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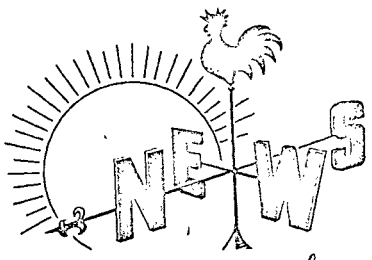

from

Around the World

Our sources of information on mushroom research are obtained from around the world in order to supply our mushroom library with comprehensive, up-to-date material on research, marketing, mechanization etc.

The following article comes from the Max Planck Institute for the breeding of cultivated plants located in Hamburg, W. Germany. Our thanks to Prof. Dr. R. v Sengbusch for the opportunity of publishing this important article in this issue.

The IIIrd stage in the development of the procedure for cultivating mushrooms on non-composted, sterile substrate.

by

W. Huhnke, G. Lemke, R. von Sengbusch

In 1965 we reported on the IInd stage in the development of a mushroom cultivation procedure on non-composted, sterile substrate (TILL Procedure, 5). In the Ist stage, TILL showed that the composting of the substrate is unnecessary for the cultivation of mushrooms and also that the spent substrate may be used again (7,9,10, 12). During the Ist stage TILL used 1 liter jars with approximately 10 kg per day. During the IInd stage, 5 liter polypropylene containers were used which required 100 kg per day. Both these procedures were only laboratory stages. The present stage of development is close to one of commercial application. Although this part of the IIIrd stage offers no final form for production on a large scale, it at least demonstrates the possibilities for a practical application of the procedure.

Cultivation rooms

We have altered our experimentation center completely to suit the new procedure. The previous cultivation rooms retained their basic shape: 4,70 m x 12,50 m, with an average height of 3,50 m, containing 288 trays with 1/2 sq.m surface area and holding 25-27 kg. These rooms are fully air-conditioned and the temperature can be raised or lowered as required. The relative humidity can be regulated as well. In contrast to the procedures in general use, the pasteurizing room is only used for the preparation of the casing soil.

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Equipment

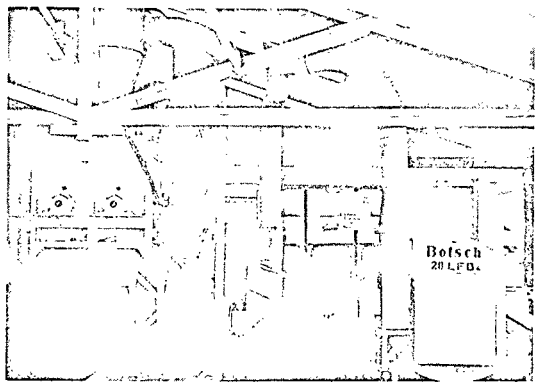

Fig. 1 Machines for preparing the substrate

The following equipment was obtained for the preparation of the nutritive substrate:
1. a chaff-cutter with a capacity of approximately 2000 kg/h for chopping the straw.

2. a hammer mill with a capacity of approximately 300 kg/h for grinding the chaff.
3. a 1000 liter mixing machine (working on a counter-current principle) to mix the substrate and add water.
4. two tunnel-autoclaves with a capacity of 7 cu. meters for sterilizing the substrate.
5. an oil-fired boiler producing a maximum of 800 kg/h of steam required for sterilizing.

Contents of the nutritive substrate

1. Carbohydrates in the form of straw, particularly wheat-straw, meadow hay or field hay or alfalfa hay.
2. Protein in the form of cotton-seed meal, soya bean meal, wheat-bran, alfalfa meal etc.
3. Peat and straw meal to increase the absorbency of moisture.
4. Calcium carbonate to regulate the pH value.
5. Water (70% of the prepared nutritive substrate).

Containers used in sterilization and cultivation

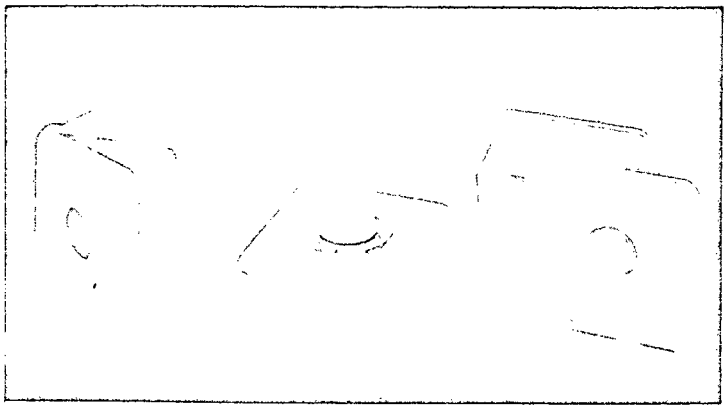

Fig. 2 Polypropylene container = 5 liters contents: 2 kgs nutritive substrate

The first type of container used for cultivation was of polypropylene and, with a capacity of 5 liters (Fig. 2 &3), could be filled with 2 kg of substrate. These containers, with tightly fitting lids, gave good results with regard to the growth of mycelium and protection against infection. At a later date larger polypropylene containers were used

