IN VITRO STUDIES ON SPECIES AND MUTANTS OF AGARICUS, CANTHARELLUS, LEPISTA, PLEUROTUS, AND VOLVARIELLA

Thesis for the Degree of Ph. D.

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Paul Albert Volz

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ABSTRACT

IN VITRO STUDIES ON SPECIES AND MUTANTS
OF AGARICUS, CANTHARELLUS, LEPISTA,
PLEUROTUS, AND VOLVARIELLA

by Paul Albert Volz

Twelve representatives of the Agaricales were selected for nutritional work and related studies. Those included were Cantharellus clavatus Fries, three strains of C. cibarius Fries, Agaricus campestris L. ex. Fries, A. bisporus (Lange) Singer plus two of its mutants, Lepista nuda (Bull. ex. Fries) Cooke, Pleurotus ostreatus (Jacq. ex. Fries) Kummer, Volvariella volvacea (Bull. ex. Fries) Singer and one of its mutants. The fungi were grown in Humfeld's medium with variations of carbon and nitrogen sources. A total of 11 monosaccharides, 7 disaccharides, 1 trisaccharide, 7 polysaccharides, 9 organic acids, 2 sugar alcohols, and miscellaneous compounds were added singly to Humfeld's medium as carbon sources at an equivalent carbon concentration. Urea was the nitrogen source. A few carbohydrates were added to the basal medium at three different carbon concentrations, and glucose was selected for growth curve studies.

Fourteen ammonium and nitrate nitrogen sources, sixteen amino acids or related compounds were added singly to the basal medium in place of urea. Mycelial growth comparisons were made for each of the 12 selected fungi. Vitamin and growth hormone studies were also made.

Observations by electron and light microscopy were made on mycelium grown in Humfeld's media and hyphae from fructifications formed in culture.

Four of the 12 fungi were grown on 15 spawn media. The same four were subjected to various temperatures while growing on rye grain. Morphological characteristics and colony measurements were made of the 12 fungi when grown on 20 selected agar media.

IN VITRO STUDIES ON SPECIES AND MUTANTS OF AGARICUS, CANTHARELLUS, LEPISTA, PLEUROTUS, AND VOLVARIELLA

By

Paul Albert Volz

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Dedication

to a patient parent
Mrs. Frieda C. Volz

and

to a pertinent professor
Dr. Arthur G. McQuate

whose persuasion formulated this work.

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INTRODUCTION

The purpose of this work was to determine how well various sources of carbon, nitrogen, vitamins, and growth hormones were utilized by some Agaricales species. The fungi were further subjected to variations in carbohydrate concentrations for possible substantial mycelial growth differences in submerged culture. Fungi selected for the studies include: Cantharellus clavatus Fries, C. cibarius Fries, Lepista nuda (Bull. ex. Fries) Cooke, Pleurotus ostreatus (Jacq. ex Fries) Kummer, and Agaricus campestris L. ex. Fries collected in nature. The cultivated mushrooms, Volvariella volvacea (Bull. ex Fries) Singer and Agaricus bisporus (Lange) Singer, were also employed in these nutrient studies, along with pleomorphic and rhizomorphic mutants of A. bisporus, and one mutant of V. volvacea. Three strains of C. cibarius were selected to determine if nutritional requirement differences occur for that species.

Various materials were selected as possible sources of spawn media, and optimal temperatures were determined for rye grain spawn. Different agar media were utilized for morphological descriptions of the colonies.

Representatives of the wild genera and V. volvacea were subjected to environmental conditions suited for mushroom production of A. bisporus to learn if fructifications would occur under such conditions.

Some anatomical work was done on hyphae grown in submerged culture and on hyphae removed from fruit bodies grown in culture.

All fungi selected were edible and had pleasing flavors; the wild species have a potential use in commercial production.

LITERATURE REVIEW

Ultrastructural details of electron micrographs were identified according to nomenclature of current literature (18, 30, 61, 85, 87, 168, 187, 188, 277, 284). Moore and McAlear (187) were first to identify the septal pores of Basidiomycetes; structural details were further clarified by other workers. The dolipore septum or septal pore apparatus consists of the pore bounded by the discontinuous septal pore cap. The latter term has replaced the earlier two dimensional concept of the parenthesome, now the cap is considered to be a dome-shaped, perforated membrane, surrounding the septal pore. When first described, the structural complex of the pore was thought to prevent interchange of cellular organelles. Various intermediate stages interpreted as steps in the breakdown of complex to simple pores have been seen by Giesy and Day (85) which may facilitate migration of cell parts. Structures frequently found within the hyphal cells include mitochondria, nuclei, vacuoles, and endoplasmic reticulum.

Among growth promoting substances, gibberellin has been a widely used material with Agaricales species. An increase of mycelial growth and basidiocarp production

was noted when gibberellin was added to Agaricus compestris compost (3). Gibberellin in any concentration hindered growth of Fomitopsis annosa (191), Amanita caesaria, A. rubescens, A. muscaria, Boletus lutens and B. bicolor (248). High concentrations of 3-indoleacetic acid (heteroauxin) proved toxic and low concentrations failed to induce a growth stimulation for Pleurotus corticatus (137), Lentinus omphalodes (74), Collybia velutipes (9), and Schizophyllum commune (230), but markedly increased growth of Psalliota hortensis f. albida in the presence of vitamins (29, 62). Schizophyllum commune mycelium was strongly inhibited by 2,4-dichlorophenoxyacetic acid while Pleurotus ostreatus and Marasmius rotula had an increase of spore germination and hyphal growth (129). Inhibition responses were noted with 3-indolepropionic acid (74), α naphthalene acetic acid, and 2,4,5-trichlorophenoxyacetic acid (230). Of 53 phenolic compounds tested on Polyporus schweinitzii, vanillin, vanillic acid, isoeugenol, ferulic acid and 3,4-dimethoxycinnamic acid showed high growth promoting activities (243). Increased mycelial production was noted with: three unidentified growth promoting substances for Poria ambigua isolated in accordance to light or dark conditions (238); Norit A filtrates of tomatoes or beech wood for Stereum fasciatum, S. subileatum, Hydnum coralloides and Peniophora coccineofulva (241); an M-factor isolated from roots of tomato and pea for Rhizopogon roseolus, roots of

tomato and wheat for Boletus variegatus and B. elegans, and roots of only pea for Russula xerampelina (182); and two unidentified growth promoting substances in malt extract for Polyporus schweinitzii (239) and Fomes igniarius var. populinus (89).

Growth of representatives of the Agaricales was first developed with media composed of extracts and wastes of plant or animal material. Yeast and malt extract in concentrations of 2 to 8% in agar or added to other nutrients have increased mycelial growth. Using malt or yeast extract, good mycelial growth was obtained with Poria ambigua (238), Formes lignosus (231), F. igniarius (89), F. annosus (45), Pellicularia koleroga (170), Coprinus fimetarius (66), Polyporus abietinus (79), P. schweinitzii (239, 243), P. cinnabarinus and P. sanguineus (163), Pholiota aurea (13), Stereum gausapatum (98), Agaricus blazei (23), Lenzites sepiaria, L. tigrinus and Polystictus versicolor (153, 198). The two extracts also produced good mycelial growth in mass scale studies involving several genera of the Agaricales conducted by Robbins and Kavanagh (245), Law (135), Leonian and Lilly who included the species Pleurotus corticatus (138), Jennison (113), Robbins and Hervey (237), Fries who used three Tricholoma species (70), Humphrey and Siggers who included Pleurotus ostreatus (110), Yusef (287), and Mikola (183). Fries noted that species of Boletus, Craterellus and Hydnum

produced better mycelial growth in 2.5% malt extract agar in the presence of living Torulopsis sanguinea (72). Robbins and Hervey (235,237) found only 41 of 82 species, mostly Agaricales, were not stimulated by malt extract and 11 had injurious effects. For Polyporus tulipiferus and P. vulpinus, Yusef (287) found the beneficial effect of malt extract was largely the result of thiamine and amino acids in the natural product.

Malt and yeast extracts at times retarded fruiting for Schizophyllum commune (220), but typical fructifications occurred in test tubes with the agar surfaces facing downwards instead of upwards as did cultures of Lenzites saepiaria, Hohenbuehelia reniformis, and Pyconoporus cinnabarius. Long (70) found no relationship of fructifications with horizontally and vertically placed cultural tubes. Coprinus sassii fruited well on yeast extract (19) while malt extract provided a suitable medium for Collybia velutipes (8, 216, 279), Polyporus fumosus but not P. adustus or Lenzites trabea (38), Paxillus panuoides (59), Polyporus brumalis (216, 217), Fomes ulmarius, Poria ambigua (154), and several other bracket fungi in experiments by Papazian (209) and Etter (55).

Other substrates and extracts have been used for hyphal growth of the Agaricales. In addition to malt, Block et al. (23) used orange juice, corn steep liquor, and citrus press water for submerged growth of Agaricus blazei while

Mathew (170) selected onion, carrot, oat, bean, and potato extracts for growth of Pellicularia koleroga. Styer (267) utilized peach gum and pectin, wheat bran, wheat straw, peat moss and lignin for growth of Agaricus bisporus; in addition to various leaves, soils, beans, sugar beets, potato and corn meal selected by Duggar (51) for growth of A. bisporus as well as Pleurotus ostreatus, Tricholoma personatum and 16 other species. Stereum gausapatum (99) grew well on potato extracts, Coprinus and Boletus species on string bean or sugar beet decoctions (50), Polyporus amarus on incense-cedar heartwood extract (36), P. zonatus, P. fuliginosus, P. annosus on pine saw dust (233), Stereum sanguinolentum on balsam fir heartwood and less on sapwood (54), Fomes annosus on various wood extracts (88) and extracts of a bacterium or Trichoderma when added to a solution of various salts (46), Fomes pini on extracts of pine xylem dead several years or recently killed by weevils and not other agents (48). La Fuze (130) made extracts of several woods and obtained good growth with Polyporus betulinus, Fomes pinicola and Polystictus versicolor, while Melin (172, 173), cultured several mycorrhizal fungi isolated from pine and spruce, Badcock (15) utilized sawdust for sporocarp production of Pleurotus ostreatus, P. euosmus, P. lingalilis, and 77 other wood-rotting species. Bechmann (17) grew luxuriant mycelial cultures of Agaricus campestris on orange, banana and tomato extracts

while Humfeld (106) obtained good growth on wart agar, liquid media of asparagus-butt juice or press juice from pear waste. Potato dextrose agar produced dense hyphal colonies of many Agaricales, including Pleurotus ostreatus (98, 122).

Robbins and Hervey (237) selected 82 species, mostly Agaricales, and obtained good growth with beech wood extracts except with Polyporus rutilans and Poria carbonica, and tomato extract, and with tomato extract except with Fomes ulmarius and Stereum rufum. The authors (239) also obtained growth increases of Polyporus schweinitzii by using tomato juice, coconut milk, casein, cork, agar and gelatine. In experiments involving many Agaricales species, Jennison (113) obtained good growth with extracts of potato, soybean meal, bran, corn steep liquor, molasses and gluten; Ferguson (58) utilized bean stems or pods, sugar beets, beef broth for Pleurotus ostreatus, Tricholoma personatum, Agaricus campestris plus 40 other Agaricales; in addition Long (156) used celery, alfalfa, parsnip, prune, beet, and corn meal extracts in agar.

Mycelia of large numbers of Agaricales were grown on various media and decoctions of fresh vegetables and dried fruits by Robbins and Hervey (241), Zeller et al. who included Pleurotus sapidus (290), Robak (234), Lyman (161), Falanghe et al. who selected Cantharellus cibarius and Tricholoma nudum plus other species (57), and Cartwright and Findley who selected Pleurotus ostreatus among their isolates (39).

The earliest studies of mycelial growth were carried out by Eidam (52) in 1875 with Agaricus coprophilus on manure decoction, Wehmer (283) in 1895 with Pleurotus ostreatus grown in a sugar solution, Brefeld between 1877 and 1889 who grew over 160 species of Agaricales in various decoctions (58). The first purely synthetic medium for Agaricales mycelium was developed by Raulin in 1869 composed of ammonium nitrate, ammonium phosphate, ammonium sulfate, zinc sulfate, iron sulfate, magnesium carbonate, potassium carbonate, potassium silicate, sucrose, tartaric acid, and water (222). Before 1860, Woronin grew Exobasidium, and Hoffman worked with growth of Agaricus campestris on undefined media (58), while Constantin grew Polyporus squamosus in culture around 1890 (43).

Various decoctions served as media for fructifications of several Agaricales. Findley (59) fruited Lenzites saepiaria on apple juice agar while Hawker (95) produced fruit bodies of Armillaria mellea, Collybia velutipes, Fomes fraxineus, Hydnum coralloides, Schizophyllum commune and Sphaerobolus stellatus on media with extracts of potatoes or lentils. Sporophores of Coprinus cubensis (119) grew on potato dextrose agar, beef extract or peptone; PDA also proved beneficial for fruiting of Agaricus leucotrichus (186) and Lentinus tuber-regium (80). The earliest sporocarp production studies on undefined media include those done by

Constantin in 1891 with Nyctalis lycoperdoides (42), Reess in 1876 with two Coprinus species (223), VanTieghem in 1875 with Collybia velutipes (275) and Labourdette in 1861 with Agaricus campestris (131).

When powdered pine, spruce or juniper wood was added to a medium containing malt extract, corn starch, sphagnum moss, and corn meal, sporophores of Pleurotus ostreatus and species of Polyporus, Coprinus, Xerotus, Pholiota, Ganoderma, Lentinus and Trametes occurred (55). Beech sawdust with additions of maize meal, bone meal, or cotton wool produced fructifications of Lentinus, Phellinus, and Polystictus (14). Alicbusan and Ela (6) produced Volvariella volvacea sporocarps on numerous media including ipil-ipil leaves, coir dust, wheat bran, brewer's dried grain, coffee pulp, bean leaves, corn cobs, leaflets of madre de cacao, tobacco midribs, unhulled rice, cow and horse manure. A mixture of horse dung and sawdust covered with a 6 inch soil layer produced fruit bodies of Coprinus comatus after 10 months hyphal growth (190). Borzini utilized synthetic media to fructificate Agaricus campestris, Tricholoma species, Volvaria volvacea, and Tuber melanogaster (28). Block et al. cultivated Agaricus campestris on composted gum-wood sawdust and Pleurotus ostreatus on balsa-wood sawdust fortified with oatmeal or soybean meal (25). P. ostreatus is able to grow on other woods (125, 198, 259) and on dead receptacles of that species (228).

Various garden soils influenced fructification of Tricholoma nudum (44) and Volvaria volvacea (31), while compost or horse dung extracts and salts in the medium developed fruit bodies of Agaricus leucotrichus (186), Coprinus sassii (19), and C. sterquilinus (189). A nutrient depleted medium is generally an inductive agent for fungal fructifications or a medium suited for less mycelial growth (9), or portions of a colony where aerial growth is the least (156).

Carbon dioxide appears to play a role in basidiocarp development for several species of Agaricales. Respiratory CO_2 in sealed chambers arrests fruiting of Schizophyllum commune at the primordia stage (102, 193, 194), reduces pilei expansion of Collybia velutipes (155, 216), inhibits fruit body production of Polyporus palustris (60), and produces shorter stipes and small pilei of Agaricus bisporus (132). Wherever Agaricus campestris is grown, substances of volatile nature accumulate and become detrimental to fructification (167). Respiration of S. commune was increased with sucrose, certain hexoses, D-xylose, acetate and ethanol whereas certain other pentoses and amino acids had little effect, and D-glucose or acetate produced incomplete oxidation thus varying CO_2 release and fruit body forms (193). Certain nitrogen sources activate homodikaryotic fruiting in S. commune (102).

The temperature and pH influence production of fruit bodies. The optimum temperature for best fruiting was 25° C for Coprinus lagopus (27, 166, 276), Cyathus stercoreus (157), Armillaria mellea (225), Poria ambigua (240), Schizophyllum commune (93), 30-45° C for Volvariella volvacea (2, 6), 26-31° C for Pleurotus ostreatus on PDA and 22-29° C on rye grain (132), 31° C for Agaricus campestris on PDA and and 22-26° C on rye grain (24), 22-27° C for Polystictus versicolor, Polyporus annosus, Pleurotus ostreatus and Psalliota bispora grown on a basal medium of glucose, vitamins and salts (125), 15° C for Polystictus versicolor on male extract and peptone agar (16). Aschan-Aberg learned that Collybia velutipes developed good fruit bodies at 25° C in darkness for one week followed by 15° C in light (8, 9), or 20° C without temperature fluctuations (10), while Kinugawa stated that a minimal duration for fruiting was 2 days at 5° to 10° C followed by 15° C or at alternating temperatures of 5° and 20° C (123). The optimal initial hydrogen ion concentrations were recorded as follows for a few species: C. velutipes, 5.2-7.2 (8, 9, 215); P. ostreatus, 5-6.2 (24); Armillaria mellea, 5.0 (225); Agaricus campestris, 5.5-6.0 (64). The terminal pH was generally somewhat lower.

The presence of light functions in fruit body formation of several species. Long (156) believed the presence of light is essential to the production of sporophores when grown on artificial media while the character of the substratum plays only a very minor role in sporophore initiation.

He obtained 629 sporophores in the light and only 11 in darkness from the following genera: Coprinus, Daedalea, Exidea, Fomes, Ganoderma, Irpex, Lentinus, Lenzites, Marulius, Panus, Pleurotus, Polyporus, Polystictus, Poria, Stereum and Trametes. In darkness, Polyporus farlowii, P. cinnabarius, and Trametes serialis developed sporophores but the latter produced no spores. Stipe growth occurs in darkness but light is required for pilei expansion and normal spore production of Pleurotus, Polystictus (125), Collybia velutipes (2, 8, 155, 216), and Psilocybe coprophila (86). The blue light of the spectrum is positive while red light is negative for sporocarp development of Poria ambigua (238, 240), Coprinus lagopus (27, 253), and Collybia velutipes (11). Long stipes, small caps and no gills form with Coprinus lagopus in yellow green light as in the dark (27). In contrast to Long, Block (24) found that Pleurotus ostreatus fruited quite normally in the dark, while Koch (125) stated that mycelial growth of Psalliota bispora was inhibited by light. Koch (125) also showed light was not required by Polyporus and Psalliota for normal development and maturity, and Ferguson (58) believed light inhibited spore germination of Agaricus campestris. Stipe elongation of Collybia velutipes and Polyporus brumalis occurs in pure culture as light intensity is reduced (216). Other authors also suggested light was needed for normal fruiting body formation of Collybia velutipes (9, 215), Coprinus lagopus (276), Polyporus palustris (60), Schizophyllum commune (102, 194, 220), Lentinus lepideus (33),

Polyporus brumalis (217), Pleurotus ostreatus (15,210), Polystictus versicolor, Trametes serialis and Fomes pinicola, Polyporus schweinitzii (15), and Lentinus tuber-regium (80). Coprinus lagopus will fruit in continuous light or in brief exposures to light of one second at 250 ft c or 5 seconds at 0.1 ft c, but not in darkness (166). The amount of light energy required for fruiting of Cyathus stercoreus is a constant 17200 ft c hours at a light intensity of 240 ft c (157). Other conditions needed for fruiting are: genetic inheritance (86), rich, well aeriated medium, high relative humidity (15), and accumulation of metabolic products (220).

Gravity was shown to effect sporocarps in culture. The stipe of Polyporus brumalis (217) and sporocarp of Pleurotus ostreatus (24) are - geotropic and + phototropic, the phototropism can mask the geotropism. Amanita phalloides and A. crenulata stipes are - geotropic while the caps are + geotropic (265). Pleurotus ostreatus fruiting on popular logs is not modified by position of log but by direction of gravity (271).

When grown on a defined medium, variations of nutrients have shown variations in the ability of fruiting. The following carbohydrates produced fructifications in media with the following Agaricales: Schizophyllum commune - sucrose, maltose, trehalose, cellobiose, glucose, fructose, mannose, galactose, xylose, mannitol, glycerol, ethanol, while acetate and citrate appeared inhibitory (195); Coprinus sassii, C.

heptemerus, C. congregatus - maltose, sucrose, but glucose only developed a luxurant mycelial growth (20); Coprinus lagopus - cellulose, glucose, maltose, fructose, starch, while negative results developed with galactose, lactose, glycerol, sodium oleate, sodium potassium tartrate (165,278); Pleurotus ostreatus - corn steep liquor, molasses, glucose, cellulose, peptone, while starch was negative (24,124); Collybia velutipes - sucrose, glucose, while maltose was poor (121,215); Polystictus versicolor - fructose, glycerin, mannitol, maltose, xylose, glucose, saccharose, and starch (125). Glucose was the carbohydrate source singly selected by authors for sporocarp production media along with a nitrogen source and a variety of salts (96,220,240) for Phlebopus lignicola (207), P. sulphureus (206), Coprinus ephemerus (96), Xerocomus badius and X. illudens (208), Suillus rubinellus (164), Polystictus versicolor (125), and Poria ambigua (238,240).

The following fungi were able to utilize the listed nitrogen sources and produce sporocarps in culture; Schizophyllum commune - asparagine, urea, glutamine, glutamic acid, serine, alanine, arginine, tryptophan, ammonium phosphate, complex nitrogen sources and ammonium salts (195); Coprinus lagopus - organic, ammonium, and nitrate nitrogen (165,250,278); Pleurotus ostreatus - casamino acids, peptone, sodium nitrate, ammonium sulfate (24,125); Collybia

velutipes - asparagine, ammonium tartrate (8,215); Polystictus versicolor - ammonium chloride, ammonium tartrate, arginine, asparagine, glycine, glutamic acid (125).

Vitamins have increased fruit body production of several Agaricales. Those affected and the vitamins involved are: Schizophyllum commune - thiamine (93,220); Agaricus campestris - thiamine, biotin (273); Poria ambigua - the known B vitamins (240); Polystictus versicolor - thiamine (125); and thiamine for Coprinus ephemerus (96,214), C. lagopus (165,278), C. sassii (19), Pleurotus ostreatus (24,125), Poria ambigua (238), and Polyporus palustris (60). In decreasing order, nicotinic acid, pantothenic acid, biotin, thiamine, riboflavin, and pyridoxine increased yields of Agaricus bisporus when added to mushroom beds (103,134,224) and a 78% greater yield occurred with a combination of riboflavin, nicotinic acid and pantothenic acid (103). None of the B vitamins had an effect on fruiting of Collybia velutipes (8).

Miscellaneous substances increasing fructifications of Agaricales include four fatty acids (linoleic, palmitic, oleic and stearic acid) for Polyporus schweinitzii (243). Mycelium inhibitors, rose bengal, tergitol-7, aerosol, and sodium lauryl sulfate, initiates earlier and more profuse fruiting of Schizophyllum commune (220).

Vitamins added to basal media composed of carbon, nitrogen and salts greatly increased mycelial growth and dry weights of the Agaricales. The organisms heterotrophic for thiamine or the pyrimidine component of thiamine include one report of 41 species or strains of Polyporus and Fomes (287,288), Fomes lignosus (231), F. annosus (226), Polyporus abietinus (79), Polystictus versicolor (125), Paxillus prunulus, Amanita pantherina, Tricholoma albobrunneum, T. flavobrunneum, T. vaccinum, T. fumosum, T. pessundatum, T. imbricatum, Boletus luteus, B. variegatus, B. piperatus, B. granulatus, Cenococcum graniforme (68,176,177,178,201), several species of Polyporaceae (254), Stereum murraii (236) Collybia tuberosa (138,245), Coprinus sp. (245), and Marasmius fulvobulbillosus (148). Thiazole is probably synthesized by the fungi but pyrimidine must be supplied by other sources.

If both pyrimidine and thiazole are present, thiamine can be synthesized by Polyporus zonatus (254), Amanita pantherina (178), Marasmius alliaceus, M. rotula, M. scorodonius (68,148).

In experiments involving many fungi, thiamine deficiency has also been noted by Lindeberg (149) for species of Clitocybe, Collybia, Lepiota, Mycena, Hypholoma, Pholiota, Flammula, Panus, Hydnum, Clavaria, Stropharia, Tubaria; by Fries (75) for species of Peniophora, Panaeolus, Panus, Polyporus, Lentinus, Nyctalis, Omphalia, Trametes,

Stropharia, Hydnum, Collybia, and Sistrotrema; and by Jennison et al. (115) for species of Daedalea, Fomes, Hydnum, Lentinus, Lenzites, Polyporus, Peniophora, Poria, Ptychogaster, and Trametes. Several other authors have noted thiamine deficiency for: 8 Coprinus sp. (65), 15 Marasmius sp. (148), 12 Polyporus sp. (70), 19 Mycena sp. (73), 4 Polyporus sp. (140), Collybia velutipes (140,215), 6 Clavaria sp., 4 Boletus sp., Lactarius deliciosus, Tricholoma imbricatum, Psalliota arvensis, Collybia dryophila, Mycena epipterygia, Stropharia aeruginosa (174), Marasmius foetidus, Boletus variegatus (151), B. granulatus (181), B. elegans, Clitopilus prunulus, Tricholoma imbricatum, T. pessundatum (177,180,245), Lenzites betulina, Fomes annosus (35,53), F. igniarius, Stereum frustulosum, Hydnum erinaceus, Polyporus spraguei (199), P. anceps (212), P. zonalis, P. dryadeus, Coniophora olivacea (204), Coprinus lagopus, Nyctalis sp., Pleurotus corticatus (138), P. ostreatus (24,129,274), Lentinus omphalodes (74), Cortinarius glaucopus (176), Lenzites trabea (141), Stereum gausapatum (101), Schizophyllum commune (244), and Armillaria mellea (84).

Essentially no requirements for vitamins, particularly thiamine autotrophy, occurred with Lactarius deliciosus, Tricholoma albobrunneum (177), Coprinus curtus (68), Poria monticola, Polyporus balsameus (204), Lenzites seapiaria (126,262), Agaricus campestris (244), Psalliota bispora (125),

Pleurotus ostreatus and Lenzites trabea (140). In contrast other workers found thiamine beneficial for strains of Agaricus bisporus (63), P. ostreatus and L. trabea (24,129,141,274).

The thiazole moiety of the thiamine molecule is required and pyrimidine therefore is synthesized for light emission of Collybia velutipes (1), and for mycelial growth of C. velutipes (169), Stereum frustulosum (200), Fomes noxius (232). Members of the Agaricales found to be heterotrophic for thiamine and nicotinic acid include Polyporus abietinus (79), Pholiota aurea (13). Polyporus texanus is heterotrophic for thiamine and pantothenic acid, and P. immitus is heterotrophic for thiamine and adenine (288). Best mycelial growth occurred with thiamine and biotin for Marasmius foetidus, M. androsaceus, and M. perforans, very poor growth developed while vitamins were used singly or with inositol (145).

A combination of thiamine and biotin best stimulated hyphal growth of Psalliota hortensis f. albida (62), Polyporus palustris, P. tulipiferus (114), and Pellicularia koleroga if thiamine was added to glucose or sucrose, or if biotin was added to sucrose medium but not glucose (170). Collybia dryophila (150), C. velutipes (169) also had increased mycelial growth on riboflavin (280), along with Trametes serialis (262), Psalliota campestris (124,273). Marasmius androsaceus (148), and Tricholoma albobrunneum (177).

Thiamine, inositol and biotin benefitted Polyporus adustus, P. abietinus, Fomes pinicola, Tricholoma nudum (126,262), and Pellicularia filamentosa (256). Biotin alone in media was heterotrophic for hyphal growth of Fomitopsis annosa (191), Marasmius androsaceus (147), and Daedalea quercina (140).

Tricholoma imbricatum is partially heterotrophic for panthotenic acid (201) while T. fumosum and Lactarius deliciosus are partially deficient for nicotinic acid (175,176,201). A mixture of 12 vitamins added to a basal medium increased hyphal growth of 41 Polyporus and Fomes genera (235). Lactarius species were unable to utilize thiamine or biotin but had excellent growth increases with inositol, calcium, pantothenate, p-aminobenzoic acid and good growth with folic acid, nicotinic acid, pyridoxamine, pyridoxine and riboflavin (112). Khudiakov and Vozniakovskaia (121) obtained good hyphal growth with Boletus edulus, B. luteus, B. luridus, and B. variegatus in a medium containing nicotinic acid, p-aminobenizoic acid, riboflavin, calcium pantothenate, biotin, and thiamine. Pyridoxine, inositol and biotin singly act as accessory factors for growth of Ganoderma lucidum (140).

Lilly and Barnett (140) believe it is entirely possible under varying environmental conditions that some fungi would be deficient for one or more growth substances and that other isolates of the same species would differ in their nutrient requirements.

Hydrogen ion concentration of liquid media for mycelial production of Agaricales has been investigated by a few authors. The optimal pH for several cultures was observed as follows: Pleurotus ostreatus, 5.5 (274); Polyporus zonatus, 5.5; P. fuliginosus, 4.5; P. annosus, 5.5 (233); Psalliota bispora f. avellanea, 6.0-6.8 depending on substrate (273); 8 Tricholoma sp., 4.0-5.0 (201); Fomes annosus, 4.6-5.6 (53, 272); Pholiota aurea, 5.0-6.4 (13); Coprinus cubensis, 6.9 (119); Polyporus betulinus, Fomes pinicola, Polystictus versicolor, 3-4.5 (130); Collybia velutipes, 6.5-6.9 (279), and 6.0 for maximum luminescence (1); Agaricus campestris, 4.5 (109); Boletus elegans, 4.5 (105); Marasmius alliaceus, 5.2; M. foetidus, 3.1; M. graminum, 6.9 (146); a Coprinus sp., 5-7 and 8.0 (69); Stereum murraii, 5.3-6.1 (236); 8 Coprinus sp., 7.8-8.2 (65); Agaricus blazei, 5.5 (23); Coprinus cubensis, 6.9 (119); Psalliota bispora, 6.0 (64), 6.8 (273), 6.3-6.7 (133).

Early pH investigations by Schmitz (251), Wolpert (285), Rumbolt (247), and Meacham (171) indicated optimum growth range between 3.5 and 6.5 for Polystictus versicolor, P. adustus, Schizophyllum commune, Lenzites sepieria, Pleurotus ostreatus, Pholiota adiposa, Armillaria mellea and Fomes pinicola, F. roseus. Except for S. commune and P. ostreatus, mycelial growth was checked in alkaline solutions and inhibited at pH 3.0.

Hacskaylo et al. (91) found that the greater the relative amount of growth of an isolate at succeeding temperatures, the lower the final pH of the corresponding solution. Of 135 Agaricales species, the raising, lowering or stabilization of the initial pH occurs according to individual species, strain, even isolate, and media (184, 273). Wang and Miles (282) learned with Schizophyllum commune that changes in pH and total acidity corresponded roughly to the growth of mycelium, an increase in mycelial dry weight was accompanied by a decrease in pH and an increase in the total acidity of the culture medium.

Optimum temperatures for mycelial growth were determined for several Agaricales by various workers. Amanita rubescens, and Russula emetica grew best in submerged culture at 29° C, Suillus punctipes between 24-29° C, and S. luteus 18-24° C (91). Other temperature optima are: Amanita muscaria 25° C (205), Fomes annosus 23° C (53), Coprinus cubensis 35° C (119), Paxillus panuoides 25° C (59), Agaricus campestris 25° C (109, 266), Schizophyllum commune, Polyporus oblique, P. albellus, P. tulipifera, Lenzites trabea 25° C (144), a Coprinus sp. 25° C (69), Polyporus abietinus 25° C, Mycena flavo-alba 15° C (77), Armillaria mellea 25° C (225), 19 Mycena sp. 20° C (73), 25 and 31° C for different Polyporus abietinus strains (79), Psalliota bispora 24° C (273), 26.7° C (51), 25° C (132), 13-21° C recorded by various authors reported in early literature (273).

Nutrient media for Agaricales usually have glucose as the carbon source if physiological studies are involved (1, 21, 35, 63, 69, 92, 94, 105, 108, 109, 112, 116, 121, 125, 146, 196, 197, 199, 201, 202, 203, 205, 212, 225, 236, 264, 268, 269, 288) while a few authors selected sucrose (101, 215, 279) and maltose (19, 130, 160, 250).

In carbon studies, some researchers identified useful carbon sources by analysizing the presence of sugars or enzymes in basidiocarp fractions (34, 128, 135, 136, 221, 289) or liquid cultures (252), however, all others carried on such experiments by varying nutrient media. The monosaccharides glucose, fructose, mannose, or xylose provided greatest hyphal growth for species of Coprinus (68, 119), Lactarius (112), Collybia (1, 215, 279), Stereum, Hydnum, Polyporus, Polystictus, Fomes (77, 90, 100, 125, 130, 143, 199, 212), Pholiota (13), Schizophyllum (143, 196, 269, 282), Lenzites (143), Boletus (105, 175), Mycena (77), Armillaria (225, 252), Pleurotus (125, 274), and Agaricus (26, 81, 107, 109, 266). Fructose-1,6-diphosphate had a very striking growth promoting effect on Boletus and Collybia (197), and all species of Agaricales used by Lilly and Barnett grew more rapidly and produced more mycelium on L- compared to D- arabinose (26, 144, 269).

Among disaccharides, maltose, lactose or sucrose were found most usable by several authors for Agaricales

(13, 26, 77, 81, 100, 105, 107, 109, 112, 119, 130, 143, 196, 212, 215, 225, 252, 266, 267, 269, 279, 289). Collybia was usually not able to utilize disaccharides (1), but Plunkett (215) noted that sucrose equalled glucose as the best carbon source in hyphal production.

Raffinose was usable for Polyporus, Fomes, Armillaria, Daedalea (130, 143, 252), Lenzites (143, 289), Stereum (100), Schizophyllum, Collybia (143, 196, 269), Coprinus (119), Polystictus (130).

According to Stoller (264), there should be an application of agricultural practices to mushroom culture of establishing NPK and C/N ratios due to the principle of limiting factors. Depending upon what nitrogen source is selected, the carbon source is then utilized in A. bisporus cultures (26).

A review of literature indicates the polysaccharides dextrin, starch, and xylan proved good carbon sources for the Agaricales Boletus, Lepiota (94, 105), Polyporus, Daedalea, Armillaria (130, 212, 252), Lenzites (289), Stereum (100), Fomes, Polystictus (125, 130), Coprinus (68, 119), Pholiota (13), Lactarius (112), Pleurotus (125, 274), Agaricus (81, 94, 107, 109, 133, 266, 267). Cellulose or lignin was utilized by Armillaria (225, 252, 285), Lenzites (285, 289), Stereum (100), Coprinus (68), Polyporus, Polystictus (37, 83, 285),

Paxillus (59), Fomes (53), Marasmius (148), Tricholoma (175, 201, 227), Pleurotus, Pholiota (37, 285), and Agaricus (26, 133, 266, 267, 281).

Moderate or poor mycelial growth developed with organic acids (26, 51, 77, 105, 112, 274), and sugar alcohols (13, 26, 27, 143, 196, 227, 269).

Nitrogen utilization studies for hyphal growth are limited in comparison to other nutrient studies with species of Agaricales. Amino acids, proteins, ammonia, ammonium salts, peptone and urea all serve as useful nitrogen sources for Agaricales species (22, 23, 26, 51, 63, 82, 107, 109, 111, 133, 266, 267, 273). Ammonium salts and nitrates induced poor growth with Pleurotus while peptone, urea and amino acids were best (92, 125, 138, 139, 262, 274). Peptone also was very useful as a nitrogen source for other Agaricales (13, 84, 100, 101, 105, 119, 130, 199, 225). Greatest hyphal growth was obtained with amino acids and urea while ammonium salts increased growth, nitrates were slightly or not utilized by species of Polystictus (125), Tricholoma (175, 201, 202, 227), Armillaria (84, 225), Mycena (73, 77), Polyporus (77, 115, 130) Stereum (100, 101, 236), Fomes (115, 130, 232), Hydnum, Lentinus, Lenzites, Peniophora, Poria, Ptychogaster, and Trametes (115). Noecker (199) found an ammonium source for nitrogen was slightly better for hyphal growth than an amino acid for species of Stereum, Hydnum, Polyporus, and Fomes.

It was assumed by most workers that only the L- isomers in the racemic amino acid mixtures were utilized, although some of the D- isomers may be utilized also (117, 227), the L- form was more useful than the D-form (116). D-alanine was used by Lenzites trabea (117), and Psalliota campestris can hydrolize both optical isomers of proline, alanine and leucine (219).

Ammonium salts produced optimum growth while amino acids and nitrates were of less use for species of Boletus (105), Collybia (1, 8, 138, 215, 279, 280), Marasmius (148). Several amino acids were found useful as the sole nitrogen source for Marasmius (152). Ammonium salts also proved productive for Lactarius species (112), however, 18 amino acids used singly or in combination increased the hyphal yield (179). Vinasse, a fermentation product of sugar beets, high in ammonium salts was useful for Boletus, Agaricus, Tricholoma but useless to Cantharellus and Collybia (56). Having an unknown metabolic role, ferulic acid greatly increased hyphal growth of species of Corticium, Fomes, Hydnum, Hymenochaete, Peniophora, Polyporus, Poria, and Stereum (242).

In nucleic acid nutritional studies with fungi, Fries found an adenine deficiency for Lenzites betulina, L. abietina, Polyporus abietinus, P. cervinus, P. annosus (75), adenosine deficiency for Mycena species, and a slight growth promoting effect by hypoxanthine, guanine and granosine for species of Polyporus (76).

Amino acids and ammonium salts induced greatest hyphal growth while nitrates were poor for Schizophyllum (196, 269).

Nitrates, nitrites and urea were more useful than amino acids to Coprinus (119, 138) while amino acids were superior to ammonium salts (68, 139, 262), particularly useful was asparagine (214) and DL-methionine (67). Using 8 Coprinus species, Fries found that ammonium salts seemed to give better growth than asparagine (65), and obtained physiological mutations induced by nitrogen mustard that had differing amino acid requirements (66). Physiological mutants produced from oidia, basidiospores, by use of ultra-violet light, and nitrogen mustard developed arginine - less, methionine - less, adenine - less, cytidine - less, uridine - less, amino acid - less, and nucleic acid - less strains of Collybia velutipes (7).

MATERIALS AND METHODS

Nine homobasidiomycetes were used in this study. Initial transfers of eight of them were made aseptically from pileus tissue. The ninth, Pleurotus ostreatus was grown from spores streaked on agar media. The two mutants of Agaricus bisporus and one of Volvariella volvacea occurred in stock cultures. All stocks were transferred every ten weeks.

The collection sites and dates for the Cantharellus specimens are as follows: C. clavatus (C-63-8) on the Michigan State University campus, July 8, 1963; C. cibarius (C-63-15) from a forest east of Marquette, Michigan, August 2, 1963; C. cibarius (C-63-86) in the University of Michigan Savoy Forest near Pellston, Michigan, August 26, 1963; and C. cibarius (C-63-96) in a jack pine grove west of Marquette, September 21, 1963. The cultures were maintained on 2% malt agar (see appendix for media contents).

Agaricus campestris (A-63-136) was collected on the grounds of Walnut Hills Golf Club, East Lansing, Michigan, September 25, 1963. A white strain of A. bisporus (A-63-137) was obtained from the Mushroom Growers Association, Niles, Michigan, July 12, 1963. The mutant forms occurred naturally

in vitro from this culture. Agaricus bisporus rhizomorph (A-63-139), a mutant, produced abnormal numbers of rhizomorphic strands in comparison to the parent colony. A second mutant, A. bisporus pleomorph (A-63-138) developed a cottony aerial culture. Three of the Agaricus cultures were maintained on medium A while the rhizomorphic form was kept in stock on 2% malt agar. The latter originated from a parent culture grown on medium A.

Lepista nuda (L-63-133) was collected at the University of Michigan Biological Station, Pellston, Michigan on September 14, 1963. The spore isolates of Pleurotus ostreatus (P-63-51) were from a spore print of a basidiocarp grown on beech near Tahquamenon, Michigan, August 8, 1963. Volvariella volvacea (V-63-135) was obtained from the University of the Philippines; V. volvacea mutant (V-63-134) is a white colony form of the commercial Philippine mushroom. All were kept on 2% malt agar in stock.

The description of the fungi used follows: Cantharellus clavatus Fries, the pig's ear mushroom (218, 259, 260), has a broad pileus, which becomes funnel-shaped and lobed (Figure 1). The stipe and pileus are in distinguishingly fused, both tapering downward, with a dry surface which later becomes scaly. The pileus surface remains smooth. Ridge-like gills are decurrent, extending almost to the stipe base, freely anastomosing by means of

interconnecting "veins". The tissue is firm and white to yellowish brown. The spore print is ochraceous, from four-spored basidia. The hymenium is without cystidia. The immature grayish to purplish tinted basidiocarp fades to a dull brown with age. The stipe is compound, fusing together at the base, and solid becoming hollow. In Michigan the species is found during late summer and fall, in a conifer forest habitat.

Cantharellus cibarius Fries, the chanterelle mushroom (218, 259, 260), has a broad, flat to slightly depressed pileus with an inrolled margin which becomes wavy and lobed (Figure 2). The surface is dry, covered with a thin coat of fine fibrils. The flesh is thick, white to pale yellow. When young, the basidiocarp is yellow, becoming bright yellow to orange with age. Lamellae are decurrent, dichotomously forked, narrow and have interconnecting "veins". The basidia have four spores, and the spore print is yellow. The stipe is thick, solid and white. The species is found in summer and early fall, singly or in clumps, and generally in a conifer forest.

Agaricus campestris L. ex. Fries is the common pink gilled field mushroom (120, 185, 218, 259). The glabrous pileus is convex with a flattened center (Figure 3). It is white colored, later turning slightly yellow to light brown. A slightly fibrillose condition was noted. The flesh is white. The gills are free, thin, crowded, wide,

rosy when young and turning pink to dark brown at maturity. The basidia are four-spored, clavate, and have dominant sterigmata. The spore print is dark brown. The cylindrical stipe is thick, solid, tapered downward, smooth, becoming hollow. A ring is found slightly higher than the stipe center; its margin is ragged. In late summer to fall, the species is found in open areas such as pastures or meadows. Its smell is acidulous.

Agaricus bisporus (Lange) Singer (185, 257) is the cultivated mushroom most commonly grown in the western hemisphere. The cap is convex with a slightly flattened center, white or cream, finely squamulose, with a thick margin slightly fibrillose to striate or crenate. The gills are free, crowded, narrow, fimbriate edged, pink to brown at maturity. Numerous cystidia are on the gill edge. The spore print is sepia to dark brown and the basidia are two-spored. The flesh is white to light tan in color, at times slightly pink. The stipe is white to tan, solid to fibrous to hollow with age, and slightly flocculose below annulus. The ring is white, thick, narrow and striate above. The stipe base is slightly expanded, tapering upwards. The odor at first is pleasant and mild, becoming acidulous with age. Wild strains of A. bisporus are found during summer and fall in rich soil near manure heaps, in gardens and parks.

Lepista nuda (Bull. ex Fries) Cooke (162, 218, 258, 259), the wood blewit, has a smooth, moist cap which is a slightly convex disc or plane, thick, very solid, pale blue gray to purple (Figure 4). Some have a violaceus margin and a brownish disc with fine hairs present. The flesh is tinted lavender, white or gray. The free lamellae are crowded, broad, violaceous to white to brown with age and are easily separated from the cap. The spore print is white with a slight salmon tint. The stem is short, solid, slightly tapered at the apex from a thickened base. The surface possesses fine hairs and is blue to violet in color. It is found in late summer and fall in hardwood forests, in piles of decaying leaves or soils with a high organic content.

