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ORIGINAL PAPER

Production of *Agaricus blazei* ss. Heinemann (*A. brasiliensis*) on different casing layers and environments

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Abstract To investigate the yield and precocity of *Agaricus blazei* using to different casing layers and cultivation environments, five casing layers were prepared with soil (different textures), wood charcoal, and calcitic lime. After colonization, the composts were placed in two growing rooms (controlled environment and plastic greenhouse) and cased. The cycle was 120 days. Yield and precocity data were evaluated in a factorial combination (5 soil types $\times 2$ cultivation environments) and 8 replications. The results showed low yields when cultivated in a controlled room (1.55 kg of mushrooms per box) and yield values of different soils ranged between 1.61 and 1.88 kg of mushrooms per box. The precocity values of the different soils and environments ranged between 62 and 51% in the first 50 days of production. The various soil types did not differ statistically for yield values (kg) and the plastic greenhouse provided higher yields. The texture of the different soils and environment directly influenced precocity in *A. blazei* yield.

Keywords *Agaricus blazei* · Medicinal mushroom · Casing layer · Cultivation environment · Yield

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Introduction

The mushroom *Agaricus blazei* has been grown commercially for the last 20 years. There are reports in the literature that this species occurs naturally in Brazil and it was first found in São Paulo State (1970s), Piedade county, in the mountainous region of the Atlantic Rainforest (Iwade and Mizuno 1997; Wasser 2002; Dias et al. 2004). However, the literature is a quite controversial, since other citations and articles report that *A. blazei* is a synonym of the species *Agaricus subrufescens*, which was previously collected and identified by Peck in 1800 (Kerrigan 2005).

Little is known about *A. blazei* cultivation technology, both the commercial and the experimental scales, quite differently from *Agaricus bisporus*, for which the cultivation technology is still in development.

As a result, composting, spawning, casing layers and colonization, control of pests and contaminants have been studied and adapted from *A. bisporus* (Donini et al. 2006; Kopytowski Filho et al. 2006; Andrade et al. 2007; Freire et al. 2007; Nascimento and Eira 2007; Silva et al. 2007; Kopytowski Filho et al. 2008; Zied and Minhoni 2009). Other studies are also cited too about cytological and genetic characterization of this mushroom (Colauto et al. 2002; Dias et al. 2008).

Like any basidiomycetes, the physiology, nutrition and development of *A. blazei* are specific to certain environmental variables (temperature, RH%, O_2 and CO_2 contents) and should be detailed in scientific studies, in order to obtain higher yields in a shorter period. Therefore, this research aimed to investigate the yield and the precocity as a function of different casing layers (five soil types) and cultivation environments (controlled room and plastic greenhouse).

Materials and methods

The experiment was carried out in the facilities of the Mushroom Research Center of the São Paulo State University, Botucatu-SP, Brazil. The commercial strain used was ABL 04/49 (isolated from samples of mushrooms commercially cultivated in São José do Rio Preto County, SP, Brazil and stored in the culture collection of the Mushroom Research Center of FCA/UNESP.

The spawn substrate used consisted of triticale (*Triticum secale*) grains, gypsum, and calcitic lime. The grain were previously cooked and to it was then added 20 g lime kg^{-1} and 160 g gypsum kg^{-1} (Andrade et al. 2008) and it was then autoclaved for 3 h at 121°C. After cooling down, the substrate was inoculated according to methodology presented by Zied and Minhoni (2009).

The C/N initial ratio of the compost was 36.5/1 (Kopytowski Filho et al. 2006). The compost was sampled before pilling, in the beginning of Phase I and at the end of Phase II. Chemical analyses (including the ingredients for preparing the compost) were carried out at the Laboratory of Fertilizers of the FCA/UNESP (Table 1).

Phase I was carried out in a covered wharf. The ingredients used in compost were Coast-Cross straw (*Cynodon dactylon*), sugar cane bagasse, gypsum and calcitic lime. At the end of Phase I, the compost was transferred to polypropylene boxes for pasteurization and conditioning in a tunnel (Dalsem Mushrooms). Pasteurization was performed at 59 ± 1°C for 12 h, and conditioning at 47 ± 1°C for 12 days.

