THE UNIVERSITY OF ASTON IN BIRMINGHAM

THE CASING LAYER IN THE CULTURE OF *AGARICUS BISPORUS*, THE CULTIVATED MUSHROOM

by

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SUMMARY

The casing layer is an essential component of the system employed in the culture of *Agaricus bisporus*.

The literature appropriate to the casing layer is fully reviewed, including aspects relating to fructification and morphogenesis in *A.bisporus*, together with an appraisal of the various media employed, their properties and functions, and the commercial significance of the casing layer.

Equipment is described for use in experiments in mushroom culture, based on a scaled-down version of normal growing technique, allowing the analysis of both weights and number of fruitbodies forming, which was useful in assessing the effects of different casing treatments.

The basic steps in the production of fruitbodies in *A.bisporus* are described, including a photographic study of the colonisation of casing and fructification.

Various alterations to the physical structure of peat/chalk casing mixtures were found to have an effect on fructification; those causing an opening-out of the casing structure tended to give better yields, especially in the early stages of production. It was shown that, in order to obtain greater yield through casing amendment, fructification must be stimulated, giving increased numbers of fruitbodies, disproportionate to their total weight and consequently of lower mean weight.

A synthetic casing medium based on the light glass-like mineral, perlite, was developed. The best formula obtained was - 1 part perlite: 1 part montmorillonite clay (by weight): 3 parts 0.01% glucose solution. Perlite/montmorillonite casing could be improved by adding compost colonised by mycelium of *A.bisporus*, or adding a peat-chalk casing extract.

Perlite was also found to be suitable for admixture with the standard casing medium and a mixture of equal parts by volume performed as well as the peat/chalk casing normally used.

KEY WORDS

CASING MUSHROOM

Agaricus bisporus

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Declaration.

No part of this work "The casing layer in the culture of *Agaricus bisporus*, the cultivated mushroom" was performed in collaboration with others, except where clearly stated. This work has not been submitted for any other award.

R. G. Steane

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

1. INTRODUCTION

The process by which Agaricus bisporus the cultivated mushroom is commercially grown relies on two component media.

Agaricus bisporus normally derives its nutrition from a pasteurised substrate prepared by aerobic fermentation - a "peak heated" compost, based on cereal straws and animal dung, or nitrogenous substitutes. The mycelium colonises the substrate by three-dimensional growth, but the thallus remains vegetative and undifferentiated.

In order for fructification to occur, a second component is necessary: the casing layer. As its mycelium grows out from the compost into this medium, a transition occurs to the sexual stage of the life cycle, and A. bisporus undergoes differentiation into fruitbodies.

Casing is also the name given to the practice, in mushroom growing, of spreading damp soil or a soil-like substance in a thin layer over the surface of the compost colonised by mycelium. Its purpose is to bring about the production of mushrooms, and as such it represents an essential part of the process, because in its absence fruiting does not occur.

This substance is also known as casing soil, the casing layer or simply as casing. In older texts it is referred to as "capping", and its translation in French is "gobetage", in German "Deckerde", and in Dutch "dekaarde".

Casing is normally applied ten to fourteen days after inoculation of the compost, when the mycelium or "spawn" of the mushroom has grown or "run" completely through it, although casing may take place earlier, even at the same time as inoculation. The first fruitbodies are then harvested about 21 days later, following a drop in temperature from about 24°C for the incubation phase to about 17°C for the cropping phase. Thereafter, fruitbodies mature almost simultaneously in "flushes" or "breaks" at approximately weekly intervals, depending on environmental conditions.

The casing layer thus represents a subject of considerable biological interest since the major morphological changes which mark the transformation from the asexual to the sexual phase of development of A. bisporus occur within this layer. The mushroom fruitbody or sporophore consists of stipe, pileus and spore-bearing tissue, and displays a considerably more complex structure than the undifferentiated mycelium.

Casing is not simply an adjunct to the commercial culture system, but rather a necessity and also a prime determinant of its productivity. Agaricus bisporus is increasingly cultivated on an extensive scale in many different parts of the world, and since casing is one of the major requirements for acceptable production, the availability of suitable casing media may be a limiting factor in the commercial exploitation of mushroom growing.

Associated with productivity, some quality characteristics of the crop can also be related to some properties of the casing layer.

In view of both the biological and commercial interest in the casing layer, this study was undertaken with the objective of reviewing and integrating existing knowledge of the casing layer and its relationship to the process of fruitbody formation in A. bisporus. Much of the information available is somewhat empirical or possibly irrelevant to the normal methods adopted by commerce, and some is contradictory. Comparative experimental work is also scarce, and in view of this, it was necessary to devise a laboratory method of experimentation which could be related to commercial practice.

Emphasis has been given to some physical characteristics of casing media, and possible areas for further research are identified. Improvements to and substitutes for the conventional casing media may thus be possible in the future.

2. LITERATURE REVIEW.

A. Fruitbody formation in *Agaricus bisporus*.

(i) Morphogenesis and physiology of fruitbody formation.

Casing normally provides a medium in which the development stages between vegetative mycelium and the mature fruitbody can occur, and provides physical support for the fruitbodies.

Descriptions of the morphological changes involved in the differentiation of the hyphae to the various stages culminating in the production of the mature fruitbody have been given by Atkinson (1906), Sass (1929), Hein (1930a,b), Styer (1930), Colson (1935), Sarazin (1952), Garrett (1954), Tschierpe (1959), Mathew (1961), Singer (1961), Flegg (1962a), and Lambert (1962).

The first signs of hyphal differentiation to be observed are an aggregation of hyphae in both compost and casing, to form mycelial strands. In the compost, their function is to transport nutrients most efficiently (Garrett, 1954, Lambert, 1962) and larger cells may actually store food reserves (Hein, 1930a). Strand growth is more pronounced in wetter composts (Hein, 1930a, Mathew, 1961, Flegg, 1962a).

The network of fine hyphae and mycelial strands in the compost is continuous with a similar network in the casing. Here, however, strand formation is more pronounced, probably because the casing layer has a high moisture content. (Flegg, 1962a, Mathew, 1961). The growth and further development of mycelial strands in the casing was found by Flegg not to depend on a well-established strand network in the compost.

However, the colonisation of the casing layer by mushroom mycelium and its formation into strands, is seen as a necessary preliminary to fruitbody formation.

Garrett (1954), considering mycelial strands in a soil sandwiched between a "food base" of mycelium colonised (manure) spawn and an

uninoculated compost, suggested that strands served the function of increasing "inoculum potential", by pooling the resources of individual hyphae enabling the colonisation of more distant substrates. However, the relevance of this work to strand formation under normal conditions is unclear. Clearly, transport of nutrients is a function of these mycelial strands, but whether this is important during the first stage of colonisation of casing is uncertain.

Styer (1930) noted increased strand formation in wetter substrates- he used filter paper - and Hein (1930a) observed strand formation mainly in wetter composts, with filamentous mycelial growth in drier composts. Mathew (1961) obtained better strand formation on the wet walls of tubes than in Petri-dishes. Using sterilised silica sand and a complete nutrient solution he concluded that strand formation in mycelia growing out from a food base was inversely correlated with the concentration of free nutrients in the surrounding medium. However, he obtained better strand development using non-sterile sand.

Long and Jacobs (1968) showed that growth of hyphal strands of A. bisporus into normal unsterilised, or sterilised, casing was a process requiring carbon dioxide (CO_2), the growth rate being proportional to the CO_2 content of the surrounding air.

Rasmussen (1959) stated that the tendency of the mycelium within casing to reanastomose following disturbance was reduced once the vegetative stage had given way to the fructification stage. In one of his experiments on nutrient translocation, Lambert (1962) noted that a considerable amount of nutrients had been passed through a small aperture in a barrier separating two sections of compost. Within this hole, 1 inch in diameter, had developed a bundle of very thick "rhizomorphs" (mycelial strands) and thinner strands could be seen converging onto this from both sides of the barrier. He concluded that the network of "rhizomorphs" was adapted to translocating nutrients and was formed in response to stimuli from

growing sporophores.

However Grainger (1962) from a field study of a small number of fruiting structures of Phallus impudicus and the associated mycelial network (which could be easily excavated, measured and analysed) concluded that the thicker mycelial strands were less efficient at producing fruitbodies, i.e. they gave smaller ones. It is unclear whether this was due to inefficiency in substrate utilisation or in translocation, (or indeed whether it is relevant in the present context). Mathew (1961) described morphogenesis of mycelial strands in A. bisporus, and concluded that it resembled A. campestris, Merulius lacrymans and Phymatotrichum omnivorans.

Butler (1957) described this sort of strand as " a loose federation of hyphae, increasing in thickness by accretion of hyphae from the base", and distinguished between this and the rhizomorph of Armillaria mellea for example which is a " fully autonomous apically growing organ". Using M. lacrymans Butler (1958) described how strands were built up by growth and branching of thin-walled thigmo-tropically sensitive "tendril" hyphae in association with wider main hyphae. This type of strand was found in mycelium growing from a wood food base onto glass slides, incubated axenically in moist containers, and also in aerial hyphae above agar medium, but not in direct contact with agar.

At or near the surface of the casing, nodules are produced by thickening, rarely at the tips but usually at lateral branches of the mycelial strands (Hein, 1930b).

At this point, fruitbody formation is said to have been induced or "initiated" - the nodules or swellings are known as "initials". This stage is characterised by very little internal differentiation.

A slightly more advanced stage is known as a primordium or pinhead. Here a distinction is visible between the parts which will become stalk (or stipe) and cap (or pileus) - this shows as a slight

constriction around the equator of the primordium, giving it a "cottage bun" like appearance.

Initiation was shown by Tschierpe (1959) to be a process independent of gravity - however the subsequent development shows definite geotropic responses.

Usually more primordia are formed than actually develop (Hein, 1930b) The primordium stage was said by Tschierpe (1973) to constitute a possible stage of rest.

From sporophore transplantation experiments, Sinden, Tschierpe and Hauser (1962) showed that maturing primordia suppressed the formation or development of further primordia, probably by hormonal action rather than by simply monopolising nutritional reserves.

Weaver et al.(1970) showed that a pigment inhibiting respiration was produced at the stage of pileus formation in Agaricus bisporus.

Manachere and Robert (1972), studying the fruiting rhythm of Coprinus congregatus hypothesised the existance of two phases in sporophore formation; the first being the development of primordia up to a certain stage at which future development is inhibited, and the second being a continuation of that development, resulting from the removal of the restraint caused by the presence of the preformed primordia. However they could not conclude whether the flushing phenomenon could be attributed to simultaneous initiation of all primordia followed by rhythmical maturation of a proportion of these, or to rhythmical repetition of the whole initiation sequence.

From a morphogenetic point of view, the developmental stages which the mushroom primordium undergoes have been studied extensively, particularly in older texts.

The universal veil splits almost imperceptibly into the general and partial veils, and the rudimentary hymenium forms as an annular mass of tissue which then develops into the gills, with the associated formation

of a cavity below them. Parallel hyphae forming the stipe tissue expand, mostly in length, (Bonner, Kane and Levy, 1956) together with expansion of the pileus which ruptures the partial veil and exposes the gills.

Singer (1961) describes the development of the fruitbody as hemiangiocarpous, in that the hymenium covering the lamellae is formed internally and only exposed at maturity.

Work on the regeneration of individual parts of the fruitbody show that the various parts of the mushroom differ in their regenerative powers, according to the complexity of their organisation.

Sinden et al. (1962) could anastomose vegetative mycelium with caps isolated from stalks, but these did not develop further, and Singer (1961) stated that lamellar regeneration would not occur except in contact with "the lamellar zone", and that orientation of these lamellae was disrupted. Such disruption of normal development is also known to be caused by contamination of casing material with mineral oils, resulting in the condition known as "rose comb".

This predetermination of the fate of different parts of the fruitbody and the inflexibility imposed by differentiation contrast with the ease by which they revert to vegetative mycelium. This is the basis of Duggar's (1905) technique for obtaining cultures from mushroom fruitbodies.

In commercial production, development of the pinheads takes them to the stages known as buttons, cups and flats, which are the main grades into which mushrooms are divided before being marketed. In the first category, the stipe and pileus are of approximately equal diameter; in the second stalk growth and an opening out of the cap occur, stretching the partial veil until it breaks, to give the "open cup", the gills showing as pink; the final stage results from continued upward curvature of the cap and spore production, causing darkening of the gills to deep chocolate brown, eventually achieving a concave top.

Flegg and Gandy (1956) defined the normal range of size and shape in the various developmental stages of the mushroom sporophore.

The feasibility of picking at different stages varies according to considerations of economy and hygiene, and can affect total yields and pattern of cropping considerably.

Cooke and Flegg (1965a) showed that individual mushrooms increased in weight as they grew in size from "buttons" to "cups" and opened to become "flats". Hence obviously greater weight yields result from picking individual mushrooms or flushes of mushrooms at more mature stages. Additionally, it was shown that if a crop was consistently picked at a mature stage, the number of mushrooms was reduced, disproportionately to the increase in weight yield, resulting in an increased mean size of individual mushrooms. The period between harvesting was, of course, increased.

Kneebone and Mason (1962) obtained similar results but found little difference in yields between mushrooms picked at the stages characterised by partially and tightly stretched veil. The effect on yield of different picking stages is particularly relevant with regard to comparisons made between yields obtained by different experimenters.

