

481

**The Mushroom Department of
the Max-Planck-Institut Für
Kulturpflanzenzucht**

THE MUSHROOM DEPARTMENT OF THE MAX-PLANCK-INSTITUT FÜR KULTURPFLANZENZUCHTUNG*

By **Professor Dr. Reinhold von Sengbusch** (*Director*)

We have been working with the cultivated mushroom since 1956. In many countries mushroom cultivation has been greatly extended in the meantime and a number of problems have been dealt with by various research centres.

The mushroom does, however, still pose a lot of unsolved problems. International co-operation in this field is therefore particularly desirable. The highlights of this co-operation are the international congresses and symposiums taking place every three years.

We are very happy to belong to the big family of people who are interested in the mushroom. The membership of the International Commission enables me to do active work in this respect.

We are particularly delighted and honoured that this year's mushroom congress takes place in Hamburg, and we shall take advantage of this opportunity for showing our Institute to all participants. In the following report we propose to give you a brief introduction into the things you could see here.

The following departments are concerned with the cultivated mushroom:

1. *Mushroom Genetics and Breeding* under the direction of **Dr. Gerda Fritsche**.
2. *Mushroom Spawn Production* under the direction of Dipl. rer. hort. **Gertraud Lemke**.
3. *Mushroom Cultivation* under the direction of **Walter Huhnke**.

The division into departments was made for reasons of organisation. The departments work in close contact with each other. Many problems are examined in team work by several departments at the same time.

DEPARTMENT OF MUSHROOM GENETICS AND BREEDING

In this department basic questions that are important for breeding work are examined, and new strains are bred.

(A) Breeding work with strains having normal fruit bodies

The new cultivation procedure (Till-procedure) developed by **Till** and after his death by the team **Huhnke, Lemke** and **Von Sengbusch**, has been used for testing new strains since March 1967.

The new procedure which works with an unvarying substrate due to preparation according to a recipe, eliminates many uncertainties which had so far been a handicap for breeding work.

Single spore cultures and, more rarely, multiple spore cultures are examined. About 7,000 single spore cultures have been treated since March 1967. This large amount of material gradually shrinks by passing the following trials:

1. Elimination of all cultures with insufficient mycelium growth (test in one Petri dish each, with wheat-agar nutrient medium). Cultures showing poor growth are tested for fruit body shape (mutants).

*Published by kind permission of *AMI News*, and the author, and written prior to the 1968 International Congress.



Fig. 1
Three year old child with a
fruit body of strain 59c
weighing 1,750 gm.

2. Selection of cultures with particularly fast growth on substrate, early yield, large amount of fruit bodies, and fruit bodies with a new shape (cultures in one $\frac{1}{2}$ l. glass each, on Till-substrate). The examination is only very rough but it enables us to test a great number of strains and thus to find the special types which usually occur very rarely. Only about 25% of the strains undergo further testing.
3. Examination for earliness and yield (cultures in 2 plastic containers each, with 2 kg. of weighed Till-substrate. Only 15 to 20% of the strains undergo further testing).
4. Another test for earliness and yield (cultures in 2 trays each, with 25 kg. of Till-substrate. The strains that are superior to the standard varieties undergo further testing).
5. Renewed test for earliness and yield, and for quality of fruit body (cultures in 5 trays each, with 25 kg. of Till-substrate. Classification of the fruit bodies).

The strains that have passed this last phase are repeatedly tested again; in case of positive results the number of trays is increased. From phase 3 onwards the cropping work is done in co-operation with the Department of Mushroom Cultivation. Spawn production is carried out from phase 4 onwards in a specially established Department.

(B) Work with mutants, in particular with strain 59 c, a strain with clump-shaped fruit bodies

A report on strain 59 c was already published in *Der Champignon* (No. 31, March 1964). 59 c produces clump-shaped fruit bodies which reach weights up to 1.8 kg. (Fig. 1). They can be sliced and fried like steaks. Smaller specimens can be ground and used as soup powder. The fruit bodies of 59 c have a very good flavour.

At the beginning the yield of 59 c was very low. It produced only 30 to 40% of the yield of normal strains which were tested at the same time.