The temperature of the compost was lowered to 23°C for inoculation in smaller polyethylene boxes, containing 12–12.5 g of fresh compost and 150 g of spawn. The boxes were randomly arranged in the incubation room during 13 days at 28 ± 1°C.

Table 1 Formulation of the compost, C/N ratio of the ingredients and the compost Phase I and II

Materials	kg			C/N ratio
	Dry weight	N content	C content	
Sugarcane bagasse	887	3.37	488.35	144.9/1
Coast-cross straw	649	10.45	333.22	31.9/1
Soybean meal	74	5.15	33.12	6.4/1
Urea	10	4.50	2.70	0.6/1
Calcitic lime	40	–	–	–
Gypsum	20	–	–	–
Total	1,680	23.47	857.39	
Initial C/N ratio (phase I)				36.5/1
Final C/N ratio (phase II)				23.7/1

The casing layer consisted of five soils with different textures (Table 2), added of 25% charcoal and calcitic lime. All casing layers were moistened to 35% and pasteurized at 62°C for 8 h.

After 15 days of spawn run, the composts were cased (5 cm depth) and the first flush started on day 18 after casing.

Figures. 1 and 2 highlight the climatic factors during the 120 days of cultivation phase. In the controlled room, the variables were managed and controlled in order to obtain six flushes, quite differently from the plastic greenhouse, where environmental variables were partially controlled and harvests occurred according to the weather factors of each particular day or week.

The first harvest started 20 days after casing and kept for more 100 days. The mushrooms were harvested manually, followed by the scraping of the bottom of the stalk to remove casing layer residues. The mushrooms were placed in a plastic bag identified with the box number of the replication.

Yield was determined by the weight of the mushrooms harvested per box:

$$Y = \frac{r1 \pm r2 + r3 + r4 + r5 + r6 + r7 + r8}{8} \text{ (kg)}$$

where $r1$ to $r8$ is the weight of fresh mushrooms harvested in each one of the eight replications of each treatment.

Precocity was determined using the following methodology: in the beginning of the first flush, the harvesting period was divided into two parts, i.e., the first mushrooms were harvested on the 20th day after adding the casing layer. The experiment consisted of a 100-day harvesting period, divided into two periods (first 50 days and last 50 days).

$$\text{PC} = \frac{Y_1}{Y_t} \times 100(\%)$$

where PC precocity (%), Y_1 yield in the first 50 days after fruiting (g), Y_t total yield (g).

The data were submitted to analysis of variance (Anova) and the means were compared by Tukey test (5%), using the SAS software.

Table 2 Texture of soils used as casing layer

Soil	g kg^{-1}				
	Coarse sand (CS)	Fine sand (FS)	Total sand (TS)	Clay	Silt
1	32.50	126.83	159.33	567.33	273.33
2	117.33	240.16	357.50	471.16	163.83
3	160.50	302.16	462.50	411.33	126.16
4	215.33	341.50	556.66	352.66	90.66
5	258.00	384.50	642.83	293.16	64.00