The difference between the nutritional requirements of the two phases of the mushroom life cycle are well known.

On the one hand, vegetative growth has lent itself to studies involving defined liquid media, Block, Stearns, Stephens and McCandless (1957), Fraser and Fujikawa (1958) whereas only limited application has been possible with the production of fruitbodies on such media impregnated into carrier materials in a hydroponic-like culture system (Bretzloff, 1962, Smith and Hayes, 1972).

From analyses of compost constituents, Gerrits (1968) and Gerrits, Bels-Koning and Muller (1965) showed that lignin was utilised by the mushroom during spawn-running, as suggested by Waksman and

Nissen (1931 and 1932), whereas during cropping, cellulose and pentosan tended to be consumed. This switch was not due to the exhaustion of lignin.

Turner (1968, 1974) performed determinations of the activity of phenoloxidase enzymes in mushroom composts and casings, and found that compost was characterised by a high concentration of laccase (lignin-decomposing enzyme) during mushroom production whereas laccase activity in casing was low. Conversly, tyrosinase activity was high in casing and low in compost.

This was taken to reinforce Lindeberg's (1950) finding that extracellular laccase was characteristic of mycelium, and intracellular tyrosinase was present in fruitbodies, while both were active in mycelial strands.

In later work, Turner, Wright, Ward, Osborne and Self (1975) showed that this enzyme pattern was not merely a reflection of the contrast between the compost and casing, but between the precropping and cropping phases.

The transition between the phases was marked by an "enzyme switch", from predominantly laccase activity to cellulase activity, a single transition at the stage of maturation of the first flush accompanied by cyclical production of ethylene as each successive flush matured. This change was detectable first at the compost/casing interface and proceeded downwards throughout the compost, and the "switch" was speeded up by the transplantation of mushrooms from other beds.

Hence a hormonal or diffusible "message" appeared to be involved, affecting not only the casing but altering the metabolism of the entire mycelium within the compost.

(ii) Theories of fructification in Agaricus bisporus

a) Action of gases and volatile substances

Early investigations showed that factors concerned with the action of ventilation operated at the surface of the bed, i.e. on the casing and mushrooms themselves.

Lambert (1933) discovered that accumulation of carbon dioxide in the air above a mushroom bed to levels of 1% by volume or higher caused abnormal growth, stunting or even death of fruitbodies and that this effect was due primarily to the carbon dioxide surrounding the sporophores, not in the interstices of the compost in the bed.

More accurate studies by Tschierpe (1959) fixed the maximum CO_2 concentration permissible as 0.2%, above which abnormal growth occurred.

Thus the casing layer came to be regarded as a medium allowing gaseous exchange to occur by diffusion. That is to say, it formed an interface between compost and air, and the relevance of conditions within the casing layer assumed fundamental importance. Pizer and Leaver (1947) had stressed the importance of contact between casing soil or mycelium therein and air. However Tschierpe (1959) postulated that the function of the casing was to produce a CO_2 gradient from the air in the compost to the air above it and Long (1968) interpreted this as meaning that fructification took place at a certain point in the region of the CO_2 partial pressure gradient.

Additional information presented by Tschierpe and Sinden (1964) showed that for most mushroom strains maximum initiation of fruiting occurred between 0.03 and 0.1% CO_2. In practical terms, this is only very slightly higher than the normal concentration of CO_2 in the atmosphere. Exposure to these levels of CO_2 could also induce fruiting even when mycelium had been previously subjected to higher CO_2 levels, which were normally unfavourable to mushroom production. They stated that a CO_2 concentration of 0.08% in the air of the cropping room was the highest tolerable level which did not tend to reduce yields or

The work of Long and Jacobs (1968) confirmed these results.

Despite this attention to carbon dioxide, other investigators reasoned that the mushroom crop might be expected to produce other volatile metabolites, presumably in quantities proportional to the output of CO_2, although perhaps in much lower, even undetectable, quantities, and that these effects attributed to carbon dioxide might actually be due to other compounds.

Mader (1943) argued that in Lambert's (1933) experiments, the effect of accumulated gases from the respiration of the mushroom (of which only CO_2 was measured) was greater than the effect of an artificial mixture with a similar concentration of CO_2. He postulated the existence of a substance acting as a stimulant to fructification when present in small amounts, yet in larger amounts arresting sporophore growth and preventing fructification. The accumulation of such a substance was favoured by conditions in underground mines, but did not have such a marked effect on yield under "mushroom house" conditions, presumably due to better ventilation.

Experiments followed, involving specially constructed chambers linked to pumps, which could be used to "wash" the atmosphere by passing it through substances designed to remove the volatile inhibitor/ stimulator. On the basis of apparently successful fruiting when alkaline potassium permanganate and mineral oil were used in air washing, Mader concluded that an unsaturated hydrocarbon was responsible. This experiment in particular was questioned by Stoller (1945) and Schisler (1957) on the grounds that a partial vacuum must have resulted due to absorption of CO_2, so that Mader's apparatus could not have been air-tight.

However, Mader also claimed tohave obtained fruiting by passing air through activated charcoal. This was partly borne out by Sinden in unpublished experiments reported by Schisler (1957). Here again, recirculation of air through activated charcoal alone brought about fruiting, even though it apparently did not absorb carbon dioxide.

Much of Stoller's work on abnormal growth forms of the mushroom (1945, 1962 a, b) probably suffered from the lack of suitably precise techniques for the determination of CO_2, and consequently he, too, attributed some morphological effects to gases other than CO_2 simply because he could not detect this gas in his apparatus, i.e. it was below 0.2%. He hypothesised the existence of a volatile substance with a lower rate of diffusion than CO_2, but which was produced together with CO_2 by the mushroom. When present in "large" amounts as in the compost, it was said to stimulate mycelial growth and act as a defence substance against attack by competing micro-organisms, but initiate mushroom fructification when present in small amounts, as in the casing.

Accordingly, Stoller saw the function of the casing as being to provide "an alkaline oxygenated medium" for the destruction of this volatile substance, which he later (1954) suggested might be a quinone, formed by the breakdown of lignin.

Linked with this hypothesis Stoller (1952 c) mentioned the addition of substances to alter the redox potential of the soil which, he claimed, increased yields, yet he did not state clearly whether reducing or oxidising conditions were preferable in the casing layer.

Schisler (1957) rejected Stoller's theory on the grounds that he had obtained fruiting by casing with silica sand kept suitably moist, and that redox reactions could not be expected to occur in this medium. He proposed the existence of a high molecular weight hormone-like substance of low volatility which was produced by the mycelium. The function of the casing layer was to inhibit sufficiently the volatilisation and/or diffusion of the material so that a certain concentration was obtained to provide the stimulus for fruiting "in the mycelial network of the compost". Excess concentrations of these substances could adversely affect strands or rhizomorphs in the casing layer.

This conclusion was based on experiments in which a small degree of initiation was obtained by watering uncased spawn-run compost. The

water formed a barrier to the volatilisation of this postulated substance.

Interestingly, it seems that, based on occasional observations that condensed water dripping onto beds brought about mushroom formation, Stoller (1954) had obviously tried watering uncased compost, but without effect.

However Flegg (1960) who also obtained fruiting on watered compost, argued that he had leached away soluble salts from the compost surface, bringing about fruiting by relief from "water stress".

In more recent work, Lockard and Kneebone (1962) succeeded in identifying by flame ionisation gas chromatography five extra metabolites produced by the mushroom mycelium. As well as CO_2 and water vapour, ethylene, acetaldehyde, acetone, ethanol and ethyl acetate - were shown to be present. Subsequent findings cast doubts on the purity of these cultures so that some of these gases may be artifacts. Visscher (1975b) cites Tschierpe and Sinden (1965) and Richter (1967) as indicating that many of these compounds were the product of anaerobic growth.

Nevertheless, these findings were partly confirmed by Tschierpe and Sinden (1965) who noted that under commercial conditions acetone accumulated after casing, and was subsequently reduced to a low level. However, they could not detect any effect on fruiting or strand formation when acetone, ethanol, acetaldehyde and ethyl acetate were applied to mushroom cultures in a continuous air stream.

Subsequently, Staueble and Rast (1971) identified in addition the following compounds: ethyl methyl ketone, isobutanol, n-butanol, iso-amyl alcohol, amyl alcohol, acetic acid and valeric acid. Such characterisation of volatile substances produced by the mycelium of the mushroom in its growth obviously suffers more from problems of technique than the isolation of substances responsible for the odour of mushroom fruitbodies as performed by Cronim and Ward (1971).

Other phases of the mushroom life cycle are affected by volatiles produced during mycelial growth; the stimulatory effect of mushroom

mycelium on mushroom spore germination as noted by Ferguson (1902) and Losel (1964) was later shown (Losel 1967) to be due to isovaleric acid, and Rast and Staueble (1970) elucidated the biochemical mechanism underlying this effect - the removal of a carbon dioxide self-inhibitor in a CO_2 fixation reaction.

Turner, Wright, Ward, Osborne and Self (1975) identified many mushroom metabolites including carbonyl sulphide which might be considered to be an artifact of their technique, which involved autoclaving the cultures "to increase the quantities of gas present". They concluded that ethylene was the only gas showing a pattern of production corresponding to cropping. Escobar (1970), however, had concluded that ethylene was irrelevant to the mushroom life cycle. Nevertheless, ethylene is known to be produced by, and have physiological effects on, a wide range of plants, and especially fungi (Ilag and Curtis, 1968, Tabak and Cooke 1968, Fries, 1973, Pratt and Goeschl, 1969).

b) Involvement of micro-organisms.

The preoccupation with the metabolic products of the mushroom was a consequence of the belief that it was the sole or at least the predominant present in the culture system. However other micro-organisms were known to be involved in the processes of mushroom culture such as composting (Hayes 1969) and the decidedly unsterile nature of both compost and casing was illustrated by the variety of fungal and bacterial pathogens which could affect the mushroom, particularly given conditions only slightly departing from those considered favourable to mushroom production (Kneebone and Merek, 1961, Sinden, 1971).

The involvement of other micro-organisms in the fruiting of the mushroom and attempts to explain their role in relation to the function of the casing were initially taken to be irrelevant to and possibly opposed to the "metabolite" theories of inhibitors, stimulators, and gaseous gradients.

Lambert and Humfield (1939) and Pizer and Leaver (1947) had noted decreased yields with heat-sterilised soils. Stoller (1954) attributed this to toxic substances.

Eger (1961) showed that the mushroom would not fruit in pure culture, and described a simple proceedure, the "Halbschalentest", in which compost and casing were placed in different halves of a divided Petri-dish. This system had the advantage that it could be totally sterile and both the compost and casing could be autoclaved. The compost was then inoculated with pure cultures of mushroom mycelium, which grew across into the casing. Only unsterilised casing or autoclaved casing inoculated with a suspension of a productive casing gave fruiting, which seemed to be associated with the inhibition of growth of the mushroom mycelium. The effectiveness of this laterally placed casing soil was taken to cast doubts on Tschierpe's CO_2 gradient hypothesis.

Thomas, Mullins and Block (1964) confirmed Eger's work by their own modification of the Halbschalentest, and concluded that the micro-organisms involved did not modify the gaseous environment, neither did a CO_2 gradient occur, nor was it likely that volatile stimulators or inhibitors were responsible. However, their work could not be taken to rule out these explanations on a highly localised and intimate basis if, for example, the operation of these factors was on a much smaller scale than could be observed in the Petri-dish microenvironment.

Eger never apparently went as far as to identify the specific bacteria responsible for the induction of fruiting, stating that mixed bacterial populations were the most effective stimulators. In a summary of her work (1972) she stressed the differences between the microflora of productive and unproductive mushroom culture systems. Bacteria present in the former, which could induce primordium formation, increased in numbers after casing, apparently utilising volatile mushroom metabolites. Sterile culture filtrates of these bacteria were without effect, and activated charcoal was the only material which could mimic the effect of

bacteria, presumably a consequence of its absorptive properties. Different mushroom strains were found to vary in their sensitivity to bacterial induction.

O'Donoghue (1962a) observed sporophore formation on grain spawn contaminated with Actinomycetes, which were confined to cottony nodules at the base of the sporophores. Three species of Streptomycetes were isolated.

Park and Agnihotri (1969a) tested several soil bacteria- notably Arthrobacter terregens, Rhizobium meliloti and Bacillus megaterium, which they said stimulated sporophore formation, and also claimed that metabolites of these bacteria "triggered" sporophore formation, since culture filtrates were apparently stimulatory. Furthermore (1969b) they claimed to obtain comparable results by the addition of biotin. This work could not be repeated by Eger (1972).

Using selective techniques Hayes, Randle and Last (1969) isolated bacteria from normal casing soil microflora which could utilise known metabolites of the mushroom as sole carbon sources.

Several bacteria identified as close to Pseudomonas putida and Group IV Pseudomonads were the most effective in causing fruiting in the mushroom when applied as pure cultures to sterile casing overlying compost containing aseptically-grown mushroom mycelium. This took place in flasks provided with a throughput of cooled and humidified air, filtered to sterility.

Hayes et al. concluded that the function of the casing was to provide a substrate which these bacteria could successfully colonise together with the mushroom.