The yield was considerably increased through selection of big fruit bodies which were propagated by tissue cultures. It now corresponds to the yields of the best normal strains and was at times up to 30% higher.

Unfortunately, the tissue cultures of 59 c easily degenerate into a preliminary form with poor yield, 59 b. Our breeding work is now focused on

keeping up the high yield. Hyphal tips are taken off under the microscope and multiplied individually. We hope to find some hyphal tips among the great number, which do not contain the genes of the preliminary low-yield form 59 b.

Another aim of our breeding work with 59 c is the qualitative improvement of the fruit body. The fruit body should be firm, its surface ought to be as smooth as possible, and it should have a pure white inside. We hope to achieve this by means of selection.

As we mentioned before, the numerous new single spore cultures are tested for mutants. So far several single spore cultures have been found which produce deformed fruit body initials of type 59 b (Fig. 2). Fruit bodies of type 59 b have, however, not yet been found (puff-ball shaped).

Pin-shaped fruit bodies having spontaneously occurred in the culture bed of a normal strain, were propagated by tissue cultures. The new fruit body shape was maintained (Fig. 3).

(C) Experiments on Maintenance Questions

A series of experiments was completed in this field. Three articles on this work were published in the periodical *Der Zuchter*, the first one deals with 'Propagation by mycelium transfer', the second with 'Propagation by tissue cultures', and the third with 'Propagation by multispore cultures'. Summaries of the three contributions will be published in *Der Champignon* in the near future.

Further work on the question of maintenance deals with the influence of the nutrient medium on the mycelium. Mycelium that had degenerated into a fluffy type with poor yield was included into the trials. Part of this work will be reported at the VIIth International Congress on Mushroom Science. The experiments are being continued.

(D) Experiments on the Question of Combination Breeding

Great progress in plant breeding has been made by crossing different

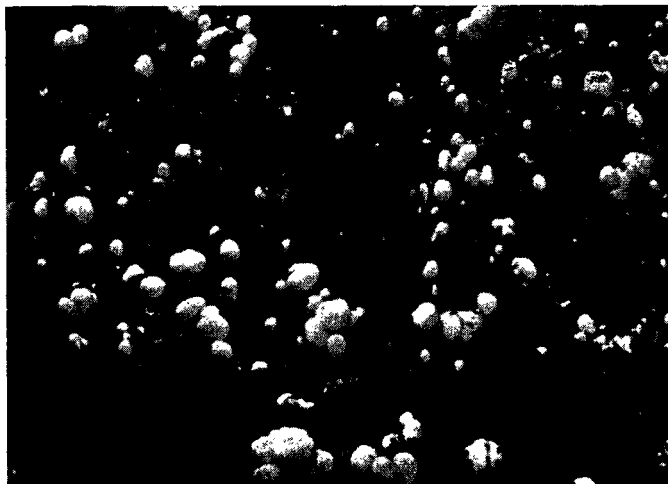


Fig. 2
Deformed fruit body initials of type 59 b

(contd. on page 516)

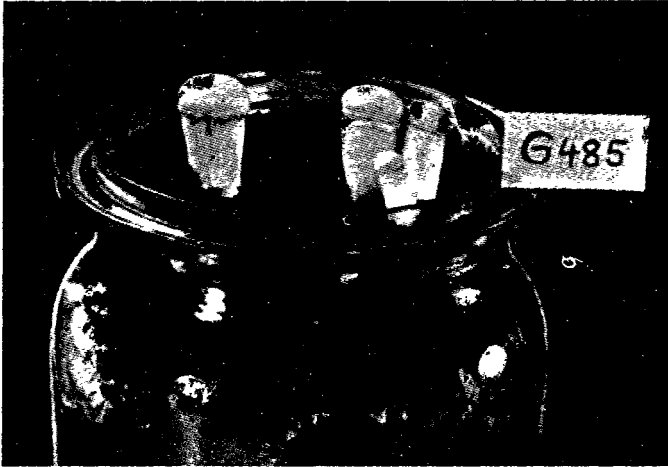


Fig. 3
Pin-shaped fruit bodies

strains, thus combining certain characteristics in their common offspring.

