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PRODUCTION FLUSH OF *Agaricus blazei* ON BRAZILIAN CASING LAYERS

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ABSTRACT

This study aimed to verify the biological efficiency and production flushes of *Agaricus blazei* strains on different casing layers during 90 cultivation days. Four casing layers were used: mixture of subsoil and charcoal (VCS), lime schist (LSC), São Paulo peat (SPP) and Santa Catarina peat (SCP); and two genetically distant *A. blazei* strains. The fungus was grown in composted substratum and, after total colonization, a pasteurized casing layer was added over the substratum, and fructification was induced. Mushrooms were picked up daily when the basidiocarp veil was stretched, but before the lamella were exposed. The biological efficiency (BE) was determined by the fresh basidiocarp mass divided by the substratum dry mass, expressed in percentage. The production flushes were also determined over time production. The BE and production flushes during 90 days were affected by the strains as well as by the casing layers. The ABL26 and LSC produced the best BE of 60.4%. Although VCS is the most used casing layer in Brazil, it is inferior to other casing layers, for all strains, throughout cultivation time. The strain, not the casing layer, is responsible for eventual variations of the average mushroom mass. In average, *circa* 50% of the mushroom production occurs around the first month, 30% in the second month, and 20% in third month. The casing layer water management depends on the casing layer type and the strain. Production flush responds better to water reposition, mainly with ABL26, and better porosity to LSC and SCP casing layers.

Key words: production flush, *Agaricus brasiliensis*, casing layer, water management, *Agaricus subrufescens*.

INTRODUCTION

Agaricus blazei Murrill *sensu* Heinemann (16) is a basidiomycete from Brazil (1) that was reclassified by Wasser *et al.* (35) as *Agaricus brasiliensis* Wasser *et al.*. That classification was contested by Kerrigan (20) who suggested the name *Agaricus subrufescens* Peck, published in 1894. Whether or not *A. brasiliensis* and *A. subrufescens* are the same species, they are *nomen illegitimum* according to the Index Fungorum (17), because those names had already been published by Fries in 1830 (15) and by Ellis and Everhart in 1893 (10), respectively. In this study, this basidiomycete will be referred as *A. blazei*.

A. blazei has immunomodulating and antitumor activities

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(18, 22, 26, 27) and it is a culinary mushroom (11). Despite its importance, few studies have been done about the effect of casing layers on basidiocarp mass, production flush, water management and productivity. In general, the same techniques utilized for casing layers of *Agaricus bisporus* cultivation are used for *A. blazei* production in Brazil (1).

The casing layer is one of the most important phases of *A. bisporus* cultivation, and it is the variable that is responsible for the induction of fructification (23). The function of a casing layer is to protect the compost from drying, pests and diseases, and provide physical support and gas exchange for the development of the basidiocarps (14). A casing layer generally consists of a mixture of peat and lime added after the substrate mycelial colonization (7, 28). However, there is an environmental pressure against peat extraction for agricultural use because it is a natural carbon reserve, and it affects the fragile, but ecologically and archeologically important, swampy ecosystem (2). Peat availability is a great concern in some regions around the world, and some research efforts have been devoted to searching other materials that may be used as a substitute or in combination with peat (5). Furthermore, in several mushroom production areas in the world, mainly in the southern hemisphere, there are no large peat sources available, and then local soil is used as a casing layer (33). However, peat is still a predominant casing layer worldwide, and it is a great challenge to find a substitute that is available in volume and low cost to meet the demands of mushroom production.

Lime schist is a clay sedimentary rock from intermediate layers of limestone mines, basically formed by calcium and magnesium carbonate salts, about 35-65% clay and 65-35% carbonate (30). Calcite limestone is used in big scale to adjust acid soils, commonly found in Brazil (24); it is abundant and easy to find, and has low cost (25). Lime schist is a non-carbon source, mineral sub product of calcite limestone, and it is not used to correct soil pH due to its reduced solubility in acid solutions (6). Although it is a source of magnesium, it has low solubility for magnesium reposition, making it an inert sub product of the calcite limestone industry (25). Colauto *et al.* (5) have already reported the use of lime schist as an alternative to peat for casing layer in the *A. blazei* cultivation in Brazil.