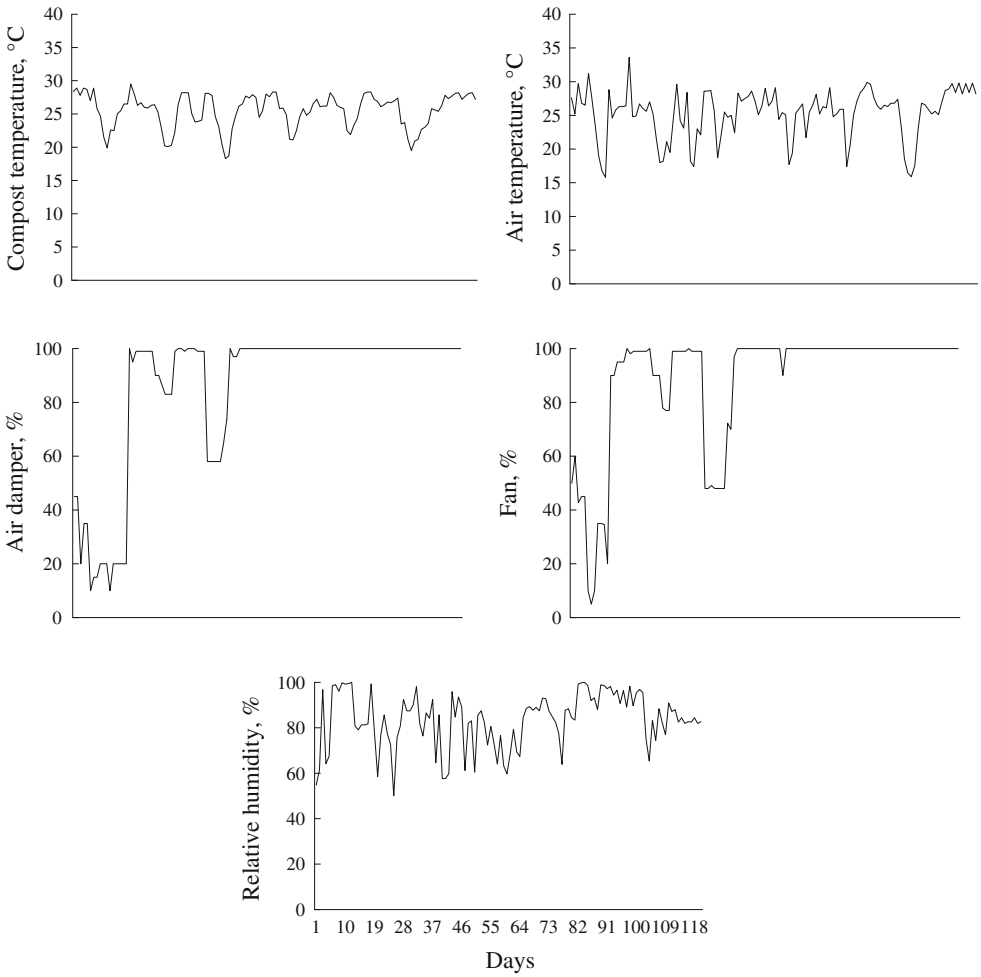

Fig. 1 Temperature data for the compost and the environment, relative humidity, air damper flushing and fan, conducted during cultivation in the controlled room

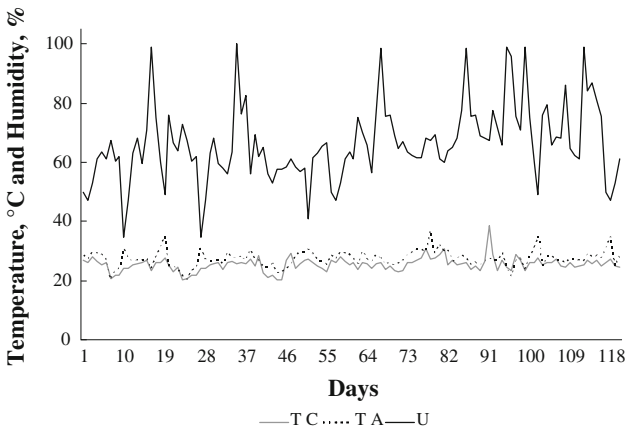

Fig. 2 Behavior of environment variables during production phase in the plastic greenhouse (*TC* compost temperature, *TA* air temperature and *U* relative humidity), over 120 days

The whole experiment lasted 187 days (Fig. 3), involving all cultivation steps: production of spawn; composting process; inoculation and spawn run; casing layer; and growing phase.

Results

According to Table 3 the yields did not differ statically for casing values with the different soils, however it was verified that there was a tendency for higher yields when using soil 1 in a controlled room (1.7 kg) and soil 4 in a plastic greenhouse (2.08 kg).

It was observed that the plastic greenhouse showed higher yield (1.94 kg) than the controlled environment (1.55 kg). This factor significantly affected yield so much that the commercial greenhouse had a 20.1% higher than controlled environment.

It is believed that this positive result for the greenhouse occurred due to the weather season in which the experiment was carried out (120 days of production phase), from 09/29/2006 to 02/01/2007, where the local climatic conditions directly influenced mushroom yield in the plastic greenhouse (Fig. 2). Little variation in compost temperature (20.3–30.8°C) and typical cool days (days 23–26; 4–45; and 98–99) that lingered for 40–48 h were important for pinheading.