Pleurotus ostreatus (Jacq. ex. Fries) Kummer, the oyster mushroom (162, 218, 259), is found on aspen, beech and sometimes elm in Michigan (Figure 5). The pileus is oyster shell-shaped, smooth, moist, white becoming brown or gray. The flesh is thick, white and soft. The gills are decurrent, broad, quite distant, continuing to stem base, white becoming light yellow. The stipe is lateral, short or nearly absent, large, solid, and white. The spore print is lilac gray. Pleurotus is found in spring or fall, during extended moist weather.

Volvariella volvacea (Bull. ex. Fries) Singer (97, 162, 255, 257) is the paddy straw mushroom, and is

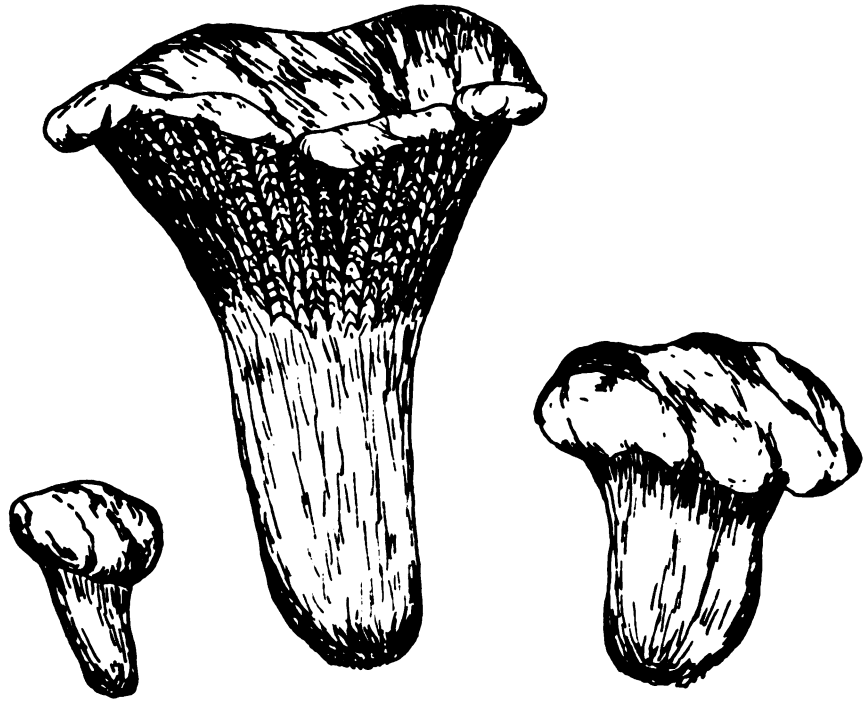
cultivated on a small scale in the Philippine Islands and other tropical areas (Figure 6). The pileus is broad, ovoid, convex, whitish to gray brown with dark gray silky fibrils. It has a subumbonate disc and the flesh is white. The gills are broad, free, close, and white becoming salmon. Conspicuous cystidia are present. The basidia are four-spored. The spore print is dull pink to brownish pink. The stipe is off white to dull brown, firm but soon becoming hollow, fibrillose, very slightly attenuated upwards. A distinctly large volva is present which is irregularly lobed, membranous, floccose, brownish. Its natural habitat is wood, soil, dung, where it sporulates from June to September singly or in tufts throughout tropical Asia.

All experiments were done in replicates of two or three, occasionally as many as five. Submerged culture experiments were carried out in a constant temperature room of 74° F.

The inoculum grew on stock plate cultures for three weeks, after which a 1.5 inch square section of the colony was removed and blended one minute with 55 ml distilled water in a 350 ml Monel metal semi-micro Waring Blendor or a Barocilicate glass Jap Blendor. Five ml of the blended mycelium was pipetted into 250 ml Erlenmeyer flasks or 250 ml DeLong Culture Flasks containing 50 ml test medium and then placed on New Brunswick Gyrotary Shakers at 180 RPM. Flasks were stoppered with cotton, covered with aluminum foil,

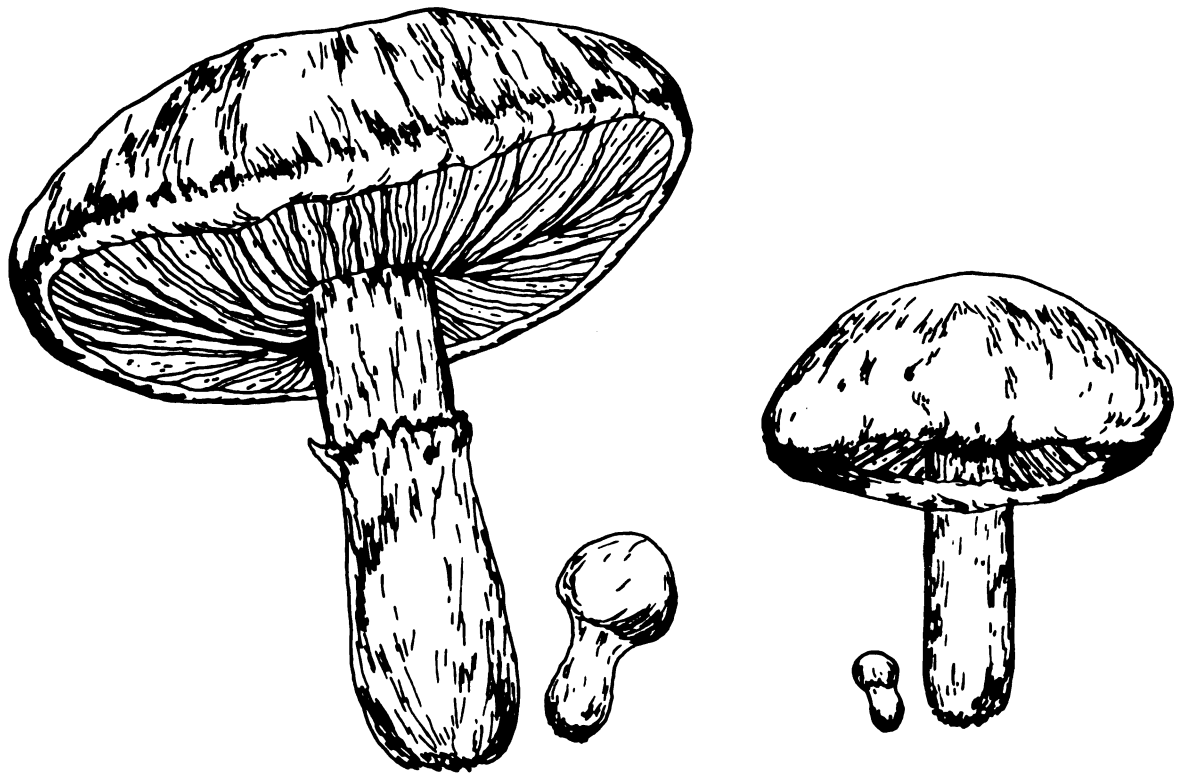
Cantharellus clavatus

Figure 1.



Cantharellus cibarius

Figure 2.

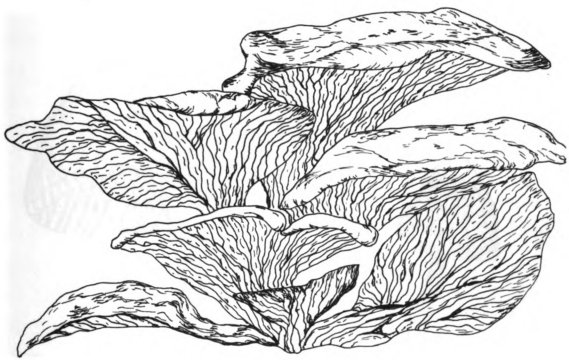


Agaricus campestris

Figure 3.

Lepista nuda

Figure 4.



Pleurotus ostreatus

Figure 5.

Volvariella volvacea

Figure 6.

or Morton culture tube closures. The flasks were autoclaved twenty minutes at fifteen pounds pressure per square inch, 121° C before inoculations were made. Experiments were carried out under aseptic conditions. A modified Humfeld's basal medium (109) was used for the shake cultures and substitutions were made according to the experiments involved. The ingredients of Humfeld's medium are: glucose, 50 g; KH_2PO_4, 0.87 g; $MgHPO_4$, 0.40 g; $CaCl_2 \cdot H_2O$, 0.37 g; H_2SO_4(2N), 5.1 ml.; trace element solution, 20.0 ml ($FeCl_3 \cdot 6H_2O$, 0.5 g; $MnCl_2 \cdot 4H_2O$, 0.36 g; $ZnCl_2$, 0.20 g; $CuSO_4$, 0.05 g; distilled H_2O to make one liter), 1,000 ml distilled H_2O. One g urea was added per liter for the nitrogen source. Of 1, 2 or 3 g urea per liter, one g produced the greatest mycelial weight with most of the twelve fungal cultures. Triplicate petri dish cultures of the twelve fungi were also made using Humfeld's medium plus 30 g agar per liter, with 1, 2 or 3 g urea per liter. Greatest colony growth also occurred with one g urea per liter. Potassium phosphate was dissolved in the sulfuric acid and 50 ml distilled H_2O before being added to the Humfeld medium. The pH was adjusted to 4.5 with NaOH (1N). Using No. 501 7.5 cm diameter Sargents filter paper upon harvest, the mycelium was vacuum filtered, washed, then dried twelve hours or more in an 85° C oven. Filter papers with the mycelium were individually suspended in 25 or 50 ml beakers in the oven to enhance drying.

To determine the most advantageous pH level with Humfeld's medium for all the cultures, a range of 4.5 to 7.2 was used. Duplicates of the twelve fungi were run in Humfeld's medium at pH 4.5, 5.5, 6.5 and 7.2, ten and twenty days each.

The density of the inoculum was checked using a Howard mold count chamber, and the average positive percentage of 25 fields. One field was considered positive if two or more hyphal strands were within that field. The percentages of the twelve fungal inocula were as follows: C-63-8, 76%; C-63-15, 84%; C-63-86, 80%; C-63-96, 90%; A-63-136, 84%; A-63-137, 76%; A-63-139, 80%; A-63-138, 84%; L-63-133, 84%; P-63-51, 76%; V-63-135, 76%; V-63-134, 84%.

Hyphal diameters of the inocula were within the following micron ranges: C-63-8, 1.73-3.46μ; C-63-15, 1.73-3.46μ; C-63-86, 1.73-3.46μ; C-63-96, 1.73-3.46μ; A-63-136, 1.73-3.46μ; A-63-137, 2.5-3.46μ; A-63-139, 3.46μ; A-63-138, 1.73-5.19μ; L-63-133, 1.73-3.46μ; P-63-51, 1.73μ; V-63-135, 3.46-6.92μ; V-63-134, 1.73-3.46μ. The fungi were also checked for frequency of clamp connections with various carbon sources in Humfeld's medium.

Using various carbohydrates, eight grams carbon per liter was substituted for glucose in Humfeld's medium. Fifty ml medium adjusted to pH 4.5 with NaOH(1N), and 5 ml blended inoculum were added to the 250 ml Erlenmeyer flasks.

Triplicate cultures of the twelve test organisms were incubated on rotary shakers for ten and twenty days each. Data were recorded as gram weight of the dried mycelium. The carbon sources included eleven monosaccharides: D-arabinose, DL-arabinose, L-arabinose, D-fructose, D-galactose, D-glucose, D-mannose, L-rhamnose, D-ribose, L-sorbose, and D-xylose; seven disaccharides: D-cellobiose, α lactose, β lactose, D-maltose, and sucrose; one trisaccharide: raffinose; seven polysaccharides: cellulose, dextrin, glycogen, inulin, pectin, starch, and xylan; and nine organic acids: citric acid, galacturonic acid, DL-malic acid, oxalic acid, pyrogallic acid, β resorcylic acid, succinic acid, tannic acid, and D-tartaric acid. DL-malic acid was Seitz filtered and pipetted aseptically to the individual sterile rotary shaker culture flasks. Water filtrates of casein and xylan were used and one gram cellulose was measured individually for each flask. Other carbon sources used included i-inositol, D-mannitol, pectinol A (a commercially prepared substance of di- and polygalacturonic acid), D-sorbitol, D-glucosamine hydrochloride, and fructose-1,6-diphosphate.

Several carbohydrates were used at 25, 50 and 75 g per liter. D-galactose, D-fructose, α lactose, sucrose, starch, i-inositol and D-sorbitol were added as the carbon source to Humfeld's medium adjusted to pH 4.5. Averaged duplicate dry mycelial weights of the twelve fungi were taken at the end of both ten and twenty days.

Glucose utilization by the twelve fungi in Humfeld's medium at pH 4.5 were recorded as averaged mycelial dry weight of duplicate cultures at the end of ten, twenty, thirty and forty days each. Growth rate studies were also included for these data.

Fourteen day dry mycelial weights of the twelve fungi in replicates of three were averaged for several ammonium and nitrate nitrogen sources: ammonium bicarbonate, ammonium chloride, ammonium citrate diabasic, ammonium nitrate, ammonium sulfate, ammonium tartrate, calcium nitrate, cobaltous ammonium sulfate, cobaltous nitrate, cupric nitrate, magnesium nitrate, potassium nitrate, sodium nitrate and uranium nitrate. With a nitrogen equivalent based on one gram urea per liter, 0.47 g nitrogen per liter was used for each of the above compounds. The pH was adjusted to 4.5 for Humfeld's medium, with glucose as the carbon source at concentrations of 8 g carbon per liter. The pH was also taken at the end of fourteen days. Several nitrogen sources were not autoclaved with the medium, but were added aseptically to each 250 ml culture flask after Humfeld's medium was sterilized. The inorganic nitrogen sources Seitz filtered included ammonium bicarbonate, ammonium citrate diabasic, ammonium tartrate, cobaltous nitrate, cupric nitrate, magnesium nitrate and uranium nitrate. Urea was also Seitz filtered.

Seventeen amino acid and related nitrogen sources at 0.47 g nitrogen per liter were used in Humfeld's medium, pH4.5. Dry mycelial weight of the twelve fungi were averaged from replicates of three rotary shaker culture flasks each, grown in 50 ml of medium using 5 ml inoculum. At the end of fourteen days the pH was taken. Amino acid compounds for this study included DL-alanine, L-asparagine, L-aspartic acid, betaine hydrochloride, creatine, cysteine hydrochloride, L-cystine, L-glutamic acid, glycine, D-histidine, DL-isoleucine, DL-methionine, L-proline, DL-threonine, DL-tryptophane, DL-valine and peptone.

Volvariella volvacea (V-63-135), Lepista nuda (L-63-133), Cantharellus cibarius (C-63-86) and Pleurotus ostreatus (P-63-51) were selected from the twelve fungal cultures for vitamin studies. The vitamins selected include p-aminobenzoic acid, ascorbic acid, biotin, calcium pantothenate, niacin, niacinamide, pyridoxine hydrochloride, riboflavin, rutin and thiamine. Replicates of three were made of each vitamin at 0.5 mg per liter of glucose-casein hydrolysate with agar (104, 142). The media contained: glucose, 25 g; casein (vitamin free), 2 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 1.0 g; fumaric acid, 1.32 g; $Na_2CO_3 \cdot H_2O$, 1.12 g; $FeSO_4$, 0.2 mg; $ZnSO_4$, 0.2 mg; $MnSO_4$, 0.1 mg; highly purified oxoid code No. L13 agar No. 3, 20 g; distilled H_2O, 1,000 ml. Twenty ml media per 25 mm test tube were

used as agar slants and hyphal growth was measured in mm, six times at two day intervals, from point of inoculation to colony margin. Colony characteristics were compared at two weeks.

The four fungi selected for vitamin studies were also grown in 2% malt agar with 0.1%, 0.01% and 0.001% gibberellic acid, kinetin, 2, 4-dichlorophenoxy acetic acid, indoleacetic acid and indolebutyric acid each in replicates of three. The growth hormones were dissolved in three ml acetone before adding to the 2% malt agar. Twenty ml agar was poured into plastic petri plates, and the colony radius was measured every two days from point of inoculation to the margin. Due to difficulty in controlling pH, the results were negative except at the 0.001% concentration.

Volvariella volvacea (V-63-135), Lepista nuda (L-63-133), Cantharellus cibarius (C-63-86) and Pleurotus ostreatus (P-63-51) were chosen for the spawn experiment. The following substrates were used: flax, lupine, millet, milo, orange cane, rape, sorghum, spelt, sudan grass, vetch, wheat, rye and sunflower seeds. Other media included ground corn cobs, spruce needles, rye plus 2 g glucose per flask and rye plus 30 ml Humfeld's medium without glucose per flask. The spawn was incubated at 24° C; additional rye spawn was grown at 12, 18, 30 and 36° C. Twenty-five grams of grain, or enough to fill flasks one-fourth full, 30 ml distilled H_2O, or enough water to adequately cover the

grain, and 0.3 g calcium carbonate were added to 125 ml Erlenmeyer flasks in replicates of four. The initial pH reading was 5.7. Larger quantities of spawn medium in proportions to the above were placed in 500 ml Erlenmeyer flasks and milk bottles, in replicates of two to five. The media were autoclaved twenty minutes, vigorously shaken to loosen and mix grain with the calcium carbonate, then inoculated. The hyphal inoculations were made from mycelium growing on 2% malt agar blocks cut out from cultures. One two cm square agar block was transferred aseptically to each flask. A two week period was allotted for spawn growth; rapid developing cultures were allowed to grow up to three months for possible fructifications. Sterile distilled water was added periodically to the three month old cultures to prevent drying.

Using fourteen day old rye spawn, the same four fungal strains mentioned above were sown in mushroom houses suited to the conditions of Agaricus bisporus. Wooden boxes one foot square and eight inches in depth were constructed and made in replicates of five for each of the four fungi. Mushroom production methods were similar to those described by Atkins (12) and Richards (229).

The horse manure and wheat straw was composted ten days and underwent three turns for aeration and thorough mixing with the 28 pounds calcium carbonate per ton compost.

At the end of ten days, the compost was placed in the wooden boxes, autoclaved forty minutes at 121° C, fifteen pounds pressure per square inch. Boxes were placed in a mushroom house just completing sweat out, the final fermentation to reduce the ammonia concentration, reduce contamination, lower the insect population and eliminate excess moisture. Inoculum was added and allowed to grow three weeks at 65° F before one-half inch layer of casing soil was spread over the compost surface to supposedly enhance fructification. Beds were watered occasionally with a fine spray to prevent drying of casing soil and compost.

In another experiment, two strains of Pleurotus ostreatus (P-63-51) and (P-64-6) each were inoculated on 2% malt agar petri plates, and placed in dark, light, inverted and upright positions. Half the inverted plates contained 10% KOH in the petri dish cover. They were allowed to grow and checked for fruiting initials.

The electron micrographs were taken with a Philips EM-100B. Material was fixed a few seconds in 5% $KMnO_4$ then dehydrated for five minutes each in 30, 50, 70 and 85% ethanol, for ten minutes each in 95% ETOH and three changes of absolute ETOH. Two fifteen minute changes of propylene oxide followed, then material remained for one hour in a mixture of propylene oxide and an Epon 812 resin mixture. The Epon 812 resin mixture was composed of equal amounts of

mixture A: Epon 812 (Shell Oil Company), 62 ml; dodecenyl succinic anhydride (DDSA hardener), 100 ml; and Mixture B: Epon 812, 100 ml; methyl nadic anhydride (MNA accelerator), 89 ml. The propylene oxide and Epon 812 mixture was drained and replaced by the Epon 812 mixture. After six hours, the first Epon change was centrifuged and pipetted off. A second change of Epon was added to the material and remained overnight. Material was then transferred with an inoculation loop to No. 1 gelatin capsules which were half filled with a resin mixture of five ml each of mixtures A and B plus 0.15 ml of tridimethylaminomethylphenol (DMP accelerator). Capsules were polymerized twelve hours each at 35, 45 and 60° C. Sections were cut on a Porter-Blum Ultramicrotome using glass knives, then were placed on specimen grids covered with a parlodion film. The procedures were formulated according to Luft (158, 159) and Pease (211).

Light microscopy procedures were carried out according to Sass (249) and Johansen (118) with modifications. Using a suction pump, material was killed and fixed twenty-four hours in Craf (equal parts of solution A: chromic acid, 1 g; glacial acetic acid, 7 ml; distilled H_2O, 92 ml; and solution B: neutral formalin, 30 ml; distilled H_2O, 70 ml). The dehydration method was as follows: 50% ethanol and tertiary butyl alcohol (distilled H_2O, 50 ml; 95% ETOH, 40 ml; TBA, 10 ml), two hours; 70% ETOH and TBA (distilled H_2O, 30 ml; 95% ETOH, 50 ml; TBA, 20 ml), twelve hours;

85% ETOH and TBA (distilled H_2O, 15 ml; 95% ETOH, 50 ml; TBA, 35 ml), two hours; 95% ETOH and TBA (distilled H_2O, 5 ml; 95% ETOH, 40 ml; TBA, 55 ml) 2 hours; 100% ETOH and TBA (absolute ETOH, 25 ml; TBA, 75 ml) plus a few grains of erythrosin, 2 hours; three changes of TBA, 3 hours each; TBA and paraffin oil, equal parts, 12 hours. Vials were then filled one-third with tissuemat (56.5° C melting point), allowed to cool, then filled another one-third with material to be imbedded plus the TBA and paraffin oil. Four changes of tissuemat were made in four hour intervals with the paraffin oven at 70° C. Embedding was done from the fifth change of tissuemat. Sectioning was done on a rotary microtome.

Paraffin ribbons of the material were floated on microscope slides using a modified Sass (249) adhesive 3 (solution A: gelatin, 1 g; sodium benzoate, 0.5 g; distilled H_2O, 100 ml; solution B: chromium potassium sulfate, 1 g; formalin, 10 ml; distilled H_2O, 90 ml) mixing one part of solution A with six parts of solution B.

A modified Conant's quadruple staining procedure was used for the sections of fruiting bodies grown in culture. Slides were passed through the following Coplin jar series in three minute intervals: two changes of xylene, 100%, 95%, 70%, 50% ETOH; 1% safranin in 50% ETOH, two hours; 30% ETOH, distilled H_2O, three minutes each; 1% aqueous crystal violet, one-half minute; distilled H_2O, one minute, 30%, 50%, 70%, 95%, 100%, 100% ETOH, two minutes each; nine

parts clove oil saturated with orange gold and one part 1% fast green in absolute ETOH, two minutes; four baths of clove oil and orange G, two minutes each; three changes of xylene, three minutes each. Clarite was the mounting medium. Mycelium from submerged cultures were directly mounted in lactophenol cotton blue for light microscopy.

The twelve fungal cultures were grown on various Difco media (49) for identification purposes. The following were used: tryptic soy agar, blood agar base, nutrient agar, rice agar, Russian medium, Russian medium minus casein and tryptophan, Russian medium minus vitamins, potato dextrose agar, prune agar, tomato juice agar special, mycological agar, brewer anaerobic agar, corn meal agar, mycosel agar, liver spleen glucose agar, chlamydospore agar, thioglycollate medium, micro assay culture agar, violet red bile agar, O. Modess (12) agar; see appendix. Replicates of three were made of each media for the four fungi and allowed to grow three weeks in petri plates.

RESULTS

Effect of pH on the Cultures

The pH of Humfeld's medium was varied to determine the most acceptable level for all 12 fungal cultures. The greatest mycelial dry weight with Lepista at ten days was with pH 7.2; 4.5 was optimum at twenty days. Pleurotus was best at pH 6.5 at ten days, and 7.2 at twenty days. Volvariella had good growth at pH 4.5, ten and twenty days, as did all four Cantharellus species. A pH of 6.5 or 7.2 produced the best growth with the Agaricus species. Dry mycelial weight differences within the pH range tested were not great, a pH of 4.5 was selected for the later performed submerged culture experiments.

Utilization of Monosaccharides

The experimental results in Table 1 present the utilization of eleven monosaccharides by the twelve fungi selected for study. Each fungal strain had certain submerged growth characteristics in Humfeld's medium that were described with the corresponding carbohydrate, and nitrogen sources.

Cantharellus clavatus (C-63-8) produced no great increase in growth with monosaccharides in comparison to the control (Table 1). Each sugar did produce slightly

higher dry weights than Humfeld's medium minus carbohydrate. The mycelium in shake culture did not form compact pellets, instead only individual strands and occasionally loosely interwoven clumps formed. D-arabinose, DL-arabinose and D-fructose produced similar low dry weight readings, slightly higher than control. L-arabinose, D-glucose and D-xylose gave the next highest dry weights followed in succession by D-ribose, D-galactose, D-mannose, L-rhamnose and L-sorbose. The latter gave the highest dry weight reading at 20 days. A one mg decrease in dry weight between the 10 and 20 day readings occurred only with DL-arabinose and D-fructose, all others gave higher readings at 20 days than 10 days.

Wide variations in dry weight readings occurred with C. cibarius (C-63-15) using monosaccharides as the carbon source in comparison to C. clavatus. Good pellet formation developed particularly at 20 days for several cultures having high dry weights. D-ribose and D-galactose produced the least hyphal growth; D-arabinose, L-arabinose, D-fructose, L-rhamnose and L-sorbose gave moderate growth results within the same dry weight range at 10 and 20 days. D-mannose and D-xylose had good mycelial growth while DL-arabinose and D-glucose produced nearly identical results, and had the highest dry weights. Hyphal growth was loosely organized, no pellets formed.

The C. cibarius strain (C-63-86) was quite comparable to C. clavatus in utilizing monosaccharides. Growth with D-arabinose, DL-arabinose, and D-mannose produced slight autolysis as did the control. No growth occurred with D-fructose. A group of monosaccharides which produced a moderate amount of growth include D-arabinose, DL-arabinose L-arabinose, D-glucose, D-mannose, D-ribose and L-sorbose. In increaseing order, L-rhamnose, D-xylose and D-galactose produced the highest dry mycelial weights. Few pellets formed in submerged growth.

C. cibarius (C-63-96) was consistently much better in hyphal production compared to the other Cantharellus species; approximately six to eight times greater dry weights were recorded with the monosaccharides. L-rhamnose was poorly utilized by the tenth day, and was the only monosaccharide to show a weight increase between 10 and 20 days; autolysis occurred in all other carbon cultures as the peak in the growth phase must have been passed. D-arabinose, DL-arabinose, and D-ribose had dry mycelial weights within 200 to 300 mg. Ten day mycelial weights were above 300 mg and 20 day weights were above 200 mg for D-fructose, D-galactose, D-glucose, D-mannose, L-sorbose and D-xylose. Maximum mycelial growth recorded was at 10 days with L-arabinose as the carbohydrate source in Humfeld's medium. Excellent hyphal pellet formation occurred in each monosaccharide medium at both 10 and 20 days, and a large mycelial ring grew

on the flask side at the height of the media splash levels produced by the shaker motion. All cultures also developed a water soluble yellow-green pigment in solution, a common characteristic of only the C. cibarius (C-63-96) strain. The greater the mycelial growth, the more pronounced was the pigment coloration.

D-fructose, D-glucose and D-mannose were exceptionally good monosaccharide carbon sources for Agaricus campestris (A-63-136), while the other sources were not as good from a concentration of 8 g carbon per liter of media. Hyphae grew in submerged culture as well formed pellets and occasionally a mycelial ring developed on the flask wall if good growth occurred.

Poor growth occurred with A. bisporus (A-63-137) grown in Humfeld's medium using D-arabinose, DL-arabinose, L-rhamnose or D-ribose; moderate growth with L-arabinose, D-galactose and D-xylose; and slightly better growth with D-glucose, D-mannose and L-sorbose. The best growth formed in the medium with D-fructose, particularly at 20 days. Hyphae did not form pellets; only occasional flask rings grew, while hyphae in submerged culture remained single stranded.

The pleomorphic form of A-63-137, A. bisporus pleomorph (A-63-138) showed similarities to its parent culture. D-arabinose, DL-arabinose, D-galactose, L-rhamnose, D-ribose and L-sorbose produced poor growth. L-arabinose

and D-xylose gave similar results while D-fructose produced good growth. These were all under 100 mg dry weight at both 10 and 20 days. Best growth occurred with D-glucose and D-mannose, both resulted in over 20 mg of mycelial dry weights at 20 days incubation. No pellet formation resulted in the hyphal growth, but frequent flask mycelial rings occurred. Most of the carbohydrate cultures having poor growth also showed autolysis or reduced mycelial dry weight at 20 days compared to 10 days incubation.

The rhizomorphic form of A-63-137, A. bisporus rhizomorph (A-63-139) also basically followed its parent in carbohydrate utilization. D-arabinose had the least mycelial growth of the monosaccharides. DL-arabinose, L-arabinose, D-galactose, L-rhamnose, and L-sorbose gave moderate growth; cultures grown in D-fructose, and D-xylose showed good growth at 20 days. The same monosaccharides, D-glucose and D-mannose, produced the highest dry weight readings particularly at 20 days with the pleomorphic strain resembling that of the parent strain but at a much lower value. D-fructose and L-ribose were not as readily utilized as by the parent strain. No distinctive submerged culture growth was evident, hyphae remained single stranded both at 10 and 20 days growth.

Lepista nuda (L-63-133) had poor growth at the end of both 10 and 20 days with D-arabinose, D-ribose and D-xylose as the monosaccharide carbon source at 8 g carbon

per liter of Humfeld's medium. L-arabinose, D-galactose, L-rhamnose and L-sorbose formed fair growths of L. nuda in submerged culture while DL-arabinose showed good growth, particularly at 20 days incubation. Excellent dry weights, three to five times greater than other monosaccharides, were obtained by using D-fructose, D-glucose and D-mannose as the carbon source at 8 g per liter in Humfeld's medium. The latter three carbohydrates formed good pellets in submerged growth while mycelium in other carbon media tended to remain single stranded.

Pleurotus ostreatus (P-63-51) grew poorly in D-arabinose, DL-arabinose, D-galactose and D-glucose, but slightly better with D-mannose and D-ribose. Fair growth occurred with L-arabinose and D-xylose while D-fructose, D-sorbose and L-rhamnose produced the most mycelium. No significant hyphal weight differences occurred between the monosaccharides giving the poorest and best results with P. ostreatus, the difference was no greater than 25 mg. A few loosely woven pellets formed in submerged culture and some flask mycelial rings developed.

Like Pleurotus, Volvariella volvacea (V-63-135) showed no significant growth increases with monosaccharides in Humfeld's medium. Quite poor growth occurred with D-arabinose, DL-arabinose, D-fructose, D-ribose and D-xylose. Slightly higher dry mycelial weights were obtained with L-arabinose, D-galactose, D-glucose and D-mannose. Hyphal

growth was fair with D-sorbose while L-rhamnose was best. Submerged growth was quite distinct compared to other genera grown in shake culture. One large loosely interwoven mass of hyphae formed. Chlamydospore masses produced the characteristic gold-brown to tan color.

V. volvacea mutant (V-63-134) developed smaller masses of hyphae but no chlamydospores compared with V. volvacea (V-63-135). Growth differences indicated by the poorest and greatest dry mycelial weight were not great. Cultures grown in D-arabinose, D-fructose or D-sorbose produced poor growth while DL-arabinose, L-arabinose and D-glucose were only slightly better. D-galactose, D-mannose and D-ribose showed fair dry mycelial weights. L-rhamnose and D-xylose were the best monosaccharides for carbon utilization.

Utilization of Disaccharides and a Trisaccharide

Seven disaccharides and one trisaccharide were substituted in Humfeld's medium as the carbohydrate source at 8 g carbon per liter (Table 2). With Cantharellus clavatus (C-63-8), the eight sugars produced similar dry weight levels, all apparently equal in usability, 20 to 30 mg dry weight above control. No pellets formed; some loose masses of hyphae organized in submerged culture.

C. cibarius (C-63-15) produced the least growth with α lactose and raffinose while β lactose, D-maltose

Table 1.--The utilization of monosaccharides* by twelve fungi grown in submerged culture reported as averaged mycelial dry weight in milligrams of three replicates at the end of both 10 and 20 days.

	Days	D-arabinose	DL-arabinose	L-arabinose	D-fructose	D-galactose	D-glucose	D-mannose	L-rhamnose	D-ribose	L-sorbose	D-xylose	Carbon Free Control
Cantharellus clavatus C-63-8	10	19.7	23.7	26.6	21.4	27.7	26.6	29.8	29.5	23.9	27.4	28.7	14.8
	20	21.2	22.5	31.6	20.6	36.4	30.2	35.0	36.4	35.0	38.3	30.9	14.5
C. cibarius C-63-15	10	34.7	46.9	26.1	23.0	22.8	46.7	30.6	32.5	15.5	39.7	37.9	18.1
	20	45.4	102.4	51.2	45.1	23.5	108.1	69.3	45.8	34.4	41.6	85.8	25.9
C. cibarius C-63-86	10	32.4	27.9	26.0	19.7	25.9	25.0	32.9	27.4	21.1	22.8	36.1	21.8
	20	24.4	26.2	27.1	21.9	45.7	30.6	28.1	34.2	32.7	28.7	37.1	19.2
C. cibarius C-63-96	10	208.4	260.4	382.9	318.6	303.7	338.3	347.1	74.4	298.3	318.4	323.2	18.8
	20	147.5	219.0	319.9	251.4	245.0	280.6	275.9	88.4	228.9	214.9	253.8	17.1
Agaricus campestris A-63-136	10	31.7	35.8	43.4	295.5	33.8	110.2	41.7	32.5	36.7	35.5	39.1	24.5
	20	34.2	35.6	32.4	271.3	38.5	212.2	191.9	46.5	34.9	34.0	39.8	25.8
A. bisporus A-63-137	10	21.5	30.2	37.5	29.6	29.2	31.5	49.8	28.4	26.7	36.2	31.5	16.5
	20	22.6	29.2	43.0	91.8	43.9	53.9	53.9	27.9	27.4	59.0	41.3	14.0
A. bisporus, pleomorph A-63-138	10	34.3	39.9	47.1	30.9	27.5	100.9	76.9	31.5	40.1	27.1	33.2	13.0
	20	38.1	37.2	40.2	77.9	39.4	224.0	221.1	33.2	27.2	32.7	48.9	12.1
A. bisporus, rhizomorph A-63-139	10	23.0	29.0	32.3	21.2	27.9	34.9	38.2	28.3	48.4	24.8	35.8	16.8
	20	20.9	30.4	28.8	38.7	28.8	52.3	53.4	31.1	30.7	31.8	46.8	19.4

Table 1.--Continued.

	Days	D-arabinose	DL-arabinose	L-arabinose	D-fructose	D-galactose	D-glucose	D-mannose	L-rhamnose	D-ribose	L-sorbose	D-xylose	Carbon Free Control
Lepista nuda	10	33.4	37.3	70.3	183.6	31.1	120.3	215.7	44.7	25.9	32.5	31.7	35.1
L-63-133	20	30.6	93.4	50.1	172.2	41.6	269.8	340.1	25.3	31.5	45.5	26.4	33.1
Pleurotus ostreatus	10	26.2	28.7	37.5	44.8	29.8	27.7	32.7	31.2	35.4	43.6	36.8	17.5
P-63-51	20	28.7	28.6	38.9	24.4	31.2	31.2	33.4	41.4	27.3	49.5	37.6	27.3
Volvariella volvacea	10	27.9	29.8	38.6	29.4	41.8	38.6	43.4	40.4	28.1	33.0	30.0	17.9
V-63-135	20	33.1	33.5	41.6	29.5	32.3	43.1	43.1	57.0	34.3	52.7	39.5	29.3
V. volvacea, mutant	10	22.1	25.2	16.3	15.9	23.3	24.0	26.8	32.8	23.6	20.2	34.8	19.6
V-63-134	20	20.1	22.4	26.7	16.2	31.3	24.9	28.5	36.7	31.6	23.3	42.9	15.5

*Carbon concentration 8 g per liter.

developed fair growth. Sucrose, D-cellobiose in that order produced better growths with the C-63-15 strain. No pellets formed; only isolated hyphae grew in submerged shake culture.

C-63-86 strain of C. cibarius also showed moderate growth with most carbohydrates listed in Table 2. All disaccharides were utilized at about the same rate; dry mycelial weights at 10 and 20 days were similar for each carbon source. The trisaccharide, raffinose, produced the highest growth reading, particularly at the 20 day reading. A few small pellets grew in culture.

The third strain of C. cibarius, C-63-96, again produced much greater dry weights than did the other two strains. Numerous compact pellets and hyphal flask rings formed in each culture. In the presence of β lactose, 3 to 5 times less hyphal growth occurred than with α lactose, D-cellobiose, D-maltose, sucrose or raffinose. Sugars of the latter group had dry weights exceeding 200 or 300 mg and all exhibited autolysis by a decreased hyphal weight at 20 days compared to 10 days. A water soluble yellow-green pigment was present in most media.

Best growth for Agaricus campestris (A-63-136) was achieved with raffinose, the disaccharides all produced less than half the mycelial dry weight of the trisaccharide. Only a small number of loose pellets grew in the cultures

except in Humfeld's media using raffinose or α lactose, D-cellobiose and sucrose.

Raffinose and D-cellobiose produced excellent hyphal growth with A. bisporus (A-63-137), particularly at 20 days incubation. Sucrose, followed by α lactose, β lactose, and D-maltose produced moderate amounts of growth at both 10 and 20 days. With the best carbohydrate sources, some pellets formed in submerged culture and mycelial rings on sides of flasks developed.

The pleomorphic strain of A-63-137, A. bisporus pleomorph (A-63-138) was very similar in its usage of carbohydrates of high molecular weights to its parent except with D-cellobiose which produced more than twice the amount of mycelial dry weight, particularly in 20 days. Hyphae grew in submerged culture as a loose mass.

A. bisporus rhizomorph (A-63-139) differed slightly in carbon utilization of carbohydrates (Table 2) in comparison to the parent culture, A-63-137. D-cellobiose was still the best carbon source in this sugar group, but dry weight results with D-cellobiose were not nearly as great as were the results of the other A. bisporus strains. Submerged cultures grew in Humfeld's media as large loosely woven pellets.

Lepista nuda (L-63-133) pellet formation was much greater in disaccharide media than monosaccharide media. Sucrose, α lactose and β lactose produced the least amount

of hyphal growth while D-maltose was a moderately good source of carbon. Raffinose was good and D-cellobiose was excellent as carbon sources at both 10 and 20 days incubation.

Best mycelial growth for Pleurotus ostreatus (P-63-51) was produced on D-cellobiose, β lactose and D-maltose. Moderate growth resulted when cultures were grown in Humfeld's media with 8 g carbon per liter of α lactose, sucrose or raffinose. Pellet formation was poor except for D-cellobiose; other media produced only hyphal flask rings or submerged isolated strands.

Volvariella volvacea (V-63-135) grew best on D-cellobiose while D-maltose was a fairly good growth promoter for both V-63-135 and V. volvacea mutant (V-63-134). Raffinose and other disaccharides listed in Table 2 showed a moderate growth pattern. D-cellobiose was only of moderate value to the mutant form, V-63-134. In comparison to V. volvacea, the growths of the mutant were less with each respective carbon source except D-cellobiose.

Utilization of Polysaccharides

Seven polysaccharides were selected for carbon utilization by the 12 fungi, and the results are tabulated in Table 3. Cantharellus clavatus (C-63-8) was readily able to utilize cellulose in Humfeld's medium while only moderate growth was achieved with dextrin, glycogen, inulin, pectin, starch or xylan, although pectin was better than other carbohydrates of this group. Hyphae grew singly as submerged strands and did not produce pellets.

Table 2.--The utilization of disaccharides* plus one trisaccharide by twelve fungi grown in submerged culture, reported as averaged mycelial dry weight in milligrams of three replicates at the end of both 10 and 20 days.

	Days	D-cellobiose	α lactose	β lactose	D-maltose	sucrose	raffinose	Control (-carbon)
Cantharellus clavatus C-63-8	10	32.9	31.2	28.2	38.5	27.7	28.3	14.8
	20	32.1	29.2	31.9	28.6	32.8	36.1	14.5
C. cibarius C-63-15	10	48.3	26.1	32.1	39.1	39.4	28.2	18.1
	20	97.1	28.9	34.9	32.1	52.5	21.9	25.9
C. cibarius C-63-86	10	26.3	31.5	28.0	35.5	32.1	24.7	21.8
	20	32.8	23.8	38.2	34.3	28.8	106.2	19.2
C. cibarius C-63-96	10	262.4	227.1	41.9	353.4	360.4	329.1	18.8
	20	216.6	167.3	54.7	264.7	274.7	250.3	17.1
Agaricus campestris A-63-136	10	48.7	50.1	35.9	39.6	40.3	114.1	24.5
	20	50.4	44.1	29.7	31.6	43.5	112.4	25.8
A. bisporus A-63-137	10	35.2	31.0	29.5	30.3	41.3	33.7	16.5
	20	103.8	29.8	32.5	37.5	41.6	92.3	14.0
A. bisporus, pleomorph A-63-138	10	58.5	28.1	52.7	53.6	43.2	32.5	13.0
	20	246.4	26.6	32.8	45.9	36.1	70.1	12.1
A. bisporus, rhizomorph A-63-139	10	26.7	30.3	28.2	43.9	43.1	29.4	16.8
	20	44.3	27.5	32.1	31.9	29.0	30.7	19.4

Table 2.--Continued.

	Days	D-cellobiose	α lactose	β lactose	D-maltose	sucrose	raffinose	Control (-carbon)
Lepista nuda	10	131.4	45.6	38.9	53.8	48.4	117.3	35.1
L-63-133	20	332.5	58.9	52.3	95.3	55.1	107.1	33.1
Pleurotus ostreatus	10	42.8	28.0	27.3	51.3	30.1	31.2	17.5
P-63-51	20	51.7	26.6	45.7	32.9	30.3	28.9	27.3
Volvariella volvacea	10	47.6	37.4	35.1	41.7	34.3	41.3	17.9
V-63-135	20	59.6	36.2	35.8	44.6	43.7	30.4	29.3
V. volvacea, mutant	10	24.0	24.7	25.9	37.2	29.3	23.0	19.6
V-63-134	20	28.4	22.3	33.7	36.9	33.7	19.8	15.5

*Carbon concentration 8 g per liter.

C. cibarius (C-63-15) grew fairly well on inulin and xylan, and well at 20 days with dextrin, glycogen, pectin and starch. Greatest growth occurred with cellulose as the carbon source in 10 days. No pellets formed; submerged hyphae and thin hyphal flask rings developed.

Similarly with the other Cantharellus strains, C. cibarius (C-63-86) grew best in Humfeld's medium with 1 g of cellulose per flask. Moderately good growth was obtained with pectin, starch, dextrin, glycogen, and inulin. Xylan produced the poorest results similar to the latter two species. Pellet formation commonly occurred; hyphae were loosely woven, however.

Polysaccharide utilization was a bit modified with C. cibarius (C-63-96) in comparison to the other Cantharellus species. Dextrin and starch were excellent carbon sources; glycogen and cellulose were also good. In contrast inulin, pectin and xylan were fair carbon sources. Dense pellet formation was common and the yellow-green water soluble pigment occurred frequently in the Humfeld and polysaccharide media.