Although new casing layers are being tested as a substitute to mushroom cultivation (5), there is not enough knowledge about the production flushes of Brazilian casing layers which is essential to the production management in order to obtain quality, uniformity, yield, nutrient addition and economic determination of the final harvest time of mushroom cultivation. Thus, the objective of this study was to verify the biological efficiency and production flushes of *A. blazei* strains on different casing layers throughout 90 days of cultivation.

MATERIALS AND METHODS

The experiments were carried out in the Sector of Biotechnology for Mushroom Cultivation of the Department of Plant Production at the Universidade Estadual Paulista (UNESP) – Campus of Botucatu, School of Agronomical Sciences. ABL 99/26 and ABL 99/29 *A. blazei* strains from the culture collection of the Sector of Biotechnology for Mushroom Cultivation were referred as ABL26 and ABL29, respectively, in this experiment. Colauto *et al.* (4) reported that those strains presented the highest genetic distance using RAPD.

Four casing layers for mushroom production in Brazil were evaluated: a mixture (7:3) of red yellow podsol from B horizon and charcoal (VCS), used as control, lime schist (LSC), also known as schist, from intermediate layers of limestone mines, São Paulo peat (SPP) from the Instituto Agronômico de Campinas and Santa Catarina peat (SCP) from COMINAS Mining S/A. LSC was immersed for one hour in slow flow running water and all other raw materials were used as found in nature.

The inoculum was made of pre-cooked wheat grains at 100 ºC for 40 min mixed to $CaCO_3$ (1%) and autoclaved at 121 ºC for 40 min. After cooling, ABL26 and ABL29 strains that had been grown in malt extract agar were transferred to the

autoclaved grains and kept at 28 ºC, in the dark, according to Colauto *et al.* (3).

For composting, the raw material was sugarcane (*Saccharum officinarum*) bagasse (500 kg), *Brachiaria* sp grass (800 kg), coast cross (*Cynidon dactylon*) grass (2200 kg), soybean (*Glycine max*) bran (140 kg), urea (50 kg), ammonium sulphate (50 kg) and gypsum (30 kg), followed by pasteurization and conditioning, according to Eira *et al.* (9) and Colauto *et al.* (5). Then, the substrate (8.0 kg) was transferred to a cultivation plastic box (55 cm long, 35 cm wide and 24 cm high) and homogenized with 80 g of inoculum. Substrate colonization occurred in the dark for 30 days at 25 ºC ± 2 ^{o}C and 90% ± 8% relative humidity. Ten boxes were used (replications) for each treatment, in a completely random factorial design of 2 x 4 (strains x casing layers).

The casing layers were saturated with water and the pH was adjusted to 7.0 with $CaCO_3$. After that, it was pasteurized at 60 ^{o}C for six hours, then cooled and added to the colonized substrate at 4 cm. When the mycelium surfaced the casing layer, there was induction of primordia increasing the ventilation but keeping humidity, reducing temperature to 20 ^{o}C and adding water to the casing layers. After the beginning of the basiciocarp production, the temperature was kept at 23 ^{o}C ± 2 ^{o}C, and the mass and the number of fresh basidiocarps were measured daily for 90 days. The moisture of basidiocarps and substrate was measured by drying at 105 ^{o}C until constant mass. The mushrooms were picked up when the veil was stretched, but before the lamella were exposed. The mushroom production was evaluated by the biological efficiency (BE) of the fresh basidiocarp mass divided by the substratum dry mass, expressed in percentage, and the production flushes were also determined over time production. The differences among the averages were determined by the variance analysis and Tukey's test ($p \leq 0.01$).

