Optimization of cultivation techniques improves the agronomic behavior of Agaricus subrufescens

Arturo Pardo-Giménez1, José Emilio Pardo2, Eustáquio Souza Dias3, Danny Lee Rinker4, Cinthia Elen Cardoso Caitano5 & Diego Cunha Zied5✉

New species of medicinal mushrooms have emerged over the past several decades, such as the Sun mushroom, Agaricus subrufescens. Horticultural improvements are required to shift its cultivation from small-scale local production to large-scale international production. The research reported here evaluated the agronomic behavior and the chemical characteristics of the Sun mushroom as a function of i) nutritional supplementation ii) ruffling of the casing layer and iii) the temperature management on the primordia induction and reduction of the crop cycle. Supplementation was beneficial for yield, unit mushroom weigh and decrease in time to first harvest. Supplementation improved biological efficiency with Champfood providing a yield increase of 15% over the non-supplemented compost. Among the supplements only Promycel increased the individual mushroom weight. Ruffling overall improved the yield in the 2nd and 4th flush. Already biological efficiency was greater by 21%. The highest yield harvested in any single day in the crop occurred in 3rd flush with the amount of 2.484 kg of mushrooms per m² for the rapid induction method. Still the biological efficiency was not significantly affected by the mushroom induction temperature method. Only the fat content of the mushrooms was positively affected by the rapid induction of primordia. Champfood supplement promotes a reduction in the value of earliness and an increase of 1st flush yield. The ruffling technique provided an increase in biological efficiency due to the great number of mushrooms harvested. Rapid primordia induction allowed the crop cycle to end 3 days earlier than the slow primordia induction, providing a higher production rate.

New species of medicinal mushrooms have emerged over the past decades. One such mushroom is the Sun mushroom, Agaricus subrufescens Peck, Agaricus brasiliensis Wasser et al. and Agaricus blazei (Murrill) ss. Heinemann1–4. This species has been cultivated on a small-farm scale in Brazil for many years. In 2017 Royse et al.5 reported that world mushroom production is divided among several genera: Lentinula (22%), Pleurotus (19%), Auricularia (18%), Agaricus (15%), Flammulina (11%), Volvariella (5%) and others (10%). In the discussion of Agaricus these authors did not mention the Sun mushroom. In 2018 Sanchez et al.6 noted that the only country in the Americas that cultivated the Sun mushroom was Brazil. Agaricus subrufescens along with Pleurotus eryngii, Flammulina velutipes and other non-Agaricus represented only 6% of the total production in Brazil.

Changes in horticultural technologies are required to shift production from small-scale local producers to large-scale commercial production with international markets. Two main challenges in the cultivation of Sun mushroom are the compost nutrition and its long cultivation cycle7,8.

Production of A. subrufescens generally follows that of Agaricus bisporus. Despite this, the cultivation of the button mushroom has a much shorter cultivation cycle and a much higher yield. Therefore, the two species can be grown using similar technology, however, the results are not the same. In A. bisporus cultivation, hybrid strains, are grown preferentially, because of the high quality and yield of the fruit-bodies. An important advance in this area occurred with the development of the well-known Horst® U-strains, on crossing white strains with off-white strains9. The spawn cultures used in A. subrufescens cultivation are from wild strains, which have not yet undergone a breeding process, as has already been the case with the main cultivated mushrooms species10.

1Centro de Investigación, Experimentación y Servicios del Champiñón (CIES), Quintanar del Rey, Spain. 2Escuela Técnica Superior de Ingenieros Agrónomos, Universidad de Castilla-La Mancha, Albacete, Spain. 3Universidade Federal de Lavras, Departamento de Biologia, Lavras, Brazil. 4University of Guelph, Vineland Campus, VinelandStation, Guelph, Canada. 5Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Tecnológicas (FCAT), Dracena, Brazil. ✉e-mail: dczied@gmail.com
Llarena-Hernández et al. analyzed European wild strains, which showed agronomic parameters superior to those of Brazilian strains. Two strains showed similar production to the button mushroom, however, after a long cultivation cycle. In addition, these strains showed greater difficulty in colonizing the compost. Another important observation was that the same strain showed a wide variation in productivity between different experiments. Then, the cultivation of the almond mushroom is still complex and even though some hybrids have been tested, no commercial company offers spawn for growers. Nowadays, strains are circulating through private collections and are generally not widely available. Moreover, Wisitrassameewong et al. did not exclude the fact that A. subrufescens might be a complex of species. Therefore, given all the above considerations, there is no doubt that there is still a long way to go to make the cultivation of this mushroom as efficient as was achieved for the button mushroom.

The almond mushroom is cultivated on farms where there are multiple crops at different stages in protected structures. Raw materials consist typically of a cereal straw (eg., wheat, barley, rice) that provide carbohydrate and lignin. These bulk materials are low in nitrogen and the compost is enriched through animal litter (eg., chicken or turkey manure) and other available nitrogen sources. Gypsum (CaSO₄) is added to conserve nitrogen, balance the pH and serve as a flocculant. These heterogeneous materials are mixed, hydrated and composted for 2 to 3 weeks. After this period, the compost is pasteurized and further composted under strict environmental controls. The composting process is stopped once the ammonia has disappeared. At this time this substrate may be supplemented with additional nutrient products (as per the research in this paper) by thoroughly incorporation. Spawn (mycelia of the fungus colonized on sterile grain) is mixed into this substrate. For 3 to 4 weeks the mycelia colonize the substrate (a.k.a. spawn run). At the end of this growth, the substrate may be supplemented as well. Then, the surface of the colonized substrate is top-dressed with a layer of material(s) (a.k.a. casing layer) buffered with lime that aids in the induction of the mushroom primordia. Within 7 to 15 days mushroom formation (a.k.a. primordia induction or initiation) is initialized through changes in substrate and air temperatures and gaseous management. Once the mushrooms form and are market ready, they are harvested by hand. Mushrooms grow in cycles (a.k.a. flushes). The crop is terminated after a set period time that has been determined to be economically viable.