Ethanol was shown by Hayes and Nair (1976) to be the most active single component of the known volatile metabolites of A.bisporus in stimulating bacterial members in a casing soil simulation.

Curto and Favelli (1972) tested the effect on cropping of a range of different organisms, bacteria and yeasts in pure and mixed cultures,

applied as a suspension before and after casing, and yields were increased by 30% or more in some cases. Urayama (1967) obtained similar stimulation in mycelial growth, earlier initiation and increased yields when a bacterium Bacillus psilocybe isolated in earlier studies (Urayama, 1957) on fruiting in Psilocybe panaeoliformis was applied to mushroom cultures, but it was reported that later this isolate had lost its power (Hayes, Randle and Last, 1969).

Smith and Hayes (1972) and Hume and Hayes (1972) extended earlier work and combined some of the features of Eger's Halbschalentest on a small scale and in Petri-dishes, with the use of defined media. Different culture systems suitable for contrasting strains were described, which permitted production of fruitbody primordia on agar media by the interaction of the mushroom with the bacterium Ps.putida.

The effectiveness of this technique and the stimulatory action of Ps.putida was confirmed by Arrold (1972).

Hayes (1972) in a summary of the nutritional requirements in the different stages of the life cycle, concluded that fruitbody formation is more nutritionally demanding than mycelial growth. He discovered that although some metal ions were inhibitory to fruitbody formation, several iron-containing compounds stimulated primordium formation at certain concentrations. EDTA, a chelating agent, stimulated primordia formation at low concentrations but at high concentrations inhibited mycelial growth, an effect which could only be reversed by the addition of ferrous ions.

Hayes argued that iron was unavailable in the calcareous neutral or alkaline casing soil, and that organic matter, with its ion-complexing properties, corresponded to his agar plus EDTA at inhibitory concentrations. He went on to suggest that Ps.putida or similar organisms function to release iron for purposes of growth of the mushroom, associated with fructification.

Subsequently Hayes and Nair (1976) linked with the various technical problems of studying fruiting in artificial conditions the capacity of strains of A.bisporus to adapt to these culture conditions.

Couvy (1974) also stated that most commercial strains of the mushroom did not normally fruit under aseptic conditions, although some strains apparently did, which may explain her earlier (1972) claim of aseptic fruiting of the mushroom on a compost medium lacking glucose. She assayed the fructification-stimulating power of the total casing soil microflora, concluding that the greatest activity is present in soils exposed to mushroom mycelium for 15-20 days. Sterile filtered casing soil extracts apparently stimulated fruiting in one out of five mushroom strains tested.

Several different strains of bacteria (Rhizobium and Pseudomonas species) were tested for their action on initiation in several mushroom strains, the results indicating that the mushroom strains employed seemed to be critical under her experimental conditions, since some strains did not respond at all to bacterial induction. However, those that did so showed a graded response according to the bacterium; the most effective Pseudomonad was Ps.putida, followed by a strain of Ps.fluorescens. Ps.tolaasii, and another unidentified Pseudomonad were less stimulatory.

Eger (1972) dismissed as being due to chance contamination earlier reports of fruiting under aseptic conditions by Schisler (1957), Koch (1958) and Lockard and Kneebone (1962) and also expressed scepticism about Park and Agnihotri's work (1969a,b). Whilst confirming some of the results of Hayes, Randle and Last (1969) she expressed some dissatisfaction about the sporophore-inducing powers of some isolates of Ps.putida obtained from culture collections. She also suggested that acetone might play a role in selecting for bacteria in the casing.

Smith and Berry (1974) mentioned an unconfirmed report of the production of an extract of Ps.putida which could stimulate sporophore production on a defined medium. Preliminary investigations of its nature apparently indicated that the active component was a chelating agent, and they stated that"sporophore production can be obtained by growing vegetative mycelium on malt agar then allowing it to spread over agar containing either aureomycin, 8,OH-quinoline sulphate or EDTA. These three compounds are all chelating agents. Preliminary results indicated that the critical ion being chelated is iron!".

During a general discussion following a seminar on the casing layer, and in reply to a suggestionby Storey that condensation of water might occur within the casing layer, Hayes (1974) postulated that this might create conditions of oxygen stress, in which Pseudomonas sp. might be expected to "solubilise" and reduce iron. He hypothesised the existence of zones of differing aeration in the casing layer, and, despite suggestions that the presence of such a layer might be detrimental to mushroom production, stressed the possibility that a "water-logged" oxygen-deficient zone may be of significance, especially as regards the relative location of fruiting within the casing layer.

Ottow and Glathe (1971) had noted that certain facultatively anaerobic bacteria including Pseudomonads could reduce iron under waterlogged conditions.

In addition Hayes explained that his Petri-dish technique for obtaining primordia could not be directly related to the effect of the casing layer, principally because it required different environmental conditions, notably humidity. Although this artificial system (Hume and Hayes 1972) differed markedly from that employed in normal mushroom growing, he did state that evaporation or drying out of the medium seemed to be implicated in both.

c) Joint approaches - microbial/gaseous interactions

Hayes, Randle and Last (1969) and Eger (1972) attempted to reconcile the two confronting opinions on the initiation of fruiting: the "microbial metabolism", and the "volatile organic compounds" theories, by a single hypothesis, because bacteria initiating fruiting are able to utilize mushroom metabolites as a sole carbon source, presumably reducing them to non-inhibitory levels.

Such integrated approaches involving a study of the microbial involvement in fruiting, taken together with a consideration of the gaseous environment of the casing, have so far been most promising.

Long and Jacobs (1968) studied the response of the mushroom to different levels of carbon dioxide, passed down through the casing and compost enclosed in a tubular chamber in such a fashion as to flush out any volatiles originating in the compost. They concluded that CO_2 was the only gas that need be involved in fruiting in normal non-sterile casing.

They found that CO_2 was required for the growth of hyphal strands into the casing, whether it was sterile or non-sterile. In sterile casing, the rate of strand growth was proportional to CO_2 concentration up to 6700p.p.m., not increasing above that level. By contrast, in non-sterile casing, hyphal growth was proportional to CO_2 concentration up to 104 p.p.m. and between 104 and 1000 p.p.m. a retardation of strand growth occurred, which was correlated with the production of sporophore initials. Above 1000 p.p.m. strand growth again increased to levels comparable with sterile casing. Maximum sporophore initiation occurred between 340 and 1000 p.p.m. CO_2, and CO_2 was also required for the early stages of sporophore development.

These observations were entirely in accord with the gradient hypothesis of Tschierpe (1959) and within the range stated by Tschierpe and Sinden (1964). However, the involvement of casing soil microflora was obviously very important, since fruiting did not occur in their

absence. Long and Jacobs suggested that they might function in disturbing the mechanism of CO_2 fixation in the mycelial strands.

San Antonio and Thomas (1972) showed that for small inocula of hyphal fragments, hyphal growth was inhibited in the absence of CO_2, and that some CO_2 was required for growth.

Le Roux and Couvy (1972) showed that mycelial strands were more active in CO_2 fixation than vegetative mycelium, principally due to increased urea cycle activity.

These studies are in addition to quite comprehensive kinetic studies of CO_2 fixation in fruitbody tissue by Wehrli and Rast (1967) and in cell free extracts of sporophores by Rast and Bachofen (1967 a, b), and Bachofen and Rast (1968).

In an extension of their previous work, Long and Jacobs (1974) used the same experimental system to investigate the mechanism by which activated charcoal simulates microbial action in fruitbody initiation, as reported by Eger (1961).

Under suitable CO_2 concentrations, growth rate of mycelial strands in charcoal amended sterile casing approximated to that obtained with non-sterile peat casing, andagain fruitbody formation occurred in conjunction with a checking of hyphal growth, although the optimum CO_2 levels for this seemed to differ somewhat.

CO_2 concentrations above the levels considered optimum for fruitbody formation gave proportional increases in growth rate, at least from 1000 to 6700 p.p.m., presumably due to fixation of CO_2, inducing the switch from the vegetative state to the reciprocal state of fructification. Le Roux and Couvy showed that CO_2 fixation does occur at 640 p.p.m.

Apparently exogenous nutrients around the growing hyphae nullified the responses to these CO_2 levels, since mycelium growing in compost was insensitive to the CO_2 levels used by Long and Jacobs, which suggests that the work of Tschierpe (1959) and San Antonio and Thomas (1972) who used nutrient media, is not relevant in the context

of the casing environment.

The action of the bacteria on the fungal growth rate was suggested by Long and Jacobs to be a continuous removal of metabolites leaked or secreted from the hyphal tip, in response to which the mushroom hyphae would cease to grow and fruitbodies would eventually form. Angeli-Couvy (1975) confirmed this effect of activated charcoal, and suggested that ethanol might be involved. Both of these hypotheses had been put forward by Eger (1961).

Long (1968) reported that temporary checking of mycelial growth would not suffice to bring about fructification. He observed that bacteria grew alongside the mushroom hyphae, suggesting the existence of a hyphosphere analogous to the rhizosphere of higher plants. He concluded that the relationship between the mycelium and its associated microflora must be intimate.

Nair and Hayes (1974, 1975) and Nair, Short and Hayes (1976) stressed that under the conditions prevailing in the alkaline casing layer, an equilibrium was established between gaseous carbon dioxide, carbonic acid, and the bicarbonate ion, so that study should centre not on the concentration (partial pressure) of carbon dioxide in the casing layer, but rather on the amount (activity) of bicarbonate ion present. The bicarbonate ion represented the species or effective form of carbon dioxide operative at the cellular level.

They interpreted the effect of various CO_2 levels on fruiting not with regard to the mushroom mycelium, as Long and Jacobs had done, but concentrated on the effect of the bicarbonate ion on the microbial populations of casing soils. Amendment with various levels of bicarbonate increased the numbers of Pseudomonads in the casing soil, up to 10 days after casing. A higher percentage of ferric oxide-reducing Pseudomonads was isolated from the bicarbonate treated soils than from controls. A distinction was drawn between the ability of the bacteria to reduce relatively insoluble ferric oxide and the relatively soluble ferric chloride, since some bacteria reduce iron

only when it is in solution. Of eight Pseudomonad strains isolated, only three were said to reduce ferric oxide, but all reduced ferric chloride.

Nair and Hayes (1975) established a relationship between yield of mushrooms and numbers of Pseudomonads in the casing layer.

(iii) Comparison of fruiting in Agaricus bisporus with other fungi

In some respects, Agaricus bisporus represents an anomaly, when compared with other fungi.

Its fruiting is unaffected by light, which Plunkett (1956, 1958) showed to be necessary in Polyporus brumalis and Flammulina (Collybia) velutipes, and this is the case with many other fungi.

Generally , wood-decaying "bracket" fungi respond to different environmental stimuli for fruiting than coprophilous and soil and litter agarics. Badcock (1943) found that the former required a high atmospheric relative humidity, but not total saturation, providing a low but steady evaporation rate. Plunkett (1958) linked this with transport of dissolved minerals up the stalk of the developing fruitbody, and also showed that with Polyporus brumalis high atmospheric levels of carbon dioxide did not have an adverse effect on the process.

Nevertheless, some wood-decaying fungi have been induced to fruit by a process broadly comparable to casing. Singer (1961) cites the example of a polypore Polyporus corylinus or P.brumalis which in the Lazio part of Italy is "cultivated" by partially burning naturally-infected hazel stumps which are then covered with a thin layer of earth. However, it is unclear whether this constitutes an improvement over a similar technique used in other parts of the Mediterranean to induce fruiting in Polyporus tunetanus, in which the earth is omitted.

Possible explanations for those practices may be based on the water holding capacity of the soil or charred wood, which may in turn relate to the practice in the production of the Shiitake Lentinus edodes of

soaking the bed-logs to bring about fruiting.

Alternatively, there may be a link with the observation that certain fungi, e.g. the Morel Morchella esculenta often fruit on charred ground (Ramsbottom, 1972, Watling, 1973). In this case, it could conceivably be explained by the removal of competition in the sterilised soil, or by the release of mineral nutrients such as potassium (Delmas, 1974).

Fruitbody formation in coprophilous fungi in general is affected by carbon dioxide concentrations in the same way as in Agaricus bisporus. That is to say, stipe elongation and reduction in size of the pileus is noted at higher levels (Plunkett, 1958).

This may be explained as an adaptation to ensure that spores are released above ground level, and a similar reasoning may account for the requirement for light to inititate fruiting.

Plunkett (1958) observed that in the agaric Flammulina velutipes, which grows on tree trunks and stumps wide variations in relative humidity (evaporative drying rate) had little effect on fruitbody formation, but extreme drying conditions caused arrest of fruitbody growth.

Roberts (1951) showed that translocation up the stipe of a developing fruitbody of an agaric was an active process unrelated to evaporative transpiration.

In fungi generally it is necessary for reserve food substances to be accumulated in the mycelium, prior to fruitbody formation (Madelin, 1956, a, b, 1960 Gruen, 1969, 1972). Smith and Berry (1974) state that both carbohydrate and nitrogen are required for initiation, but that only carbohydrate is necessary for growth of primordia.

It appears that most of the well-studied fungi can fruit axenically (Eastwood, 1949, Volz and Beneke 1969, Gruen 1969, 1972) but there are reports concerning requirements for the presence of contaminating micro-organisms in order to bring about fruiting in other, mainly "lower", fungi (Heald and Pool, 1908, Manning and

Crossan, 1966).

