PRODUCTION OF *Panus strigellus* SPAWN USING THE INTERNAL SHEATH OF PEACH PALM (*Bactris gasipaes*) AS A SUBSTRATE

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SUMMARY

Mycelial growth takes place at elevated temperatures in Panus strigellus*, which makes it a promising species for cultivation in tropical regions. The use of locally available substrates is the first step in cost-effective mushroom production. Peach palm,* Bactris gasipaes *is grown in palm agribusinesses in Amazonas State, Brazil; one of the waste products of this crop is its protective internal sheath (PPIS). The potential use of PPIS for* P. strigellus *spawn production was evaluated through the analysis in Petri dishes of the effects of substrate moisture and sawdust supplementation levels. Mycelial growth was evaluated by measuring the growth (cm/day) and vigor of the colony. A humidity of 60% and supplementation of* Simarouba amara *sawdust with PPIS in a 10:1 ratio was considered the most suitable combination. This formulation was applied for spawn preparation in polypropylene bags. Spawn production of* P. strigellus *was successfully achieved after 25 days of incubation at 35°C.*

USO DE LA VAINA INTERNA DEL PIJUAYO (*Bactris gasipaes*) PARA LA PRODUCCIÓN DE SEMILLA-INÓCULO DE *Panus strigellus*

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RESUMEN

Panus strigellus *presenta crecimiento micelial en temperaturas elevadas, favoreciendo el desarrollo de su cultivo en regiones tropicales. Obtener substratos localmente disponibles es el primer paso para el cultivo rentable de setas. El pijuayo,* Bactris gasipaes*, es cultivado en las agroindustrias de palmito en el Estado de Amazonas, Brasil; uno de los residuos originados de la palmera es la vaina interna protectora (PPIS). Se evaluó en placas de Petri el potencial de uso de la PPIS para la producción de semilla-inóculo de* P. strigellus*, analizando el efecto de la humedad del substrato y los niveles de suplementación de aserrín por PPIS. El crecimiento micelial fue evaluado por la medida de la colonia en cm/día y el vigor. La humedad de 60% y* Simarouba amara *suplementada con PPIS en la proporción 10:1 fue considerado como el mejor resultado. Esta formulación fue aplicada en la elaboración de la semilla-inóculo de* P. strigellus *con éxito después de 25 días de incubación a 35°C.*

Introduction

In Brazil, heart of palm is extracted from several genera and species of palm. Although *Euterpe edulis* Mart. ('juçara') and *E. oleracea* Mart. ('açaí') have been used for heart of palm production, more heart of palm has been produced (Clement and Bovi, 2000) using *Bactris gasipaes* Kunth or 'peach palm', known locally as 'pupunha', as 'pijuayo' in Peru, 'pijiguao' in Venezuela, 'pejibaye' in Costa Rica and Nicaragua, 'chontaduro' in Colombia and Ecuador, 'tembé' in Bolivia, and 'pibá' in Panama (http://en.wikipedia.org/Bactris_gasipaes).

Bactris gasipaes is the only domesticated Neotropical palm whose starchy-oily fruits are subsistence products, and heart of palm production is an expanding agribusiness (Silva and Clement, 2005). The micro-businesses of peach palm in Amazonas State, including the Manaus region, produced 45.1ton of heart of palm in 2012 (IDAM, 2012). The protective internal sheath of *B. gasipaes* is discarded during the palm core production process, generating large amounts of unused residue. Some studies have examined the use of these residues in mushroom cultivation. Tonini *et al.* (2007) investigated the use of *E. edulis* sheaths as a medium for the cultivation of *Lentinula edodes* (Berk.) Pegler in axenic culture and obtained successful basidiomata production. Sales-Campos and An-

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USO DA BAINHA INTERNA DA PUPUNHEIRA (*Bactris gasipaes*) PARA PRODUÇÃO DE SEMENTE-INÓCULO DE *Panus strigellus*