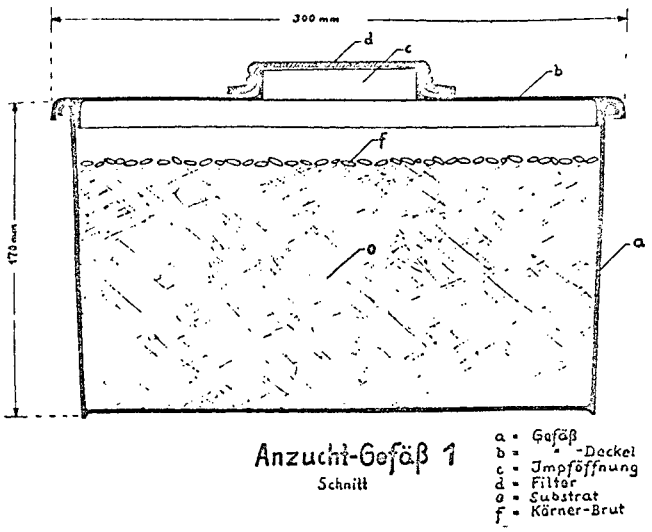

Fig. 3 See Fig. 2
Section of the polypropylene container = 5 liters contents: 2 kgs nutritive substrate

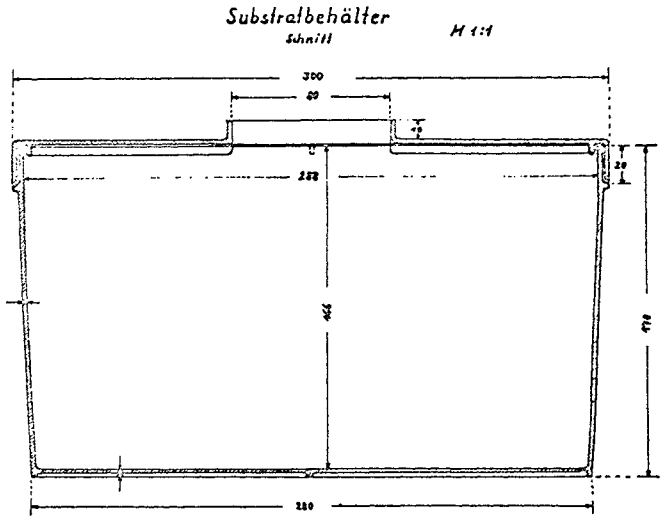

Fig.5 See Fig. 4
Section of the polypropylene container = 10 liters contents: 4 kgs nutritive substrate

which had a capacity of 10 liters and could hold 4 kg of substrate. During the early experiments with these containers it became clear that the lid was not properly sealed to the container. This source of infection was avoided by sealing the lid down with adhesive tape. The opening in the top of the lid used for inoculation and for the exchange of gases was sealed with foil like the jars in the earlier stages. Experiments using tissue paper as a means of sealing the containers showed that not only did these stoppers encourage a faster through-spawning of the substrate because of the improved exchange of gases but that the risk of infection was not increased by them.

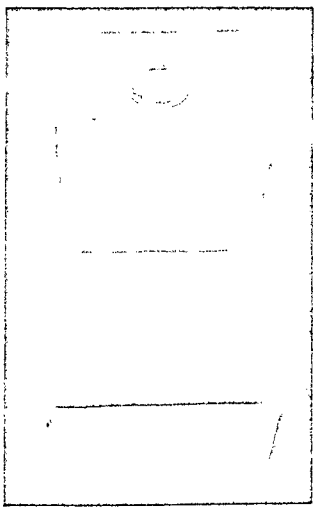

Fig. 4 Polypropylene container = 10 liters contents: 4 kgs nutritive substrate

Transport of containers

Our cultivation rooms have capacity for 288 trays containing 7,5 tons of nutritive substrate. We require about 1,800 cultivation trays to fill one room. In order to simplify the transportation of this large number of containers, trolleys were built capable of holding 30 containers in 5 tiers. The containers are loaded onto the trolleys after being filled with nutritive substrate. Each autoclave holds 6 trolleys with 180 containers, i.e. about 750 kg of nutritive substrate. (Fig.6).