Often these have been in somewhat artificial conditions, and have usually been ascribed to the production of growth factors, vitamins etc., required by the fungus.

Urayama (1957) found that under certain circumstances pinhead formation in Psilocybe panaeoliformis could be stimulated by the presence of bacteria of the genus Bacillus. This organism, which was originally isolated as a contaminant of the culture medium, seemed to have its effect by producing a metabolite reversing the inhibition of pinhead formation due to excess nitrogen in the medium. The same bacterial strain had similar effects on other hymenomycetes, including Agaricus bisporus (Urayama, 1967).

The genus Agaricus was subdivided by Singer (1961, 1951) into several taxonomic classes.

Within the class "Campestres" he included the cultivated mushroom Agaricus bisporus, also Agaricus bitorquis, increasingly cultivated under similar conditions, and the field mushroom Agaricus campestris, which despite indications to the contrary in older texts, (Lambert, 1929, Ferguson, 1902) has probably never been cultivated under normal conditions (Sarazin, 1951, Cayley, 1937, Kligman, 1943). Agaricus subperonatus, which may have been cultivated, is also in this group.

Agaricus bitorquis (syn. edulis, rodmani) forms hyphal masses in pure culture which may be equivalent to primordia or initials, and are taken to signify fertility in genetic studies (Fritsche, 1976, Raper, 1976). Other evidence that this species may not require a microbial stimulus for fruiting, is afforded by occasional observations of the formation of complete fruitbodies in pure culture spawn bottles.

Nevertheless, when this mushroom is grown (Poppe, 1972, Vedder, 1975, Barnard, 1977) a casing layer is employed exactly as in the cultivation of Agaricus bisporus.

Sarazin (1951) reported that he had successfully cultivated the 4-spored Agaricus campestris: "ordinary" compost (for A.bisporus) was unsuitable, but a straw compost fermented at low temperatures, mixed with soil, was satisfactory under otherwise normal conditions of cave culture. Conversely he had been able to establish spawn of the bispored cultivated mushroom in sheltered grassy situations outdoors, and by this method he produced fruitbodies of A.bisporus in the same habitat as A.campestris.

Within his class "Arvenses" Singer (1961) included Agaricus arvensis and A.sylvicola. He expressed respectful scepticism towards reports by Sinden and Treschow that A.arvensis had been grown commercially, since he knew of no examples of cultivable species outside his section "Campestres". However Zadrazil et al. (1973) and Couvy (1973 a) stated that this species would fruit axenically, on uncased compost.

Nevertheless, Couvy (1973 b) considered that both A.sylvicola and A.arvensis could be useful in studies on the fundamentals of fruiting in mushrooms. A.syvicola would form primordia axenically under laboratory conditions, the extent and timing of their production being related to the sugar content of the culture media employed. This species has, however, been grown under conditions comparable to the ordinary mushroom production, and following the normal casing procedure, fruitbodies were formed (T. G. Denham, W. Darlington & Sons Ltd., personal communication).

Couvy (1973 a) found that in Agaricus arvensis grown axenically on compost in flasks or Petri-dishes, maximum growth rate was achieved at 25°C in darkness. Early stages of primordia formation were substantially unaffected by temperature or lighting, but further development of these primordia required a drop in temperature of 16°C, and for complete fruitbody maturation an alternation of 12 hours light and 12 hours darkness was necessary. The ecological significance of these findings seems extremely clear-cut, in that they reflect the

contrast between conditions below and above ground, but also there is a direct parallel between these parameters and those aimed for in the production of Agaricus bisporus, with the exception of light. In Agaricus arvensis, carpophores which formed in total darkness had a reduced cap and often a long stalk, which may represent an adaptation to ensure that spores are liberated above ground.

Couvy (1973 a) apparently observed an effect of light on Agaricus bisporus, whereby primordium number was increased by exposure to light.

This suggestion that initiation could be sub-divided into several phases, each with differing environmental requirements, is also explored in hypothetical terms by Manachere and Robert (1972).

In the course of studies on several isolates of the litter and soil-inhabiting fungi, Lepista nuda and Lepista saeva, Wright (1976) attempted to bring about fruiting in these species.

On the laboratory scale, no evidence was discovered for the involvement of other micro-organisms in fruiting. Rather, a certain amount of nutrient or mycelial reserves were required; also a steady low temperature ($12^{o}C$) was necessary, and fruiting took a long time.

On a larger scale, spawn of some strains of L.nuda grew into "ordinary" mushroom compost, which was subsequently subjected to "casing" by a variety of substrates, the most successful of which was a standard peat/chalk casing mixture. The mycelium having grown through this casing formed patches of dense growth similar to "overlay!" in mushroom cultivation; such areas were unproductive, exactly as would be the case with Agaricus bisporus.

However, the extended time scale of this experiment suggests that the prime function of this casing was to minimise drying out of the culture.

Thus it may be seen that the casing procedure has parallels in other situations regarding fungal fruiting, even in fungi where fruiting depends on factors apparently not operating in Agaricus bisporus.

B. Applied aspects of casing techniques in commercial mushroom growing

(i) Historical review of casing techniques and experimentation

Mushroom growing techniques have evolved considerably since the first chance production of mushrooms, probably in hotbeds in France early in the Seventeenth Century (Ramsbottom, 1972). From that time onwards, a series of empirical discoveries on casing materials and associated techniques have made many contributions to our knowledge of the properties of casing which are required. The historical development of currently used casing materials and methods is presented here.

The earliest descriptions of the methods of mushroom growing (de Tournefort, 1707) mentioned the use of a 2 cm layer of top soil mixed with limestone quarry debris, applied 2 - 3 weeks after spawning, in the French system of underground culture. Robinson (1891) and Falconer (1910) advised English and American growers to case 4 - 10 days after spawning, using 2 - 3 inches of rich garden soil, although Jackson (1909) and Duggar (1915) preferred a thinner layer of lighter soil. Passecker (1932) recommended casing later, 10 - 14 days after spawning, and he suggested the use of subsoil, to minimise contamination by the pathogen Mycogone perniciosa, preferring a sandy loam.

Ware (1935) who was the first to experiment with casing soils advocated the addition of lime to casing soil. Lambert and Humfield (1939) also carried out experiments on optimum depth, time and pH of casing. They advocated limestone instead of lime, and advised medium-to-heavy-textured loam soils due to their properties of water retention, and stated that clay soils could be satisfactory if they contained enough organic matter and had a good enough structure. Different watering regimes were shown to have an effect. Heat sterilisation of casing soil (to eradicate Mycogone) also reduced mushroom yields.

Pizer and Leaver (1947) found from a survey of casing soils used on different farms that, all other factors being equal, the casing soil had a large effect on the efficiency of the mushroom production, and accounted for the great variation in yields between the thirty-nine farms studied. Soils had a characteristic productivity, and a fixed type of mycelial growth was always observed in them. Their characteristic productivities were retained when the soils were layered on top of one another - the top layer dictating the yield obtained. Thus formation of sporophores was seen as a process requiring contact between the soil or the mycelial strand of the mushroom with air. However the productive potential of these soils was not linked to any of the usual characteristics of soil fertility or structure. Pizer and Leaver also stated that the only reliable method of assessing the value of the material for casing was by direct trial.

Work along similar lines by Borzini (1949) confirmed these conclusions, and was also taken to show the existence in casing soils of substances causing mushroom formation.

Courtieu (1949) mixed six contrasting soils of different geographical origin in various proportions, and tried to relate cropping yields to the physical and chemical analysis of the mixtures. He derived an ideal range of pH (7.2 - 8.2), organic nitrogen (0.07 - 0.18 parts per thousand) and "active calcium" content (20 - 50 parts per thousand), but came to no conclusion on the effect of physical texture of the casing types. There was a slight correlation in the cases of calcium, potassium, and phosphate between the levels of mineral in the casing and in the mushrooms derived from them.

Edwards (1949) stated that casing in addition to supporting mushrooms physically and holding water, had to allow the passage of air. He concentrated on the use of peat to increase water-holding capacity, but warned that water was also easily lost from peat, and

also stated that peat added to soil had an aerating effect, keeping soil structure "open" and preventing caking.

This was a consequence of work at Cheshunt before the war, reported by Bewley (1946, 1950). Here yield increases resulted from amending ordinary soil with peat, sand, gravel and vermiculite.

Bels-Koning (1950) using different soils, as well as artificial mixtures, concluded that, provided the material allowed sufficient aeration, the better the water holding capacity, the better the yield. Satisfactory results were obtained with vermiculite, and even cloth and paper. Powdered bricks worked exceptionally well, so that the casing layer appeared to make no nutritional contribution to the mushroom. Different cultural conditions and management practices were combined with these casing treatments as a result of which Bels Koning concluded that a certain degree of air movement was necessary to establish a state of slight but continuous evaporation, in order to bring about fruitbody formation.

Middlebrook and Storey (1950), in a comparative study of cropping in three different types of mushroom house suggested that the major controlling factor was rate of air change, indicating that high humidity, rather than some other volatile products determined mushroom productivity. This was interpreted in terms of Mader's (1943) claim of the existence of an unidentified volatile substance produced by the mushroom crop which acted as a stimulator or inhibitor of fruiting according to its concentration, hence the requirement for ventilation.

Many mixtures of different soils and other artificial substrates were studied by Edwards and Flegg, and their results were published in a series of reports of the Mushroom Research Association, Yaxley, between 1947 and 1953.

They considered especially -

(1) Water holding capacity, over a range of different water tensions (PF).

(2) Pore space, especially of peat mixtures, and its relation to the rate of gaseous exchange (Flegg, 1954 a).

Partly as a result of this work, 1954 saw a widespread adoption of Sphagnum peat as the major constituent of casing materials in this country. Peat had the advantage of relative uniformity, did not need such careful management, especially watering, and was also fairly free from mushroom pathogens.

Stoller (1952, b, c, d; 1954) claimed to have independently recommended the use of peat as a basis for casing. However, he favoured non-fibrous peat neutralised with quite small amounts of limestone and gypsum, but rejected fibrous peat (advised by the Mushroom Research Association) as unsuitable. He also drew attention to the high cation exchange capacity of peat.

For most growers in Britain today, the variability in cropping of the type referred to by Pizer and Leaver (1947), for instance, has been largely eliminated since the use of soil as a casing material has been superseded.

Studies which have been carried out in Ireland by An Foras Taluntais in conjunction with Bord Na Mona (the Irish Peat Development Authority) have included experiments on physical formulations and pasteurisation of Sphagnum peat, and additions of gravel (O'Donoghue, 1962 b), the effect of wetting agents (O'Donoghue, 1963), and different neutralising agents and depths of casing (MacCanna, 1966). Similar studies with peat carried out at the Fairfield Horticultural Station (1964) suggest that about two inches is the optimum depth with peat casing.

MacCanna (1969) attributed the almost exclusive use of Sphagnum peat to the limited experimentation with other types. O'Donoghue (1963), however, found that Phragmites peat out-yielded Sphagnum peat, but only

if gravel was added. Previously (1962 b) she showed yield differences between Phragmites peat dug from different levels. Clearly effects due to the physical structure of this type of peat are involved, and what little experience has been gained with this peat is of little significance.

Experiments with sedge peat were mentioned by Atkins (1972), who pointed out that ground chalk was found by workers at Stockbridge House to be preferable to lump chalk. Barnard (1974) obtained good yields with sedge peat especially in the early flushes, combined with uniform cropping across the bed. However the structure of the peat soon deteriorated with the formation of a "pan", so that difficulties were experienced in watering.

The adoption of peat as a casing in Great Britain prompted interest in peat mixtures, organic soils and soil conditioners by American investigators.

Rao and Block (1962) compared mushrooms grown on a clay soil from Pennsylvania with those grown on a mixture of equal volumes of peat and sand. Due to an arithmetical error in their statistics, they dismissed as insignificant a 40% higher yield on the peat based casing, and concentrated on the difference in shape of the resulting mushrooms. The peat casing gave cleaner heavier mushrooms, with tapering stipes, so that a 7% greater yield could be expected, due to reduced loss in trimming!

Reeve, Backes, Murphy, Schramer and Vollbrecht (1959) found no significant effect on yield by adding high molecular weight soil conditioning chemicals or inorganic salts (potassium and sodium chlorides, potassium carbonate and sodium sulphate) to a mineral casing soil, but in some cases size of mushrooms was increased significantly. However small scale experiments involving muck soils (high in organic matter) gave yield increases over mineral soils, but attempts to simulate the mixtures of German peat and English chalk adopted by British growers gave little improvement over their standard mineral soil. When

extended to commercial scale, such increases could not be repeated, suggesting that experience acquired with one type of casing cannot be applied to another. In particular, Stoller (1952 b) emphasized the importance of adequate air movement in conjunction with peat casings.

It should be stressed that casing is a process of primary importance in mushroom production. In the absence of casing, fruitbody formation does not occur. Mushrooms are formed on or in the casing layer, although they derive their nutrition from the compost below.

