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Original Article

Casing layer and effect of primordia induction in the production of *Agaricus subrufescens* mushroom

Emerson Tokuda Martos,[a] Diego Cunha Zied,[b,*] Pedro Paulo Gadoni Junqueira,[a] Danny Lee Rinker,[c] Romildo Da Silva,[a] Rômulo César Clemente Toledo,[d] Eustáquio Souza Dias[a]

[a] Universidade Federal de Lavras (UFLA), Departamento de Biologia, 37200-000, Lavras, MG, Brazil
[b] Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Tecnológicas (FCAT), 17900-000, Dracena, SP, Brazil
[c] Department of Plant Agriculture, University of Guelph, 4890, Guelph, ON, Canada
[d] Instituto Federal do Triangulo Mineiro, Campus de Ituiutaba, 38305-200, Itaiutaba, MG, Brazil

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ABSTRACT

Agaricus subrufescens growers have faced difficulties in standardizing and maintaining optimal production yield, even when they produce or acquire quality substrate, as cultivation success is also related to the quality of the casing layer and the production environment. The production of *A. subrufescens* was evaluated using different casing layers and methods for primordia induction. Three experiments were carried out: 1) to evaluate the effect of dolomitic limestone in the casing layer; 2) to evaluate the effect of different combinations of mineral and organic materials used as the casing layer; and 3) to evaluate the effect of temperature in primordia induction with two commercial strains. The results demonstrated that an increase in the limestone concentration in the casing resulted in a superior yield (16.7%). Casing layer combinations using organic substrate + sand (proportion 1:1, volume to volume) resulted in a greater yield (19.2%). Temperature did not affect primordia induction.

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Introduction

The *Agaricus subrufescens* mushroom is native to Brazil and was identified in the 1960's, but more recently has been compared to *A. bisporus*, which has been cultivated for centuries (Dias, 2010). Even though these species present different morphologies, the cultivation of *A. subrufescens* is possible using the production technology already consolidated for *A. bisporus*. However, *A. subrufescens* must be evaluated in order to reach the maximum yield in short time of harvest. To achieve a high yield it is necessary to use an adequate casing layer which may supply environmental conditions favorable to mushroom fructification (Colauto et al., 2011).

The casing layer to be added on the substrate must not be toxic and has to not have a sandy texture; however it has to be porous to facilitate gas exchange, or else enable appropriate aeration, water holding capacity and also capacity to liberate water without changing its structure (Zied et al., 2012). Bacterial activity also plays an important role in the fruiting process (Pardo et al., 2002). In Brazilian cultivation of *A. subrufescens*, a soil B horizon with an open clay texture is used empirically and is mixed several times with other substrates, such as washed sand, vegetable coal and rice husk, which offer desirable physicochemical characteristics. The diversity of soils available with different physical, chemical and biological characteristics is a limiting factor for the standardization of the soil as a casing layer (Siqueira et al., 2009; Dias et al., 2013).

The climatic conditions in each region are other factor which must be considered, and can be controlled when cultivation is made in a sheltered environment. According to the literature, the relative humidity of the air must be close to 70% and temperature between 25 °C and 30 °C (Dias, 2010). However, it has been recommended to reduce the temperature to 20 °C, followed by increasing to 26 °C for pinning (Zied and Minhoni, 2009). It is worthwhile noting that keeping the temperature in the range 21–27 °C is favorable to cultivate this mushroom (Colauto et al., 2010b).

Considering the unavailability of a standardized casing layer and a methodology involving an ideal temperature for *A. subrufescens*

* Corresponding author.
E-mail address: dczied@gmail.com (D.C. Zied).

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cultivation, this work aimed to evaluate production using different casing layers and methods for primordia induction.

Materials and methods

Three experiments were carried out: 1) to evaluate the effect of dolomitic limestone in the casing layer; 2) to evaluate the effect of different combinations of mineral and organic materials to be used as the casing layer; and 3) to evaluate the effect of temperature in primordia induction using two commercial strains.

Compost was produced according to conventional procedures (Phase I and II), formulated from methodology described by Siqueira et al. (2011), using an initial nitrogen concentration at 1.5%, inoculated with approximately 3% of "spawn", which was evenly mixed into the substrate and incubated for 30 d at 25 °C. When colonization was completed, the compost was leveled and covered with 5 cm of casing layer. After casing layer colonization, the CO_2 concentration was kept below 1000 parts per million during all cultivation cycles and the humidity was always kept above 70% while allowing the temperature to vary naturally (18–30 °C), except in the third experiment where primordia induction was tested.

Mushrooms were picked whenever they reached maximum size before opening of the pilei. The casing layer residue at the base of each mushroom picked was removed with a brush, and mushrooms were weighed for later calculation of the production parameters following the methodology presented by Pardo-Giménez et al. (2011). The results were analyzed using Tukey's test with $p \leq 0.05$.

First experiment: effect of limestone concentration

An Oxisol horizon B with sandy loam texture was used as the casing layer. Soil was collected, sun-dried and sieved to eliminate roots and clods greater than 1 cm. Based on the results of soil analysis, five concentrations of dolomitic limestone (percentage relative to the total volume of the casing layer) were determined to be mixed in the casing (Table 1), which were incubated for 90 d and irrigated every 7 d to facilitate the limestone reaction.