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RESUMO

Panus strigellus *apresenta crescimento micelial em temperaturas elevadas, favorecendo o desenvolvimento do cultivo em regiões tropicais. Obter substratos disponíveis localmente é o primeiro passo para cultivo rentável de cogumelos. A pupunha,* Bactris gasipaes*, é cultivado nas agroindústrias de palmito no Amazonas, Brasil; um dos resíduos da palmeira é sua bainha interna protetora (PPIS). Neste trabalho foi avaliado o potencial de uso do PPIS para a produção de semente-inóculo de* P. strigellus*. Em placas de Petri foram analisados o efeito da umidade do substrato e níveis de suplementação de serragens por PPIS. O crescimento micelial foi avaliado pela medida da colônia em cm/dia e vigor. A umidade de 60% e* Simarouba amara *suplementada com PPIS na proporção 10:1 foi considerada o melhor resultado. Esta formulação foi aplicada na elaboração da semente-inóculo em sacos de polipropileno. Obteve-se com sucesso a semente-inóculo de* P. strigellus *após 25 dias de incubação a 35°C.*

drade (2010) studied *Panus lecomtei* (Fr.) Corner (*Lentinus strigosus* Fr.) mycelia growth on *B. gasipaes* stipes.

Panus strigellus (Berk.) Overh. has very interesting prospect for the development of mushroom cultivation in the Amazonas State, considering its characteristics of mycelial growth at 35-40°C and accentuated *umami* taste (Vargas-Isla and Ishikawa, 2008). Screening has been conducted using 11 forest species and regional fruit residues of the Central Amazon to produce the spawn of *P. strigellus*; among these, *Simarouba amara* Aubl. ('marupá') sawdust and *Astrocaryum aculeatum* Meyer ('tucumã') fruit shell formulations presented the best alternatives for *P. strigellus* spawn production (Vargas-Isla *et al*., 2012). However, the fruit shell residues are only produced in small amounts by fruit processing facilities and are thus unlikely to be available on the scale required for spawn production.

The main steps for mushroom production are eight, as follows. 1) Stock culture is first grown on sterilised media in Petri plates and/or in tubes. 2) Spawn or inocula are prepared, where the spawn are any form of mycelia that can be dispersed into a substrate. The most common forms of spawn substrate are grain or sawdust; cereal grains such as wheat (*Triticum aestivum* (L.) Thell.) may be also used for spawn production. The next step is the exponential expansion of the mycelial mass. The pure culture is transferred to sterilised grain. Then, sawdust spawn are inoculated with grain spawn into the sawdust formulation. 3) The substrate is formulated, usually using a carbon source such as sawdust, sugar cane (*Saccharum officinarum* L.) bagasse, or corn (*Zea mays* L.) cobs, supplemented with a nitrogen source such as cereal bran. 4) Spawn are inoculated into the substrate of mushroom culture. 5) Spawn running. 6) Fruiting induction, e.g. by thermal shock, mechanical shock, injury and illumination. 7) Fructification. 8) Harvesting.

The spawn constitutes the base for the commercial cultivation of edible mushrooms, and spawn production is the main challenge faced by commercial mushroom producers. The current study evaluated formulations using the *B. gasipaes* internal sheath (PPIS) as an alternative for use in a substrate for *P. strigellus* spawns.

Material and Methods

Microrganism

The *P. strigellus* INPACM 1464 culture from the Coleção de Microrganismos de Interesse Agrossilvicultural, Instituto Nacional de Pesquisas da Amazônia (INPA), was used. The stock culture and inocula for the experiments were the same as those reported by Vargas-Isla *et al*. (2012).

Residues for substrate formulation

Bactris gasipaes stipes (1.50m height; n= 5) were cut on Campus III, INPA, to obtain PPIS. The samples were weighed and divided into stipe, leaves, external and internal sheath, and heart of palm. Following dehydration at 65°C during 48h, PPIS was crushed in an industrial blender (LSP-04, Siemsen Ltd., Brusque-SC, Brazil) and then transferred and ground in a Willye TE-680 (Tecnal, Piracicaba-SP, Brazil) grinder, using a 0.5mm-mesh sieve. Sawdust of *S. amara* and *Hymenolobium petraeum* Ducke ('angelim-pedra'), *A. aculeatum* fruit shell (TFS), and rice bran (*Oryza sativa* L.) were used for comparison. Each residue was oven-dried at 65°C with air circulation and stored in plastic bags at room temperature (~25°C).