Commercial cultivation of A. bisporus utilizes compost supplementation, a ruffling of the casing layer (mixing of mycelium and materials in situ), and various schema of temperature manipulation for primordia initiation. Supplementation is the incorporation of high nitrogen materials into the compost at spawning or casing to improve yield. Compost supplementation provides nutrients to the developing mushroom, resulting in increased productivity up to 30% with a shortening cultivation cycle.

Ruffling is a process whereby the Agaricus mycelium which is growing into the casing is thoroughly mixed through the whole casing depth. This process allows for more accurate control of the first flush and uniformity of production due to the diffusion of CO₂ and the admission of O₂ when the growing room is aerated. Recommendations for the initiation of fructification and the temperatures used for the Sun mushroom vary.

Some authors recommend the reduction of the temperature to approximately 20°C for primordia induction while others maintain the temperature constant at 23°C and 24–26°C. Primordia initiation and harvest intervals are temperature sensitive. Its management is fundamental for the timing of the flushes for harvest. In this manuscript we report production and crop responses from nutritional supplementation of compost at spawning, ruffling of the casing layer, and various temperature management schema for induction of primordia in the medicinal mushroom A. subrufescens.

Materials and methods

Experimental design. The experimental factorial design was a 4 × 2 with six replicates completely randomized. Factor 1 had four kinds of supplements (three commercial supplements and a non-supplemented control). For Factor 2 the casing was either ruffled or not. Factor 3 used two mushroom production chambers to achieve two schemes of primordia induction conditions. Ninety-six small trays (0.145 m² surface area) were randomized, equally divided between two identical growth chambers and arranged on two levels.

Spawn. A. subrufescens strain ABL 99/30 (Centro de Estudos em Cogumelos, FCAT-UNESP, Brazil) was selected for the experiment. This strain, collected in Piedade (São Paulo, Brazil) in 1999, is characterized by its medium to small size fruit bodies, strong texture, high yield and precociousness, reduced time to first harvest, and slightly lower fructification temperature. Spawn was prepared according to Andrade et al. in these steps: production of subculture from culture bank, production of mother spawn, and production of grain spawn for compost inoculation. Compost was inoculated with grain spawn at a rate of 15 g kg⁻¹ of fresh weight of compost.

Substrates and supplements used. Commercial Phase II compost used for production of A. bisporus that was based on wheat straw and chicken manure (Compost Villacasa S.L., Casasimarro, Cuenca, Spain) was used as substrate for the Sun mushroom production. Along with the non-supplemented compost, three commercial products with high protein content were evaluated as nutritional supplements for mushroom cultivation: Promycel® 600 (Amycel Europe, Vendôme, France), Champfood® (ChampFood International, Vierlingsbeek, The Netherlands) and Calprozime® (Calliope, Breziers, France). The physical, chemical and biological characteristics of the compost and supplements were determined according to Zied et al. (Table 1).

Sun mushroom trials were carried out in two 20 m² experimental growth chambers equipped with automatic control of temperature, relative humidity and carbon dioxide. At spawning, the compost was thoroughly incorporated with 10 g kg⁻¹ of Promycel 600, Champfood or Calprozime. Trays were cased 13 days after spawning with a peat-based commercial mixture, Euroveen® (Euroveen BV, Grubbenvorst, The Netherlands) to the depth of 4 cm.

Materials and methods

Experimental design. The experimental factorial design was a 4 × 2 with six replicates completely randomized. Factor 1 had four kinds of supplements (three commercial supplements and a non-supplemented control). For Factor 2 the casing was either ruffled or not. Factor 3 used two mushroom production chambers to achieve two schemes of primordia induction conditions. Ninety-six small trays (0.145 m² surface area) were randomized, equally divided between two identical growth chambers and arranged on two levels.

Spawn. A. subrufescens strain ABL 99/30 (Centro de Estudos em Cogumelos, FCAT-UNESP, Brazil) was selected for the experiment. This strain, collected in Piedade (São Paulo, Brazil) in 1999, is characterized by its medium to small size fruit bodies, strong texture, high yield and precociousness, reduced time to first harvest, and slightly lower fructification temperature. Spawn was prepared according to Andrade et al. in these steps: production of subculture from culture bank, production of mother spawn, and production of grain spawn for compost inoculation. Compost was inoculated with grain spawn at a rate of 15 g kg⁻¹ of fresh weight of compost.

