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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY-BULLETIN NO. 85.

B. T. GALLOS AY Chief Chureca.

THE PRINCIPLES OF MUSHROOM GROWING AND MUSHROOM SPAWN MAKING.

BY

B. M. DUGGAR,

PROFESSOR OF BOTANY IN THE UNIVERSITY OF MISSOURI, AND COLLABORATOR OF THE BUREAU OF PLANT INDUSTRY.

VEGETABLE PATHOLOGICAL AND PHYSIOLOGICAL INVESTIGATIONS.

ISSUED NOVEMBER 15, 1905.



WASHINGTON:

GOVERNMENT PRINTING OFFICE. 1905.





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Book 286









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A FINE BED OF MUSHROOMS GROWN FROM SPAWN OF PURE-CULTURE ORIGIN.

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B. T. GALLOWAY, Chief of Bureau.

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BUREAU OF PLANT INDUSTRY.

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B. T. GALLOWAY,

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 ^a Detailed to Seed and Plant Introduction and Distribution.
 ^b Detailed to Bureau of Chemistry.
 ^c Detailed from Bureau of Chemistry.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., August 21, 1905.

Sir: I have the honor to transmit herewith a paper entitled "The Principles of Mushroom Growing and Mushroom Spawn Making," and to recommend that it be published as Bulletin No. 85 of the series of this Bureau.

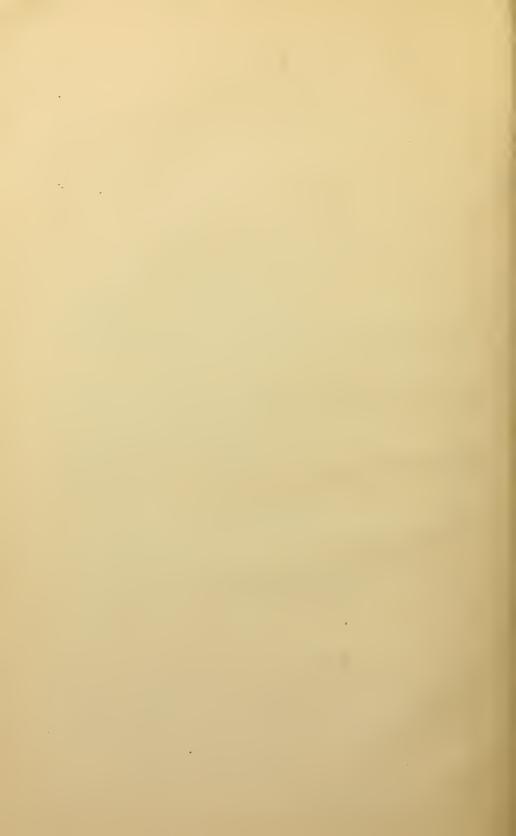
This paper was prepared by Dr. B. M. Duggar, Professor of Botany in the University of Missouri and Collaborator with the Office of Vegetable Pathological and Physiological Investigations of this Bureau. Under the direction of the Pathologist and Physiologist, Doctor Duggar has been engaged for several years in the investigation of mushroom culture in all of its phases, and great advances have been made, especially in the production of purer and better spawns.

The accompanying illustrations are necessary to a complete understanding of the text of this bulletin.

Respectfully,

B. T. Galloway, Chief of Bureau.

Hon. James Wilson, Secretary of Agriculture.



PREFACE.

The bulletin submitted herewith presents the results of the work up to the present time on the problems of mushroom culture and spawn making. The first publication on the subject from the standpoint of pure culture was Bulletin No. 16 of the Bureau of Plant Industry. This was followed by a Farmers' Bulletin (No. 204) on mushroom culture, presenting the results of our work for the use of the practical grower. As an outcome of the work Doctor Duggar has already accomplished, spawn of pure-culture origin is now being produced on a very large scale by several growers and is giving excellent results. This method enables the grower to improve and maintain the most desirable varieties of mushrooms in the same manner as is possible with other plants propagated from cuttings or buds. Information which would enable a grower to accomplish this has not been up to this time available. The general method of securing pure cultures as here described will enable the experimenter to cultivate spawn of other edible species of mushrooms in case it should be found desirable to cultivate them. The methods described differ radically from any hitherto used. They are of more general application and give far better results.

For the past three years this work has been carried on in cooperation with the University of Missonri, Doctor Duggar having left the Department to accept the professorship of botany in that institution. We wish to express our appreciation of the facilities furnished by the university for continuing this work.

Albert F. Woods, Pathologist and Physiologist.

Office of Vegetable Pathological and Physiological Investigations,

Washington, D. C., June 16, 1905.



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THE PRINCIPLES OF MUSHROOM GROWING AND MUSHROOM SPAWN MAKING

INTRODUCTION.

For a number of years there has been an increasing demand in the United States for information concerning mushroom growing. In the horticultural and agricultural press many individual practices have been presented; but in order to give rational encouragement to mushroom growing in favorable sections of this country it was recognized at the outset of the investigations undertaken by the writer that much experimental work would be required. Bearing upon the culture of Agaricus campestris a number of physiological questions were demanding attention, for it was desirable to ascertain (1) the conditions of spore germination, in order that "virgin" spawn might be propagated and the principle of selection attempted; (2) the relation of this fungus to nutrients, or a determination of the substances or compounds which might best serve as food materials; and (3) the relation of the growing mycelium and of mushroom production to temperature, moisture, and other conditions of the environment. In the next place it would be necessary to determine the application of any physiological principles established to the practice of mushroom growing and mushroom spawn making.

In connection with a presentation of the results of the experimental work ^b it seems desirable to include also a more or less comprehensive account of the present status of mushroom growing at home and abroad.

^a Throughout this paper the writer has employed the generic name Agaricus in the sense in which it is usually understood by those interested in the practical side of the work.

^b During 1903—4 the writer was assisted in the experimental work by Mr. A. M. Ferguson, instructor in botany in the University of Texas, at that time special agent of the Department of Agriculture, and during 1904–5 similar assistance has been rendered by Mr. L. F. Childers, student assistant. Through the assistance thus given it has been possible to complete an unusual amount of experimental work, only a portion of which can be described in detail, although it has all been taken into consideration in the conclusions drawn.

It is not possible at this time to give more than a few brief suggestions concerning the possibility of cultivating other edible species than Agaricus campestris. The determination of the fundamental needs of diverse species will require study during a term of years.

GENERAL CONSIDERATIONS.

The propagation of Agaricus campestris does not seem to have been undertaken to any extent by the ancient Greeks or Romans. The occasional references to mushrooms in the classics are very general, as a rule, and do not suggest that artificial propagation was attempted. In the vicinity of Paris Agaricus campestris has been cultivated for several centuries, and the plants have certainly been sold on the open market quite as long. It has not been possible to ascertain whether the methods now in vogue are essentially the same as those employed a few centuries ago. It is very probable, however, that the methods have been gradually improved. It would appear that the cultivation in caves is comparatively recent. The earliest records obtainable concerning the cultivation of mushrooms in the underground quarries indicate that this practice was not common previous to the nineteenth century.

Mushrooms are to-day extensively grown in England and France, and to a limited extent in Belgium, in Germany, and in many other Paris remains, however, the center of commercial production. In the vicinity of that city the culture of mushrooms is now almost entirely confined to the underground limestone quarries or cement mines. The caves used for this purpose are termed "carrières" or "champignonières." These caves may consist of a labyrinth of galleries, or halls, ranging from 5 to 50 feet in width and from 5 to 30 feet in height. In some regions the earth is practically honeycombed by them, and the extent of the cave space used by the larger growers may be measured by miles. For the most part the ventilating system is perfect, every cave system possessing numerous air shafts, protected at the surface by wooden towers. Artificial partitions in the caves themselves enable the operator to control the ventilation. Until recent times the cultural methods have been more or less sacredly guarded by the growers, and even to-day it is not easy to get permission to make a casual visit to the champignonières. In many cases the work has been followed from generation to generation within the same family. There are at present, however, large corporations in control of some of the most famous caves.

^a In a painting of the early seventeenth century (that of a Fishmonger's and Ponlterer's Shop, by Jordaens and Van Utrecht, in the Gallery of Old Pictures, Brussels) Agaricus campestris and Boletus are shown on sale as a conspicuous part of a market scene.

In the United States fresh mushrooms have only recently been of any importance commercially, although florists and gardeners of English and French training have long been successful growers on a small scale. Nevertheless, during the past decade or so, the record of failures has been most conspicuous, and it is certain that of the many who attempted this work only a few, relatively, were uniformly successful.

The conditions under which mushrooms may be successfully grown are limited, and intelligent attention is therefore essential. It must be said, moreover, that the majority of failures may be directly traced to erroneous ideas as to the cultural requisites, or to a reckless disregard of conditions. The essential conditions will be subsequently defined in detail, but it may be stated here that failures are usually due to one or more of the following causes: (1) Poor spawn; (2) very poor manure; (3) unfavorable temperature; and (4) heavy watering during the early stages of growth.

Under suitable conditions mushrooms may be grown with assurance of success. Ordinarily they are grown only where the conditions may be controlled, and success should therefore be invariable.

MARKET CONDITIONS.

In the vicinity of Paris the mushroom industry has been remarkably developed during the past eight or ten years. The total product sold through the central market of Paris in 1898 was nearly 4,000,000 pounds; the quantity for 1900 is given as approximately 8,500,000 pounds, and for 1901 nearly 10,000,000 pounds. These figures show most convincingly the present status of the mushroom industry in France. It may be safely assumed that more than one-third of this quantity is consumed in a fresh state in and about the city. The growth of the canning industry during this period has also been remarkable. In 1898 about 1,800,000 pounds were preserved, while in 1901 the canned product amounted to nearly 6,200,000 pounds. During 1901 the approximate monthly production of mushrooms ranged from 651,000 pounds to 985,000 pounds, from which it is evident that these caves yield heavily throughout the year. In some instances growers are able to get a crop every four or five months.

It is extremely difficult to estimate the quantity of mushrooms grown in the United States. It is certain, however, that the production has increased very greatly, and particularly within the last four or five years. In the vicinity of several of our larger cities there are to-day individual growers who produce more than the total commercial output in the neighborhood of those cities ten years ago.

There is now a very good open market for fresh mushrooms in a few of the larger cities, although many large growers continue to

sell entirely by contract or by special orders to hotels and restaurants. With such an enormous comparative consumption of the canned product, there is every reason to believe that fresh mushrooms can be sold in much greater quantity as soon as this product becomes a certain factor in the market. With canning factories to take the surplus product, growers could afford to accept a smaller margin of profit, and this would place mushrooms within reach of many who may not be able to purchase them at present average prices. Agaricus campestris and its varieties and allied species are perhaps the only fresh mushrooms commonly salable in the markets of American cities. Throughout practically the whole of Europe several other species are legitimate market products. The more delicate or fleshy forms of the latter are sold as fresh mushrooms; others are dried. and some of these, being tougher, are used only for soups, sauces, and gravies. Besides the various species of truffle and morel, any special mention of which will be omitted here, the French market to-day legalizes the sale of five or six other species of mushrooms.

GERMINATION STUDIES.

Review of earlier work.—In a small way the germination of the spores of Basidiomycetes has received attention from the earliest times. A complete historical review of the literature dealing with spore germination will be found in Bulletin No. 16 of the Bureau of Plant Industry. It will be seen that most of the early work furnishes only incidental references to spore germination. By far the most important contributions made by early workers to this particular subject were several papers by Hoffmann.^a It is not to be expected that the method employed by him would yield accurate results. Nevertheless, the work of Hoffmann is comprehensive for that time. Brefeld,^b in his extensive reports upon the Basidiomycetes, gives the results of germination studies with a large number of the fleshy fungi. More than 200 species were used in his various experiments, and successful germination is recorded for about 160 species.

In 1898 the writer became interested in some attempts to germinate the spores of certain Basidiomycetes. Subsequently the problem received incidental attention in connection with some general studies on the physiology of spore germination.^c The work progressed only

^a Hoffmann, H. Ueber Pilzkeimungen. Botan. Zeitg., 19:209–214, 217–219. 1859. Beiträge zur Entwickelungsgeschichte und Auatomie der Agaricineen. Botan. Zeitg., 18:389–395, 397–404. 1860. Untersuchungen über die Keimung der Pilzsporen. Jahrb. f. wiss. Botanik, 2:267–337. 1860.

b Brefeld, O. Botanische Untersuchungen über Schimmelpilze. Basidiomyceten, I, Bd. I, H. 3. 1877. Untersuch. a. d. Gesammtgebiete der Mykologie. Basidiomyceten, II, H. 7; III, II. 8. 1888–89.

^c Duggar, B. M. Physiological Studies with Reference to the Germination of Certain Fungous Spores. Bot. Gaz., 31:38-66.

far enough to suggest that an investigation of the factors influencing germination might yield studies of special interest. During 1900-1901 Dr. Margaret C. Ferguson undertook a systematic investigation of the relation of stimuli to germination in certain species. The results a have made it evident that the problems involved are not the well-known simple nutrient or physical factors. Miss Ferguson spent much time in experimenting with a great variety of nutrient media and special stimuli. Several thousand cultures were made. In the majority of these cultures Agaricus campestris was used, and it is shown that from the known ecological relationships of this fungus one could not possibly predicate the probable stimulus for germination. In fact, with no known nutrient medium or special chemical stimulus employed, was there anything more than erratic germination. Nevertheless, the work was finally very successful in the discovery that almost-a perfect percentage of germination could be secured by the influence of the living hyphæ of Agaricus campestris upon the spores, as announced in the statement that "if a few spores are able to germinate under the cultural conditions. or if a bit of the mycelium of Agaricus campestris be introduced into the culture, the growth resulting will in either case cause or make possible the germination of nearly all the spores of the culture, provided, of course, that the other conditions are not such as to inhibit germination."

The stimulus would seem to be of enzymatic nature. No other mycelium tested produced a similar effect. This was a distinct advance in our knowledge of factors influencing germination. The stimulus, however, could only be looked upon as perhaps a substitution stimulus. It did not seem possible that it could obtain in nature, nor could it be looked upon as wholly satisfactory from a practical point of view.

Miss Ferguson's results offered encouragement; but, nevertheless, the problems with Agaricus campestris and related species were left open for further investigation. It should, perhaps, be emphasized that prior to 1902 no method had been published, so far as can be learned, whereby one might be able to obtain with uniformity the germination of Agaricus campestris. It is quite certain that Chevreul and others obtained at best only erratic results. Nevertheless, as early as 1893 Costantin and Matruchot b announced that a method had been developed by them whereby they were able to germinate the

^a Ferguson, M. C. A Preliminary Study of the Germination of the Spores of Agaricus Campestris and Other Basidiomycetous Fungi. Bulletin No. 16, Bureau of Plant Industry, U. S. Dept, Agriculture, pp. 1–43. 1902.

b Costantin and Matruchot. Nouveau procédé de culture du champignon de conche. Compt. Rend. de l'Acad. des Sci., 117 (2): 70-72. (Compare, also, Bul. Soc. de Biol., 2 December, 1893.)

spores and to grow in pure culture the mycelium of Agaricus campestris. Information concerning the details of the method employed was avoided in the reports of this announcement and in subsequent references to the process.^a In the first announcement the method is stated as follows:

Method followed.—The spores are collected free from contaminations, and in order to preserve them in that condition are sown on a certain sterilized nutritive medium. We obtain in this manner a twisted mycelium which constitutes pure spawn. By repeated cultures on an identical substratum the spawn can be multiplied indefinitely, and is transferred at a proper time to sterilized manure, where it develops abundantly in several weeks. At that stage it possesses the characteristic appearance and odor of natural spawn. It can then be sown in a bed of ordinary manure, to which it adheres and where it grows and fruits normally.

In the later paper cited, writing of the recent improvements in mushroom culture, Costantin expresses himself as follows:

We have succeeded in manufacturing an artificial spawn obtained from the spore germinated on a medium free from contamination. It is then pure spawn. We can state further that it is virgin spawn.

In 1897 Répin ^b claimed to have independently arrived at results similar to those obtained by Costantin and Matruchot. Concerning his germination studies he says:

It is only recently that the study of this question has been renewed, independently and simultaneously by Costantin and Matruchot.