Agaricus campestris (A-63-136) grew well on cellulose, particularly at 10 days incubation. Good hyphal production was obtained with dextrin, pectin or starch with 20 days growth while only moderate growth occurred with glycogen, inulin and xylan. Mycelium grew as isolated strands in submerged culture.

A. bisporus (A-63-137) and one of the two mutants from this culture, A. bisporus pleomorph (A-63-138) were similar in carbon utilization of polysaccharides. Cellulose and xylan produced excellent hyphal growth, however cellulose was not as readily utilized; xylan doubled the dry mycelial weight between 10 and 20 days incubation. Pectin produced good growth, but only moderate growth occurred with dextrin, glycogen, inulin or starch. Some pellet production resulted in the submerged cultures of A-63-137, but A-63-138 hyphae grown in Humfeld's media formed only a singular dense interwoven mass of mycelium in each flask.

The second mutant of A-63-137, A. bisporus rhizomorph (A-63-139) differed slightly from its parent and the pleomorphic mutant in utilization of polysaccharides. Cellulose was readily used by the rhizomorph mutant, but no hyphal growth with xylan as the carbon source in Humfeld's medium occurred. Mycelium remained single stranded in submerged culture at both 10 and 20 days incubation, no pellets formed.

Lepista nuda (L-63-133) had excellent growth of pellet and flask ring formation with pectin and xylan, particularly at 20 days incubation. Cellulose gave good mycelial growth at 20 days but was quite poor with only 10 days incubation. Starch resulted in moderate growth at

both incubation times. Moderate growth developed from dextrin, glycogen or inulin in Humfeld's medium at 8 g carbon per liter.

Cellulose produced the best growth in Pleurotus ostreatus (P-63-51) shaker cultures. Good growth was obtained with glycogen, pectin, or xylan as carbon sources in Humfeld's medium. Moderate growth resulted with dextrin and starch while inulin was poor for hyphal growth in submerged culture. Loose pellets developed in media with glycogen or pectin; other cultures grew as isolated mycelial strands.

Growth was extremely good with Volvariella volvacea (V-63-135) in Humfeld's media and 8 g carbon per liter glycogen, at both 10 and 20 days incubation. Cellulose and starch produced good hyphal growth but proved to be only about a third as much mycelium as glycogen. Moderate growth occurred with dextrin, inulin and pectin while xylan was not utilized.

Xylan was also not utilized with V. volvacea mutant (V-63-134) in mycelium production. Best growth at both 10 and 20 days incubation was with cellulose in Humfeld's medium. Moderately poor growth resulted with dextrin, glycogen, inulin, pectin or starch.

One g of cellulose, insoluble in water, was added to each flask of 50 ml Humfeld's medium then 1 g was subtracted from each dry weight reading of mycelium obtained

at both 10 and 20 day incubations. Inaccurate data interpretation may have resulted with cellulose as a carbon source. All other polysaccharides as other carbon sources were in concentrations of 8 g carbon per liter of Humfeld's medium.

Utilization of Organic Acids

The utilization of nine organic acids was compared for each of the twelve fungal strains by dry mycelial weights at the end of 10 and 20 days incubation (Table 4). A pH reading at the end of 20 days for each culture was taken; the initial pH was 4.5.

In comparison to the other fungal cultures, C. cibarius (C-63-96) showed the most variation in the pH of the medium after 20 days incubation. Terminal pH readings with citric acid for eleven fungal strains were 2.0, while one (C-63-96) was 7.0; DL-malic acid used in the medium had a pH of 4.0 for 11 cultures, 9.6 for one (C-63-96); oxalic acid terminal pH was 8.0 for one culture (C-63-96), 7.6 for other cultures; succinic acid in the medium for C. cibarius (C-63-96) had a 20 day pH of 10.6, about 6.0 for other strains; a pH of 9.6 for D-tartaric acid was recorded for C-63-96, 3.0 for the other cultures. Pyrogallic acid and tannic acid had terminal pH readings for all 12 cultures equal to the 4.5 initial readings. Pyrogallic acid medium was 8.0 at 20 days while galacturonic acid medium had varied terminal pH readings.

Table 3.--The utilization of polysaccharides* by twelve fungi grown in submerged culture, reported as averaged mycelial dry weight in milligrams of three replicates at the end of both 10 and 20 days.

	Days	cellulose	dextrin	glycogen	inulin	pectin	starch	xylan	Control (-carbon)
Cantharellus clavatus C-63-8	10	101.2	28.8	30.0	29.0	48.6	30.9	20.5	14.8
	20	116.3	33.2	31.2	29.3	45.7	31.0	15.5	14.5
C. cibarius C-63-15	10	103.0	46.7	29.5	29.5	52.1	44.1	32.9	18.1
	20	73.8	54.4	52.2	36.7	81.5	56.7	42.6	25.9
C. cibarius C-63-86	10	93.7	25.4	24.3	26.6	45.6	28.3	13.2	21.8
	20	117.3	33.4	26.3	39.4	45.2	43.9	18.8	19.2
C. cibarius C-63-96	10	89.5	285.0	128.2	22.8	55.7	285.6	37.3	18.8
	20	125.4	228.7	99.7	36.2	52.3	275.6	71.0	17.1
Agaricus campestris A-63-136	10	95.6	39.6	21.7	33.1	28.9	52.9	18.2	24.5
	20	23.0	42.1	26.5	31.4	45.3	49.7	32.5	25.8
A. bisporus A-63-137	10	96.9	30.8	23.2	32.8	60.1	33.9	58.6	16.5
	20	133.5	32.4	27.1	31.7	86.3	35.6	136.9	14.0
A. bisporus, pleomorph A-63-138	10	86.9	27.5	28.6	32.4	63.8	32.1	67.3	13.0
	20	89.9	24.9	27.4	39.6	55.4	34.4	120.6	12.1
A. bisporus, rhizomorph A-63-139	10	95.0	27.4	34.3	31.3	45.4	32.2	19.0	16.8
	20	135.8	27.5	42.4	29.9	67.2	34.8	27.4	19.4

Table 3.--Continued.

	Days	cellulose	dextrin	glycogen	inulin	pectin	starch	xylan	Control (-carbon)
Lepista nuda	10	11.4	47.3	44.6	29.9	98.0	63.6	99.7	35.1
L-63-133	20	113.8	50.8	47.4	45.9	151.4	71.8	188.3	33.1
Pleurotus ostreatus	10	77.4	42.2	64.6	29.0	50.9	37.2	66.9	17.5
P-63-51	20	112.2	30.6	63.8	30.0	59.7	44.1	39.7	27.3
Volvariella volvacea	10	81.2	52.0	246.3	32.7	36.9	69.0	23.4	17.9
V-63-135	20	118.6	62.4	267.6	37.2	50.6	85.6	40.8	29.3
V. volvacea, mutant	10	59.1	25.4	21.6	26.5	29.7	32.6	14.5	19.6
V-63-134	20	112.6	26.3	28.9	28.1	36.9	33.1	14.3	15.5

*Carbon concentration 8 g per liter.

Cantharellus clavatus (C-63-8) had poor to fair mycelial growth in citric acid, DL-malic acid, oxalic acid, pyrogallic acid, β resorcylic acid, succinic acid and D-tartaric acid. Good growth was obtained with galacturonic acid and tannic acid.

Mycelial growth with C. cibarius (C-63-15) was poor to fair in Humfeld's media containing pyrogallic acid, D-tartaric acid, citric acid, oxalic acid, β resorcylic acid or tannic acid while moderately good growth was obtained with galacturonic acid, DL-malic acid or succinic acid. C. cibarius (C-63-86) developed poor hyphal growth in media with citric acid, DL-malic acid, β resorcylic acid and pyrogallic acid. It showed good growth with galacturonic acid and succinic acid. C. cibarius (C-63-96) grew well with citric acid, DL-malic acid, succinic acid or D-tartaric acid. Hyphal density in the shaker cultures with these media reduced nutrient movement greatly. Humfeld's medium with galacturonic acid, pyrogallic acid or tannic acid produced moderate mycelial growth; little growth developed in media containing oxalic acid or β resorcylic acid. Mycelium of the four Cantharellus species developed good hyphal pellets only in cultures with high mycelial dry weights; other media produced only isolated hyphal strands.

Agaricus campestris (A-63-136) grew fairly well with galacturonic acid or tannic acid. It had moderately

poor growth with citric acid, oxalic acid or D-tartaric acid, but grew quite poorly with DL-malic acid, pyrogallic acid, β resorcylic acid or succinic acid.

A. bisporus (A-63-137) had moderately poor growth in Humfeld's media containing citric acid, DL-malic acid, pyrogallic acid, succinic acid, oxalic acid, β resorcyllic acid, or D-tartaric acid media while good growth formed in media with galacturonic acid or tannic acid. The pleomorphic strain of A. bisporus, A. bisporus pleomorph (A-63-138), grew poorly in DL-malic acid in Humfeld's medium, slightly better in all the other organic acids, with good growth in tannic acid. The rhizomorphic strain, A. bisporus rhizomorph (A-63-139) had little or no growth in media containing citric acid, DL-malic acid, pyrogallic acid, succinic acid or D-tartaric acid; fair growth developed in galacturonic acid, β resorcylic acid or tannic acid; good growth occurred in oxalic acid particularly at 10 days incubation. Only the succinic acid medium had good pellet formation for the Agaricus cultures.

Lepista nuda (L-63-133) grew exceptionally well in Humfeld's medium containing galacturonic acid both at 10 and 20 days incubation. Moderate hyphal growth developed in media with citric acid, pyrogallic acid, β resorcylic acid, tannic acid or D-tartaric acid, and poor or no growth occurred with DL-malic acid, oxalic acid or succinic acid. Pleurotus ostreatus (P-63-51) showed only moderate mycelial

growth with organic acids, with tannic acid giving the highest mycelial weights both at 10 and 20 days incubation and pyrogallic acid showing the least growth. D-galacturonic acid and succinic acid were the only media to support pellet formation for both Lepista and Pleurotus.

Volvariella volvacea (V-63-135) grew moderately well in the medium containing galacturonic acid, and quite poorly or not at all in pyrogallic acid or D-tartaric acid. Dry mycelial weights obtained with citric acid, DL-malic acid, oxalic acid, β resorcylic acid, succinic acid or tannic acid were only moderately good. The mutant form, V. volvacea mutant (V-63-134) showed no growth in media containing DL-malic acid, pyrogallic acid or succinic acid, moderate growth with citric acid, galacturonic acid, oxalic acid, β resorcylic acid or D-tartaric acid, and best growth with tannic acid. Volvariella mycelium grew in submerged culture as masses of interwoven hyphae.

Miscellaneous Carbon Sources

Miscellaneous carbon sources, including two sugar alcohols, were substituted for glucose at 8 g carbon per liter in Humfeld's medium, and hyphal dry weights were taken of the 12 fungi at 10 and 20 days incubation (Table 5). With Cantharellus clavatus (C-63-8) and two of the three C. cibarius isolates, C-63-15 and C-63-86, the ability to use these carbon sources was nearly the same, except C.

Table 4.--The utilization of organic acids* by twelve fungi grown in submerged culture, reported as averaged mycelial dry weight in milligrams of three replicates at the end of both 10 and 20 days.

	Days**	citric acid	galacturonic acid	DL-malic acid	oxalic acid	pyrogallic acid	β resorcylic acid	succinic acid	tannic acid	D-tartaric acid	Control (-carbon)
Cantharellus clavatus C-63-8	10	20.8	38.2	17.5	28.0	20.2	23.4	25.5	39.8	23.9	14.8
	20	23.6	33.7	18.7	20.0	20.2	23.3	44.6	11.9	29.8	14.5
	pH	2.0	2.0	4.0	7.6	4.5	8.0	6.0	4.5	3.0	
C. cibarius C-63-15	10	20.4	34.5	48.5	27.2	20.5	24.9	46.8	28.2	20.5	18.1
	20	25.7	48.5	55.0	20.0	13.9	21.1	38.9	23.7	17.9	25.9
	pH	2.0	1.6	4.0	7.6	4.5	8.0	7.0	4.5	3.0	
C. cibarius C-63-86	10	18.4	58.1	17.0	24.0	24.4	20.2	29.1	15.8	22.4	21.8
	20	22.1	44.1	12.5	18.0	19.5	21.7	36.3	10.4	18.8	19.2
	pH	2.0	4.0	4.0	7.6	4.5	8.0	6.0	4.5	3.0	
C. cibarius C-63-96	10	277.3	26.4	275.3	25.0	33.2	22.9	119.9	46.5	302.3	18.8
	20	222.8	35.1	206.7	20.3	26.5	21.4	92.3	32.5	223.9	17.1
	pH	7.0	4.5	9.6	8.0	4.5	8.0	10.6	4.5	9.6	
Agaricus campestris A-63-136	10	21.8	40.3	14.1	26.6	17.9	19.2	11.3	44.8	21.7	24.5
	20	24.3	25.7	10.1	17.1	16.3	17.2	15.0	48.8	20.0	25.8
	pH	2.0	2.0	4.0	7.6	4.5	8.0	6.0	4.5	3.0	
A. bisporus A-63-137	10	19.7	31.5	14.7	23.3	19.0	28.5	20.4	40.4	19.8	16.5
	20	20.1	46.5	11.0	18.1	16.7	19.3	12.8	43.6	24.7	14.0
	pH	2.0	3.0	4.0	7.6	4.5	8.0	5.7	4.5	3.0	

Table 4.--Continued.

	Days**	citric acid	galacturonic acid	DL-malic acid	oxalic acid	pyrogallic acid	β resorcylic acid	succinic acid	tannic acid	D-tartaric acid	Control (-carbon)
A. bisporus, pleomorph A-63-138	10	15.7	33.7	22.0	28.3	24.5	33.9	33.6	55.4	30.1	13.0
	20	24.1	26.6	17.0	22.7	29.5	25.1	17.7	48.7	20.8	12.1
	pH	2.0	2.0	4.0	7.6	4.5	8.0	6.0	4.5	3.0	
A. bisporus, rhizomorph A-63-139	10	20.9	36.5	17.7	46.6	17.6	27.4	16.9	24.9	21.9	16.8
	20	19.4	28.9	11.0	19.7	15.7	21.4	14.5	10.3	18.7	19.4
	pH	2.0	3.0	4.0	7.6	4.5	8.0	5.7	4.5	3.0	
Lepista nuda L-63-133	10	20.3	195.6	12.7	24.1	33.9	28.7	20.8	38.4	25.5	35.1
	20	27.7	202.9	17.2	16.2	29.0	21.7	22.4	43.4	24.5	33.1
	pH	2.0	2.0	4.0	7.6	4.5	8.0	6.0	4.5	3.0	
Pleurotus ostreatus P-63-51	10	19.5	24.5	34.5	27.9	20.9	24.7	22.8	36.3	25.4	17.5
	20	24.9	25.7	25.4	20.1	15.1	22.8	25.4	32.5	21.9	27.3
	pH	2.0	2.0	4.0	7.6	4.5	8.0	6.0	4.5	3.0	
Volvariella volvacea V-63-135	10	18.3	35.0	21.4	26.2	16.7	27.3	26.5	34.8	21.2	17.9
	20	25.2	33.9	34.0	23.6	14.9	21.2	27.3	14.8	18.9	29.3
	pH	2.0	1.6	4.0	7.6	4.5	8.0	6.0	4.5	3.0	
V. volvacea, mutant V-63-134	10	21.0	18.9	17.6	24.6	20.4	31.4	8.6	39.2	25.1	19.6
	20	27.6	24.0	8.0	19.4	14.1	19.8	9.8	20.6	19.5	15.5
	pH	2.0	2.0	4.0	7.6	4.5	8.0	5.7	4.5	3.0	

*Carbon concentration 8 g per liter.

**pH was taken at 20 days.

cibarius (C-63-15) grew much better than the rest with fructose-1,6-diphosphate. Casein was poorly utilized as were i-inositol, D-mannitol, D-sorbitol and D-glucosamine hydrochloride. Pectinol A was fairly good while fructose-1,6-diphosphate produced excellent mycelial growth, the latter far surpassing dry weight readings of hyphae grown in the other carbohydrates at 8 g carbon per liter of Humfeld's medium. Pellet formation developed in submerged growth with all three culture strains when grown in pectinol A, casein and fructose-1,6-diphosphate. The other nutrient flasks had only isolated hyphal growth.

Cantharellus cibarius (C-63-96) differed from the other Cantharellus culture strains in that again large quantities of mycelium were produced. All carbon sources produced well formed hyphal pellets, and the best sources also developed hyphal flask rings at media splash levels. The yellow-green pigment in solution was easily recognized. Hyphae grown in Humfeld's medium with D-mannitol or D-sorbitol grew extremely fast. Mycelial production in pectinol A or fructose-1,6-diphosphate was also very great. Moderately large amounts of mycelium were obtained from i-inositol and D-glucosamine hydrochloride while casein was a poor source. Additional carbon sources were selected for utilization by C. cibarius (C-63-96). The 10 and 20 day dry mycelial weights in mg for the carbon sources not recorded

in Table 4 were for 10 and 20 days respectively: D-trehalose, 299.2, 217.0 mg; glucose-1-phosphate, 49.5, 67.9 mg; glucuronolactone, 179.5, 232.3 mg.

Agaricus campestris (A-63-136) grew poorly in Humfeld's media with casein or D-glucosamine hydrochloride and moderately in i-inositol. D-mannitol, pectinol A and D-sorbitol produced good mycelial growth while fructose-1,6-diphosphate yielded excellent hyphal production at both 10 and 20 days incubation. Pellets formed only in cultures containing D-mannitol or fructose-1,6-diphosphate; hyphae grew in strands in submerged cultures of the other miscellaneous carbon sources.

Abundant mycelium was produced in media containing pectinol A or fructose-1,6-diphosphate, while good growth with D-mannitol or casein developed as submerged hyphae for A. bisporus (A-63-137). Moderate growth was obtained from Humfeld's media with 8 g carbon per liter i-inositol or D-sorbitol and poor growth developed when casein was used. Hyphae grew as a mass in submerged culture at times producing rings on flask sides. The pleomorphic mutant of A-63-137, A. bisporus pleomorph (A-63-138), had growth responses similar to the parent strain although pectinol A was not utilized as well.

A. bisporus rhizomorph (A-63-139), a mutant of A-63-137, had a similar growth pattern to the parent strain except pectinol A and casein were able to be utilized nearly as well.

Both pectinol A and fructose-1,6-diphosphate were excellent carbon sources for growth of Lepista nuda (L-63-133) at 8 g carbon per liter. D-mannitol and casein produced good yields of mycelium at 10 and 20 days incubation while only moderate results were obtained with i-inositol, D-sorbitol or D-glucosamine hydrochloride. Good pellet formation was obtained with pectinol A and fructose-1,6-diphosphate; other hyphal cultures produced only submerged mycelial masses.

Pleurotus ostreatus (P-63-51) grew exceptionally well in Humfeld's medium with 8 g fructose-1,6-diphosphate carbon per liter. Good hyphal growth was produced with pectinol A while moderate mycelial production occurred with i-inositol, D-mannitol, D-sorbitol or casein. D-glucosamine hydrochloride was not utilized. Good pellets formed if the carbohydrates were readily usable by the mycelium.

Volvariella volvacea (V-63-135) and V. volvacea mutant (V-63-134) showed differences in abilities to utilize carbohydrates listed in Table 5. Fructose-1,6-diphosphate was the best carbon source for both cultures; pectinol A was also an excellent carbon source for V-63-135 but was only moderately useful for V-63-134, the mutated colony. For both strains, i-inositol, D-mannitol and D-sorbitol produced some hyphal growth in submerged culture while D-glucosamine hydrochloride and casein were poor

carbon sources. Minute hyphal pellets grew in cultures using the best two carbon sources; other miscellaneous carbon sources produced submerged masses of interwoven hyphae.

Table 5.--The utilization of miscellaneous carbon sources* by twelve fungi grown in submerged culture, reported as averaged mycelial dry weight in milligrams of three replicates at the end of both 10 and 20 days.

	Days	i-inositol	D-mannitol	pectinol A	D-sorbitol	D-glucosamine hydrochloride	fructose -1,6-diphosphate	casein
Cantharellus clavatus C-63-8	10	35.6	33.9	95.2	25.9	23.6	154.1	30.0
	20	42.5	37.8	109.8	30.9	42.4	191.8	57.5
C. cibarius C-63-15	10	36.8	37.5	71.3	50.9	25.6	315.1	24.9
	20	44.0	49.7	116.3	47.7	56.1	317.6	19.8
C. cibarius C-63-86	10	30.6	31.8	112.0	31.1	18.1	115.3	17.4
	20	39.2	42.2	126.8	35.7	49.9	183.8	45.1
C. cibarius C-63-96	10	234.3	410.5	346.7	424.5	259.0	329.4	53.5
	20	194.3	340.1	298.2	307.4	214.2	396.5	56.6
Agaricus campestris A-63-136	10	39.4	44.8	47.9	47.9	20.7	115.3	19.0
	20	36.1	41.3	50.1	44.7	22.1	144.7	21.3
A. bisporus A-63-137	10	28.7	41.7	81.8	30.5	18.1	118.2	49.3
	20	34.3	41.5	156.1	33.1	23.0	120.3	31.1
A. bisporus, pleomorph A-63-138	10	22.8	50.9	53.2	30.3	14.6	96.5	35.6
	20	35.4	45.9	111.9	28.6	18.3	102.9	42.1
A. bisporus, rhizomorph A-63-139	10	27.5	37.7	46.3	28.9	31.1	95.0	19.0
	20	43.3	44.7	39.7	32.9	22.5	93.0	20.6

Table 5.--Continued.

	Days	i-inositol	D-mannitol	pectinol A	D-sorbitol	D-glucosamine hydrochloride	fructose -1,6-diphosphate	casein
Lepista nuda L-63-133	10	39.6	76.5	290.0	13.8	31.2	159.3	40.0
	20	55.6	44.5	234.1	51.1	32.9	249.1	68.1
Pleurotus ostreatus P -63-51	10	56.2	43.2	88.2	32.5	19.9	189.4	31.1
	20	28.9	48.8	68.9	32.9	21.5	264.4	36.0
Volvariella volvacea V-63-135	10	23.9	40.3	109.4	44.9	18.1	154.5	54.6
	20	38.9	49.2	121.1	50.9	46.6	293.3	69.7
V. volvacea, mutant V-63-134	10	23.6	30.0	50.4	26.6	19.5	95.0	20.7
	20	26.4	24.0	38.3	25.6	22.4	198.6	16.4

*Carbon concentration 8 g per liter.

Clamp Connections in Hyphae in Submerged Cultures

The frequency variation of clamp connections with various carbon sources in Humfeld's medium was checked for the twelve fungal cultures. L-arabinose, casein, D-mannitol, pectin, L-sorbose produced no clamp frequency differences in the inocula. Agaricus and Volvariella were without clamp connections, Lepista had a moderate number, Pleurotus had a slightly higher frequency of clamps while Cantharellus produced clamps at almost all cross walls. When mycelium was transferred from the above carbon source media to 2% malt agar for Cantharellus, Pleurotus and Volvariella species, medium A for Lepista, and allowed to grow three weeks, no change in clamp connection numbers was noted. Hyphal clamp connections can be seen in mycelium of C. cibarius (C-63-86) at all cross walls when grown in Humfeld's medium and L-sorbose (Figure 7).

A characteristic of Volvariella in either agar or liquid media is the numerous spherical chlamydospores which gives the cultures the common tan color (Figure 8).

Cell anatomy of some mycelium grown in submerged culture was further studied with the aid of the electron and light microscopes. Distinct nuclei were seen in Lepista nuda (L-63-133) hyphae grown for 20 days in submerged culture of Humfeld's medium with glucose (Figure 9). Numerous vacuoles were present in Cantharellus cibarius (C-63-86)

mycelium grown in Humfeld's medium with glucose, at 20 days incubation (Figure 10). Mitochondria were identified in hyphal cells of Volvariella volvacea (V-63-135), grown in Humfeld's medium with glucose (Figure 11).

It is well known that fungi belonging to the Agaricales are able to produce crystals of calcium oxalate in culture (47). Crystal formation was seen on mycelium of Cantharellus cibarius (C-63-96) when grown in Humfeld's medium with glucose (Figure 12), mycelium of Pleurotus ostreatus (P-63-51) when grown in Humfeld's medium with L-sorbose (Figure 13), and Cantharellus cibarius (C-63-86) mycelium when grown in Humfeld's medium with casein (Figure 14).

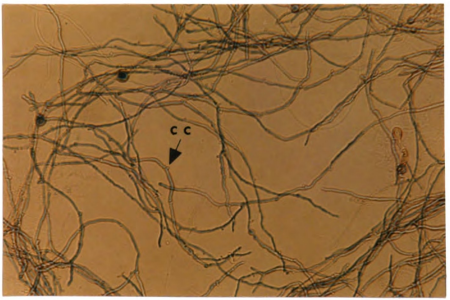


Figure 7.--Cantharellus cibarius (C-63-86) hyphae with clamp connections at all cross walls, grown 20 days in submerged culture, using Humfeld's medium with L-sorbose (cc, clamp connection). x 1,400

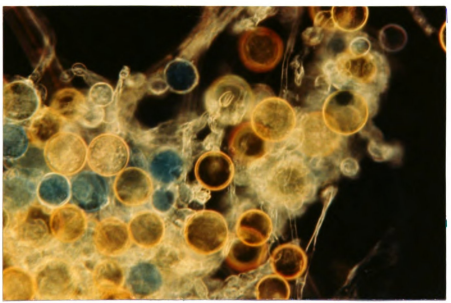


Figure 8.--Volvariella volvacea (V-63-135) grown on 2% malt agar for 15 days, showing aerial hyphae with characteristic spherical chlamydospores. x 4,000

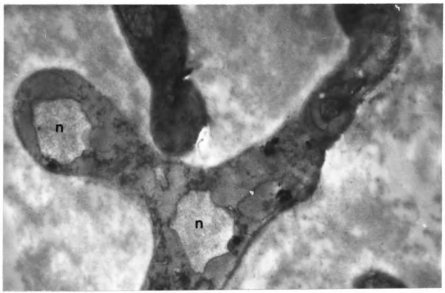


Figure 9.--Lepista nuda (L-63-133) hypha with two dominant nuclei, from a submerged culture, grown 20 days in Humfeld's medium with glucose (n,nuclei). x 17,500

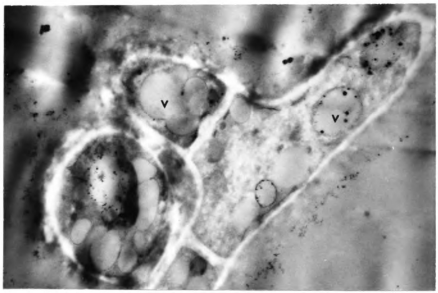


Figure 10.--Cantharellus cibarius (C-63-86) hyphae showing several vacuoles, from a submerged culture, grown 20 days in Humfeld's medium with glucose (v, vacuoles). x 14,800

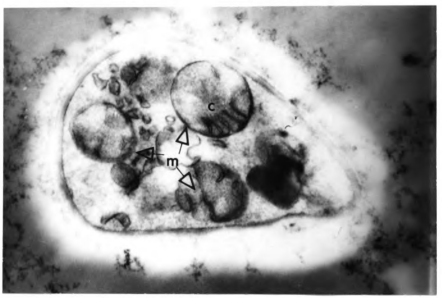


Figure 11.--Volvariella volvacea (V-63-135) hyphal cross section, showing three mitochondria with cristae, grown for 20 days in Humfeld's medium with glucose (m, mitochondria; c, cristae). x 27,400

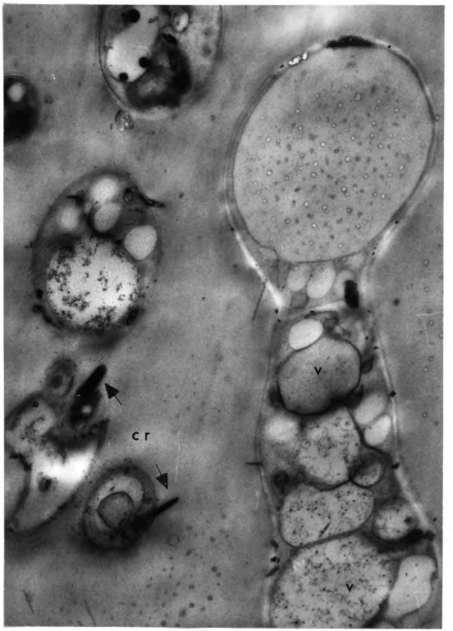


Figure 12.--Cantharellus cibarius (C-63-96) hyphae with numerous vacuoles and crystal formation, from a submerged culture, grown 20 days in Humfeld's medium with glucose (cr, crystal; v, vacuole). x 12,600

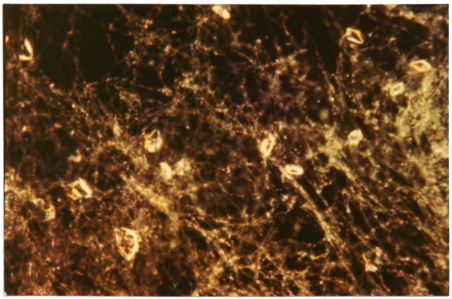


Figure 13.--Crystal formation with Pleurotus ostreatus (P-63-51) hyphae grown 20 days in submerged culture, using Humfeld's medium with L-sorbose. x 900

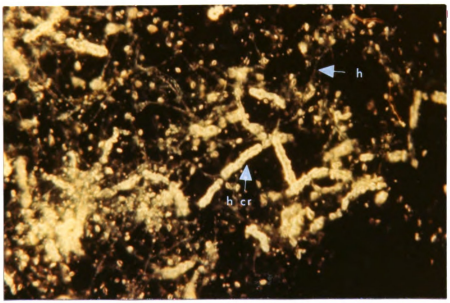


Figure 14.--Cantharellus cibarius (C-63-86) production of crystals along hyphae grown 20 days in submerged culture, using Humfeld's medium with casein (h, hypha without crystals; h cr, hyphae bordered by crystals). x 1,000

Utilization of Carbohydrates at Various Concentrations

Cantharellus cultures were grown in 25, 50 and 75 g of selected carbohydrates per liter of Humfeld's medium as an indication of concentration tolerance levels. As indicated in Table 6, media were made of D-galactose, D-fructose, αlactose, sucrose, starch, i-inositol and D-sorbitol. Cantharellus clavatus (C-63-8) produced the greatest mycelial dry weight at 75 g per liter with D-galactose, D-fructose, starch, sucrose and i-inositol. Mycelial production leveled off at 50 g per liter and showed little variation between 50 and 75 g carbohydrate per liter if αlactose, sucrose or D-sorbitol were used.

C. cibarius (C-63-15) grew most successfully at 75 g per liter of sucrose, starch, with moderate growth for αlactose, i-inositol or D-sorbitol. There was little difference in dry mycelial weight at 50 and 75 g per liter with D-galactose or D-fructose. Cantharellus cibarius (C-63-96) grew much more profoundly with most carbon sources used at concentrations of both 50 and 75 g carbohydrate per liter at both 10 and 20 days incubation (Table 6).

There is generally a leveling off of hyphal growth with many of the carbon sources between 50 and 75 g per liter at 10 days incubation as compared with 25 and 50 g per liter. In some cases a surge of growth can also be seen at 20 days incubation. A large increase in mycelial

weight occurs with the majority of carbohydrates between 50 and 75 g per liter. The mycelial weight increase between 25 and 50 g at 20 days incubation is generally not quite as substantial as between 25 and 50 g.

Table 7 records utilization of the 7 carbohydrate sources at 25, 50 and 75 g per liter selected for the Agaricus species. The four Agaric cultures were grown under the same experimental conditions for 10 and 20 days. Agaricus campestris (A-63-136) has greater hyphal growth at 75 g per liter of Humfeld's medium for all eight carbon sources at 20 days incubation, while sucrose was best at 10 days. Mycelial dry weights at 10 days were higher at 50 instead of 75 g per liter with sucrose and starch.

A. bisporus (A-63-137) was quite inconsistent in carbohydrate concentration requirements. D-fructose, α-lactose, starch and D-sorbitol produced the greatest hyphal growth at 75 g per liter at both 10 and 20 days incubation. I-inositol at 50 g per liter gave the best hyphal yields at both 10 and 20 days, while 50 g per liter at 20 days and 75 g per liter at 10 days gave the best results for D-galactose. Sucrose at 20 days with 25 g per liter was the best concentration.

The pleomorphic mutant of A-63-137, A. bisporus pleomorph (A-63-138) had greater growth consistency at similar carbohydrate concentrations than its parent.

Table 6.--The utilization of carbon sources at concentrations at 25, 50 and 75 grams per liter grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates at the end of both 10 and 20 days for C. clavatus, and three strains of C. cibarius.

		Cantharellus clavatus C-63-8			C.cibarius C-63-15			C. cibarius C-63-86			C. cibarius C-63-96		
	Days	25	50	75	25	50	75	25	50	75	25	50	75
D-galactose	10	33.3	30.8	41.0	25.5	36.7	36.3	547.8	781.7	785.4	467.9	803.5	803.6
	20	34.5	39.5	48.2	65.8	72.9	64.2	482.6	697.3	891.7	368.7	650.8	893.4
D-fructose	10	44.5	49.8	61.2	32.8	39.1	60.9	514.2	1303.4	1360.7	494.3	1076.3	1281.4
	20	40.2	33.3	50.8	42.6	69.9	14.2	555.5	963.4	1343.7	526.8	982.0	1215.7
α lactose	10	32.7	43.7	53.6	20.2	29.3	48.9	36.2	36.0	54.1	131.7	136.3	93.3
	20	23.8	64.0	49.5	24.5	40.7	55.9	29.6	54.3	128.4	352.8	433.9	607.9
sucrose	10	48.3	45.1	61.8	46.8	96.4	98.9	559.3	859.1	1074.6	547.3	930.3	905.8
	20	27.4	46.5	36.0	94.5	162.1	276.1	468.4	825.7	1125.1	463.7	813.0	926.3
starch	10	32.0	63.1	64.5	24.7	74.2	305.6	557.6	1091.4	1373.1	561.4	1007.3	1268.1
	20	39.8	63.6	99.1	50.6	103.9	136.2	611.8	1189.6	1609.0	524.8	983.6	1300.6
i-inositol	10	27.1	37.7	46.1	29.4	51.7	71.8	26.6	35.8	41.4	331.1	644.4	777.8
	20	36.9	49.5	63.8	31.5	50.5	50.0	31.6	37.6	69.0	243.2	420.0	749.6
D-sorbitol	10	26.3	31.9	35.8	31.8	39.8	52.0	26.2	32.6	38.8	487.8	756.3	759.9
	20	30.1	47.4	43.7	34.5	44.7	87.7	26.6	38.7	43.8	380.8	845.6	679.2

Seventy-five g per liter gave the best growth at 20 days incubation with D-fructose, α-lactose, sucrose, starch, i-inositol and D-sorbitol. D-galactose produced nearly the same dry mycelial weights at 50 and 75 g per liter at 20 days incubation.

A. bisporus rhizomorph (A-63-139) had some carbohydrate utilization concentration tolerances in growth patterns similar to the parent culture, A. bisporus (A-63-137). Starch, α-lactose and D-sorbitol cultures grew the best mycelium at 75 g per liter, both 10 and 20 days. Sucrose in Humfeld's medium cultures produced greatest hyphal growth at 25 g carbohydrate per liter at 20 days incubation. I-inositol at 50 g per liter, 20 days incubation, produced high mycelial yields. Compared to the parent strain, the rhizomorphic form differed with the following mycelial dry weight results: D-galactose had little variation between 25, 50 or 75 g per liter both 10 and 20 days growth; hyphal formation was best in D-fructose at 50 g per liter, 10 days and 75 g per liter, 20 days; α-lactose produced highest yields at 75 g per liter, both 10 and 20 days incubation, and did not utilize sucrose as readily.

The utilization of carbon sources at concentrations of 25, 50 and 75 g per liter in Humfeld's medium by Lepista, Pleurotus and Volvariella are reported in Table 8. Lepista nuda (L-63-133) had greater hyphal growth both at 10 and 20 days incubation using D-fructose, α-lactose, starch,

Table 7.--The utilization of carbon sources at concentrations of 25, 50 and 75 grams per liter grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates at the end of both 10 and 20 days for *A. campestris*, and three strains of *A. bisporus*.

		Agaricus campestris A-63-136			*A. bisporus* A-63-137			*A. bisporus*, pleomorph A-63-138			*A. bisporus*, rhizomorph A-63-139		
	Days	25	50	75	25	50	75	25	50	75	25	50	75
D-galactose	10	62.7	115.9	99.6	35.6	35.8	76.5	45.6	37.8	37.2	29.5	29.5	39.1
	20	52.8	116.5	151.5	72.2	98.0	59.1	71.3	75.7	74.3	52.3	32.0	35.8
D-fructose	10	59.2	76.3	254.0	66.9	63.3	120.8	119.1	107.9	201.0	57.8	74.7	46.1
	20	70.0	206.3	267.0	83.1	179.7	187.2	136.1	194.7	243.8	34.8	98.6	134.2
α lactose	10	24.9	42.3	41.5	32.3	34.2	96.2	38.2	62.0	96.4	31.4	55.3	72.6
	20	27.6	43.0	85.4	51.0	79.1	85.5	38.6	63.9	105.8	29.7	77.4	104.7
sucrose	10	43.0	81.8	201.1	46.5	87.1	71.7	68.0	92.5	94.1	36.7	49.5	41.2
	20	38.5	57.4	69.5	139.6	48.6	81.8	50.4	60.2	202.4	83.6	34.2	47.3
starch	10	45.9	84.1	82.1	46.3	75.4	128.0	65.5	44.2	219.2	32.3	71.8	90.7
	20	56.0	74.2	124.3	53.2	88.3	112.1	40.7	107.8	319.9	34.0	103.4	167.9
i-inositol	10	36.9	49.0	73.8	36.0	69.1	57.7	27.4	42.1	63.6	31.4	56.2	68.5
	20	43.0	65.4	87.4	59.2	81.3	70.8	40.3	62.6	66.9	39.1	227.9	61.3
D-sorbitol	10	38.8	40.0	45.9	30.1	37.2	43.6	31.4	38.6	48.2	30.4	35.0	41.5
	20	52.7	49.4	66.7	31.1	41.2	48.1	35.1	45.8	50.9	35.9	35.0	47.6

i-inositol, or D-sorbitol at 75 g per liter. D-galactose was most effective at 50 g per liter, 20 days. Sucrose at 50 g per liter, both 10 and 20 days produced the highest mycelial dry weights for that particular carbohydrate.

Pleurotus ostreatus (P-63-51) showed maximum growth at 20 days incubation, 75 g per liter with D-galactose and αlactose, and in 10 days for starch. D-fructose was moderately good in 10 days at 75 g per liter. Moderate hyphal growth differences were not great at 50 and 75 g for i-inositol, 20 days growth.

The Volvariella strains showed some data similarities in Table 8. In both cases sucrose and starch were utilized much more rapidly by the parent strain, maximum growth occurred at 75 g per liter at 10 instead of 20 days incubation for the mutant. The V-63-134 mutant had good yields at 75 g per liter with i-inositol, D-sorbitol, αlactose, D-fructose and D-galactose. Fifty g per liter, 10 days; and 75 g per liter, 20 days produced fairly good hyphal growth with V-63-135 in D-galactose, D-fructose, αlactose and i-inositol. V-63-135 growing in D-sorbitol developed the peak in a moderate amount of hyphal growth at 10 days incubation in 75 g per liter, and 50 g per liter at 20 days incubation.

In general, mycelium showing poor growth in various carbohydrate media frequently produced cytoplasmic coagulation. Also, chlamydospores were more frequent, shorter hyphal

Table 8.--The utilization of carbon sources at concentrations of 25, 50 and 75 grams per liter grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates at the end of both 10 and 20 days for L. nuda, P. ostreatus, and two strains of V. volvacea.

		Lepista nuda L-63-133			Pleurotus ostreatus P-63-51			Volvariella volvacea V-63-135			V. volvacea, mutant V-63-134		
	Days	25	50	75	25	50	75	25	50	75	25	50	75
D-galactose	10	32.7	38.0	45.4	32.9	47.1	53.6	41.6	54.6	10.3	22.0	29.3	33.3
	20	58.4	65.9	54.8	38.1	88.8	231.7	39.2	76.2	110.9	22.3	26.2	35.9
D-fructose	10	303.6	334.1	338.3	69.9	58.8	100.3	45.8	81.3	69.6	30.4	48.2	59.5
	20	320.5	426.8	462.5	51.0	51.6	74.2	48.2	61.3	71.2	25.9	30.6	36.9
α lactose	10	37.4	65.9	53.9	41.4	58.3	43.7	36.5	59.9	56.7	33.6	37.0	44.4
	20	58.4	69.6	81.3	42.0	39.7	170.4	44.1	76.0	116.8	29.4	39.0	56.5
sucrose	10	56.8	119.8	114.4	37.2	64.7	73.0	41.0	53.2	99.6	29.5	34.8	40.8
	20	55.8	88.0	84.2	46.1	38.6	65.9	44.9	64.0	88.3	23.6	32.6	35.2
starch	10	45.8	96.9	124.5	26.8	38.6	247.6	78.8	137.4	325.1	33.7	37.1	72.9
	20	50.5	128.5	177.5	23.4	107.3	104.1	61.8	95.5	247.7	33.0	63.5	80.1
i-inositol	10	30.8	37.2	78.4	35.1	41.0	54.9	28.9	66.8	60.7	22.6	41.3	46.7
	20	39.1	49.3	52.8	38.9	62.5	61.3	37.3	48.0	52.7	32.3	43.3	56.3
D-sorbitol	10	40.5	50.0	57.2	28.9	37.8	51.3	33.5	43.0	51.3	33.8	41.7	50.4
	20	51.3	56.9	61.0	39.5	43.0	51.4	35.5	66.8	51.4	47.9	29.8	52.6

cells were present, there were more frequent cross walls, and hyphal walls were less parallel.