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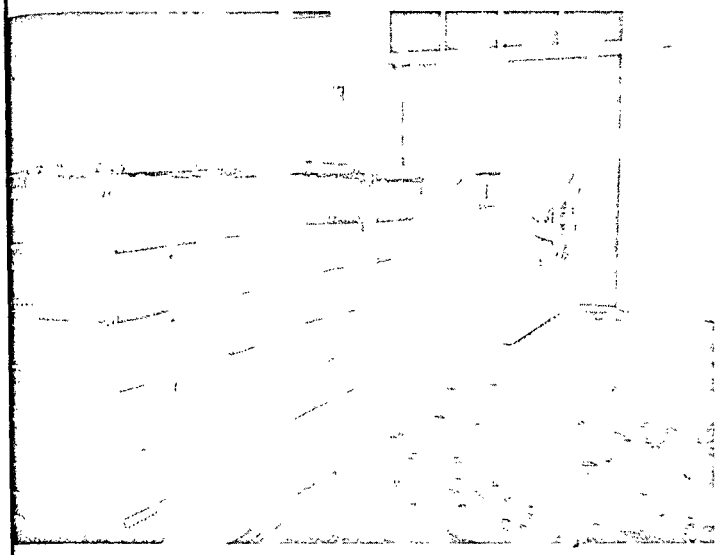

Fig. 6 Trolleys with 30 x 10 liter containers x 4 kgs = 120 kgs carting to autoclave

Rapid inoculation is made possible by removing the containers from the trolleys in groups of three after autoclaving (Fig.7). The trolleys

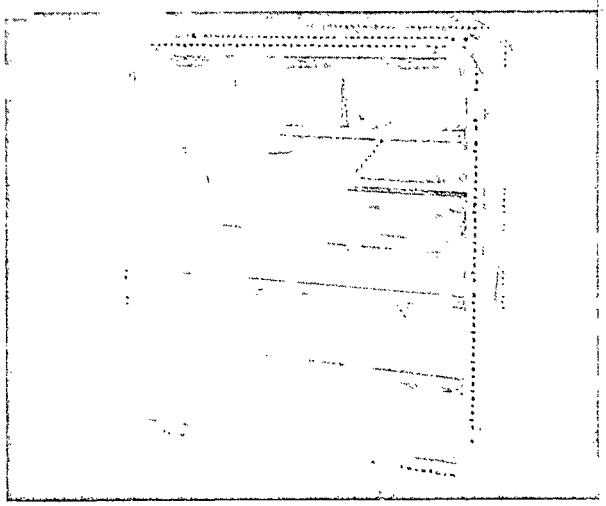

Fig. 7 Trolley with 30 containers (10 liters)

with the inoculated containers are then wheeled to the cultivation room where they are stacked in pairs by means of a fork-lift truck (Fig.8). After the mycelium has spread through the substrate the containers are checked for completed through-spawning and infection, and those which are not completely through-spawned or show infection are eliminated. The trolleys are then wheeled to the mixing machine where the substrate is shaken up and mixed, according to plan, with some form of protein and thereby enriched.

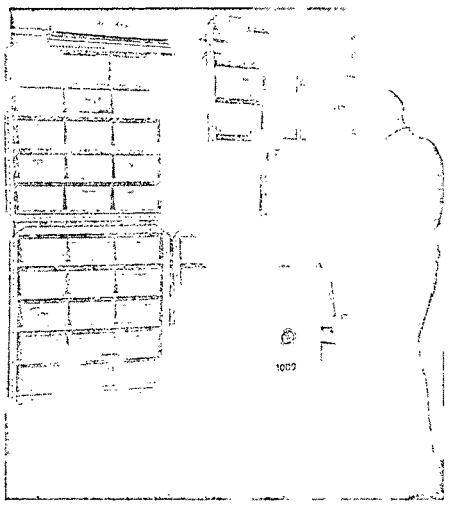

Fig. 8 Spawn-running room (Containers on trolley) Piling up the trolleys with the fork-lift truck.

Autoclaving

The nutritive substrate has a very high carbonic acid content after autoclaving (up to and above 8%), as we determined in the first experiments. When the carbonic acid content reaches this level, the formation of mycelium is restricted. In order to reduce the amount of carbonic acid which collects in the substrate and in the empty parts of the containers during autoclaving, the steam is constantly

being replaced by fresh steam. This allows the carbonic acid formed to be continuously siphoned off with the steam and the concentration of acid is thus kept at a low level.

Another way to reduce the amount of carbonic acid after the autoclaving period, lies in the type of cooling employed. The faster cooling occurs, the more intensive the exchange of gases between the interior of the container and the fresh air entering the autoclave to equalize the pressure. At this point it is particularly important for the fresh air to enter the autoclave through a bacteria-filter.

The temperature during autoclaving is between 121°-130°C. It takes 3 hours for the center of the substrate to reach the required temperature in the autoclave. This temperature is then maintained for another 2 hours before the cooling-down process is started. The cooling-down process entails circulating cold water in the outer shell of the autoclave. Cooling takes about 7-12 hours, according to the temperature of the cold water available, so that the whole process of autoclaving is only practicable once per day. With an optimum cold water temperature and a night shift, however, the operation could be carried out twice in 24 hours. After cooling the temperature in the nutritive substrate must have fallen to at least 33°C. Experiments have shown that for mushroom mycelium, temperatures in excess of 35°C are lethal (6).