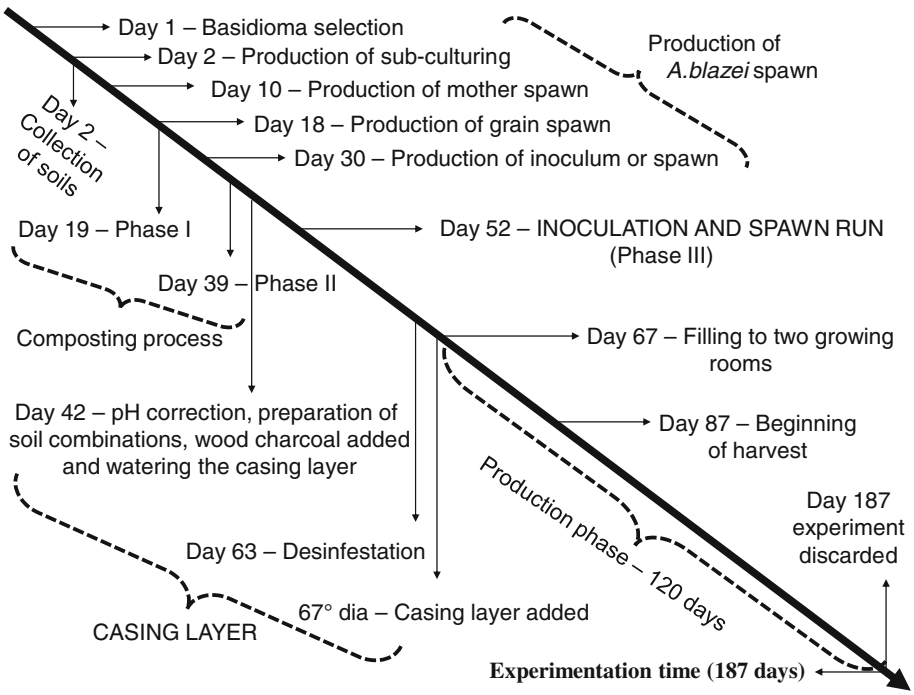

Fig. 3 Description of activities performed along 187 days of experimentation

Table 3 Yield (kg mushroom per box) obtained with different soil types and cultivation environments in the end of harvest (120 days)

Soil	Environment		Mean[a]
	Controlled room	Plastic greenhouse	
1	1.70	1.84	1.77a
2	1.62	1.89	1.75a
3	1.38	2.03	1.71a
4	1.68	2.08	1.88a
5	1.35	1.88	1.61a
Mean[a]	1.55B	1.94A	
LSD	0.16[b]		0.36[c]
CV (%)		29.89	

Each box containing 12–12,5 kg de fresh compost

[a] Means followed by the same letter do not differ from one another (Tukey, 5%)

[b] Value obtained from the various soil types

[c] Value obtained from the various environment types

Precocity was correlated between time of pinheading and mushroom yield, Fig. 4 shows a vertical line in the center to demonstrate the time established to evaluate precocity (70 days after casing). The upper part of the graphs show four values (yield, %); those on the left-hand side refer to the first 50 harvesting days in the Controlled environment (D) and in the Plastic Greenhouse (P), while those on the right-hand side refer to the last 50 harvest days in Controlled environment (D) and in the Plastic Greenhouse (P).

For all casing layers the yield was higher in the first 50 harvesting days. Soil 1 showed the highest precocity in both environments (D-62% and P-57%), while soil 2 showed the lowest precocity in both environments (D-53% and P-51%). Soil 1 had better results than soil 2, in the order of 14.5 and 10.5%, for cultivation in the controlled room and in the plastic greenhouse, respectively.

With regard to cultivation environment, soils 1, 2, and 4 provided highest precocity in the controlled room, in the order of 62, 53, and 55%, respectively. Soils 3 and 5 did not show differences regarding cultivation environments and both reached 55% of the yield in the first 50 harvesting days. It becomes clear that cultivation environment, together with soil texture, directly influence yield precocity in *A. blazei*.