Utilization of Glucose at Various Concentrations

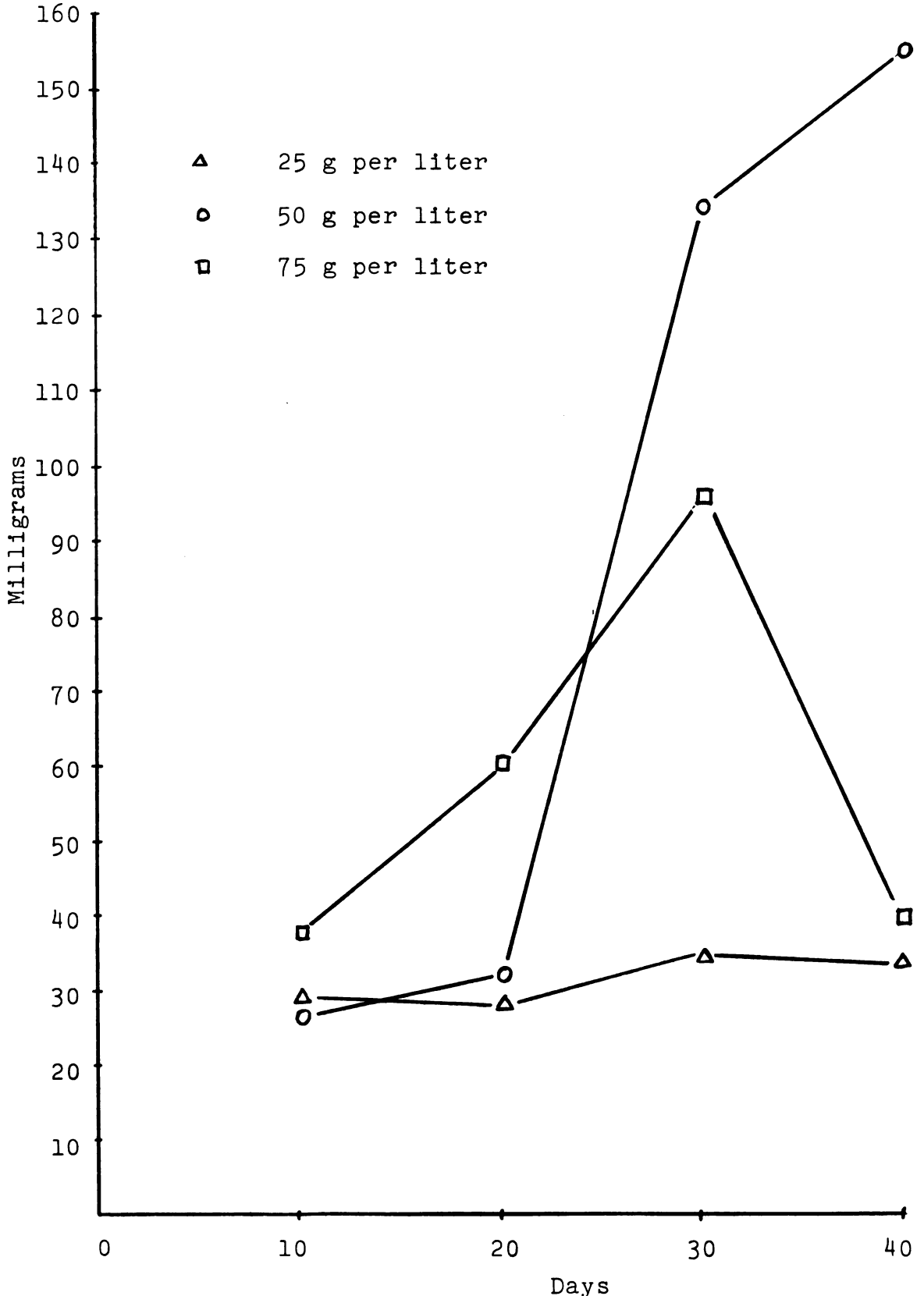
Growth curves were made of the twelve fungi grown in 25, 50 and 75 g glucose per liter of Humfeld's medium and incubated for 10, 20, 30 and 40 days each. The data, averages of two replicates each (Table 9), were plotted on graphs 1 through 12. Cantharellus clavatus (C-63-8) grown in submerged culture of 25 g glucose per liter produced little hyphal growth over a 40 day period, dry weight remained about equal to the dry weight of the inoculum (Graph 1). At 50 g glucose per liter, a rapid growth increase occurred between 20 and 30 days then leveled off after 30 days incubation. A steady hyphal growth increase developed in flasks of 75 g glucose per liter until a steep growth decrease (autolysis) occurred after 30 days after reaching a growth peak of about 95 g dry mycelial weight. A maximum growth of 155 g dry weight occurred at 50 g glucose per liter, 40 days incubation and the minimum growth of 30 g dry weight occurred at 10 days incubation, 25 g glucose per liter.

C. cibarius (C-63-15) mycelium grown in submerged culture at 25 and 50 g glucose per liter produced similar results at 10, 20, 30 or 40 days incubation (Graph 2). Dry

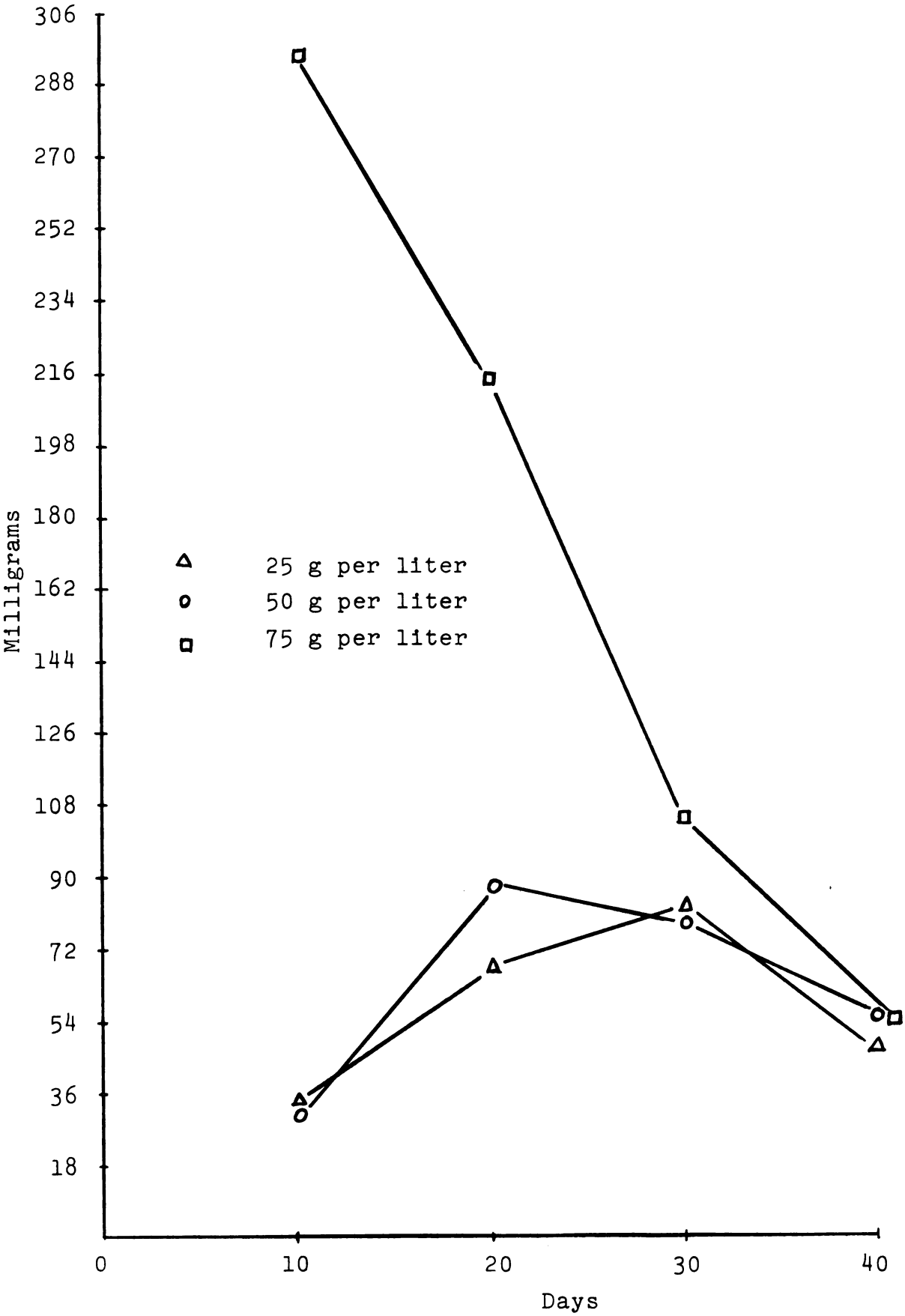
Table 9.--The utilization of glucose by twelve fungi grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates at the end of 10, 20, 30, and 40 days.

Culture	Grams per Liter	Days 10	20	30	40
Cantharellus clavatus C-63-8	25	29.4	28.5	35.4	34.0
	50	27.7	32.1	134.7	155.7
	75	38.9	61.3	96.7	40.9
C. cibarius C-63-15	25	35.2	68.0	83.2	47.8
	50	34.8	88.0	79.3	56.6
	75	95.4	214.6	104.6	55.5
C. cibarius C-63-86	25	24.3	22.3	38.8	35.9
	50	28.5	39.5	46.4	42.1
	75	35.5	54.5	42.3	52.2
C. cibarius C-63-96	25	433.2	372.7	340.1	317.5
	50	805.7	704.3	603.1	535.9
	75	1031.2	938.5	851.3	778.4
Agaricus campestris A-63-136	25	111.8	247.1	259.2	238.5
	50	470.2	253.8	310.3	224.6
	75	432.5	408.9	291.4	314.9
A. bisporus A-63-137	25	36.5	44.3	81.4	90.5
	50	32.9	51.2	67.2	213.9
	75	37.7	45.1	85.7	87.7
A. bisporus, pleomorph A-63-138	25	35.8	100.8	171.9	307.5
	50	31.2	121.8	364.6	363.2
	75	46.5	46.8	283.1	361.0
A. bisporus, rhizomorph A-63-139	25	29.0	27.6	36.4	40.7
	50	34.6	39.0	44.3	146.9
	75	41.4	32.4	121.6	108.5
Lepista nuda L-63-133	25	155.3	241.2	322.7	397.3
	50	115.3	240.9	390.8	461.9
	75	114.8	155.4	386.1	451.8
Pleurotus ostreatus P-63-51	25	35.7	37.2	27.2	28.7
	50	38.9	99.2	86.9	175.7
	75	47.7	71.3	282.0	216.9
Volvariella volvacea V-63-135	25	34.5	30.9	39.3	39.8
	50	57.6	54.6	44.5	33.2
	75	89.2	97.2	65.2	54.5
V. volvacea, mutant V-63-134	25	20.7	21.9	33.7	19.3
	50	24.2	31.1	60.9	23.0
	75	32.7	51.3	180.0	268.7

Graph 1.--Cantharellus clavatus (C-63-8) utilization of glucose at concentrations of 25, 50 and 75 grams per liter grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates each at the end of 10, 20, 30 and 40 days incubation.



Graph 2.--Cantharellus cibarius (C-63-15) utilization of glucose at concentrations of 25, 50 and 75 grams per liter grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates each at the end of 10, 20, 30 and 40 days incubation.

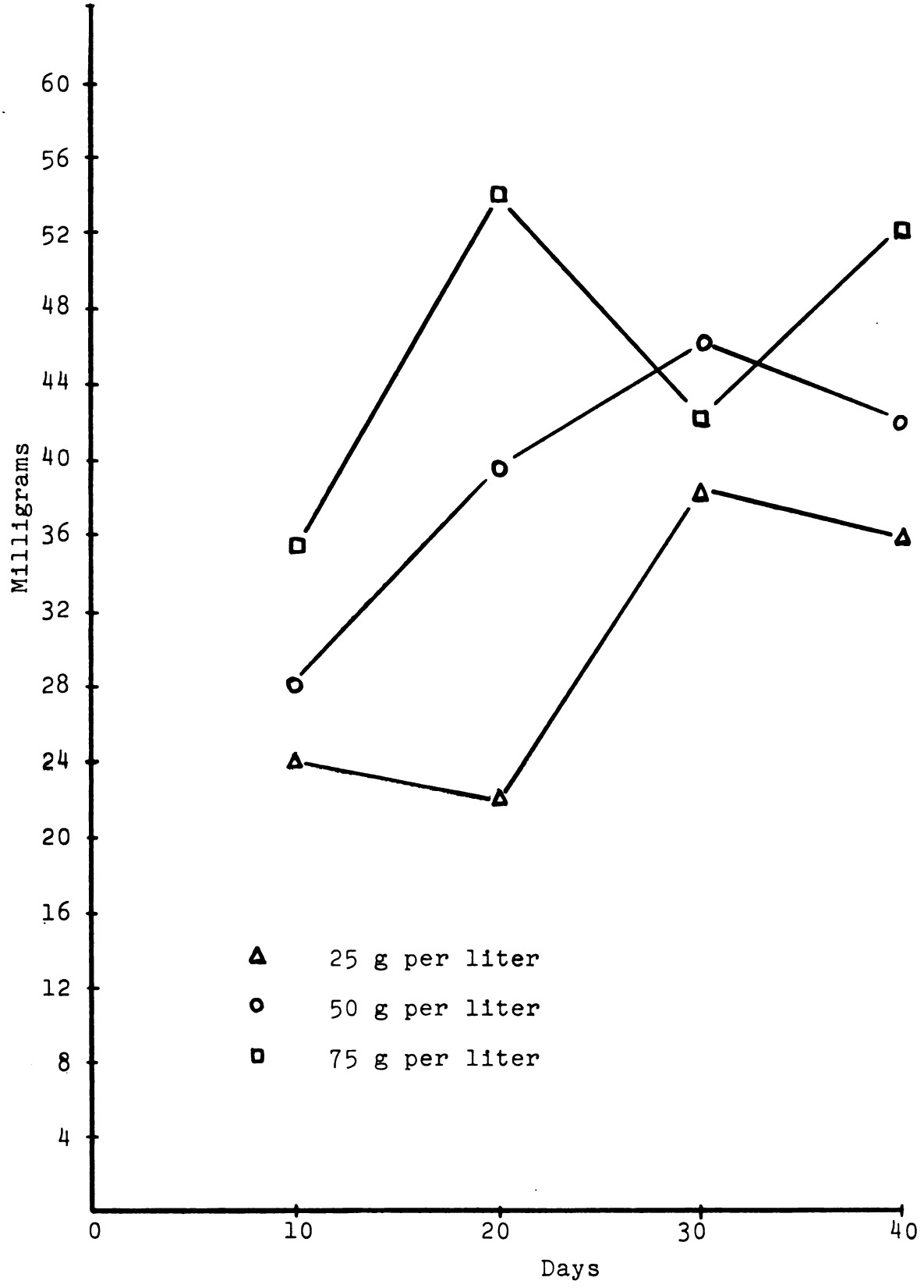


mycelial weights increased to 20 days, leveled off between 20 to 30 days, then decreased after 30 days growth. At 75 g glucose per liter, hyphal growth decreased from a maximum of 300 mg dry weight at 10 days to a minimum dry weight at 40 days incubation that was similar to the 40 day results of the other two glucose concentrations. A tremendous surge of growth probably occurred during the first 10 days incubation at 75 g glucose per liter. The maximum growth for C-63-15 at all three concentrations occurred at 10 days.

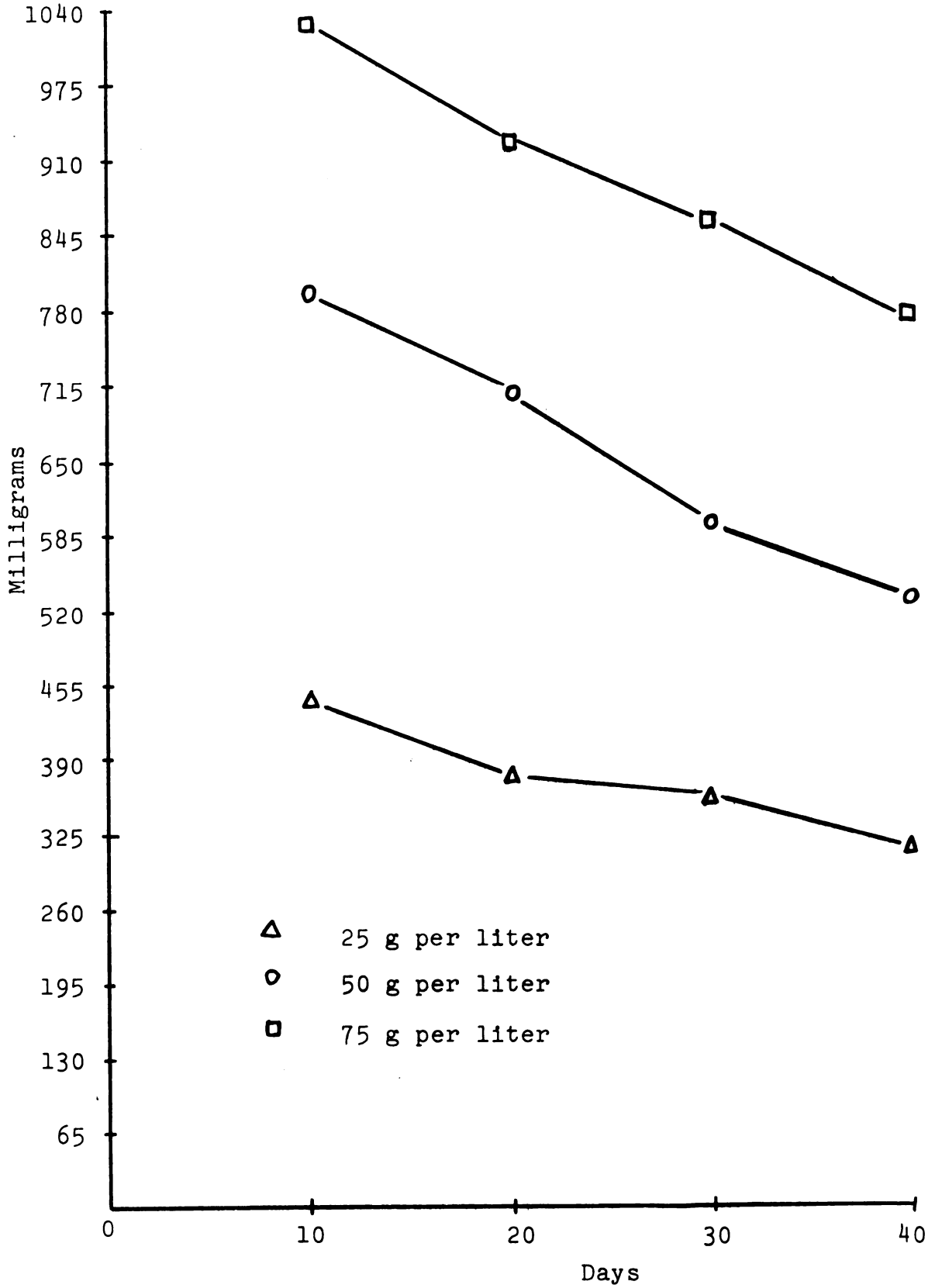
C. cibarius (C-63-86) grown in 25 g glucose per liter had a slight growth decrease at 20 days incubation but increased to 30 days when another decrease followed to 40 days incubation (Graph 3). At 50 g glucose per liter, growth steadily increased up to 30 days then decreased as with 25 g glucose per liter. Hyphal growth in 75 g glucose per liter greatly increased between 10 and 20 days incubation, decreased between 20 and 30 then increased once again at 40 days incubation. The similar growth decrease then occurred both in 25 and 75 g glucose media, however, at 75 g this phenomoma occurred 10 days later than in the 25 g medium. The greatest growth for C-63-86 strain occurred at 20 days incubation, 75 g glucose while the least growth occurred at 20 days, 25 g glucose per liter.

In Graph 4, dry mycelial weight of C. cibarius (C-63-96) is plotted to determine the growth curves at various glucose concentrations during 10, 20, 30 or 40 days

Graph 3.--Cantharellus cibarius (C-63-86) utilization of glucose at concentrations of 25, 50 and 75 grams per liter grown in submerged cultures, reported as averaged mycelial dry weight in milligrams of two replicates each at the end of 10, 20, 30 and 40 days incubation.



Graph 4.--Cantharellus cibarius (C-63-96) utilization of glucose at concentrations of 25, 50 and 75 grams per liter grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates each at the end of 10, 20, 30 and 40 days incubation.

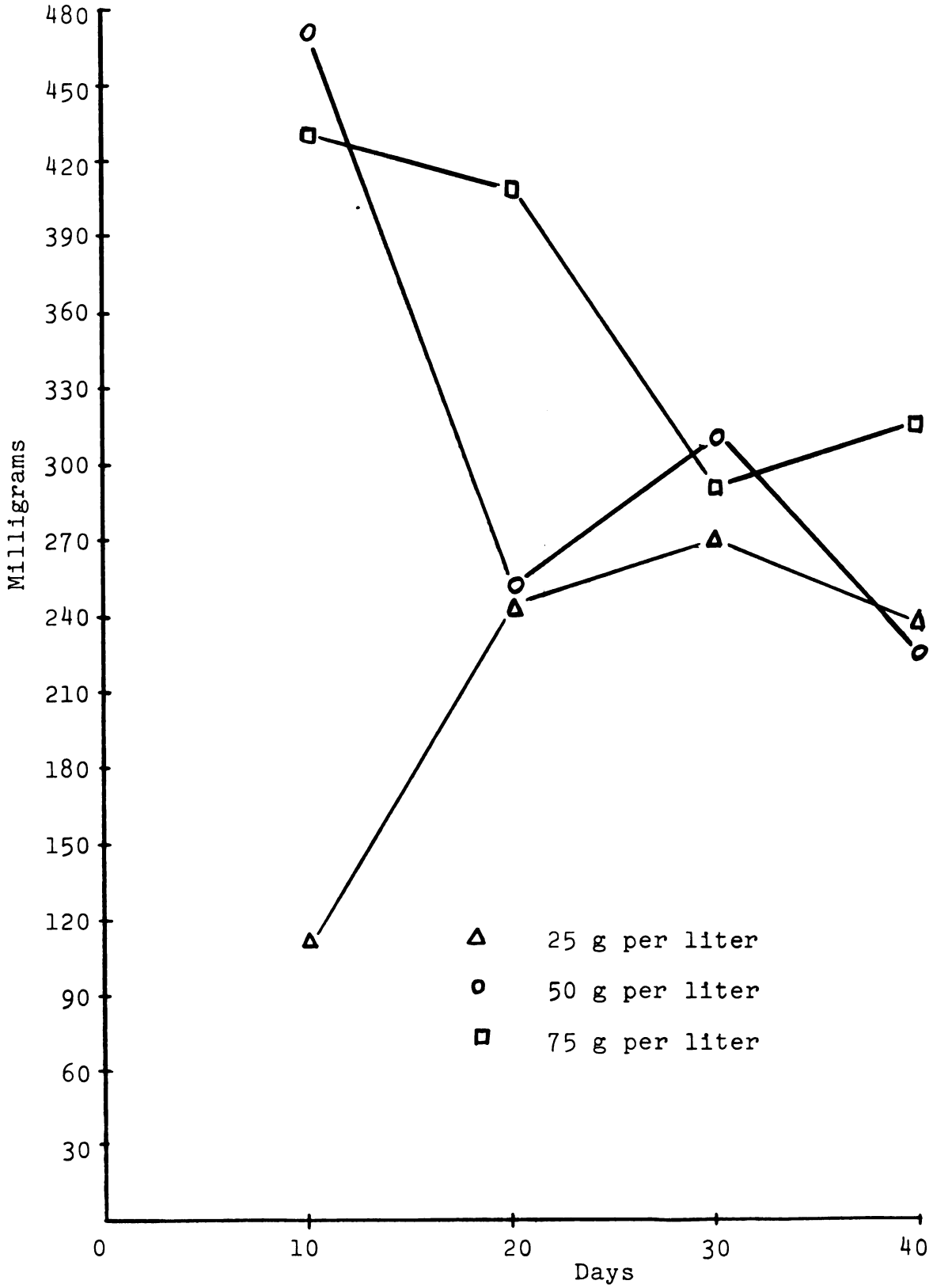


incubation. At each glucose concentration of 25, 50 and 75 g per liter, hyphal growth reached a high at 10 days incubation which was followed by a gradual but steady decrease at 20, 30 and 40 days. The greatest dry mycelial weight was at 10 days, 75 g glucose per liter, and the smallest dry weight occurred at 40 days incubation, 25 g glucose media.

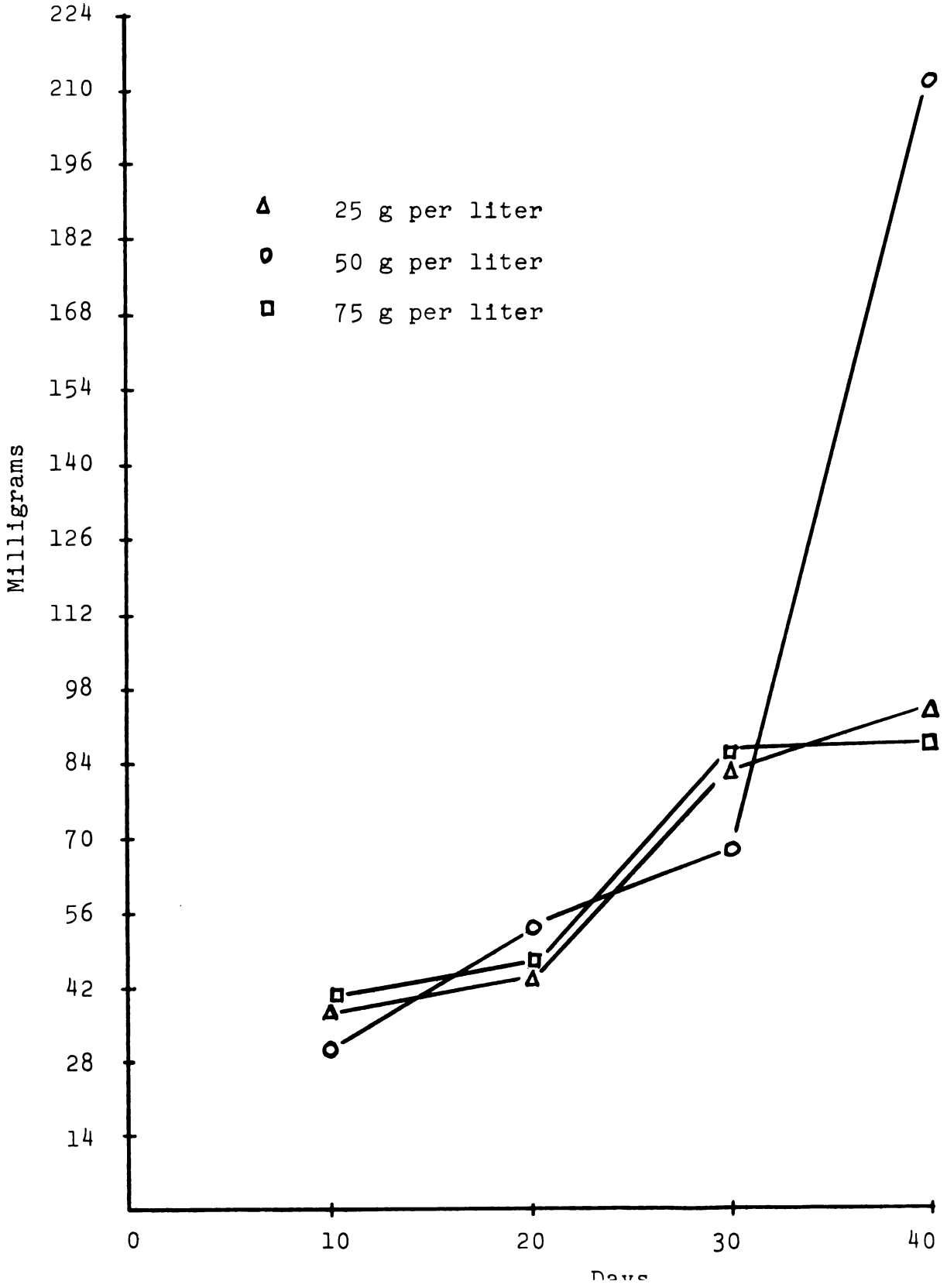
Mycelial growth for Agaricus campestris (A-63-136) in varied glucose media is pictured in Graph 5. A great increase of growth in media containing 25 g glucose per liter occurred between 10 and 20 days incubation then leveled between 20 and 30 days followed by a decrease of dry weight at 40 days incubation. At 50 g glucose per liter, the highest growth at 10 days was followed by a rapid decrease to 20 days, then a slight hyphal dry weight increase to 30 days and again a decrease at 40 days. Growth in 75 g glucose per liter was somewhat similar to growth in the 50 g per liter medium, with a growth peak at 10 days followed by a slight decrease at 20 days, then a greater decrease at 30 days. A slight increase of growth occurred at 40 days in the 75 g medium. The least growth of submerged hyphae occurred at 25 g carbohydrate per liter, 10 days incubation; the greatest was at 10 days 75 g glucose per liter.

The utilization of glucose for A. bisporus (A-63-137) is diagramed in Graph 6. At 25 and 75 g, dry mycelial

Graph 5.--Agaricus campestris (A-63-136) utilization of glucose at concentrations of 25, 50 and 75 grams per liter grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates each at the end of 10, 20, 30 and 40 days incubation.



Graph 6.--Agaricus bisporus (A-63-137) utilization of glucose at concentrations of 25, 50 and 75 grams per liter grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates each at the end of 10, 20, 30 and 40 days incubation.

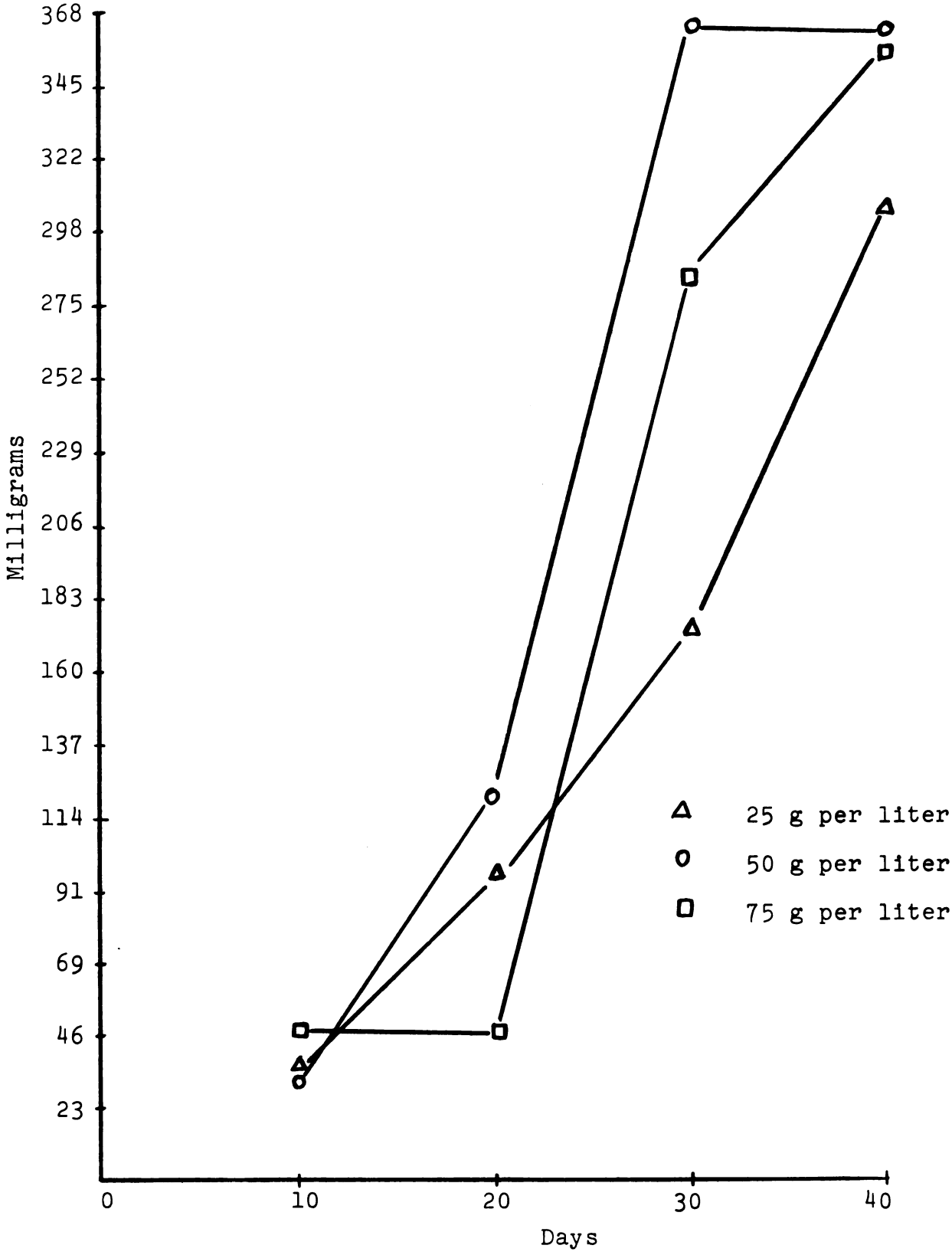


weights were almost identical. A slight increase in growth occurred between 10 and 20 days incubation, a greater increase followed at 30 days, and a lesser increase occurred at 40 days incubation. Submerged growth in 50 g glucose per liter gradually increased up to 30 days incubation then greatly increased to a maximum dry weight reading at 40 days of over 200 mg. The minimum growth occurred at 10 days incubation, 50 g glucose per liter.

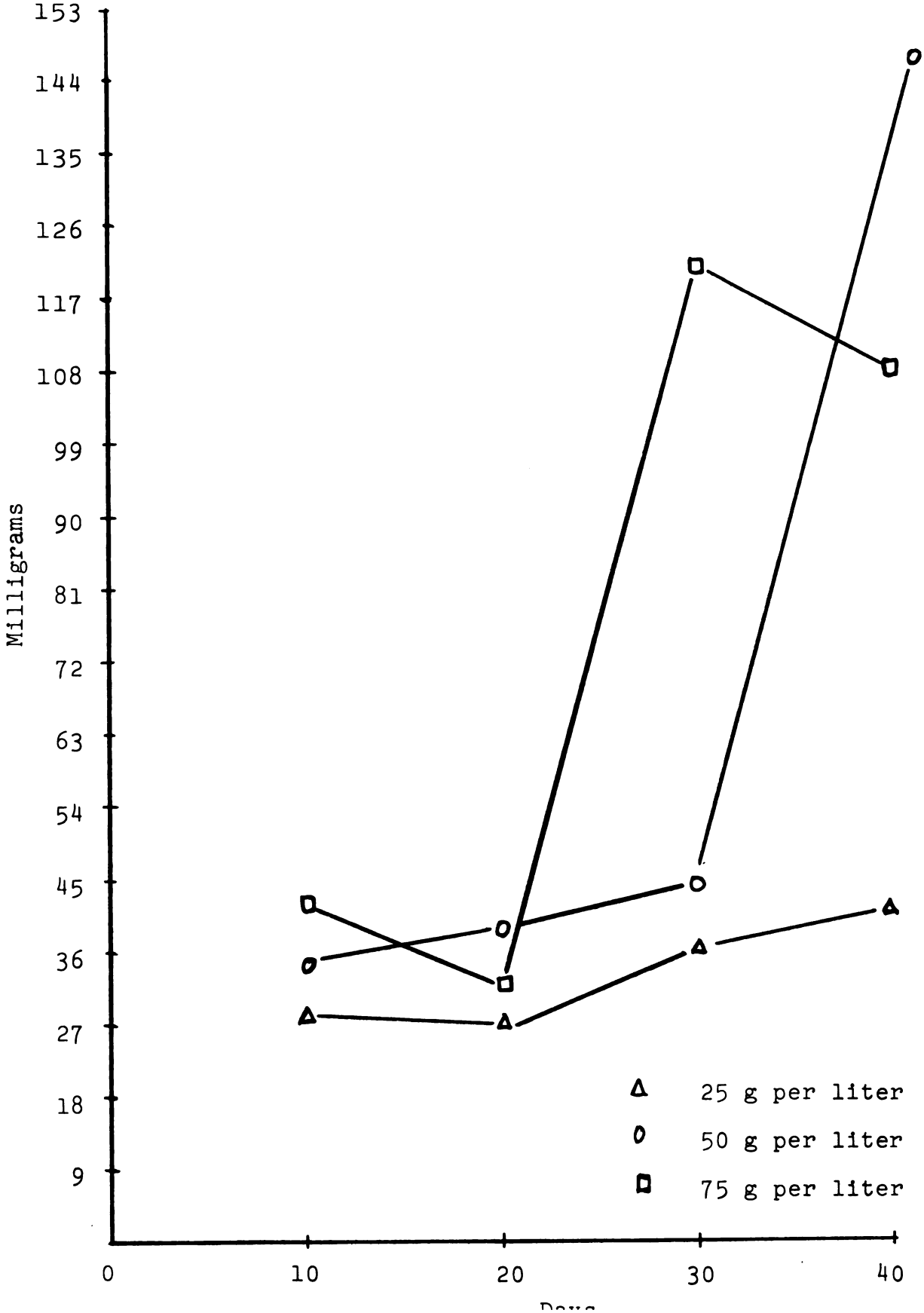
A. bisporus pleomorph (A-63-138) growth curves in glucose and Humfeld's media are plotted in Graph 7. At 25 g per liter, hyphal growth steadily increased from 10 to 40 days incubation; the greatest increase occurred between 30 and 40 days. Mycelial growth at 50 g per liter showed a rapid increase from 10 to 30 days but then remained stationary between 30 and 40 days. Dry mycelial weights were also stationary when grown in 75 g glucose per liter, 10 and 20 days incubation. After 20 days, growth rapidly increased up to 30 days, then slightly lessened. Mycelial dry weight was the smallest at 10 days, 50 g glucose per liter, and the greatest at 30 days, 50 g.

Graph 8 represents submerged hyphal growth of A. bisporus rhizomorph (A-63-139) in Humfeld's glucose media at the three selected carbohydrate concentrations and four growth periods. In 25 and 50 g glucose per liter, growth slightly increased between 10 and 30 days incubation, a slight decrease was registered in the 25 g medium between

Graph 7.--Agaricus bisporus pleomorph (A-63-138) utilization of glucose at concentrations of 25, 50 and 75 grams per liter grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates each at the end of 10, 20, 30 and 40 days incubation.



Graph 8.--Agaricus bisporus rhizomorph (A-63-139) utilization of glucose at concentrations of 25, 50 and 75 grams per liter grown in sumberged culture, reported as averaged mycelial dry weight in milligrams of two replicates each at the end of 10, 20, 30 and 40 days incubation.

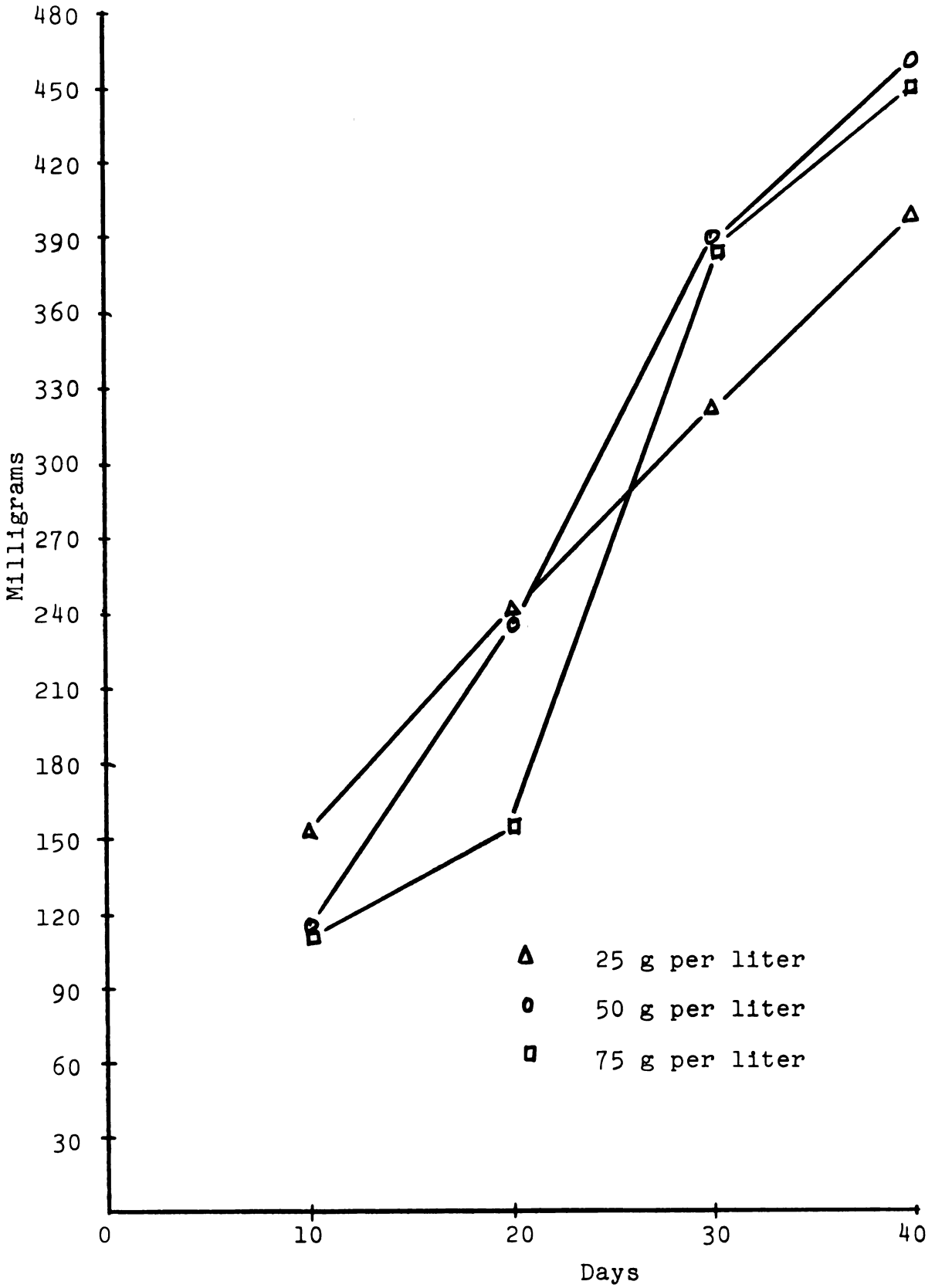


10 and 20 days. Between 30 and 40 days, hyphal growth continued the gradual increase in 25 g glucose per liter but greatly increased in the 50 g per liter medium. A slight decrease occurred between 10 and 20 days growth in 75 g glucose per liter. A drastic increase, parallel to the one in the 50 g glucose medium, occurred between 20 and 30 days incubation with 75 g per liter. A mycelial reduction followed after 30 days. The smallest dry mycelial weight was at 20 days, 25 g glucose, and the greatest dry weight developed in the 50 g media, at the 40 day reading.