Substrates and supplements used. Commercial Phase II compost used for production of A. bisporus that was based on wheat straw and chicken manure (Compost Villacasa S.L., Casasimarro, Cuenca, Spain) was used as substrate for the Sun mushroom production. Along with the non-supplemented compost, three commercial products with high protein content were evaluated as nutritional supplements for mushroom cultivation: Promycel® 600 (Amycel Europe, Vendôme, France), Champfood® (ChampFood International, Vierlingsbeek, The Netherlands) and Calprozime® (Calliope, Breziers, France). The physical, chemical and biological characteristics of the compost and supplements were determined according to Zied et al. (Table 1).

Sun mushroom trials were carried out in two 20 m² experimental growth chambers equipped with automatic control of temperature, relative humidity and carbon dioxide. At spawning, the compost was thoroughly incorporated with 10 g kg⁻¹ of Promycel 600, Champfood or Calprozime. Trays were cased 13 days after spawning with a peat-based commercial mixture, Euroveen® (Euroveen BV, Grubbenvorst, The Netherlands) to the depth of 4 cm.
Rapid induction of primordia was accomplished by reducing the temperature to 20 °C in two schemes (Fig. 1A). Slow induction of primordia was managed by reducing the compost temperature by 2 °C d−1 until 28 °C was achieved (day 29)24. Rapid induction of primordia was accomplished by reducing the temperature to 20 °C in a day25. It was kept at this temperature for 4 days and then increased to 28 °C in one day (day 26). Drop air temperature to 20 °C for fruiting and compost temperature of 26 °C and 700 ppm CO2 during the harvest were used to generate Pearson Product Moment correlations between the values for earliness, biological efficiency, number and weight of mushrooms with the chemical characteristics of the supplements.

**Results and discussion**

Production parameters. Mushrooms were harvested daily at their optimal commercial development stage prior to opening of the veil. The measured or calculated agronomic behavior (earliness, biological efficiency, production rate, number of mushrooms, unitary weight and yield per area) were assessed on untrimmed mushrooms. Whereas, proximate analysis (dry matter content of harvested mushrooms, protein, fat, carbohydrate and fiber) were evaluated on trimmed mushrooms of the peak harvest day of first flush. Earliness, or days to the first harvest, was expressed as the number of days between casing and the beginning of the first flush harvest. Biological efficiency, an estimation of mushrooms’ ability to convert substrate into fruiting bodies, was calculated prior to opening of the veil. The measured or calculated agronomic behavior (earliness, biological efficiency, number and weight of mushrooms with the chemical characteristics of the supplements).

| Characteristics       | Compost  | Promycel 600 | Champfood | Calprozime |
|-----------------------|----------|--------------|-----------|------------|
| pH (1.5, w/v)         | 7.54     | 5.82         | 6.19      | 8.95       |
| Moisture (g kg⁻¹)     | 684.0    | 119.0        | 144.2     | 109.7      |
| Total nitrogen (g kg⁻¹) | 20.5    | 82.1         | 82.7      | 64.8       |
| Protein (g kg⁻¹)      | 128.1    | 513.0        | 516.6     | 405.1      |
| Ash (g kg⁻¹)          | 273.6    | 64.3         | 65.1      | 117.5      |
| Organic matter (g kg⁻¹) | 726.5   | 935.7        | 934.9     | 882.5      |
| C/N                   | 20.6     | 6.6          | 6.6       | 7.9        |
| Crude fibre (g kg⁻¹)  | 276.8    | 97.7         | 65.5      | 144.9      |
| Crude fat (g kg⁻¹)    | 3.9      | 31.2         | 10.0      | 16.5       |
| N-free extract (g kg⁻¹) | 317.6   | 402.3        | 342.8     | 316.1      |
| Hemicellulose (g kg⁻¹) | 178.1   | 188.7        | 342.2     | 227.9      |
| Cellulose (g kg⁻¹)    | 131.3    | 50.0         | 60.1      | 95.0       |
| Lignin (g kg⁻¹)       | 268.9    | 132.3        | 91.3      | 123.3      |
| Neutral-det. sol. (g kg⁻¹) | 148.3   | 564.6        | 441.3     | 436.4      |

Table 1. Chemical characteristics of compost and supplements.
Increased production from supplementation is the commercially desirable outcome. Total production over four harvest flushes showed a significant increase in yield by Champfood over any other treatment. The gain in production was realized primarily in the first flush (Fig. 2). The other flushes did not demonstrate significant yield responses for supplementation. Rankings among treatments for progressive accumulated yield (data not shown) was consistent throughout the trial. The positive effect of supplementation on yield (up to 50%) of the Sun

| Treatment       | Earliness (days from casing) | Biological efficiency (kg dt⁻¹ compost) | Number of mushroom (m⁻²) | Weight of mushroom (g) |
|-----------------|------------------------------|----------------------------------------|--------------------------|------------------------|
| Non-supplemented| 28.7 b                       | 53.71 ab                               | 533                      | 23.0 b                 |
| Promycel 600    | 27.3 a                       | 52.75 b                                | 464                      | 26.5 a                 |
| Champfood       | 27.2 a                       | 60.65 a                                | 558                      | 25.9 ab                |
| Calprozime      | 27.6 a                       | 58.18 ab                               | 555                      | 24.6 ab                |
| Mean            | 27.7                         | 56.33                                  | 528                      | 25.0                   |

Table 2. Agronomic behavior of non-supplemented and supplemented compost (Factor 1). For each factor within a column, values followed by a different letter are significantly different at 5% level according to Tukey’s HSD test. The absence of letters in the column indicates non-significance.
mushroom has been reported by other authors as well. They have noted that the positive response of supplementation is also mushroom strain related\(^30\). In a study specifically regarding the economic aspects of compost supplementation for *Agaricus bisporus* production, Randle and Smith\(^31\) estimated that the cost of supplementation is covered by a yield increase of 1.5 kg m\(^{-2}\). In our study, untrimmed yields over four flushes were non-supplement (12.01 kg m\(^{-2}\)), Promycel (12.11 kg m\(^{-2}\)), Calprozime (13.36 kg m\(^{-2}\)) and Champfood (13.91 kg m\(^{-2}\)). In our study, the only supplement that would be economically viable would be the Champfood.

