substrate and temperature on hyphal growth and fruit body production of Australian *Lepista* isolates. Results indicate that Australian isolates have the capacity to colonise appropriate substrate more rapidly than French isolates, and suggest that a colder (12°C) temperature may be more beneficial to fruit body initiation. The experiments also made it possible to conclude that the addition of 10% straw to compost was not beneficial to the fruiting of Australian isolates.

This information will form the basis for the development of viable commercial cultivation techniques for the exotic/wild mushroom industry. The cultivation requirements and growth rates of the Australian strains differ in several respects from those of their French counterparts. The differences suggest that a shorter production cycle than the 14 weeks currently achievable with French isolates will be possible.

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