RESULTS AND DISCUSSION

The control treatment of VCS presented the lowest BE when compared to other casing layers for both strains, while LSC and SCP had the highest BE throughout 90 cultivation days (Table 1). Moreover, around 57% of total production occurred in the first month for LSC, SCP and SPP, but VCS produced only 42% of its total production at that time. So, although VCS is the most used casing layer in Brazil, its BE was the lowest among casing layers tested in this experiment (Table 1). Physical and chemical characteristics as casing layer depth, chemical and microbial composition, moisture content, and porosity, play important roles in the yield and quality of mushrooms (19, 29). Colauto *et al.* (5) reported the physical and chemical characteristics for the materials used as casing layer in this study. LSC, SPP and SCP presented bigger particle size than VCS (5). Thus, LSC, SPP and SCP have less compaction tendency than VCS, facilitating mycelial growth (21) due to the higher air availability. The low compaction tendency of casing layers is very important to avoid the formation of anaerobic systems (8), contaminant growth (32) and stroma formation (13), mainly after water addition. Also LSC, SPP and SCP have a better balance between total porosity and micro porosity than VCS (5) corroborating the low compaction tendency of the casing layer with more empty spaces for gas exchanges and oxygen reserve (31). These characteristics could explain the better values of BE for LSC, SPP and SCP found in this work after 90 cultivation days, whereas VCS presented the worst result. They could explain as well the reduction of production along cultivation with 53.2% in 30 days, 22.1% in 60 days and 24.7% in 90 days of cultivation for ABL26 strain (Table 1). A yield reduction was observed as well after 30 days of cultivation for ABL29 strain (Table 1). It is important to know about basidiocarp production percentage along cultivation to decide the economical moment to stop cropping and the moment to invest in improvements for mushroom production.

When analyzing the BE for each strain, it was lower for ABL29 but the total mushroom production was anticipated reaching 91.8% at 60 cultivation days with LSC. This has not

happened for the ABL26 strain, which reached 76.8% under the same condition (Table 1). It seems that ABL29 has a short production period, around 60 cultivation days, whereas ABL26 could last until 90 days. For ABL26, mainly with LSC, SCP and SPP, two clear production flushes occurred until 30 days of production but for VCS only one flush occurred (Fig. 1). Zied *et al.* (34) studied different casing layers to produce *A. blazei* and reported three distinct production flushes until 90 cultivation days. The first flush started at 37 cultivation days; however, casing layers did not affect BE and production flush. In this study, after 30 cultivation days, the production flushes were less distinct, although a final flush occurred between 70 and 80 days of cultivation. For ABL29, the flushes were not so clear with small increases on mushroom mass production along the time (Fig. 2). This distinct behavioral characteristic can be explained by the higher genetic distance between the strains. Colauto *et al.* (4) used 20 primers to study polymorphism of five (including ABL26 and ABL29) *A. blazei* strains. ABL26 and ABL29 showed higher genetic variability by RAPD. It was possible to verify during the cultivation that basidiocarps had distinct morphological differences (photos not showed). It is possible that these genetic variability and morphological and behavioral characteristics of these strains, isolated from growers in Brazil, are the result of genetic recombination and/or mutations processes, considering that many growers used an open-air cultivation system for this mushroom in Brazil.

The LSC, SPP and SCP casing layers anticipated basidiocarp production and produced more distinct flushes for both strains while VCS produced less distinct and delayed flushes along the cultivation time (Fig. 1 and 2), explaining the good porosity of LSC, SPP and SCP casing layers. Thus, during 90 days, the BE and production flushes were affected by the strains as well as by the casing layers.