There is nothing unusual in the germination of the spores of Agaricus. Spores can be germinated on media such as used in bacteriology, on wet sand, or in moist air as well as on mannre. Without doubt, germination is not produced with the same spontaneity and rapidity as in the case of the spores of lower fungi, which fact makes it necessary to promote the process by some artifices, but they are only sleight-of-hand tricks, variable according to the operators, and which are acquired after some unsuccessful attempts. The spores which should germinate (and these are always in the minority) begin by swelling. This very simple method makes it possible to obtain virgin spawn at pleasure. It is applied industrially in the manufacture of spawn of Agaricus from cultures which I have made.

So far as the writer has been able to ascertain, therefore, no description of the method employed by the above writers is to be found. The report of Miss Ferguson's work is accordingly the only available scientific record defining the conditions under which germination had been constantly obtained up to this time.

Experimental work.—The writer has been able to confirm Miss Ferguson's work repeatedly, and at the same time numerous series of experiments have been made to test further the possibility of influence-

^a Constantin, J. La culture du champignon de couche et ses récent perfectionnements. Extrait du Revue Scientifique. April, 1894.

 $[^]b\,{\rm R\acute{e}pin},$ C. Le blanc vierge de semis pour la culture du champignon de couche. Revue Générale des Sciences. (September 15, 1897.)

ing germination by chemical stimuli. In distilled water, on the one hand, and in plant decoctions (such as decoctions of beans, sugar beets, mushrooms, potatoes, etc.) and in bouillon, on the other hand, there have been tested a large number of inorganic and organic salts, carbohydrates, nitrogenous compounds, and active enzymes.

The results of one series of experiments are tabulated in detail. In general, it has been found that dulcite, monobasic magnesium phosphate, magnesium phosphate, magnesium potassium ammonium phosphate, ammonium molybdate, magnesium lactophosphate, dibasic calcium phosphate, and other salts, especially phosphates, have in one medium or another been more or less effective as stimuli for germination. Unfortunately, none of the substances mentioned, apparently, are very strong stimuli; they are unable to cause invariable germination in all nutrient media. Moreover, in subsequent series, where the conditions have been the same, within experimental possibilities, wholly analogous results have not always been obtained. No account has been taken, however, of the particular variety of Agaricus campestris from which the spores were obtained, and it may be that this will influence the results.

It is to be noted from the following table that Miss Ferguson's method of employing living bits of mycelium was modified by the use of small pieces of the inner tissue of a young mushroom taken under sterile conditions. It was found that often a new growth of mycelium was developed from this tissue. Whenever this growth appeared, the influence upon spores in the drop culture was, as might be expected, the same as had been demonstrated for the living mycelium. Frequently a few spores germinated within from three to five days. The most interesting conclusion, however, which could be drawn from the cultures in which small bits of tissue were used was the following: Under favorable conditions a small piece of the inner growing tissue of a mushroom is capable of producing a mycelium with great readiness. This fact has been utilized, as shown in detail later, in the development of a new and effective method of securing pure cultures of fleshy fungi in general.

TABLE		E I.—Extent of germination.
No.	Media.	After 3 days.

No.	Media.	After 3 days.	After 5 days.
1 2 3 4 5 6 7 8 9	Distilled water Bouillon per cent KH.PO4 per cent K4.PO4 per cent K4.PO4 per cent K4.PO4 per cent N4.PO4 per cent N4.PO4	do do do	As before. Do. None. Do, Do, Do. Do. Do. Do. Do. Do. Do.
10	$ \begin{array}{c} \stackrel{?}{\downarrow} \text{ per cent } (NH_4)_2 HPO_4. \\ \stackrel{?}{\downarrow} \text{ per cent } (NH_4)_2 HPO_4 \text{ in bouillon.} \end{array} $	dodo	Do. Do.

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Table I.—Extent of germination—Continued.

	1	1	
No.	Media.	After 3 days	After 5 days.
11 12	$\frac{1}{2}$ per cent $MgH_4(PO_4)_2$	10 spores {a-1 spore b-None	50 per cent. 3 per cent. None.
13	per cent MgHPO,		Do. 1 per cent.
. 14	‡ per cent MgHPO4 in bouillon	a-10 spores	Germinated spores badly injured.
15 16	† per cent Mg(NH ₄)PO ₄ † per cent Mg(NH ₄)PO ₄ in bou-	None	None. As before; injured.
- 17 18	illon. $\frac{1}{2}$ per cent MgK(NH ₄)PO ₄ $\frac{1}{2}$ per cent MgK(NH ₄)H ₂ (PO ₄) ₂ in	Few sporesdo	5 per cent. Do.
19	bouillon. $\frac{1}{2}$ per cent $(NH_4)_2C_4H_4O_6$	(a-None	Few spores.
20	$\frac{1}{2}$ per cent $(NH_4)_2C_4H_4O_6$ in bouillon.	10 spores	10 per cent. As before; injured.
21	per cent magnesium lactophosphate.	do	
22 23	phate in bouillon.	Nonedo	2-5 per cent.
24	per cent Ca ₂ H ₂ (PO ₄) ₂ in bouillon	10 spores	Injured.
24 25	1 ner cent KCHO₀	None	1-2 per cent
26	per cent MgHPO, in bouillon.	10 spores	10-50 per cent.
27	per cent MgHPO3 in bouillon	do	1 per cent.
28	in mushroom decoction.		•
29 30	† per cent KH ₂ PO ₄ in mushroom decoction. † per cent K ₂ HPO ₄ in mushroom	None	None. Do.
31	decoction.	Few spores	
32	room decoction.	1 or 2 spores	Few spores.
33	room decoction.	Few spores	•
34	room decoction.	10-50 per cent	2-5 per cent. 10-20 per cent.
35	† per cent Mg(NH ₄)PO ₄ in mushroom decoction.	Few spores	
36	¹ / ₄ per cent (NH ₄) ₂ C ₄ H ₄ O ₆ in musli- room decoction.	2 per cent	Contaminated; 50 per cent, but injured.
37	‡ per cent magnesium lactophosphate in mushroom decoction.	10 spores	3-5 per cent; injured.
38	$\frac{1}{2}$ per ceut $Ca_2H_2(PO_4)_2$ in mushroom decoction.	5–8 per cent	10 per cent.
39	per cent KCHO ₂ in mushroom decoction.	2–3 per cent	10-20 per cent.
40	½ per cent MgHPO ₃ in mush- room decoction.	}1-2 per cent	a=5 per cent. b=Contaminated
41	Decoction of mushrooms	{a-2 per cent b-Yery few spores	2 per cent.
42	Living tissue of mushroom in mushroom decoction.	a-Few spores a b-None b	12 spores.
43	do	$\begin{cases} a - \text{Few spores} a \\ b - \text{None} b \end{cases}$	As before. None.

 $[^]a$ ln this cell the tissue developed a new growth, b No growth from tissue introduced.

On account of the fact that magnesium phosphite and magnesium potassium ammonium phosphate had in most cases proved to be stimuli for germination, experiments were next made to determine the efficiency of these salts on various media, as indicated in the table on the following page.

Table II.—Efficiency of salts on various media.

Nature of compost.	Appearance after 25 days.	Nature of compost.	Appearance after 25 days.
manure. a Do. b	Fine growth. One, fair growth; one,	Well-rotted cow manure, b Penty mold a Do, b Maryland peata Do, b Well-rotted Ginkgo leaf mold, a Do, b Cotton-seed motes a Do, b	No growth. Do. Do. Do. Do. Do.

^a Watered with concentrated solution of magnesium phosphite.
^b Watered with strong solution of magnesium potassium ammonium phosphate.

Large test tubes were used in these experiments, and duplicate cultures were made in every instance. From these and from numerous other cultures it was ascertained that germination could not be obtained invariably, even on favorable media and under pure-culture conditions, by the use of these partial stimuli. Nevertheless, the percentage of failures has usually been small. By means of the stimulus given by magnesium phosphite it has also been possible to get growth from the spores in test-tube cultures with gray filter paper as the solid substratum and various plant decoctions and culture solutions as the nutrients. Details of these results, however, may be omitted.

In many eases it has been possible to obtain growth from the spores by the use of the stimulating salts which have been mentioned in connection with the germination studies. Where it is desired to make experiments along this line the writer has found it more practicable to use spores from a mushroom as young as possible. If one takes a mushroom just at the time that the veil is breaking, inoculations may be readily made from the spores and few contaminations will result. In this case, by means of a sterile needle, or scalpel, a few spores may be removed from the spore-bearing, or gill, surface and these may be transferred to the tubes in the same way as were bits of the fresh tissue. It is also possible to secure a spore print from a mushroom the gill surface of which has not been exposed to germs of the atmosphere. In the latter case it is desirable to remove stem and partial veil, peel off the incurved edges of the cap which have been in contact with the soil, and place the cap, gill surface downward, in a sterilized dish or on sterile paper. If this is then kept free from dust, a spore print may be obtained, which should not be contaminated by foreign germs. This print may then be used in making a large number of spore cultures.

Experiments were also made in which pots of unsterile composts and manures were inoculated, on the one hand, with spores, and, on the other hand, for control purposes, with spawn from pure cultures. The duration of the experiments was two months. Some of these pots were watered with a mineral nutrient solution including as one constituent magnesium phosphite, designated X, others with the same solution to which was also added a small quantity of dried blood, designated Y, and the remainder with pure water. The results are tabulated as follows:

Table III.—Extent of growth of spores and spawn in pot	TABLE III	.—Extent o	growth o	f spores and .	spawn in nots
--------------------------------------------------------	-----------	------------	----------	----------------	---------------

	Cattle manure, old.	Fresh stable manure and sand.	Stable manure.	Old stable manure and sand.	Old stable manure.	Fertilizer
Spores	∫a-Good }b-None	Nonedo	Slight	None	Nonedo	Y_{\bullet}
Spores	$b = \begin{bmatrix} a - Good \dots \\ b - Do. \end{bmatrix}$	Very good .	do	do	do	X. X.
Spawn	a—Slight b — Do.	Slightdo	None Slight	do	do	Υ. Υ.
Spawn	b—None b —Do.	Nonedo	Good Slight	do	do	X. X.
Spawn	$b = None \dots$	Slightdo	None	do	do	None. Do.

TISSUE CULTURES.

The suggestion which had presented itself of using bits of living tissue from a sporophore instead of spores seemed also, from general observations, to be of sufficient importance to warrant a thorough trial. During moist weather, or in a moist cellar where mushrooms are being grown, one will frequently find that an injury in a young mushroom is rapidly healed by a growth of hyphæ from the edges of the injured area. The same thing had been noted in the open in the case of puffballs. In many instances, moreover, pure cultures of fungi in other groups have been obtained by the use of small bits of a sclerotial mass of tissue. Accordingly, a young sporophore of Agaricus campestris was obtained, and after breaking it open longitudinally a number of pieces of tissue from within were carefully removed with a sterile scalpel to a sterile Petri dish. A number of cultures were then made by this tissue-culture method on a variety of nutrient media, such as bean pods, manure, leaf mold, etc. From this and from numerous other similar tests it was ascertained that when the mushrooms, from which the nocules of tissue are taken, are young and healthy, there is seldom an instance in which growth does not result. It was easily shown that failure to grow was generally due to the advanced age of the mushroom used, to an unfavorable medium, or to bacterial contamination.

The first successful pure cultures were made by this method during the early spring of 1902 from mushrooms grown indoors. During the following summer, or as other fleshy fungi appeared in the open, cultures were made from other forms in order to determine the general applicability of the method. The experiments were successful in most cases, although it was found almost impossible to obtain certain species of fungi in a condition young enough to be free from bacterial infestation. In general, the method seemed to commend itself strongly as a means of procuring pure cultures of desirable edible species, particularly of those species the spores of which could not be obtained pure or which could not be readily germinated.

During the two subsequent seasons this method has been employed with a great variety of fungi representing many natural orders. No systematic endeavor has been made to determine the limitations of the tissue-culture method as applied to Basidiomycetes, but, incidental to the general studies, cultures have been made from forms differing very widely, not only in relationship but also in texture and in habitat.

In all there is a record of 69 species having been tested upon one or another medium. In a few cases the cultures have invariably been contaminated, and it is to be supposed, perhaps, that the plants were collected in a condition too old for the purpose in hand. In only about ten forms has it seemed that there is no evident reason for the failure to develop mycelium. Of the remainder fully 40 have grown promptly on the media employed. The table following indicates the names of the species employed and the results obtained. It must be said, however, that cultures of a number of species were made of which no record was kept; among these, also, some grew and some failed.

Table IV.—Results obtained from different species.

Fungus.	Num- ber of cul- tures.a	Substratum.	Result.
Agaricus arvensis Agaricus campestris (various varieties). Agaricus fabaceus Agaricus fabaceus Agaricus fabaceus var Agaricus placomyces. Agaricus villaticus Amanita frostiana Amanita muscaria Amanita verna Amanitopsis vaginata Armillaria mellea Boletinus porosus Boletus selleus Boletus felleus Boletus miniato-violaceus Boletus miniato-violaceus Boletus miniato-violaceus Calvatia craniiformis Calvatia cyathiforme Calvatia cyathiforme Calvatia rubro-flava Cantharellus cibarius	Tew. 1 2 2 2 2 2 2 2 1 2 2 Few. Few. Few.	Beans, manure, leaves, etc. Beans Beans, manure, leaves, etc. do do Beans Manure, leaves, etc. Leaves Beans do do Beans, leaves, dead wood, etc. Beans do do Beans, leaves, etc. Beans, leaves, etc.	Rapid growth. Contaminated. Rapid growth. Do. Do. Some growth. Rapid growth. Contaminated. Do. Do. Bapid growth. No growth. No growth. No growth. Contaminated. Rapid growth. Do. Contaminated. Rapid growth. Do. Do. Do. Do. No growth.

Table 1V.—Results obtained from different species—Continued.

Fungus.	Num- ber of cul- tures.	Substratum.	Result.
6044 a mail a 400 a 4 a mai		D	
Clitocyhe illudens	2 2	Beaus	Some growth.
Clitocybe sp.?		do	Rapid growth.
Clitopilus prunulus	Few.	Beans, manure, etc	
Collybia platyphylla	1 1	Beans	No growth.
Collybia radicata	Few.	do	Good growth.
Collybia velutipes	1	do	Do.
Coprinus atramentarius	\propto	Beans, leaves, manure, etc	Rapid growth.
Coprinus comatus	000	Beans, manure, leaves, etc	Do.
Coprinus fimetarius	Few.	Beans, leaves	Do,
Coprinus micaceus	000	Beans, leaves, manure, etc	Do.
Cortinarius armillatus	1	Beans	Contaminated.
Cortinarius castaneus	1	do	Do.
Cortinarius sp.?	Few.	Beans, leaves, manure	Good growth.
Daedalia quercina	Few.	Beans, leaves, manure, etc	Rapid growth.
Hydnum caput medusae	1	Beans	Good growth.
Hydnum coralloides	2	do	Do.
Hydnum erinaceum	2	do	Do.
Lactarius corrugis (?)	Few.	Beans and leaves	Slight growth, one.
Lactarius piperatus	\propto	do	No growth.
Lactarius volemus	1	Acid beans	
Do.	\propto	Beans	No growth.
Lepiota americana	1	do	Do.
Lepiota morgani	2	do	Some growth.
Lepiota procera	000	Beans, leaves, etc	Rapid growth.
Lepiota rhacodes	Few.	do	Do.
Lycoperdon gemmatum	2	Beans	Good growth.
Lycoperdon wrightii Morchella esculenta	1	Sod	Do.
	2	Beans and leaves	Do.
Pluteus cervinus	Few.	do	Some growth.
Pleurotus ostreatus	\propto	Beans, leaves, manure, etc	Rapid growth.
Pleurotus ulmarius	1 1	Beans	Do.
Pholiota adiposa	1	Beans and leaves	No growth.
Polyporus betulinus	1	Beans	Slow growth.
Polyporus intyhaceus	9	Poons and lugran	Do,
Polyporus sulphureus Polystictus cinnabarinus	2 2	Beans and leaves	Rapid growth.
Russula adusta	î	do	Good growth.
Russula emetica	000	Beans	No growth.
Russula emetica	α	Beans, etc	Often contaminated
Russula sp	Few.	Beans	but some grew.
Russula sordida	Tew.		No growth.
Russula virescens	Few.	do	Do. Do.
Secotium acuminatum.	rew.	do	
Strobilomyces strobilaceus	1	do do	Slow growth. No growth.
Stropharia sp.	1	do	Contaminated.
Tremella mycetophila	1	do	No growth.
Tricholonia personatum	1	Beans and manure	Good growth.
Tricholoma russula	2	Beaus and leaves	Do.
A Fredorita I ussuit	4	Deaus and leaves	10.
	1	1	

It is not to be understood that the failures recorded in the foregoing table indicate that these species will not grow. The evidence is that they did not grow upon the media used, and it is very probable that most of these could be propagated in culture by this method if a systematic attempt were made to determine what substrata are desirable. The writer believes that this statement holds true particularly in the case of certain species of Boletus. No attempt was made to cultivate Boleti in any other way than upon bean pods. A few mycelial threads were developed in such cases, but these failed to grow upon the bean, apparently dying before even the nutrients in the fragment of tissue were exhausted.