Centesimal analysis

The centesimal composition analysis of the residues was conducted in accordance with the AOAC (1998) methodology (n= 3).

Substrate humidity

For the humidity measurement, sawdust of *H. petraeum* mixed with rice bran (sawdust:supplement 10:1 w/w) was used and distilled water was added to the formulation to reach 30, 40, 50, 60 and 70% hydration (w/v) prior to distribution into Petri plates (15 ±1 g/plate) and sterilized 1h at 121°C. Following sterilization, one mycelial disk was deposited in the center of the plate containing the formulation (Vargas-Isla *et al*., 2012). Incubation temperature was 35°C.

Mycelial growth was evaluated by measurement colony diameter change (cm/day) and colony vigor was visually evaluated and classified as (+) thin, (++) medium or (+++) dense.

Substrate formulations

The experiment with different substrate formulations was carried out in two stages. First, the PPIS and *S. amara* sawdust were mixed and supplemented separately with TFS and rice bran in proportions of 10:1 and 5:1 (w/w), and mixed with distilled water to ~60% hydration (w/v). Pure *S. amara* sawdust was used as a control. Second, sawdust of *H. petraeum* and *S. amara* was prepared and mixed separately with PPIS and rice bran (1:1 and 10:1 w/w), with distilled water added to ~60% hydration (w/v). The formulations were then distributed on Petri plates (15 ±1 g/plate) and sterilized. The experiment was evaluated after five days using the criteria of substrate humidity test of mycelial growth (growth measurement in cm/day and visual determination of colony vigor).

Statistical analysis

Each experiment was conducted twice using five replicates. Analysis of variance (ANOVA) was used to examine the results of the experiments, and the averages were compared using the Tukey test at the 1% level of significance.

TABLE I
DISTRIBUTION OF FRESH MATERIAL OBTAINED FROM *Bactris gasipaes* *

	Parts of palm				
	Stipe	Leaves	External sheath	Internal sheath	Heart of palm
Fresh weight (kg)	17.9	5.5	1.7	2.4	0.7
Percentage	63.5	19.5	6.0	8.5	2.5

*Data shown represent the average of five samples of ~1.5m height.

TABLE II
COMPOSITION (% DRY MATTER) OF THE RESIDUES OBTAINED IN MANAUS, AM, BRAZIL *

Residues	Ash	Protein	Lipids
Astrocaryum aculeatum fruit shell	3.28 ±0.049	5.55 ±0.041	24.29 ±0.081
Bactris gasipaes internal sheath	3.79 ±0.040	3.65 ±0.046	0.88 ±0.062
Oryza sativa bran	3.28 ±0.407	13.07 ±0.701	14.76 ±0.002
Simarouba amara sawdust	0.13 ±0.012	1.34 ±0.034	0.42 ±0.0003

*Data shown represent the average of three samples according to AOAC (1998) methods.

Spawn production

S. amara sawdust supplemented with PPIS (10:1) was placed in polypropylene packing (23×36cm) with 800g of wet substrate with a respirator, using a ring of PVC tubing with 3cm height × 5cm diameter and hydrophobic cotton for gas exchange (Vargas-Isla *et al*., 2012). The substrate package was sterilized for 1h at 121°C. Following sterilization, one mycelial disk was deposited in the center of the plate containing the formulation (Vargas-Isla *et al*., 2012). Incubation temperature was 35°C. After 25 days, the colonized substrates were taken out of the packing and cut for visual observation (n= 5).

Results and Discussion

Table I lists the quantity and distribution of heart of palm and residue obtained from *B. gasipaes*. The total residue is equivalent to 97.5%, of which the internal sheath residue represents 8.5%. Considering these data and the 2012 production of heart of palm from *B. gasipaes* in the Amazonas State, we estimated that over 1768ton of residue has been discarded, indicating that this residue is readily available. The fresh mushroom marketing in the Manaus region is based on two species: shiitake (*L. edodes*) and shimeji (*Pleurotus* spp.) imported from Sao Paulo State.