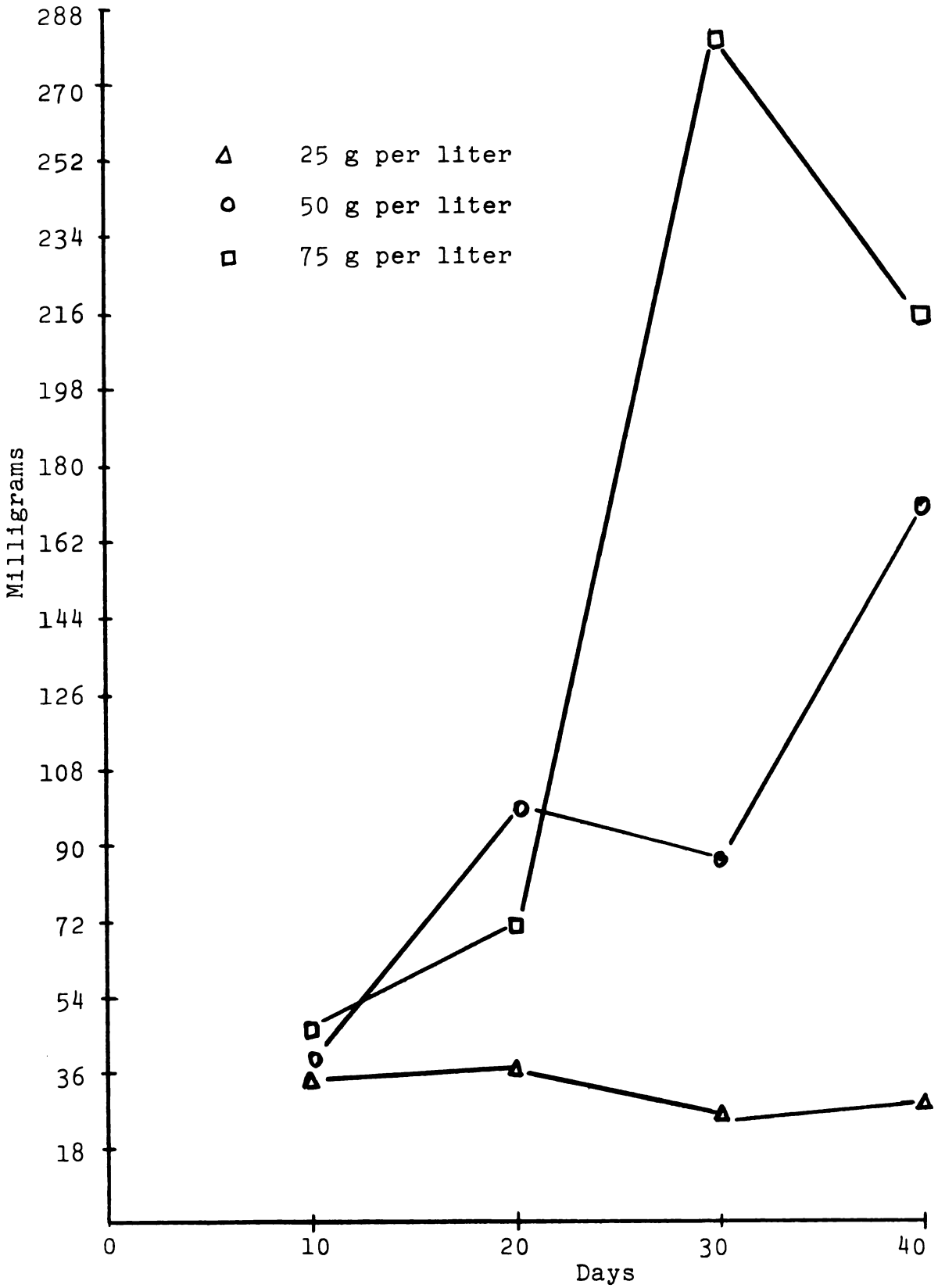
Lepista nuda (L-63-133) grew at a steadily increasing rate in submerged culture of 25 g glucose per liter (Graph 9). At 50 g per liter, hyphal growth was more rapid than at 25 g per liter for the first 30 days incubation, then the growth increment lessened. Mycelium in Humfeld's medium with 75 g glucose per liter had a gradual dry weight increase between 10 and 20 days incubation, greatly increased from 20 to 30 days then again lessened after 30 days. Least hyphal growth occurred at 10 days, 75 g glucose per liter; greatest growth occurred at 40 days incubation, 50 g glucose medium.

In Graph 10, the hyphal growth rate in submerged shake culture of various glucose concentrations in Humfeld's medium are reported for Pleurotus ostreatus (P-63-51). At 25 g glucose per liter, growth remained stationary between 10 and 40 days incubation, no change beyond the weight

Graph 9.--Lepista nuda (L-63-133) utilization of glucose at concentrations of 25, 50 and 75 grams per liter grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates each at the end of 10, 20, 30 and 40 days incubation.



Graph 10.--Pleurotus ostreatus (P-63-51) utilization of glucose at concentrations of 25, 50 and 75 grams per liter growth in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates each at the end of 10, 20, 30 and 40 days incubation.

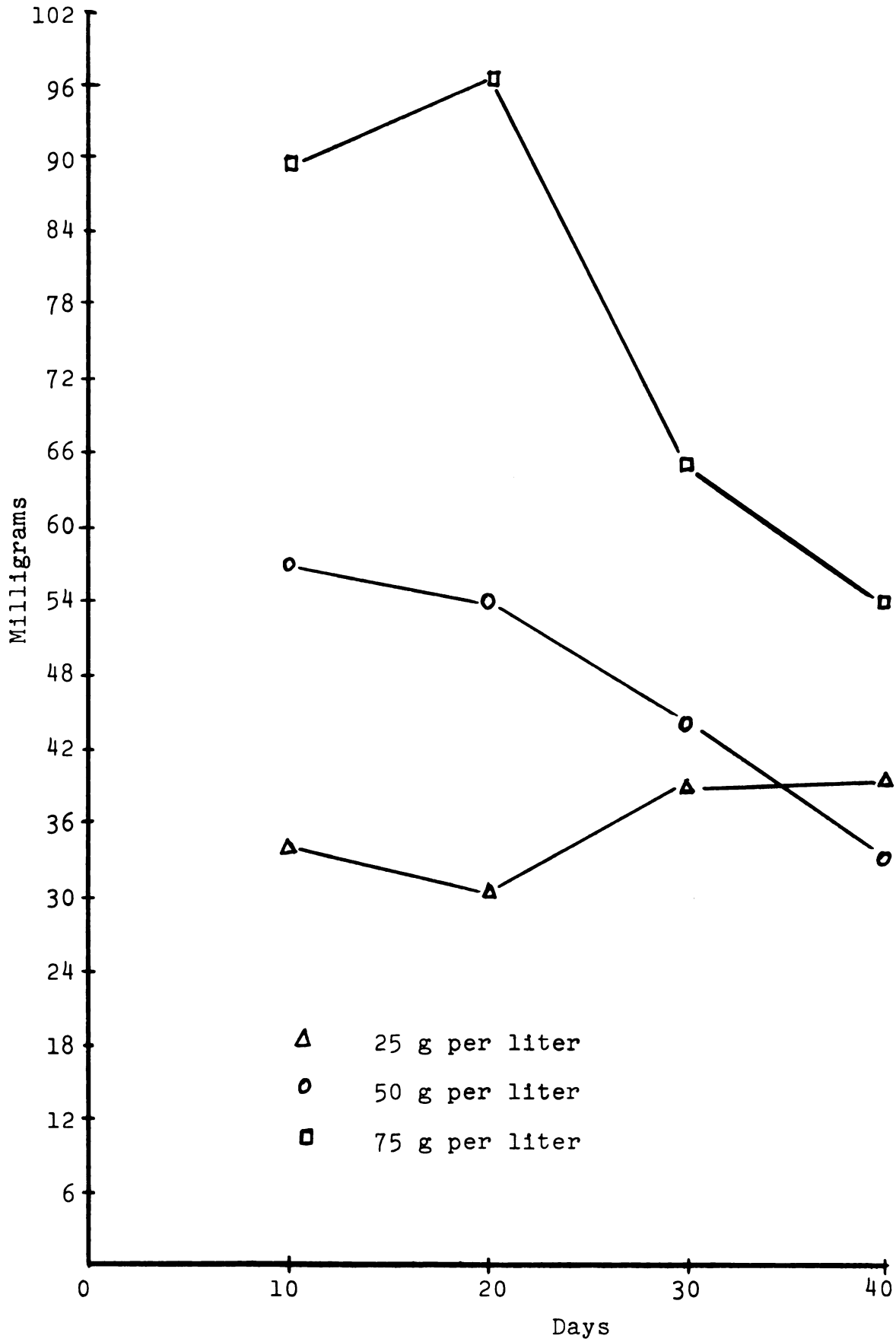


of inoculum. Mycelium growing in 50 g glucose concentration produced a moderate dry weight increase between 10 and 20 days, decreased slightly from 20 to 30 days, then again increased at 40 days incubation. Hyphal growth slightly increased between 10 and 20 days, greatly increased between 20 and 30 days, then decreased after 30 days at 75 g glucose per liter.

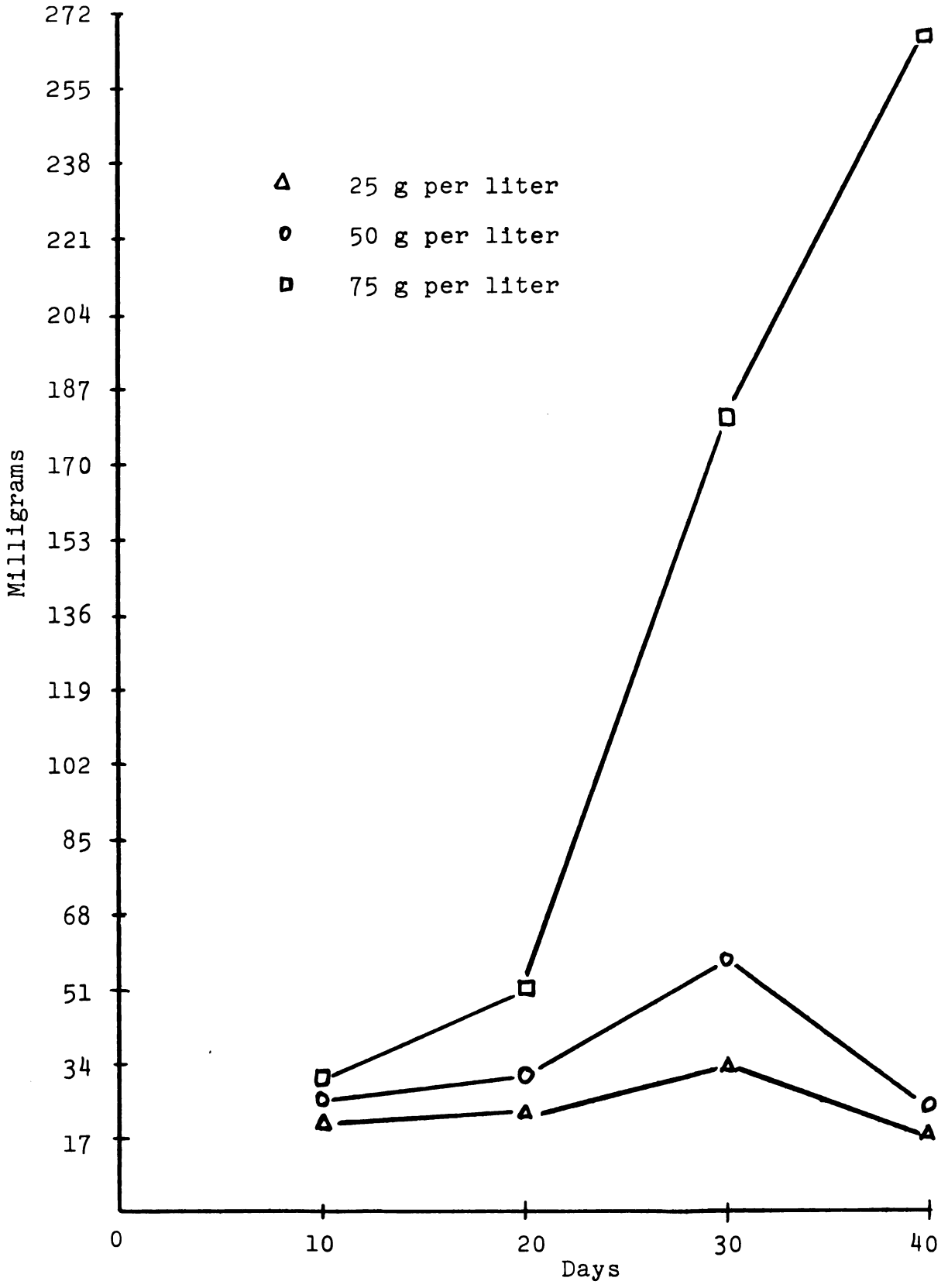
Volvariella volvacea (V-63-135) glucose utilization is shown in Graph 11. Hyphal growth in 25 g glucose per liter varied only slightly; a small dry weight decrease occurred between 10 and 20 days incubation, an increase followed between 20 and 30 days then leveled off after 30 days. At 50 g glucose per liter, a gradual growth rate decrease began at 10 days and increased in intensity until a low at 40 days incubation was reached. Growth in 75 g glucose per liter began at a high of about 90 mg mycelial dry weight, increased up to 20 days incubation then dramatically decreased after 20 days. Least growth occurred at 20 days, 25 g glucose per liter, greatest occurred at 20 days, 75 g glucose medium.

At 25 and 50 g glucose per liter of Humfeld's medium, hyphal growths for V. volvacea mutant (V-63-134) were the same (Graph 12). A slight increase in dry weight occurred up to 30 days incubation, then a decrease followed after 30 days. Mycelial production in 75 g glucose medium increased slightly from 10 to 20 days and greatly increased

Graph 11.--Volvariella volvacea (V-63-135) utilization of glucose at concentrations of 25, 50 and 75 grams per liter grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates each at the end of 10, 20, 30 and 40 days incubation.



Graph 12.--Volvariella volvacea mutant (V-63-134) utilization of glucose at concentrations of 25, 50 and 75 grams per liter grown in submerged culture, reported as averaged mycelial dry weight in milligrams of two replicates each at the end of 10, 20, 30 and 40 days incubation.



at 30 and 40 days incubation. Greatest dry mycelial weight was registered at 75 g glucose per liter, 40 days; least growth occurred at 10 days, 25 g glucose.

Utilization of Nitrogen

Sixteen amino acids and related compounds were added singly in concentrations of 0.47 g nitrogen per liter to Humfeld's medium for each of the 12 fungal strains. Mycelium from the submerged cultures was harvested at the end of 14 days, and the terminal pH was read (Table 10). Control shaker culture flasks had no nitrogen source added to the medium and a pH of 4.5. Cantharellus clavatus (C-63-8) produced mycelial dry weights equal to control with almost all amino acids added to Humfeld's medium, but moderate growth was obtained with L-proline and good growth resulted with peptone. Terminal pH was between 3.0 and 4.5 for all cultures.

C. cibarius (C-63-15) had little or no growth in L-asparagine or D-histidine; and grew moderately in media containing DL-alanine, betaine hydrochloride, creatine, cysteine hydrochloride, L-cystine, glycine, L-glutamic acid, DL-isoleucine, DL-methionine, DL-tryptophane or DL-valine. Highest dry weight was obtained from mycelium grown in peptone; L-aspartic acid, L-proline or DL-threonine also gave good results. The final pH for C-63-15 cultures were 3.0 to 3.5 in media containing DL-isoleucine, DL-methionine, DL-threonine or DL-valine, 5.6 for creatine, 7.0 for peptone,

Table 10.--The utilization of amino acid and related nitrogen* sources by twelve fungi grown in submerged culture, reported as averaged mycelial dry weight in milligrams of three replicates at the end of 14 days.

	Days**	DL-alanine	L-asparagine	L-aspartic acid	betaine hydrochloride	creatine	cysteine hydrochloride	L-cystine	L-glutamic acid	glycine
Cantharellus clavatus C-63-8	14	21.8	19.9	15.8	27.1	27.2	25.1	17.8	18.0	25.0
	pH	4.0	4.5	4.5	4.0	4.5	4.0	3.5	4.0	4.0
C. cibarius C-63-15	14	41.2	17.8	74.1	39.0	30.7	35.2	28.5	65.3	56.4
	pH	4.0	4.0	4.5	4.0	5.6	4.0	4.0	4.0	4.0
C. cibarius C-63-86	14	25.9	15.9	18.5	23.0	39.0	25.0	18.5	15.9	37.8
	pH	4.5	4.5	4.5	4.0	4.5	4.0	3.5	4.0	4.0
C. cibarius C-63-96	14	340.6	217.1	424.0	387.5	81.1	218.3	53.3	464.2	291.1
	pH	5.5	7.0	7.0	5.6	5.6	3.0	1.9	6.8	6.8
Agaricus campestris A-63-136	14	182.0	355.6	278.6	49.5	48.0	14.2	28.3	607.4	311.1
	pH	4.0	4.0	6.4	4.0	5.0	4.0	3.0	6.4	4.0
A. bisporus A-63-137	14	41.5	18.8	13.6	29.8	43.1	11.9	18.4	13.9	29.1
	pH	4.0	4.5	4.0	4.0	5.0	4.0	3.0	4.0	4.5
A. bisporus, pleomorph A-63-138	14	53.9	38.0	22.3	46.4	83.9	30.5	30.0	28.1	42.3
	pH	4.5	6.0	4.5	4.0	5.6	4.0	3.0	4.0	6.0
A. bisporus, rhizomorph A-63-139	14	25.6	23.1	12.9	25.6	21.2	10.3	19.8	12.5	37.9
	pH	4.0	4.5	4.0	4.0	5.6	4.0	4.0	4.0	4.5

Table 10.--Continued.

	Days**	D-histidine	DL-isoleucine	DL-methionine	L-proline	DL-threonine	DL-tryptophane	DL-valine	peptone	control
Cantharellus clavatus C-63-8	14	16.5	24.4	20.7	51.7	19.0	27.6	22.9	119.8	21.0
	pH	4.5	4.0	4.0	4.0	4.5	4.0	4.0	4.5	4.0
C. cibarius C-63-15	14	18.4	63.8	53.7	98.5	87.7	48.6	49.8	154.3	18.5
	pH	4.0	3.0	3.5	4.0	3.5	4.0	3.5	7.0	4.
C. cibarius C-63-86	14	14.6	36.1	20.3	54.5	20.3	33.1	25.0	138.5	27.5
	pH	4.5	4.0	4.5	4.0	4.5	4.0	4.0	6.8	4.0
C. cibarius C-63-96	14	90.8	274.2	121.6	338.0	323.2	262.9	355.8	320.6	78.2
	pH	4.0	6.0	3.0	7.0	7.0	5.0	6.8	4.5	4.0
Agaricus campestris A-63-136	14	31.4	34.2	24.4	48.6	71.6	28.9	63.8	479.5	48.5
	pH	4.0	4.0	4.5	4.0	4.5	4.0	4.0	4.0	4.5
A. bisporus A-63-137	14	19.5	22.8	24.0	45.0	19.2	24.7	29.4	240.0	22.0
	pH	4.5	4.5	4.0	4.0	4.5	4.0	4.0	4.0	4.0
A. bisporus, pleomorph A-63-138	14	25.2	43.2	51.6	81.7	39.3	28.6	62.1	197.2	62.8
	pH	4.5	4.5	4.0	4.0	4.5	4.0	4.0	4.5	4.5
A. bisporus, rhizomorph A-63-139	14	18.5	23.5	20.0	70.7	21.5	27.2	29.2	127.5	29.0
	pH	4.0	4.5	4.5	4.0	4.5	4.0	4.5	4.5	4.5

Table 10.--Continued.

	Days**	DL-alanine	L-asparagine	L-aspartic acid	betaine hydrochloride	creatine	cysteine hydrochloride	L-cystine	L-glutamic acid	glycine
Lepista nuda	14	157.5	75.9	99.0	50.7	51.9	18.4	31.5	115.8	418.1
L-63-133	pH	4.5	4.0	4.5	4.0	5.0	3.5	4.0	4.5	4.5
Pleurotus ostreatus	14	87.5	34.1	49.7	29.7	33.9	35.8	18.8	27.5	22.2
P-63-51	pH	4.0	4.0	4.0	4.0	5.6	3.5	4.0	4.0	4.5
Volvariella volvacea	14	49.3	80.0	36.1	29.3	34.1	44.3	19.0	47.1	43.6
V-63-135	pH	4.0	4.0	4.0	4.5	5.6	3.5	4.0	4.0	4.5
V. volvacea, mutant	14	15.3	17.9	13.2	20.3	24.0	23.6	19.4	16.0	17.3
V-63-134	pH	4.0	4.5	4.0	4.5	5.6	3.5	4.0	4.0	4.5

Table 10.--Continued.

	Days**	D-histidine	DL-isoleucine	DL- methionine	L-proiine	DL-threonine	DL-tryptophane	DL-valine	peptone	control
Lepista nuda L-63-133	14	20.6	46.2	102.4	52.0	42.0	41.3	65.8	236.8	41.9
	pH	4.0	4.0	4.0	4.5	4.5	4.0	4.0	4.0	4.5
Pleurotus ostreatus P-63-51	14	24.6	31.3	33.3	30.3	43.0	38.9	28.3	84.3	21.4
	pH	4.0	4.5	4.5	4.0	4.5	4.0	4.0	4.0	4.5
Volvariella volvacea V-63-135	14	25.9	51.4	35.9	68.5	28.3	31.1	45.6	195.2	39.8
	pH	4.0	4.0	4.0	4.0	4.5	4.0	4.0	4.0	4.5
V. volvacea, mutant V-63-134	14	17.6	16.1	19.1	38.0	20.8	33.8	20.4	87.1	13.3
	pH	4.5	4.5	4.5	4.0	4.5	4.0	4.0	4.0	4.5

*Nitrogen content based on 0.47 g per liter, the amount in one g of urea.

**pH was taken at 14 days.

and 4.0 to 4.5 for all other amino acid media. C. cibarius (C-63-86) had growth comparable to control in Humfeld's media for a number of the amino acids, and other related compounds. Moderate hyphal growth resulted with creatine, glycine, DL-isoleucine, L-proline, and DL-tryptophane while the best growth was obtained with peptone at 14 days incubation. The pH at 14 days was between 3.5 and 4.5 with all cultures except peptone which was 6.8.

Excellent mycelial growth for C. cibarius (C-63-96) resulted in media containing 0.47 g nitrogen per liter of DL-alanine, L-aspartic acid, betain hydrochloride, L-glutamic acid, glycine, DL-isoleucine, L-proline, DL-threonine, DL-tryptophane, DL-valine or peptone. Moderately heavy growth developed in media with L-asparagine, cysteine hydrochloride, D-histidine, DL-methionine while little or no growth occurred in cultures with creatine or L-cystine. The terminal pH was 4.0 or below for cysteine hydrochloride, L-cystine, D-histidine and DL-methionine, 4.5 for peptone, and 5.0 to 7.0 for the remaining amino acid media. C. cibarius (C-63-96) produced loosely organized pellets and hyphal flask rings in the media containing amino acids. The other Cantharellus strains developed minute pellets in media containing L-glutamic acid, L-asparagine, betaine hydrochloride, L-proline or peptone.

Agaricus campestris (A-63-136) had exceptionally good growth in media containing L-glutamic acid, dry mycelial

weight at 14 days was above 600 mg. Dry weights were between 180 to 480 in media with DL-alanine, L-asparagine, L-aspartic acid, glycine or peptone. Moderate growth was obtained with DL-threonine or DL-valine, all other amino acids produced dry weights equal to control or less. The pH remained between 4.0 and 4.5 for all cultures except for drops to 3.0 for L-cystine, 5.0 for creatine and 6.4 for L-aspartic acid and L-glutamic acid.

A. bisporus (A-63-137) could not utilize any amino acids except DL-alanine, creatine, L-proline or the related betaine hydrochloride and peptone. A moderate mycelial growth at the end of 14 days occurred. Terminal pH was 4.0 to 4.5 except for creatine at 5.0 and L-cystine at 3.0. A. bisporus pleomorph (A-63-138) grew best in the medium with peptone, moderately well only in creatine or L-proline and equal to control in all other amino acid media. A. bisporus rhizomorph (A-63-139) had moderate growth with glycine and L-proline, the best growth with peptone and no growth in the remaining amino acid media. The two Agaricus mutants had final pH readings of 4.0 to 4.5 except 5.6 for both mutants in the medium with creatine, and 6.0 for the pleomorphic strain in L-asparagine and glycine. A. campestris (A-63-136) formed hyphal pellets in most amino acid cultures while A. bisporus (A-63-137) and its two mutants produced few pellets.

Glycine and peptone both produced excellent hyphal growth in submerged culture for Lepista nuda (L-63-133) when added in concentrations of 0.47 g nitrogen per liter. Good growth at 14 days incubation was also obtained with DL-alanine, L-aspartic acid, L-glutamic acid and DL-methionine. The remaining amino acid nitrogen sources produced growth near to control or less. The pH remained between 4.0 to 4.5 for all cultures except pH 5.0 for creatine and 3.5 for cysteine hydrochloride. L. nuda in submerged culture containing peptone produced a purple color characteristic of the basidiocarp. In other cultures, pellet and flask rings were very common.

Pleurotus ostreatus (P-63-51) had good growth in media containing DL-alanine or peptone, and no growth with L-cystine, glycine or D-histidine. Moderate mycelial development was obtained with the other amino acids added individually to Humfeld's media. The pH at 14 days remained at 4.0 to 4.5 for all media except 3.5 for cysteine hydrochloride, and 5.6 for creatine. Pleurotus grew as loose interwoven hyphal masses in amino acid media.

Volvariella volvacea (V-63-135) and V. volvacea mutant (V-63-134) both grew best in peptone, while V-63-135 also grew well in L-asparagine. Moderate growth for both strains resulted with cysteine hydrochloride, L-proline and DL-valine; V-63-135 produced moderate growth

also in DL-alanine, L-glutamic acid, glycine and DL-isoleucine; while mutant strain V-63-134 grew moderately in media containing betaine hydrochloride, creatine or DL-tryptophane when results were compared to control. No growth equivalent to control was obtained with L-aspartic acid, L-cystine, D-histidine and DL-methionine for both Volvariella strains. Poor growth for V. volvacea (V-63-135) also developed in betaine hydrochloride, DL-threonine or DL-tryptophane, and for V. volvacea mutant (V-63-134) in DL-alanine, L-asparagine, L-glutamic acid, glycine and DL-isoleucine. The pH remained at 4.0 to 4.5 except for 3.5 for cysteine hydrochloride and 5.6 for creatine. Hyphae of the Volvariella cultures grew as submerged mycelial masses in the amino acid media.

Several ammonium and nitrate compounds were selected for nitrogen utilization in Humfeld's medium by the twelve fungi and compared to urea; results were recorded in Table 11. Dry mycelial weights of three replicates for each nitrogen source and culture were averaged at the end of 14 days incubation. Only compounds producing hyphal growth greater or equal to that of urea medium are considered here, and terminal pH changes from the initial 4.5 are mentioned. The nitrogen content for each medium was 0.47 g per liter, or the equivalent amount of nitrogen in one g urea.

Table 11.--The utilization of ammonium and nitrate nitrogen sources in comparison to urea* by twelve fungi grown in submerged culture, reported as averaged mycelial dry weight in milligrams of three replicates at the end of 14 days.

	Days**	ammonium bicarbonate	ammonium chloride	ammonium citrate	ammonium nitrate	ammonium sulfate	ammonium tartrate	calcium nitrate	cobaltous ammonium sulfate	cobaltous nitrate	cupric nitrate	magnesium nitrate	potassium nitrate	sodium nitrate	uranium nitrate	urea
Cantharellus clavatus C-63-8	14	41.6	24.6	27.5	27.9	26.8	25.6	26.5	29.0	14.5	21.7	24.8	27.5	25.1	15.1	31.4
	pH	7.0	4.5	5.0	4.5	4.5	4.5	4.5	4.5	4.5	3.5	4.5	4.5	4.5	1.9	6.4
C. cibarius C-63-15	14	43.0	55.7	75.0	55.8	62.8	65.5	25.9	29.4	16.6	24.3	22.4	21.2	26.8	11.9	57.8
	pH	7.0	4.5	3.5	3.0	3.0	3.0	4.5	4.5	4.5	3.5	4.5	4.5	4.5	1.9	6.4
C. cibarius C-63-86	14	44.7	26.9	28.4	28.8	31.3	49.5	34.3	33.3	16.3	25.7	43.7	23.3	37.8	16.2	58.2
	pH	7.0	4.5	5.0	4.5	4.5	4.5	4.5	4.5	4.5	3.5	4.5	4.5	4.5	1.9	6.4
C. cibarius C-63-96	14	45.5	323.6	313.8	267.6	330.9	352.7	376.3	344.0	377.6	323.4	370.0	325.0	331.8	30.0	295.3
	pH	7.6	1.9	6.8	7.0	1.9	4.5	6.4	1.9	6.4	5.0	7.2	7.2	7.0	1.9	7.0
Agaricus campestris A-63-136	14	51.3	38.8	166.8	49.9	48.8	156.6	96.4	37.5	24.9	33.3	42.8	56.2	57.0	34.8	160.2
	pH	7.0	4.5	3.5	4.5	4.0	3.5	4.5	4.5	4.5	3.5	4.5	4.5	4.5	1.9	6.8
A. bisporus A-63-137	14	41.6	26.1	26.9	31.7	29.4	43.1	32.8	31.0	21.5	24.3	30.9	30.3	42.7	8.0	53.6
	pH	7.0	4.5	6.8	4.5	4.5	5.0	4.5	4.5	4.5	3.5	4.5	4.5	4.5	1.9	6.4
A. bisporus, pleomorph A-63-138	14	56.3	41.3	32.4	44.6	29.4	36.4	33.6	28.9	28.2	12.6	75.1	34.7	75.2	21.9	36.6
	pH	7.0	4.5	5.0	4.5	4.5	5.0	4.5	4.5	4.5	3.5	5.0	4.5	4.5	1.9	6.4
A. bisporus, rhizomorph A-63-139	14	34.8	19.5	21.4	24.5	24.4	36.8	23.9	29.9	18.3	19.7	20.7	21.5	25.5	22.8	36.6
	pH	7.0	4.5	6.8	4.5	4.5	5.0	4.5	4.5	4.5	3.5	4.5	4.5	4.5	1.9	6.4
Lepista nuda L-63-133	14	291.5	36.8	20.1	48.9	37.8	78.1	34.0	25.5	16.0	20.8	53.1	40.4	43.1	12.8	286.0
	pH	4.0	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	3.5	4.5	4.5	4.5	1.9	6.0
Pleurotus ostreatus P-63-51	14	48.5	46.0	82.2	38.0	39.8	53.1	39.5	33.1	21.2	27.6	30.3	31.0	43.4	15.2	91.9
	pH	7.0	4.5	6.8	4.5	4.5	4.5	4.5	4.5	4.5	3.5	4.5	4.5	4.5	1.9	6.4
Volvariella volvacea V-63-135	14	46.5	31.9	51.6	48.0	43.1	54.9	66.2	29.9	19.0	22.0	40.3	47.6	49.5	15.9	49.1
	pH	7.0	4.5	4.5	4.0	4.5	4.5	4.5	4.5	4.5	3.5	4.5	5.0	4.5	1.9	6.4
V. volvacea, mutant V-63-1[illegible]	14	47.5	22.4	24.5	15.3	12.6	26.1	26.4	28.5	18.9	22.0	[illegible]	24.2	29.8	16.5	26.2
	pH	7.6	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	3.5	[illegible]	4.5	4.5	1.9	6.0

*Nitrogen content based on 0.47 g per liter, the amount in one g of urea.

**pH was taken at 14 days.

Cantharellus clavatus (C-63-8) was able to utilize only ammonium bicarbonate, and produced a higher dry mycelial weight than with urea. The former had a dry weight of 41.6 mg, the latter was 31.4. At 14 days, the pH with ammonium bicarbonate was 7.0, and 6.4 with urea, other nitrogen source media remained close to the original pH or lower. Few mycelial pellets formed; hyphae remained single stranded in submerged culture.

Dry mycelial weight for C. cibarius (C-63-15) was higher in cultures of ammonium citrate, ammonium sulfate or ammonium tartrate than with urea; the respective 14 day pH readings were 3.5, 3.0 and 3.0. Other terminal pH readings were generally 4.5 or higher. The other ammonium compounds produced moderate submerged hyphal yields, while nitrates were not utilized. Urea in Humfeld's medium gave greater hyphal growth than any of the inorganic nitrogen source media for C. cibarius (C-63-86). Hyphal pellets developed only in the urea medium, all other cultures produced hyphal strands.

An opposite effect was registered with C. cibarius (C-63-96) compared to the other Cantharellus strains. All the nitrogen sources, except ammonium bicarbonate and uranium nitrate, were utilized by the mycelium producing dry mycelial weights around 300 mg and above at 14 days incubation. Terminal pH readings were quite varied. Except

for ammonium bicarbonate, ammonium nitrate and uranium nitrate, mycelium of C. cibarius (C-63-96) developed dense pellet formation in submerged culture and grew thick flask rings at splash levels.

Urea, ammonium citrate, calcium nitrate, and ammonium tartrate each produced good mycelial dry weights at 14 days with Agaricus campestris (A-63-136), while other inorganic nitrogens were not utilized. Urea had a terminal pH of 6.8, while the other three cultures were 3.5 to 4.5. The pH values for the other media remained near 4.5 except for pH 1.9 with uranium nitrate and pH 7.0 with ammonium bicarbonate, a consistency with the two latter inorganic nitrogens found in all 12 fungal cultures with one exception. Hyphal pellets formed in the cultures which produced good mycelial dry weights.

A. bisporus (A-63-137) had greatest hyphal growth in urea in Humfeld's medium while all the other nitrogen sources had less dry mycelial weights. Both mutant strains of A. bisporus had a reduced mycelial dry weight in the urea medium compared to the parent culture. A. bisporus pleomorph (A-63-138) utilized most of the ammonium compounds at about the same rate as urea, all producing similar dry weights. Magnesium nitrate and sodium nitrate media both produced over twice the amount of hypha as did urea, other nitrate sources were poor sources. With A. bisporus rhizomorph (A-63-139), only the ammonium tartrate

flasks had hyphal growth equal to urea cultures, all other inorganic nitrogen sources were not good sources. The pH values for media supporting good growth with A. bisporus and its mutants remained around 4.5 to 5.0 except for the Humfeld's medium with ammonium bicarbonate. Both mutants had poor pellet formations in media containing an inorganic nitrogen source.

Lepista nuda (L-63-133) grown in urea or ammonium bicarbonate had dry mycelial weights around 290 mg at the end of 14 days incubation. Terminal pH was 6.0 for the urea medium, and 4.0 for ammonium bicarbonate. Very poor growth occurred in all other inorganic nitrogen sources. Pleurotus ostreatus (P-63-51) grew well in urea and ammonium citrate, and had terminal pH readings of 6.4 and 6.8 respectively. All other ammonium and nitrate nitrogen sources produced minimal growth results and the pH readings remained at 4.5 except for the usual low pH of uranium nitrate and ammonium bicarbonate. Inorganic nitrogens supporting good growth developed moderate pellet formation.

Volvariella volvacea (V-63-135) was able to utilize ammonium bicarbonate, ammonium citrate, ammonium nitrate, ammonium tartrate, calcium nitrate, potassium nitrate, and sodium nitrate and produced similar mycelial dry weights as urea did at 14 days incubation. Except for pH 6.4 in the urea medium and pH 7 in ammonium bicarbonate medium, all other good hyphal producing media remained at

pH 4.5. Ammonium sulfate, and magnesium nitrate media were moderately good growth promoters for V-63-135. V. volvacea mutant (V-63-134) grew best in ammonium bicarbonate, and well in urea, ammonium chloride, ammonium citrate, ammonium tartrate, calcium nitrate, cobaltous ammonium sulfate, cupric nitrate, magnesium nitrate, potassium nitrate, and sodium nitrate. Terminal pH readings at the end of 14 days for inorganic nitrogen source media with the Volvariella mutant remained at the initial 4.5 except for ammonium bicarbonate. V. volvacea (V-63-135) grew in submerged culture as a mass of interwoven hyphae. V. volvacea (V-63-134) grew only as isolated hyphal strands.

Vitamin Requirements

In Table 12, ten vitamin sources are listed that were each added in concentrations of 0.5 mg per liter to glucose casein hydrolysate with purified agar. Four fungi, Volvariella volvacea (V-63-135), Lepista nuda (L-63-133), Cantharellus cibarius (C-63-86) and Pleurotus ostreatus (P-63-51), were selected for this study. Hyphal growth measurements in mm at two day intervals after inoculation were taken for each of the four fungi growing on each of the 10 vitamin media and control, and averaged from replicates of 3 cultures.

V. volvacea (V-63-135) had growth differences during the first two days of incubation but then growth

Table 12.--Hyphal growth in millimeters at two day intervals after inoculation on agar media containing vitamins.

	Days	p-amino-benzoic acid	ascorbic acid	biotin	calcium panto-thenate	niacin	niacina-mide	pyridoxine hydro-chloride	riboflavin	rutin	thiamine	control
Volvariella volvacea V-63-135	2	5	6	8	6	6	5	1	1	3	2.5	1
	4	14	13	14	14	14	13	14	14	12	11	12
	6	23	27	28	24	23	25	26	25	23	23	24
	8	43	44	42	41	41	39	42	43	36	33	34
	10	62	65	57	50	58	48	54	55	51	52	53
	12	86	88	76	67	78	65	80	80	72	75	65
Lepista nuda L-63-133	2	2.5	2	2	2.5	2.5	2	3	2.5	2.5	3	3
	4	5	4	4	4	4	4	5	5	5	6	5
	6	6.5	6	5	6	5	5	6	6	6	7	6
	8	12	8	10	12	12	10	7	8	9	12	9
	10	15	12	15	17	15	15	13	13	15	14	12
	12	20	16	19	23	19	17	16	17	19	19	17
Cantharellus cibarius C-63-86	2	1	0.5	0.3	1	0.5	0.5	0.5	0.5	0.5	1	0.5
	4	4	2	3	5	3	1	1	2	3	3	1
	6	6	4	9	10	7	3	3	3	3.5	6	2
	8	13	6	12	16	11	4	6	9	4	13	3
	10	15	9	15	17	13	7	10	13	9	16	3.5
	12	16	16	18	23	15	9	16	19	14	22	4
Pleurotus ostreatus P-63-51	2	1.5	2	2.5	2	1.5	2	1.5	1.5	1.5	1.5	1.5
	4	6	6	7	8	6	6	6	7	8	8	5
	6	14	14	15	15	12	10	11	11	12	18	9
	8	20	23	22	29	22	14	19	19	22	28	12
	10	32	36	32	37	31	22	23	24	25	40	21
	12	45	43	39	51	41	35	35	32	38	51	30

rates became equalized. P-amino benzoic acid, ascorbic acid, biotin, calcium pantothenate, niacin and niacinamide media had initial hyphal growth, during the first two days after inoculation, of about 5 to 8 times greater than control. Pyridoxine hydrochloride, riboflavin, rutin or thiamine media had hyphal growth rates equal to hyphae grown in the medium without a vitamin source. Calcium pantothenate and niacinamide had a slightly reduced growth rate compared with other vitamins, they equaled growth in the control medium.

Hyphal growth of L. nuda (L-63-133) and P. ostreatus (P-63-51) showed no significant growth rate differences on any of the 10 vitamin agar media when compared to the control culture. C. cibarius (C-63-86) had approximately similar hyphal growth for all vitamin media except control and niacinamide. Mycelium grown on the latter two agar media grew only half or one fourth as fast as mycelium on the other nine vitamin media.

Cantharellus cibarius (C-63-86) appears to be the only one of the four fungi selected for vitamin study that showed a dependency upon vitamins for mycelial growth.

Growth Hormones

Five growth hormones; gibberellic acid, kinetin, 2,4-dichlorophenoxy acetic acid, indoleacetic acid, and indolebutyric acid, were each added in concentrations of 0.1%,

0.01% and 0.001% to 2% malt Oxoid agar no. 3 and then inoculated with V. volvacea (C-63-135), L. nuda (L-63-133), C. cibarius (C-63-86) or P. ostreatus (P-63-51). Hyphal measurements in mm were taken seven times at two day intervals. Growth hormones at 0.1% concentration killed the inoculum due to difficulty in controlling the pH, concentrations of 0.01% limited growth also due to the low pH factor compared with control media, with or without acetone. No growth differences were noted in the two control media; acetone was used as solvent for the hormones. At 0.001% concentration, no hyphal growth differences were noted with Lepista, Cantharellus or Pleurotus in any of the growth hormone media as compared to the two controls for each species. V. volvacea (V-63-135) had an increased growth with four of the hormones especially gibberellic acid, and a retardation with another. Growth readings in mm for Volvariella were as follows at 0.001% hormone concentration at the second and fourth day of incubation respectively: gibberellic acid, 22, 34; 2,4-dichlorophenoxy acetic acid, 15, 22; indoleacetic acid, 19, 25; indolebutyric acid, 18, 23; and kinetin, 1.4, 9; compared to control with acetone, 9, 26; and without acetone, 9, 27. After four days incubation, hyphal growth on all hormone cultures simulated development of the control cultures and in eight days covered the entire agar surface of the petri plates.

Spawn Media

Table 13 indicates the suitability of spawn media for growth of V. volvacea (V-63-135), L. nuda (L-63-133), C. cibarius (C-63-86) and P. ostreatus (P-63-51), incubated 2 weeks at 24° C unless otherwise indicated. V. volvacea (V-63-135) had no hyphal growth on seeds of rape, vetch, rye at 12° C or spruce needles; poor growth with flax, milo, rye at 18° C or sunflower; moderate growth with orange cane, spelt, sudan grass or rye with glucose; and heavy hyphal growth on lupine, millet, sorghum, wheat, rye in Humfeld's medium, rye at 24° C, 30° C, 36° C and ground corn cobs.

L. nuda (L-63-133) had no growth with spawn media of rape, vetch, rye at 36° C or spruce needles; poor growth with flax, lupine, millet, rye at 12° C or sunflower seeds; moderate growth with milo, sorghum, rye with glucose, rye in Humfeld's medium,rye at 18° C, 24° C, 30° C or ground corn cobs; heavy mycelial growth with orange cane, spelt, sudan grass or wheat.

C. cibarius (C-63-86) had no growth on vetch, rye at 36° C, or ground corn cobs; poor growth on rape, sorghum, rye at 12° C or spruce needles; moderate growth on lupine, orange cane, wheat or sunflower; heavy growth with flax, millet, milo, spelt, sudan grass, rye with glucose, rye in Humfeld's medium, rye at 18° C, 24° C or 30° C.

P. ostreatus (P-63-51) had hyphal growth on spawn media of vetch; poor growth with rape, rye at 12° C, 36° C,

Table 13.--Suitability of spawn media for growth of four selected fungal cultures.*

Substrate**	Volvariella volvacea V-63-135	Lepista nuda L-63-133	Cantharellus cibarius C-63-86	Pleurotus ostreatus P-63-51
flax	1	1	3	3
lupine	3	1	2	3
millet	3	1	3	3
milo	1	2	3	3
orange cane	2	3	2	3
rape	0	0	1	1
sorghum	3	2	1	3
spelt	2	3	3	3
sudan grass	2	3	3	3
vetch	0	0	0	0
wheat	3	3	2	3
corn cobs, ground	3	2	0	3
spruce needles	0	0	1	1
sunflower seeds	1	1	2	3
rye	3	2	3	3
rye, with glucose	2	2	3	3
rye, with Humfeld's medium minus carbohydrate	3	2	3	3
rye, 12°C	0	1	1	1
rye, 18°C	1	2	3	3
rye, 30°C	3	2	3	3
rye, 36°C	3	0	0	1

*Cultures grown for 2 weeks.

