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EFFECTS OF SYNTHETIC NUTRIENT CARRIERS ON THE FRUITING OF *PLEUROTUS OSTREATUS* VAR. *COLUMBINUS*

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Abstract

Pleurotus ostreatus *var.* columbinus *was cultivated on two kinds of perlite-defined substrates (PDS). In a first experiment, PDS with either cellulose, sugar, nitrogen or mineral-starvation were tested using pure washed mycelium as inoculum in reference to grain spawn and compared with a wheat-straw substrate. Cellulose, nitrogen and minerals were found to be essential compounds for* Pleurotus *fruiting. When grain spawn was used as inoculum, nitrogen and minerals supplied by the grain were sufficient for fruiting, in spite of nitrogen and mineral-starvation. Protein content of fruitbodies increased when grown on PDS inoculated with spawn only. In a second experiment, PDS as synthetic substitutes for wheat straw and grass hay were inoculated with pure washed mycelium and compared to natural substrates. In spite of mineral and sugar supplement, synthetic substitutes for wheat straw gave a fruitbody yield lower than that recorded on natural wheat straw. The protein content of fruitbodies harvested from all substrates did not vary.*

Key words: Pleurotus ostreatus var. *columbinus*, perlite-defined substrates, fruitbody yield, protein content.

INTRODUCTION

Careful control of nutritional factors in mushroom cultivation substrates may increase yield and protein content of fruitbodies (Tshinyangu, 1994). Many lignocellulosic materials are generally used as substrates for cultivation of *Pleurotus*, a saprobiotic edible mushroom. The nutrient composition of the substrate is one of the factors limiting the saprobiotic colonization of cultivated mushrooms and particularly the fruiting of *Pleurotus*. However, the substrate composition can vary, depending on substrate nature and storage conditions. Consequently, fruitbody yield and protein content of *Pleurotus* could be affected. When supplementation is assayed with natural substrates, it is not possible to detect which nutrient is limiting if the pre-existing substrate nutrients are unknown.

Several inorganic supporting materials, like perlite, vermiculite, glass-wool and pumice stones (Ballero *et al.*, 1990; Miles & Chang, 1987), have been used in mushroom cultivation. To those, supporting material complex organic products, such as olive wastes, are added (Ballero *et al.*, 1990). To acquire a good knowledge of the efficiency of nutrients, it is necessary to study nutrient effects by growing mushrooms on chemically defined substrates. Perlite-based substrates have been tested as synthetic substrates for *Pleurotus* cultivation (Kerem & Hadar, 1993).

In this investigation, perlite-defined substrates containing all supposed essential nutrients, but using no complex products, were tested. The intention was to define which nutrients are necessary for *Pleurotus* fruiting on perlite-defined substrates and under what concentration. Pure washed mycelium of *Pleurotus ostreatus* var. *columbinus* was used for inoculation in reference to grain spawn. A simulation of wheat straw and grass hay substrates was also performed with perlite-defined substrates to see whether fruitbody yield and protein content of *Pleurotus* on synthetic substitutes might approach those observed on two natural substrates.

METHODS

Pleurotus ostreatus var. *columbinus* MUCL 28785 from the Mycothèque de l'Université Catholique de Louvain collection was tested on two series of perlite-defined substrates in glass bottles (9·5 cm diameter × 12·5 cm high), as described below. Inoculum was either spawn on wheat grain or pure washed mycelium from submerged liquid culture.

Production of inoculum

Spawn was prepared by the method described by Senyah *et al.* (1989). Wheat grain was heated in water at 80°C until it became soft (approximately 50% moisture). The grain was then decanted, air-dried and mixed with 1% (w/w) $CaCO_3$ and $CaSO_4$

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before being autoclaved in polypropylene bags (12 cm diameter × 30 cm high) at 121°C for 30 minutes. On cooling, the grain was inoculated with pieces (8 mm diameter) of a 14-day agar-plate culture of *Pleurotus* and incubated at 25°C for 14 days in darkness.

Pure washed mycelium was prepared as described by Leathnam (1983). A piece of 14-day agar-plate culture of *Pleurotus* was submerged in 30 ml of sterile liquid medium (Cailleux *et al.*, 1974) in a static 500-ml Erlenmeyer flask. After 7 days of incubation at 25°C, fungal biomass was collected by decantation, washed twice and homogenized in 100 ml of sterile distilled water.