Sinden, Tschierpe and Hauser (1962) suggested that the size of individual mushrooms produced by a given system was dependent mainly on the number of fruitbody initials formed, and that yield depended on the amount of nutrients stored in the mycelium and available for growth. Reeve, Backes, Murphy, Schramer and Vollbrecht (1959) showed that overall yield was not reduced by blanking out a large proportion of the producing area of their beds with bricks. Lambert (1962) obtained a similar effect when large areas of the casing were rendered toxic to mushroom mycelium with excess fungicide, as did Flegg and Ganney (1974) when parts of the casing were sprayed with sodium chloride solution. This technique has been tried as a means of restricting cropping to certain areas of the bed, and for this purpose experiments involving replacement of the centre of the casing with polythene have been attempted.

The practice of leaving a section of bed uncased has, however, been shown on some occasions to reduce yield (Allen, 1976).

In practical terms, therefore, the casing layer acts as a means by which the nutritional reserves in the compost are utilised and converted into fruitbodies.

(ii) Casing media currently employed

Despite continued interest in the structure of peat/sand mixtures as components of "compost" or media for horticultural crop production (Puustjarvi, 1968, Olsen, 1968, Boggie, 1971, Bunt, 1974) mixtures incorporating sand or vermiculite together with peat, as initially used by Edwards and Flegg (1954) at the Mushroom Research Station are seldom used in mushroom growing today.

Sphagnum peat, usually in the form of "Irish Moss Peat", has come to be used almost exclusively in Great Britain today, despite the reservations about its suitability expressed by Atkins (1972) and Barnard (1974). Its high cost is apparently worthwhile in view of its relative freedom from mushroom pathogens and relative uniformity in regard to its physical characteristics, pore space and water holding capacity. However, in view of the various treatments to which different growers subject their peat, there must be considerable latitude.

Other sources of Sphagnum peat employed in this country include Germany, Finland and Russia. Native sedge peat is used by some growers, either as a component of the casing mixture or on its own. This peat, which is darker and more decomposed, has a higher pH, so that less neutralising material is required.

It normally requires sterilisation because it harbours greater populations of eelworms and potentially pathogenic fungi. The natural pH of Sphagnum peat is around 4-5, so neutralising materials must be used. "Lime", i.e. slaked lime or calcium hydroxide, is only used very rarely, but this term is sometimes used to describe the various forms of calcium carbonate or "carbonate of lime" which are used.

In the South of England quarried amorphous sedimentary chalk is the normal substance, and in Sussex Duncton chalk is widely used. In the North of England dolomitic limestone with a partly crystalline structure is used.

The chalk is normally delivered in bulk having been crushed, and screened within rough limits. Commercial grades usually used are so-called $\frac{1}{4}$, $\frac{1}{2}$ or $\frac{3}{8}$ inch "to dust", although lumps or "kibbled" grades are occasionally used. Finer chalks may be obtained ready bagged at greater cost. There is some evidence that "flour" chalk may be used at lower rates (Allen 1976), but this form of chalk makes little contribution to the bulk of the mixture, necessitating the increased use of peat which is more expensive. It also costs considerably more than coarser types of chalk, but is said to possess the advantages of sterility, ease of mixing, close grading of particle size and apparently high water holding capacity, due to porosity of particles (Gibson, 1975).

The amount of chalk used varies widely. Atkins (1974) states that most growers use peat and ground limestone in equal quantities by weight, but some use lump chalk (up to 1 inch to dust), usually on an equal volume basis. Nevertheless large variations are possible. The extent of this variability is well illustrated by the results of a survey by Ganney and Richardson (1974) showing the constituent ratios, rates of application and moisture contents of casing samples from 12 different farms. Indeed the results of physical and chemical analyses were so varied that these investigators could draw no conclusions from them.

The amounts of chalk used in casing mixtures are generally in excess of those required for neutralisation purposes. This excess may contribute to improved physical structure as well as increasing the bulk density of the mixture.

British growers do not normally subject Sphagnum peat-based casings to any form of sterilisation. Steam treatment is thought to have an adverse effect on structure of these media, but methyl bromide fumigation (Hayes 1970) is sometimes used.

Britain differs from other countries in that peat casings are used almost exclusively.

Much experimentation in mushroom growing is reported from the United States of America, and in particular Pennsylvania, where ordinary loam soils - "mineral soils" are widely used. Nevertheless, in parts of the U.S.A. "muck soils" high in organic matter are employed as casing, and Sphagnum peat is also used. Stoller (1952 d) reported on the use of "agricultural" (non-fibrous) peat.

It is standard practice in some areas of the U.S.A. to use decomposed spent compost as a casing medium. This is spread on the land and a crop such as hay or alfalfa is grown on it before it is skimmed up and used. Most of these methods necessitate sterilisation by steam, steam-air mixtures (Baker and Olsen, 1960) or chemical methods, such as "vapam".

In Gossau, Switzerland, casing soil is recycled (Alderton, 1972a). The soil is weathered for one year, the loss in the process being made up by a mixture of spent compost weathered for two years and limestone. Again pasteurisation is necessary.

According to Shandilya (1978) well-composted farmyard manure has been found to be satisfactory as a casing material in parts of India. In tropical countries high temperatures can cause rapid decomposition of used compost and other, sometimes rather novel, materials which could then be used as casing media. In a discussion Rodwell (1977) described the use in Kenya of coconut husks, also sisal waste, and by-products from the tea and sugar-processing industries, as casing constituents.

However, in countries where mushroom growing is established on a small scale only, it is normal for ordinary field soil (usually subsoil) to be used as the basis of casing.

In Australia, native soils are sometimes used for casing, although peats - either native or imported - have gained prevalence.

Visscher (1975a) reported that 90% of mushroom growers in Holland used a casing mixture of 65% black non-fibrous peat, 25% Sphagnum peat, 5% sand and 5% marl to adjust the pH. This was later superceded by a mixture comprising of peats plus "limecakes" - waste chalk from sugar

refineries.

Gapinsky and Gierszynski (1973) stated that peats, especially so-called "high" ones constituted the basic casing medium in the production of cultivated mushrooms in Poland.

In France, mushrooms are produced on casings with a high proportion of calcareous matter, so that they resemble chalky soils. Most mushrooms are grown in caves which have a constant high humidity and low temperature and consequently lower evaporation rate, so that thinner casing layers may be achieved.

Small quantities of peat, usually 10-20% by volume of Dutch Sphagnum peat, are added for water retention to crushed limestone, often obtained from the operation which was the original purpose for quarrying the cave. In the Loire valley, the limestones used known as tuffeaux, of sedimentary origin but consolidated with calcite, require crushing, but the softer unconsolidated faluns from further South are merely screened before use (Bazergue and Laborde, 1976).

Other substances which have been tested as casings on a commercial scale include bark (Allen, 1976), the plastics Styromull and Hygromull (Steineck, 1970, Visscher, 1973, 1975 a), polyurethane crumb foam and perlite (Barnard, 1974, Stoller, 1969). Hayes, Yeo, Cresswell and Jakeman (1978) showed that certain waste products of the paper industry could serve as satisfactory casings, and Stoller (1978) described and patented a casing mixture apparently as effective as peat, comprising a mixture of shredded newspaper, chalk, water and activated carbon. In later work, Stoller (1979) reported that undecomposed steam-sterilized spent compost could also be used as a casing material, after the soluble salts in it were removed by leaching.

(iii) Physical and chemical characteristics

a) Water holding capacity and water supply

The casing layer acts as a reservoir of water, not only preventing desiccation of the compost surface, but also providing a major proportion of the water content of the fruitbodies and contributing to the conditions required for their production. According to Bels-Koning (1950), as high a water holding capacity as possible was required, without interfering with gaseous exchange, which prompted Edwards and Flegg (1954) to state that casing must have an optimum water content since it conflicted with the requirement for a high pore space.

Flegg (1956) also interpreted Bels-Koning's results as suggesting that a transfer of water from casing to compost might take place.

Later, Flegg (1960) implicated water-holding capacity of the casing layer as being the factor inducing fructification. He hypothesised that fruiting was brought about in the casing because of its low "water stress", since additions of salts to the casing caused reductions in a yield. He reasoned that compost had too high a concentration of salts and that the water in the casing functioned to bring about the conditions necessary for fruiting.

Whether or not an actual contribution to the mushroom crop is made by the water in the casing is no longer in debate. Burrows (1951) had argued that water loss from the compost more than adequately accounted for the water contained in the crop, and Gerrits (1968) stated more positively that all the water needed by the fruitbodies was taken up from the compost, and watering the casing only supplied the water lost by evaporation.

On the other hand, Edwards and Flegg (1954) obtained increased yields with wetter casings, and showed that cropping could be retarded by delaying the first watering after casing. Reeve, Backes and Schramer (1959) obtained better yields with moister casings, especially those kept constantly moist, and observed that drier casings gave smaller mushrooms, in larger numbers. They obtained a direct

correlation between the moisture content of the mushrooms and the treatments applied to the casings on which they grew. Flegg (1965) obtained higher yields on casings watered by capillarity (1962 b), which maintained higher moisture levels than hand-watered controls. This technique was shown to have no effect on numbers of mushrooms forming, merely on the mean weight of individual mushrooms. Their increased water content (about 1%) accounted for the yield increases.

Sinden, Tschierpe and Hauser (1962) in describing a special technique of transplanting sporophores, stated that mushrooms developing from normally-induced primordia transplanted onto uncased compost, contained greater percentages of dry matter, which they interpreted as demonstrating the importance of casing as a water reservoir for the sporophores.

In a discussion, Flegg (1974 a) expressed the opinion that mushrooms probably obtained water from both compost and casing, and that the balance of these factors would be affected by growing conditions.

As regards the optimum regime of application of water to the beds, no critical optima have been defined. Reeves, Backes and Schramer (1959) stated that best yields resulted from maintaining the optimum water content of their soil (between 65 and 85% of the field capacity) but noted that wide variations in quantity and pattern of application of water gave similar results.

Flegg (1974 a and b, 1975) showed that casing should be applied wet and kept wet by frequent and regular waterings, with no distinction between the precropping and cropping periods. He also drew attention to the slight loss of productivity resulting from areas of "overlay" or dense non-fructifying mycelium when the casing was kept drier during the precropping phase, and the consequent loss in quality due to overcrowding on the other parts of the bed. He calculated typical figures showing the sources of water available to supply the mushroom growing system, and the demands made on this, including the

relative importance of evaporation and wastage.

San Antonio and Flegg (1964) showed that, in the course of its growth up to the cup stage, a mushroom may have transpired half its weight in water.

b) Physical structure

Another major variable property of the casing material in its percentage pore space - that is to say the proportion of its volume occupied by gas. This is to a large extent inversely correlated with its water-holding capacity, because air can be displaced from the pores between the particles, which indeed is the basis for its determination.

Although casing materials with high water holding capacity are preferred by growers, Flegg (1954 a) showed that this might conflict with the requirement to allow metabolic gases, notably carbon dioxide, to diffuse out of the casing layer during cropping. Flegg derived a set of theoretical values for diffusion rate based on assumptions of the pore space of casing mixtures under differing conditions of carbon dioxide production and casing moisture. Diffusion rate varied with aggregate particle size which was directly related to pore space. Watering the casing reduced the diffusion rate in proportion to the reduction in pore space.

It was shown that, under normal conditions, the possible rate of diffusion through a reasonably open-textured soil was quite sufficient to remove all the carbon dioxide as it was produced, without reaching a high concentration in the bed.

However, apart from a few limited investigations (Kim, 1974, Hayes and Shandilya, 1977), the pore space of casing media has received little attention.

Nevertheless, other aspects of the importance of the physical structure of the casing continue to be stressed.

MacCanna and Flanagan (1972) devised the method of "spawned casing"

whereby spawn-run compost, mixed into the casing at the rate of 5% by weight, brought about earlier cropping. This was attributed by them to assisted colonisation of the casing layer, and they noted improvements in quality of the mushroom crop, since mushrooms formed consistently on the surface and crop management was considerably eased.

This technique has been employed semi-commercially on several occasions, with varying results (Coates-Smith, 1972; Ganney and Stanley-Evans, 1973; Barnard, 1974). The main objection seems to be the danger of infection of the mycelium in the casing with virus or bacterial disease such as "mummy" while it is still in an actively growing stage, although MacCanna (1973) envisaged major alterations to the growing cycle of mushroom farms operating this technique. The advantage seems to be one of quality and uniformity of cropping, the resulting mushrooms being well spaced out and clumping being eliminated.

Nair and Hayes (1974) associated the effects of the procedure with modifications of the physical structure of the casing by the strands of straw in the compost, which they simulated using glass fibre strands added to the casing. Evidence was presented that the precropping period was reduced by this method and the application of spawned casing altered its aeration properties.

In the same context, one may consider the results of work by Flegg (1954 b) in which unspawned compost was mixed with casing in different proportions. In some cases, adding compost had beneficial effects on yields and numbers of mushrooms produced, possibly due to the effects of the compost on pore space of the casing, or by increasing the area of contact between soil and mycelium. Some experiments, however, gave drastically lower yields.

In Holland it is common practice to rake or ruffle the casing surface after partial mycelial colonisation even drawing strands of compost up into the casing, similar to "spawned casing". Visscher (1975 b) distinguished between the vegative (colonisation) phase and the

reproductive (cropping) phase. The former required a more compact structure, and the latter a more open structure to the casing, which was achieved by this practice.