Table 1. Percentage of total basidiocarp mass production (TP) and biological efficiency (BE) of *Agaricus blazei* ABL26 and ABL29 strains in function of casing layers of lime schist (LSC), São Paulo peat (SPP), Santa Catarina peat (SCP) and mixture (7:3) of subsoil and charcoal (VCS), during 90 cultivation days

Cultivation until:		**ABL26**					**ABL29**				
		LSC	**SCP**	**SPP**	**VCS**	**(Average)**	**LSC**	**SCP**	**SPP**	**VCS**	**(Average)**
30 days	TP	59.7	54.2	57.2	41.9	(53.2)	62.5	59.7	48.9	41.8	(53.2)
	BE	36.1^{a}	29.1^{b}	24.5^{b}	13.3^{d}	(25.8)	23.9^{b}	24.5^{b}	18.3^{c}	9.3^{d}	(19.0)
60 days	TP	76.8	77.7	74.4	72.2	(75.3)	91.8	86.9	86.3	76.9	(85.5)
	BE	46.5^{a}	41.8^{ab}	31.9^{b}	23.0^{c}	(35.8)	35.0^{b}	35.7^{b}	32.3^{b}	17.1^{d}	(30.0)
90 days	TP	100.0	100.0	100.0	100.0	(100.0)	100.0	100.0	100.0	100.0	(100.0)
	BE	60.4^{a}	53.7^{ab}	42.9^{b}	31.8^{c}	(47.2)	38.2^{b}	41.1^{b}	37.5^{bc}	22.3^{c}	(34.7)

*Different letters indicate significant differences according to Tukey's test ($p \leq 0.01$).

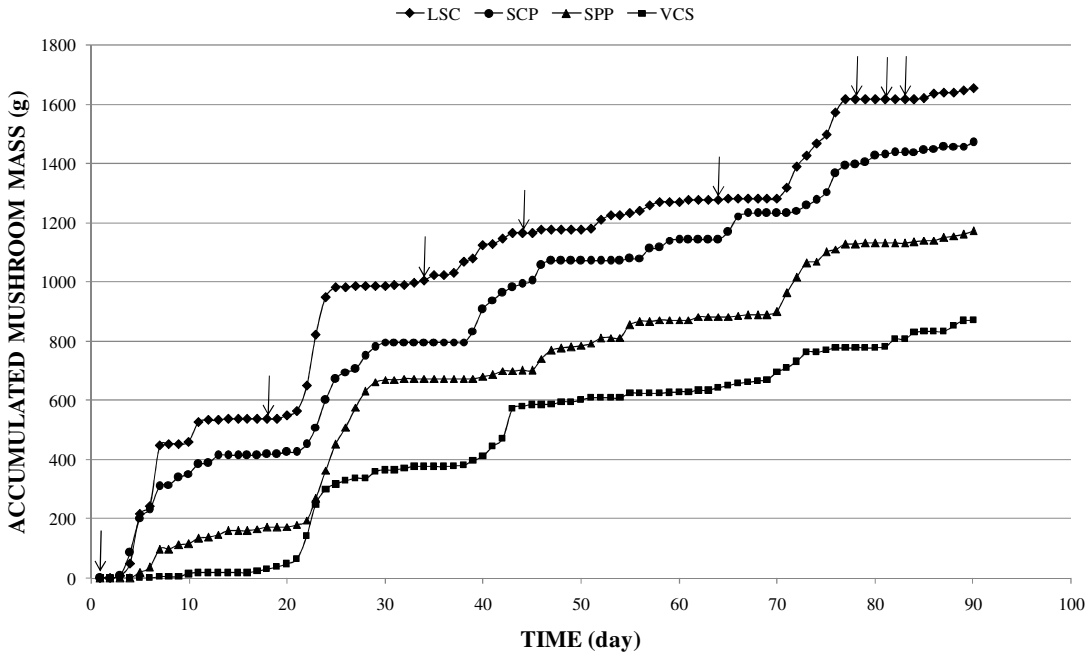

Figure 1. Production flush and accumulated mass of fresh mushrooms of *Agaricus blazei* ABL26 strain on the casing layers of lime schist (LSC), São Paulo peat (SPP), Santa Catarina peat (SCP) and mixture (7:3) of subsoil and charcoal (VCS), during 90 cultivation days. Arrows indicate the time in which water was added to casing layers.