It is interesting to note that many of the fungi which have given good growth have not hitherto been grown in pure culture. Accord-

^a Costantin et Matruchot. Sur la production du mycelium des champignon supérieurs. Extrait Compt. Rend. d. Séances de la Soc. de Biologie. January,

ing to Costantin and Matruchot, Van Tieghem (1876) produced the mycelium of Coprinus in pure culture. Later, Brefeld a accomplished the same result with many species of Coprinus, and also with Armillaria mellea. Costantin has also published a number of brief papers, or announcements, of successful cultures upon artificial media of the mycelium of several fleshy fungi. Besides Agaricus campestris, he has grown the mycelium of Amanita rubescens, Armillaria mellea, Collybia velutipes, Lepiota procera, Marasmius oreades, Tricholoma nudum, Pleurotus ostreatus, Pholiota negerita, Coprinus comatus, Polyporus tuberaster, P. frondosus, Hydnum coralloides, Morchella esculenta, and perhaps a few others. He has also grown to maturity sporophores of Agarieus eampestris, Coprinus comatus, and Tricholoma nudum. Unfortunately, Costantin seldom indicates the substratum upon which his cultures were made. Falck b reports having produced in culture the sporophores of Collybia relatives, Phlebia merismoides, Hupholoma fasciculare, Chalymotta campanulata, and Coprinus ephemerus in his studies upon the connection of oidial stages with perfect forms of the Basidiomycetes. In the work of the writer so far no special attempt has been made to obtain the sporophores of the fungi cultivated except in the case of Agaricus campestris. Nevertheless, the following species have fruited in pure culture upon the media indicated:

	Medium.
Agaricus campestris	Manure.
Agaricus fabaceus	Manure.
Agaricus amygdalinus	Manure.
Armillaria mellea	Beans.
Bovistella ohiensis	Soil.
Calvatia cyathiforme	Soil.
Calvatia rubro-flava	Soil.
Cortinarius sp	Soil.
Coprinus comatus	Leaves.
Coprinus fimetarius	_Leaves.
Coprinus solstitialis (?)	Leaves, etc.
Daedalia quercina	Leaves, etc.
Hydnum coralloides	Beaus.
Lycoperdon wrightii	Soil.
Pleurotus ostreatus	Beans and manure.
Pleurotus ulmarius	Manure.

In some instances the sporophores have been minute, owing to the small quantity of the culture medium.

a Brefeld, O. Unters. aus d. Gesammtgebiete d. Mykologie, 8, 9, 10.

b Falck, R. Die Cultur der Oidien und die Rückführung in die höhere Fruchtform bei den Basidiomyceten. Cohn's Beiträge zur Biologie der Pflanzen, 8: 307-346 (Pls. 12-17).

From the standpoint of obtaining pure cultures, the tissue-culture method is capable of very general application. Three considerations render it particularly important, as follows: (1) When a suitable culture medium is at hand, a pure culture may be obtained almost invariably from a young, healthy plant. (2) Cultures may be made from fungi the spores of which have never been brought to germination. (3) Pure cultures are made by direct inoculation; that is, dilution cultures are rendered wholly superfluous. In the case of Agaricus campestris and other Basidiomycetes, in which the gillbearing surface is protected until the spores are produced, it is possible, with the precautions previously mentioned, to obtain the spores pure, or practically pure, and at the same time in considerable quantity. This is not possible with the great majority of fleshy fungi, which are truly gymnocarpous. Again, members of the genus Coprinus are deliquescent, and here it is impracticable to secure spores by the spore-print method. In the Lycoperdaceæ and other Gasteromycetes it has been found that bacteria are frequently present in the tissues by the time the spores are formed, and, even if the spores could be germinated, direct cultures would perhaps be seldom possible. By the tissue method it is only necessary that the plant shall be so young that the cells of the tissue are capable of growth and that there are no foreign organisms present in the tissue. In this connection it may be stated that in the Phallinea, Hymenogastrinea, and Lycoperdinea no representative has been germinated, while in the Plectobasidineæ germination is known only in the case of Sphaerobolus stellatus and Pisolithus crassipes.

When the natural conditions of germination shall have been more definitely ascertained, direct spore-culture methods should in practice, perhaps, replace the pure tissue-culture methods in making virgin spawn. This would render unnecessary a tedious portion of the work, and the process of spawn making would be thereby made less expensive.

A discussion of the respective practical merits of the spore and tissue methods would not be complete without reference to the comparative vigor, or productive power, of the resulting mycelium. In the growth of the mycelium no difference could be detected. The writer has also grown mushrooms from spawn produced by both of these methods; but the results do not indicate that there is any advantage for the one over the other. It is believed, therefore, that the processes of basidial and spore formation are in this regard relatively unessential, or at least do not intensify whatever invigoration may, in general, result from mere sporophore production. It is to be expected, perhaps, that any and all cells of the sporophore may be invigorated by whatever is to be gained by the assemblage, or concentration, in the differen-

NUTRITION. 23

tiated sporophore, of food products collected by a ramifying mycelium. According to the studies of Harper,^a Maire,^b and others, there is no sexual fusion in the case of the Basidiomycetes which have been studied. Two nuclei are present in the cells of the sporophore, but these are associated conjugate nuclei, and the fusion of these in the basidium is generally considered in no sense an act of fertilization, but rather a form of nuclear reduction. Maire states that the cells of the mycelium obtained by the germination of the basidiospore are uninucleate. It has not yet been ascertained when or how the binucleate condition arises.

NUTRITION.

Although Agaricus campestris has been cultivated for so long a time, it does not seem that it has previously been subjected to careful experimentation from the point of view of nutrition. The belief generally prevalent is that the most essential factor in the nutrition of the mushroom is the "ammonia" of the manure or compost. Again, it is claimed that organic waste products, such as those indicated, must undergo a process of fermentation, or "preparation," in order to furnish the necessary nutrients for the growing mycelium. This idea, as will be seen later, is merely based upon casual observations "in nature," and it is found wholly erroneous when tested for its fundamental worth by the elimination of other factors of the compost environment.

Growth on manure and other complex media.—Early in this investigation it was ascertained that the mycelium of Agaricus campestris in pure cultures would grow luxuriantly on fresh stable manure, and as a rule upon the same product in any stage of fermentation or decomposition. In some instances, undoubtedly, fresh manure may contain injurious compounds; somewhat oftener the same is true for the fermented product. In some instances it is desirable to dry or thoroughly air the fresh manure before use. Fresh manure from grass-fed animals is not to be recommended. The mycelium also grows luxuriantly on bean stems or pods, on half-rotted leaves of deciduous trees, on rich soil, on well-rotted sawdust, and on a variety of other substances. It does not grow readily upon peaty products.

Some of the more promising edible species were cultivated in various media in order to obtain an idea of the comparative value of these media in furnishing a nutrient to particular forms. It is not possible, of course, to base definite conclusions upon results obtained

^a Harper, R. A. Binucleate cells in certain Hymenomycetes. Bot. Gaz., 53: 1-25, 1902.

 $[^]b$ Maire, R. Recherches cytologiques et taxonomiques sur les Basidiomycetes. Bul. Soc. Myc. de France, $18:1{-}209,-1902,$

from pure cultures, since the presence of particular foreign organisms in the substratum under natural conditions is perhaps quite as important a consideration as that of the specific nutrient value of the substratum. The following results are, however, suggestive:

1. Agaricus campestris.

Leaves-good growth throughout.

Soil—fair growth, with tendency to become threaded early.

Manure-good growth throughout.

Beans—good growth throughout.

Sugar beet—fair growth, spreading very slowly.

Potato—slight growth, spreading very slowly.

Corn meal—slight growth, spreading slowly, soon becoming brown.

2. Agaricus fabaceus.

Leaves—very good growth, rapidly filling tube.

Soil—good growth, but slower than the above.

Manure—good growth, but slower than the above.

Beans—very dense growth, soon filling whole tube.

Sugar beet—good growth; somewhat less rapid and abundant than the above.

3. Agaricus villaticus.

Practically the same as Agaricus eampestris.

4. Agaricus fabaceus var.

Practically the same as Agaricus fabaceus.

5. Bovistella ohiensis.

Leaves-good growth throughout.

Soil-growth throughout, but sparse and threadlike.

Mannre-good growth throughout.

Beans—good growth; appressed.

Sugar beet-very slight growth.

6. Calvatia cyathiforme.

Leaves—very good growth throughout.

Soil-good growth; quite as rapid as above.

Manure—practically no growth.

Beans—good growth, but spreads very slowly.

Sugar beet-slight growth.

7. Calvatia craniiformis.

Practically the same as above.

8. Calvatia rubro-flava.

Practically the same as in the other species of this genus, but spreads somewhat more slowly on soil.

9. Coprinus atramentarius.

Leaves-very good growth throughout.

Soil-slight growth.

Manure-fair growth, but very slow.

Beans-very good growth.

10. Coprinus comatus.

Leaves-very good growth throughout; rapid.

Soil-good growth.

Manure-very good growth throughout; rapid.

Beans-very good growth throughout; rapid.

Sugar beet-very slow growth.

11. Lepiota rhacodes.

Leaves-very good growth.

Soil-slight growth.

Manure—slight growth.

Beans—very good growth throughout.

Sugar beet-very good growth throughout.

12. Morchella esculenta.

Leaves-very good growth; mycelium never dense.

Soil-very little growth.

Manure-very slight growth.

Beans-very good growth.

Sugar beet—good growth, but slower than above.

13. Pleurotus ostreatus.

Leaves—very good growth: rapid.

Soil-fair growth.

Manure-good growth.

Beans-very good growth; rapid.

Sugar beet-slight growth; very slow.

14. Pleurotus ulmarius,

Practically the same as Pleurotus ostreatus.

15. Polyporus sulphureus.

Leaves-fair growth; abundant, filling tube.

Soil—fair growth.

Manure—fair growth, but very slow.

Beans—very good growth, rapidly filling tube.

Sugar beet—fair growth; much lighter mycelium than the above, with prompt oidial development.

16. Tricholoma personatum.

Leaves-very good growth throughout.

Seil-very good growth throughout.

Manure—growth slow, but eventually good,

Beans-good growth throughout.

Plates II, III, and IV show some of the more important of these species.

Taking into consideration the variable quality of the stable manner which may be obtained at all seasons; the value of half-rotted decidnous leaves as a substratum for Basidiomycetes is worthy of special emphasis. The writer has found such material more readily sterilized than manner, and usually more prompt than the latter to give growth.

In order to test in pure cultures the probable effect of fertilizers as indicated by any marked increase in the rapidity of growth of the mycelium, experiments were made by adding a small quantity of ordinary nutrient salts to test tubes containing manure. A chlorid and a nitrate of the following salts were employed, viz, ammonium, calcium, magnesium, and potassium. In addition, dibasic potassium phosphate and also sodium chlorid, as well as control cultures, were used. Three tubes were employed with each of the compounds mentioned. There was no marked difference in the amount or rapidity of the growth noted, as found by comparing the averages of growth.

It seemed possible, however, that some slight advantage resulted from the calcium compounds, but there was no pronounced benefit in any tube. Further reference is made to the use of nutrient salts in mushroom growing in another chapter.

Growth on chemically known media. - In an attempt to determine somewhat more accurately the value of different compounds as nutrients, particularly carbohydrate and nitrogenous substances, several series of extensive tests have been made with Agaricus campestris, and also with Agaricus fabaceus and Coprinus comatus. These fungi do not grow readily in liquid media, and it has been difficult to obtain a wholly reliable and satisfactory substratum, one which would itself be practically pure, or well known, chemically, and at the same time effective for its purpose. After unsatisfactory attempts with various gelatinous solid media, with charcoal, etc., it was decided that the commercial gray filter paper had more to recommend it than any other substance suggested. Accordingly, all experiments were made in Erlenmeyer flasks of 150 c. c. capacity, and in each flask was placed about 6 grams of this paper wadded into pellets. The latter was moistened in each ease with the nutrient solution used. The flasks were subsequently sterilized in the autoclave and then inoculated with a very minute fragment of straw with the fresh mycelium from a pure culture on manure.

Tabulation of special results.—In the following tables are given the results of two out of several series of experiments, which have been conducted in order to throw some light on the point just discussed. These tables include, also, many cultures on media of unknown composition.

Table V.—Results of growth on media—First series of experiments.

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Table V.—Results of growth on media—First series of experiments—Continued.

	Medium.	Extent of growth.
1	Dextrose, 1‡ per cent	Slight.
Sk.	Solution A and mannite, 1\(\frac{1}{2}\) per cent.	Do.
1		
Į.	Mannite, 1½ per cent	(Contaminated with Asper
)	Solution A and maltose, 1½ per cent.	gillus. Fair growth, ye. lowish in color.
ŀ	Maltose, 1} per cent	Pair.
1	Solution A and potassium tartrate, ‡ per cent	Slight. Do.
í	Potassium tartrate, † per cent	
1	Solution A and magnesium tartrate, } per cent	Do
Ì	Magnesium tartrate, ‡ per ceut	
1	Solution A and ammonium tartrate, ‡ per cent	Do.
Į	Ammonium tartrate, ‡ per cent.	Do.
,		1 50.
Ì	Solution A and potassium lactate, ‡ per cent.	Do.
j	Potassium lactate, ‡ per cent	Slight to fair.
J	Solution A and magnesium lactate, ‡ per cent	Do.
1	Solution A and magnesium lactate, ‡ per cent Magnesium lactate, ‡ per cent Solution A and ammonium lactate, ‡ per cent	$\left\{\begin{array}{c} D_0, \\ D_0, \end{array}\right.$
ĺ		
	Ammonium lactate, } per cent	Slight.
	Solutiou A and calcium hippurate, ‡ per cent	Very good.
1	Calcium hippurate, ‡ per ceut	Slight.
ĺ	Solution A and asparagin, ½ per cent	[] D0.
ļ		201
į	Asparagin, ‡ per cent	1
ì	Solution A and peptone, ‡ per cent	i Do
	Peptone, ‡ per cent	Very slight. Do.
	Solution A and casein, per cent	Do.
	Casein, per cent	
	Solution A and pepsin, ‡ per cent	Slight to fair.
Ì	Pepsin, ‡ per cent	D .
ŀ	Solution B	Do.
Į	Solution B and asparagin, ‡ per cent	Do. Slight.
Į	Solution B and peptone, I per cent	Nery slight,
Ų		(Very good.
k	Solution B and casein, † per cent	Čulture lost.
	Solution B and pepsin, ‡ per cent	Do. Slight to fair.
ļ	Bouillon	Do. Very good,
ļ	Bean decoction) Do.
į	Beet decoction	Good to very good. Do.
F	Manure decoction	Very good. Do.
1	Manure	Do. Do.
,	Wheat straw	Lost. Do.
1	Solution A and wheat straw	l Do.
1	Solution B and wheat straw	Do. Do.
1	Solution B and NH ₄ NO ₃ and cane sugar.	Slight to fair.
ľ	Solution B and cane sugar and Ca(NO ₃) ₂ , ½ per cent	Very slight.

Table V.—Results of growth on media—First series of experiments—Continued.

No.	Medium.	Extent of growth.
56a 56b 57a	Solution B and sugar and Mg(NO ₃) ₂ , ‡ per cent	(Very slight. Do.
57a 57b 58a 58b	Solution B and Sugar and NH ₄ Cl, ‡ per cent Solution B and NH ₄ NO ₃ , ‡ per cent	Do. Do. Do.
59a 59b 60a 60b	Solution B and Ca(NO ₃₎₂ , ½ per cent Solution B and Mg(NO ₃) ₂ , ½ per cent	Do. Do. Do.
61a 61b	Solution B and NH ₄ Cl, ‡ per cent	Do. Slight to fair Do.
62a 62h	Mushroom decoction	Do. Do.

Table VI.—Results of growth on media—Second series of experiments.