**24°C unless indicated.

Key: no growth 0
poor growth 1
moderate growth 2
heavy growth 3

or spruce needles; and heavy growth with all other substrates listed. Examination of V. volvacea (V-63-135) inoculum on spruce needles, vetch or rye at 12° C spawn media showed that hyphae were dead, showing coagulated protoplasm. The Volvariella inoculum on rape produced an extremely dense mycelial growth over the inoculum plug and 2 mm of the adjacent rape seeds indicating nutrients were supplied by the agar plug. Mycelium growing on spawn media supporting poor growth was not dense, the growth was light and wispy over the grain surface and throughout the spawn medium. Occasional aerial or submerged dense tan hyphal areas appeared; these were composed of chlamydospore masses. The spawn cultures of moderate growth had a greater mycelial density, more aerial growth, some hyphal tufts and larger chlamydospore masses. Some flasks, with heavy growth produced dense tan mycelial mats over the surface and throughout the spawn, growth density was equal to basidiocarp tissue in some areas. Aerial hyphae in flasks of heavy growth filled the entire flask volume. Rye and wheat spawn developed the best growth for Volvariella.

L. nuda (L-63-133) grown on spawn media supporting poor growth had white cottony mycelial development over the surface with no aerial growth. There was little growth into the grain from the culture surface. Moderate growth cultures produced thin mycelial growth throughout all the

spawn media, surface growth became more dense and slight aerial hyphal development occurred. Heavy growth yielded a mycelial mass over and throughout all the spawn media, but no tissue formation. Some aerial cottony growth developed over a portion of the surface areas. Best growth was supported on spelt, sudan grass or orange cane spawn.

C. cibarius (C-63-86) hyphal inoculum was killed on vetch spawn, ground corn cobs or rye at 36° C. Media supporting poor growth had cottony, thin growth over and through all spawn, at times wispy tufts appeared on the spawn surface. Moderate growth spawn had dense hyphal development over and throughout the media, some solidation, little aerial hyphal production, and was tan to cream in color. Dark brown exudate droplets appeared on the culture surface of several spawn media. Heavy growth cultures had very dense hyphal growth; tissue formation was common throughout the media. The surface again did not support aerial growth, only a dense mycelial mat was present, exudate droplets formed small surface pools of dark tan liquid (Figure 15). Rye and Humfeld's medium, and spelt spawn developed a mass of fructifications after 12 weeks incubation (Figure 16). There was little differentiation in the round dense bodies; no lammae formed.

P. ostreatus (P-63-51) hyphal inoculum was killed on vetch spawn. Flasks with poor growth showed thin white mycelial development over and throughout the media and had

15.

16.

Figure 15.--Cantharellus cibarius (C-63-86) exudate formation with three month old culture, growing on millet grain.

Figure 16.--Cantharellus cibarius (C-63-86) fructifications on rye grain with Humfeld's medium minus carbohydrate, three month old culture.

a somewhat cottony surface. Only rye at 36° C, rye at 12° C, spruce needles or rape spawn had poor growth, all other media supported heavy mycelial growth. Extremely dense hyphal growth developed, solidation was common on surface and throughout the media, rhizomorphs were abundant. Aerial growth at times filled the entire flask volume and grew out of the cotton stoppered flasks. Fructifications formed within 2 to 6 weeks on sudan, orange cane, millet, rye, rye with 2 g glucose per flask, rye with Humfeld's medium without glucose, or ground corn cob spawn media. Basidiocarps grown on corn cobs had large stipes and small caps (Figure 17) while other fructifications were more equally proportioned such as those grown on 2% malt agar slants (Figure 18). An interesting observation concerning Pleurotus fructifications, when inoculations were made on 2% malt agar in petri plates, is that only rudimentary basidiocarp initials appeared and were composed of undifferentiated mycelium (Figure 19). At times only rhizomorphs formed on the 2% malt agar petri plates (Figure 20). A microscopic view of early rhizomorph formation can be seen in A. bisporus rhizomorph (A-63-139) material (Figure 21). The cultures not forming fructifications developed small droplets of yellow exudate on the colony surface.

High temperatures of 36° C or above killed mycelium of Cantharellus, Lepista and Pleurotus but Volvariella showed the greatest hyphal growth at 36° C. Volvariella

17.

18.

Figure 17.--Pleurotus ostreatus (P-63-51) basidiocarp formation on ground corn cobs, six weeks.

Figure 18.--Pleurotus ostreatus (P-63-51) fructification on a 2% malt agar slant, six weeks old.

Figure 19.--Pleurotus ostreatus (P-63-51) basidiocarp initials on 2% malt agar, five weeks old.

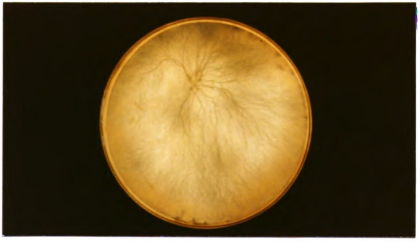


Figure 20.--Pleurotus ostreatus (P-63-51) development of rhizomorphs on 2% malt agar in a six week old culture.

hyphae was extremely dense, large tissue areas formed throughout the rye spawn medium, and chlamydospore production was at its greatest. Cantharellus formed fructifications on rye spawn at 18° C, and did not develop at 12°, 24°, 30° or 36° C. Pleurotus fructifications on rye spawn formed between 18° and 24° C only. C. cibarius (C-63-86) also produced immature fructifications on 4% and 6% malt agar petri plates at 4 weeks incubation. The structures were columnar projections 2 cm in length and 2 mm in diameter growing from the agar surface and were composed of psuedoparenchyma and compact hyphae.

The fructifications produced in culture were examined cytologically. Pleurotus ostreatus (P-63-51) basidiocarp grown on a 2% malt agar slant formed mature hymenia complete with basidiospores (Figure 22). Tissue removed from a Pleurotus fructification revealed a typical basidiomycete dolipore septa with parenthesomes (Figures 23 and 24). A simple pore between two adjacent cells was found in tissue from Cantharellus cibarius (C-63-86) (Figure 25).

P. ostreatus (P-63-51) was inoculated on 2% malt agar plates and incubated in inverted or upright positions, with or without 10% KOH present in the petri dish cover, in light or in dark. Fructifications were produced in cultures placed in continual light, inverted, without KOH; continual light, inverted, with KOH; and in natural light, alternating periods of light and dark, no KOH, inverted plates. Pleurotus cultures developed aerial tufts and a cottony surface in

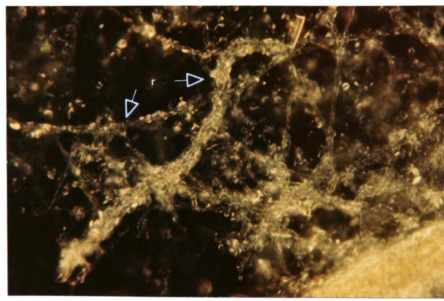


Figure 21.--Agaricus bisporus rhizomorph (A-63-139) showing early formation of rhizomorphs (r, rhizomorphs). x 3,400

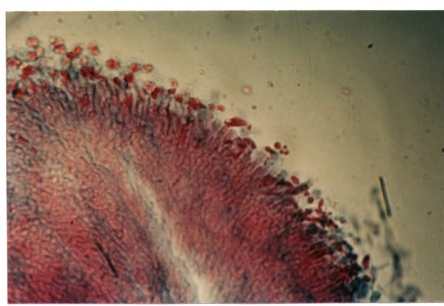


Figure 22.--Hymenium and basidiospores of Pleurotus ostreatus (P-63-51) grown on 2% malt agar slant. x 2,600

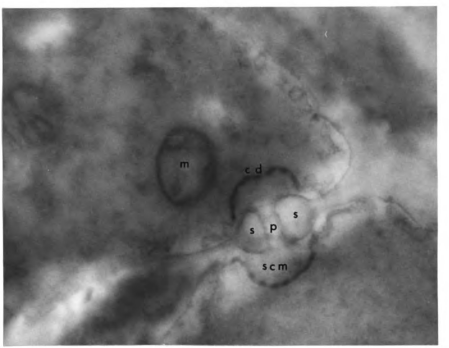


Figure 23.--Pleurotus ostreatus (P-63-51) showing one dolipore septum in cross section from a fruiting body (s, septal swelling; p, pore in septal pore apparatus; scm, subcap matrix; cd, discontinuous pore cap; m, mitochondrion). x 33,000

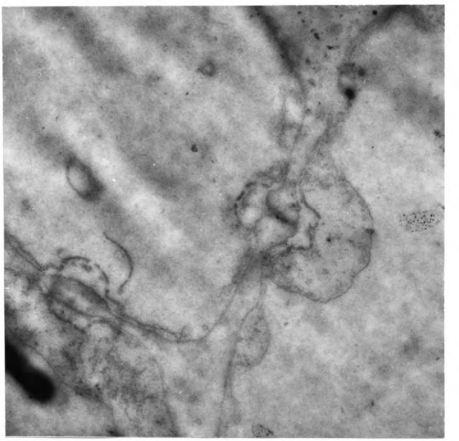


Figure 24.--Pleurotus ostreatus (P-63-51) cell with dolipore septa on adjacent walls from a fructification grown on ground corn cob spawn medium. x 23,375

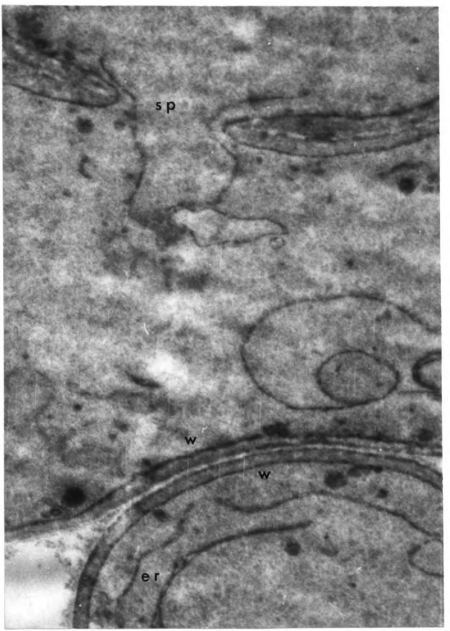


Figure 25.--Cantharellus cibarius (C-63-86) adjacent cells isolated from a fruiting body showing a simple pore septum (sp, simple pore; er, endoplasmic reticulum; w, walls of adjacent cells). x 56,500

continual light, right side up, no KOH; continual dark, inverted plates or right side up, with or without KOH; in natural light, alternating periods of light and dark, inverted, with KOH; and natural light, alternating periods of light and dark, plates right side up, no KOH. Hyphal growth in petri plates containing 10% KOH was slightly less than plates without KOH. Potassium hydroxide has the capacity of absorbing CO_2 but no relation to fructification was noted when the level of CO_2 was changed.

P. ostreatus was inoculated into autoclaved beech logs by filling 2" holes, 5" apart, 1 1/2" deep with rye spawn then covering the inoculum with drill shavings and taping to hold in place. After 2 weeks at 34° C incubation, the wood chips were covered with a dense hyphal growth. Mycelium slowly grew over the log and in 3 months, basidiocarp initials appeared. The fructifications grew to one inch in height, but did not differentiate from the cylindrical hyphal mass. Old decomposed beech logs were also inoculated, mycelial growth was greater and developed faster but the same abnormal fructifications appeared. Logs containing rhizomorphs were transported from the collection site near Rose City and placed in a protected area near a building in East Lansing over winter. The following spring and fall, a number of Pleurotus sporophores developed.

V. volvacea (V-63-135), L. nuda (L-63-133), C. cibarius (C-63-86) and P. ostreatus (P-63-51) were selected for mushroom house studies and were grown under conditions

suited for Agaricus bisporus production, using rye spawn. Volvariella had some mycelial growth over the compost, but with the addition of the casing soil, no further growth occurred. Lepista produced a dense hyphal growth over the compost surface having a purple tint typical of Lepista basidiocarps. A mycelial mat also covered the casing soil but no fructification initials formed. The Cantharellus inoculum did not grow on the compost. After Pleurotus mycelium grew at a fast rate within and on the compost surface, tissue formation developed. When casing soil was added, mycelial growth on the soil surface was quite good but basidiocarp button stages failed to appear.

Colony Morphology on 20 Selected Media

Tables 14 through 25 briefly describe morphological characteristics and colony measurements of the twelve fungi when grown on twenty selected agar media (appendix). The basis for use of these 20 selected agar media was to pick out colony characteristics that could be used to recognize a specific strain or possible variants in these strains. The cultures were incubated four weeks before descriptions were made. Following the descriptive tables, Cantharellus cibarius (C-63-86), Lepista nuda (L-63-133), Pleurotus ostreatus (P-63-51) and Volvariella volvacea (V-63-135) are each pictured growing on four selected media (Figures 26-29).

Table 14.--Colony morphology of Cantharellus clavatus (C-63-8) grown on twenty agar media for one month.

Blood agar base: no growth.

Brewer anaerobic agar: diameter of colony 1.8 cm, tan color, dense growth, slightly course texture, irregular margin.

Chlamydospore agar: diameter of colony 7.2 cm, light growth, smooth surface with some aerial tufts, irregular margin, cream color, agar color change from blue to pink adjacent to colony.

Corn meal agar: diameter of colony 7.4 cm, dense growth, white color, numerous aerial tufts, cottony texture, smooth margin.

Liver spleen glucose agar: no growth.

Micro assay culture agar: diameter of colony 6.8 cm, waxy texture, moderate growth, aerial tufts at colony margin, cream color.

Mycological agar: diameter of colony 7.4 cm, dense growth, matted texture, many aerial tufts, cream color, smooth margin.

Mycosel agar: no growth.

Nutrient agar: entire petri plate covered with moderate hyphal growth, smooth surface, white color.

O. Modess agar: entire petri dish covered, dense growth, granular texture, white color.

Table 14.--Continued.

Potato dextrose agar: entire petri dish surface covered, white color, aerial tufts, cottony surface, dense growth.

Prune agar: entire plate covered, tufted, slightly aerial, white color, granular to cottony texture.

Rice extract agar: poor hyphal growth over entire surface, cottony texture, white color, some small dense aerial tufts, large amount of subsurface growth.

Russian medium: diameter of colony 6 cm, tan color, margin smooth, dense surface growth with tufted clumps, slightly matted.

Russian medium minus casein and tryptophan: moderate surface growth, diameter of colony 4 cm, smooth surface, no aerial development.

Russian medium minus vitamins: diameter of colony 5 cm, tan color, smooth margin, dense growth, slightly matted.

Thioglycollate medium: diameter of colony 7.2 cm, brown to black color, dense growth, matted cottony texture.

Tomato juice agar special: entire petri plate covered, dense growth, matted cottony texture, light cream color.

Tryptic soy agar: no growth.

Table 14.--Continued.

Violet-red bile agar: diameter of colony 3.5 cm, agar color change from purple to pink, waxy colony surface, irregular margin, white to yellow color, white aerial hyphae, moderate growth.

Table 15.--Colony morphology of Cantharellus cibarius (C-63-15) grown on twenty agar media for one month.

Blood agar base: diameter of colony 4.5 cm, dense hyphal growth, cream color, smooth margin and surface growth.

Brewer anaerobic agar: diameter of colony 6.8 cm, cream color, moderate growth, smooth margin and surface.

Chlamydospore agar: diameter of colony 5.3 cm, smooth margin and surface, dense growth, cream to white color.

Corn meal agar: entire petri plate covered, moderate growth, rough texture, white color, no aerial development.

Liver spleen glucose agar: diameter of colony 2 cm, smooth surface and margin, cream color, slightly waxy texture.

Micro assay culture agar: entire plate covered, waxy surface, some colony folds radiating from point of inoculation, cream color.

Mycological agar: entire petri plate covered, dense growth, waxy surface texture, cream color, smooth margin.

Mycosel agar: no growth.

Nutrient agar: diameter of colony 4.5 cm, dense growth, cream color, smooth texture and margin.

O. Modess agar: entire petri dish covered, growth dense, light cream color, smooth surface.

Table 15.--Continued.

Potato dextrose agar: entire petri dish covered, moderate growth, cream color, smooth texture, no aerial hyphae.

Prune agar: entire petri dish covered, moderate growth, cream color, surface slightly rough with some hyphal tufts.

Rice extract agar: entire petri dish covered, thin surface growth, smooth texture, cream color, no aerial growth.

Russian medium: diameter of colony 6.5 cm, moderate surface growth with some subsurface growth, cream to yellow color, smooth texture and margin.

Russian medium minus casein and tryptophan: diameter of colony 4.2 cm, poor growth, cream color, smooth margin.

Russian medium minus vitamins: diameter of colony 5.0 cm, moderate surface growth, cream color, smooth texture and margin.

Thioglycollate medium: entire petri plate covered, dense growth, cream color, waxy texture, smooth margin.

Tomato juice agar special: entire petri plate covered, dense growth, cream to white color, matted cottony surface, slightly aerial.

Table 15.--Continued.

Tryptic soy agar: diameter of colony 3.5 cm, waxy surface, moderate subsurface growth, some colony folds, white color, smooth margin.
Violet-red bile agar: diameter of colony 4.5 cm, waxy texture, dense growth, folded and heaped surface, cream color, smooth margin.

Table 16.--Colony morphology of Cantharellus cibarius (C-63-86) grown on twenty agar media for one month.

Blood agar base: diameter of colony 4.5 cm, smooth texture and margin, surface folds, white color, moderate growth.

Brewer anaerobic agar: diameter of colony 2.5 cm, tan color, smooth texture and margin, poor growth density.

Chlamydospore agar: diameter of colony 6.8 cm, blue color of agar changed to pink, many aerial hyphal tufts, rough texture, white color, moderate growth, smooth margin.

Corn meal agar: diameter of colony 7.5 cm, many hyphal tufts, slightly aerial cottony texture, moderate growth, white color.

Liver spleen glucose agar: diameter of colony 2.2 cm, dense growth, white color, matted surface growth, margin smooth.

Micro assay culture agar: diameter of colony 4.9 cm, many aerial tufts of hyphae, dense growth, cream color, red to brown color for reverse surface of colony, smooth margin.

Mycological agar: diameter of colony 7.3 cm, numerous small hyphal tufts, very dense growth, cream to yellow color, smooth surface, irregular margin.

Table 16.--Continued.

Mycosel agar: no growth.

Nutrient agar: diameter of colony 6 cm, dense hyphal growth, granular texture, white color, smooth margin, no aerial growth.

O. Modess agar: diameter of colony 5 cm, smooth surface and margin, tan color, dense hyphal growth.

Potato dextrose agar: entire petri dish covered, numerous aerial tufts, very dense growth, white color, matted cottony texture.

Prune agar: entire petri dish covered, very dense growth, white color, numerous aerial hyphal tufts, fine cottony texture.

Rice extract agar: entire petri dish covered, thin growth, smooth surface, no aerial growth, moderate subsurface hyphal growth, white color.

Russian medium: diameter of colony 7 cm, smooth to granular texture, small aerial hyphal wisps radiating from colony center, white color, smooth margin.

Russian medium minus casein and tryptophan: diameter of colony 5 cm, granular texture, dense growth, smooth margin, surface growth only, white color.

Russian medium minus vitamins: diameter of colony 6 cm, granular texture, moderate growth, white color, smooth margin.

Table 16.--Continued.

Thioglycollate medium: diameter of colony 5.3 cm, tan color, moderate growth, smooth margin and surface, no aerial growth.
Tomato juice agar special: entire petri dish covered, light cream color, very dense growth, rough texture, many small aerial tufts, reverse surface dark brown.
Tryptic soy agar: diameter of colony 6 cm, white color, dense growth, smooth surface with some folds, no aerial development.
Violet-red bile agar: diameter of colony 3.5 cm, gray color, reverse surface black, matted cottony texture, some aerial growth, smooth margin, surface folds.

Table 17.--Colony morphology of Cantharellus cibarius (C-63-96) grown on twenty agar media for one month.

Blood agar base: diameter of colony 4 cm, smooth texture, and margin, folds and breaks, cream to white in color, thin growth.

Brewer anerobic agar: diameter of colony 5.4 cm, smooth texture and margin, dense growth, folded surface, cream color.

Chlamydospore agar: diameter of colony 2.0 cm, white color, dense cottony growth, blue color of agar cleared around colony to pink, reverse colony surface black, irregular margin.

Corn meal agar: diameter of colony 7.2 cm, slight waxy texture, some aerial hyphal tufts, cream to white color, smooth margin.

Liver spleen glucose agar: diameter of colony 7.4 cm, smooth surface and margin, moderate growth, white color, extremely folded surface.

Micro assay culture agar: diameter of colony 6.8 cm, dense growth, white color, smooth texture with some folds.

Mycological agar: diameter of colony 7.6 cm, slightly folded, rough surface, heaped center, white color, smooth margin.

Table 17.--Continued.

Mycosel agar: diameter of colony 6.8 cm, dense surface growth, slightly folded, smooth to waxy at circumference, center of colony white and periphery cream.

Nutrient agar: diameter of colony 5 cm, white color, dense growth, some subsurface growth, fairly smooth margin.

O. Modess agar: diameter of colony 4 cm, white color, slightly cottony surface, moderately dense growth, smooth margin.

Potato dextrose agar: entire petri plate covered, smooth surface, light cream color, no aerial growth.

Prune agar: entire petri dish covered, white to cream color, dense growth, waxy texture, no aerial growth.

Rice extract agar: entire petri plate covered, cream color, thin growth, some aerial tufts.

Russian medium: diameter of colony 4.5 cm, light tan color, circumference of colony darker brown, dense growth, rough texture.

Russian medium minus casein and tryptophan: diameter of colony 3 cm, tan color, moderate growth, rough texture, smooth margin.

Table 17.--Continued.

Russian medium minus vitamins: diameter of colony 4 cm, tan color, smooth margin, moderate growth, rough texture.
Thioglycollate medium: diameter of colony 6.4 cm, dense growth, smooth surface and margin, some folds, cream color.
Tomato juice agar special: diameter of colony 7.2 cm, dense growth, cream to white color, matted cottony texture, smooth margin.
Tryptic soy agar: diameter of colony 6 cm, smooth paper-like texture, surface folds, margin smooth, densely compact growth.
Violet-red bile agar: diameter of colony 4.8 cm, waxy texture, heaved center, some folds, tan color.

Table 18.--Colony morphology of Agaricus campestris (A-63-136) grown on twenty agar media for one month.

Blood agar base: no growth.

Brewer anaerobic agar: diameter of colony 1.2 cm, cream color, waxy texture, smooth margin.

Chlamydospore agar: diameter of colony 2 cm, white color, cottony texture, aerial hyphae, blue color of agar around colony cleared to pink, smooth margin, reverse surface of culture black.

Corn meal agar: diameter of colony 7 cm, white color, moderately dense, some aerial hyphal strands, smooth margin.

Liver spleen glucose agar: diameter of colony 0.5 cm, cream color, waxy texture, dense growth, heaped.

Micro assay culture agar: diameter of colony 0.9 cm, tan color, waxy surface, heaped, dense hyphal growth.

Mycological agar: diameter of colony 6.5 cm, dense growth, smooth surface and margin, brown color.

Mycosel agar: no growth.

Nutrient agar: diameter of colony 2 cm, white color, dense growth, some aerial hyphae, smooth margin, granular texture.

O. Modess agar: diameter of colony 3 cm, waxy texture, smooth surface and margin, white color.

Table 18.--Continued.

Potato dextrose agar: diameter of colony 7.4 cm, cream color, smooth surface and margin, moderately dense growth.

Prune agar: diameter of colony 4.2 cm, white color, dense growth, some aerial tufts, rough texture.

Rice extract agar: entire petri dish covered, white color, rhizomorphs present, moderate growth, some aerial development.

Russian medium: diameter of colony 3 cm, buff color to dark brown at circumference, dense growth, smooth margin, granular texture.

Russian medium minus casein and tryptophan: diameter of colony 1.5 cm, buff color, granular texture, smooth margin, moderate growth.

Russian medium minus vitamins: diameter of colony 0.5 cm, tan color, some aerial tufts, dense surface growth, much subsurface hyphal growth.

Thioglycollate medium: no growth.

Tomato juice agar special: diameter of culture 4 cm, dense growth, heaped center, some surface folds, matted cottony texture, white to gray color.

Tryptic soy agar: no growth.

Violet-red bile agar: diameter of colony 0.7 cm, waxy texture, heaped surface, cream to tan color.

Table 19.--Colony morphology of Agaricus bisporus (A-63-137) grown on twenty agar media for one month.

Blood agar base: diameter of colony 0.2 cm, cottony surface, slightly elevated, tan color, reverse surface white, smooth margin.

Brewer anaerobic agar: diameter of colony 2.9 cm, cream to white color, cottony texture, smooth margin, tan color pigment diffused in agar.

Chlamydospore agar: diameter of colony 2.4 cm, cottony surface, moderate growth, blue color of agar changed to pink around colony, smooth margin, cream color.

Corn meal agar: diameter of colony 6.6 cm, dense growth, matted cottony texture, white color, some aerial hyphal tufts, smooth margin.

Liver spleen glucose agar: diameter of colony 2.5 cm, matted cottony surface, dense growth, cream color, smooth margin.

Micro assay culture agar: diameter of colony 4.8 cm, waxy surface, cream color, reverse surface of colony brown, smooth margin.

Mycological agar: diameter of colony 6.5 cm, cream to white color, diffused tan pigment in agar, rhizomorph formation, tufted aerial hyphae.

Mycosel agar: no growth.

Table 19.--Continued.

Nutrient agar: diameter of colony 5 cm, white color, sparce growth, smooth surface and margin.

O. Modess agar: entire plate covered, thin growth, numerous aerial hyphal tufts, white color.

Potato dextrose agar: diameter of colony 5.8 cm, many aerial tufts, moderately dense growth, cottony texture, smooth margin, white color.

Prune agar: diameter of colony 6.9 cm, white color, matted texture, smooth margin, some aerial hyphae.

Rice extract agar: entire petri plate covered, white color, rhizomorph formation, moderate growth, some aerial growth.

Russian medium: entire plate covered, cream to white color, some rhizomorph formation, few aerial hyphal tufts.

Russian medium minus casein and tryptophan: diameter of colony 6 cm, white color, dense growth, cottony texture, smooth margin.

Russian medium minus vitamins: diameter of colony 4 cm, large amount of subsurface growth, irregular margin, cottony surface, white color.

Thioglycollate medium: no growth.

Tomato juice agar special: diameter of colony 5.6 cm,

Table 19.--Continued.

matted cottony texture, cream to white color, dense growth, reverse surface of colony dark brown, smooth margin.
Tryptic soy agar: diameter of colony 2 cm, brown color, granular texture, reverse surface of colony dark brown, smooth margin.
Violet-red bile agar: diameter of colony 2.5 cm, smooth surface and margin, cream color, slightly folded, moderate growth.

Table 20.--Colony morphology of Agaricus bisporus pleomorph (A-63-138) grown on twenty agar media for one month.

Blood agar base: diameter of colony 5 cm, dense growth, tan color, some aerial growth, smooth margin, granular texture.

Brewer anaerobic agar: diameter of colony 4.5 cm, cottony texture surface, cream color, reverse surface of colony brown, dense growth, smooth margin.

Chlamydospore agar: diameter of colony 6.2 cm, few aerial hyphal tufts, white color, blue agar color change to pink adjacent to colony, irregular margin.

Corn meal agar: entire petri plate covered, aerial hyphae fills petri plate volume, white color, dense growth.

Liver spleen glucose agar: diameter of colony 5.4 cm, tan color, matted cottony texture, dense growth, smooth margin.

Micro assay culture agar: diameter of colony 5.5 cm, tan color, waxy surface, heaped center, reverse side of colony brown color, smooth margin.

Mycological agar: entire petri plate surface covered, dense cottony growth, cream to white color.

Mycosel agar: no growth.

Nutrient agar: diameter of colony 4.5 cm, white color, dense growth, some aerial hyphae, rough surface, smooth margin.

Table 20.--Continued.

O. Modess agar: entire petri plate covered, moderate aerial development, white color, rough surface.

Potato dextrose agar: entire petri plate covered, white color, moderate growth, some aerial tufts, rough texture.

Prune agar: entire petri plate covered, densely aerial hyphae, petri volume filled with hyphae, white color.

Rice extract agar: entire petri plate covered, several rhizomorphs developed, some aerial cottony growth, not dense, white color.

Russian medium: entire petri plate covered, cottony texture, a few rhizomorphs, white color, moderate growth.

Russian medium minus casein and tryptophan: entire petri plate covered, white color, moderate growth, cottony texture, no rhizomorphs.

Russian medium minus vitamins: diameter of colony 5 cm, smooth margin, matted cottony texture, white color.

Thioglycollate medium: no growth.

Tomato juice agar special: diameter of culture 7.2 cm, cream color, dense cottony growth, aerial hyphae.

Tryptic soy agar: diameter of colony 1 cm, tan color, reverse colony surface brown color, dense growth, smooth margin, cottony texture.

Table 20.--Continued.

Violet-red bile agar: diameter of colony 4.1 cm, colony perimeter waxy, cottony textured center, white color, irregular margin.

Table 21.--Colony morphology of Agaricus bisporus rhizomorph (A-63-139) grown on twenty agar media for one month.

Blood agar base: diameter of colony 1 cm, dense growth, tan color, smooth margin, granular texture.

Brewer anaerobic agar: diameter of colony 3.7 cm, tan color, brown pigment diffused into agar, dense growth, irregular margin, matted texture.

Chlamydospore agar: diameter of colony 4.9 cm, blue color of agar changed to pink adjacent to colony, thin surface growth, irregular margin, cream color.

Corn meal agar: diameter of colony 6 cm, cream to white color, moderate growth, numerous aerial tufts of hyphae, irregular margin.

Liver spleen glucose agar: diameter of colony 3.7 cm, tan color, dense surface growth, some rhizomorph formation, irregular margin.

Micro assay culture agar: diameter of colony 3.2 cm, dense growth, waxy texture, tan color, smooth margin.

Mycological agar: entire petri dish surface covered, numerous rhizomorphs, some basidiocarp initials, very dense growth, tan color.

Mycosel agar: no growth.

Nutrient agar: diameter of colony 7 cm, white color, moderately dense growth, matted cottony texture, some rhizomorphs, smooth margin.

Table 21.--Continued.

O. Modess agar: entire petri dish covered, white color, some aerial hyphal tufts, moderate growth, cottony texture.

Potato dextrose agar: diameter of colony 6.4 cm, dense growth, smooth to waxy texture, cream color, smooth margin.

Prune agar: entire petri dish covered, numerous rhizomorphs, good growth, white color, some aerial hyphae, rough texture.

Rice extract agar: entire petri plate covered, moderate growth, good rhizomorph formation, some aerial rhizomorphs, irregular margin, some subsurface growth, cream color.

Russian medium: entire petri plate covered, matted cottony texture, some rhizomorphs, some submerged hyphal patches in agar.

Russian medium minus casein and tryptophan: diameter of colony 5 cm, moderate growth, rough texture, cream color, irregular margin.

Russian medium minus vitamins: diameter of colony 6 cm, rough surface growth, irregular margin, subsurface hyphal growth.

Thioglycollate medium: no growth.

Table 21.--Continued.

Tomato juice agar special: diameter of colony 6.2 cm, brown color, reverse side of colony dark brown, dense growth, somewhat aerial, rough texture.
Tryptic soy agar: diameter of colony 2 cm, waxy surface with folds, smooth margin, some subsurface growth.
Violet-red bile agar: diameter of colony 3.1 cm, smooth texture and margin, no aerial growth, dense development, tan color.

Table 22.--Colony morphology of Lepista nuda (L-63-133) grown on twenty agar media for one month.

Blood agar base: entire surface of petri dish covered with thick growth, some aerial hyphae, tan color, reverse colony surface dark brown, rough texture.

Brewer anaerobic agar: entire petri plate covered, buff color, matted cottony texture, moderate growth.

Chlamydospore agar: diameter of colony 5.2 cm, some rhizomorphs, moderate growth, aerial cottony hyphae, blue color of agar adjacent to colony changed to pink, irregular margin.

Corn meal agar: diameter of culture 7.4 cm, cream to white color, extremely aerial, not dense, little surface growth.

Liver spleen glucose agar: diameter of colony 5.7 cm, tan to cream color, extremely dense growth, matted cottony texture, smooth margin.

Micro assay culture agar: entire petri dish surface covered, waxy surface, violet to black color, pigment diffused into agar, smooth margin.

Mycological agar: entire petri dish covered, dense growth, tan to yellow color, matted to rough texture, some aerial hyphae.

Mycosel agar: no growth.

Nutrient agar: growth over entire petri plate, white color, abundant aerial growth, cottony texture.

Table 22.--Continued.

O. Modess agar: entire petri dish covered, thin growth, cottony texture, no aerial hyphae, white color.

Potato dextrose agar: entire petri plate covered, white color, moderate growth, slightly aerial, somewhat cottony.

Prune agar: entire surface of petri dish covered, moderate growth, tan to white color, some aerial hyphal tufts.

Rice extract agar: entire surface of petri plate covered, thin growth, white color, abundant aerial hyphae, some subsurface growth.

Russian medium: entire petri dish surface covered, white to tan color, thin growth, cottony texture, aerial development, no subsurface hyphae.

Russian medium minus casein and tryptophan: entire petri plate covered, white color, moderate growth, cottony texture, aerial hyphae.

Russian medium minus vitamins: entire petri dish covered, white color, moderate growth, cottony texture, aerial hyphal development.

Thioglycollate medium: entire petri plate covered, dense growth, matted cottony texture, cream to white color.

Table 22.--Continued.

Tomato juice agar special: diameter of colony 6.2 cm, cream color, brown pigment diffused in agar, cottony texture, reverse surface of colony dark brown, densely aerial, irregular margin.
Tryptic soy agar: entire petri dish covered, dense growth, white color, small cottony tufts, dark brown pigment diffused into agar, reverse surface of colony brown to black.
Violet-red bile agar: diameter of colony 3.3 cm, waxy surface, smooth margin, some folds, central area violet black, circumference cream color.

Table 23.--Colony morphology of Pleurotus ostreatus (P-63-51) grown on twenty agar media for one month.

Blood agar base: diameter of colony 3 cm, thin growth, subsurface mycelium present, tan color, irregular margin.

Brewer anaerobic agar: diameter of colony 6.9 cm, small aerial hyphal tufts, dense growth, white color, rough texture.

Chlamydospore agar: diameter of colony 4.5 cm, white color, aerial hyphal tufts, blue color of agar changed to pink adjacent to colony, moderately dense growth, irregular margin.

Corn meal agar: entire petri plate covered, aerial hyphal tufts, white color, thin surface growth.

Liver spleen glucose agar: entire petri plate covered, yellow to white color, dense surface growth, slightly aerial, rough texture.

Micro assay culture agar: entire petri plate covered, cream color, matted cottony texture, numerous aerial tufts, rough texture, growth over petri dish sides, yellow exudate drops on colony surface.

Mycological agar: entire petri dish covered, very dense growth, growth over sides of plate, cottony texture, yellow exudate drops on surface, white to yellow color.

Table 23.--Continued.

Mycosel agar: no growth.

Nutrient agar: entire petri plate covered, white color, dense surface growth, rough texture.

O. Modess agar: entire petri plate covered, tan to white color, moderate growth, rough texture, not aerial.

Potato dextrose agar: entire surface of petri dish covered, dense growth, white color, rough texture, some aerial hyphae.

Prune agar: entire petri dish surface covered, moderate growth, aerial hyphae, white color, rough texture, some mycelium growing over petri dish side.

Rice extract agar: entire petri dish covered, thin growth, several aerial hyphal tufts, white color, rough texture.

Russian medium: entire petri plate covered, dense growth, white color, tufts of aerial hyphae.

Russian medium minus casein and tryptophan: entire petri plate covered, dense growth, white color, tufted aerial growth.

Russian medium minus vitamins: entire petri plate covered, no aerial hyphae, subsurface growth, moderate growth, rough texture.

Thioglycollate medium: diameter of colony 6.8 cm, white color, cottony growth, aerial hyphal tufts.

Table 23.--Continued.

Tomato juice agar special: entire petri plate covered, very dense growth, cottony, yellow to white color, hyphal growth out of plate, yellow droplets of exudate on surface.
Tryptic soy agar: diameter of colony 5 cm, white color, dense growth, rough texture, smooth margin, aerial hyphal tufts.
Violet-red bile agar: diameter of colony 4.8 cm, buff color, waxy to smooth texture, no aerial growth, irregular margin.

Table 24.--Colony morphology of Volvariella volvacea (V-63-135) grown on twenty agar media for one month.

Blood agar base: entire petri dish covered, cottony texture, white color, moderate aerial growth, poor surface growth.

Brewer anaerobic agar: entire petri dish covered, tan color, moderate growth, some aerial growth.

Chlamydospore agar: diameter of colony 3.2 cm, wispy aerial growth, poor surface growth, white to tan color, irregular margin, purple color of agar adjacent to colony changed to pink.

Corn meal agar: entire petri plate surface covered, many aerial hyphal tufts white to tan color, moderate growth.

Liver spleen glucose agar: entire petri plate surface covered, dense aerial growth filling plate, tan color, grew out of dish.

Micro assay culture agar: entire petri dish covered, dense surface growth, very dense aerial cottony development, tan color, grew out of dish.

Mycological agar: entire petri plate covered, dense aerial growth, tan color, cottony texture, grew out of plate.

Mycosel agar: very thin growth on agar surface.

Nutrient agar: entire petri plate covered, light tan color, moderately dense aerial growth.

Table 24.--Continued.

O. Modess agar: poor surface growth covering petri plate.

Potato dextrose agar: entire petri plate covered, moderate growth, some aerial hyphae, cream color, colony radiating from inoculation point.

Prune agar: entire petri dish covered, moderate growth over surface, fair development of aerial hyphae, tan color.

Rice extract agar: entire petri dish covered, moderate surface growth, several dense aerial areas, tan to white.

Russian medium: sparce surface growth over entire petri plate, aerial hyphal tufts, tan color, some subsurface hyphal growth.

Russian medium minus casein and tryptophan: sparce surface growth, some aerial tufts, tan to white color, some subsurface growth.

Russian medium minus vitamins: light growth over petri dish agar surface, greater submerged growth than surface development, some aerial hyphal strands.

Thioglycollate medium: entire petri dish surface covered, dense aerial hyphal growth, tan color, plate volume filled with hyphae.

Table 24.--Continued.

Tomato juice agar special: entire plate covered, very dense aerial development, tan color, hyphal growth out of plate.
Tryptic soy agar: entire petri dish covered, sparce growth, moderate aerial hyphae, tan color.
Violet-red bile agar: diameter of colony 6.1 cm, very dense surface growth, loose cottony texture, tan color, irregular margin.

Table 25.--Colony morphology of Volvariella volvacea mutant (V-63-134) grown on twenty agar media for one month.

Blood agar base: entire petri dish surface covered, white color, dense growth, matted cottony texture, somewhat aerial.

Brewer anaerobic agar: entire petri plate surface covered, moderately aerial growth, white to tan, matted cottony texture.

Chlamydospore agar: very thin mycelial growth over petri plate, white color, some aerial hyphae.

Corn meal agar: entire surface of petri plate covered, rough texture, aerial hyphal tufts, white to tan color.

Liver spleen glucose agar: entire petri plate covered, cream to white color, dense growth, matted cottony texture.

Micro assay culture agar: entire petri dish surface covered, very dense growth, aerial hyphae present, cottony texture, some fructification initials.

Mycological agar: entire petri dish surface covered, rough texture, white color, dense growth.

Mycosel agar: no growth.

Nutrient agar: thin hyphal growth over entire petri dish surface, white color, not aerial.

Table 25.--Continued.

O. Modess agar: sparce surface growth over entire plate, no aerial hyphae, tan color.

Potato dextrose agar: entire petri plate covered, moderate growth, some aerial hyphae, cream color.

Prune agar: diameter of colony 7.2 cm, moderate surface growth, granular texture, smooth margin, slightly cottony, cream color.

Rice extract agar: moderate growth over entire petri plate surface, some subsurface mycelium, some aerial tufts of hyphae, white color.

Russian medium: entire petri plate surface covered, white color, thin growth, some dense aerial hyphal growths.

Russian medium minus casein and tryptophan: entire petri dish surface covered, white color, thin growth, some aerial hyphal masses.

Russian medium minus vitamins: very thin hyphal growth over petri plate surface, white to cream color, no aerial hyphae.

Thioglycollate medium: entire petri dish surface covered, dense growth, tan color, matted cottony texture, some aerial hyphae.

Table 25.--Continued.

Tomato juice agar special: entire petri plate surface covered, matted cottony texture, cream color, some fructification initials.

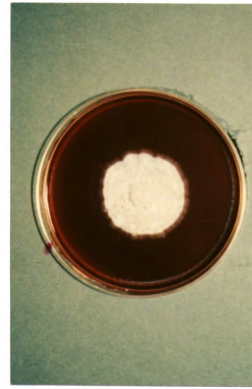
Tryptic soy agar: sparce hyphal growth over plate surface, white color, some aerial hyphal strands.

Violet-red bile agar: diameter of colony 6 cm, extremely dense growth, aerial mycelium, several fruiting initials, white color.

a.

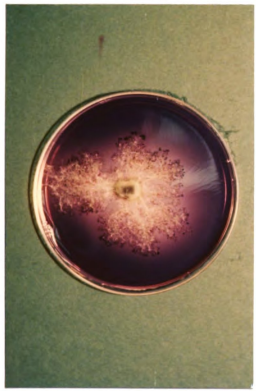
b.

c.



d.

Figure 26.--Cantharellus cibarius (C-63-86) growing on the following media: (a) Potato dextrose agar; (b) Mycological agar; (c) Micro assay culture agar; (d) Violet-red bile agar.



a.

b.

c.

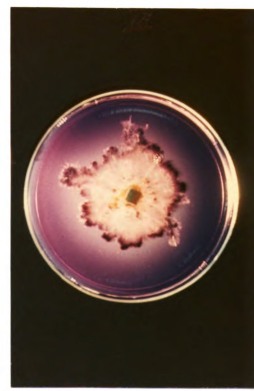
d.

Figure 27.--Lepista nuda (L-63-133) growing on the following media: (a) Chlamydospore agar; (b) Tomato juice agar special; (c) Violet-red bile agar; (d) Thioglycollate medium.

a.

b.

c.



d.

Figure 28.--Pleurotus ostreatus (P-63-51) growing on the following media: (a) Tomato juice agar special; (b) Brewer anaerobic agar; (c) Potato dextrose agar; (d) Chlamydospore agar.

a.

b.

c.

d.

Figure 29.--Volvariella volvacea (V-63-135) growing on the following media: (a) Mycological agar; (b) Violet-red bile agar; (c) Micro assay culture agar; (d) Prune agar.

No growth of C. clavatus (C-63-8) occurred on blood agar base, liver spleen glucose agar, or tryptic soy agar while the three strains of C. cibarius (C-63-15, C-63-86, and C-63-96) had growth. The C-63-96 strain was the only one of the four Cantharellus cultures to grow on mycosel agar. Cantharellus clavatus (C-63-8) did not grow on tryptic soy agar while all three C. cibarius strains did. Cantharellus clavatus (C-63-8) had the ability to cause color change from purple to pink in violet-red bile agar, the three C. cibarius strains did not. Only C. cibarius (C-63-15) did not produce an agar color change from blue to pink using chlamydospore agar, the three other Cantharellus strains did. All four strains had colony color differences when grown on violet-red bile agar but the morphology was similar. The three C. cibarius strains grew as dense cream to tan colored colonies with a smooth surface or margin on thioglycollate medium while C. clavatus (C-63-8) produced a dense cottony colony, brown to black in color.