Second experiment: effect of different casing layer materials

Possible alternatives were tested to replace the material traditionally used in the casing layer. Commercial substrates used in the production of vegetables seedlings, containing coconut fiber and carbonized pine bark as the main ingredients, were used in different combinations (Table 1). The composition of the organic substrate according to the manufacturer was: carbonized pine bark, binders, coconut fiber, vermiculite and rice husk. Sand granules were 0.2–2.0 mm.

Third experiment: effect of temperature in the primordia induction

Two commercial strains of *A. subrufescens* were used (CS12 and ABL 04/49). Colonized substrates were conditioned in plastic boxes, leveled and covered with 5 cm of the same soil used in previous experiments, with 5% limestone and 25% pulverized vegetable charcoal added (Siqueira et al., 2009). The temperature was kept in the range 21–26 °C for 15 d until the casing layer was totally colonized. After the casing colonization, the boxes were transferred to a room with controlled temperature at 16 °C (±1 °C) and humidity above 70%, for different intervals (Table 1). Control parcels were kept at 21–26 °C during the complete cultivation cycle. After cooling, the boxes were transferred to the cultivation chamber again, together with the control treatment. This procedure was repeated at each flush with intervals of 30 d.

Results and discussion

First experiment: effect of limestone concentration

Limestone addition to the soil used as a casing layer had a positive effect on the mushroom yield of *A. subrufescens*, with a production response due to increasing the amount of limestone up to 30% (Table 2). According to Zied et al. (2012), previous correction of soil pH used as a casing layer is necessary for successful cultivation. However, most producers do not carry out a pH correction based on the chemical analysis of the soil, adopting proportions of limestone without considering the chemical characteristics of the soil used. The results obtained in the current study demonstrated that the addition of limestone must be made to increase the saturation of bases to values close to or greater than 70% and the pH to values above 6.0. Generally, the literature cites pH 7.0 as ideal for the casing layer (Colauto et al., 2010a). A significant difference was observed after the pH reached 6.3 resulting in a greater yield. For *Agaricus bisporus* cultivation, Zied et al. (2011) proposed that casing layer pH levels of 6.8, 7.8 and 9.0 were the minimum, optimal and maximum, respectively.

Table 1
Details of experimental design and treatments using two commercial strains of *A. subrufescens* (CS12 and ABL 04/49).

Experiment	Treatment	Number of repetitions	Experimental design	Analyzed variable
First	0% dolomitic limestone (control) 10% dolomitic limestone 20% dolomitic limestone 30% dolomitic limestone 40% dolomitic limestone	5	Randomized delineation	pH Yield Biological efficiency
Second	100% soil (control) 50% soil + 50% coconut fiber 50% soil + 50% organic substrate[a] 50% coconut fiber + 50% washed sand 50% organic substrate[a] + 50% washed sand 100% coconut fiber 100% organic substrate[a]	5	Randomized delineation	Earliness Yield Biological efficiency
Third	0 d at 16 °C − CS2 (control) 4 d at 16 °C; CS2 6 d at 16 °C; CS2 8 d at 16 °C; CS2 0 d at 16 °C − ABL 49 (control) 4 d at 16 °C; ABL 49 6 d at 16 °C; ABL 49 8 d at 16 °C; ABL 49	6	Randomized delineation in double factorial	Earliness Yield Biological efficiency

[a] Organic substrate compound of carbonized pine bark, binders, coconut fiber, vermiculite and rice husk.

Table 2
Production parameters in first and second experiments (see Table 1).

Treatment	First experiment		
	pH	Yield (%)	Biological efficiency (kg/dry tonne)
0% dolomitic limestone (control)	4.5^{c††}	11.4^{b}	37.9^{b}
10% dolomitic limestone	5.5^{b}	12.1^{b}	40.1^{b}
20% dolomitic limestone	6.0^{ab}	13.8^{b}	45.9^{b}
30% dolomitic limestone	6.3^{a}	16.7^{a}	55.5^{a}
40% dolomitic limestone	6.7^{a}	16.8^{a}	56.1^{a}
	Second experiment Earliness (days after casing)	Yield (%)	Biological efficiency (kg/dry tonne)
100% soil (control)	39^{b††}	3.5^{b}	8.7^{b}
50% soil + 50% coconut fiber	56^{c}	3.9^{b}	9.8^{b}
50% soil + 50% organic substrate‡	27^{a}	3.7^{b}	9.1^{b}
50% coconut fiber + 50% washed sand	27^{a}	9.3^{b}	23.2^{b}
50% organic substrate‡ + 50% washed sand	42^{b}	19.2^{a}	48.4^{a}
100% coconut fiber	27^{a}	6.0^{b}	14.9^{b}
100% organic substrate‡	29^{a}	3.2^{b}	8.1^{b}

††Media followed by same lowercase, superscript letter in a column do not differ statistically according to Tukey's test, $p \leq 0.05$.
‡Organic substrate compound of carbonized pine bark, binders, coconut fiber, vermiculite and rice husk.