In a paper published in *Der Zuechter* in 1964, it was proved that crossings can be carried out with the cultivated mushroom too. A summary of this paper appeared in *Der Champignon*, No. 55, 1966. The new combinations, however, appeared only very rarely in the experiments. It is, therefore, extremely important to recognise new combinations as early as possible. Only then can the great number be tested which is necessary for finding the rare new types.

A characteristic which is apparently useful for early selection was discovered. Strains with cream fruit bodies differed from strains with white fruit bodies in their mycelial growth on two particular nutrient mediums (biomalt-agar and compost-agar).

Experiments are at present going on with a white and a cream single spore culture. Spores were obtained from white and cream fruit bodies which were standing closely together in mixed culture, and single spore cultures were raised. They are tested for behaviour of the mycelium and colour of their fruit bodies. Particularly interesting are the single spore cultures which differ from the parent mushroom in their mycelium growth on the mediums.

DEPARTMENT OF MUSHROOM SPAWN PRODUCTION

The main task of this department is to provide the Cultivation Department with sterile grain spawn of proprietary strains which have previously been developed and tested in the Department of Genetics and Breeding, i.e. large-scale propagation and maintenance of productive strains. The preliminary propagation of mycelium in the agar tube is kept under control in each further phase of propagation, the grain spawn which is produced out of it (**Fig. 4 and 5**) is transferred together with commercial varieties for yield comparison.

It is only when these tests have shown positive results and no change in

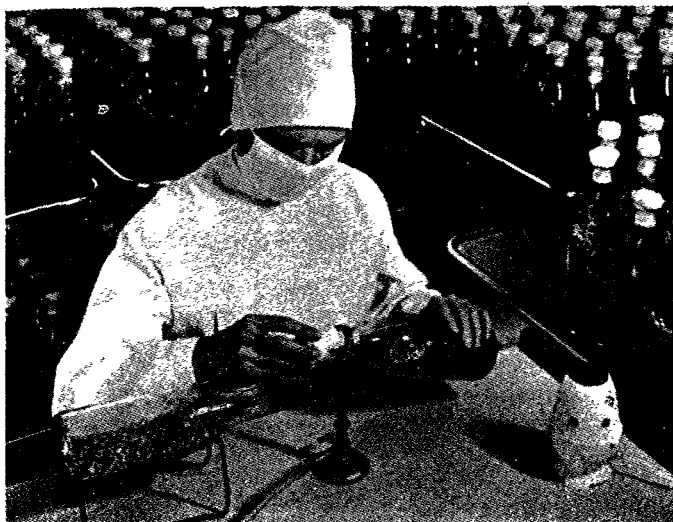


Fig. 4



Fig. 5

A glance into an incubation room

the mycelium or decrease in productive capacity can be observed, that spawn for use is produced out of the tested mycelium stocks on a large scale.

We use wheat (Canadian origin, 'Manitoba', with small grains, very glutinous) for our grain spawn. The wheat is boiled, mixed with gypsum and whiting (13 gm. gypsum + 3 gm. whiting per kg. of boiled grains), filled into 1 l. milk bottles, and autoclaved.

New containers for spawn production were tested, a number of experiments were in particular carried out with the plastic material Polypropylene as foil in the form of bags and moulded as to form containers. But since the French enterprise Somycel has a patent for the production of spawn in plastic containers which is also valid in Germany, these promising experiments had to be discontinued.

A mixture of Perlite (carrier substance) and wheat brans (nutrient substance) was tested as a new spawn substrate on a mainly inorganic basis. This substrate has various advantages compared with grain spawn:

1. lower material costs
2. sterile raw material
3. constant quality of the raw material
4. easier production, without boiling
5. longer shelf-life of the finished spawn

Otherwise it can be used just like grain spawn. A patent for it is pending.

Since the Till-procedure requires sterile spawn, all measures for sterility control were checked scrupulously and various disinfectants were tried.

It turned out that alcohol is inefficient; Bacillol-Spray (Dr. Bode & Co., Hamburg), on the other hand, proved to be a very useful disinfectant. The losses in spawn production amount to less than 1%.