Agaricus campestris (A-63-136), the wild strain, did not grow on blood agar base and tryptic soy agar while A. bisporus and its mutants had some growth. The colony of A-63-136 was waxy and smaller on brewer anaerobic agar, liver spleen glucose agar, O. Modess agar, and violet-red bile agar, compared to the larger cottony colonies of the three A. bisporus strains on the same media. The pleomorphic strain of A. bisporus, A-63-138 , grew more vigorously and

more densely on most all agar media compared to the parent strain, rhizomorphic strain, and A. campestris. The pleomorphic strain was less pigmented than the other strains. Agaricus campestris (A-63-136) had a brown colony circumference not found with the three A. bisporus strains on mycological agar. Less growth occurred with all four strains when casein and tryptophan or vitamins were eliminated from the Russian medium.

Lepista nuda (L-63-133) produced rhizomorphs on chlamydospore agar. Waxy colonies formed on micro assay culture agar and violet-red bile agar instead of the usual cottony textured colonies. Colony colors were violet to black on micro assay culture agar, white aerial growth occurred and a brown pigment diffused into the tryptic soy agar giving a reverse black colony surface. Tan or cream surface growth occurred and the reverse surface was brown on blood agar base and tomato juice agar special, while growth on most other agars was white.

Pleurotus ostreatus (P-63-51) changed the blue color of chlamydospore agar to pink adjacent to the colony. Numerous yellow exudate drops formed on the colony surface when grown on micro assay culture agar, mycological agar, and tomato juice agar special. Colony color was white to yellow on most of the 20 agars, colony texture also had little variation.

Volvariella volvacea (v-63-135) grew quite well on most of the agar media and produced dense aerial hyphae while growth of the mutant strain, V-63-134, was very much reduced. Mycosel agar supported no growth for the mutant but some growth for the parent. The parent was able to produce a color change from purple to pink with chlamydospore agar while the mutant did not. The tan color of aerial hyphae, indicating chlamydospores, was present with the parent strain on chlamydospore agar, liver spleen glucose agar, mycological agar, nutrient agar, prune agar, rice extract agar, Russian medium, tomato juice agar special, tryptic soy agar, and violet-red bile agar while the V. volvacea mutant (V-63-134) strain had a cream to white color and an absence of chlamydospores. Fructification initials occurred with the mutant strain on tomato juice agar special and violet-red bile agar while initials were absent with the parent strain.

DISCUSSION

The purpose of this work was to determine first the carbohydrate and nitrogen requirements of three wild mushrooms Lepista nuda, Cantharellus clavatus, and C. cibarius which are considered desirable for adaptation to commercial mushroom production. Volvariella volvacea, a mushroom grown commercially on rice straw in the Philippines, with little or no information on nutritional requirements, was also used in this study. A wild strain of Agaricus campestris and A. bisporus, the commercial mushroom, were also compared.

Additional investigations were made on the effect of other factors on the growth and fructification of these organisms including: growth hormones, vitamins, types of spawn media, temperature, and environmental conditions in a mushroom house.

Based on reports in the literature, a pH of 4.5 was selected for all growth studies in submerged culture. Urea was the nitrogen source in all liquid culture experiments except for studies in the utilization of nitrogen sources. Humfeld and Sugihara (109) found that the utilization of urea does not change the pH of the medium sufficiently to have an unfavorable effect on growth. In

media studies involving fungi, a high pH affected solubilities while acidity affects the entry into the cell of metabolites (41). Lilly and Barnett (143) kept the initial pH of media low to avoid isomerization of closely related monosaccharides such as glucose, fructose and mannose. Bohus (26) believes the hydrogen ion concentration on the medium determines what carbon and nitrogen sources will be at the disposal of the species, effects changes of colloids within the fungal cell and determines cell membrane permeability to the components of nitrogen source mixtures. Volvariella and Cantharellus species grew more favorably in acid media than alkaline, while Agaricus and Pleurotus species grew in only mildly acidic media. Lepista produced better mycelial growth in acid conditions in those experiments which lasted for shorter periods of time.

The pH changes in media during the course of the experiment were caused by fungal metabolites (142). Fungi are able to produce acids, or bases such as ammonia from deamination of amino acids and proteins, thus changing the environmental pH. The utilization of cations such as the ammonium ion tends to drive the pH down resulting in a more acid medium; utilization of nitrate ion or other anions increases the hydroxyl ion concentration to create a more basic medium.

Investigating the most useful carbon sources for mycelial growth of Cantharellus clavatus (C-63-8) showed that fructose-1,6- diphosphate produced the highest yields

followed by pectinol A, and pectin. The organic acids galacturonic and tannic acid were best suited for a 10 day period of hyphal growth, while succinic acid was best for 20 days incubation for C. clavatus.

All three strains of C. cibarius utilized fructose-1,6-diphosphate as the best carbon source. However, strains of C. cibarius had variation in selectivity of the remaining best carbon sources. Raffinose, a trisaccharide with one sucrose linkage to melibiose, is usually utilized readily by the majority of the fungi (Cochrane, 41); however, only C-63-86 strain readily utilized this carbon source. Strain C-63-96 had remarkable growth rates compared to the other C. cibarius strains, and also produced a water soluble yellow-green pigment. Extensive dry mycelial weights resulted from utilization of D-mannitol, and D-sorbitol. Many other carbon sources were readily utilized by the strain, indicating greater adaptability to growth under natural conditions.

The wild strain of Agaricus campestris (A-63-136) had greatest hyphal growth with D-fructose, followed by D-glucose, fructose-1,6-diphosphate and raffinose. Similarly, for the cultivated A. bisporus (A-63-137) parent and pleomorphic (A-63-138) strains, fructose-1,6-diphosphate, pectinol A, pectin, xylan, and D-cellobiose were excellent carbon sources. The utilization of D-cellobiose by the cultivated strain A-63-137 as well as the pleomorphic form A-63-138

indicates that both produced the required enzymes to separate the β glucosidic linkage in cellobiose. The rhizomorphic strain of A. bisporus, A-63-139, differed from the other two strains in that it could not utilize xylan, and also in that growth was much less with D-cellobiose and pectinol A. The utilization of pectin as a carbon source was previously reported by Treschow (273). He also reported that glucose, xylose, L-arabinose, and oxalate were good carbon sources. The rhizomorphic strain was similar to the parent and differed from the pleomorphic form by not utilizing well the monosaccharides D-glucose and D-mannose. The pleomorphic mutant, A-63-138, was similar to the parent A. bisporus (A-63-137) in the degree of utilization of organic acids, the rhizomorphic mutant, A-63-139, was more readily able to utilize oxalic and less capable of utilizing tannic acid.

Results obtained with the A. bisporus strain and and mutants agreed with those reported by Cochrane (41) in that glucose, fructose, galactose, xylose, L-arabinose, some mannitol, maltose, and sucrose were good carbon sources. Differences in carbon source usefulness reported by Garibova (81) indicated that race a utilized glucose, starch and maltose best, race b used glucose and starch, while race c used starch, glucose and xylose. Styler (266, 267) found with his A. bisporus strains that xylose, dextrose and maltose were the good carbon sources. Bohus (26) found that only those polysaccharides which give the proper monosaccharides

by hydrolysis were suitable carbon sources for the cultivated mushroom. He obtained best growth with his A. bisporus strains by using D-xylose, D-glucose, D-fructose, and hemicellulose, while organic acids and other carbon sources were poor. All sugars, starch, hemicellulose, cellulose and lignin were utilized by the A. bisporus strain selected by Lambert (133). As can be seen from the reports in the literature on carbon utilization by strains of A. bisporus, there is some variation in the results probably due to variations in strains and environmental conditions. This was evident in the variations in carbon utilization of the three strains studied.

Waksman (281) and Kliushnikova (124) found that A. campestris was able to utilize hemicellulose, but found differences in the usability of lignin. In an early work by Duggar (51), all carbon sources were poorly used due to the high osmotic concentrations reached in his experiments. Humfeld and Sugihara (107, 109) selected several carbon sources and obtained similar results with their A. campestris strain as did others. However, in these experiments, hexoses pentoses, disacchardes, soluble starch and dextrin were utilized by A. campestris. In the wild strain of A. campestris (A-63-136) similar results were found except that monosaccharides were the best carbon source.

Lepista nuda (L-63-133) (=Tricholoma personatum) had exceptionally good growth in media containing D-mannose, D-glucose, D-fructose, or galacturonic acid. Several

Tricholoma species selected by Melin (175) and Norkrans (201) also grew best with the same three sugars. Tricholoma fumosum (175) was reported as a strong cellulose decomposer while T. nudum (227) was not able to use cellulose. Other carbon sources such as starch, raffinose, and inulin varied greatly in their usefulness for different Tricholoma species. Tricholoma flavobrunneum (201) failed to utilize cellobiose but other species of the same genus grew well with it as the sole carbon source. In order of decreasing yield of dry mycelial weights for T. nudum, Reusser et al. (227) selected the following carbon sources: pyruvate, xylan, sucrose, maltose, D-mannose, raffinose, D-arabitol, L-rhamnose, D-galactose, D-fructose, D-xylose, dextrin, glucose, D-ribose, cellobiose, sorbitol, L-arabinose, starch, dulcitol, mannitol, inulin, salicin, lactose, L-sorbose, and cellulose.

Pleurotus ostreatus (P-63-51) developed approximately three times greater mycelial growth with fructose-1,6-diphosphate than with any other carbon source. D-fructose, D-sorbose, D-cellobiose and D-maltose were of some use to P. ostreatus while all other mono-, di-, and trisaccharides were of little importance. In comparison, Block, Tsao and Han (24) found corn steep liquor best, molasses moderate, glucose fair, and corn starch poor for mycelial growth of P. ostreatus. It is interesting that D-sorbose was utilized by P. ostreatus (P-63-51) as it is

not utilized by most fungi and is definitely toxic to some (Cochrane, 41).

The mono-, di-, and trisaccharides were of minor importance to both Volvariella volvacea (V-63-135) and the mutant strain V-63-134. Fructose-1,6-diphosphate was readily utilized by both strains. Glycogen and pectinol A were excellent carbon sources for the parent strain, while the mutant strain assimilated these poorly. A change in the ability to assimilate glycogen by the mutant strain indicates that this organism can not utilize starch readily. Fungi unable to use glycogen would not be able to utilize starch as insofar as is known the same enzymes attack both polysaccharides (Cochrane, 41). The parent produced better hyphal growth in media containing galacturonic, oxalic or succinic acids while the mutant strain could not utilize these particular acids. Succinic acid was not utilized by V. volvacea mutant (V-63-134).

The twelve test organisms I mentioned under "Materials and Methods" were grown in eight different carbon sources at various concentrations to determine if the carbon source in the basal medium had been a limiting factor. The Cantharellus species grew increasingly better with greater amounts of available carbon except for C. cibarius (C-63-15) in the presence of D-fructose. Agaricus bisporus pleomorph (A-63-138) was the only Agaricus strain able to grow best with all selected sugars at the highest

concentrations indicating the carbon source may still be a limiting factor in this case. The other three strains all were varied in utilization of the different sugars. Pleurotus ostreatus (P-63-51) grew at higher carbon levels slightly better than Lepista nuda (L-63-133).

Concerning carbon utilization, Perlman (213) listed three spheres of influence affecting the utilization of energy sources by fungi. They include the physical availability of carbon source to fungal cells; cultural conditions such as the presence of other nutrients in the media, aeration, incubation temperature, concentration of nutrients; and adaptation of the strain to the substrate. Fungal cells utilize disaccharides in one of two ways (41, 246): by saccharases splitting the compound molecule on the outer surface of the cell to monosaccharides which are then transported into the cell by carrier systems for monosaccharides, or by direct movement of disaccharides into the cell by specific membrane transport systems. Lilly and Barnett (142) found the availability of carbohydrates to depend upon the production of the necessary extracellular hydrolytic enzymes. Intermediate metabolic products should also serve as a source of carbon for the fungus. The composition, structure and configuration of organic compounds affect utilization, but the effects of these factors are different for different fungi (142). The metabolic pathways of carbohydrates and their components differ, depending upon the environmental

conditions and the fungus involved. The growth promoting effect of organic acids, added to the buffer capacity of media, act as chelating agents, and compensate for a deficiency in the cyclophorase system (78). Variation in carbon utilization by the twelve test organisms may be explained by some of these conditions just mentioned.

Growth curve studies of the twelve fungal strains and mutants at various concentrations of glucose produced interesting results. Cantharellus clavatus (C-63-8) at 25 g per liter had little mycelial growth indicating carbon was a limiting factor. Maximum growth occurred at 30 days with 75 g per liter, then autolysis followed. At 75 g per liter growth was the greatest for C. cibarius (C-63-15), the peak occurred during the first 10 days of growth, then autolysis occurred. In contrast, Cantharellus cibarius (C-63-86) developed the typical lag, log, peak and decline sigmoid phases at 25 g glucose per liter, but had little or no noticable lag phase at 50 or 75 g.

No typical growth curves formed with the wild strain of Agaricus campestris (A-63-136). At 25 g glucose per liter, an extended peak phase occurred before autolysis. A rapid growth increase developed during the first few days in 50 or 75 g glucose per liter, both concentrations had a slight increase in hyphal growth following a sharp decrease, probably due to autolysis. In contrast, extended sigmoid curves developed for A. bisporus (A-63-137) in 25 and 75 g

glucose per liter. At 25 g per liter, the glucose concentration was not sufficient for growth of the rhizomorphic mutant, but greatly increased hyphal growth for the pleomorphic form. A similar growth delay occurred for A. bisporus rhizomorph (A-63-139) at 50 g glucose per liter as was formed with the parent strain, but the extended lag phase was absent with the pleomorph mutant at this glucose concentration.

A sigmoid curve was produced at 75 g glucose per liter for Lepista nuda (L-63-133), but only the growth rate increase phase occurred between 10 and 40 days at 25 or 50 g glucose per liter. Pleurotus ostreatus (P-63-51) did not have enough carbonaceous material present at 25 g glucose per liter for sufficient growth, but at 75 g glucose per liter produced a sigmoid curve. For Volvariella volvacea at 75 g, the parent strain had autolysis occur at 20 days while the mutant began a steady growth increase at 20 days after a lag phase.

Several nitrogen sources were selected from ammonium and nitrate compounds, and amino acids to determine which is best suited for each of the twelve fungal strains and mutants. Peptone was by far the best source of nitrogen for hyphal growth of Cantharellus clavatus (C-63-8). Nitrates were not utilized and ammonium only in the form of ammonium bicarbonate was usable. Amino acids were good nitrogen sources, especially L-proline.

The three C. cibarius strains varied in their nitrogen utilization requirements as they did for carbon sources. Urea was better utilized by C. cibarius than C. clavatus. Inorganic nitrogen sources produced growth better than urea for the three C. cibarius strains. Peptone was a good nitrogen source and a number of the amino acids equaled or produced greater mycelial growth than urea. As with carbon sources, mycelial growth with various nitrogen sources for C-63-96 was several times greater compared to growth of the other C. cibarius strains.

On the basis of experimental condition, Agaricus campestris (A-63-136) readily utilized urea as a nitrogen source as well as ammonium citrate, ammonium tartrate, peptone and a number of amino acids. Less mycelial production occurred when arginine was used although Iwanoff and Toschewikowa (111) reported a marked mycelial growth increase occurred. Similarly, Humfeld and Sugihara (107, 109) found ammonium nitrogens, urea, peptone, amino acids and monosodium glutamate good nitrogen sources for A. campestris. In an early work by Duggar (51), A. campestris grew fairly well with asparagine, urea, peptone, casein and albumen. Koch (125) found that Psalliota (=Agaricus) campestris could utilize urea, arginine, asparagine, aspartic acid, ammonium tartrate and alanine which were some of the best nitrogen sources in these studies, however arginine was not as readily utilized by the strains studied.

Agaricus bisporus and the two mutants produced greatest mycelial dry weights when grown in peptone as the nitrogen source. Urea was a better nitrogen source for the parent strain than for either the pleomorphic or rhizomorphic mutant, and had greater growth than with any inorganic nitrogen source. The pleomorph form (A-63-138) excelled in hyphal growth with ammonium compounds, while ammonium tartrate was the only ammonium nitrogen source to equal urea for mycelial production with the rhizomorphic strain. Some of the amino acids were better than urea in mycelial production for the two mutant strains, A-63-138 (pleomorph), and A-63-139 (rhizomorph). Similar results reported by Styler (266, 267) indicated A. bisporus utilized ammonium salts, glycine, asparagine, peptone, proteins, and urea as good nitrogen sources.

According to Garibova (82), A. bisporus races can be separated according to preference for individual amino acid nitrogen sources: race a utilizing glycine, α alanine for race b, and asparagine for race c. Jennison and Perritt (117) found Psalliota (=Agaricus) campestris utilized the L-isomer (natural) of amino acids better or exclusively, while the D-isomer (unnatural) may actually be inhibitory. However, both isomers of proline, alanine and leucine can be utilized. In these studies, D-histidine was not usable as a nitrogen source by A. campestris and the three strains of A. bisporus. The A. bisporus strain studied by Fraser

(63) developed greater growth responses when amino acids were used in different combinations, but growth was promoted to some degree when phenylalanine, methionine, proline, tryptophane, and tyrosine were used singly.

Fungi utilizing nitrate nitrogen must be able to reduce the nitrogen to the oxidation level of ammonia (142). Ammonium salts or nitrates are suitable sources of nitrogen although with nitrates there may be a lag in growth before the necessary enzymes concerned are produced (192). Bohus (26) found that the source of nitrogen for A. bisporus is important for carbon utilization and the ammonium nitrogen utilization is modified by pH. The cation (NH_4^+) is the same in every ammonium compound; utilization depends on the anion. Media can become exceedingly acid if ammonium ions are greater than anion uptake. The rate of amino acid utilization is a specific character of strains. Treschow (273) reported Psalliota (=Agaricus) bispora f. avelanea was unable to use nitrate nitrogen, grew moderately in ammonium nitrogen, and grew best in amino acids. This is similar to the growth responses for the A. bisporus strains.

Both urea and peptone were good as nitrogen sources for Lepista nuda (L-63-133) (=Tricholoma personatum). Ammonium bicarbonate and glycine were excellent sources; other useful amino acids include DL-alanine, L-glutamic acid, and DL-methionine. Melin (175) found nitrate nitrogen was poor for eight Trichomoma species, but inorganic

and organic ammonium salts were easily used. Twenty-one amino acids, plus potassium nitrate, ammonium sulfate, ammonium nitrate, ammonium tartrate, ammonium phosphate, ammonium chloride, and urea were useful for T. nudum (Reusser, et al., 227). Falanghe et al. (57) found ammonium acetate, ammonium tartrate and ammonium sulfate in decreasing order of usefulness for T. nudum. Falanghe (56) increased growth of A. campestris, T. nudum, but not Cantharellus cibarius with vinasse, a fermentation product of beet sugar high in ammonium salts. Glutamic and aspartic acid along with their corresponding keto acids accelerated growth of seven Tricholoma species; T. gambosum and T. imbricatum were greatly stimulated by glutamine and asparagine (Norkrans, 202). Ammonium and organic nitrogen are assimilated by all Tricholoma species, nitrate nitrogen by T. nudum only, and various amino acids stimulate growth of different species (Norkrans, 201). Urea was the best nitrogen source for T. fumosum and T. vaccinum. The utilization of nitrogen sources by T. nudum is in general agreement with the results reported in the literature for T. nudum and other species of Lepista as urea was usually the best nitrogen source, followed by amino acids, then ammonium salts, and with the nitrate form a poor source.

Peptone and urea were the best nitrogen sources for Pleurotus ostreatus (P-63-51), closely followed by ammonium citrate, and DL-alanine. The other compounds including

amino acids were of little value as nitrogen sources. Tsao (274) and Block et al. (24) noted nitrate and ammonium salts produced poor growth with P. ostreatus while peptone and casamino acids were quite good for hyphal growth, which is similar to the results for nitrates and peptone for strain P-63-51. Koch (125) selected asparagine, glutamic acid, glycocoll, and alanine as nitrogen sources for a strain of the same species. Leonian and Lilly (138) found the following amino acids useful for P. ostreatus: D-arginine, L-aspartic acid, D-glutamic acid, glycine, DL-alanine, DL-isoleucine, β alanine, L-histidine, L-hydroxyproline, L-proline, L-tryosine, and DL-serine, while those not well utilized included L-tryptophane, DL-valine, and DL-leucine. Hacskaylo et al. (92) found asparagine best, ammonium nitrogen good, ammonium sulfate poor and nitrate nitrogen poor for growth of a strain of P. ostreatus. Pleurotus corticatus had better growth with several amino acids but could not utilize ammonium nitrate (138, 139, 262), a pattern that is followed by some of the P. ostreatus strains.

Several inorganic nitrogen sources better for hyphal growth than urea for Volvariella volvacea (V-63-135) and the mutant V-63-134 included: ammonium bicarbonate, ammonium citrate, ammonium tartrate, calcium nitrate, potassium nitrate and sodium nitrate. The mutant strain could utilize cobaltous ammonium sulfate as well as urea while the parent strain could not. Similarly, the parent

strain of V. volvacea could utilize ammonium salts better than the mutant. Peptone produced the greatest hyphal growth for both parent and mutant of any nitrogen source tested. Amino acids equivalent to urea for growth for the V-63-135 strain include: DL-alanine, L-asparagine, L-proline, and DL-tryptophane. The utilization of inorganic and organic nitrogen sources by this organism would account for the adaptability to the method of cultivation in the Philippines and other areas. Compounds utilized such as nitrates and amino acids are usually in plant material used in the compost for cultivation of these mushrooms.

The shorter carbon chain length amino acids generally were more readily utilized by the strains studied. Jennison, Newcomb and Henderson (116) concluded from their studies with amino acids with A. campestris that the shorter 3-4 carbon chain length--glycine, valine, α alanine--averaged more growth than the 5 or 6 carbon acids--leucine, isoleucine, and norleucine. The shorter chain acids containing an OH group--serine and threonine--were considerably poorer; those containing sulfur--cystine, cysteine, methionine--were even more poorly assimilated. The heterocyclic tryptophane, histidine, proline, and hydroxyproline followed no pattern. Jennison et al.(116, 117) listed the reasons for poor growth to be pH concentration, buffer action, redox potential, osmotic concentration, lack of minerals or vitamins, inadequate kinds and amounts of carbon or nitrogen, unfavorable

balance of constitutents, incubation time and synergistic action of organic acids. Changes in amino acid requirements may be induced by mutation in fungi but are uncommon in nature, when they occur naturally they are more often partial rather than absolute requirements (192). The variations in amino acid requirements between the parent and mutants may be due to mutation for A. bisporus and due to strain variation that occurred in nature for Pleurotus ostreatus. The many nutrilites present in malt or yeast extract (see Appendix) serve as good constituents of basal or stock culture media.

No vitamin heterotrophy was noted for Volvariella volvacea, Lepista nuda, Cantharellus cibarius or Pleurotus ostreatus under cultural conditions selected for the experiments. Hyphal length in mm was greater the first two days of growth for V. volvacea with 6 of the 10 vitamins added singly to the agar media, but then all growth thereafter equaled control. The other three fungal strains had no growth effects with vitamins. The vitamins act as coenzymes in fungal metabolism and may be synthesized or exogenously supplied by the organism (192). Absolute vitamin deficiencies in fungi are not known to be influenced by the environment, while conditioned deficiencies may be affected either by nutritional or physical environment factors such as temperature, composition and pH of the medium (142). Tsao (274), Kurancowa (129), and Block, et al. (24) found

thiamine increased hyphal growth of P. ostreatus, and Koch (125) added the vitamin to a medium for fructification of that species. No observed difference was noted with the addition of thiamine for P. ostreatus, however, the period of time was not sufficient to determine if fructification was affected. Lilly and Barnett (140) believed the vitamins to be autotrophic for P. ostreatus but it is entirely possible under other environmental conditions that a deficiency would evolve. Thiamine was found necessary for P. corticatus (138). Melin and Norkrans (178) used thiamine and pyrimidine to increase hyphal growth of Tricholoma albobrunneum. Melin et al. (177, 178) believed thiamine, pyrimidine or pyrimidine-thiazole heterotrophy existed for several Tricholoma species. Tricholoma imbricatum is partially heterotrophic for pantothenic acid, T. fumosum is partially deficient for nicotinic acid, T. fumosum grew equally well in thiazole and pyrimidine or thiamine while 4 other Tricholoma species grew better in thiazole + pyrimidine, pyrimidine, or thiamine (176). Thiamine, biotin, and inositol greatly increased growth of T. nudum (126).

Psalliota hortensis f. albida increased vegetative growth in media containing thiamine and biotin (Fraser, 62). Treschow (273) increased hyphal growth of Psalliota bispora f. avellanea also with thiamine and biotin. Fraser (63) found only thiamine increased hyphal growth of A. bisporus. Robbins and Kavanagh (244) saw no growth effect upon Agaricus

campestris with these vitamins. On the contrary, Hoffman and Hunt (103) increased mushroom yields of Agaricus bisporus with nicotinic acid, pantothenic acid, biotin, thiamine or pyridoxine added singly to the medium or with riboflavin + nicotionic acid + pantothenic acid, or thiamine + nicotinic acid in combination. There was no growth effect on the wild strain of Agaricus campestris, A. bisporus or the two mutants with vitamin B_1 in the experiments, corresponding to the results reported by Robbins and Kavanagh (244).

Growth hormones were of no significance to the 12 selected fungal strains at low concentrations while at high concentrations growth was inhibited apparently due to pH changes in the medium. Fraser (62) noted that Psalliota hortensis f. albida had a marked growth increase with IAA in the presence of thiamine and biotin. Heteroauxin is a growth inhibiting substance for Pleurotus corticatus (137), and 2,4-D increased germination and lengthened spore viability of P. ostreatus (129).

In selecting various seeds and other material as spawn media for growth of V. volvacea, L. nuda, C. cibarius, and P. ostreatus, different temperatures were selected to determine the best suited range for hyphal growth on rye grain. Optimal temperature for hyphal growth of the organisms were as follows: V. volvacea, 30-36°C; L. nuda, 18-30°C; C. cibarius, 18-30°C; P. ostreatus, 18-30°C. Except for

Volvariella at 36° C, growth was inhibited. Many plant seeds furnish essential nutrients for good spawn production of the four genera. When C. cibarius was grown on rye or spelt spawn media, immature fructifications occurred after three months. Immature basidiocarps formed for P. ostreatus within two to six weeks on several spawn media. Changes in the environment might trigger the maturation of the basidiocarps.

Mushroom house environmental conditions suited for production of Agaricus bisporus were unfavorable for basidiocarp development of the selected strains of Volvariella, Pleurotus, Cantharellus or Lepista. Only hyphal growth occurred. Additional variations in the conditions in the mushroom house, such as changes in temperature, modifications in compost, type of casing soil, light, and moisture are just some of the important factors that need further investigation with respect to the fructification problem.

SUMMARY

The ability of Cantharellus clavatus Fries, three strains of C. cibarius Fries, Lepista nuda (Bull. ex. Fries) Cooke, Pleurotus ostreatus (Jacq. ex. Fries) Kummer, Agaricus campestris L. ex. Fries, A. bisporus (Lange) Singer and pleomorphic and rhizomorphic mutants of A. bisporus, Volvaviella volvacea (Bull. ex. Fries) Singer and one mutant of V. volvacea to utilize various nutrilites was studied. Agaricus bisporus and Volvariella volvacea have been grown on a commercial basis. The others are considered as desirable species for cultivation by the mushroom industry. A pH of 4.5 was selected for the growth studies in submerged culture since several of the 12 species and mutants produced optimum growth at that pH level, at a temperature range of 18-30° C. Volvariella was able to grow up to 36° C.

For the carbon and nitrogen studies, fructose-1,6-diphosphate and peptone, respectively, were generally the best sources for mycelial growth of Cantharellus clavatus and three strains of C. cibarius. C. clavatus (C-63-8) produced optimum mycelial growth with pectinol A as the carbon source and L-proline for the nitrogen source. The sugar alcohols and amino acids were utilized best for C. clavatus. C. cibarius (C-63-15) grew well with pectinol A and L-proline.

Cantharellus cibarius (C-63-86) grew best with pectinol A as the carbon source and with urea as the nitrogen source. The third strain, C-63-96, was the only one to produce a water soluble yellow-green pigment in submerged culture. It also had much greater mycelial growth than other Cantharellus strains, and grew best with fructose-1,6-diphosphate and peptone.

Agaricus campestris (A-63-136) grew best with D-fructose and L-glutamic acid, while sugar alcohols and amino acids were good for mycelial growth. A. bisporus (A-63-137) best utilized xylan as the carbon source with peptone, urea, and L-proline as the best nitrogen sources. The polysaccharides and inorganic nitrogen sources were slightly better for mycelial production of strain A-63-137. The pleomorphic mutant, A-63-138, grew best with D-cellobiose and creatine. The rhizomorphic strain, A-63-139, utilized all carbon and nitrogen sources about equally well.

Lepista nuda (L-63-133) produced greatest mycelial growth when D-mannose was used as the carbon source and glycine was the nitrogen source. A number of amino acids were good nitrogen sources.

Pleurotus ostreatus (P-63-51) grew best with fructose-1,6-diphosphate and urea as the carbon and nitrogen sources. The polysaccharides were good carbon sources while a number of nitrogen sources were readily utilized.

Volvariella volvacea (V-63-135) produced maximum growth with fructose-1,6-diphosphate as the carbon source, and peptone as the nitrogen source. Other good carbohydrate sources were polysaccharides while amino acids and inorganic nitrogen compounds were equally good nitrogen sources. The mutant, V-63-134, of V. volvacea (V-63-135) grew best with the same carbon and nitrogen groups as did its parent, but less vigorously.

Growth curves were made for each organism grown over a period of 40 days in three different concentrations of glucose. In most strains used, 75 g of the carbohydrate source per liter was sufficient for maximum growth of the organism.

P-aminobenzoic acid, ascorbic acid, biotin, calcium pantothenate, niacin, and niacinamide produced an accelerated hyphal growth rate for V. volvacea for the first 4 days after inoculation; then growth leveled off to the control rate. The other organisms were not affected by the vitamins.

The growth hormones selected were of no significance to the test organisms. Several agar media were selected for growth of the 12 fungi, and morphology of these colonies was described.

Several spawn media were selected and utilized by V. volvacea (V-63-135), L. nuda (L-63-133), C. cibarius (C-63-86), and P. ostreatus (P-63-51). Immature fructifications occurred with C. cibarius (C-63-86) when grown on

rye or spelt spawn. No tissue differentiation developed within the fruiting-body. P. ostreatus (P-63-51) produced basidiocarps on several spawn media. The basidiocarps differentiated into modified stipes, and pilei with lamellae and spores. Light and electron microscopy identified dolipore septa in mycelium of Pleurotus, a simple pore in Cantharellus, crystals in both genera, and other cellular organelles in Volvariella and Lepista.

The same four fungal strains selected for spawn studies were subjected to growth conditions suitable for A. bisporus mushroom production on horse manure compost. No fructifications occurred. The mycelial mat of Lepista nuda which developed over the compost had a light blue coloration similar to normal basidiocarps. Pleurotus and Volvariella developed some hyphal growth but Cantharellus did not grow.

APPENDIX

The following stock culture media are those mentioned in the text but not identified as to contents. The accompanying number in parentheses refers to the bibliography.

Medium A (263):

glucose	15.00 g
sucrose	15.00 g
Bacto peptone	5.00 g
Bacto yeast extract	5.00 g
magnesium sulfate	0.50 g
potassium phosphate, di-H	1.00 g
potassium chloride	0.50 g
ferrous sulfate	0.01 g
agar	15.00 g
distilled water	1,000.00 ml

2% Malt agar (4):

malt extract	25.00 g
agar	15.00 g
distilled water	1,000.00 ml

The twelve strains were grown on the following agar media for culture identification.

Blood agar base (49):

infusion from heart muscle	375.0 g
tryptose	10.0 g
sodium chloride	5.0 g
agar	15.0 g
distilled water	1,000.0 ml

Brewer anaerobic agar (49):

Bacto yeast extract	5.0 g
Bacto tryptone	5.0 g
proteose peptone no. 3	10.0 g
sodium chloride	5.0 g
sodium thioglycollate	2.0 g
sodium formaldehyde sulfoxylate	1.0 g
resazurin	0.002 g
agar	20.0 g

Chlamydospore agar (49):

ammonium sulfate	1.0 g
monopotassium phosphate	1.0 g
biotin	5.0 mg
trypan blue	0.1 g
purified polysaccharide	20.0 g
agar	15.0 g
distilled water	1,000.0 ml

Corn meal agar (49):

corn meal infusion	50.0 g
agar	15.0 g
distilled water	1,000.0 g

Liver spleen glucose agar (49):

beef liver infusion	250.0 g
beef spleen infusion	250.0 g
proteose peptone	10.0 g
dextrose	10.0 g
agar	20.0 g
distilled water	1,000.0 ml

Micro assay culture agar (49):

Difco proteose peptone no. 3	5.0 g
Bacto yeast extract	20.0 g
Bacto dextrose	10.0 g
monopotassium phosphate	2.0 g
sorbitan monooleate complex	0.1 g
agar	10.0 g
distilled water	1,000.0 ml

Mycological agar (49):

Bacto soytone	10.0 g
Bacto dextrose	10.0 g
agar	15.0 g
distilled water	1,000.0 ml

Mycosel agar (49):

phytone	10.0 g
Bacto dextrose	10.0 g
actidione	0.4 g
chloromycetin	0.05 g
agar	15.5 g
distilled water	1,000.0 ml

Nutrient agar (49):

Bacto beef extract	3.0 g
Bacto peptone	5.0 g
agar	15.0 g
distilled water	1,000.0 ml

O. Modess agar (184):

potassium phosphate	0.5 g
magnesium sulfate	0.5 g
ammonium chloride	0.5 g
ferric chloride (1% sol)	10 drops
glucose	5.0 g
malt extract	5.0 g
agar	20.0 g
distilled water	1,000.0 ml

Potato dextrose agar (49):

infusion from potatoes	200.0 g
Bacto dextrose	20.0 g
agar	15.0 g
distilled water	1,000.0 g

Prune agar (49):

infusion from prunes	36.0 g
agar	15.0 g
distilled water	1,000.0 ml

Rice extract agar (49):

extract from white rice	20.0 g
agar	20.0 g
distilled water	1,000.0 ml

Russian medium (121):

ammonium nitrate	1.0 g
potassium phosphate	1.0 g
magnesium sulfate	0.5 g
calcium chloride	0.1 g
sodium chloride	0.1 g
casein hydrolysate with 0.025 g tryptophan	5.0 ml
ferrous chloride	0.6 mg
cupric sulfate	0.23 mg
sodium borate	0.045 mg
sodium molybdate	0.043 mg
manganese chloride	0.046 mg
zinc sulfide	4.3 mg
nicotinic acid	1.0 mg
para-amino-benzoic acid	1.0 mg
riboflavin	1.0 mg
ca-pantothenate	1.0 mg
biotin	1.0 mg
thiamine	1.0 mg
glucose	15.0 g
agar	20.0 g
distilled water	1,000.0 ml

Russian medium minus casein and tryptophan.

Russina medium minus vitamins.

Thioglycollate medium (49):

Bacto yeast extract	5.0 g
Bacto casitone	15.0 g
Bacto dextrose	5.0 g
L-cystine	0.25 g

sodium chloride	2.5 g
thioglycollic acid	0.3 mg
agar	0.75 g
distilled water	1,000.0 ml

Tomato juice agar special (49):

tomato juice (400 ml)	20.0 g
Bacto peptone	10.0 g
Bacto peptonized milk	10.0 g
agar	20.0 g
distilled water	1,000.0 ml

Tryptic soy agar (49):

Bacto tryptose	17.0 g
Bacto soytone	3.0 g
Bacto dextrose	2.5 g
sodium chloride	5.0 g
potassium phosphate	2.5 g
agar	15.0 ml
distilled water	1,000.0 ml

Violet-red bile agar (49):

Bacto peptone	10.0 g
Bacto lactose	10.0 g
Bacto bile salts	1.0 g
Bacto yeast extract	5.0 g
agar	15.0 g
neutral red	0.05 g
crystal violet	0.004 g
distilled water	1,000.0 ml

Components of Difco malt extract:

A dehydrated barley infusion containing diastase, dextrin, dextrose, protein and salts from barley.

Analysis of Bacto yeast extract,
furnished by Difco Laboratories, Detroit, Michigan:

Percent		
	ash	10.10
	total N	9.18
	chloride	0.19
	total sulphur	1.39
PPM		
	lead	16.00
	arsenic	0.11
	manganese	7.80
	zinc	88.00
	copper	19.00
Percent		
	phosphorus	0.89
	iron	0.028
	silicon dioxide	0.052
	potassium	0.042
	sodium	0.32
	magnesium	0.03
	calcium	0.0406
	arginine	0.78
	aspartic acid	5.1
	glutamic acid	6.5

Percent	
glycine	2.4
histidine	0.92
isoleucine	2.9
leucine	3.6
lysine	4.0
methionine	0.79
phenylalanine	2.2
threonine	3.4
tryptophane	0.88
tryosine	0.60
valine	3.4
Micrograms per gram	
pyridoxine	20.0
biotin	1.4
thiamine	3.2
nicotinic acid	279.0
riboflavine	19.0
folic acid	0.3

Analysis of Bacto peptone,

furnished by Difco Laboratories, Detroit, Michigan:

total nitrogen	16.16%
primary proteose N	0.06%
secondary proteose N	0.68%
peptone N	15.38%
ammonia N	0.04%
free amino N	3.20%
amide N	0.49%
mono-amino N	9.42%
di-amino N	4.07%
tryptophane	0.29%
tyrosine	0.98%

cystine	0.22%
organic sulphur	0.33%
inorganic sulphur	0.29%
phosphorus	0.22%
chlorine	0.27%
sodium	1.08%
potassium	0.22%
calcium	0.05%
magnesium	0.056%
manganese	nil
iron	0.0033%
ash	3.53%
lead	15.00 ppm
arsenic	0.09 ppm
zinc	18.00 ppm
copper	17.00 ppm
silicon dioxide	0.042%
arginine	8.00%
aspartic acid	5.90%
glutamic acid	11.00%
glycine	23.00%
histidine	0.96%
isoleucine	2.00%
leucine	3.50%
lysine	4.30%
methionine	0.83%
phenylalanine	2.30%
threonine	1.60%
valine	3.20%
pyridoxine	2.50 µ per g
biotin	0.32 µ per g
thiamine	0.50 µ per g
nicotinic acid	35.00 µ per g
riboflavine	4.00 µ per g

BIBLIOGRAPHY

1. Airth, R. L., and Foerster, G. E. 1965. Light emission from the luminous fungus Collybia velutipes under different nutrient conditions. Am. J. Bot. 52: 495-505.

2. Akai, S. 1940. The effect of light upon the formation of fruit-bodies in Collybia velutipes. Bot. Zool. 8:687.

3. Aleksandrov, F. A. 1964. Vliyanie gibberellina na rost i urozhai shampin' ona. (Effect of gibberellin on the growth and yield of field mushroom.) Bot. Zhurnal 49:1056-1057.

4. Alexopoulos, C. J., and Beneke, E. S. 1962. Laboratory Manual for Introductory Mycology. Burgess Publishing Co., Minneapolis, Minn. 199 p.

5. Alicbusan, R. V. 1963. Letter of correspondence.

6. Alicbusan, R. V., and Ela, V. M. 1961 Mushroom culture. Technical Bulletin 5. Univ. of the Philippines, College of Agriculture. 17 p.

7. Aschan, K. 1952. Studies on dediploidisation mycelia of the basidiomycete Collybia velutipes. Svensk Bot. Tidskr. 46:366-392.

8. Aschan, K. 1954. The production of fruit bodies of Collybia velutipes. I. Influence of different culture conditions. Physiol. Plant. 7:571-591.

9. Aschan-Akerg, K. 1958. The production of fruit bodies in Collybia velutipes. II. Further studies on the influence of different culture conditions. Physiol. Plant. 11:312-328.

10. Aschan-Akerg, K. 1960 (a). Studies on mono-and di-caryotic mycelia of Collybia velutipes. Physiol. Plant. 13:280-297.

11. Aschan-Akerg, K. 1960 (b). The production of fruit bodies in Collybia velutipes. III. Influence of the quality of light. Physiol. Plant. 13:276-297.

12. Atkins, F. C. 1961. Mushroom growing today. Faber and Faber Ltd., London. 186 p.

13. Bach, E. 1956. The agaric Pholiota aurea, physiology and ecology. Dansk Bot. Arkiv 16 (2):1-220.

14. Badcock, E. C. 1941. New methods for the cultivation of wood-rotting fungi. Trans. Brit. Mycol. Soc. 25:200-205.

15. Badcock, E. C. 1943. Methods for obtaining fructification of wood-rotting fungi in culture. Trans. Brit. Mycol. Soc. 26:127-132.

16. Baybliss, J. S. 1908. The biology of Polystictus versicolor (Fries). J. Ec. Biol. 3:1-24.

17. Bechmann, E. 1929. Untersuchungen über die Kulturfahigkeit des Champignons (Psalliota campestris). Ztschr. f. Bot. 22:289-323.

18. Berliner, M. D., and Duff, R. H. 1965. Ultrastructure of Armillaria mellea hyphae. Can. J. Bot. 43:171-172.

19. Bille-Hansen, E. 1953 (a). Fructification of coprophilous Coprinus on synthetic medium. Physiol. Plant. 6:523-528.

20. Bille-Hansen, E. 1953 (b). Fructification of three coprophilous species of Coprinus using glucose, sucrose, or maltose as carbon source. Bot. Tidsskr. 50:81-85.

21. Block, S. S. 1959. Developments in the production of mushroom mycelium in submerged culture. Mushroom Science 4:287-293.

22. Block, S. S. 1960. Developments in the production of mushroom mycelium in submerged culture. J. Biochem. Microbiol. Technol. 2:243-252.

23. Block, S. S., Stearns, T. W., Stephens, R. L., and McCandless, R. F. J. 1953. Mushroom mycelium. Experiments with submerged culture. J. Agr. Food Chem. 1:890-893.

24. Block, S. S., Tsao, G., and Han, L. 1959. Experiments in the cultivation of Pleurotus ostreatus. Mushroom Science 4:309-325.

25. Block, S. S., Tsao, G, Lunghwa, H. 1958. Production of mushrooms from sawdust. J. Agar. and Food Chem. 6:923-927.

26. Bohus, G. 1959. Investigations concerning the life processes of the cultivated mushroom. Mushroom Science 4:86-131.

27. Borriss, H. 1934. Über den einfluss Äusserer Faktoren auf Wachstum und Entwicklung der Fruchtkörper von Coprinus lagopus. Planta 22:644-684.

28. Borzini, G. 1949. Ricerche sullo Psalliota campestris Fr. e notizie sulla fungicoltura in Italy. Atti Ist. Bot. Univ. Lab. Crittogamico Pavia Suppl. Agli Atti Ser. 5:1-26.

29. Bouillenne-Walrand, M., and Evrard, L. 1950. Influence des hormones d'application ajoutées en diverses concentrations aux milieux de culture du mycelium de Agaricus hortensis (Cooke), f. albida Lange. Bull. Soc. Roy. Sci. Liége 12:539-545.