No.	Medium.	Extent of growth.
la lb	Fresh horse manure (grass-fed animals)	(None. Slight.
2a 2b	Fresh horse manure, thoroughly washed, residue only used	Contaminated,
3a 3b	Filtrate, or liquid resulting from washing No. 2.	Good. Contaminated.
4a 4b	Decoction of fresh horse manure, as in No. 1	Fair.
5a 5b	Fermented horse manure, thoroughly washed	Very good. Do.
6a 6h	Filtrate or washing from No. 5	Slight.
7a 7b	Rotted stable manure	Good. Contaminated.
8a 8b	Decoction of green timothy hay	Good.
9a 9b	Residue from decoction in No. 8	None. Do.
10a 10b	Strong bean juice	Slight. Contaminated.
lla	Weak bean juice	Good,
12a 12b	Strong decoction of mushrooms	Slight.
13a	One-half strength decoction of mushrooms	Slight. Coutaminated.
13b 14a 14b	Weak decoction of mushrooms.	
15a	Oat straw	Contaminated, Slight.
15b 16a	Wheat straw	Good.
16b 17a	Corn meal	Fair.
- 17b 18a	}{ gram cane sugar in 25 c. c. solutioù A	Slight. Confined to nocules.
18b 19a	} gram milk sugar in 25 c. c. solution A	Slight throughout.
19b 20a		1 120.
20b 21a	description of the state of the	Slight, but contaminated, Slight at top.
21 b 22a	1.0	Confined to nocules.
23a 23b	d gram glucose in 25 c. c. solution A	
24a 24b	}4 gram dextrose in 25 c. c. solution A	Fair throughout.
25a 25b	aram mannite in 25 c. c. solution A	(Fair.) Contaminated.
26a 26b	aram glycogen in 25 c. c. solution A	
27a 27b	gram maitose in 25 c. c. solution A	Slight at top.
28a 28b	aram levulose in 25 c. c. solution A	Slight. Slight at top.
29a 29b	H gram glycerin in 25 c. c. solution A	Confined to nocules.

Table VI.—Results of growth on media—Second series of experiments—Cont'd.

	Medium.	Extent of growth.
:	gram potassium tartrate in 25 c. c. solution A.	(Veryslight; contaminated
Í	gram magnesium tartrate in 25 c. c. solution A	Confined to nocules.
H	gram potassium lactate in 25 c. c. solution A	Do.
$\left\{ \right\}$	gram potassium lactorhosphate in 25 c. c. solution A	Slight at top.
' K		Do.
	gram magnesium eitrate in 25 c. c. solution A	Do. Do.
- K	degram magnesium malate in 25 c. c. solution A	Do.
ij	gram calcium hippurate in 25 c. c. solution A	I Do.
	‡ gram asparagin in solution A	Slight. Do.
3 1.	‡ gram urea in solution A	Confined to nocules.
1	gram peptone in solution A	Fair at top.
- 15	gram casein iu solution A	Fair throughout.
Ì	gram benzoic acid in solution A	None. Do.
	gram benzoic acid in solution A	j Do.
. }	Solution A.	Confined to uocules.
Ų	Solution B	Do.
1		Do. Do.
Į.	Distilled HO	Do. Fair throughout.
Ì	Decoction from productive old bed	Do.
	Oak sawdust, only slightly rotted	Confined to nocules. Do.
ĺ	Gluten meal and water	Good throughout.
1	Cotton-seed meal and water	Contaminated.
- 131	Cotton-seed meal	Slight at top. Contaminated.
-lj	gram asparagin in solution B	Slight at top.
}	gram asparagin in solution B.	Do. Fair.
1	gram urea in solution B	None,
{		Slight at top.
ſ	gram urea in solution B	Do. Slight throughout.
}	½ gram urea in solution B	Do.
}	gram peptone in 25 c. c. solution B	Slight at top. Do.
}	gram peptone in 25 c. c. solution B	Do. Do.
	gram peptone in 25 c. c. solution B	Fair at top.
13	gram peptone and 18 gram NaNO3 in solution B	Fair throughout.
	gram casein in 25 c. c. solution B	(Very slight.
1	gram casein in 25 c. c. solution B	Fair at top.
3	% gram casein in 25 c. c. solution B.	Slight at top. Fair throughout. Do.
Ŋ		Do. Slight at top.
{	‡ strength albumen (egg)	Do. Good throughout.
}	Oil meal and water	Do.
ر	White pine shavings.	Very small area, but copous. Do.
}	White pine shavings with hean decoction	Do. Do.
}	Asbestos with bean decoction	Confined to nocules.
	Old flake spawn	Very good. Do.

It is not possible here to enter into a detailed discussion of the results, but attention is directed to the fact that under ordinary conditions Agaricus campestris does not give a copious growth when nitrogen is furnished from an inorganic salt and carbon in the form of the well-known sugars. Calcium hippurate in a solution of the necessary salts has almost invariably given better growth than other organic salts and carbohydrates. In general, casein has been a better source of carbon, or of earbon and nitrogen, than other proteids.

When the manure is of good quality it furnishes, in pure cultures, a source of necessary nutrients, whether fresh or fermented, whether as a decoction or an infusion (a cold aqueous extract).

Acid and alkaline media.—Manure which has undergone fermentation for a few weeks is usually slightly acid in reaction. Under certain conditions of fermentation the acidity is increased, and this is probably an important factor in making the manure from animals fed with green foods less valuable for mushroom work. Some acid tests were made of beds which had failed to yield satisfactory results, and in many instances it was found that the acid content was much above the normal. A small series of experiments was therefore instituted to determine the relative amount of acidity or of alkalinity most favorable for the growth of the spawn under pure-culture conditions. In this test there were also included several other edible fungi, the results of all of which are included in the table below. These experiments were made in large test tubes, and in such a test it was impracticable to determine absolute acidity or alkalinity, and from the results only a rough qualitative comparison could be anticipated. Potassium hydrate and lactic acid were used as reagents. The duration of the experiments was one month, and duplicate cultures were used in every instance.

Although the results are not wholly uniform, it may be inferred that in the case of Agaricus campestris a marked acidity of the medium would be unfortunate; Calvatia cyathiforme, on the other hand, seems to have grown somewhat better, in general, in the more acid media; Coprinus comatus grows under a wider range of conditions; and Coprinus atramentarius, in this instance, thrives in an alkaline medium. Further tests on a quantitative basis are required before definite conclusions may be drawn. This matter will also receive further attention when facilities are at hand for undertaking to better advantage than has yet been possible the practical growing of the other species, besides Agaricus campestris, included in this test.

Table VII.—Results of tests of acidity and alkalinity.

	Nature of	re of Extent of growth.								
Medium.	stable compost.			Coprinus co- matus.	Coprinus atra- mentarius.					
4 drops KHO	Fresh Rotted		Very slight	Slight	1 good, 1 very slight. Contamina- ted.					
2 drops KHO	Fresh Rotted	l good, l fair Very good.		l very good, l excellent. Very good	Very slight. Good					
1 drop KHO	Fresh Fresh		Very slight None	do	Excellent.					
Control	Rotted	l fair. Veryslight	ted.	None	None.					
1 drop acid	Fresh	l contami- nated, l very good	1 slight, 1 good.	Excellent	Very slight.					
2 drops acid	(Rotted (Fresh (Rotted	do	Very slight do	do None	Do. Do. Do.					

TEMPERATURE AND MOISTURE.

The temperature factor is, next to that of good spawn, perhaps the most important in mushroom growing. It has been frequently stated that mushroom growing is not profitable when the temperature may not be maintained more or less continuously at from 50° to 60° F. It is very probable that the exact temperature which may be considered an optimum will vary somewhat in different sections of the country. It will be noted later in detail that the temperature factor acts not so directly upon the growth of the spawn or the production of mushrooms as indirectly to render some other conditions of the environment injurious. It is best to consider that in practice the optimum temperature for mushroom growing varies from 53° to 58° F.

When the matter of temperature was first under consideration, a series of pure cultures of Agaricus campestris was placed at different temperatures in the laboratory in order to determine the rapidity of growth. It was soon found that a temperature above 60° F, and, indeed, as high as from 80° to 85° F,, was much more favorable to rapid growth than a lower temperature, provided, of course, that the higher temperature did not encourage a too rapid drying out of the culture. It was soon definitely ascertained that the conditions of pure-culture growth are essentially different from those attending the growth of mushroom spawn in the bed. This was perhaps best indicated by comparing spawn grown in pots at 85° F, under impure conditions with similar spawn grown at 50° F. At the former temperature, even though the conditions of moisture were properly maintained, there was little or no growth. Foreign fungi, molds, and bacteria, as well as insects, were, however, abundant. At the lower temperature there was little or no evident appearance of other fungi, molds, or insects;

yet the mushroom spawn grows slowly and continuously so long as other conditions are maintained. From numerous experiments of this nature it is apparent that the temperature relation is one which is governed by the competition to which the mushroom spawn is subject in the bed. This is, of course, wholly in accord with the results obtained from the study of the relative growth made by mushroom spawn in fresh and composted manures.

The statement previously made, therefore, that the optimum temperature may vary slightly in different localities is true on account of the fact that the mites, insects, and other animal pests of mushroom growing may vary considerably in different localities, or under different conditions, even though there may not be a great variation, perhaps, in the bacterial and fungus flora of the compost upon which the mushrooms are grown. Certain insects, for example, are more abundant in a moist climate, but if special precautions can be taken to eliminate all such pests, the growth problem is confined to the interrelation existing between the mushroom spawn and the microscopic flora of the compost. Mushrooms grown in the open will probably show greater variation with reference to the temperature factor than those grown in caves or cellars.

While a number of interesting problems would be presented by a study of the interrelation of the mushroom mycelium with that of other microscopic fungi present in the compost, these are matters of detail; and it has been wholly impossible thus far to give any attention to suggestions which have been furnished by the experimental data. It may be possible that other species of mushrooms are more independent of insects and other microscopic fungi, and such fungi may therefore be more suitable for cultivation at high temperatures than is Agaricus cam pestris or any of its close allies. A considerable effort is being made to obtain spawn of certain species of Agaricus, and also of other edible mushrooms which make their appearance during the warm weather. At this time, however, it is not possible to say what results of value may be anticipated from this line of work.

The direct effect of a temperature above the optimum upon the sporophores is manifest through lengthening of the stipes and rapid expansion of the caps, ordinarily accompanied by toughness and decreased size. In other words, the lower grade market product is produced at the higher temperature.

The moisture factor is also one of importance. It is undesirable that the place in which mushrooms are grown should be very damp, or dripping with water. Nevertheless, a fairly moist condition of the atmosphere should be maintained throughout the growing and productive period. There should be a gradual but slight evaporation from the surface of the beds, and sufficient ventilation to insure this

is believed to be essential. It is certain that in poorly ventilated caves mushrooms do not succeed. On the other hand, in a dry atmosphere, or exposed to drying winds, mushroom beds soon cease to bear, while such sporophores as are developing may have their caps cracked and torn.

Mushrooms are grown in cellars, caves, or specially constructed houses largely on account of the fact that temperature and moisture are then practically under control. The nature of the structure or cellar which is constructed for mushroom growing must be determined, therefore, not merely by its expense, but by the effectiveness of the structure in regulating the factors indicated under the particular climatic conditions.

It is not possible at this time to discuss cellar or house construction, and the accompanying illustration of mushroom houses (Plate VI, fig. 1) must suffice to give an idea of the types which are in use.

PREPARATION OF THE COMPOST.

It is not to be understood that there is one and only one method of preparing compost for mushroom growing. Nor is it always necessary that the compost shall be in one particular stage of fermentation or decay. In fact, every change of condition elsewhere may necessitate a similar change in the amount of fermentation which may be most desirable. At the outset it should be understood that it is not the "fermentation" which is absolutely essential." The

^a Répin, I. c. (See translation in The Garden (London), February 5, 1898. Special reprint, pp. 10–16.) Here it is stated that "manure is rendered capable of supplying nutriment suitable for mushrooms only by means of fermentation;" further, that "all the higher orders of mushrooms, the spores of which I have succeeded in causing to germinate, have a sterile spawn of a similar nature." Again, the conclusion is expressed somewhat indefinitely that manure is "rendered suitable" by means of chemical combustion, which is said to proceed rapidly only at a temperature above 178° F.; that it is not the soluble substances in the manure which are valuable, but rather the cellulose matter, together with the necessary salts.

In this connection it is of interest to note that the material constituting many of the beds in the experimental cellar at Columbia, Mo., were fermented at comparatively low temperatures. A complete temperature record was kept of 18 small compost piles in which special kinds of manure were prepared, and in only one instance was the temperature in any pile more than 140° F. In some cases 120° F, was the maximum attained.

Répin implies that mushrooms will not grow in manure until there has been effected "the destruction of all the soluble organic matters, which disappear through the agency of bacteria or are consumed in the process of oxidation." Very simple nutrition experiments clearly demonstrate that these conclusions are erroneous.

It may be stated, however, that peculiarities appear when the fresh manure contains certain compounds which render it injurious; for example, the mycelium does not grow readily in pure culture upon fresh manure from animals fed almost wholly on green forage. Such manure is improved by fermentation.

"fermentation" is of itself a minor matter. In pure cultures, where sterile media are employed, mushroom spawn starts slowly, but finally grows best, in general, upon fresh (wholly unfermented) manure. It grows least well, or, rather, less densely, so far as tested, on very well fermented manure. This certainly indicates that it is not fermentation which is ordinarily advantageous. In practical mushroom growing, however, it is not possible to deal with pure cultures; and, therefore, other conditions of the environment must be correspondingly changed. The rapid oxidation action of bacteria, and perhaps of independent ferments, upon manure causes a considerable rise of temperature. At the higher temperatures (which may be maintained as long as there are present rapidly oxidizable food products) bacterial action is vigorous, and is unquestionably injurious to mycelial development. Wholly aside from the rise of temperature accompanying their activities, bacteria are otherwise injurious. In fact, manure which is put to ferment in a small test tube shows little or no rise of temperature above that of the place in which it is incubated. Nevertheless, the mycelium of the mushroom will not grow under such conditions. Rapid bacterial action is therefore prejudicial. Under those conditions where bacterial action is not rapid, fresh manure might be used to advantage; in other words, if the beds are so constructed that the manure ferments very gradually, without either excessive bacterial action or rise of temperature, then spawning might be made in fresh manure.

The old belief that rotten manure does not have the necessary strength—that is, does not produce so vigorous a mushroom growth as that which has been less transformed by bacterial action—has been confirmed by practical experiments. This loss of effectiveness is probably due, in part, to a change in texture or to other physical changes. In well-rotted manure there is ample food material to support a very good growth of mycelium in pure cultures. This has been chemically proved by sterilizing such manure and growing mushroom spawn upon it in pure culture. Nevertheless, by comparing (in Table VIII) No. 12 with Nos. 13, 14, and 15, it will be seen that beds prepared with well-fermented manure and left for some time before spawning do not yield so well. It is believed that here the physical condition has much to do with the result.

The latter does not by any means invalidate the following practice, which has commended itself to some very successful growers: The manure is piled in very large compost heaps, where it is kept moist and is turned only once or twice. It ferments very slowly. Then it is carted into the cave, or mushroom house, long before it could be considered in proper condition to be spawned. The beds (usually flat when this is the procedure) are made immediately. These are fairly well moistened and compressed, then left to undergo a gradual

fermentation, which may require a month. When the manure shows a tendency to fall to the temperature of the room it is spawned. Meanwhile, it will doubtless be found that a heavy crop of some small species of Coprinus will have appeared. The presence of this fungus is not injurious, but rather it may be taken as an indication that the conditions are favorable.

Ordinarily the manure is obtained as fresh as possible. It should include the straw used in bedding the animals, and the quality of the straw will determine to some extent the value of the manure. straw of cereals is far better than that of most other grasses. The more resistant straws seem greatly to improve the texture of the compost for mushroom purposes. Commercially it is a mistake to attempt to get the manure free from straw. If fresh manure is not obtainable, that which has been trampled by the animals is ordinarily rich, well preserved, and desirable. It ferments best in large piles, and these may be of considerable extent, about 3 or 4 feet deep throughout. If not uniformly moist the material should be sprinkled. At no time is a very heavy watering desirable. In from four days to a week or more the compost should be turned, or forked over, and a second turning will be required a week or ten days later. Water should be added only when necessary to maintain a moist (but not a wet) condition. With this amount of moisture, and with the piles deep enough to become fairly compact as a result of their own weight, there will be little danger of any injurious fermentation. During the normal fermentation the temperature may rise higher than 150° F. In from fifteen to twenty-one days or more, depending upon the conditions, the temperature will begin to fall, and the compost may be used in the construction of the beds. When used in the beds, it has ordinarily lost all objectionable odor, and the color of the straw has changed from yellow to brown. In figure 2 on Plate V is shown a shed in which the manure is composted during the summer.