Nevertheless, the presence of mycelium itself clearly has an effect on the physical structure of the casing. Edwards and Flegg (1952) observed that the presence of mycelium increased the stability of soil aggregates to water.

Hayes and Nair (1976) showed that the addition of 5% (w/w) gravel to a peat casing caused a stimulation in productivity similar to but less than that obtained with "spawned casing" or glass-fibre amended peat. Bewley (1950), O'Donoghue (1963) and Gardner and Davies (1962) had obtained yield increases by amending various types of casings with gravel or stones at higher levels.

Gapinsky and Gierszinski (1973) reported that cropping on certain types of peat casings was improved by the addition of sand. Kim (1974), on the other hand, noted an adverse effect when sand was added to clay loam casing soils.

(c) pH

In the case of pH, international, and methodological variations have combined to confuse consideration of an optimum level for this factor.

For example, Courtieu (1949) advised a pH of between 7.2 and 8.2 although his graph suggested a wider range was possible. Bels-Koning (1950) arrived at an optimum pH of 8 to 9. De Kleermaker (1954) concluded that the optimum range was 8.0 to 8.2. However Allison and Kneebone (1962) considered that a pH range of 5.5 to 7.5 was satisfactory for both casing and compost. Pizer and Leaver (1947) stated that soils with pH above 5.4 were satisfactory, but increasing the pH did not necessarily have much effect.

An explanation may be that whereas Courtieu and Pizer and Leaver

used ordinary mineral soils and soil mixtures, Bels-Koning used ordinary phosphate buffers to alter the pH of her ground bricks and vermiculite peat mixtures. De Kleermaker used clay river deposits and lime, and Allison and Kneebone used peat, charcoal, aluminium sulphate and ground limestone in combination with a loamy mineral soil.

In addition, pH tends to alter during cropping. When alkaline soils are used this normally means that the pH falls to approximately neutrality. Courtieu, however, showed that even acid soils become more alkaline during this phase, so that the mushroom probably tolerates a wider range in pH of casing soils, and possibly may alter the pH of the medium to suit itself. Allison and Kneebone stated that as long as the mushroom would grow through it, the pH of the casing was relatively unimportant.

d) Ionic inter-relations

For vegative growth of Agaricus bisporus, several elements are required. Calcium was shown by Sinden (1936) and Treschow (1944) to be necessary for mycelial growth, and Pizer (1937) recommended the addition of gypsum (calcium sulphate) to composts in order to improve their physical texture. Stoller (1940) contended that in spawn production it was necessary to add salts of aluminium, iron manganese chromium or zinc to counteract the high pH caused by addition of calcium hydroxide.

Treschow (1944) noted that the phosphate ion could easily become inhibitory in laboratory media, and that iron was required for mycelial growth, but could observe no effect of other trace elements.

The involvement of chemical factors in the action of casing and fructification has proved to be more difficult to study. Pizer and Leaver (1947) noted little correlation between the available potassium and phosphate content of casing soils, and the yields obtained from them. Nevertheless Courtieu (1949) showed that the potassium and

phosphoric acid content of mushrooms varied according to the levels of these substances in the casing soils on which they were produced, although Bels-Koning (1950) stated that casing made no significant contribution to the nutrition of the mushroom.

Flegg's (1961 a) work on the conductivity of the casing layer showed that salts generally had a gross effect on fruiting in the mushroom; this might conceivably be explained by osmotic effects, or the finding of Wodinsky and Frazier (1950) that aerobic pseudomonads were extremely sensitive to water potential, might be invoked to explain these results.

More specifically, Hayes (1972) has implicated iron in its ferrous state as being involved. Long and Jacobs (1974) could see no justification, but Long (1968) had noted that EDTA had several complex effects on primordium formation, as did phosphate buffers, although these were obviously localised effects, occurring only in non-sterile conditions. Nair and Hayes (1974) showed that the bicarbonate ion had an effect on bacterial populations in the casing layer and later work (Nair and Hayes, 1975) showed a relationship between high levels of pseudomonads and high yields of mushrooms.

Casing, being a mixture of peat or soil with an excess of chalk or limestone, can be considered as similar to a calcareous soil.

Bradfield (1941) stated that in such soils under normal conditions practically all the calcium in solution is in the form of calcium bicarbonate, and that the activity of (soluble) calcium is hence equal to that of the bicarbonate ion, which is directly proportional to the atmospheric carbon dioxide content, above pH7.

However, Whitney and Gardner (1943) showed that even the pH of a soil could vary greatly in proportion to the partial pressure of carbon dioxide.

In practical terms, the excess calcium carbonate represents a large buffering capacity, and variations in atmospheric carbon dioxide

above the casing layer are probably reflected by corresponding variations in the bicarbonate equilibrium within the water film of the casing layer (Ponnamperuma, 1967).

These equilibria interact with others. For example, by the solubility product principle, iron and phosphate ion activities are affected by calcium and bicarbonate activities. Brown (1960) showed that chlorosis or lack of iron in calcareous soils was not directly due to bicarbonate, but rather to the combined effects of phosphate and calcium.

In aerobic conditions iron is converted by oxidation/reduction reactions to its insoluble ferric form. It may thus be nutritionally unavailable to the mushroom.

Similarly, manganese has been shown to be only mobilised in anaerobic conditions in certain field soils.

(iv) Pathology

Being the medium on which and within which mushrooms are formed, the casing layer plays a major part in the etiology of various mushroom "diseases" - bacterial and fungal disorders, and can also support the development of certain pests, notably flies and nematodes. The reader's attention is drawn to the works of Kligman (1950), Kneebone and Merek (1958), Hussey, Read and Hesling (1969), Atkins and Atkins (1971), Sinden (1971), Atkins (1974), Vedder (1974) and Fletcher and Atkinson (1977).

The causative organism of the major bacterial disease of the mushroom - brown or bacterial "blotch", Pseudomonas tolaasii is closely related to the stimulatory Pseudomonas putida and appears to originate within the casing layer, or at least the casing harbours an inoculum once infection has occurred (Sinden, 1971).

In Sinden's opinion the occurrence of this disease is essentially a cultural problem in that high relative humidities and fluctuating

temperatures can lead to the formation of a film of condensed moisture on the mushrooms themselves, so that this condition is closely linked with the watering of the casing layer and the associated management practices - bringing into crop and climate control during cropping.

The means by which the growing system becomes initially infected has received little attention, however; Sinden suggests that the causative organism of "blotch" might be a form of Ps.fluorescens adapted to pathogenicity on the mushroom by association with mushroom tissue.

Nevertheless, certain casing components (peats) seem to be more prone to this problem. Possibly this is merely a reflection of the numbers or types of bacteria native to those materials: and this may again be due to the conditions to which these materials were subjected prior to their use in casing mushroom beds (Steane and Hayes, 1978).

In particular, the relationship between Ps.tolaasii and Ps.putida could be clarified, together with the further elucidation of the mode of action of Ps.putida in stimulating mushroom fruitbody formation. For example it would be worthwhile to know whether bacterial stimulation is necessary at only one point at the start of the mushroom life cycle, or recurrently for each flush. Since Ps.tolaasii and Ps.putida are so closely related, it is unlikely that selective chemical control measures could be developed to eliminate the one whilst having no effect on the other, so that there would be considerable commercial significance in the answer to these questions.

This organism has also been studied by Gandy (1967, 1968) and Nair and Fahy (1972, 1973) who have developed a technique of biological control for this disease by inoculation of casing with antagonistic organisms, including other Pseudomonads.

Pseudomonads are widely implicated in many other mushroom diseases, for example "mummy disease" (Schisler, Sinden and Siegel, 1967) and "drippy gill" (Young, 1970). "Pseudomonas fluorescens" has also been isolated from mushrooms infected with "bacterial pit".

The major fungal pathogens of the mushroom crop, Verticillium fungicola, (synonym V.malthousei), and Mycogone perniciosa which cause "dry bubble" and "wet bubble" respectively, and the much less common related species Verticillium psalliotae and Mycogone rosea, are associated mainly with developing mushroom sporophores, and although their conidiospores germinate within the casing layer, each pathogen has no observable effect on the mushroom mycelium, nor do they form extensive mycelial growth of their own on the casing.

Hypomyces rosellus (syn. Dactylium dendroides), H.aurantius, and Didymocladium ternatum are responsible for certain "mildew" diseases in which mushrooms are engulfed by a fluffy mycelial welt, causing them to rot. Mycelium of these organisms will grow over the casing surface, often quite rapidly. Mortierella baineri, which causes "shaggy stipe" may also grow to some extent on casing after infecting mushrooms (Fletcher 1973).

Other competitor or facultative disease organisms such as the green mould Trichoderma species, "lipstick" mould ("Geotrichum") Sporendonema purpurescens, which can be found on or in the casing layer can usually be traced back to the compost below. In some cases it is an over-simplification to describe them either as competitors or pathogens, or indeed as indicators of shortcomings in materials or conditions provided for mushroom growing, "weed moulds".

Some organisms originating in the compost appear to be especially virulent at the compost-casing interface, such as "False Truffle", Diehliomyces microsporus, and various yellow moulds such as Chrysosporium luteum and C.sulfureum which may be responsible for "mat disease". Indeed it has been suggested that the mushroom itself is at a competitive disadvantage whilst growing through the casing layer, hence its susceptibility to many diseases in this region.

An organism thought until recently at least, not to have many major ill effects on mushroom production is the mould, extremely common

on peat casings, known as "Botrytis" or "Brown mould" probably Chromelosporium ollare (Hennebert and Karf, 1975). Other names for it are Ostracoderma terrestre, (Peziza ostracoderma), Phymatotrichum sp., Plicaria fulva, (Peziza atrovinosa) and Botrytis gemella. This well-known organism (despite its aliases) is often found on uncolonised patches of casing, and its presence has been noted on virus infected areas of bed (Atkins, 1969), probably as a secondary coloniser. Although it will also grow on compost it seems to prefer the casing surface especially if wet or in humid conditions and will often be seen spreading out over it from a box leg or side. It becomes less prevalent as cropping proceeds, probably due to lowering of humidity. Although mushrooms will grow through it, Fletcher (1977) has noted that even this mould may effect cropping yields.

In commercial practice, fungicides are commonly added to the casing medium. Because of their minimal effects on yield, certain compounds of the benzimidazole group with higher specific activity against lower fungi than Basidiomycetes have become popular (Gandy, 1971) but in many cases resistant strains of Verticillium sp. have been selected by this means. Also in some cases control of Mycogone sp. by these fungicides has been reduced by bacterial degradation of these compounds within the casing layer (Fletcher, Mountfield, and Butler, 1976, Siegel, 1975). Dutsch (1975) has also noted that organic matter in casings can inactivate these compounds. Fungicides with a less specific action have not suffered to the same extent, but have been found to be more likely to affect cropping of the mushroom as well as growth of pathogenic moulds.

The main insect pests of the mushroom crop are three types of flies:

(i) sciarids (Lycoriella auripila, L.solani and Bradsysia brunnipes)

(ii) phorids (Megaselia halterata and M.nigra)

(iii) cecids (Heteropeza pygmaea, Mycophila speyeri, M.barnesi and other species).

The first two types mostly lay their eggs on mushrooms or casing; most pass through at least the last part of their larval stages within the casing layer. The paedogenetic cecids may even be introduced, as larvae or as a specialised resting stage, in peat used for casing.

Again control of these pests may be achieved by the addition of various insecticides to the casing.

Eelworms which are important in mushroom growing can be of either the fairly inocuous saprophagous type (various Rhabditis spp. and Panagrolaimus spp.) or of the two parasitic (fungal-feeding) species Ditylenchus myceliophagus and Aphelencoides composticola.

Hesling and Gaze (1975) stated that these eelworms may be introduced to the mushroom crop in peat used for casing and although peat usually has very low numbers of these animals present, changes in peat production techniques or sources of supply may alter this situation at times.

Fungicides and insecticides may be added to the casing mixture (Alderton 1973, Peake, 1972, Ganney and Atkins, 1972) although this may to some extent affect yields or alter patterns of production, (Wuest, Cole and Patton, 1972, Gandy and Spencer, 1976, Wyatt, 1978).

(v) Commercial aspects of casing

a) Crop management

The various constituents of casing may be mixed by different means. In many cases a modified rotating concrete mixer is used to blend the peat and chalk, together with the addition of water, usually in metered quantities, and any adjuncts such as pesticides.

In most modern mushroom farms casing is applied automatically to trays by means of a special machine operating as part of a conveyor belt process line. Trays pass below the unit, and the casing is dropped in an even layer usually from 1 to 2 inches thick, onto them from a revolving belt or drum moving at the same speed. In farms operating the shelf system, the casing is applied to the surface of the compost inside the growing house. Even this process can be automated.

In smaller farms, a wooden former or a system of planks may be used to apply casing to a uniform depth, and even when mushrooms are grown in plastic bags, a casing layer of even thickness and compaction is aimed for.

Once cased, the culture may be transferred directly to the cropping area or to specialised "holding rooms","set back rooms" or "pinning rooms" equipped with relatively sophisticated air conditioning and heating systems to provide the necessary environmental conditions required during the next phases of growth. The cased beds may be watered again, especially if the casing is applied dry by machinery. Upward growth of mycelium then occurs through the casing layer in a 2-dimensional manner. In the intensive culture system, this phase - "holding", "spawn run into casing", or "case-running" takes place under conditions similar to spawn running: incubation at 24°C (75°F) bed temperature or higher, with no ventilation if possible. When this phase is completed and the mycelium has penetrated virtually to the surface of the casing, usually after 6-9 days, the initiation of fruiting is brought about by sudden extensive ventilation with cold fresh air.