Inoculation

The inoculation with grain-spawn is carried out through the opening in the lid of the substrate container. The spawn amounts to 2,5% of the weight of the substrate. With regard to inoculation there are no essential changes from the method described in our earlier paper.

One additional checking procedure was introduced to ensure that the spawn and nutritive substrate were in order Sterile substrate in 1 liter jars sealed with foil is inoculated with samples of spawn of the type to be used. After inoculation the spawn and substrate are mixed by being shaken up. If stored at a room temperature of 24°C, the spawn develops in a few days and the quality of its growth may be classified. If the mycelium develops poorly or not at all, it is probable that the spawn is defective of the nutritive substrate has not been degassed sufficiently.

Through-spawning

In our first experiments in the IIIrd stage with non-sealed, 10 liter containers, and without the above-mentioned precautions for removing the carbonic acid during autoclaving, we

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had to reckon with losses of up to 40% caused by infections, poor mycelium growth or insufficient through-spawning. This was reduced to 2-3% after removing the source of infection and lowering the carbonic acid content before inoculation. As a result of all the measures taken, the time required for through-spawning was reduced from 8-10 weeks to 4-8 weeks.

Shaking-up, mixing and enriching

The through-spawned nutritive substrate is shaken up in the mixing machine i.e. it is broken up mechanically and mixed at regular intervals with substances containing protein which increases the nutritive content (8). The substances containing protein are: cotton-seed meal, wheat-bran, alfalfa meal, soya bean meal etc. The cultivation trays are then filled with the enriched substrate which is immediately covered with casing soil. The trays are then placed in the cultivation room. It is not yet clear whether we could add the protein to the substrate even before autoclaving and still achieve the same yields as when the addition is made after through-spawning. So far, experiments giving protein enrichment before autoclaving have not been particularly successful. We assume firstly, that the protein, by being heated in the autoclave, deteriorates in quality and secondly, that the increase in the amount of protein leads to an increase in the amount of carbonic acid formed. This affects the pH value adversely. It is not yet certain how far a simultaneous increase in the amount of lime would neutralize this tendency. A report on experiments dealing with this question will be made elsewhere.

The effect of shaking-up

A comparison was made between the yields from substrate (without protein enrichment) which had been shaken up and through-spawned and that which had not been shaken up (Table 1). From this it became clear that an increased yield can normally be achieved by the shaking-up process alone.

CLASSIFIED COLUMN

FOR SALE — 28¼ acres land located Limestone Road, Hockessin, Delaware. Suitable for future development. Property has: 4 double and 1 single mushroom houses containing approximately 34,000 square feet of bed space, good condition and presently being operated; dirt shed, two level barn, 2 homes, 1 trailer, 2 dump trucks, 1 pickup truck, tractor, hay baler, spreader, other miscellaneous farm equipment. For information, phone Wilmington, Delaware. Area Code 302 656-1691 from 10 AM to 4 PM. Monday through Friday.

WANTED — Spawn technician. Need someone familiar with making spawn and all phases of operating a spawn plant. Preferably one with a B.S. degree, having some training in microbiology. Resume held in strictest confidence. If interested write to Department M. American Mushroom Institute. V.F.W. Bldg., Kennett Square, Pa. 19348, giving full particulars.

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LARGE Midwest mushroom producer has opening for an experienced mushroom grower. This opening offers a permanent position with excellent benefits and advancement possibilities to a qualified man. Please send resume including references, age and expected salary to box O-1.

Firstly the yields from shaken-up substrates start earlier than those from non-shaken-up material. Clearly not all mushroom strains react as strongly to the process. But the fact that shaking-up generally has a positive effect on the yield is of value, as only in this way is the mixing for enrichment with protein possible. Enrichment would be practically impossible if the material were not shaken up beforehand.

Results of enrichment after through-spawning

After the enrichment of the through-spawned nutritive substrate with protein and the subsequent casing, the

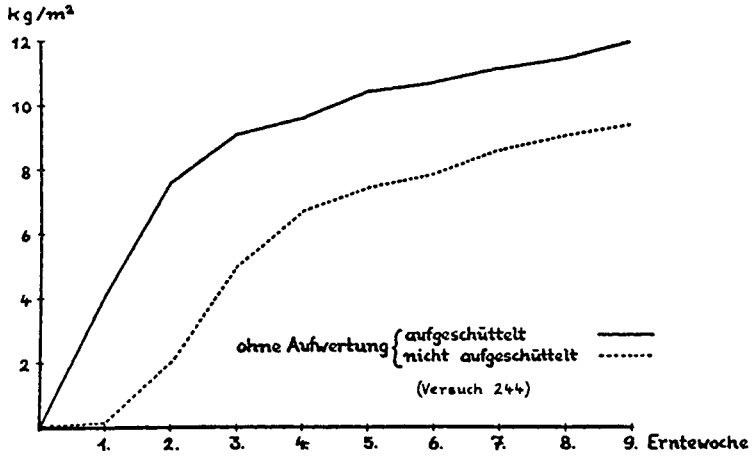

Table 1. The effect on the yield of shaking up and not shaking up through-spawned substrate.