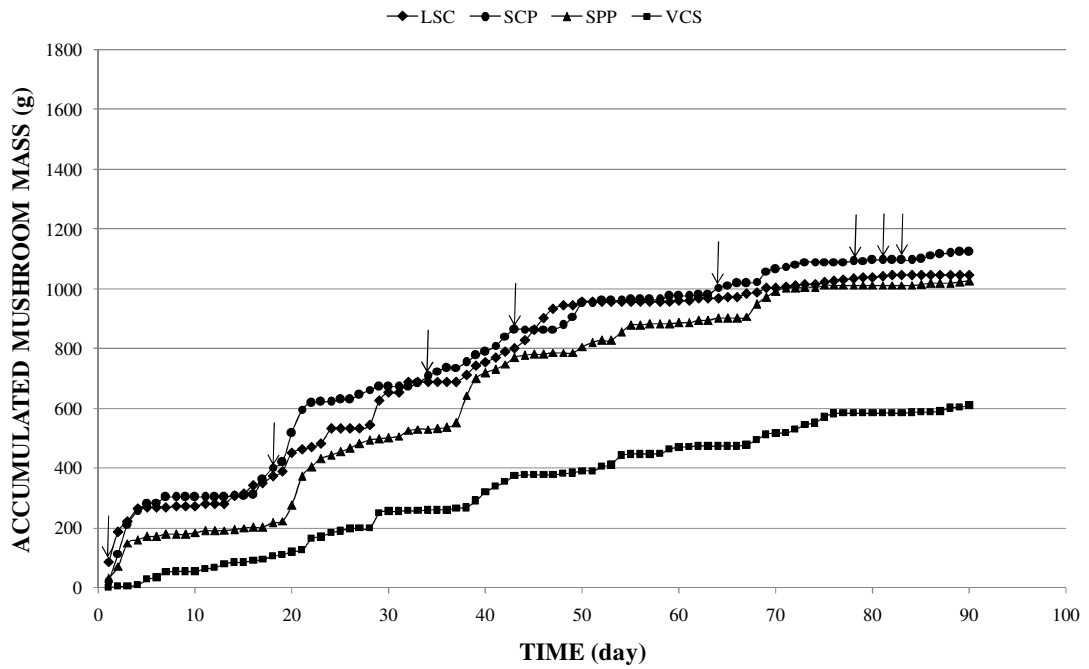

Figure 2. Production flush and accumulated mass of fresh mushrooms of *Agaricus blazei* ABL29 strain on the casing layers of lime schist (LSC), São Paulo peat (SPP), Santa Catarina peat (SCP) and mixture (7:3) of subsoil and charcoal (VCS), during 90 cultivation days. Arrows indicate the time in which water was added to casing layers.

Water was added every time the casing layers had visual signs of lack of water that initially occurred in the period of 16 days, and then it has not showed any regular periods during the cultivation (Fig. 1 and 2). At the end of the cultivation, water addition was frequent due to the low retention capability of casing layers mainly after 76 days. Two to five days after water addition to casing layers, mushroom production was stimulated (Fig. 1 and 2), although this was more evident in ABL26 than ABL29, mainly in the first flushes; it was important to adjust water management for each strain. Also *A. blazei* was induced to produce mushrooms two to five days after water addition, mostly until second flush. It shows that the fungi respond to fructification induction when water is available, similarly to what happens in nature after rain. The best regular responses of production flush to water addition happened when LSC or SCP was used as casing layer, and no regular response was observed with VCS, indicating that the adjustment depends on the casing layer type and the strain (Fig. 1 and 2). At the end of the cultivation time (90 days), the total mass of the substrate was reduced in 65% with no differences ($p \leq 0.01$) among casing layer types. That indicates that the casing layers had similar capacity to avoid substratum water loss. Moreover, comparing the differences of BE among casing layers, it is possible to infer that the casing layer with higher BE, such as LSC for ABL26 and LCS and SCP for ABL29 (Table 1), provided more water to the basidiocarp, suggesting that the water in the casing layer was responsible for the improvement of the mushroom production. Colauto *et al.* (5) also reported that LSC, SPP and SCP have higher capacity to keep water linked into physical-chemical structure than VSC. This is an important characteristic because it may avoid sudden humidity variations in the casing layer, improving the micro environment stability and fungus adaptation. A casing layer may provide up to 37% of water to mushrooms, and reduce the water demand from the substrate which can not be easily replaced (12, 19). The higher capacity to keep water of LSC, SPP and SCP could explain the results in this study, where different values for BE were obtained among tested casing layers, but the final total mass of the substrate (data not showed) was equal ($p \leq 0.01$), corroborating that the casing layer, not the substratum, is the main water supplier for the basidiocarp production. Besides, after 90 cultivation days, the production flushes were probably reduced because of the substratum nutrient decrease and the loss of the physical-chemical structure of LSC, SPP and SCP, which reduced both the water capacity maintenance and gas exchange.