As stated in Farmers' Bulletin No. 204:

It is the custom with some growers to mix a small quantity of loam, about one-fourth, with the mannre. This enables one to use the manure earlier; and, indeed, under such circumstances it may sometimes be used with but little or no composting. Nevertheless, the majority of growers have obtained greater success by the use of the manure alone, and this is also the writer's experience. Very well-rotted compost should not be used in mushroom growing if large and solid mushrooms are desired. When sawdust or shavings are employed for bedding the animals, the composting may require a somewhat longer period.

It has been the experience of some of the most successful growers that the use of shavings for bedding material in the stables does not injure the value of the product for mushroom work. The presence of a large amount of sawdust is, however, objectionable so far as the writer's experience goes. Compost containing much sawdust is

necessarily very "short," and therefore the physical condition is not the most favorable for Agaricus campestris.

In another chapter attention is called to the fact that the value of the manure depends to a considerable extent upon the feed given the animals. It would not be wise to depend upon that obtained from stables in which hay and green foods are used to too great an extent. Moreover, it is not believed that compost made from the manure of cattle barns is in mushroom growing as desirable as stable manure.

In some cities the municipal ordinances require that the manure shall be promptly removed from the feeding stables or that it shall be disinfected. In the latter case crude carbolic acid, or even corrosive sublimate, may be used to secure this end. Manure thus disinfected is, of course, undesirable for mushroom work. For the same reason the manure of veterinary hospitals is of questionable value.

It is not wholly improbable that some other waste products of the farm, field, and forest may be utilized in mushroom growing; nevertheless, no such product has yet been found which, under the conditions of the experiment, has yielded sufficiently to make it of special interest in growing Agaricus campestris. Among the products which have been tested, either alone or in conjunction with some commercial fertilizer, are the following: Leaves of decidnous trees, needles of conifers, sawdust, cotton-seed hulls, cotton seed, corn stover, sorghum stover (or bagasse), rotten hav, sphagnum, and yeddo fiber. The writer is convinced that greater profit may be anticipated, for the present, at least, if the culture of Agaricus campestris is confined to manure; and if other edible forms which grow in the woods are used in beds of leaves, etc., as indicated elsewhere in these pages, it is quite possible that such a fungus as Coprinus comatus may be grown successfully in this latter way. It may, however, be too much to hope that the morel may also be thus made amenable to culture, although leaf mold is in nature the favorite habitat of this fungus.

From the prompt and abundant growth of Agaricus campestris on half-rotted leaf mold in pure cultures, it was thought that mushrooms might be grown to advantage upon this product. The practical experiments made to test this point are distinctly discouraging, as shown by reference to No. 17, Table VIII; Nos. 3 and 4, Table IX, and No. 11, Table X.

For the most part manure may be composted in the open air. It may, however, be prepared with greater uniformity under cover. During midsummer, protection may be desirable on account of drying out, while in the winter it is more important in case of excessive cold. If it is necessary to compost manure during the winter, moreover, the piles should be of considerable depth.

INSTALLATION OF BEDS.

In making the beds, as well as in other phases of mushroom work, regard must be had for all environmental conditions. The type of bed should be determined by convenience, and the size, to a certain extent, by the temperature to which the beds may be exposed. The flat bed, frequently referred to as the English type, is more commonly employed in the indoor work in England and America. With this type merely the entire floor space may be utilized, as illustrated in the frontispiece, Plate I, or the beds may be arranged in tiers of shelves. In figure 1 on Plate V a view may be had of the supports for shelf beds in a large commercial house. In this house there is the greatest economy of space. The shelf system gives the greatest amount of bed space and is certainly most economical where the floor space is an important factor. Such beds do not require great depth, but merely sufficient to insure an ample development of spawn. They should be from 8 to 10 inches deep after being firmed or compressed.

The ridge-bed system is employed almost exclusively in the caves about Paris. This system is also in use in open-air culture. It may be used to advantage in low cellars, caves, or houses when labor is not too expensive. Ridge beds increase slightly the surface area and permit of easy passage from one part of the cave to another. The size of such beds in caves, or under other conditions where the temperature remains practically uniform, should be not more than 2 feet wide at the base and 15 inches high, tapering gradually to the top when compressed. Slanting beds are commonly employed next to the walls. Large beds are desirable under changeable open-air conditions.

The prevalent opinion among amateurs that the bed should always be deep enough to maintain a considerable heat is believed to be erroneous. Grown under more or less uniform conditions, mushrooms seem to require no bottom heat, and the bed should fall to the temperature of the room some time after spawning. Bottom heat, and hence large beds, are, however, desirable when sudden changes of weather would so reduce the temperature of the bed as to delay growth. Under similar conditions, as well as in dry air, mulching may be required.

As previously stated by the writer in Farmers' Bulletin No. 204 of the Department of Agriculture—

In any case, the manure is made up in the form of the bed desired and should be firmed, or compressed, to some extent immediately, in order to prevent drying out and burning when the secondary fermentation takes place. At this time the manure should be neither wet nor dry, but merely moist. The only practical test of the proper moisture content of the manure which can be relied upon is when, upon compression, water can not readily be squeezed out of it.

SPAWNING AND CASING THE BEDS.

From what has been said concerning the temperature requirements. it will be evident that spawn should not be inserted in the beds until the temperature has fallen low enough to insure successful competition on the part of the mycelium with other organisms. In many articles on mushroom growing it has been suggested that beds may be spawned when the temperature has fallen to about 90° F. From experience and observation, the writer can only conclude that such a temperature is frequently fatal, and it is believed that the temperature of the beds should be permitted to fall to 70° F, before being spawned. In fact, the most successful results have been obtained at temperatures from 65° to 70° F. It was formerly believed that if the spawn were inserted at 90° F, this higher temperature incited the rather dormant mycelium to rapid and vigorous growth. It is clear, however, that the rapid development of new mycelium from the pieces of spawn brick inserted is not so important a factor as suitable conditions for continued growth. If the temperature falls rapidly from 90° F. after spawning, however, no injury may result. Nevertheless, it is to be considered an unfortunate condition.

The bricks of spawn may be broken into from ten to twelve pieces, from 1\(\frac{1}{2}\) to 2 inches square. These pieces may be inserted about 1 inch beneath the surface of the manure. In flat beds they may be placed from 10 to 12 inches apart throughout the bed, and in ridge beds the pieces should be inserted on each side alternately, one near the top and the next near the bottom. It is well to insert the pieces vertically, as the mycelium does not then seem so readily to suffer damping off. After spawning, the beds should again be firmed, and they are then ready to be cased or loamed whenever this process may seem most desirable. At the time of spawning the beds should be in the best condition possible for the growth of the mycelium. Delay in growth at this time is one of the surest indications of a light yield. If the bed contains the proper amount of moisture, and if the walls and floors of the house or cellar are sprinkled occasionally, so as to maintain a moist condition of the atmosphere, it is possible to avoid wholly the use of water upon the beds immediately after spawning. In no case should a bed recently spawned be heavily watered. surface may be sprinkled, if there is a tendency toward drying out. The same test for moisture content as has been outlined previously in these pages in the chapter on preparing the manure should be followed. The beds should become gradually somewhat drier, however, during the growth of the spawn.

The absolute water content for the bed at the time of spawning should be about 40 per cent, although this will vary considerably, according to the conditions, and especially with relation to the quantity of straw in the manure.

If the spawn grows rapidly at first and spreads throughout the bed, it will not be injured by a slight drying out, or by a temperature even as low as 32° F. On the other hand, a continuous high temperature for several days, or excessive watering, is sure to result in an irreparable injury. In several instances where the experimental beds of the writer have been made during the late autumn, and where a vigorous growth of spawn has been secured before the advent of the coldest weather, the beds have remained unproductive throughout the winter months, or so long as the temperature remained intermittently below 40° or 50° F. With warmer weather, these beds have come into bearing several months later, and where the temperature has then remained favorable for some time a good yield has been obtained. In this case, moreover, the bed will bear much longer at a temperature of 60° F., or above, than if the temperature has been constantly in the neighborhood of 60° F, throughout the growing season of the spawn. As a rule, beds thus filled with spawn and then subjected for a time to cold conditions yield at the outset much larger mushrooms than beds exposed to a more constant temperature, even if this constant temperature may be the optimum.

At any rate, the beds must be "cased" as soon as convenient after the spawn is inserted. As a rule, one should wait from one to two weeks in order to be sure that the spawn is growing. Casing consists in applying to the bed a layer of loam from 1 to 1½ inches deep. In France the casing soil consists usually of calcareous earth, sometimes mixed with loam. Ordinary loam of almost any quality will suffice. This should be secured in advance, and it is well to protect it from the weather, so that at a convenient time it may be worked over and, if necessary, screened, in order to free it from large pebbles or trash. When the loam is applied, it should, on ridge beds, be carefully firmed. When cased a bed should require watering for the most part merely to maintain a moist surface.

MUSHROOM GROWING.

EXPERIMENTS AT COLUMBIA, MO.

The practical experiments in mushroom growing which have been undertaken at Columbia, Mo., were designed, in the first place, to determine the exact effect of conditions upon the growth of mushrooms, and in the second place to test or immediately apply the results obtained or suggested by the laboratory work. The effects of temperature, moisture, etc., have already been discussed, and the conclusions drawn have been based upon the most careful observations of the experimental beds, as well as upon the evidence which has been obtained by a personal study of the conditions in commercial mushroom houses and caves both at home and abroad. It is needless to give in detail the record of all failures or of poor yields

invariably obtained when the conditions were unfavorable—that is, when they were beyond the limits which have been more or less definitely stated as requisite. On the other hand, the results which are given do not represent the best yields obtained; they are those which seem to be most instructive.

The experimental work has been seriously handicapped in one particular. With only one set of experiments (those recorded in Table VIII) has it been possible to maintain a temperature constantly between 50° and 60° F. Unfortunately a north basement room which gave those results during the winter of 1903—4 has not since been available for the work. The results are, however, comparative when not absolute.

The results given in Table VIII are referred to in various parts of this bulletin. Attention should be directed to the fact that many of these beds were yielding well when the experiment was necessarily closed to make room for a second series of experiments planned during the same winter. Beds Nos. 6, 9, 13, 25, and 40, for instance, each yielded between 8 and 15 ounces the day the experiment was closed, while beds Nos. 2, 10, 14, 23, 26, 30, and 37 each yielded 1 pound or more on the same day.

It is to be noted that a considerable number of beds in this series produced more than 1 pound per square foot, and some nearly 2 pounds for a similar area. It is certain that some beds would have yielded more than 2 pounds if they could have been permitted to produce longer.

Table VIII.—Yields of experimental mushroom beds.

Number of the experi- mental bed.	Material used in the bed.	Source of the spawn.	Number of days to produce mushrooms.	Yield in ounces first 30 days.	Yield in ounces second 30 days.	Total yield in ounces at close of experiments.	Area in square feet per bed.	Yield in ounces per square foot.
1	Fermented horse manure.	Alaska, old	27	53	54	107	6	18,0
2 3	dodo	Old American made English, current year market	104 51	20 7		20 7	6 6	$\frac{3.6}{1.0}$
4 5	dodo	product. English, 2 years old. English, 1 year old.				0	6 6	0.0
6	do	Alaska, U.S. Department of Agri- culture.	51	47	68	115	6	18.8
7	do	Bohemia, U. S. Department of Agriculture.	53	48	17	65	5	13.0
8	do	Mixed varieties, U.S. Department	51	78	34	113	6	18,6
9	do	Bohemia, U. S. Department of	68	102		102	6	17.0
10	do	Bohemia, U. S. Department of	46	71	65	136	6	22.6
11 12	Formented horse manure (bed left for 2 months before	Agriculture, heavy spawning. Agaricus amygdalinus, old. Bohemia, U. S. Department of Agriculture.	61	5		0 5	6 6	0.0 0.8
9 10 11	do	of Agriculture. Bohemia, U. S. Department of Agriculture, light spawning. Bohemia, U. S. Department of Agriculture, heavy spawning. Agaricus amygdalinus, old. Bohemia, U. S. Department of	68 46	102 71		102 136 0	6 6	

Table VIII.—Yields of experimental mushroom beds—Continued.

Number of the experimental bed.	Material used in the bed.	Source of the spawu.	Number of days to produce mushrooms.	Yield in ounces first 30 days.	Yield in ounces second 30 days.	Total yield in ounces at close of experiments.	Area in square feet per bed.	Yield in ounces per square foot.
13	Fermented	Bohemia, U. S. Department of	49	110	50	160	б	27.7
14	horse manure.	Agriculture.	61	241	40	281	12	23.4
15 16	Leaf mold	Calvatia cyathiforme				0	6	0,0
17	do	Bohemia, U. S. Department of Agriculture.				0	6	0,0
18	Fermented sta- ble manure; bed fairly com-	Alaška, U. S. Department of Agriculture.	48	118	71	189	9	21,0
19	pact. Fermented sta-	do	5 3	93	30	123	6	20.5
20	ble manure.	Bohemia, U. S. Department of	48	101	39	140	6	23.3
21	do	Agriculture. Var.y, U. S. Department of Agri-	53	96	37	133	6	22.2
2:2	do	culture. American commercial more than				0	6	0.0
23	đo	1 year old. American commercial, Bohemia	53	111	58	164	8	20.5
24 25	do	-p.do	51	46	67	113	9	15.2
		Agriculture. Loose cakes; dried.	46	20	50			12.0
26	do	Bohemia, U. S. Department of Agriculture. Loosecakes; dried. Bohemia, U. S. Department of Agriculture. Watered freely late.	49	74	75	159	6	26, 6
27	do	Bohemia, U. S. Department of Agriculture. Watered freely. Bohemia, U. S. Department of	49	42	51	93	6	15, 5
28	do	Bohemia, U. S. Department of Agriculture.	46	89	30	119	6	19.8
29 30	Fermented sta-	do	55 51	90 129	55 146	145 275	8 9	18.1 30.5
	ble manure and 5 pounds cot-		.,,					
31	ton-seed meal.	English communical Ct. Louis				0		0.0
	Fermented sta- ble manure.	English commercial, St. Louis				0	6	0.0
32 33	do	English commercial, New York Bohemia, Americal commercial	42	70	32	102	6	$\frac{0.0}{17.0}$
34 35	do	Alaska, American commercial	46	- 70	31	101	6 8	$\frac{16.7}{0.0}$
36	Fermented sta- ble manure and cotton-seed	Bohemia, U. S. Department of Agriculture.	53	47	\$#6	143	9	15. 9
37	hulls. Fermented sta-	do	61			104	6	17.3
5,	ble manure; bed heavily compressed.							
38	do	Var., U.S. Department of Agri- culture.	46	58	46	104	6	17.3
39	Fermented sta- ble manure and sphagnum.	do	46	11	11	22	6	3, 7
40	Fermented	do	50	44		44	6	7.3
41		do	46	18	39	57	8	7.1
1	ble manure, cotton-seed hulls, and cot-							
4.2	ton-seed meal. Fermented cot- tou-seed hulls and cotton-	Bohemia, U. S. Department of Agriculture.	55			5	9	0.6
43	seed meal. Manure mold	do				2	6	
43	Sod	Calvatia cyathiforme. Pure cul-	1			0	6	0,0
		tures.	***			- 1		
45	Old compost, left 2 months be- fore spawning.	Bohemia, U. S. Department of Agriculture.	52			5	9	0,6

The series of experiments outlined in Table IX followed directly upon the series given in Table VIII. The beds in the first series were made in midwinter, and as the manure had been well fermented there was little or no rise of temperature after the beds were made. The spawn was therefore inserted at an unusually low temperature. During thaws in the late winter there was considerable seepage through the walls of the room. Some of the wall beds—Nos. 14 to 21—were seriously damaged, but although beds Nos. 7 to 13 were also wall beds seepage was not evident in this region. Within about thirty days after vigorous mushroom production began in this series the basement was flooded, and the work was therefore brought to an abrupt close. The yield up to that time is given, however, since in this series there are included many fertilizer tests.