In 1966 experiments for sterilising nutrient media with ethyleneoxide were started. Instead of autoclaving, cold sterilisation by the action of gas was carried out with

- (a) grain as spawn substrate, and
- (b) Till-substrate as culture medium

The trials gave positive results. Full sterility was achieved. The spawn developed normally and the thus sterilised nutrient substrate produced the same good yields as the autoclaved controls. New possibilities of using cheap containers for spawn production which need not be heat-proof present themselves here.

Questions of grain spawn storage were examined intensively. When wheat grain spawn was kept in cold storage at + 2° Celsius, important physiological differences between white and cream varieties were noted. Cream varieties could be kept in cold storage for only up to 2 months without difficulties, whereas white varieties could be stored for 4-6 months. Alcohol develops as a metabolic product in the cream varieties after longer cold storage of comparatively old spawn, or even relatively fresh spawn after reduction of the air supply and this probably leads to the destruction of the mycelium. In white varieties under the same conditions we were never able to find any measurable traces of alcohol. The spawn of white varieties remained active and showed good growing results after transfer, whereas the spawn of cream varieties was severely damaged. These experiments are carried on in order to obtain further results which will be equally interesting to spawn makers and mushroom growers as spawn consumers.

MUSHROOM CULTIVATION DEPARTMENT

In 1956 the Mushroom Cultivation Department started with cultivation experiments using the traditional cultivation method still applied all over the world, in which horse manure or materials like straw, hay, maize, or

other cellulose materials are converted into a nutrient compost for mushrooms by means of a composting process. One result of this work was, among other things, 'The Active Mycelium Cultivation Method' (Huhnke, W. and R. V. Sengbusch: *Das Aktivmycel-Spickverfahren als Grundlage für das Aktivmycel-Anbauverfahren*, *Deutsche Gartenbauwirtschaft*, 8, Heft 11, 1961).

When it became obvious in the course of the first years of experiments that decisive progress was not to be expected with the traditional cultivation methods on the basis of compost, an entirely new thing was tried with a view to making mushroom cultivation independent of the uncertainties and difficulties of compost preparation.

In 1960 Till began to investigate the question whether the activity of micro-organisms during composting is an indispensable prerequisite for making productive nutrient substrate for mushrooms. He was able to prove that it is possible to do without composting. He raised fruit bodies on sterilised nutrient substrate in glasses. After Till's death the procedure which was named after him was developed by the team Huhnke, Lemke, Von Sengbusch, at first under laboratory conditions.

In the second phase it was already possible to improve the procedure to such an extent that the average yields of practical mushroom growing were surpassed (average yields of more than 12 kg./m² from 50 kg. of substrate/m²). Reports on the results obtained were published (Huhnke, W., G. Lemke, and R. V. Sengbusch: *Die Weiterentwicklung des Till'schen Champignon-Kulturverfahrens auf nicht kompostiertem sterilem Nährsubstrat* (II. Phase), *Gartenbauwissenschaft* 30, 189-207, 1965).

The 'Till-procedure' which had so far been developed under laboratory conditions, could only be made practicable and useful for mushroom growing, if it was improved and if the yields achieved under laboratory conditions could be maintained under practical conditions.



Fig. 6
Machinery for preparing 'Till-substrate'
(chopping, mixing, filling)

The experimental department 'Mushroom Cultivation' created the preconditions for a further development of the 'Till-procedure' by rebuilding, and by providing the necessary technical facilities (Fig. 6). In the third phase, which begins here, experiments were going on with 1.5 tons of sterilised nutrient substrate per day. This quantity on five working days per week was sufficient for filling one cultivation room with 7.5 tons of substrate (300 trays, 0.5 gm. per 25 kg. of substrate).

When the initial difficulties had been overcome, the mushroom yields of the laboratory phase could not only be reached, but even surpassed due to improvements in the consistency of the substrate, a modified autoclaving technique, and the creation of more favourable growing conditions.

It was confirmed that it is actually possible to obtain average yields of 17.5 kg./m² from 50 kg./m² of nutrient substrate with the 'Till-procedure' under practical conditions, i.e. a yield of 35% of the nutrient substrate weight. These results are thus more than 50% higher than comparable crops achieved with the usual cultivation procedures on composted nutrient substrate.