With reference to tables 1 3,4,6,7,8, m^2 - yields: 50 kgs substrate/m^2 : trimmed mushrooms (15% wastage)

temperature of the substrate increases (6). In the cultivation trays this temperature can become lethal to mycelium growth. The

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increase in temperature is presumably caused by increased bacterial activity. During the growing of mycelium similar rises in temperature occur, though at this stage the lethal amount is never exceeded (relevant results will be published elsewhere, 6). The temperature should be kept within the toleration limits of the mushroom mycelium by cooling the rooms, either with air from outside or with air-conditioning. If enrichment by various forms of protein is not given, then the harmful rises in temperature do not take place. The rises in temperature are smallest with material that has not been shaken up and enriched (Table 2).

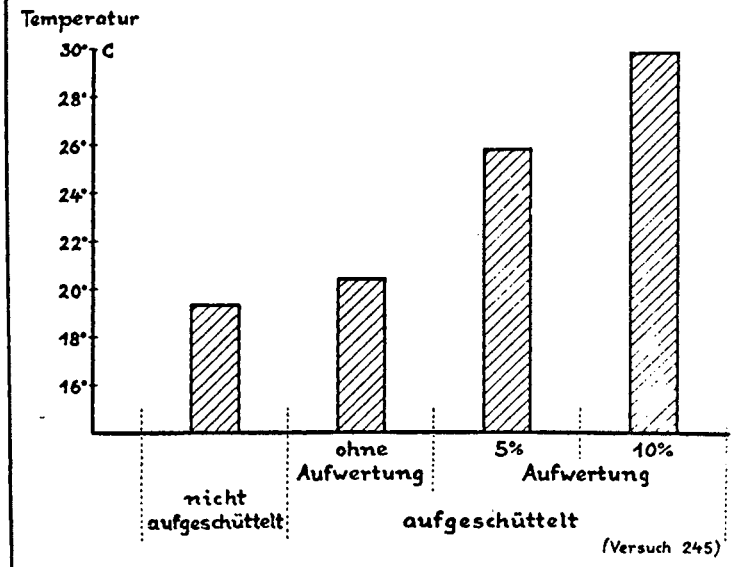


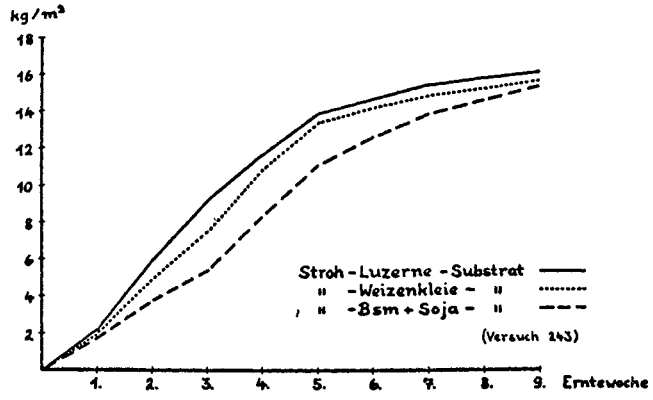
Table 2. Temperature increase in through-spawned substrate after shaking-up and enrichment.

Yields depending on the sort of substances containing protein being used

It could be observed that the substitution of the various substances containing protein used in the production of nutritive substrate had only a small effect on the yield (Table 3a), while the addition of various kinds of protein during enrichment had a greater effect on the height of the yield (Table 3b). A complementary effect was also observed. If alfalfa is used in preparing the nutritive substrate, then wheat-bran or cotton seed meal are effective as subsequent enrichment. A similar increase in yield however, is not achieved if alfalfa is used in both the preparation and the subsequent enrichment of the substrate.

The prospects of raising the yield by increased protein enrichment

Enrichment with increased amounts of protein brings about a rise in yield. We do not as yet know, whether the maximum amount of protein enrichment has already been reached. The temperature which is reached after enrichment sets a natural limit to the amount of enrichment possible (Table 4). Besides this we determined that by increasing the size of the cultivation containers and consequently the substrate content, as well as by using thicker layers, the danger of the lethal temperature being exceeded, increases also. The result of this proportional increase is that as a rule, the relative yield (based on the amount of substrate) in small containers is higher than that in the larger cultivation trays (Table 5). The danger from excessive temperatures increases with the protein enrichment and the size of the container. Only when the temperature is fully controllable can the highest yields be obtained with added protein enrichment and larger containers.