Second experiment: effect of different casing layer materials

The time observed to the start of fructification (earliness) varied in the range 27–42 d. The onset of fructification occurred earlier in treatments that had received coconut fiber alone and in combination with sand (27 d), followed by organic substrate at 29 d after the addition of the casing layer. A mix with soil and organic substrate also frutified earliers (27 d) (Table 2).

Conversely, although resulting in later fructification, the combination of organic substrate + sand produced the best results in mushroom yield and biological efficiency, being clearly different from all other treatments, with a yield of 19.2% and 48.4 kg/dry tonne, respectively. Harvests were divided into four flushes; each flush was defined as an interval of 30 d. The second best result (not significant, but meaningfully different from the first) was obtained with a mixture of coconut fiber + sand; this result suggested that sand is a good material to be used in combination with material with a low density (organics) in the casing layer.

Pure sand was not considered a suitable material as the casing layer because it does not have good water holding capacity, which is a factor of great importance in the formation of fructification bodies, since water accumulated in the casing layer is extremely important for mushroom development and when it is not efficiently retained, more frequent irrigation is necessary and this in turn increases the probability of waterlogging of the cultivation compost, favoring anaerobic conditions (Colauto et al., 2010b).

Zied et al. (2014) studied the effect of four casing layers and observed that in a climatic-controlled chamber the different casings did not affect the yield, but in a greenhouse covered with plastic film, a superior yield resulted from a casing using soil + charcoal and soil + bark. Pardo-Giménez et al. (2014) verified that casings based on peat (Euroveen and Infertosa) had a better yield than casings based on mineral soil.

According to Pardo-Giménez et al. (2014), values of electric conductivity above 1600 μS/cm in the casing layer promoted a considerable reduction in mushroom yield. A combination of casing layers used in the experiment resulted in values below 1600 μS/cm; however, this did not appear to have any influence on the yield.

Third experiment: effect of temperature in the primordia induction

Based on the first flush, it was possible to verify the influence of the genetic characteristics of the strain and the temperature reduction for primordia induction only for the CS2 strain (Table 3). Generally, the ABL 49 strain was more productive in relation to the CS2 strain. These results confirmed the study by Llarena-Hernández et al. (2014) who stated that due to the great genetic distance between strains of *A. subrufescens*, each isolate has a different yield and varied responses to environmental characteristics, such as cultivation temperature. It was also evident that in both strains, the best yield occurred in the second and fifth flushes, demonstrating that the cultivation time for *A. subrufences* may be extended for up to 2 mth, subject to there being no pest or disease infestation. These results demonstrated the absence of such mushroom production technology. Compost technology and better formulation may explain this long cultivation crop cycle.

Different thermal induction treatments in relation to periods (days) did not interfere with the yield and biological efficiency of mushrooms of *A. subrufescens*. The treatment at 16 °C for 8 d required more energy and this is still not a viable procedure for Brazilian conditions. Braga et al. (2006) evaluated different cultivation environments (plastic greenhouse, bamboo rustic environment and climatized chamber) and verified that the best mushroom yield was produced using the plastic greenhouse. According to these authors, their results obtained may be explained by the greater oscillation in the plastic greenhouse, which resulted in a greater thermal increase, which might be responsible for inducing fructification.

In a similar experiment, Zied and Minhoni (2009) evaluated mushroom production in a controlled environment in contrast with a plastic, rustic greenhouse and observed that the cultivation in the rustic greenhouse produced 19.2% more production compared to

Table 3
Production parameters in third experiment (see Table 1) using two commercial strains of *A. subrufescens* (CS12 and ABL 04/49).

	Third experiment					
	Earliness (days after casing)		Yield (%)		Biological efficiency (kg/dry tonne)	
	CS2	ABL 49	CS2	ABL 49	CS2	ABL 49
Control	29A^{a†}	29B^{a}	7.8A^{b}	16.6A^{a}	19.4A^{b}	41.7A^{a}
4 d at 16 °C	46B^{b}	21A^{a}	10.4A^{b}	16.0A^{a}	26.6A^{b}	40.0A^{a}
6 d at 16 °C	47B^{b}	21A^{a}	8.8A^{b}	15.2A^{a}	21.6A^{b}	38.0A^{a}
8 d at 16 °C	56C^{a}	45C^{a}	10.8A^{b}	15.8A^{a}	26.6A^{b}	39.6A^{a}

†Media followed by the same capital letter in a column (primordia induction) and the same lowercase superscript letter in the line (strains) do not differ statistically according to Tukey's test, $p \leq 0.05$.

the controlled environment. These results may indicate that the ideal conditions of temperature, humidity and ventilation for producing this mushroom still need better definition.

Therefore, it was concluded that an increase in the limestone concentration resulted in a greater yield (16.7%). However, combinations of casing layer using organic substrate + sand, in the proportion of 1:1 (v:v) produced a greater yield (19.2%). Finally, there was no observed effect of temperature on primordia induction.

Conflict of interests

The authors declare no conflict of interest.

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