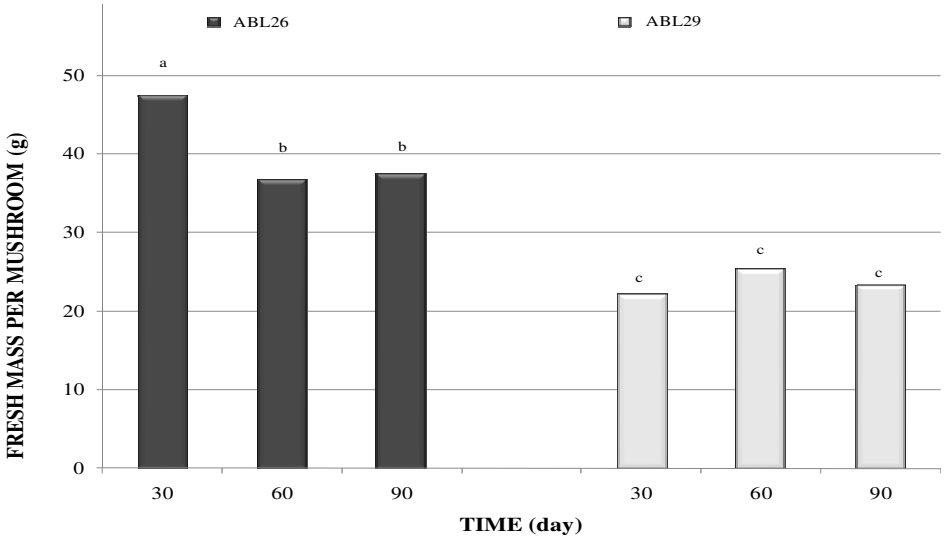

Figure 3. Average fresh mass per mushroom of *Agaricus blazei* ABL26 and ABL29 strains at 30, 60 and 90 days of cultivation. Different letters indicate significant differences according to Tukey's test ($p \leq 0.01$).

ABL26 produced bigger mushrooms (average of 37.4 g) and ABL29 smaller ones (average of 23.2 g) for all casing layers. This indicates that the basidiocarp mass is a genetic characteristic with low influence from the casing layer environment. However, when the average fresh mass per mushroom is analyzed along the cultivation time, there are two distinct results (Fig. 3). First, the average fresh mass per mushroom was reduced for ABL26 ($p \leq 0.01$) after 30 cultivation days, without further reduction; second, ABL29 kept the same mass per mushroom along cultivation time (Fig. 3). Thus, the strain, not the casing layer type, is responsible for the average mushroom mass along cultivation time.

In conclusion, the BE and production flushes during 90 days were affected by the strains as well as by the casing layers. The ABL26 and LSC produced the best BE of 60.4%. Although VCS is the most used casing layer in Brazil, it is inferior to other casing layers, for all strains, throughout cultivation time. The strain, not the casing layer, is responsible for eventual variations of the average mushroom mass. In average, *circa* 50% of the mushroom production occurs around the first month, 30% in the second month, and 20% in third month. The casing layer water management depends on the casing layer type and the strain. Production flush responds better to water reposition, mainly with ABL26, and better porosity to LSC and SCP casing layers.

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