Thus the bed temperature falls quickly, ideally to about 20°C (68°F) and the air temperature is maintained at 15.5 - 17.5°C (60-63°F) for the rest of the cropping cycle.

In the older system, a more gradual cooling down and less ventilation are used, pinning occurring gradually as the mycelium nears the surface of the casing.

Pinning is considered by most to be complete by the 14th or 15th day after casing. Watering is generally withheld from the developing mushroom crop until the majority of pinheads have reached a certain size, depending on the strain of spawn used, by which time it is assumed that they are well enough formed to withstand such treatment.

The art of the grower depends to a large extent on the correct appraisal of the various factors. One is the determination of the correct time to apply ventilation, bearing in mind the ability of some strains under certain circumstances to continue to grow on vegetatively for some time. If this is done too early it usually means that initiation occurs deep in the casing, thus giving mushrooms which are dirty in appearance. If too late, it could result either in excessive formation of "overlay" or non-productive areas covered by mycelium which resists penetration by water, or in the formation of pinheads on the very top of the casing material, usually over-abundant and over-exposed to the action of water and ventilation, hence tending to become moribund. Other fine points are the amount of ventilation required, and the watering requirements during the individual phases.

b) Commercial trends

In general, the mushroom industry represents itself as being highly mechanised, "tray" farms operating automated process lines for major operations; tray filling, spawning, casing and emptying and hence rely heavily on forklift trucks for the transport of trays between special rooms according to the various processes.

In addition some farms have installed "picking lines" where trays are brought to the picking staff, but particularly where the farm was not specifically designed with this development in mind, the strain of extra handling has not been found to be worthwhile (Alderton 1972 a, Pinkerton, 1973). The temptation was to fill the growing houses with boxes so tightly packed that it was necessary to unstack the whole house and put it through the picking line even when only a few mushrooms required picking; or if the beds needed watering. Not only was mechanical damage to the trays increased, but crop damage frequently occurred, and disease was spread by this technique.

Nevertheless, in view of the continuing rises in picking costs, it seems that the small step from picking lines to completely automated (mechanised) picking could eventually occur. Prototype machines have already been experimented with by Persson (1972, 1973) and Sinden, as quoted by Alderton (1972 b).

Vedder (1977) described two types of machine developed for the Dutch system of shelf beds which operate by means of an oscillating knife moving across the beds. Some British growers have adopted a fully mechanised version of this system. Preliminary results indicate that the mushrooms produced by this process are not of a high enough quality for the fresh market. However, the system has its advantages in that problems associated with picking (not only cost, but also availability of labour) are often the main limiting factors on the production of individual farms. However, with growers being urged to concentrate on quality, the economics of a lower quality product with low production costs could become favourable. The product of mechanical harvesting does however lend itself to being processed. In Britain at the current time with most growers placing emphasis on the fresh market there is little interest in this development, although the reverse is true on the continent, especially in Holland.

In these cases, great stress is placed in manipulating the crop to a suitable stage to undergo the mechanical harvesting process, since practically an entire flush is removed at one time. Thus all mushrooms must be of the same size and at the same stage of development, and with pronounced elongation of the stipe, to allow a certain latitude in setting the level of the cutting blade. Practically, this calls for slight overdevelopment of some of the crop to maximise the number of mature mushrooms harvested.

The resulting crop of mushrooms can thus consist of mixed grades and sizes, together with odd pieces of mushrooms sliced haphazardly. The possibility of virus and other disease is greatly increased under these conditions.

In conjunction with this development increased control over cropping seems to be necessary. Manipulation of ventilation is practised, in order to increase the length of stalks due to the accumulation of carbon dioxide, as described by Lambert (1933).

The methods of controlled cropping advocated by Flegg (1970, 1972), Flegg and Ganney (1974) and Tschierpe (1973) rely mainly on fine control of environmental conditions, which is difficult on some farms. A major advantage of the shelf system is that it apparently gives rise to less variability in cropping than does the tray system (Middlebrook 1977).

Flegg's (1961 b) experiments in which the addition of soluble salts to the casing layer increased the mean size of individual mushrooms at the expense of yield indicate that there is some possibility of manipulation of cropping patterns by this, somewhat uncertain, means.

The mushroom industry strives especially hard to achieve "uniformity" of cropping. This uniformity can be considered to have three aspects: the first with respect to time (simultaneous flushing on all beds) the second with respect to area of the beds (elimination of clumping and "edgebreaks", and adequate spacing between mushrooms)

and lastly in the uniformity of size of mushrooms.

It seems reasonable to assume that all of these factors might prove to be amenable to genetic manipulation, or at least selection by spawn producers.

Whereas the first will probably be mainly achieved by manipulating environmental conditions and reducing variability of the overall system by attention to detail, the second and third will probably result from a consideration of the physical nature of the casing layer, and its interaction with biological and chemical aspects.

In conclusion, an acceptable casing medium might be considered to possess the following properties:

1) A fairly high water-holding capacity is necessary so that water for the mushroom crop can be retained within the casing layer and not pass through into the compost below, waterlogging the surface and killing the mycelium. Ideally it should absorb water easily when applied and release it only slowly by evaporation.

2) Its physical texture should be even and open so as to allow diffusion of metabolic gases, notably carbon dioxide, out of the compost. Watering should not substantially affect this property, either by sealing the pore spaces or causing a deterioration in the aggregate structure of the casing material.

3) Its reaction should be neutral or slightly alkaline (pH 7-8) and it should contain few soluble salts (low salinity or conductivity).

4) It should be free from bacterial and fungal disease organisms, animal pests and weed seeds, and material which could decompose and attract these organisms. It must not, however, represent a medium inhospitable to colonisation by the mushroom mycelium and its associated stimulatory microflora.

However, these properties interact with environmental conditions as well as management practices. Casing thus cannot be considered in

isolation from other components of the culture system. Hence, there is a certain latitude in the acceptable range of these factors.

Also, due to the background of the fruiting and developmental processes, there is the possibility of a compensation reaction which may to some extent limit the probability of improved results from different casing media.

This research had as its aims:

1) to evolve a culture system in which experiments with mushrooms can reliably be carried out and which reflected normal growing conditions;

2) to investigate the influence of casing structure on productivity in the cultivated mushroom, and to observe the means by which these factors have their effects;

3) to look into the possibility of finding a replacement, substitute or extender for peat.

The study therefore centred on casing materials rather than the mechanisms underlying fruitbody formation and flushing. A series of yield experiments were carried out which involved different formulations of normal peat-chalk casings together with some substances thought to represent promising substitutes. Standard commercial spawn strains and substrates were used throughout.

3. MATERIALS AND METHODS

(i) Compost and spawn

Normally, compost was inoculated with a culture of *Agaricus bisporus* on arrival at the laboratory. Grain spawn (mycelium growing on sterilised rye or millet) was added, usually at the rate of 1% by weight. When required, the insecticide Diazinon, in the form of 5% active ingredient granules, was also mixed in at the rate of 0.2% by weight.

Compost was mixed before, and in the course of this "spawning" operation, and again after the mycelial colonisation was practically complete (normally about 14 days at 24°C). When compost was received already inoculated and colonised by mycelium ("spawn-run compost") it was especially important to break up and remix it, because of variations in compost texture and mycelial density. Normal commercial spawning rates are 0.3 - 0.5% by weight.

In most cases mycelial growth took place in bulk in a tub. In order to reduce variability, all receptacles for each experiment were filled together, then individually made up to contain the same weight of compost, and compressed well. After a further 2-3 days at 24°C, strong and uniform regrowth of mycelium occurred, and the cultures were cased.

Initially, it was agreed that all cropping experiments would be carried out using spawn of Darlington's strain 621, in conjunction with compost prepared by W. Darlington & Sons Ltd. at Luckfield Nurseries, Angmering. However, various commercial spawn strains and substantially normally prepared composts from several different sources, some unspecified, were used, having been peak-heated at their place of manufacture and transported to the laboratory in polythene bags.

Mushroom growing is normally a mixed culture system. Occasionally, various weed moulds and pests were encountered, in the compost, but these problems were common to all cultures used in individual experiments, so that they should have had little or no effect on the comparisons drawn from those experiments.

(ii) Casing

It was important that casing media should be well mixed, and experiments were planned so that enough casing for each treatment was made up in single batches, except in the example of admixtures of minor components to a standard mixture, when an adequate amount of the common formula was prepared to cover all the variations.

Sometimes it was best to mix casing ingredients in the dry state before adding water - for example peat and fine chalk - but where there were large differences in bulk densities and particle sizes of components, it proved better to mix already wetted ingredients - such as peat and lump chalk or gravel.

Initially, casing mixtures were made on a volume basis, but mainly due to problems with the compressibility of peat, it was later found preferable to standardise on proportions by weight, with occasional reference to volumetric relationships.

Peat casings were generally brought close to their water-holding capacity before application. This necessitated the addition of varying quantities of water because different batches of peat and chalk varied in their moisture content. However enough water was added to result in slight runoff from the mass. When spread in a thin layer on the compost, this runoff did not occur because of reduced compression.

Casings were applied at a depth of $1\frac{1}{2}$-$1\frac{3}{4}$ inches (3.8-4.4 cm) and standardised by weight.

In the case of small rates of admixture of substances to standard casings, these were added to a fixed weight of standard casing, so that the total amount of the major components of the casing mixture was kept constant.

The proportions of peat to chalk used in "standard" peat/chalk casings varied. In early experiments a ratio of 1.5:1 peat: chalk was used, but a 1:1 ratio by weight was later used as a standard. The amount of water added at mixing varied according to the moisture content of the peat, but enough was added to be easily expressed when small samples

were lightly squeezed between the fingers. It was normal to add a quantity of water approximately equivalent to three times the weight of peat. The moisture content of casings was on average about 70% on wet weight at application. Subsequent waterings could have temporily increased this percentage, but peat will hold over $4\frac{1}{2}$ times its own weight of water, so that saturation would not be attained until a percentage moisture greater than 80% was obtained. Normal tap water was used.

Peat, chalk and sometimes prepared casing were obtained from W. Darlington & Sons Ltd., and occasionally from other mushroom farm sources. Medium grade Irish moss peat was used exclusively.

Morden R ground chalk was provided by Melbourn Chemicals Ltd., of Esher, Surrey.

Glass fibre strands were sections of sleeving as used for insulating electronic components, obtained from Turner Brothers Asbestos, Glass Fibre Division, of Rochdale, Cheshire. Glass fibre "staples" were obtained from the Strand Glass Company, Sparkhill, Birmingham.

"Synthetic" casing mixtures were composed of simple ratios of ingredients by (air dry) weight.

The substance perlite was normally wetted with three times its own weight of water before being mixed with other substances and used as a casing. It was obtained in the first instance from W. Darlington & Sons Ltd., and then from Perlite Industries Ltd. of Belper, Derbyshire and Tilling Construction Ltd. of Oxford.

Samples of clay minerals were obtained initially from the University of Aston Geology Department, and then from Steetley Minerals Ltd., Milton Keynes Buckinghamshire.

Laboratory reagents used as casing constituents were of the "general purpose reagent" grade.

(iii) Watering

Large boxes were watered using a watering can and small pots using a syringe, but in most cases a polyethylene wash-bottle was used. Various methods of computing watering requirements based on evaporative loss of casing samples, and watering by means of a capillary wick fed from a container kept at a constant level of water were tried. However these methods could not be relied upon to maintain a constant moisture content in the casing for long enough periods. Consequently, watering was varied according to subjective judgements of the amount required to keep stable conditions, as noted by the physical appearance, colour and dryness of the casing surface.

It was normal to water casings two or three times between application and ventilation for fruiting, then to cease until primordia were well formed. Prior to picking a flush, watering was necessary at least once a day. Watering requirements varied between different casings, and between experiments, due to variations in climate.

(iv) Containers

Various receptacles were used as experimental units for compost and casing.

Early experiments were carried out using wooden boxes 18 inches square and 5 inches deep (45.7 x 45.7 x 12.7 cm). These could accommodate about 14 kg of compost, leaving space for casing, but used in conjunction with perspex rims 3 inches (7.6 cm) deep, about 18 kg compost.

Polypropylene trays 17 by 23 inches and 4 inches deep (43.2 x 58.4 x 10.2 cm) were occasionally used but since they could only take 8 kg compost, they were found to be unsatisfactory.

For most experiments, however, plastic boxes were used. These were of two types, each with the top dimensions $6\frac{1}{2}$ inches (16.5 cm) square; since the sides tapered somewhat, this was taken to be equivalent to

about $\frac{1}{4}$ square feet. Polyethylene boxes, bought as frozen food containers, were found to be of a useful depth - $7\frac{1}{2}$ inches (19.0 cm), containing 2 kg compost, and these came with snap-on lids, but could not withstand sterilisation by heat. Polypropylene boxes with the same surface area and only $5\frac{1}{2}$ inches (13.7 cm) deep were also used. These were autoclavable, but had no lids and held less compost - 1.3 kg.