An interesting question is “which characteristic of the supplement influences production”? Several authors have indicated that the supplement used in the production of *Agaricus* should be rich in N (vegetable protein)\(^32,33\). Others have noted that the supplement should have low levels of P, K and Mg\(^30\). The supplements evaluated varied substantially among their chemical characteristics. Promycel 600 and Champfood supplements were about 21% higher in total nitrogen than Calprozime. However, Promycel 600 was lower in hemicellulose and cellulose but higher in lignin. Champfood was lower in crude fat and Calprozime higher in pH, ash and fiber (Table 1). In our study we observed a negative correlation between biological efficiency and fat content of the supplement (r = −1.00 and P = 0.0034) (Fig. 3). In this sense, we suggest that future experiments be carried out using nutritional supplements with high protein and low fat content, chemical characteristic similar to that observed by Champfood.

Finally, the commercial *A. bisporus* compost used in the present research was poor, with values of 2.05% N content and C/N ratio of 20.6/1. Some of the literature report higher N values such as N = 2.6 and lower C/N ratio = 16/1\(^34\). *A. subrufescens* is highly sensitive to ammonia in compost, and when the initial concentration of nitrogen is high, there is a more intense production of ammonia, depending on the degradation of protein\(^35\). Thus, organic supplements rich in N can improve the quality of the compost, avoiding the excessive application of mineral N during the composting phase I, which can reduce the losses of ammonia volatization.

Supplementation significantly decreased the time (earliness) to first harvest. All the supplements evaluated significantly reduced this time by over one day (Table 2). Although all supplements decreased the time to first harvest, only Champfood demonstrated a significant increase in yield over all other treatments (Fig. 2; Table 2).

Earliness is an important parameter in improving the mushroom technology. Lower earliness values were observed in the cultivation of *A. bisporus*, ranging from 19.0 to 21.7 days, and the addition of supplements in the compost had no significant influence on earliness\(^36,37\), different from the results obtained in this manuscript which the supplementation had significant influence on earliness. Dias et al.\(^38\) (as ourselves, Table 2) observed that overall production in *A. subrufescens* was greater when the time to harvest (earliness) was shorter.

Compost supplementation increased individual mushroom weight, however, only Promycel was significant (Table 2). Greater individual mushroom weight is extremely important for the Sun mushroom since the product is valued for its larger size, washed by-hand and dehydrated. A larger mushroom makes the handling process more efficient. The strain used in this study (ABL 99/30) strain is characterized as a smaller one\(^20\) which can be improved through addition of a compost supplement at spawning.

**Factor 2 (ruffling technique).** The ruffling technique significantly improved yield, biological efficiency and number of total mushrooms (Table 3). Biological efficiency was greater by 21%. This gain in productivity came from significantly higher yields on the second and fourth flushes (Fig. 4). This fact occurs naturally in the cultivation of *A. subrufescens*, specifically, when a flush (2nd) present good yield, the subsequent one (3rd) tends to present less yield. The importance of the ruffling technique becomes essential to leave the casing layer with a lower mycelium density, which allows a better gas exchange until the end of the harvest period (4th flush). Despite the significant increase in total mushrooms through ruffling the casing layer, the individual mushroom weight was not significantly influenced by the ruffling technique.
Dias et al. 38, after ruffling three casing materials (sphagnum peat moss, loam soil, coconut coir), observed that a loam soil casing was highly superior to either peat or coir. Soils typically have a more dense compact structure than peat or coir. In contrast, in the cultivation of *A. bisporus* loam soil was used early in its commercial development. However, in more recent years materials or blends (such as certain peat mosses and sugar beet lime) that provide low density, good water holding capacity and yet an open texture to reduce buildup of carbon dioxide have been used16,39,40.

### Table 3. Agronomic behavior of non-ruffled and ruffled casing layer (Factor 2). For each factor within a column, values followed by a different letter are significantly different at 5% level according to Tukey’s HSD test. The absence of letters in the column indicates non-significance.

| Treatment       | Earliness (days from casing) | Biological efficiency (kg dt⁻¹ compost) | Number of mushroom (m⁻²) | Weight of mushroom (g) |
|-----------------|-----------------------------|----------------------------------------|--------------------------|------------------------|
| Non-ruffled     | 27.7                        | 50.99 b                                | 491 b                    | 24.7                   |
| Ruffled         | 27.7                        | 61.66 a                                | 564 a                    | 25.3                   |
| Mean            | 27.7                        | 56.33                                  | 528                      | 25.0                   |

### Figure 3. Correlations between biological efficiency and the contents of fat.

![Correlations between biological efficiency and the contents of fat.](image1)

### Figure 4. Yield obtained by flushes with non-ruffled and ruffled casing layer (Factor 2). For each flush, values followed by a different letter are significantly different at 5% level according to Tukey’s HSD test.