Table IX.—Yields of experimental unshroom beds in a north basement room, 1904.

Number of the experimental bed.	Bedding material and fertilizer.	Spawn used.	Number of days to first picking.	Yield, in ounces, per bed.	Area of bed in square feet.
1	Stable manure and cotton-seed Bohulls,	ohemia, U. S. Department of Agriculture.	48	33	6
2		do	48	91	6
3	Leaf mold and stable manure		48	38	6
4	Ctable meaning and in become		48	36	6
5 6	Stable manure and sphagnum Stable manure and cotton-seed	do	61 61	64 64	6
О	meal.		OT	0.4	9
î		do	66	73	6
8	Stable manure, timothy fed	do	73	2	6
9	do	do		0	6
10	Stable manure, clover fed	. do		1	6
- 11	Stable manure, bran fed	do		3	6
12			66	84	6
13	Stable manure, corn fed	- do '	54	109	6
14a 15a	stable manure, corn reddo	do	71 68	12	6
150 16a	Stable manure, oats fed	do		3	6
17a	dodo		80	14	6
180		do	71	24	6
190	do	.do	66	40	6
200	do		71	17	6
210	do		68	55	6
	do		48	61	6
23	Stable manure and complete fer	do	64	ãõ	6
	tilizer: KCl, 1 ounce; KNO ₃ , 1				
24	ounce; bone meal, 7 ounces.	. do	64	30	6
24	Stable manure and incomplete fer- tilizer: NaNO, 1 ounce; bone	. (10)	04	-913	0
	meal, 7 onnes.				
25	Stable manure and NaCl, 2 ounces	do	66	41	6
26	Stable manure and NaNO3, 2onnces	_do .	48	42	6
27	Stable manure and MgSO4, 2 ounces	do	66	39	6
28	Stable manure and K ₂ SO ₄ , 2 ounces	do	64	46	6
29	Stable manure and kainit, 4 ounces	do	64	62	6
30	Stable manure and CaCl2, 2 ounces	doob	64	48	6
31	Stable manure and Na ₂ HPO ₁ , 2	_do	64	65	6
1343	ounces.	do	2.4	(1	,,
32			54	41	6
33	ounces. Stable manure and NaNO ₃₁ lounce;	do	68	30 1	6
രെ	kainit, 2 ounces,		tari	CAT.	,

 $[^]a\mathrm{Some}$ of the beds in this block—Nos. 14-21 $\,$ were seriously injured by seepage water, and the results are untrustworthy.

Table IX.—Yields of experimental mushroom beds in a north basement room, 1904—Continued.

Number of the ex- perimental bed.	Bedding material and fertilizer.	Spawn used.	Number of days to first picking.	Yield, in ounces, per bed.	Area of bed in square feet.
34	Stable manure	English commercial (ordered as	68	34	6
35 36	Stable manure, lime dressing		66 68	12 8	6 6
37	Stable manure, ammonium molyb- date, ‡ ounce.	do		(2)	6
38	Stable manure, ZnNO ₃ , 1 gram	do		(?)_	6 6
39 40	Stable manuredo		68 64	77	о б
40		Agriculture.	.,,		
41	do	English commercial (New York).	41	33	6 6
42	do	Bohemia, U. S. Department of Agriculture.	64	99	n
43	do	Spawn from old bearing bed		0	6
44	do	Pleurotus ostreatus		0	6
45 46	Stable manure and sawdust	English commercial (Philadelphia) Bohemia, U. S. Department of		0	6 6
40		Agriculture.			
47	Stable manure	Var. American commercial	48	60	6
44	do	Alaska, American commercial	64	22	- 65

From the experiments given in the foregoing table further proof is furnished of the fact that stable manure alone, when of good quality, is sufficient for the growth of mushrooms. The addition of nutrient salts as fertilizers has not, on an average, given any marked increase in yield, but rather the contrary. It is hardly possible that the quantity of salts used on the beds was too little to make the effect felt. On the other hand, it was not sufficient to be injurious. It is evident from the experiment in bed No. 29, for instance, that the addition of 4 ounces of kainit could not have been injurious. In some instances the results obtained by the use of fertilizers were poorer than where the manure alone was used. This, however, the writer believes to be due largely to differences in the spawn used, or the differences in condition owing to the location of the bed, for subsequent experiments with some of the salts which seemed to be either injurious or beneficial have not wholly confirmed these results. It is to be noted, however, from the experiment in bed No. 6 of this series and also from bed No. 30, in Table VIII, that the beds treated with cotton-seed meal have invariably yielded somewhat above the average. These beds do not come into bearing quite so rapidly as those in which manure alone is used. It is thought that this is due to the fact that bacterial action is at the beginning more rapid in beds containing cotton-seed meal, and that, consequently, when this wave of bacterial growth has passed the nutrition of the spawn is favorably affected. Experiments had already indicated that manner from animals which were fed a poor diet, such, for instance, as grass or hay alone, is much less valuable than where the animals are well fed. The experiments in beds Nos. 10 to 22 were designed to test the value of some different feeds. The writer was fortunate in being able to secure manure from work animals which were being used in feeding tests where very different foods were employed. Unfortunately, however, the mushroom beds were located next to a basement wall, and in beds Nos. 14 to 21 the results were vitiated by the fact that there was considerable seepage water in that region during the thaws and heavy rains of the spring. Nevertheless, it is believed that the experiments in beds Nos. 8 to 13 are trustworthy. An attempt was made to check these results by using some of this manure in tube cultures, and it was found that the manure used in beds Nos. 8, 9, 10, and 11 particularly was unfavorable for the growth of the mycelium even in the pure cultures.

On account of its stimulating action upon the spores of Agaricus campestris a small quantity of ammonium molybdate was applied to one bed, No. 37, in order to test its effect upon the growing mycelium. Moreover, since certain salts of zinc at considerable dilution have been found to increase greatly the quantity of mycelium produced by other fungi, zinc nitrate was employed in an adjacent experiment. The results of these two tests were the same. There was a profuse mycelial development and an abundant production of small deformed sporophores.

Table X also summarizes a series of some interest. These beds were spawned early in November, 1904. Soon after the spawn began to spread throughout the beds—about December 15—the temperature of the room fell to 40° F. From that time on until March 1, 1905, the temperature was constantly below 52°, and on several occasions as low as 32° F. After two or three weeks of warmer weather the beds began to bear vigorously, and the mushrooms, particularly the first ones, were of unusual size and of excellent flavor. Numerous individuals weighed from 6 to 8 ounces immediately after the separation of the ring, and a few mature specimens ranged from 10 to 14 ounces.

Table X.—Vields of experimental mushroom beds—Third series.

Bed No.	Material constituting bed.	Spawn used.	Compar- ative yield per bed, in ounces.
5 6 7 8 9 10 11 12 13 14		U. S. Department of Agriculture, Columbia. do do do	0 70 16 49 40 57 34 54 56 31 30 3 6 60

In some publications on mushroom growing the claim is made that old or practically exhausted beds may be brought into bearing again by heavy fertilization with liquid manure or with a weak solution of potassium nitrate. From a commercial point of view, no measurable success has resulted from any trials of this nature made by the writer; consequently, it is believed that exhausted beds should be immediately disearded. From the standpoint of mushroom sanitation, this is also particularly desirable.

VARIABILITY IN MUSHROOMS GROWN UNDER DIFFERENT CONDITIONS.

The writer does not intend to discuss even in a general way the relationships of the various forms of Agaricus-that is, those that may be considered allies of A. campestris—which he has cultivated or studied in the field. Some reference to the variability of common forms should, however, be made. For a comprehensive study of species and varieties, a knowledge of European forms as well as of those found in America is essential. Authors differ so widely in their descriptions of species, as well as in their conceptions of them, perhaps, that in the absence of unlimited material nothing short of confusion results from any attempt to harmonize opinions. It is sufficiently difficult to separate what many would regard as varieties of 1. campestris from those of 1. arvensis. When specific rank is bestowed also upon such forms as A. pratensis, A. villaticus, A. magnificus, A. rodmani, etc., the difficulties are greatly increased. The writer has grown many forms of Agaricus, and, as might be expected. there seems to be no form which will remain practically constant under variable conditions. Besides general size, size of spores, etc.,

some of the characters used in separating the common forms are color of gills; character of ring, particularly as to whether single or double; shape of stipe; color and markings of pilens; color of flesh, etc. In following the development of these characters in different forms. many variations will be found. Agaricus campestris grown on composted leaves shows very little pink in the gills. The color changes rapidly from dull pinkish-brown, or almost white, to a leaden hue. Several brown-capped forms, usually considered varieties of A. campestris, never show a bright-pink surface unless produced under exceptionally favorable conditions, moist air being a sine qua non. The ring is naturally variable. In any variety of A. campestris it is not uncommon for an edge of the partial veil to remain attached to the base of the stem as a volvate line, or this line may be left at any stage during the elongation of the stem. Again, if the lower margin of the partial veil on the stipe separates slightly from the stipe, and upon drying curves slightly upward, there is an indication of a double ring. A very good double ring appeared on a number of very vigorons specimens of an undoubted variety of A. campestris during the present season. It is possible that there is a greater tendency to produce a double ring when conditions are favorable for the production of the most vigorous mushrooms. Agaricus arvensis is also very variable with respect to the formation of a double ring, as also in the persistence of the partial veil.

The shape of the stipe is in many forms dependent upon the conditions. Under favorable conditions a brown variety of A. campestris may have a very short, thickened, equal stem, when grown on manure, and practically uniform at maturity, while the same form grown on decayed leaves may show in the main a stipe with thickened base, gradually tapering to the top. The color of the cap is of undoubted value as a varietal or specific character, yet it must be remembered that whether the surface be smooth or rough, merely fibrillose, or broken into scales of definite form, may depend entirely upon whether produced in moist air or in dry air, subjected to drying after being wet, etc. The color of the flesh is also dependent, to a considerable extent, upon the conditions. A specimen grown in even fairly unfavorable conditions will show the flesh somewhat darkened, and on exposure the characteristic pink tint will not be even momentarily visible. In other words, a considerable range of variation must be anticipated, and in comparisons there should be stated very clearly the conditions under which the particular forms are produced.

THE CULTIVATION OF VARIOUS SPECIES OF MUSIROOMS.

In Table X are given the results of a single test with Agaricus arrensis, or what is supposedly a brown variety of this species, and

also of a single experiment with A. rillaticus. In both cases the yield was excellent. It is not well to draw definite conclusions from individual tests, but it is believed that both of these forms will yield profitably in general culture under conditions similar to those required for A. campestris. Plate III, figure 2, indicates the size and compactness of the mature sporophore of A. rillaticus. Moreover, both of the species above referred to are to be recommended for texture and flavor. Two forms of Agaricus fabaccus (see Pl. III, fig. 1), both with amygdaline odor and flavor, have been tried in relatively few experiments. In no case has the yield been very good, and further experiments will be required before it will be possible to state under what conditions these forms may be most successfully grown. At the Missouri Botanical Garden Prof. William Trelease has for some time grown successfully one of these varieties.

Owing to the profuse and rapid growth of the mycelium of Coprinus comatus in pure cultures, it was anticipated that it might easily be grown in beds. The few experiments thus far made indicate that in impure cultures (beds) of leaf mold the mycelium grows and spreads very slowly. Hot weather prevented the maturity of the tests, but no sporophores were produced during a considerable period. In similar experiments Lepiota rhacodes and Tricholoma personatum were used. The former has given unsatisfactory results thus far, but the latter is promising.

It is not yet time to report on the possibility of growing the better and larger species of puffballs and the morel. It has already been indicated that the mycelium of these fungi grows well in pure cultures. From the pure cultures it has also been demonstrated that spawn may be made, but it has not been determined under what conditions the fruit may be produced. Figure 1 on Plate IV shows a young specimen of one of the puffballs, Calvatia craniiformis, the spawn of which is produced with the least difficulty.

COOPERATIVE EXPERIMENTS.

During the winter of 1902-3 a small quantity of experimental spawn made by the writer was sent out to mushroom growers for trial; in 1903-4 this spawn was made in large quantity, and trial packages were sent to more than 100 growers or interested persons. At that time Farmers' Bulletin No. 204 had not been issued, and the instructions which could be furnished inexperienced growers were inadequate. Nevertheless, an attempt was made to obtain reports from all persons receiving the experimental spawn, even from those who had applied for and received spawn when the season was too far advanced for successful work except in caves and cool cellars. A number of reports were received, but, as might be expected, fully 50 per cent of these indicated that the conditions under which the experi-

ments were made were wholly unsatisfactory, and that, therefore, no favorable results could be anticipated. Among those whose reports indicated that the conditions were favorable, or fairly favorable, only a small percentage reported failures, while four-fifths of those claiming success secured yields of more than one-half pound per square foot of bed space, many obtaining more than 1 pound per square foot. In two instances a yield of nearly 2 pounds to the square foot was reported. The frontispiece, Plate I, a bed in full bearing, and Plate VII, figure 1, showing the mushrooms as prepared for market, are photographs furnished by cooperating growers who are now also making spawn of pure-culture origin. It was suggested to growers who received the experimental spawn that a comparative test of the English or other commercial spawns with that received from the Department of Agriculture would be of interest. Comparative tests were made and reported by 10 growers. In most cases the English spawn, obtained at random on the market, failed to grow. In only one case did the English spawn prove better than the pureculture product, and in this instance the spawn furnished by the Department when used was nearly one year old.

Failures may always be anticipated when attempts are made to grow mushrooms under adverse conditions, and it must be said that greater success was obtained from the cooperative work than could have been hoped for, considering the fact that many of the persons who sent in reports were wholly inexperienced and were practically unguided.

During the present year experimental mushroom spawn has been sent to more than 200 interested persons, and this will doubtless be the last general distribution of this product by the Department of Agriculture. Representing the varieties of Agaricus campestris commonly grown, mushroom spawn of pure-culture origin is now an established market product. In order that the standard of the American spawn may be maintained, spawn makers, dealers, and growers should see to it that only the fresh, recently dried product is used.

Nevertheless, it is hoped that this cooperative work may be carried forward, looking toward the development of better varieties or the bringing into culture and the testing of new species.

CAVE FACILITIES IN THE UNITED STATES.

Cave facilities in the United States are by no means so meager as has been supposed. There are in some sections caves from which rock for Portland cement has been mined. Some of these have been utilized for mushroom growing. There are also natural caves of great extent in many of the States of the Central West—especially

in Indiana, Missouri, Kentucky, and Arkansas—as well as in Virginia.4 The difficulty is to obtain caves within a convenient distance from cities, for stable manure becomes expensive if it must be hauled many miles or transported long distances by the carload. Again, caves should be easy of access, since after each crop every vestige of soil, mamure, etc., of the preceding crop must be removed as a sanitary precaution. This is especially necessary since there is much waste space in most natural caves, and it becomes a very difficult or expensive matter to fumigate. If the cave system is extensive, it must also be possible to give it thorough ventilation. Many natural caves are the courses of subterranean streams. The latter are by no means objectionable if there is no danger from overflow. In many caves the stream has long since found a new channel and the cave is dry. Seepage water, usually accompanied by continuous stalactite and stalagmite formation, is undesirable. In some of the Eastern States coalpits or coal mines may be important for mushroom purposes. Where the coal mine is not too deep, or where perfect ventilation may be given, there is no reason why it is not entirely suitable for mushroom growing.

OPEN-AIR CULTURE.

In some sections of England and France open-air culture of mushrooms in beds is practicable during the late autumn and winter months, in which case the productive period may extend into the spring. The difficulties in the way of open-air culture are not merely those of maintaining a more or less uniform temperature, but also of maintaining practically constant conditions of moisture. For these reasons it is necessary to mulch the beds heavily with clean straw. In some instances a light mulch of straw is permitted to remain even during the period of production, for a rapid drying out of the surface would be hazardous or fatal. It is better, perhaps, to put the beds under some form of protection, such as an improvised cold frame.