Supplementation with a nitrogen source is necessary because pure *S. amara* sawdust contains only 1.34% crude protein (Table II). The PPIS contained more protein (3.65%) than *S. amara* sawdust but less protein than rice bran (13.07%). The nutritional supplements were added to increase the levels of nitrogen and useable carbohydrates because nitrogen levels in the sawdust were low, which may be a limiting factor for decay.

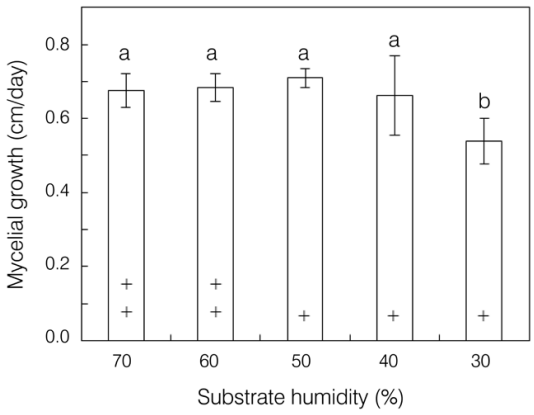

Figure 1. Effect of substrate humidity on *Panus strigellus* mycelial growth. Substrate formulation was a mixture of *Hymenolobium petraeum* sawdust and rice bran (10:1 w/w). Data shown represent the average of five replicates and two repetitions of mycelial growth (cm/day). Means indicated by the same letter are not significantly different (p<0.01) by the Tukey test. Colony vigor levels are +: thin, ++: medium, and +++: dense.

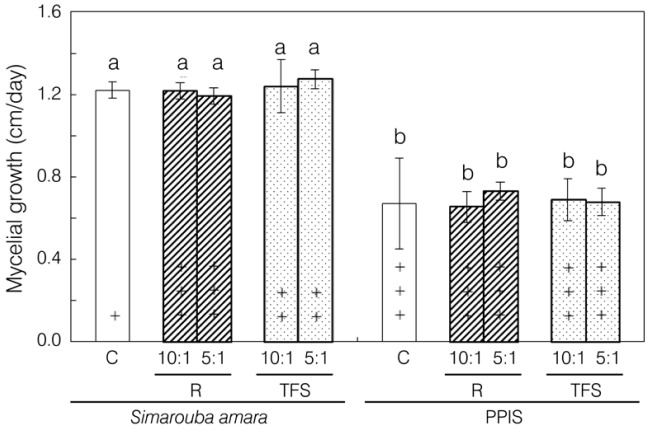

Figure 2. Effect of supplementing *Bactris gasipaes* internal sheath and *Simarouba amara* sawdust on *Panus strigellus* mycelial growth at 35°C. C: control, R: rice bran, TFS: *Astrocaryum aculeatum* fruit shell, PPIS: *B. gasipaes* internal sheath. Substrate composition was 10:1 and 5:1 (sawdust:supplement, w/w). Data shown represent the average mycelial growth (cm/day) in five replications and two repetitions. Means indicated by the same letter(s) are not significantly different (p<0.01) by the Tukey test. Colony vigor levels are +: thin, ++: medium, and +++: dense.

In Brazil, the base material in the major substrates used for edible mushroom cultivation in the south and southeast include rice straw and sugar cane bagasse for *Agaricus bisporus* (J.E. Lange) Pilát; *Eucalyptus* sp. and *Quercus acutissima* Carr. for *L. edodes*; and sugar cane bagasse (*Saccharum officinarum* L.) for *Pleurotus* spp. (Bononi and Trufem, 1986)

Alternative sources have also been evaluated, such as corn cob (Eira *et al*., 2005), elephant grass (*Pennisetum purpureum* Schum.; Donini *et al*., 2005; Bernardi *et al*., 2007), coffee husks (*Coffea arabica* L.; Fan *et al*., 2006), peach palm stipe (*B. gasipaes*; Sales-Campos and Andrade, 2010), pineapple crown (*Ananas comosus* (L.) Merr.), 'cupuaçú' fruit shell (*Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum.), banana peel (*Musa* sp.), and 'tucumã' fruit shell (Aguiar *et al*., 2011).