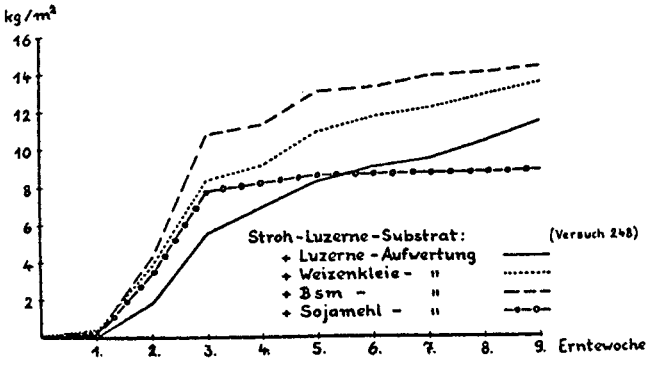


Table 3. Effect of different protein supplements on the level of yield.
a) For substrate production. With reference to tables 3a, 3b, 4:
Bsm = cotton seed meal
Soya = soya bean meal
b) For enrichment after through-spawning.

Yields dependent on strains and varieties

In order to test the yields we used our own strains and compared them with well-known varieties of mushrooms (Table 6). This showed firstly, that there are noticeable differences in

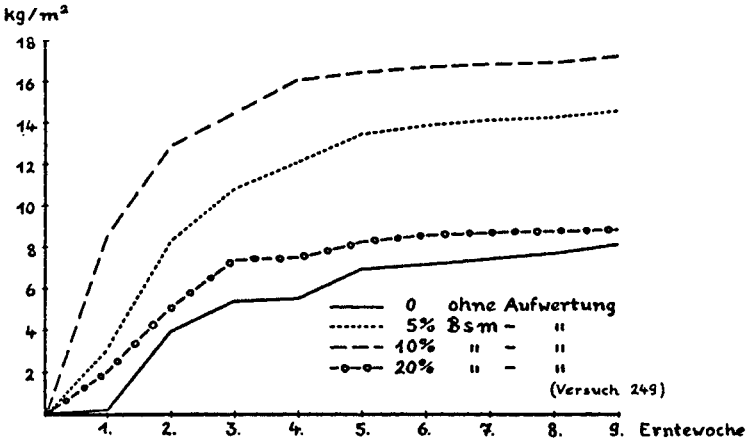


Table 4. Increasing yields by additional protein enrichment. Further protein supplements (20%) cause the lethal temperature to be exceeded and also diminish the yield.

Table 5

Temperature increase with larger containers and greater protein enrichment.

amount of substrate	Poly-bags 1,5 kg	Polypropylene containers 2 kg	4 kg	trays 25 kg
enrichment				
30 %	24,5°C	30,0°C	43,0°C	---
25 %	23,0°C	34,5°C	31,5°C	---
20 %	22,0°C	22,0°C	31,0°C	47,0°C
15 %	19,5°C	19,0°C	24,5°C	30,5°C
10 %	18,5°C	18,0°C	18,0°C	26,0°C
5 %	17,0oC	------	------	21,0°C
none	16,5°C	------	------	17,5°C

Table 5. Temperature increase with larger containers and greater protein enrichment.

the total yield after a picking period of 9 weeks, secondly that the start of the first flush can vary considerably and thirdly, that the course of the yield fluctuates too.

Varying course of yield with different strains. Economic assessment

The observations made above tend to suggest that the yield of a particular strain and the course of the harvest should no longer be characterized in the old way, i.e. by

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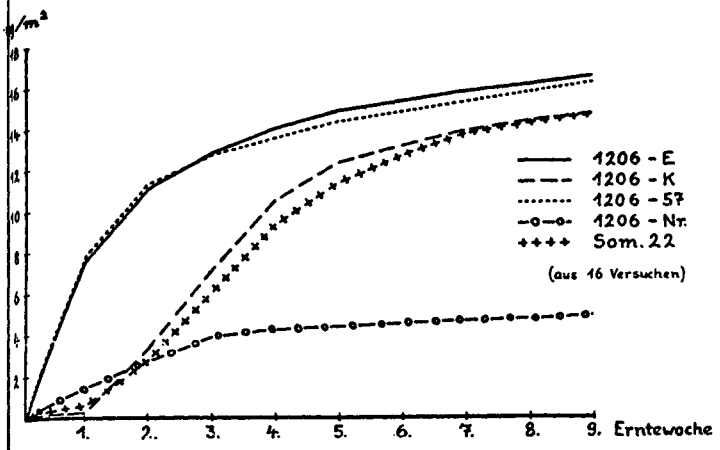

Table 6. Influence of different varities and breeding-strains on the yield.

assessing the picking period from start to finish. It would seem advisable now to show the duration and course of the yield in relation to the total time from casing. This procedure has the advantage of not only assessing the yield at its peak but also shows it in relation to the actual time under cultivation. With such a procedure one is able to calculate the true yield of the cultivation room for the space of a year, a week or a day. The actual amount of harvest then available gives the true gross income for each of these periods of time and from this the specific production cost for a corresponding length of time must be deducted.