In regions where the climatic changes are marked, open-air culture is probably not to be recommended during any season for commercial purposes. It is probable that there are some areas in the United States in which open-air culture might be practiced with profit. It has seemed that certain sections of California might be favorable for this phase of the work. In the interest of experiments

^q The writer is indebted to Prof. C. F. Marbut for the information that caves are to be expected in the Silurian limestone, which occurs particularly in the extension of the Shenandoah Valley, in the bluegrass region of Kentucky, and in the Ozark region of Missouri and Arkansas; also in the Lower Carboniferous limestone, which extends into Indiana. Kentucky, Tennessee, and Missouri.

along this line the writer has made a special attempt to acquaint himself with the conditions in that section of the country. This has seemed particularly desirable, inasmuch as fresh mushrooms could not be shipped to the far West from sections in which they are at present grown in quantity. From the information obtained it is thought that successful open-air mushroom growing might be anticipated in those sections where the average temperature is between 48° and 55° F., provided there are relatively few days when the temperature falls as low as 32° F. At the same time, open-air culture can not be recommended for those sections in which dry winds are prevalent. As a rule, during the wet or winter season the rainfall is so light that heavy mulching would probably suffice to prevent injury from excessive wetting. Nevertheless, it seems apparent that even in regions most favorable for open-air culture some inexpensive partial protection against the changes of temperature due to direct sunlight, or against heavy rainfall, would be desirable.

It was also ascertained that Agaricus campestris appears naturally in some quantity during the months of January and February, or longer, during the rainy season. This, however, is also true of other species of fleshy fungi. The large size of some of the specimens of Agaricus campestris and A. arrensis found would seem to suggest that they were produced from an unusually vigorous mycelium. This may be the result of a condition analogous to that previously mentioned, where, on account of the low temperature of the atmosphere, the spawn may develop slowly through a considerable period, and finally, under favorable conditions, sporophores of unusual size are produced.

In the following table are given the monthly mean temperatures from several representative stations in California during the years 1899 and 1900. From this table it will be seen that so far as the mean temperature is concerned Eureka and San Francisco would be especially favorable during a large portion of the year. Independence and Red Bluff are likewise satisfactory, while San Luis Obispo, Santa Barbara, Los Angeles, and San Diego show a mean which is perhaps rather too high. The moisture of the atmosphere, the prevalence of hot winds, the variation in the daily temperature, and the number of hot or cold days must all be considered. From the data obtained, the general conclusion seems to be that the most favorable regions are those where conditions correspond closely to those of Eureka and San Francisco. This, however, represents a large region, including a considerable portion of the San Joaquin and of the Sacramento valleys. In a few places experiments have already been undertaken to determine the possibilities for the development of this work, but no definite recommendations can be made until the experimental evidence is at hand. It may be said, moreover, that some of the regions which seem to be too warm for open-air culture may be especially favorable during several months at a time for mushroom growing in ordinary cellars, or in very simply constructed mushroom houses. In those sections the winter and early spring months would doubtless give the most satisfactory conditions; and this period, fortunately, corresponds with the tourist season—a season when the market demands are greatest. It is also possible that with mulching and with simple protection, mushroom growing may be successful in some of the Eastern States.

Table XI.—Mean monthly temperatures at points in California, in degrees Fahrenheit,

		Fahrei	theit.					
Month.	Eureka.		San Francisco.		San Luis Obispo.		Santa Barbara.	
JII/AICII.	1899.	1900.	1899,	1900.	1899.	1900.	City.	F.H.S.a
January	47. 5 44. 4	50, 4 48, 6	53, 0 51, 6	50.7 53,6	54.2 54.4	56.2 56.2	53.0 54.6	55. 4 58. 0
March April May	48,0 48,2 49,6	50, 5 50, 0 54, 4	52.2 54.6 52.6	55.2 54.0 57.0	54, 0 56, 4 54, 0	58.2 54.2 61.6	55.8 57.9 59.4	57.4 59.3 59.4
July	52.0 54.8 55.9	56. 2 56. 4 57. 0	56, 9 55, 9 58, 3	57. 6 58. 2 59. 7	62, 4 64, 4 64, 0	63, 9 64, 2 64, 9	62, 6 65, 5 66, 9	64, 4 68, 1 68, 9
August September October	54.8 52.0	56.6 53.8	58, 2 59, 3	63.3 58.8	65, 5 59, 6	64.4 62.8	66.1 62.6	69, 9 64, 8
November	55.9 48.0	53.3 50.8	56.8 49.6	56, 3 50, 2	57.4 54.3	55, 6 ——	59.1 55.6	64. 7 58. 4
Year	50, 9	53.2	54.9	56, 2	ā8.4	60.2	59, 9	62.3
Month.	Los A	Los Angeles. San Diego.		Independence		Red Bluff.		
мощи.	1899,	1900.	1899.	1900.	1899.	1900.	1899.	1900.
January February	56 54	58 58	56, 0 53, 4	57.1 57.2	40, 2 46, 5	46.6 48.1	48.8 51.6	48.8 51.1
March April May	57 60 60	60 57 64	56.4 58.0 58.0	59. 1 57. 1 60. 6	50.5 59.4 60.0	54.9 52.0 65.8	52, 2 60, 8 63, 2	58.6 57.6 67.0
July	65 70	67 71	61.4 65.6 65.8	63. 9 67. 1 65. 7	74.2 80.4 72.6	75. 4 79. 4 72. 4	77.9 82.0 73.8	76.8 82.6 77.0
August September October	69 70 63	68 67 64	65.5 62.7	65.3 62.8	74.6555.4	63, 5 58, 8	78.0 61.0	69.9 60.0
November December	62 58	66 60	61.0 58.7	63.7 59.7	49.4 43.1	50.4 43.4	54.4 45.5	54.8 45.4
Year	62	64	60, 2	61, 6	58.9	59.2	62.4	62.5

[&]quot;Foothills or suburbs of Santa Barbara, at an elevation of 750 feet above the city.

Occasionally one reads of successful natural cultures of mushrooms: that is, the production of this plant in pastures, lawns, etc., under more or less natural conditions. At Columbia, Mo., the writer has made numerous attempts to spawn plats in pastures and lawns; but thus far failure has attended every attempt. The spawning has, moreover, been tried at every season of the year. It is believed that in the section of the country mentioned only exceptionally favorable seasons will permit any success in this phase of open-air culture.

MUSHROOM SPAWN MAKING.

The mycelium of the cultivated mushroom has long been known commercially as "spawn." From early times it has been recognized that mushrooms may be grown from spawn, and it is quite certain that in all attempts to propagate mushrooms spawn has been used for the purpose.

In France, in England, and in other countries in which the mushroom has long been grown it is recognized that it is not profitable continually to take growing spawn from one bed to be preserved as "seedage" for the next crop. The common expression is that the spawn "runs out" in about three years. There seem to be few or no definite experiments indicating the exact conditions under which the spawn in two or three years loses the power of vigorous mushroom production. Nevertheless, it is the almost unanimous opinion of all extensive growers that there is a marked diminution in the yield after several successive propagations from the spawn in the mushroom bed. This has seemed to be true in the writer's experiments, although it must be said that accidents to experiments undertaken have made it impossible to report at this time upon the nature of this running out. That deterioration does result is apparently a fact accepted by all scientific men who have given attention to mushroom growing. It is possible, however, that under certain conditions the spawn might be repeatedly propagated without loss of prolificness. It is not necessary to enter here into a discussion of possibilities or to attempt to explain why weakening might be evident under ordinary conditions.

A "chance" method.—For practical purposes it is necessary to renew the spawn and to secure, if possible, spawn which has not previously weakened itself by the production of mushrooms—known as virgin spawn. Natural virgin spawn may be found wherever "in nature" it has been possible for the spores to germinate and to produce a mycelium. Ordinarily such so-called "spontaneous" appearances of spawn may be anticipated in compost heaps, rich garden beds, pastures near the feeding places of animals, etc.

Many attempts have been made by practical growers to develop spawn from spores, sowing the gill portions of mature mushrooms in specially constructed beds; but the results, so far as the writer is aware, have not been satisfactory. As a rule, therefore, growers have been compelled to rely wholly upon a virgin spawn which has been obtained by chance. It is said that in the vicinity of Paris some persons make a business of searching for this virgin spawn, which they sell to the growers at a high figure. It is claimed that they become so adept in detecting the differences in the character of growth, the quality of odors, etc., that they can distinguish not only

Agarieus campestris, but also some of its varieties. In England much of the virgin spawn has been obtained from pastures. Where a "spontaneous" growth of spawn is observed, trenches are dug, and these are filled with good stable manure. The latter in time becomes penetrated, and it is highly prized for cultural purposes. As a rule, the virgin spawn is used in spawning beds, which, when well penetrated, are torn down, and the whole bed used as flake spawn in spawning the general crop. Again, the virgin spawn may be used in spawning the brick, or cakes, this being the form in which English spawn is usually made. However adept persons may become in the identification of various varieties of spawn by odor, etc., this must be considered essentially a chance method.

A "selective" method.—From what has been said it will be perceived that very little advancement could be made in the selection of desirable varieties of mushrooms, in varietal improvement and the like, so long as the chance method of securing spawn should prevail. The studies in the germination of mushroom spores previously referred to were encouraged by the apparent necessity of beginning with spores from mushrooms of known qualities in order to effect improvement. In recent years the investigations of Costantin a upon spore germination have found application in a department of the Pasteur Institute. By a secret method, mycelium is grown from the spores in pure cultures. These cultures, which are, of course, pure virgin spawn, are then offered for sale to the growers. This spawn does not seem to have received deserved consideration on the part of the growers. The secret method of effecting spore germination referred to by Répin b has also been practically applied by one of the largest seed firms in Paris. In general, however, French growers have not profited so much by the new methods, perhaps partially on account of the fact that these methods are not known and partially because of the expense of the new virgin spawn. It is to be noted that these methods imply pure cultures to begin with.

The successful germination studies with chemical stimulation mentioned in this paper were soon overshadowed by the discovery of the ease of making tissue cultures. The use of the latter method has been the means of a sudden advancement in spawn making in this country during the past two years, for many practical men have been quick to see the advantages which it offers.

Pure-culture precautions.—It has already been stated that the pure-culture method of making virgin spawn is not one which will prove successful in the hands of wholly inexperienced persons, or of those who are unwilling to spend time and use the utmost care in the manipulation of the cultures and the culture material. The use of

a Costantin, J., loc. cit.

b Répin, C., loc. cit.

pure-culture methods necessitates to a considerable extent a knowledge of the bacteria and molds which are everywhere present in the air and which are especially abundant wherever there are dusty or damp, moldy conditions. The principle of making pure cultures is briefly this: The materials, or media, and all the vessels employed must be sterilized, which implies being heated at a temperature sufficient to kill all germs present in the vessels or materials used. If the vessels used are test tubes or other pieces of glassware with small mouths, they should, previous to sterilization, be plugged with cotton batting. This cotton batting prevents, when carefully manipulated, the entrance of germs from the air, and therefore keeps the vessel or medium in a pure or sterile condition. If such a vessel is opened, this should be done in a room free from currents of air or falling dust particles: and, while open, tubes and other apparatus should be held in a more or less horizontal position, so that they will be less liable to contamination. It follows, of course, that the cotton plug, if removed, should not come in contact with any unsterilized substances. If, now, a small quantity of the growing mycelium of a mushroom from a pure culture is transferred to such a sterilized tube, using for this transfer sterile needles, or scalpels, there will be little danger from foreign organisms, and the piece of mycelium inserted will therefore grow as a pure culture free from all other fungi or bacteria.

The tissue-culture method,—In making pure cultures of mushrooms, large test tubes or wide-mouthed bottles may be used. These should be carefully cleaned, and, if possible, a sterilization should be given by means of dry heat as a preliminary precaution. In this event the tubes are plugged with cotton plugs and placed in a dry oven made for the purpose. They are heated to a temperature of about 150° C., and this temperature should be maintained for nearly an hour. Ordinarily, however, in rough work it is not essential to employ this preliminary sterilization. In either case the tubes are next partially filled (about two-thirds) with the manure, or half-decayed leaves, upon which it is desired to grow the virgin spawn. A plug is inserted in each tube, and the tubes are then sterilized in a steam boiler or under pressure. If sterilized under steam pressure, as in an autoclave, it is necessary to use about 15 pounds pressure and to allow the tubes to remain at this pressure for from fifteen minutes to half an hour. If the sterilization must be effected in a boiler or in an open water bath, it can only be done at 100° C., of course; and it is then desirable to boil the tubes for at least one hour on each of two or three successive days.

With the tubes thoroughly sterile, the next step is to make the cultures or inoculations. By the tissue-culture method it is implied

that the inoculations are made from pieces of the tissue of a living mushroom. It is at this stage that selection may be made. One should procure from a bed of mushrooms in full bearing a mushroom which represents the most desirable qualities that are to be found. Size, quality, and general prolificness must all be considered, as well, also, as other characteristics in any special selections. One may desire, for instance, to select from a variety which yields throughout a long period—one which is resistant to higher temperatures, etc. Having found the mushroom from which it is desired to propagate, plants as young as possible may be used, and those which show the veil still intact are especially desirable. With a scalpel, or a pair of forceps, which has been sterilized by passing the blade through a gas flame, or even the flame from an alcohol or ordinary lamp, small pieces of the internal tissue may be removed, and these pieces transferred to the tubes, without, of course, coming in contact with any object whatever which has not previously been sterilized. It is a good idea to wash the mushroom first, so that no dust will be made. The plant may then be broken open longitudinally and bits of the internal tissue readily removed without fear of contamination when one becomes adept in this kind of manipulation. Immediately upon inoculation the cotton plug is replaced in the tube, and after all the tubes are inoculated they should be put out of the dust, preferably in a situation where the temperature is about that of an ordinary living room. In the course of several days a slight growth may be evident from the tissue if the conditions have been perfectly sterile. In the course of a week or more the growth should become very evident, and in three weeks the moldlike development of mycelium should spread to practically all parts of the medium in the tube. The method of making pure cultures and the laboratory apparatus usually involved are shown in Plate VI, figure 2.

When the tubes are thoroughly "run" the contents may be removed and used in spawning brick. The contents of a single tube may spawn several bricks when carefully employed. If no transfers are made of the growing mycelium from one lot of tubes to another, the writer has not found it at all impracticable or unfavorable to utilize this first lot of bricks later in spawning others. No further transfers, however, should be made from these bricks to others under any circumstances in spawn making. As elsewhere indicated, such a continuous transference is injurious to the vigor of the spawn and diminishes the quantity of mushrooms produced.

The commercial process.—The essentials in spawn making are (1) a uniform, compact manure brick; (2) vigorous and well-selected virgin spawn to be used in inoculating the bricks, and (3) favorable conditions for the storage of the bricks during the growth of the spawn.

It should be indicated that there is no one method of making brick spawn. The process may and will be varied by each spawn maker. Any skill or mechanical devices which will simplify or improve the process in any particular are to be recommended.

The materials entering into the composition of the brick are fermented stable manure, cow manure, and sometimes a small quantity of well-selected loam. Perhaps the chief value of these different constituents is as follows:

In the horse manure the mycelium grows most readily. The cow manure binds the materials together into compact brick. The loam, which is perhaps least essential, is supposed to prevent cracking or hardening of the surface, and therefore contributes to the appearance of the finished brick, at the same time tending to prevent rapid fermentation during growth. It also in some cases facilitates the uniform spread of the mycelium. If fresh manure is used, the necessity of using loam is perhaps to be emphasized.

In the experiments which have been made under the auspices of the Department of Agriculture these materials have been used singly and in various combinations, and it is beyond doubt that the relative proportions of these should be determined by the special conditions under which the spawn is made. Excellent results have been obtained by using a mixture of from two-thirds to three-fourths stable manure and the remainder cow manure. In this case the compost for the brick is subjected to fermentation previous to its use. When loam is employed it may be used in more or less equal proportion to the cow manure; and the quantity of stable manure should about equal that of the other two ingredients. If the straw present does not become sufficiently disintegrated during the preparation of the manure to enable one to make a smooth brick, it should be removed, in part at least.

The dry bricks ordinarily measure about $5\frac{1}{3}$ by $8\frac{1}{4}$ (to $1\frac{1}{3}$) inches. They should therefore be molded of somewhat larger size, perhaps 6 by 9 by 2 inches, since there is considerable contraction during drying. The mold consists merely of an oak frame of four pieces strongly riveted together. It may also be profitably lined with thin steel plates. In molding the brick one of two methods may be followed: (1) The compost may be thoroughly wet or puddled; then, with the mold upon a board of suitable width, the manure is compressed into it, the mold removed from the brick then formed, and the board pushed along for a succession of such impressions. The boards supporting the bricks are then disposed in racks and the bricks dried for a few days, or until they may be turned on edge for further drying out. (2) The compost may be used in a condition which is merely moist. It is compressed into the brick with some force, a mallet being often employed. The brick thus obtained is

sufficiently rigid to be immediately handled if necessary. By this method, unless the compost has been in excellent condition, the bricks are not so smooth as might be desired for commercial purposes. In some instances they have then been subjected to a repress process, an old repress brick machine being adapted for the purpose. In such cases the bricks are made thicker to begin with. The second method has been discontinued by some who at first employed it.