The tendency to low yield presented by soil 1 in the last 50 harvesting days, both in the controlled room and in the commercial greenhouse might be due to the daily irrigations of the casing layer, which apparently caused soil compaction, thus not allowing an adequate gaseous exchange for the mycelium.

Based on these results, using different soil with added 25% charcoal in both cultivation environments, the following conclusion can be drawn: *A. blazei* cultivation still has a tendency to be a long process in relation to other species of mushrooms (with cycles between 70 and 100 days). This becomes evident in view of the fact that yield values observed in the last 50 days of cultivation were relatively good, which would not justify the mushroom grower in dumping the compost 70 days after casing

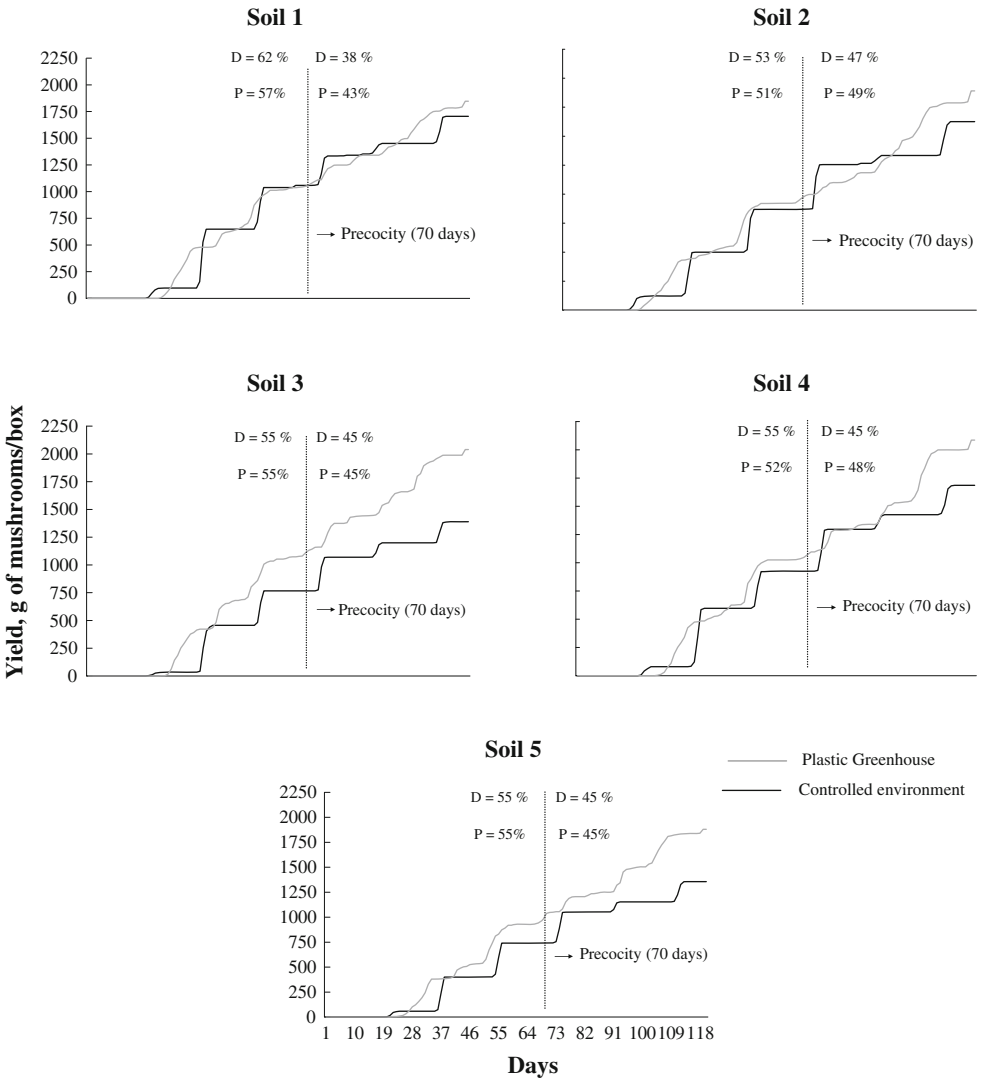

Fig. 4 Cumulative yield precocity, as a function of five casing layers and two cultivation environments (*D* controlled environment, *P* plastic greenhouse)

if only 62 and 57% of yield have been harvested in that period (soil 1).