30. Bracker, Jr., C., and Butler, E. E. 1963. The ultrastructure and development of septa in hyphae of Rhizoctonia solani. Mycologia 55:35-58.

31. Bukowski, T. 1954. Kukmák pochvatý--Volvaria volvacea (Bull. ex Fr.) Quél. nová plevelná houba na pecárkovych záhonech. Ceská Mykologie 8:1-4.

32. Buller, A. H. R. 1905 (a). The reactions of the fruit-bodies of Lentinus lepideus Fr. to external stimuli. Ann. Bot. 19:427-438.

33. Buller, A. H. R. 1905 (b). The destruction of wooden paving blocks by the fungus Lentinus lepideus Fr. J. Ec. Biol. 1:1-13.

34. Buller, A. H. 1906. The enzumes of Polyporus squamosus Huds. Ann. Bot. 20:49-59.

35. Burkholder, P. R., and Moyer, D. 1943. Vitamin deficiencies of fifty yeasts and molds. Bull. Torrey Bot. Club 70:372-377.

36. Byrnum, H. H. 1965. Effect in incense-cedar heartwood extract on growth of Polyporus amarus. Mycologia 57:642-648.

37. Campbell, W. G. 1932. The chemistry of the white rots of wood. Biochem. J. 26:1829-1838.

38. Cartwright, K. St. G. 1932. Further notes on Basidiomycetes in culture. Trans. Brit. Mycol. Soc. 16: 304-307.

39. Cartwright, K. St. G., and Findley, W. P. K. 1934. Studies in the physiology of wood destroying fungi. Ann. Bot. 48:481-495.

40. Cauchon, R. 1963. Influence of slant orientations on fruiting in cultures of Hymenomycetes. Can. J. Bot. 41:1275-1276.

41. Cochrane, V. W. 1958. Physiology of Fungi. John Wiley and Sons, Inc., New York. 524 p.

42. Constantin, J. 1891. Étúde sur la culture des Basidiomycetes. Revue Général de Botanique 3:497-511.

43. Constantin, J. 1894. Sur la culture du Polyporus squamosus et sur son Hyphomyces. Bull. de la Société Mycologique de France 10:102-106.

44. Constantin, J., and Matruchot, L. 1898. Essai de culture du Tricholoma nudum. Compt. Rend. Acad. Sci., Paris. 126:853-856.

45. Cowling, E. B., and Kelman, A. 1964. Influence of temperature on growth of Fomes annosus isolates. Phytopath. 54:373-378.

46. Curl, E. A., and Arnold, M. M. 1964. Influence of substrate and microbial interactions on growth of Fomes annosus. Phytopath. 54:1486-1487.

47. DeBary, A. 1887. Comparative Morphology and Biology of Fungi, Mycetozoa and Bacteria. Oxford Univ. Press. 525 p.

48. DeGroot, R. C. 1965. Germination of basidiospores of Fomes pini on pine wood extract media. For. Sci. 11: 176-180.

49. Difco manual of dehydrated culture media and reagents for microbiological and clinical laboratory procedures, 9th Ed., Difco Laboratories, Inc., Detroit. 350 p.

50. Duggar, B. M. 1901. Physiological studies with reference to the germination of certain fungous spores. Bot. Gaz. 31:38-66.

51. Duggar, B. M. 1905. The principles of mushroom growing and mushroom spawn making. U.S.D.A. Bur. Pl. Ind. Bull. 85:1-60.

52. Eidam, E. 1875. Zurkenntniss der Befruchtung bei den Agaricus Arten. Bot. Zeitg. 33:649-670.

53. Etheridge, D. E. 1955. Comparative studies of North American and European cultures of the root rot fungus, Fomes annosus (Fr.) Cooke. Can. J. Bot. 33:416-428.

54. Etheridge, D. E. 1962. Selective action of fungus-inhibitory properties of balsam fir heartwood. Can. J. Bot. 40:1459-1462.

55. Etter, B. E. 1929. New media for developing sporophores of wood-rot fungi. Mycologia 21:197-203.

56. Falanghe, H. 1962. Production of mushroom mycelium as a protein and fat source in submerged culture in medium of vinnasse. Appl. Microbiol. 10: 572-576.

57. Falanghe, H., Smith, A. K, and Rackis, J. J. 1964. Production of fungal mycelial protein in submerged culture of soybean whey. Appl. Microbiol. 12: 330-334.

58. Ferguson, M. C. 1902. A preliminary study of the germination of the spores of Agaricus campestris and other basidiomycetous fungi. U.S.D.A. Bur. Pl. Ind. 16:1-40.

59. Findley, W. P. K. 1932. A study of Paxillus panuoides Fr. and its effects upon wood. Ann. Appl. Biol. 19:331-350.

60. Flexer, A. S. 1963. Requirements for fruiting in Polyporus palustris. Am. J. Bot. 50:625.

61. Foerster, G. E., Behrens, P. Q., and Airth, R. L. 1965. Bioluminescence and other characteristics of Collybia velutipes. Am. J. Bot. 52:487-495.

62. Fraser, I. M. 1953. The growth-promoting effect in indole-3-acetic acid on the common cultivated mushroom, Psalliota hortensis (Cooke) Lange f. albida Lange. Austral. J. Biol. Sci. 6:379-395.

63. Fraser, I. M., and Fujikawa, B. S. 1958. The growth effect of several amino acids on the common cultivated mushroom, Agaricus bisporus. Mycologia 50: 538-549.

64. Frear, D., Styer, J. F., and Haley, D. E. 1928. A study of the effect of H-ion concentrations of the growth of Agaricus campestris. Plant Physiol. 3:91-94.

65. Fries, L. 1946. Über das Wachstum einiger Coprinus-Arten bei verschiedenen Wasserstoffionenkonzentration. Arkiv f. Bot. 32 A, no 10:1-8.

66. Fries, L. 1948. Mutations induced in Coprinus fimetarius (L.) by nitrogen mustard. Nature 162: 846-847.

67. Fries, L. 1953. Factors promoting growth of Coprinus fimetarius (L.) under high temperature conditions. Physiol. Plant. 6:551-563.

68. Fries, L. 1955. Studies in the physiology of Coprinus. I. Growth substance, nitrogen and carbon requirements. Svensk Bot. Tidskr. 49:475-535.

69. Fries, L. 1956. Studies in the physiology of Coprinus. II. Influence of pH, metal factors and temperature. Svensk Bot. Tidskr. 50:47-96.

70. Fries, N. 1938. Über die Bedeutung von Wuchsstoffen für das Wachstum verschiedener Pilze. Symb. Bot. Upsal. 3(2):1-189.

71. Fries, N. 1942. Über die Sporenkeimung bei einigen Gasteromyceten und mykorrhizabildenden Hymenomyceten. Arch. Mikrobiol. 12:266-284.

72. Fries, N. 1943. Untersuchungen über Sporenkeimung und Mycelentwicklung bodenbewohnender Hymenomyceten. Symb. Bot. Upsal. 6:1-80.

73. Fries, N. 1949. Culture studies in the genus Mycena. Svensk Bot. Tidskr. 43:316-342.

74. Fries, N. 1950 (a). Precursors of tryptophan in the nutrition of Lentinus omphalodes Fr. and some other Hymenomycetes. Physiol. Plant. 3:185-196.

75. Fries, N. 1950 (b). Growth factor requirements of some higher fungi. Svensk Bot. Tidskr. 44:379-386.

76. Fries, N. 1951. Effect of certain nucleic acid constituents on the growth of some higher fungi. Nature 168:1045-1046.

77. Fries, N. 1954. The response of some Hymenomycetes to constituents of nucleic acid. Svensk Bot. Tidskr. 48:559-578.

78. Fries, N. 1961. Growth factors: Metabolic factors limiting growth. General introduction. Handbuch der Pflanzenphysiologic. 330-400.

79. Fries, N., and Aschan, K. 1952. The physiological heterogeneity of the dikaryotic mycelium of Polyporus abietinus investigated with the aid of micrurgical technique. Svensk Bot. Tidskr. 46: 429-445.

80. Galleymore, B. 1949. The development of fructifications of Lentinus tuber-regium Fries, in culture. Trans Brit. Mycol. Soc. 32:315-317.

81. Garibova, L. V. 1964 (a). Fiziologiya pitaniya kul' turnogo shampin'ona (Agaricus bisporus Lange). I. Uglerodnoe pitanie. Ref. ZH Biol. No. 12v63.

82. Garibova, L. V. 1964 (b). Fiziologiya pitaniya kul' turnogo shampin'ona (Agaricus bisporus Lange). II. Azotone pitanie. Ref. ZH Biol. No. 18v54.

83. Garren, K. H. 1938. Studies on Polyporus abietinus. II. The utilization of cellulose and lignin by the fungus. Phytopath. 28:875-878.

84. Garrett, S. D. 1953. Rhizomorph behavior in Armillaria mellea (Vahl) Quél. I. Factors controlling rhizomorph initiation by A. mellea in pure culture. Ann. Bot. N. S. 17:63-79.

85. Giesy, R. M., and Day, P. R. 1965. The septal pores of Coprinus lagopus in relation to nuclear migration. Am. J. Bot. 52:287-293.

86. Gilmore, K. A. 1926. Culture studies of Psilocybe coprophila. Bot. Gaz. 81:419-433.

87. Girbardt, M. 1961. Licht- und electronenmikroskopische Untersuchungen an Polystictus versicolor. II. Die Feinstruktur von Grundplasma und Mitochondrien. Archiv f. Mikrobiol. 39:351-359.

88. Glaser, T., and Sosna, Z. 1956. Comparative studies on Fomes annosus from pine, spruce, and birch grown in culture. Acta Soc. Bot. Poloniae 25: 285-303.

89. Good, H. M., and Spanis, W. 1958. Some factors affecting the germination of spores of Fomes ignarius var. populinus (Neuman) Campbell, and the significance of these factors in identification. Can. J. Bot. 36:416-436.

90. Gottlieb, S., Day, W. C., and Pelczar, Jr., M. J. 1950. The biological degradation of lignin. II. The adaptation of white-rot fungi to growth on lignin media. Phytopath. 40:926-935.

91. Hacskaylo, E., Palmer, J. G., and Vozzo, J. A. 1965. Effect of temperature of growth and respiration of ectotrophic mycorrhizal fungi. Mycologia 57: 748-756.

92. Hacskaylo, J., Lilly, V. G., and Barnett, H. L. 1954. Growth of fungi on three sources of nitrogen. Mycologia 46:691-701.

93. Hafiz, A., and Niederpruem, D. J. 1963. Studies on basidiospore germination in Schizophyllum commune. Am. J. Bot. 50:614-615.

94. Halbinger, R. E. 1954. Application of the submerged mycelium method to the cultivation of edible mushrooms. Rev. Argentina Agron. 21:162.

95. Hawker, L. E. 1936. The effect of certain accessory growth substances on the sporulation of Melanospora destruens and of some other fungi. Ann. Bot. 50:699-717.

96. Hawker, L. E. 1942. The effect of vitamin B_1 on the concentration of glucose optimal for the fruiting of certain fungi. Ann. Bot. 6:631-636.

97. Heim, R. 1947. Volvariella esculenta Massee. Bull. Soc. Mycol.Fr. 63:121-126.

98. Herrick, J. A. 1939 (a). The growth of Stereum gausapatum Fries in relation to temperature and acidity. Ohio J. Sci. 39:254-258.

99. Herrick, J. A. 1939 (b). Growth and variability of Stereum gausapatum in culture. Phytopath. 29: 504-511.

100. Herrick, J. A. 1940. The carbon and nitrogen metabolism of Stereum gausapatum Fries. Ohio J. Sci. 40:123-129.

101. Herrick, J. A., and Alexopoulos, C. J. 1942. A further note on the nitrogen metabolism of Stereum gausapatum Fries. Ohio J. Sci. 42:109-111.

102. Hobbs, H., and Niederpruem, D. J. 1963. Studies on homokaryotic fruiting in Schizophyllum commune. Am. J. Bot. 50:615.

103. Hoffman, I. C., and Hunt, C. H. 1958. Effect of supplementary B-complex vitamins on yields and vitamin content of mushrooms. Ohio Agar. Exp. Sta. Bull. 815:1-27.

104. Horowitz, N. H., and Beadle, G. W. 1943. A microbiological method for the determination of choline by use of a mutant of Neurospora. J. Biol. Chem. 150:325-333.

105. How, J. E. 1940. The mycorrhizal relations of larch. I. A study of Boletus elegans Schum. in pure culture. Ann. Bot. N. S. 4:135-150.

106. Humfeld, H. 1948. The production of mushroom mycelium (Agaricus campestris) in submerged culture. Science 107:373-375.

107. Humfeld, H. 1950. The production of mushroom mycelium. U.S.D.A. Yearbook of Agriculture. 242-245.

108. Humfeld, H., and Sugihara, T. F. 1949. Mushroom mycelium production by submerged culture. Food Tech. 3:355-356.

109. Humfeld, H., and Sugihara, T. F. 1952. The nutrient requirements of Agaricus campestris grown in submerged culture. Mycologia 44:605-620.

110. Humphrey, C. J., and Siggers, P. V. 1933. Temperature relations of wood-destroying fungi. J. Agr. Res. 47:997-1008.

111. Iwanoff, N. N., und Toschewikowa, A. 1927. Über zwei Arten von Harnstoffbildung bei Champignons. Biochem. Zeitschr. 181:1-7.

112. Jayko, L. G., Baker, T. I., Stubblefield, R. D., and Anderson, R. F. 1962. Nutrition and metabolic products of Lactarius species. Can. J. Microbiol. 8:361-371.

113. Jennison, M. W. 1948. The growth of wood-rotting fungi in submerged culture. Am. J. Bot. 35:801.

114. Jennison, M. W., and Derrick, M., and Henderson, R. 1949. Growth of wood-rotting fungi in synthetic media. Bact. Proceed. 1949:66-67.

115. Jennison, M. W., Derrick, M., and Henderson, R., 1950. Nutrition of wood-rotting fungi in chemically defined media. Bact. Proceed. 1950:38-39.

116. Jennison, M. W., Newcomb, M. D., and Henderson. R. 1955. Physiology of the wood-rotting Basidiomycetes. I. Growth and nutrition in submerged culture in synthetic media. Mycologia 47:275-304.

117. Jennison, M. W., and Perritt, A. M. 1960. Physiology of wood-rotting Basidiomycetes. III. Studies on the utilization of optical isomers of amino acids. Mycologia 52: 628-635.

118. Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill Book Co., Inc., New York. 523 p.

119. Johnson, G. T., and Jones, A. C. 1941. Data on the cultural characteristics of a species of Coprinus. Mycologia 33:424-433.

120. Kauffman, C. H. 1918. Agaricaceae of Michigan, Vol. I. Wynkoop Hallenbeck Crawford Co., Lansing, Mich. 924 p.

121. Khudiakov, I. P., and Vozniakovskaia, I. M. 1951. Chistye kul'tury mikoriznykh gribov. (Pure mycorrhizal cultures.). Mikrobiologiia 20:13-19.

122. Kimura, K. 1954. On the growth rate of mycelia in the Hymenomycetes. Biol. J. Okayama Univ. 2:7-12.

123. Kinugawa, K., and Furukawa, H. 1965. The fruit-body formation in Collybia velutipes induced by the lower temperature treatment of one short duration. Bot. Mag. Tokyo. 78:240-244.

124. Klioushnikova, E., Wiatkina, A., Wassilien, A., and Zukerman, P. 1935. Mushroom culture, kinds of manure, races of Psalliota campestris and spore germination. Wiss. Ber. Moskauer Statsuniversitat. 4:216-265.

125. Koch, W. 1958. Untersuchungen über Mycelwachstum und Fruchtkörperbildung bei einigen Basidiomycetin (Polystictus versicolor, Polyporus annosus, Pleurotus ostreatus, and Psalliota bispora). Arch. f. Mikrobiol. 30:409-432.

126. Kögl, F., und Fries, N. 1937. Über den Einfluss von Biotin, Aneurin und meso--Inosit auf das Wachstum verschiedenen Pilzarten. Zeitscher. f. Physiol. Chem. 249:93-110.

127. Kuhlman, E. G., and Hendrix, Jr., F. F. 1962. A selective medium for the isolation of Fomes annosus. Phytopath. 52:1310-1312.

128. Kulaeu, I. S., Kritskii, M. S., and Belozerskii, A. N. 1960. Metabolism of polyphosphates and of some other phosphorus compounds in the development of fruiting bodies of the mushroom Agaricus bisporus L. Biokhimiia 25:563-672.

129. Kurancowa, Z. 1963. Kielkowanie zarodnikow niektorych gatunkow grzybow nalezacych do Agaricales w zaleznosci od czasu przechowywania spor, pory, roku, wplywu kwasu 2,4-dwuchloro-fenoksy-octowego i tiaminy. (Germination of spores of some species of the Agaricales and its dependence on the duration of storage of the spores, the season of the year, and the action of 2,4-D and thiamine.) Ann. Univ. Mariae Curie-Sklodowska Sect. C. Biol. 17:433-451.

130. La Fuze, H. H. 1937. Nutritional characteristics of certain wood-destroying fungi, Polyporus belulinus Fr., Fomes pinicola (Fr.) Cooke, and Polystictus versicolor. Plant Physiol. 12:625-646.

131. Labourdette, M. 1861. Nourelle method de culture de l'agaric comestible; extrait d'une note de M. Labourdette. Compt. Rend. des Seances de l'Academie des Science 53:235.

132. Lambert, E. B. 1933. Effect of excess carbon dioxide on growing mushrooms. J. Agar. Res. 47:599-608.

133. Lambert, E. B. 1938. Principles and problems of mushroom culture. Bot. Rev. 4:397-426.

134. Lambert, E. B., and Ayers, T. T. 1950. Yield responses from supplementing mushroom manure. Mushroom Science 1:61-62.

135. Law, K. 1950. Phenol oxidases in some wood-rotting fungi. Ann. Bot. N. S. 14:69-78.

136. Law, K. 1955. Saccase and tyrosinase in some wood-rotting fungi. Ann. Bot. N. S. 19:561-570.

137. Leonian, L. H., and Lilly, V. G. 1937. Is hetero-auxin a growth-promoting substance? Am. J. Bot. 24:135-139.

138. Leonian, L. H., and Lilly, V. G. 1938. Studies on the nutrition of fungi. I. Thiamin, its constituents, and the source of nitrogen. Phytopath. 28: 531-548.

139. Leonian, L. H., and Lilly, V. G. 1940. Studies on the nutrition of fungi. IV. Factors influencing the growth of some thiamin-requiring fungi. Am. J. Bot. 27:18-26.

140. Lilly, V. G., and Barnett, H. L. 1947. Growth rates and vitamin deficiencies of various fungi. Proc. W. Va. Acad. Sci. 19:27-33.

141. Lilly. V. G., and Barnett, H. L. 1948. The inheritance of partial thiamine deficiency in Lenzites trabea. J. Agar. Res. 77:287-300.

142. Lilly, V. G., and Barnett, H. L. 1951. Physiology of the fungi. McGraw-Hill Book Co., Inc., New York. 464 p.

143. Lilly, V. G., and Barnett, H. L. 1953. The utilization of sugars by fungi. W. Va. Univ. Agar. Expt. Sta. Bull. 262:1-58.

144. Lilly, V. G., and Barnett, H. L. 1956. The utilization of D- and L-arabinose by fungi. Am. J. Bot. 43: 709-714.

145. Lindeberg, G. 1939 (a). Über das Wuchsstoffbedürfnis verschiedener Arten der Pilzgattung Marasmius. Svensk Bot. Tidskr. 33:85-90.

146. Lindeberg, G. 1939 (b). Über den Einfluss der Wasserstoffionenkonzentration auf das Wachstum verschiedener Marasmius-Arten. Svensk Bot. Tidskr. 33: 341-346.

147. Lindeberg, G. 1941. Über die Wirkung von Biotin auf Marasmius androsaceus (L.) Fr. Arch. F. Mikrobiol. 12:58-62.

148. Lindeberg, G. 1944. Uber die Physiologie Ligninabbauender Boden-Hymenomyzeten. Studien an Schwedischen Marasmius-Arten. Symb. Bot. Upsal. 8(2):1-183.

149. Lindeberg, G. 1946 (a). Thiamin and growth of litter-decomposing Hymenomycetes. Botan. Notiser 1946: 89-93.

150. Lindeberg, G. 1946 (b). The effect of biotin and thiamin on the growth of Collybia dryophila Fr. Svensk Bot. Tidskr. 40:63-69.

151. Lindeberg, G., and Korjus, M. 1949. Gallic acid and growth of Marasmius foetidus. Physiol. Plant. 2:103-113.

152. Lindeberg, G., and Lindeberg, M. 1964. The effect of pyridoxine and amino acids on the growth of Marasmius perforans Fr. Arch. Mikrobiol. 49: 86-95.

153. Lindgren, R. M. 1933. Decay of wood and growth of some Hymenomycetes as affected by temperature. Phytopath. 23:73-81.

154. Lombard, F. F., and Lowe, J. L. 1960. Cultural characteristics of Fomes ulmarius and Poria ambigua. Mycologia 52:280-294.

155. Long, T. J. 1962. The effects of chloramphenicol and 8-azaguanine on normal and abnormal sporocarp development in the mushroom Collybia velutipes. Am. J. Bot. 49:655.

156. Long, W. H., and Harsch, R. M. 1918. Pure cultures of wood-rotting fungi on artificial media. J. Agar. Res. 12:33-82.

157. Lu, B. C. 1965. The role of light in fructification of the Basidiomycete Cyathus stercoreus. Am. J. Bot. 52:432-437.

158. Luft, J. H. 1956. Permanganate--A new fixative for electron microscopy. J. Biophys. and Biochem. Cytology 2:799-800.

159. Luft, J. H. 1961. Improvements in epoxy resin embidding methods. J. Biophys. and Biochem. Cytology 9:409-414.

160. Lutz, M. L. 1925. Sur la culture des champignons Hyménomycètes en milieu artificiel. Compt. Rend. Acad. Sci., Paris. 180:532-534.

161. Lyman, G. R. 1907. Culture studies on polymorphism of Hymenomycetes. Proc. Boston Soc. Nat. Hist. 33:125-209.

162. McIlvaine, C. 1900. One thousand American fungi. Bowen-Merril Co., Indianapolis. 704 p.

163. McKay, H. H. 1959. Cultural basis for maintaining Polyporus cinnabarinus and Polyporus sanguineus as two distinct species. Mycologia 51:465-473.

164. McLaughlin, D. J. 1964. Production of fruitbodies of Suillus rubinellus in pure culture. Mycologia 56:136-138.

165. Madelin, M. F. 1956 (a). Studies on the nutrition of Coprinus lagopus Fr., especially as affecting fruiting. Ann. Bot. N.S. 20:307-330.

166. Madelin, M. F. 1956 (b). The influence of light and temperature on fruiting of Coprinus lagopus Fr. in pure culture. Ann. Bot. N.S. 20:467-480.

167. Mader, E. O. 1943. Some factors inhibiting the fructification and production of the cultivated mushroom, Agaricus campestris L. Phytopath. 33:1134-1145.

168. Manocha, M. S. 1965. Fine structure of the Agaricus carpophore. Can. J. Bot. 43:1329-1334.

169. Marczynzski, Bro. R. 1943. Studies on the nutrition of Collybia velutipes (Curt.) Quel. (Homobasidiomycetes, Agaricales). Am. Midl. Natur. 30:164-170.

170. Mathew, K. T. 1952. Growth-factor requirements of Pellicularia koleroga Cooke in pure culture. Nature 170:889-890.

171. Meacham, M. R. 1918. Notes upon the hydrogen-ion concentration necessary to inhibit growth of four wood-destroying fungi. Science N.S. 48:499-500.

172. Melin, E. 1921. Über die Mykorrhizenpilze von Pinus silvestris L. und Picea abies (L.) Karst. Svensk Bot. Tidskr. 15:192-203.

173. Melin, E. 1922. Boletus-Arten als Mykorrhizenpilze der Waldbäume. Deut. Botan. Gesell. Berlin. 40:94-97.

174. Melin, E. 1946. Der Einfluss von Waldstreuextrakten auf das Wachstum von Bodenpilzen, mit Besonderer Berüksichtigung der Wurzelpilze von Baümen. Symb. Bot. Upsal. 8(3):1-116.

175. Melin, E. 1953. Physiology of mycorrhizal relations in plants. Ann. Rev. Plant Physiol. 4:325-346.

176. Melin, E. 1954. Growth factor requirements of mycorrhizal fungi of forest trees. Svensk Bot. Tidskr. 48:86-94.

177. Melin, E., und Lindeberg, G. 1939. Über den einfluss von Aneurin und Biotin auf das Wachstum einiger Mykorrhizenpilze. Botan. Notiser 1939:241-245.

178. Melin, E., und Norkrans, B. 1942. Über den Einfluss der Pyrimidin-und der Thizaolkomponente des Aneurins auf das Wachstum von Wurzelpilzen. Svensk Bot. Tidskr. 36:271-286.

179. Melin, E., and Norkrans, B. 1948. Amino acids and the growth of Lactarius deliciosus (L.) Fr. Physiol. Plant. 1:176-184.

180. Melin, E., und Nyman, B. 1940. Weitere Untersuchungen über die Wirkung von Aneurin und Biotin auf das Wachstum von Wurzelpilzen. Arch. f. Mikrobiol. 11:318-328.

181. Melin, E., und Nyman, B. 1941. Über das Wuchsstoffbedürfnis von Boletus granulatus (L.) Fr. Arch. f. Mikrobiol. 12:254-259.

182. Melin, E., and Rama Das, V. S. 1954. Influence of root-metabolites on the growth of tree mycorrhizal fungi. Physiol. Plant. 7:851-858.

183. Mikola, P. 1955. Metsämaan kantasienien puhdasviljely. (Growing forest soil Basidiomycetes in pure culture.) Karstenia 3:5-16.

184. Modess, O. 1941. Zur Kenntnis der Mykorrhizabildner von Kiefer und Fichte. Symb. Bot. Upsal. 5(1): 1-147.

185. Möller, F. H. 1949. Danish Psalliota species. Friesia 4:1-71.

186. Möller, F. H. 1962. Agaricus (Psalliota) leucotrichus Möll. I. Kultur. Friesia 7:60-68.

187. Moore, R. T. and McAlear, J. H. 1962. Fine structure of mycota. 7. Observations on septa of Ascomycetes and Basidiomycetes. Am. J. Bot. 49:86-94.

188. Moore, R. T. and McAlear, J. H. 1963. Fine structure of mycota. Fungal mitochondria. J. Ultrast. Res. 8:144-153.

189. Mounce, I. 1920. Homothallism and the production of fruit-bodies by monosporous mycelia in the genus Coprinus. Trans. Brit. Mycol. Soc. 7:198-217.

190. Mounce, I. 1922. The production of fruit-bodies of Coprinus comatus in laboratory cultures. Trans. Brit. Mycol. Soc. 8:221-226.

191. Negrutskii, S. F. 1963. The effect of vitamins B_1 and H and gibberellin on the growth of Fomitopsis annosa. Nauchn. Dokl. Vysshei Shkoly Biol. Nauki 2:144-147.

192. Nicholas, D. J. D. 1965. Utilization of inorganic nitrogen compounds and amino acids by fungi. p. 349-376. In A. S. Sussman, and G. C. Ainsworth (Ed.). The Fungi, Vol. I.

193. Niederpruem, D. 1962. Physiological studies on morphogenesis of Schizophyllum commune. Bact. Proceed. p. 61.

194. Niederpruem, D. J. 1963. Role of carbon dioxide in the control of fruiting of Schizophyllum commune. J. Bacteriol. 85:1300-1308.

195. Niederpruem, D. and Hobbs, H. 1963. Nutritional studies on morphogenesis of Schizophyllum commune. Bact. Proceed. p. 32.

196. Niederpruem, D. J., Hobbs, H., and Henry, L. 1964. Nutritional studies of development in Schizophyllum commune. J. Bacteriol. 88:1721-1729.

197. Nilsson, H. 1956. The growth-stimulating effect of fructose-1, 6-diphosphate on Boletus variegatus and Collybia velutipes. Physiol. Plant. 9:74-81.

198. Nishikado, Y. and Miyawaki, Y. 1943. Principles of artificial propagation of Pleurotus ostreatus, Hiratake mushroom. (Japanese). Rept. Ohara Agric. Inst. 35:481-484.

199. Noecker, N. L. 1938. Vitamin B_1 in the nutrition of four species of wood-destroying fungi. Am. J. Bot. 25:345-348.

200. Noecker, N. L. and Reed, M. 1943. Observations on the vitamin requirements of Stereum frustulosum (Pers.) Fr. Am. Midl. Natur. 30:171-174.

201. Norkrans, B. 1950. Studies in growth and cellulolytic enzymes of Tricholoma with special reference to mycorrhiza formation. Symb. Bot. Upsal. 11(1): 1-126.

202. Norkrans, B. 1953. The effect of glutamic acid, aspartic acid, and related compounds on the growth of certain Tricholoma species. Physiol. Plant. 6:584-593.

203. Oddoux, L. 1953. Essai de culture de 508 especes d'homobasidiomycetes. Mushroom Science 2:28-39.

204. Osborne, L. D. and Thrower, L. B. 1964. Thiamine requirements of some wood-rotting fungi and its relation to natural durability of timber. Trans. Brit. Mycol. Soc. 47:601-611.

205. Pacifici, L. R., Blomster, R. N., Ludowski, H. J., and Schwarting, A. E. 1960. Preliminary growth and nutrition studies of Amanita muscaria in submerged culture. Lloydia 23:43-50.

206. Pantidou, M. E. 1961. Carpophores of Phlebopus sulphureus in culture. Can. J. Bot. 39:1163-1167.

207. Pantidou, M. E. 1962. Cultural studies of Boletaceae carpophores of Phlebopus lignicola in culture. Can. J. Bot. 40:1313-1319.

208. Pantidou, M. E. 1964. Cultural studies of Boletaceae: Carpophores of Xerocomus badius and Xerocomus illudens in culture. Can. J. Bot. 42:1147-1149.

209. Papazian, H. P. 1950. A convenient method of growing fruiting-bodies of a bracket fungus. Bot. Gaz. 112:138.

210. Passecker, F. 1959. Kulturversuche mit Wildformen des Champignons und anderen Agaricaceen. Mushroom Science 4:476-483.

211. Pease, D. C. 1960. Histological techniques for electron microscopy. Academic Press, New York. 274 p.

212. Perlman, D. 1949. Studies on the growth and metabolism of Polyporus anceps in submerged culture. Am. J. Bot. 36:181-184.

213. Perlman, D. 1965. Chemical environment for fungal growth. Carbon sources. p. 479-488. In A. S. Sussman, and G. C. Ainsworth (Ed.). The Fungi, Vol. I.

214. Peterson, R. H. 1960. Observations on Coprinus ephemerus. Mycologia 52:513.

215. Plunkett, B. E. 1953. Nutritional and other aspects of fruit-body production in pure culture of Collybia velutipes (Curt.) Fr. Ann. Bot. N. S. 17:193-217.

216. Plunkett, B. E. 1956. The influence of factors of the aeration complex and light upon fruit-body form in pure culture of an agaric and a polypore. Ann. Bot. N. S. 20:563-586.

217. Plunkett, B. E. 1961. The change of tropism in Polyporus brumalis stipes and the effect of directional stimuli on pileus differentiation. Ann. Bot. N. S. 25:206-223.

218. Pomerleau, R. 1951. Mushrooms of Eastern Canada and the United States. Les Editions Chantecler Ltée, Montreal. 302 p.

219. Price, J. E., Levintow, L., Greenstein, L. P., Kingsley, R. B. 1950. The enzymatic hydrolysis of L- and D-amino acid amides by mushroom preparations. Arch. Biochem. 26:92-96.

220. Raper, J. R., and Krongelb, G. S. 1958. Genetic and environmental aspects of fruiting in Schizophyllum commune Fr. Mycologia 50:707-740.

221. Rast, D. 1965. Zur stoffwechselphysiologischen Bedeutung von Mannit und Trehalose in Agaricus bisporus. Eine gaschromatographische Studie. Planta 64:81-93.

222. Raulin, J. 1869. Etudes chimiques sur la vegetation. Ann. Sci. Nat., Ser. V. 11:93-229.

223. Reese, M. 1875. Über den Befruchtungsvorgang bei den Basidiomyceten. Jahrb. f. Wiss. Bot. 10:179-198.

224. Reeve. E. 1952. Influence of vitamin supplements on mushroom yields. Proc. Amer. Soc. Hort. Sci. 59:367-371.

225. Reitsma, J. 1932. Studien über Armillaria mellea (Vahl) Quel. Phytopath. Zeitschr. 4:461-522.

226. Rennerfelt, E. 1944. Die Entwicklung von Fomes annosus Fr. bei Zusatz von Aneurin und Verschiedenen Extrakten. Svensk Bot. Tidskr. 38:153-162.

227. Reusser, F., Spencer, J. F. T., and Sallans, H. R. 1958. Tricholoma nudum as a source of microbiological protein. Appl. Microbiol. 6:5-8.

228. Ricci, I. 1963. Spore germination and the development of Pleurotus ostreatus Jacq. on dead receptacles of that species. Giorn. Bot. Ital. 69:384-386.

229. Richards, A. A. 1960. Modern mushroom cultivation. W. H. & L. Collingridge Ltd., London. 96 p.

230. Richards, R. R. 1949. Responses of representative fungi to certain growth-regulating substances. Bot. Gaz. 110:523-550.

231. Riggenbach, A. 1957. Fomes lignosus, a pyrimidine-deficient fungus. Nature 180:43-44.

232. Riggenbach, A. 1958. Fomes noxius, a thiamine-deficient fungus. Nature 182:1390-1391.

233. Robak, H. 1933. On the growth of three wood-destroying Polyporeae in relation to the hydrogen-ion concentration of the substratum. Svensk Bot. Tidskr. 27:56-76.

234. Robak, H. 1942. Cultural studies in some Norwegian wood-destroying fungi. Medd. Vestl. Forstl. Forsöksstation, Bergen. 25:1.

235. Robbins, W. J. 1950. A survey of the growth requirements of some Basidiomycetes. Mycologia 42: 470-476.

236. Robbins, W. J., and Hervey, A. 1955. Growth substance requirements of Stereum murraii. Mycologia 47: 155-162.

237. Robbins, W. J., and Hervey, A. 1958. Wood, tomato and malt extracts and growth of some Basidiomycetes. Mycologia 50:745-752.

238. Robbins, W. J., and Hervey, A. 1959. Light, growth, and reproduction of Poria ambigua. Science 129:1288.

239. Robbins, W. J., and Hervey, A. 1960 (a). Growth substances for Polyporus schweinitzii. Mycologia 52:956-957.

240. Robbins, W. J., and Hervey, A. 1960 (b). Light and the development of Poria ambigua. Mycologia 52:231-247.

241. Robbins, W. J., and Hervey, A. 1963. Unidentified filtrate growth substances for several fungi. Mycologia 55:59-64.

242. Robbins, W. J., and Hervey, A. 1965. Response of eight Basidiomycetes to ferulic acid. Bull. Torrey Bot. Club 92:266-269.

243. Robbins, W. J., Hervey, A., Page, A. C., Gale, P. H., Hoffman, C. H., Moscatelli, E. A., Koniuszy, F. R., Smith, M. C., and Folkers, K. 1963. Growth factors for Polyporus schweinitzii. The identification of ferulic acid as a new cofactor. Mycologia 55:742-757.

244. Robbins, W. J., and Kavanagh, F. 1938. Vitamin B_1 or its intermediates and growth of certain fungi. Am. J. Bot. 25:229-236.

245. Robbins, W. J., and Kavanagh, F. 1942. Vitamin deficiencies of the filamentous fungi. Bot. Rev. 8:411-471.

246. Rothstein, A. 1965. Uptake and translocation. p. 429-456. In A. S. Sussman, and G. C. Ainsworth, Ed. The Fungi, Vol. I.

247. Rumbold, C. 1908. Beiträge zur Kenntnis der Biologie holzzerstörender Pilze. Naturwiss. Zeitschr. f. Forst. 6:81-140.

248. Santoro, T., and Casida, L. E., Jr. 1962. Growth inhibition of mycorrhizal fungi by gibberellin. Mycologia 54:70-71.

249. Sass, J. E. 1961. Botanical Microtechnique, 3rd Ed. Iowa State University Press, Ames, Iowa. 228 p.

250. Scheler-Correns, E. 1957. Über die Fruchtkorperbildung von Coprinus lagopus bei verschiedenen Stickstoffquellen. Arch. f. Mikrobiol. 26:52-54.

251. Schmitz, H. 1919. Studies in the physiology of the fungi. VI. The relation of bacteria to cellulose fermentation induced by fungi, with special reference to the decay of wood. Ann. Mo. Bot. Gard. 6:93-136.

252. Schmitz, H., and Zeller, S. M. 1919. Studies in the physiology of the fungi. IX. Enzyme action in Armillaria mellea Vahl., Daedalea confragosa (Bolt.) Fr., and Polyporus lucidus (Leys.) Fr. Ann. Mo. Bot. Gard. 6:193-200.

253. Schneiderhöhn, G. 1954. Das Aktionsspecktrum der Wachstumsbeeinflussung durch Licht bei Coprinus lagopus. Arch. f. Mikrobiol. 21:230-236.

254. Schopfer, W. H., und Blumer, S. 1940. Recherches sur la repartition de l'heterotrophie par rapport á l'aneurine chez les champignons. Archiv f. Mikrobiol. 11:205-214.

255. Shaffer, R. L. 1957. Volvariella in North America. Mycologia 49:545-579.

256. Sims, Jr., A. C. 1960. Effect of culture substrate on the virulence of single basidiospore isolates of Pellicularia filamentosa. Phytopath. 50:282-286.

257. Singer, R. 1961. Mushrooms and Truffles. Interscience Publishers, Inc., New York. 272 p.

258. Singer, R. 1962. The Agaricales in modern taxonomy, 2nd Ed. J. Cramer Pub., Weinheim, Germany. 915 p.

259. Smith, A. H. 1963. The mushroom hunter's field guide, revised and enlarged. Univ. of Mich. Press, Ann Arbor. 264 p.

260. Smith, A. H., and Morse, E. E. 1947. The genus Cantharellus in the Western United States. Mycologia 39:497-534.

261. Spaulding, P. 1905. Cultures of wood-inhabiting fungi. Science 21:143-144.

262. Steinberg, R. A. 1939. Growth of fungi in synthetic nutrient solutions. Bot. Rev. 5:327-350.

263. Stevens, J. A. 1957. Studies of environmental factors influencing in vitro growth of Basidiomycetes and their elaboration of biologically active substances. Ph.D. thesis, Mich. State Univ.

264. Stoller, B. B. 1954. Principles and practice of mushroom culture. Econ. Bot. 8:48-95.

265. Streeter, S. G. 1909. The influence of gravity on the direction of growth of Amanita. Bot. Gaz. 48: 414-426.

266. Styler, J. F. 1928. Preliminary study of the nutrition of the cultivated mushroom. Am. J. Bot. 15:246-250.

267. Styler, J. F. 1930. Nutrition of the cultivated mushroom. Am. J. Bot. 17:983-994.

268. Sugihara, T. F., and Humfeld, H. 1954. Submerged culture of the mycelium of various species of mushroom. Appl. Microbiol. 2:170-172.

269. Swack, N. S., and Miles, P. G. 1960. Conditions affecting growth and indigotin production by strain 130 of Schizophyllum commune. Mycologia 52:574-583.

270. Teixeira. A. R. 1960. Characteristics of the generative hyphae of Polypores of North America, with special reference to the presence or absence of clamp connections. Mycologia 52:30-39.

271. Terakawa, H. 1956. Studies on the morphogenesis in Pleurotus ostreatus. Sci. Papers Coll. Gen. Ed. Univ. Tokyo. Biol. 6:61-87.

272. Tilford, P. E. 1936. The relation of temperature to the effect of hydrogen- and hydroxyl-ion concentration on Sclerotinia fructicola and Fomes annosus. Spore germination and growth. Ohio Agr. Exp. Sta. Bull. 567:1-27.

273. Treschow, C. 1944. Nutrition of the cultivated mushroom. Dansk Botan. Arkiv. 11(6):1-180.

274. Tsao, G. T. 1963. Production of oxalic acid by a wood-rotting fungus. Appl. Microbiol. 11:249-255.

275. VanTieghem, P. 1875. Sur la fécondation des Basidiomycètes. Compt. Rend. de l'acad. d. Sci. Paris.

276. Voderberg, K. 1949. Zur Fruchtkörperbildung von Coprinus lagopus. Planta 37:149-160.

277. Voelz, H., and Niederpruem, D. J. 1964. Fine structure of basidiospores of Schizophyllum commune. J. Bacteriol. 88:1497-1502.

278. Vorderberg, K. 1948. Zum Hähr-und Wirkstoffbedarf von Coprinus lagopus. Z. Naturforsch 36:272-279.

279. Wakita, S. 1954 (a). Biochemical studies of Collybia velutipes. Effect of sucrose/$NaNo_3$ ratio on the growth of mycelia and the fructification of the fungus. J. Agr. Chem. Soc. Japan 28:577-580.

280. Wakita, S. 1954 (b). Biochemical studies on the velvet-mushroom, Collybia velutipes. Effect of amino acids and vitamins on the growth of the mycelium. J. Agr. Chem. Soc. Japan 28:707-711.

281. Waksman, S. A., and Nissen, W. 1931. Lignin as a nutrient for the cultivated mushroom, Agaricus campestris. Science 74:271-272.

282. Wang, C. S., and Miles, P. G. 1964. The physiological characterization of dikaryotic mycelia of Schizophyllum commune. Physiol. Plant. 17:573-588.

283. Wehmer, C. 1895. Notiz über die Unempfindlichkeit der Austernpilzes (Agaricus ostreatus) gegen Erfrieren. Ber. d. Deut. Bot. Gesells. 13:473-475.

284. Wells, K. 1965. Ultrastructural features of developing and mature basidia and basidiospores of Schizophyllum commune. Mycologia 57:236-261.

285. Wolpert, F. S. 1924. Studies in the physiology of the fungi. XVII. The growth of certain wood-destroying fungi in relation to the H-ion concentration on the media. Ann. Mo. Bot. Gard. 11:43-97.

286. Young, H. C., and Bennett, C. W. 1922. Growth of some parasitic fungi in synthetic culture media. Am. J. Bot. 9:459-469.

287. Yusef, H. M. 1950. An investigation of the requirements of some Hymenomycetes for specific essential metabolites. Dissertation (Pub. 1727). Columbia Univ. 168 p.

288. Yusef, H. M. 1953. The requirements of some Hymenomycetes for essential metabolites. Bull. Torrey Bot. Club. 80:43-64.

289. Zeller, S. M. 1916. Studies in the physiology of the fungi. II. Lenzites saepiaria Fries, with special reference to enzyme activity. Ann. Mo. Bot. Gard. 3:439-510.

290. Zeller, S. M., Schmitz, H., and Duggar, B. M. 1919. Studies in the physiology of the fungi. VII. Growth of wood-destroying fungi on liquid media. Ann. Mo. Bot. Gard. 6:137-142.

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