This can be expressed in another way:

Two varieties of mushroom which over 8 weeks produce approximately the same yields can be of differing value to the cultivator according to the length of time without yield, from being placed in the cultivation room till the start of the picking period, and also with regard to the course of the yield. In future a distinction will have to be made between varities with early and late starts, as well as between those which have a short and rapid yield and others whose picking period is prolonged and regular. Cultivators will have to take such characteristics into account when organizing their business and calculating profit margins.

Yields depending on the sort of multiplication of the strains used in spawn production

One of the conditions for success with our new process is absolutely sterile spawn which furthermore does not have any of the "uninhibited" characteristics. In our experiments to produce new spawn we frequently observed that most varieties and strains could deteriorate either because of mutation or through continual modification. One example of this degeneration we have called "uninhibited". In this case clumps of mycelium are formed in the bottles while the spawn is being produced. After inoculation, lumps of mycelium are formed in the substrate (Fig. 9&10) which completely or partially prevents the formation of the fruit bodies. This phenomenon has posed us a considerable task in the past few years. In producing the

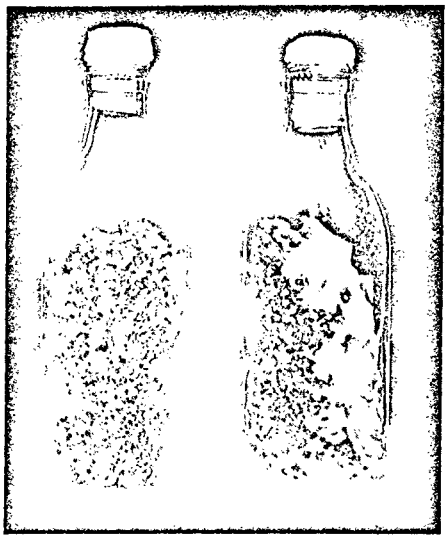

Fig. 9 Degeneration of a strain to the "uninhibited" type (right)

spawn we evolved a relatively simple system of checks to ensure the "sterility" of the spawn, though it was difficult to know when a spawn had the "uninhibited" tendency or not. As a result of constant selection we are now able to eliminate this type to a large extent.

It was for this purpose that we resolved on various types of multiplication from strains. This spawn material which was propagated in different ways was also tested for yield, among those tried being strain 1206-E, one of our own single spore cultures (FRITSCHE, v.SENGBUSCH, 1). Spores were collected from one fruit-body of this strain and different sowings of this spore print propagated separately in the normal way (1206-Numbers). A multi-spore propagation from the same fruit body was carried out on a composted substrate (1206 K), Table 7 shows the yields from different kinds

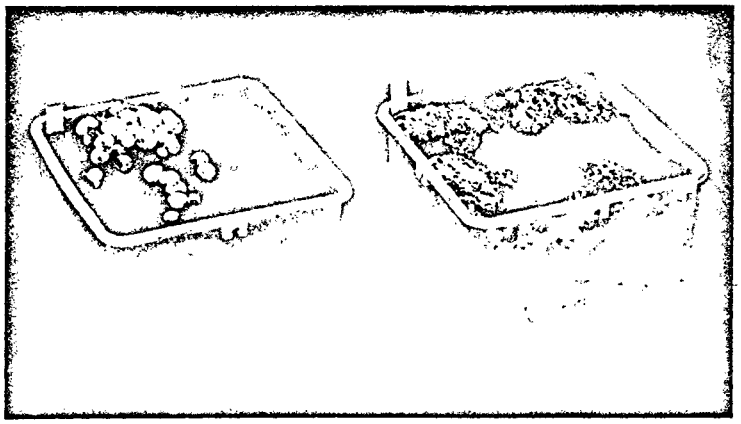

Fig. 10 Left: normal strain
Right: degenerated to the "uninhibited" type

of propagation of strain 1206-E. It was evident that 1206-Numbers and 1206-K produced very different yields and that the Numbers only produced about half of 1206-K's yield. One of the multi-spore cultures, Number 57(1206-57), had approximately the same yield as 1206-E and 1206-K. That is to say, there are multi-spore cultures of a single fruit-body from the single-spore culture 1206-E whose descendants give very high yields and others which give very low yields. This result indicates that the common assumption that "spawn from multi-spore cultures is of high quality", should be accepted with reserve. The