The cost for making a new compost, comprising labor, raw materials, electricity (composting phase II), spawn, casing layer, and 20 extra days until the first flush (excluding the cost of materials for sanitation) would make it not economically viable to dump the compost in that time. It would be worthwhile for the mushroom grower to go on with cultivation for another 50 days and then harvest 38% in the Controlled Environment and 43% in the Plastic Greenhouse of the yield using the same compost, using soil 1, which showed the best precocity observed in this experiment.

Discussion

Few countries commercially cultivate *A. blazei* (Brazil, USA, China, Taiwan and others) and little study is reported in the literature about its cultivation. However, Bittencourt (2003) stated the hypothesis that *A. blazei* yield can be increased as a function of casing layer type. The author observed significant yield differences using the following materials: Dutch peat; Santa Catarina peat; soil + charcoal (3:1, v/v); Santa Catarina peat + soil and charcoal (1:4, v/v); Santa Catarina peat + soil and charcoal (1:1, v/v); and Santa Catarina peat + soil and charcoal (4:1, v/v). But, tests with different soils have not yet been carried out.

In several countries due the difficulty in finding peat suppliers at a competitive price and with high enough quantity, the normal procedure is the use of "local soils" to make the casing layer. However, detailed description of chemical, physical, and microbiological characteristics should be available in order to obtain a casing layer with some similarities to peat (black, brown or moss). The soil itself will certainly not have the same properties as a peat, although other materials available in certain areas, with consistent supply, and reduced cost can be added to the soil

in order to provide some properties the soil does not provide.

Several experiments using charcoal and tile fragments were carried out (Andrade et al. 2007; Alonso 2002). Other materials have already been evaluated, but few studies have focused on their cost-effectiveness (Eira 2003; Cavalcante et al. 2008, Zied et al. 2009).

The soil itself has the advantage that it can be used as a casing layer due to the following factors: abundance at all seasons of the year, low cost (most times without any cost at all) and easy management. Soil collected at a 2.0-m depth is a good alternative for *A. blazei* cultivation in respect to the absence of pests and diseases of mushrooms (Zied and Minhoni 2009).

However, Oei (2003) reported that it is disadvantageous to use clay soils as casing layer, due to their rapid compaction and low water retention capacity, when compared with other materials (peat, spent compost mushroom, and coconut fiber) used as a casing layer in *A. bisporus* cultivation.

Synthetic ingredients, such as acrylamide gel can be added to the casing with soil to increase the yield and precocity results, as presented by Zied et al. (2009), who tested 70% soil + 25% charcoal + 5% calcitic lime and obtained 53% precocity, results similar to those obtained in this experiment, where values ranged from 51 to 62%. So, those authors highlighted that, when 0.5% acrylamide gel was added to the same casing layer, the precocity reached 75% in the first 50 harvesting days and the yield in the end of growth was 1.54 kg.

Pardo et al. (2002) also observed similar results to these ones when testing several casing layers, and correlated them with earliness in first harvest mushrooms. Earliness values were lower with only soil was used when compared with soil added to peat or composted pine bark. The same authors emphasized the ruffling in the casing layer showed positive influence on the beginning of the primordial formation (first flush) and precocity.

In regard to cultivation technologies influencing precocity, Siqueira (2006) tested inoculation of microorganisms on the compost (Phase II) and reported that 90% of mushrooms were harvested 91 days after casing. The same author evaluated the influence of microorganisms inoculated on the compost and their respective responses on the flushes.

Thus we concluded that various soil types did not differ statistically from one to another (Tukey, 5%) for yield values (kg); the plastic greenhouse provided higher yields (kg); The texture of the different soils and environment directly influenced precocity in *A. blazei* yield and the production phase had a tendency in being longer when soil + charcoal + calcitic lime are used as casing layer.

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