Perlite-defined substrates (PDS) with nutrient starvation

In the first experiment, five perlite-defined substrates composed of cellulose, sugar, nitrogen and/or minerals were tested for their ability to induce *Pleurotus* fruiting and to change the protein content of fruitbodies. These substrates were defined as follows: CNSM — perlite-defined substrate (PDS) contained cellulose, sugar, nitrogen and minerals; CNM — the same PDS but lacking sugar; CSM — the same PDS but lacking nitrogen; CNS — the same PDS but lacking minerals; NSM — the same PDS but lacking cellulose. L-Glutamic acid at 0·2% (w/w of fresh substrate), cellulose at 3·3% (w/w) and dextrose at 1% (w/w) were used as nitrogen and carbon sources. Minerals were used according to Cailleux *et al.* (1974) as a mixture of KH_2PO_4, 0·15% (w/w); $CaH_4(PO_4)_2$, 0·08% (w/w); $MgSO_4 \cdot 7H_2O$, 0·2% (w/w); $FeSO_4 \cdot 7H_2O$, 0·002% (w/w); $MnSO_4 \cdot 7H_2O$, 0·002% (w/w) and $ZnSO_4 \cdot 7H_2O$, 0·002% (w/w). The quantities of ingredients were calculated for 300 g of wet substrate per bottle. All ingredients except cellulose were previously solubilized in 235 ml distilled water. The pH of the nutritive solution was adjusted to 6·0 using 1 N NaOH. The nutritive solutions were added to 55 grams of perlite previously mixed with 10 g of cellulose powder in the bottle. The fruiting of *Pleurotus* on those PDS, inoculated with either pure washed mycelium or grain spawn, was compared with fruiting on natural wheat straw and grass hay.

Perlite-defined substrates (PDS) as synthetic substitutes for wheat straw and grass hay

In the second experiment, seven perlite-defined substrates were tested in comparison to substrate controls, wheat straw and grass hay. The synthetic substrates were composed of perlite supplemented with amino acids, major minerals, oligoelements, cellulose and sugars in the concentrations equal to those found in natural wheat straw and grass hay substrates (Tables 1–5). The amounts were based on the analysis of natural wheat straw and grass hay (Tshinyangu, 1994). Aspartic acid, glutamic acid, alanine, asparagine, cysteine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine were used as L-amino acids. $CaCl_2.H_2O$, $MgSO_4 \cdot 7H_2O$, KH_2PO_4, KCl and $C_2H_3O_2Na$ were used as major mineral sources. $FeSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $MnSO_4 \cdot 7H_2O$ and $ZnSO_4 \cdot 7H_2O$ were used as mineral oligoelement sources. D-Glucose, D-fructose and

Table 1. Amounts of amino acids in synthetic substitutes for wheat straw and grass hay (g/kg wet substrate, 78% water content)

Amino acid	Substitute for wheat straw (W)	Substitute for grass hay (G)	ΔA in intermediate substitutes
Aspartic acid	0·35	1·81	1·46
Glutamic acid	0·36	1·71	1·35
Alanine	0·27	1·14	0·87
Arginine	0·17	0·81	0·64
Cysteine	0·09	0·24	0·15
Glycine	0·19	0·74	0·55
Histidine	0·06	0·31	0·25
Isoleucine	0·13	0·59	0·46
Leucine	0·22	1·00	0·78
Lysine	0·17	0·79	0·62
Methionine	0·04	0·20	0·16
Phenylalanine	0·14	0·71	0·57
Proline	0·21	1·03	0·82
Serine	0·19	0·75	0·56
Threonine	0·19	0·70	0·51
Tyrosine	0·08	0·41	0·33
Valine	0·20	0·86	0·66

ΔA: Difference in amino acid added to wheat straw substitute to prepare intermediate substitute (WΔCA).