In this third phase of the 'Till-procedure' a great number of 10 l. plastic containers, as employed as models in the laboratory phase, were used during sterilisation and the subsequent sterile incubation period. Thus the usefulness of the principle of sterile procedure itself could be proved, but

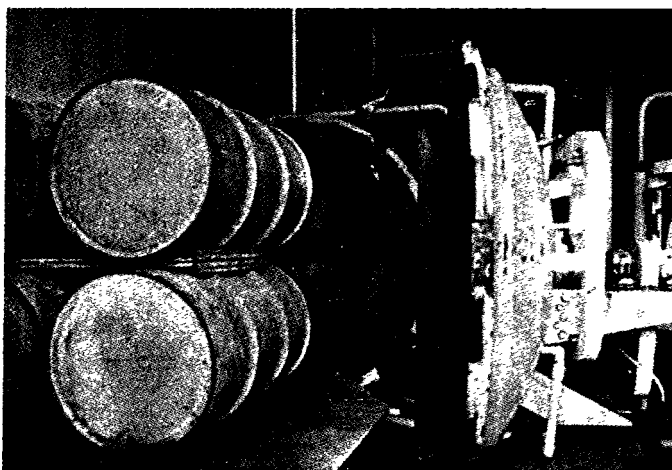


Fig. 7
Sheet-steel barrels as containers for 'Till-substrate' before autoclaving

the great number of small containers prevented a rationalised large-scale production.

In order to achieve further rationalisation of the procedure we changed from small plastic containers to 200 l. sheet steel barrels (Fig. 7). The barrels can be aired actively and in a sterile manner during the incubation period, thereby the incubation period can be reduced to 3 weeks. Apart from that, the barrels offer other possibilities of rationalisation (transport and stacking among others). Even in this IVth phase, which is already largely

adapted to practical mushroom production, the high mushroom yields of the preliminary phase of 35% of the substrate were reached. Further development towards bigger substrate containers, rapid sterilisation, improvement of mechanisation is necessary before industrial large-scale production can be undertaken. The extremely high and constant mushroom crops would warrant further efforts.

It is true that there are some difficulties which prevent the 'Till-procedure' from being put into practice on a large scale. First of all high investments would have to be made for the technical facilities, furthermore, the buildings in the existing enterprises would have to be adapted. Apart from that, the procedure requires great ability and excellent training of the technical staff.

Only very few enterprises are up to these standards. There is, however, still the possibility of co-operative work for making use of the advantages of the 'Till-procedure'.

For the above reasons, the Cultivation Experiments Department is now trying to develop an altered and simplified procedure which should enable more growers to make use of the advantages of mushroom cultivation with non-composted nutrient substrate.

In the amended procedure 'Till-substrate' is sterilised, and that is now the end of the sterile phase.

Immediately afterwards the germ and pest-free substrate is submitted to a controlled fermentation process. The fermentation leads to immunisation of the treated substrate against rival organisms of the mushroom. It is thus possible that all subsequent work and phases, such as inoculation with mushroom spawn and mycelium growth, can happen under usual conditions without any danger of infection. This simplifies the application of the system immensely. Existing facilities, such as peak-heating and incubation rooms can still be used.

This new procedure, which we call 'Huhnke-procedure', enables more growers to change over to mushroom cultivation with non-composted nutrient substrate. Our work in this direction is not yet finished, but the first results give rise to hopes that it will soon be possible to put a new, useful, and progressive procedure into practice.

Apart from the development of new cultivation procedures, other problems of mushroom culture are dealt with in the cultivation department, such as questions of climate, casing material, etc. Above all, however, the cultivation rooms provide an experimentation field for breeding new mushroom varieties and for comparative variety tests. It has become evident that the 'Till-procedure' is particularly suitable for efficiency trials for the selection of the first breeding grades, due to the fact that the nutrient substrate is reproducible and due to the yield constancy resulting from this and to its freedom from diseases. The same applies to experimental cultivation in general with the more advanced newly bred strains for the purpose of testing their suitability for cultivation. In comparative trials with good commercial varieties, significant variations in yield earliness, yield tendency as well as quality characteristics always become clearly visible on 'Till-substrate' even if many replicates are made.

Reprinted from *MGA Bulletin* 226, Oct., 1968
Printed by W. S. Maney & Son Limited, Leeds 9