![Yield obtained by flushes with non-ruffled and ruffled casing layer.](image2)

Dias et al. 38, after ruffling three casing materials (sphagnum peat moss, loam soil, coconut coir), observed that a loam soil casing was highly superior to either peat or coir. Soils typically have a more dense compact structure than peat or coir. In contrast, in the cultivation of *A. bisporus* loam soil was used early in its commercial development. However, in more recent years materials or blends (such as certain peat mosses and sugar beet lime) that provide low density, good water holding capacity and yet an open texture to reduce buildup of carbon dioxide have been used16,39,40.
The Sun mushroom has a denser mycelium than *A. bisporus* and produces a greater formation of stroma in its 65 to 120 day cultivation cycle\(^2,19,41,42\). Our casing material, used commercial in The Netherlands for *A. bisporus* production, had an open structure, a high porosity (93%), a high water holding capacity (4.92 kg kg\(^{-1}\)) and a low bulk density (0.654 g cm\(^{-3}\)).

The ruffling technique may increase the cost of production, especially if hand labor is required. Ruffling machines have been used on *A. bisporus* shelf farms for decades\(^46\). If *A. subrufescens* would be grown on a shelf system, little, if any, adaptations would be necessary.

**Factor 3 (temperature management).** After spawning, the temperature of the compost in both chambers (slow and rapid induction) ranged from 23 ± 1 °C to approximately 30 ± 0.5 °C (Fig. 1A). Heating of the compost results from the metabolic process of the mycelian colonization of the substrate with its release of CO\(_2\) and heat. After casing, the compost temperatures were maintained at 28 ± 0.5 °C for 7 days. On day 21 the casing layer was ruffled (8 days after casing) and the temperature reduction of the compost began in both chambers. Slow induction of primordia was accomplished by day 29. Whereas, the rapid initiation of primordia was complete on day 26.

Harvesting of first flush started for both chambers on day 39 and ended on day 43. Maximum production in the rapid induction occurred 2 days before the slow (Fig. 4B). Pre- and post-first flush occurred in the slow induction, outside the bulk harvest days 39–43 (green arrows in Fig. 1B). Depending on the quality of these mushrooms, this pre- and post- occurrence may not be undesirable.

In the second flush, the duration of the slow induction harvest continued for 9 days and the rapid induction for two days less. The maximum production of rapid induction occurred 4 days prior to the slow induction.

In the third flush, the duration of slow induction harvest lasted 7 days and the rapid induction lasted 6 days. The highest yield harvested in any single day in the crop occurred in this flush with the amount of 2.48 kg of mushrooms per m\(^2\) for the rapid induction method. The yield difference between primordia induction methods in this flush (3.47 kg m\(^{-2}\), rapid induction; and 2.72 kg m\(^{-2}\), slow induction) was the only significance during the experiment.

Finally, the last flush terminated 3 days earlier for the rapid induction. However, both methods were harvest for 4 days. Additional the production rate was significant higher in the rapid induction method (0.73 kg dt\(^{-1}\) d\(^{-1}\)) than in the slow induction method (0.67 kg dt\(^{-1}\) d\(^{-1}\)) (data not shown).

The speed of induction significantly affected the color of the mushroom. Primordia induction slowly had a significantly darker pileus color (L\(^*\) - 56.43, a\(^*\) - 8.55 and b\(^*\) 19.74). The color of the pileus is an indicator of the maturity of Sun mushroom. The color of the pileus lightens in color as the veil is closer to opening.

The biological efficiency was not significantly affected by the mushroom induction temperature method. Our biological efficiencies (57.84%, rapid induction; and 54.81%, slow induction) were better than other reports that managed the temperatures of primordia initiation differently. Martos et al.\(^3\) obtained a biological efficiency of 40.0, 38.0 and 39.6% when the temperature was lowered to 16 °C and then maintained for 4, 6 and 8 days, respectively. Wang et al.\(^19\) maintained a constant temperature between 24 and 26 °C with the highest biological efficiency being 41.4% over a cultivation period of 120 days of 6 flushes.

**Proximate analysis.** In general, the composition of mushrooms is water (90%), protein (2–40%), fat (2–8%), carbohydrates (1–55%), fiber (3–32%) and ash (8–10%)\(^44\). The composition of *A. subrufescens* presents low water content, crude fat, crude fiber and ash, while high protein content, total carbohydrate and mean values of available carbohydrates and energy value\(^45\). Composition reported in this paper is, in common, of the same magnitude as those reported in the literature\(^56,46,47\).