Two methods are also employed in spawning: (1) The more common method is to insert into the brick near both ends a piece of the virgin spawn obtained for the purpose. A cut is made with the knife, the spawn inserted, and a stroke of the knife effectively closes the surface. This must be done as soon as the brick can be readily handled. (2) The bricks are dried until merely moist throughout; then, on being piled, nocules of spawn are placed between successive bricks, a piece at each end. In either case the bricks are not piled for the growth of the spawn until in good condition as to moisture content. This should be determined not by the surface, but by the interior of the brick. In the pile the surface will soon become moist. When the first method is employed it is sometimes customary to spread between the layers of brick in the pile a little moist manure or sawdust. It has been determined, also, that the absolute moisture content of the brick should be about 40 per cent, which is the same as for the mushroom bed. Tests of the moisture content of bricks growing well have varied from 35 to 471 per cent.

Occasional examination should be made to determine the temperature and the extent of growth. In order that the bricks may become thoroughly penetrated, more than a month will usually be required.

The most favorable conditions for the growth of the spawn are practically the same as for mushroom growing. A fairly moist atmosphere, maintained, if necessary, by spraying, and a more or less uniform temperature (55° to 60° F.) are to be preferred. The size of the piles will depend upon the other conditions; but if there is any danger of considerable fermentative activity the bricks should be so disposed as to permit perfect ventilation between two or more adjacent rows.

When the bricks are thoroughly "run" they are dried under cover before being shipped or stored in bulk, since in a moist brick the spawn would continue to grow and would soon produce small mushrooms or else would become moldy. Well-penetrated bricks of spawn are shown in Plate VII, figure 2. The areas of mycelial growth should be evident to the eye. The growth should be moldlike, however, rather than composed of very large threads or fibers.

The suggestion made in a previous publication that mushroom spawn should be sold by the brick (with a uniform standard of size) seems to have been adopted by American makers. The trade names

suggested for the common types of Agaricus campestris in culture have also come into use. It is certain that these names, Alaska, Bohemia, and Columbia, designating respectively a white, a brown, and a more or less cream-gray form, do not include all forms in cultivation. Until a careful study has been made of varieties, however, this nomenclature will enable spawn makers to keep in mind certain types, and will make it possible for growers to ask for a spawn yielding a color demanded by their special markets.

THE VITALITY OF MUSHROOM SPAWN.

Many of the early experiments in mushroom growing undertaken by the writer were made in the hope of being able to ascertain the more frequent causes of failure and some of the chief difficulties encountered by American mushroom growers. The ordinary commercial spawn used by amateurs, that is, such as is obtainable upon the market during the winter months, was purchased wherever possible. Samples of this spawn were placed under conditions which were supposed to be most favorable for growth. Nevertheless, in the majority of cases there was no indication of the development of a new mycelium from the bricks of spawn thus obtained. From these results it was suspected that much of the spawn which reaches the amateur grower may be considerably injured, or even killed, by transportation or improper conditions of storage; for it must be supposed that most of this spawn is in good or at least fair condition when exported from Europe.

Subsequently the writer was able to look into the matter of spawn making in Europe and France, and he was convinced that the difficulty of securing good spawn in England is not a very serious factor. The same is true with reference to the material which is obtained by both extensive and small growers in France.

Special importations of some of the commercial English and French spawns were made, and this was packed, shipped, and stored under conditions as favorable as may ordinarily obtain. This spawn was imported during midwinter and stored until March or early April, when it was used in spawning some experimental beds. The conditions of the experiments were practically the same throughout, yet in not more than half the beds was there a favorable development of mushroom spawn. A distribution of the French spawn, both the commercial flake and the improved cake spawn, was made to several prominent American growers. Some of these growers experienced entire failure, while others reported that, after a slow beginning, beds spawned with this material made a good yield. The general conclusion, reenforced by observation and by the experience of practical growers, could only be that a large percentage of loss in mushroom

growing is attributable to the injury suffered by the spawn after its preparation. This conclusion has been further strengthened by the experience of the past three years. From Table VIII, beds Nos. 1, 2, 4, 5, and 30, it will be seen that, under conditions where fresh spawn has invariably made a good yield, the spawn which is more than a year old is, for the most part, seriously injured or killed. To be exact, in only one case was there any production of mushrooms by spawn which had been kept for a year or longer. It must be said that no attempt was made to keep these spawns under similar conditions or under the most favorable conditions. For the most part the spawn was stored in the dry laboratory room, in which the temperature was more or less variable, but never extreme. The old American spawn which was used in experimental bed No. 1, in Table VIII, was stored in a basement room where the average temperature was undoubtedly cooler than that of the laboratory room.

From experimental beds Nos. 1, 3, 4, and 5, in Table X, it is again seen that old spawn is unreliable. In this particular case the material was furnished by a prominent mushroom grower—an English spawn importer. This spawn had been stored in a dry house and was therefore subject to similar conditions. In Table VIII, beds Nos. 31, 32, 35, and in Table IX, Nos. 34, 41, and 45, there is further proof of the loss of vitality in the imported spawn ordinarily offered for sale in many of our cities. In these cases spawn was bought on the market just as offered for sale to the amateur buyer; "best on hand " was asked for, but no stipulation was made that is should be of recent importation, and no guaranty was asked. The tests were not, therefore, to compare the very best English with the best American spawn, but merely to secure an indication of some of the causes of failure by the purchase at random of English and French spawn on the market. Even in times past the extensive mushroom growers have either imported their spawn direct, or made sure that they were obtaining the best product that the market could furnish. Unfortunately, it has not been possible to compare, in any experiments thus far concluded, the best English with the best American spawn.

The results seem also to indicate that brick spawn maintains its vitality longer than the flake material, and that brick spawn made of loose, light material is less retentive of vitality than that made after the formula commonly followed in England. This proves to be an unfortunate factor to be dealt with in the attempt to reduce by all means the weight of the brick. The reduction in weight would be most desirable, since freight upon this material adds considerably to the price of market spawn. To the poor keeping qualities of loose spawn is perhaps due the large number of failures with French flake spawn, and perhaps also some of the failures with the

newer form of French brick spawn. The latter is made in the form of very small, thin bricks, which are unquestionably more affected by weather conditions than the larger English bricks.

These results have seemed to demand that special attention should be given to methods of spawn making in the United States in order that growers might be able to secure this product as fresh as possible. Moreover, it was desirable, as previously indicated, to attempt work leading to the selection and improvement of varieties. The success of the work in spawn making has been almost all that could have been anticipated. By the pure-culture methods described, several firms are now making grades of brick spawn which have yielded remarkably well. This fact is now thoroughly recognized by a large number of the best growers throughout the country. Probably as many as 50,000 bricks were sold during 1904, and it is perhaps to be expected that several hundred thousand will be sold during the present year.

It is to be regretted that it has not yet been possible to abandon the pure-culture process by means of which the virgin spawn is made while retaining the advantages of selection. Nevertheless, it should be remembered that the very difficulties of this process insure its use only by those who are able to give it their best attention and who will doubtless develop it to the fullest commercial extent. It has not been supposed by the writer that the work thus far accomplished will enable all mushroom growers to manufacture their own spawn with comparative ease. In other phases of horticultural work it is not so much to individual growers as to progressive seedsmen that we look for the best seed of improved varieties. The same thing apparently must be anticipated in the development of the mushroom industry. The growing of selected spawn may, in general, become a specialized process.

Nevertheless, it is believed that in time a method of spawn production from spores without pure-culture precautions will be developed. The necessity of developing immediately, or placing on a practical basis, the pure-culture process has temporarily directed the experimental work along other lines.



Fig. 1.—A Fine Cluster of Agaricus Campestris, the Horticultural Variety Columbia.

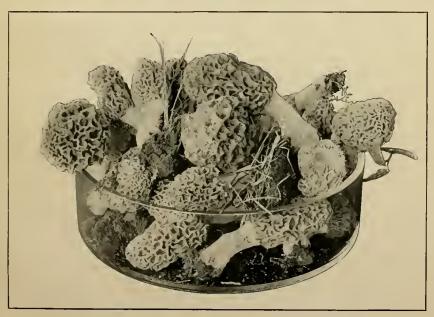


Fig. 2.-Morels (Morchella esculenta), One of the Finest Edible Fungi.





Fig. 1.—AGARICUS FABACEUS, THE ALMOND-FLAVORED MUSHROOM.

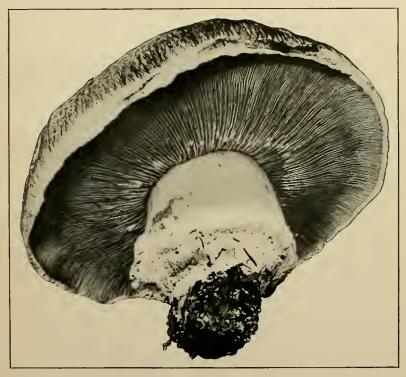


Fig. 2.—Agaricus Villaticus, a Promising Species, Fleshy and Prolific.

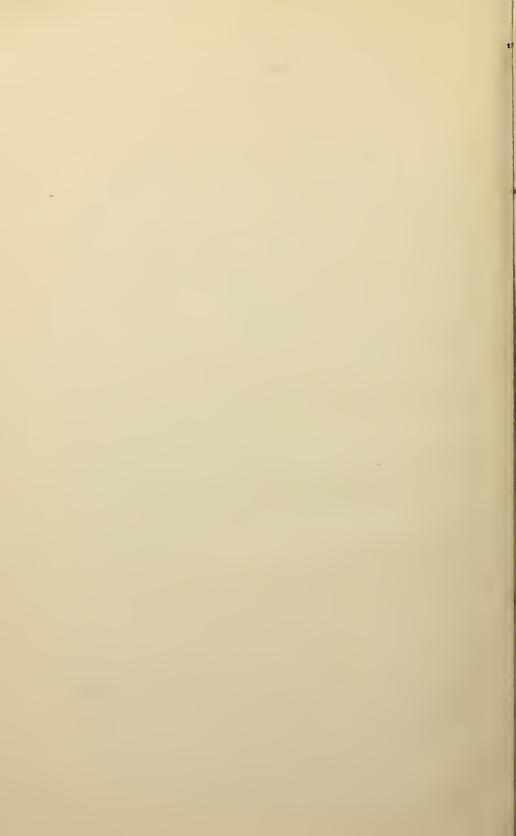




Fig. 1.—A Young Specimen of the Common Puffball (Calvatia Craniiformis).



Fig. 2.—The Oyster Mushroom (Pleurotus ostreatus), Growing on Decayed Willow Log.

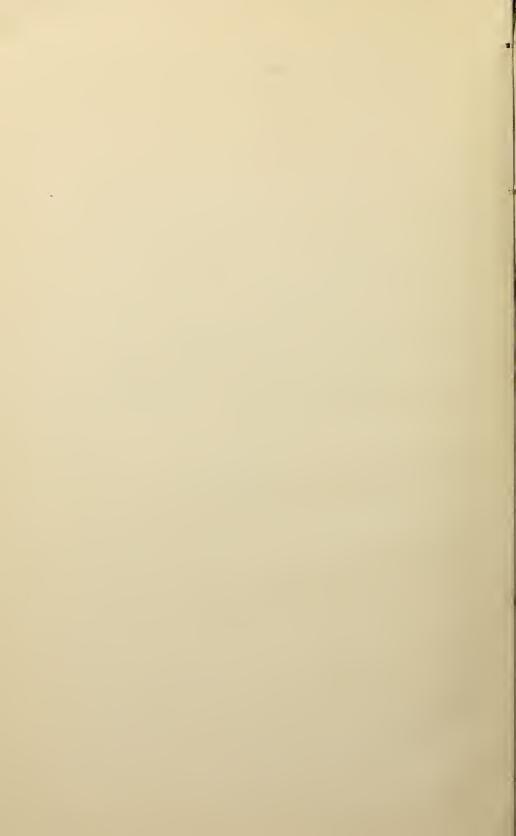




Fig. 1.—A Mushroom House Provided with Gas-Piping Framework for Shelf Beds.



Fig. 2.—The Preparation of Compost.

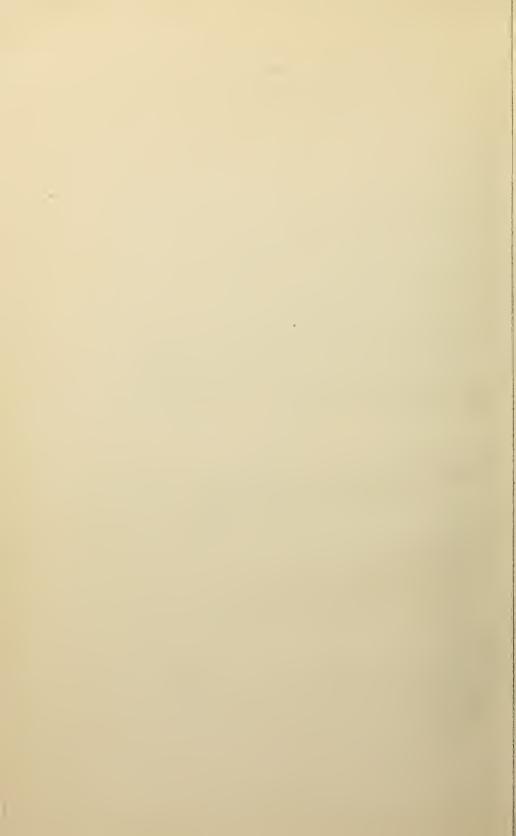
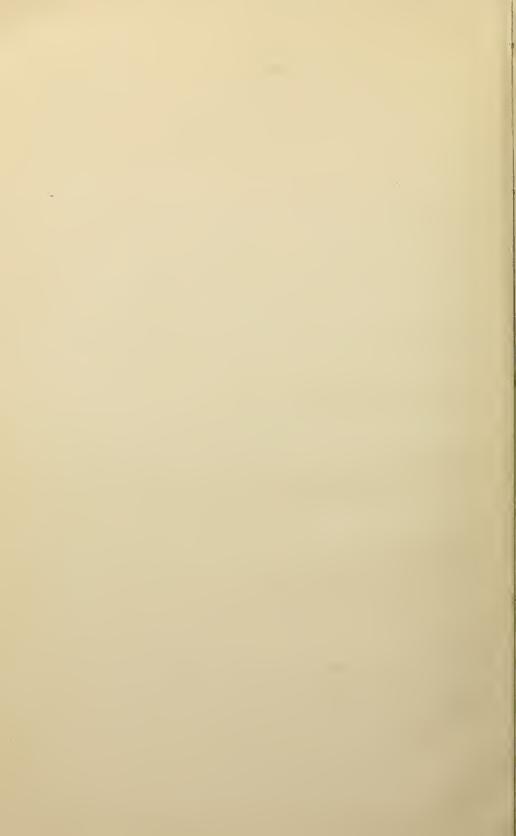




FIG. 1.—A LARGE MUSHROOM ESTABLISHMENT—A COMMON FORM OF MUSHROOM HOUSE.



Fig. 2.—The Method of Making Pure Cultures, Showing the Apparatus and Materials.



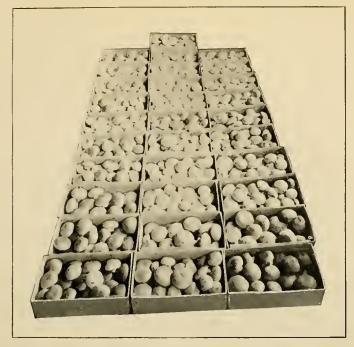


FIG. 1.-MUSHROOMS PREPARED FOR THE AMERICAN MARKET.

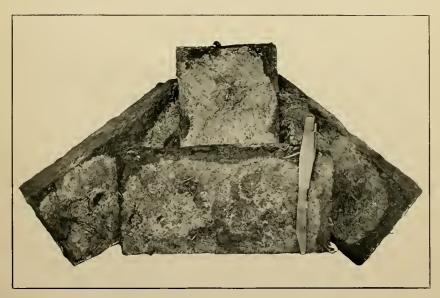


Fig. 2.—Good ("Well-Run") Mushroom Spawn, Brick Form.