Table 2. Amounts of nitrogen in the amino acids in synthetic substitutes for wheat straw and grass hay according to Table 1 (g/kg wet substrate)

Amino acid	Substitute for wheat straw (W)	Substitute for grass hay (G)	ΔN in intermediate substitutes
Aspartic acid	0·04	0·19	0·15
Glutamic acid	0·03	0·16	0·13
Alanine	0·04	0·18	0·14
Arginine	0·05	0·26	0·21
Cysteine	0·01	0·04	0·03
Glycine	0·04	0·14	0·10
Histidine	0·01	0·06	0·05
Isoleucine	0·01	0·06	0·05
Leucine	0·02	0·11	0·09
Lysine	0·03	0·12	0·09
Methionine	0·00	0·02	0·02
Phenylalanine	0·01	0·06	0·05
Proline	0·03	0·13	0·10
Serine	0·03	0·10	0·07
Threonine	0·04	0·13	0·09
Tyrosine	0·01	0·03	0·02
Valine	0·02	0·10	0·08
Total g/kg WSw	0·47	1·89	1·42
Total g/100 g DSw	0·23	0·93	0·70

WSw: Wet substrate weight; DSw: dry substrate weight; ΔN: difference in nitrogen of amino acid between W and G.

Table 3. Amounts of mineral elements in synthetic substitutes for wheat straw and grass hay (g/kg wet substrate)

Mineral element	Substitute for wheat straw (W)	Substitute for grass hay (G)	ΔM in intermediate substitutes
Calcium	0·730	0·953	0·223
Magnesium	0·121	0·300	0·179
Phosphorus	0·100	0·480	0·380
Potassium	1·746	3·589	1·843
Sodium	0·099	0·294	0·195
Copper	0·002	0·002	0·000
Iron	0·017	0·043	0·026
Manganese	0·005	0·020	0·015
Zinc	0·003	0·007	0·004

ΔM: Difference in mineral element between W and G.

Table 4. Amounts of mineral salts in synthetic substitutes for wheat straw and grass hay according to Table 3 (g/kg wet substrate)

Mineral salt	Substitute for wheat straw (W)	Substitute for grass hay (G)	ΔM in intermediate substitutes
Calcium chloride	2·68	3·50	0·82
Magnesium sulfate	0·98	2·44	1·46
Potassium phosphate	0·44	2·11	1·67
Potassium chloride	3·09	5·70	2·61
Sodium sulfate	0·35	1·05	0·70
Copper sulfate	0·007	0·007	0·000
Iron sulfate	0·085	0·214	0·129
Manganese sulfate	0·015	0·062	0·047
Zinc sulfate	0·013	0·031	0·018

ΔM: Difference in mineral salt added to wheat straw substitute to prepare intermediate substitutes (WΔCM, WΔCO).

Table 5. Amounts of carbohydrates in synthetic substitutes for wheat straw and grass hay (g/kg wet substrate)

Carbohydrate	Substitute for wheat straw (W)	Substitute for grass hay (G)	ΔS and ΔC in intermediate substitutes
Fructose	31·50	43·20	11·70
Glucose	1·44	7·57	6·13
Sucrose	1·02	0·83	−0·19
Cellulose	86·64	57·62	−29·02

ΔS: Difference in sugar; ΔC: difference in cellulose added or deducted from wheat straw substitute to prepare intermediate substitutes (WΔC, WΔCS).

maltose were used as sugars. All ingredients except cellulose were previously solubilized in distilled water. The pH of final solutions was adjusted to 6·0 by addition of 1 N NaOH, and the nutritive solution (235 ml) was added to mixtures of perlite (38 and 47 g for wheat straw and grass hay substitutes, respectively) in glass bottles to make 300 g of wet substrate at approximately 78% water content after sterilization. Intermediate synthetic substrates were prepared using perlite supplemented with cellulose, amino acids, minerals and sugars, alone or combined, in an amount equal to the positive or negative difference (Δ) between wheat straw and grass hay substrates.

Perlite-defined substrates (PDS) as synthetic substitutes for wheat straw and grass hay and intermediates substitutes were labelled as follows: W — perlite supplemented with cellulose, amino acids, sugars and minerals at the level found in wheat straw; G — perlite supplemented with cellulose, amino acids, minerals and sugars at the level found in grass hay; WΔC — the same as W but adjusted with cellulose to the level found in grass hay; WΔCM — the same as W but adjusted with cellulose and minerals (macroelements and oligoelements) to the level found in grass hay; WΔCO — the same as W adjusted with cellulose and oligoelements to the level found in grass hay; WΔCS — the same as W but adjusted with cellulose and sugars to the level found in grass hay; WΔCA — the same as W but adjusted with cellulose and amino acids to the level found in grass hay. Those substitute substrates were inoculated with pure washed mycelium only and the fruiting of *Pleurotus* was compared with that recorded on natural substrates.