The mushrooms were not significantly affected by supplementation, or presence or absence of ruffling of the casing layer. Speed of induction did significantly affect the fat content. All other parameters were not influenced (Table 4). Siqueira et al.\(^25\) evaluating composts prepared with different initial concentrations of nitrogen, found that the chemical analysis of dry mushrooms revealed not significant differences in protein, fat and ash.

| Treatment (Factors) | Dry matter (%) | Protein (Nx4.38, g kg\(^{-1}\)) | Fat (g kg\(^{-1}\)) | Carbohydrates (g kg\(^{-1}\)) | Fiber (g kg\(^{-1}\)) | Ash (g kg\(^{-1}\)) |
|--------------------|----------------|-------------------------------|-------------------|-------------------------------|-----------------|-------------------|
| Non-supplemented   | 12.47          | 283.6                         | 15.5              | 632.1                         | 62.3            | 68.8              |
| Promycel 600       | 12.39          | 272.6                         | 17.2              | 642.0                         | 62.7            | 68.2              |
| Champflood         | 12.48          | 277.3                         | 17.6              | 637.4                         | 61.8            | 67.7              |
| Calprozime         | 12.37          | 273.2                         | 17.0              | 642.3                         | 64.6            | 67.5              |
| Non-ruffled        | 12.49          | 278.8                         | 16.5              | 657.0                         | 61.7            | 67.7              |
| Ruffled            | 12.36          | 274.5                         | 17.1              | 640.0                         | 64.0            | 68.4              |
| Rapid induction    | 12.33          | 272.5                         | 18.6              | 640.9                         | 63.6            | 68.0              |
| Slow induction     | 12.52          | 280.9                         | 15.0              | 636.0                         | 62.1            | 68.1              |
| Mean               | 12.43          | 276.7                         | 16.8              | 638.5                         | 62.8            | 68.0              |

Table 4. Chemical characteristics of the mushrooms due to the supplementation of the compost (Factor 1), the used of the ruffling technique (Factor 2) and the temperature management in the induction of primordia (Factor 3). For each factor within a column, values followed by a different letter are significantly different at 5% level according to Tukey’s HSD test. The absence of letters in the column indicates non-significance.
content between the treatments, while significant differences in fiber (source of b-glucan) content were observed. Supplementation of A. bisporus compost, on-the-other-hand, increased the protein, fiber and ash content and reduced the carbohydrate content of the harvested mushrooms. The study of Eira et al. reported the influence of different strains and morphogenetic stages on the proximate analysis of A. subrufescens.

Conclusions
Champignon supplement promotes a reduction in the value of earliness and an increase of 1st flush yield. The ruffling technique provided an increase in biological efficiency due to the great number of mushrooms harvested. Rapid primordia induction allowed the crop cycle to end 3 days earlier than the slow primordia induction, providing a higher production rate.

Received: 13 December 2019; Accepted: 28 April 2020;
Published online: 18 May 2020