Considering both criteria of mycelial growth, in the present case the best substrate condition was provided by 60 and 70% humidity (p<0.01; Figure 1). The best substrate moisture depends on the mushroom cultivation species; e.g. 55-70% substrate moisture for *L. edodes* and 60-80% substrate moisture for *Pleurotus* spp. (Chang and Hayes, 1978).

The substrates formulated with *S. amara* sawdust provided the highest radial mycelial growth (cm/day) of *P. strigellus* (p<0.01; Figure 2). However, both rice bran and TFS supplementation improved the colony vigor as compared with the control *S. amara* sawdust. Although the mycelial growth on PPIS formulations supplemented with rice bran and TFS was lower, the colony vigor was at the highest level; thus, in the second experiment PPIS supplemen-

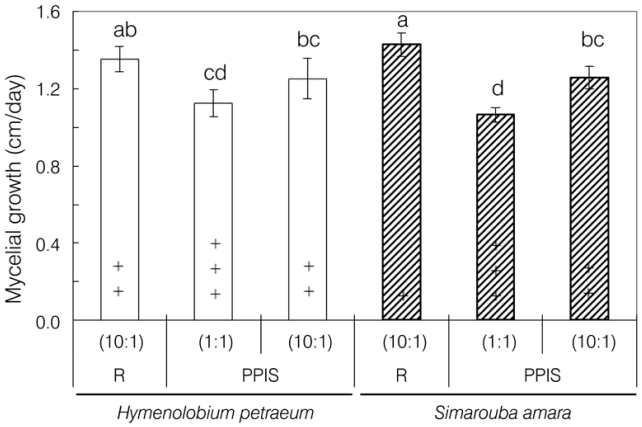

Figure 3. Effect of supplementing sawdust with *Bactris gasipaes* internal sheath on *Panus strigellus* mycelial growth at 35°C. R: rice bran, PPIS: *B. gasipaes* internal sheath. Substrate was composed of 10:1 and 1:1 (sawdust:supplement, w/w). Data shown represent the average mycelial growth (cm/day) in five replicates and two repetitions. Means indicated by the same letter are not significantly different ($p<0.01$) by the Tukey test. Colony vigor levels are +: thin, ++: medium, and +++: dense.

tation was used. The use of large amounts of cereal bran (rice, wheat and soybean) as supplements would increase the cost of substrate formulation in the region. However, regional agroforestry residues are produced in large amounts and are rarely used. For example, *A. aculeatum* fruit is consumed widely in regional dishes, and its fruit shell residue is generally not used (Vargas-Isla *et al*., 2012).

The effect of supplementing *H. petraeum* and *S. amara* sawdust with PPIS on *P. strigellus* mycelial growth was tested (Figure 3). Formulations with both sawdust and PPIS (1:1) presented the lowest radial mycelial growth (cm/day). Considering both criteria (radial mycelial growth and colony vigor), the sawdust:PPIS (10:1) formulation presented the best alternative. Fungi are carbon and nitrogen heterotrophs, so they must be supplied with both in some combined form. Nitrogen is also needed for the synthesis of other cell components (nucleic acids and chitin), and carbon is required as an energy source (Carlile *et al*., 2001). Carbon:nitrogen ratios of 10:1 or less will ensure a high protein content, whereas ratios in excess will favour the accumulation of alcohol, acetate-derived secondary metabolites, lipids, or extracellular polysaccharides (Carlile *et al*., 2001).

Regarding spawn production, *S. amara* substrates (800g) had been totally colonized by *P. strigellus* after 25 days of incubation at 35°C in the polypropylene bag.

Conclusion

The results suggest that the sawdust supplementation is necessary. The internal sheath of *Bactris gasipaes* (PPIS) is a supplementation alternative for the spawn production of *Panus strigellus*.

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