Production of fruitbodies

Substrates were sterilized in glass bottles by autoclaving at 121°C for 2 h. After cooling, three substrate replicates were inoculated with either 25 ml of pure washed mycelium or 5% (w/w) grain spawn of *Pleurotus*. Wheat straw and/or grass hay were used as control treatments in the same way as perlite-defined substrates. Inoculated substrates were incubated at 25°C for 25 days in darkness. After spawn running, all colonized substrates were subjected to fruiting in a greenhouse at 20°C and 90% relative humidity. Mature fruitbodies were first harvested approximately 40 days after inoculation and three flushes were produced with an interval of 7–10 days between flushes. Samples were weighed, dried in a ventilated oven at 40°C and ground for protein-content determination. Fruitbody yield was expressed as fresh weight of fruitbodies per kilogram of fresh substrate.

Determination of protein content

Protein content of fruitbodies was determined by multiplying the nitrogen content by 6·58, the conver-

sion factor defined for *Pleurotus* (Tshinyangu, 1994), and expressed as a percentage of dry matter of fruitbodies dried overnight at 105°C. Nitrogen content was determined by the Kjeldahl method (AOAC, 1980).

Analysis of data

Fruitbody yield and protein content were the two variables used for comparison of the substrate effects. All data were subjected to analysis of variance (one-way) using the STATITCF program (ITCF, 1991). Mean values of each variable were separated by using the Newman-Keuls test ($P<0.05$).

RESULTS AND DISCUSSION

Effect of perlite-defined substrates (PDS) with nutrient starvation on fruitbody yield and protein content

Fruitbody yield and protein content of *Pleurotus ostreatus* var. *columbinus* grown on PDS with nutrient starvation, from both kinds of inoculum, are shown in Figs 1 and 2. *Pleurotus* did not fruit from pure washed mycelium in the absence of nitrogen, minerals or cellulose (Fig. 1). Dextrose was not necessary when cellulose was present. When cellulose was absent, dextrose at the supplied amount did not induce *Pleurotus* fruiting. When cellulose was present in PDS, fruitbody production occurred but the yield was sensibly lower than that on natural wheat straw. Spawning was able to withstand mineral and nitrogen starvation and to induce fruiting, but not in the absence of cellulose. The fruitbody yield obtained on the PDS inoculated with grain spawn was similar to that on wheat straw, except in the case of mineral (CNS) and nitrogen (CSM) starvation.

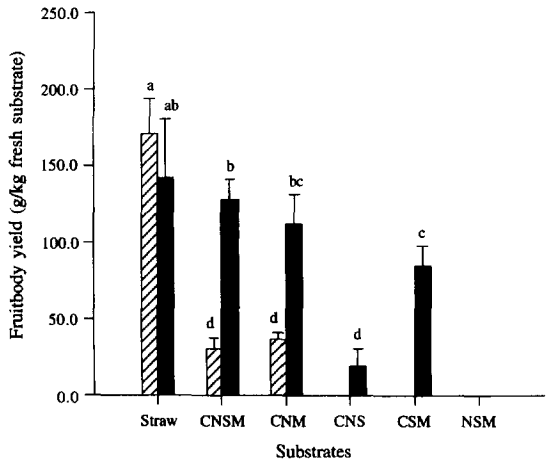

Fig. 1. Effect of perlite-defined substrates with nutrient starvation on fruitbody yield. Straw: wheat straw; CNSM: perlite-defined substrate (PDS) containing cellulose, nitrogen, sugar and minerals; CNM: the same PDS but lacking sugar; CNS: the same PDS but lacking minerals; CSM: the same PDS but lacking nitrogen; NSM: the same PDS but lacking cellulose; ▨: substrate inoculated with pure washed mycelium; ■: the same substrate inoculated with grain spawn; C: cellulose; N: nitrogen; S: sugar; M: minerals. Bars are the means (±SEM) of three replicates of each substrate. Bars with a similar letter were not significantly different using the Newman-Keuls test ($P<0.05$).