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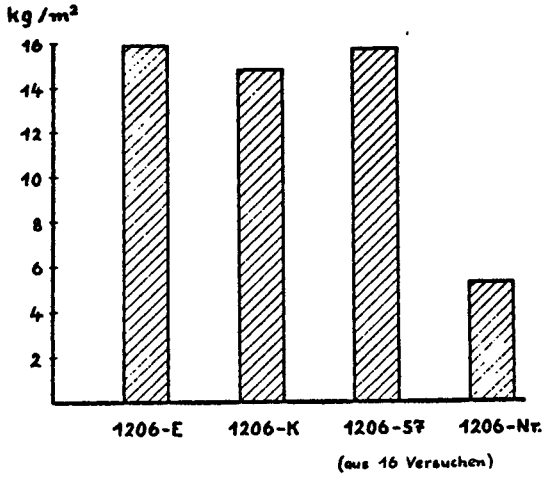

Table 7. Various kinds of propagation of multi-spore cultures from the same source bring different yields.

kind of multiplication where multi-spore cultures are based on spore mixtures from a single fruit-body, can lead to negative results. However, the example of 1206-Number 57 showed that descendants may be equal or even superior to the basic material of a single spore culture. Successive experiments using 1206-E showed that with careful propagation of a similar single-spore culture the yield can be kept at a constant level. (FRITSCHE-sustained cultivation, 2,3,4,).

Constant high yields as the criterion for the new cultivation procedure

In our paper concerning the IInd stage of the process, we reported on yields which at that time were only achieved on a small scale. In the IIIrd stage, the question was whether similar or even better yields could be obtained with large-scale cultivation.

In a series of experiments with a greater number of culture trays we obtained on six trials with strain 1206-K an average yield of 34% of the nutritive substrate = 17 kg/m^2 from 50 kg substrate per sq.m. (trimmed mushrooms, wastage 15%), (Table 8). These results from the IIIrd stage of development showed us for the first time that consistent and relatively high yields could be achieved in large-scale cultivation, provided that all the information regarding improvements in the process was utilized. The yields were in fact higher than those achieved previously.

Table 8
Constant high yields with the strain 1206-K in repeated large-scale experiments in the IIIrd stage of the "TILL Procedure".

Trial Nr.	Yield kg/m^2	% of the nutritive substrate
228	17,562	35,12
229	17,066	34,13
230	17,288	34,58
231	16,174	32,35
233	17,334	34,67
234	17,257	34,51
ø	17,113	34,23

Table 8. Constant high yields with the strain 1206-K in repeate large-scale experiments in the IIIrd stage of the "TILL Procecure".

SUMMARY

The IIIrd stage of development in the mushroom cultivation procedure on non composted sterile substrate.

The IIIrd stage of TILL's Procedure showed that a high yield might be obtained by using the procedure on a large, commercial scale. The reduction of carbonic acid, and improvements to the gas-exchange filters, as

well as in the composition of the nutritive substrate, led to shorter periods of spawn-running. There was also an increase of 40% in the yield (according to the weight of the substrate). Additional protein enrichment after the mycelium had grown had a positive effect on the yield but at the same time it increased the dangers from excessive temperatures. With suitable refrigerating methods it is possible to avoid the losses caused by overheating. Large differences in yield were found with spore seedlings of one strain. The varying course of the yield with different strains is as decisive for profitable cultivation as the height of the yield. The time during which a strain is without yield should be included with the figures showing the yield.

S. S. PIERCE TO ACQUIRE KENNETT CANNING COMPANY

Roger D. Williams, president and chief executive officer, S.S. Pierce Co., announced the signing of a letter of agreement for the acquisition of Kennett Canning Co., Kennett Square, Pa., one of the nation's major mushroom growers and packers. The acquisition places the well-known Boston-based quality grocery and liquor products firm into direct manufacturing for the first time in its 138-year-old history.

The acquisition lifts S.S. Pierce's annual sales to a rate in excess of $50 million.

Kennett Canning Company, of Kennett Square, Pa., is one of the major mushroom growing and specialty canning firms in the U.S. The company grows mushrooms in several locations in the Kennett Square-Delaware area, and in 1967 produced over four million lbs. of mushrooms. As a result of new growing techniques, Kennett has increased per square foot mushroom production to levels well above the industry average. In a modern processing and canning facility in Kennett Square, the company produces a wide variety of specialty mushroom products, such as pickled and marinated varieties, developed in its own test kitchens. Currently under development are new items in the freeze-dried category. Among the benefits anticipated as a result of the acquisition are opportunities for both Pierce and Kennett to realize maximum use of facilities and operations. Seasonal production schedules can be adapted and supplementary major product manufacturing and in-house new product development introduced.

In line with S.S. Pierce policy, Kennett Canning will be operated as an autonomous unit under the direction of Mr. Edward J. Sharpless and his associates.

DR. STOLLER SAYS

In connection with my article, "The Role of Gamma Radiation in Mushroom Growing", published in Oct. issue, Dr. Kneebone has called to my attention that I overlooked research that he published on gamma radiation of mushroom spores which he published in the MGA Bulletin Feb. 1954. Whereas

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