References
1. Kerrigan, R. W. Agaricus subrufescens, a cultivated edible and medicinal mushroom, and its synonyms. Mycologia 97, 12–24, https://doi.org/10.1080/1557236.2006.1183283 (2005).
2. Wasser, S. P. et al. Is a widely cultivated culinary–medicinal Royal Sun Agaricus (Champignon do Brazil, or the Hemimatsutake mushroom) Agaricus brasiliensis? S. Wasser et al. indeed a synonym of A. subrufescens Peck? International Journal of Medicinal Mushrooms 7, 567–511, https://doi.org/10.1615/IntJMedMushr.v7.i7.30 (2005).
3. Wistressameewong, K. et al. Agaricus subrufescens: a review. Saudi Journal of Biological Sciences 19, 131–146, https://doi.org/10.1016/j.sjbs.2012.01.003 (2012).
4. Win, T. T. & Ohga, S. Study on the cultivation of Agaricus blazei (almond mushroom) grown on compost mixed with selected agro-residues. Advances in Microbiology 8, 778–789, https://doi.org/10.4236/am.2018.810055 (2018).
5. Royse, D. J., Baars, J. & Tan, Q. Current overview of mushroom production in the world. In Edible and Medicinal Mushrooms: Technology and Applications, ed. by Zied, D. C. & Pardo-Gimenez, A. Wiley-Blackwell, West Sussex, England, pp 5–13, https://doi.org/10.1002/9781119149446.ch2 (2017).
6. Sánchez, E. J., Zied, D. C. & Alberto, E. Edible mushroom production in the Americas, in 9th International Conference on Mushroom Biology and Mushroom Products. WSMMP, Shanghai, China, pp. 2–11, (2018).
7. Eira, A. F. et al. Farming technology, biochemistry characterization, and effect of active ingredients of culinary–medicinal mushrooms Agaricus brasiliensis. S.Wasser et al. and Lentinus edodes (Berk.) Singer: five years of research in Brazil. International Journal of Medicinal Mushrooms 7, 281–299, https://doi.org/10.1615/IntJMedMushr.v7.i2.260 (2005).
8. Wang, Q., Li, B. B., Li, H. & Han, J. R. Yield, dry matter and polysaccharides content of the mushroom Agaricus blazei produced on asparagus straw substrate. Scientia Horticulturae 125, 16–18, https://doi.org/10.1016/j.scienta.2010.02.022 (2010).
9. Fritsche, G. & Sonnenberg, A. S. M. Mushroom strains. In The Cultivation of Mushrooms, ed. by van Gremse, L. J. L. D., Intelingua, Sussex, England, pp.101–123 (1988).
10. Fukuda, M., Ohno, S. & Kato, M. Genetic variation in cultivated strains of Agaricus blazei. Mycoscience 44, 431–436, https://doi.org/10.1007/S10267-003-0136-X (2003).
11. Llarena-Hernández, R. C. et al. Potential of European wild strains of Agaricus subrufescens for productivity and quality on wheat straw based compost. World Journal of Microbiology and Biotechnology 29, 1243–1253, https://doi.org/10.1007/s11274-013-1287-3 (2013).
12. Llarena-Hernández, C. R., Largeteau, M. L., Ferrer, N., Regnault-Roger, C. & Savoie, J. M. Optimization of the cultivation conditions for mushroom production with European wild strains of Agaricus subrufescens and Brazilian cultivars. Journal of the Science, Food and Agriculture 94, 77–84, https://doi.org/10.1002/jsfa.6200 (2014).
13. Savoie, J. M., Llarena-Hernández, R. C., Mata, G. & Largeteau, M. L. (2012). Cultivation of medicinal almond mushrooms, Agaricus subrufescens, in Europe. In Mushroom Biology and Bioengineering, ed. by Petre, M. & Berovic, M. C. MPress Publishing House, Bucharest, Romania, pp. 115–125 (2012).
14. Arce-Cervantes, O. et al. Alternative supplements for Agaricus bisporus production and the response on lignocellulolytic enzymes. Scientia Horticulturae 192, 375–380, https://doi.org/10.1016/j.scienta.2015.06.030 (2015).
15. Pardo-Giménez, A. et al. Effect of supplementing crop substrate with defatted pistachio meal on Agaricus bisporus and Pleurotus ostreatus production. Journal of the Science, Food and Agriculture 96, 3838–3845, https://doi.org/10.1002/jsfa.7579 (2016).
16. Pardo-Giménez, A., Pardo-González, J. E. & Zied, D. C. Casing materials and techniques in Agaricus bisporus cultivation. In Edible and Medicinal Mushrooms: Technology and Applications, ed. by Zied, D. C. & Pardo-Gimenez, A. Wiley-Blackwell, West Sussex, England, pp. 149–174, https://doi.org/10.1002/9781119149446.ch7 (2017).
17. Gregori, A., Pahor, B., Glaser, R. & Pohleven, F. Influence of carbon dioxide, inoculum rate, amount and mixing of casing soil on Agaricus blaezi fruiting bodies yield. Acta agriculturae Slovenica 91, 371–378, https://doi.org/10.2478/v10014-008-0017-2 (2008).
18. González Matute, R., Figlas, D. & Curvetto, N. Sunflower seed hull based compost for Agaricus blaezi Murrill cultivation. International Biodeterioration and Biodegradation 64, 742–747, https://doi.org/10.1016/j.ibiod.2010.08.008 (2010).
19. Wang, J. T., Wang, Q. & Han, J. R. Yield, polysaccharides content and antioxidant properties of the mushroom Agaricus subrufescens produced on different substrates based on selected agricultural wastes. Scientia Horticulturae 157, 84–89, https://doi.org/10.1016/j.scienta.2013.04.006 (2013).
20. Zied, D. C. et al. Effect of cultivation practices on the β-glucan content of Agaricus subrufescens basidocarps. Journal of Agriculture and Food Chemistry 62, 41–49, https://doi.org/10.1021/jf040358g (2014).
21. Andrade, M. C. N., Kopytowski Filho, J., Minhoni, M. T. A., Coutinho, L. N. & Figueiredo, M. B. Productivity, biological efficiency, and number of Agaricus blaezi mushrooms grown in compost in the presence of Trichoderma sp. and Chaetomium olivaceum contaminants. Brazilian Journal of Microbiology 38, 243–247, https://doi.org/10.1590/S1517-83822007000200010 (2007).
22. Zied, D. C., Pardo, J. E., Thomaz, R. S., Miasaki, C. T. & Pardo-Gimenez, A. Mycotoxicological characterization of Agaricus subrufescens considering their morphological and physiological stage of maturity on the traceability process. Biomedical Research International 127, 1–11, https://doi.org/10.1155/2017/2713742 (2017).
23. Cosyn, J. Les facteurs de la fructification de l'Agaricus bisporus (champignon de couche). Bull EFSA 14, 585–604 (1972).
24. Kopytowski Filho, J. & Minhoni, M. T. A. Produtividade e eficiência biológica da linhagem AFLB 99/30 de Agaricus blaezi em três tipos de compostos e em dois ambientes de cultivo. Energia na Agricultura 22, 65–78 (2007).
25. Colauto, N. B., Silveira, A. R., Eira, A. F. & Linde, G. A. Alternative to peat for Agaricus brasiliensis yield. Bioresource Technology 101, 712–716, https://doi.org/10.1016/j.biortech.2009.08.052 (2010).
26. Miles, P. G. & Chang, S. T. The chemical composition of fungal cells. Useful generalizations. In Mushroom biology. Concise basics and current developments, ed. by Miles, P. G. & Chang S. T. World Scientific Publishing, Singapore, pp.33–35 (1997).
27. Ankrom. Rapid determination of oil/fat utilizing high temperature solvent extraction. Ankrom Technology Method 2, AOCS Official Procedure Am 5-04. Macedon, USA, (2009).
28. Ankom. Crude Fiber Analysis in Feeds By Filter Bag Technique. Ankom Technology Method 7, AOCS Approved Procedure B4.05. Macedon, USA (2008).

29. Lau, O. Methods of chemical analysis of mushrooms. In Tropical Mushrooms. Biological Nature and Cultivation Methods, ed. by Chang, S. T. & Quimio, T. H. The Chinese University Press, Hong Kong, China, pp. 87–116 (1982).