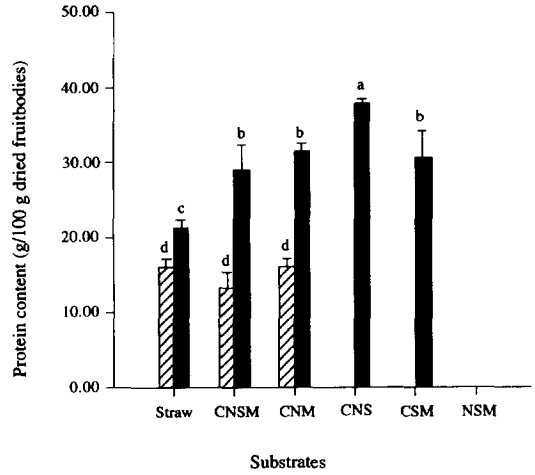

Fig. 2. Effect of perlite-defined substrates with nutrient starvation on protein content of fruitbodies. See Fig. 1 for abbreviations.

When fruitbody production occurred on PDS inoculated with pure mycelium, protein content did not vary significantly from that of *Pleurotus* fruitbodies harvested from wheat straw (Fig. 2). However, using grain spawn the protein content increased significantly in *Pleurotus* fruitbodies, whatever the PDS might be. The greatest increase was recorded on PDS with mineral starvation.

These results showed that cellulose, nitrogen and mineral sources, as used in the first experiment, were all necessary in PDS, as the absence of one of them inhibited *Pleurotus* fruiting. However, they could not be considered as essential in the sense of Arnon and Stout (1939), as their possible substitution by other compounds has not been demonstrated. *Pleurotus* has been shown to grow on chemically defined media containing different mineral and organic nitrogen sources (Cailleux *et al.*, 1974). Glutamic acid is one of the nitrogen sources that can induce growth (Hong, 1978). The substitution of cellulose by dextrose might be successful for *Pleurotus* fruiting by increasing the dextrose supply to the substrate. Leathnam (1983) has shown that fruitbodies of *Lentinus edodes* were produced only when dextrose concentration was at least 50 grams per litre. Grain spawn did not prevent the absence of fruiting on cellulose-lacking substrate. However, it allowed fruitbody production even when nitrogen or minerals were not supplied in the substrates. Inoculation of PDS lacking either sugar, nitrogen or minerals with grain spawn not only induced fruiting but also increased the protein content of the fruitbodies.

Ginterova and Maxianova (1975) have asserted that *Pleurotus* fixed atmospheric nitrogen because

the nitrogen balance was positive after its cultivation. However, Shi-Li *et al.* (1984) showed that *Pleurotus* does not fix atmospheric nitrogen. From the present investigation, it is evident that *Pleurotus* is not able to grow or fruit on artificial substrate lacking nitrogen after inoculation with pure washed mycelium and incubation in air. This demonstrates that *Pleurotus* has no ability to fix atmospheric nitrogen, even to induce mycelial growth.

Effect of synthetic substitutes for wheat straw and grass hay on fruitbody yield and protein content

Fruitbody yield and protein content of *Pleurotus* cultivated on synthetic substrates and natural substrates are presented in Figs 3 and 4. Fruitbody yield decreased significantly in *Pleurotus* grown on the synthetic substrates when compared to growth on wheat straw and grass hay (Fig. 3). Fruitbody yield decreased most on the wheat straw substitute supplemented with oligoelements only (WΔCO). Wheat straw substitute supplemented with amino acids (WΔCA) and grass hay substitute (G) did not support *Pleurotus* growth or fruitbody production. Grass hay appeared to be the best substrate for maximizing fruitbody yield, as well as protein content.

When production occurred on synthetic substitutes, protein content in *Pleurotus* fruitbodies did not change significantly in comparison with wheat straw (Fig. 4). A significant difference was observed between the protein content on synthetic substrates. Fruitbodies harvested from WΔC and WΔCM substrates had a low protein content when compared to that of those harvested from W and WΔCO.

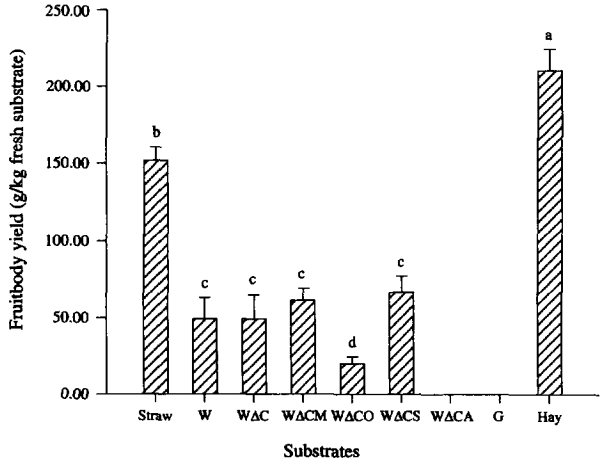

Fig. 3. Effect of synthetic substitutes for wheat straw and grass hay on fruitbody yield. Straw: wheat straw; W: perlite supplemented with cellulose, amino acids, sugars and minerals at the level found in wheat straw; WΔC: the same as W but adjusted with cellulose to the level found in grass hay; WΔCM: the same as W but adjusted with cellulose and minerals (macroelements and oligoelements) to the level found in grass hay; WΔCO: the same as W adjusted with cellulose and oligoelements to the level found in grass hay; WΔCS: the same as W but adjusted with cellulose and sugars to the level found in grass hay; WΔCA: the same as W but adjusted with cellulose and amino acids to the level found in grass hay; G: perlite supplemented with amino acids, cellulose, minerals and sugars at the level found in grass hay; Hay: grass hay.

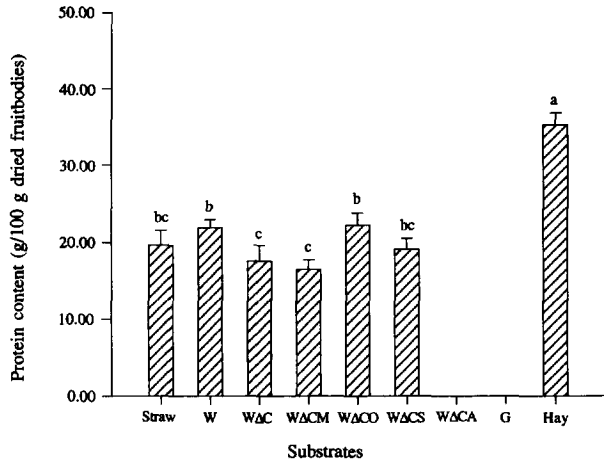

Fig. 4. Effect of synthetic substitutes for wheat straw and grass hay on protein content of fruitbodies. See Fig. 3 for abbreviations.

It can be seen from these data that the productivity of synthetic substitutes for wheat straw was very limited when compared to that of natural wheat straw. Further addition of sugars, nitrogen and minerals to the synthetic substitutes for wheat straw, so as to reach the grass hay concentrations, did not change the limited productivity of those substitutes. It can be deduced that the absence in PDS of lignin, hemicelluloses, vitamins, or other undefined compounds which are present in wheat straw, must be the limiting factor for fruitbody production. Indeed, lignin and hemicelluloses are taken up by *Pleurotus* in preference to cellulose (Moyson & Verachtert, 1992). Although vitamins and plant hormones are not essential for *Pleurotus* fruiting, they have a favourable effect on *Pleurotus* growth. Thiamine is favourable for *Pleurotus* fruiting, but it is not an absolute requirement, *Pleurotus* and other mushrooms being able to synthesize some of this vitamin (Chang & Miles, 1989).

Synthetic substitutes for wheat straw (W) and grass hay (G) contained 0·23 and 0·93% of nitrogen as free amino acids, respectively (Table 2). All substitutes containing 0·23% of nitrogen allowed growth, but gave lower fruitbody yields than those on natural wheat straw and grass hay. However, those containing more than 0·23% nitrogen had an inhibitory effect on *Pleurotus* growth. This indicates that free amino acids may have an inhibitory effect when supplied as synthetic substitutes for substrates. Indeed, in wheat straw and grass hay substrates, amino acids are not free but linked into proteins and are slowly released without inhibitory effect on *Pleurotus* growth. The synthetic substitutes (W) supplemented with amino acids cannot support growth of *Pleurotus* because a high concentration of free amino acids causes hyphal plasmolysis.

Our experiments have suggested that certain nutrients are limiting the fruiting of *Pleurotus ostrea-*

tus var. *columbinus* on synthetic substitutes for substrates. Supplementation of such substrates with proteins, lignin and vitamins might provide the answer.

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