30. Zied, D. C. et al. Using of appropriated strains in the practice of compost supplementation for Agaricus subrufescens production. Fungal Biotechnology 2, 26, https://doi.org/10.3389/fsf.2018.00026 (2018).

31. Randle, P. E. & Smith, J. F. Economic aspects of compost supplementation. Mushroom Journal 165, 297–305 (1986).

32. Royse, D. J., Sanchez, J. E., Beelman, R. B. & Davidson, J. Re-suplementing and recasing mushroom (Agaricus bisporus) compost for a second crop. World Journal of Microbiology and Biotechnology 24, 319–325, https://doi.org/10.1007/s11274-007-9473-9 (2008).

33. Royse, D. J. & Sanchez, J. E. Supplementation of 2nd break mushroom compost with isoleucine, leucine, valine, phenylalanine, Fermenten® and SoyPlus®. World Journal of Microbiology and Biotechnology 24, 2011–2017, https://doi.org/10.1007/s11274-008-9703-9 (2008).

34. Lyons, G. A., Sharma, H. S., Kilpatrick, M., Cheung, L. & Moore, S. Monitoring of changes in substrate characteristics during mushroom compost production. Journal of Agricultural and Food Chemistry 54, 4658–4667, https://doi.org/10.1021/jf052934i (2006).

35. Siqueira, F. G., Martos, E. T., Silva, E. G., Silva, R. & Dias, E. S. Biological efficiency of Agaricus brasiliensis cultivated in compost with nitrogen concentrations. Horticultura Brasileira 29, 157–161, https://doi.org/10.1590/0100-05362011000200004 (2011).

36. Navarro, M. J., Merino, L. & Gea, F. J. Evaluation of residue risk and toxicity of different treatments with diazinon insecticide applied to mushroom crops. Journal of Environmental Science and Health, Part B 52, 218–221, https://doi.org/10.1080/03601234.2017.1261555 (2017).

37. Oh, Y. I. et al. Cultural and morphological growth characteristics of a new white button mushroom cultivar ‘Saedo’. The Korean Journal of Mycology 44, 94–99, https://doi.org/10.4489/KJM.2016.44.2.94 (2016).

38. Dias, E. S., Zied, D. C. & Rinker, D. L. Physiologic response of Agaricus subrufescens using different casing materials and practices applied in the cultivation of Agaricus bisporus. Fungal Biology 117, 569–575, https://doi.org/10.1016/j.fungbi.2013.06.007 (2013).

39. Van Gils, J. J. Cultivation. In The Cultivation of Mushrooms, ed. by van Griensven, L. J. L. D., Interlingua, Sussex, England, pp. 263–308, (1988).

40. Vedder, P. J. C. Practical experience with the cac’ing technique. Mushroom Science 12, 381–385 (1989).

41. Largeteau, M. L., Llarena-Hernández, R. C. & Regnault-Roger, C. The medicinal Agaricus mushroom cultivated in Brazil: biology, cultivation and non-medicinal valorisation. Applied Microbiology. Biotechnology 92, 897–907, https://doi.org/10.1007/s00253-011-3630-7 (2011).

42. Lissecka, J., Sobieralski, K., Siwulski, M. & Isińska, A. Almond mushroom Agaricus brasiliensis (Wasser et al.) properties and culture conditions. Acta Scientiarum Polonorum-Hortorum Cultus 12, 27–40 (2013).

43. Martos, E. T. et al. Casing layer and effect of primordia induction in the production of Agaricus subrufescens mushroom. Agriculture and Natural Resources 51, 231–234, https://doi.org/10.1016/j.anres.2017.04.003 (2017).

44. Firenzuoli, F., Gori, L. & Lombardo, G. The medicinal mushroom Agaricus blazei Murrill: review of literature and pharmaco-toxicological problems. Evid.-based Complement Altern. Med 5, 3–15, https://doi.org/10.1093/ecam/nem007 (2008).

45. Chang, S. T. & Miles, P. G. Agaricus blazei and Grifola frondosa – Two important medicinal mushrooms. In Mushrooms. Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact, 2nd edition. CRC Press, Boca Raton, FL, USA, pp. 373–381 (2004).

46. Eira, A. F., Nascimento, J. S., Colauto, N. B. & Celso, P. G. Tecnologia de cultivo do cogumelo medicinal Agaricus blazei Murrill: review of literature and pharmaco-toxicological problems. Evid.-based Complement Altern. Med 5, 3–15, https://doi.org/10.1093/ecam/nem007 (2008).

47. Carneiro, A. A. J. et al. Chemical composition and antioxidant activity of dried powder formulations of Agaricus blazei and Lentinus edodes. Food Chemistry 138, 2168–2173, https://doi.org/10.1016/j.foodchem.2012.12.036 (2013).

Acknowledgements
This research was supported by the Diputación Provincial de Cuenca in Spain, and the Fundação de Amparo à Pesquisa do Estado de São Paulo in Brazil (FAPESP 2018/21492-2).

Author contributions
conception and design: A.P., D.C.Z.; acquisition, analysis and interpretation of data; statistical analysis; technical support; A.P., J.E.P., E.S.D., D.L.R., C.E.C.C.; supervising the work; drafting and critical revision of the manuscript: A.P., D.C.Z.; coordinating the research project: D.C.Z.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-65081-2.

Correspondence and requests for materials should be addressed to D.C.Z.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020