From time to time, other containers such as small pots, bulb trays and photographic cells were also used.

(v) Growing conditions

Most cropping experiments were carried out in the Aston Laboratory for the Cultivation of Edible Fungi (A.L.C.E.F.) in the basement of the George Alexander block.

This provided a cool and relatively stable temperature regime (14-22°C) and was equipped with an extractor fan system to renew the air. The walls were painted with a rubberised vapour seal and the floors covered with a polyvinyl chloride flooring as protection against water spillage and contamination.

a) Preliminary casing experiments

In early experiments, incubation was carried out in an isolation section constructed of polyethylene sheeting, heated by an electric blower heater. Cultures were afterwards arranged on steel shelves in the main laboratory area. Although this system was relatively economical in the use of floor space, with shelves at approximately 60, 120 and 180 cm above ground level, practical difficulties were encountered with the scale of, and variation within, experiments, and it did not permit environmental control.

b) Design and Construction of Laboratory Growth Cabinets for investigations into casing

A smaller scale culture system was developed and constructed, with these problems in mind. The environmental control system for the experimental plots was designed to meet the requirements of the lifecycle of Agaricus bisporus.

The growing units, boxes, trays or pots, were placed inside a cabinet based on a commercially available propagator unit (see Figure 1). An enlarged hood $49\frac{1}{2}$ inches (126 cm) wide x $25\frac{1}{2}$ inches (65 cm) deep x 24 inches (61 cm) high, made of 5 mm thick perspex was fitted to the top. The hood was sealed by means of a foam rubber strip to the base unit, but it could be easily lifted off to give access. A hinged door 24 inches (61 cm) x 12 inches (30.5 cm) in the front of the perspex hood (not shown on the diagram) was used for access for picking and watering.

For the incubation phase, temperature was maintained by a thermostat controlling power to an air warming cable wound around the edge of the perspex hood. However in action this had the effect of lowering the relative humidity.

A similar cable buried in wet sand in the propagator base had the opposite effect, so that the humidity within the cabinet could be varied by controlling the power to this cable. This effectively altered the amount of water driven off from the sand into the air above. This action was largely independent of the water content of the sand.

For the cropping phase of the life cycle, fresh air was introduced into the cabinet through a pipe with holes in its upper surface. The distribution pattern of air was as indicated on the diagram. Air left the unit via holes in each end. Conditions were substantially the same on either side of the cabinet, i.e. it was symmetrical about the air pipe.

During cropping, three cabinets were served by a single ventilation unit, which had the capacity to heat or cool air. This unit (Figure 2)

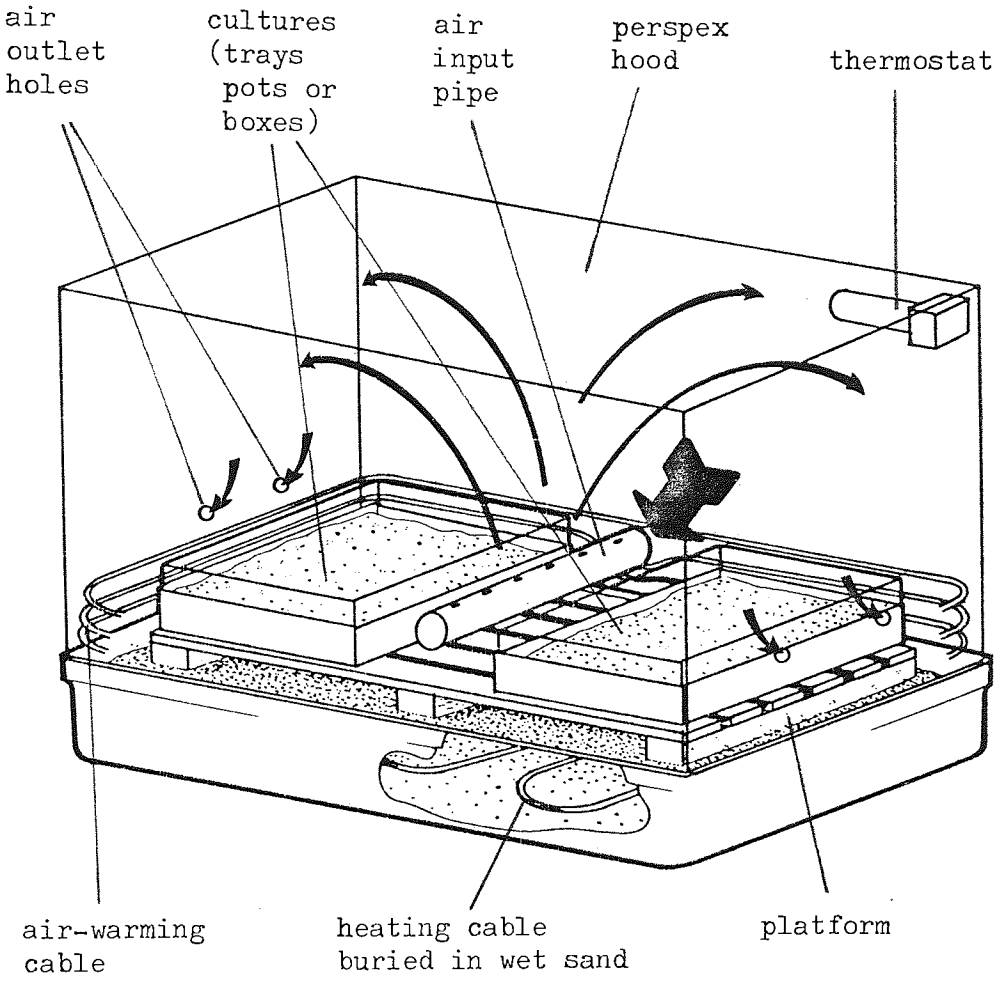


Figure 1 Laboratory growth cabinet used in casing investigations

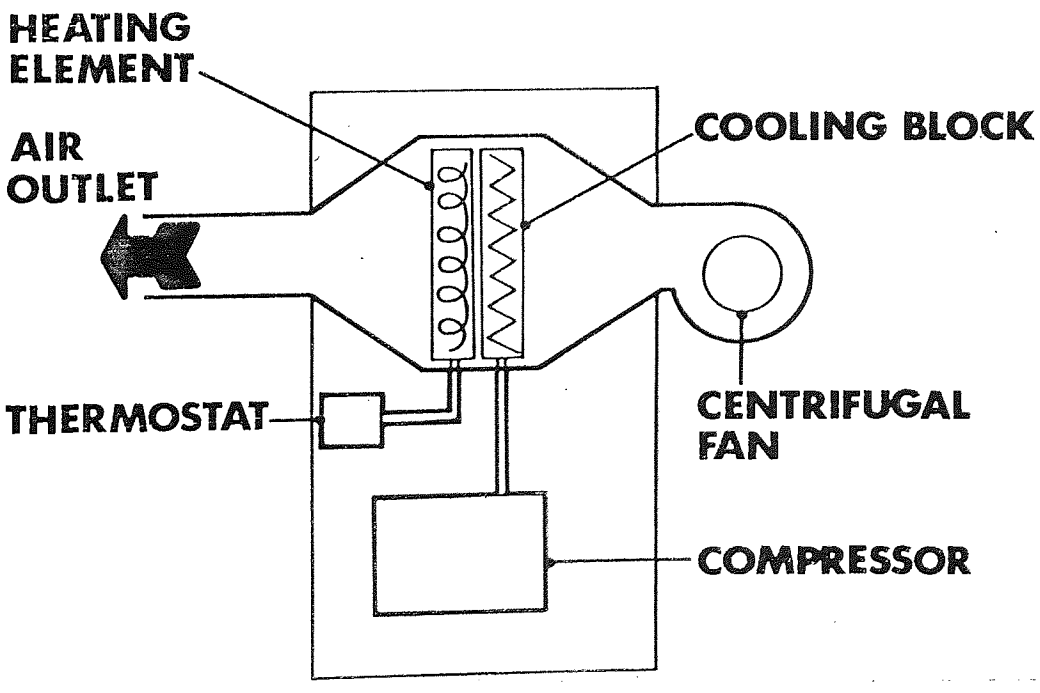


Figure 2 Ventilation unit for use during cropping (diagrammatic)

was specially constructed by a refrigeration company. Regulation of the air temperature was achieved by means of an electronic thermostat utilising a thermocouple or thermistor probe, either inserted into the airflow, or mounted within one of the cabinets. Temperature could be maintained to an accuracy of $\pm$ 1°C of the desired values.

The unit could accommodate either 2 large trays or, more usually, 18 small polyethylene boxes. The latter provided an acceptable number of replicates without producing an embarrassingly large quantity of fruitbodies. The requirements for growing substrates was also limited - 36 kg compost per cabinet being used in most experiments. Due to the closer packing of experimental containers, their arrangement on one level, and the forced air movement, variation between different positions in the cabinet was negligible compared with the system of growing in trays on shelves in the open cellar.

The system thus represented a means of producing fruitbodies of A.bisporus on a laboratory scale and in a manner comparable to commercial conditions. It also served to contain and protect against pests and diseases, which were much less prevalent than with the system previously used. The cabinet growing system has since found application in other studies involving the cultivation of A.bisporus.

c) Commercial scale testing

One experiment was performed at the Research and Development unit of W. Darlington & Sons Ltd. This was carried out under conditions very close to those used in normal growing. Standard large trays 7 ft. x 4 ft. (25 sq. ft. nominal) were filled with compost, peakheated, spawned and cased in the same way as the main production unit of Lucksfield Nurseries, Angmering. These trays were then transferred to the small growing houses of the R. and D. unit, arranged in randomised positions and then managed in exactly the same way as in the production unit.

(vi) Photography

A system of glass fronted cells was constructed allowing observation of mycelial growth from spawn run compost into the casing layer.

Dimensions were 15 cm. wide x 22 cm. tall x 7.5 cm. deep. Each cell was three-quarters filled with compost, then covered with a 4 cm. layer of casing, and the top was left uncovered to permit watering and gaseous exchange.

The cells were then placed in a cabinet and subjected to normal cultural practices. The glass front could be lifted away to permit photographs to be taken without reflections.

A series of photographs were taken using a Practica LTL single lens reflex camera fitted with 55 mm. extension tubes between the camera body and lens (Tessar 50 mm. f2.8). Illumination was mainly by means of a Sunpak Auto 28 flashgun, or occasionally by photoflood bulbs. Film used was Panchromatic-X black and white.

(vii) Counting of primordia.

In early experiments, the number of primordia developing to a visible size were counted; however this was inaccurate because many formed below the casing surface, and more became visible as the experiments proceeded. Total numbers visible were thus recorded at the stage of first picking from the first or second flush.

A more repeatable procedure was used in later experiments. Mushroom cultures in small bulb trays 20 x 6.5 x 7.5 cm. were sacrificed at intervals and dried in an oven at 105°C, allowing the whole casing layer to be removed as a solid cake. This could then be placed in a sieve and by means of a jet of water the upper layers of casing could be washed off, down to the level of the mycelial tips, which bound together the casing material. At this level, more or less spherical formless structures could be seen attached to the mycelial network in

the casing. These were taken to represent hyphal aggregates (a preliminary stage in the development of primordia). Their distribution was fairly regular, and they could be easily counted.

(viii) Expression of results of yield experiments

As far as possible, each experimental treatment was subjected to identical management. Fruitbodies developing on each experimental unit were harvested daily, weighed and counted, and cumulative totals of both weight and numbers were kept.

In most cases, experimental results were presented as yields per unit area: either as numbers or weights scaled up to kilograms of cut fruitbodies (fresh weight) per square metre.

The ends of the stalks were trimmed in accordance with normal commercial practice. The amount cut off was rather subjective, representing enough to remove adhering particles of casing material and render the picked mushrooms of a uniform appearance, but attempts were made to standardise this treatment.

In some cases the results could be presented in terms of individual flushes, but because patterns of flushing did not always coincide between treatments or replicates, it was sometimes thought to be preferable to compare treatments at 10-day intervals - usually 30, 40 and 50 days after casing. This would tend to show the speed of attainment of the final yields obtained, a factor which is of commercial significance.

Where possible, statistical analysis of yields at the stated time intervals took two forms (Fisher, 1973, Bishop 1974).

(1) simple t-tests in which means and standard deviations of treatment yields were compared individually in pairs, usually with a standard peat-chalk casing, and

(2) analysis of variance, in which similar comparisons were made between each treatment and all the treatments in an experiment, taken

together. This analysis did not indicate which particular treatments differed significantly from the rest.

In general, it was preferred to use t-tests, since by means of an Olivetti Programma 22 calculating machine, a series of t-tests could be easily carried out comparing standard casing materials with experimental ones.

Other statistical treatments - computation of mean treatment yields together with standard errors and the performance of analysis of variance leading to the determination of the least significant difference between means - were most conveniently carried out using a Casio fx 2200 electronic calculator.

(ix) Determination of the physical characteristics of casing mixtures

a) Moisture contents

Moisture contents of casing materials were determined by difference after drying the samples, in glass beakers, to constant weight in an oven at 105°C. These results were either expressed as a percentage of the fresh weight (wet weight): $\frac{\text{fresh weight - oven dry weight}}{\text{fresh weight}} \times 100\%$

or in terms of the oven dry weight: $\frac{\text{fresh weight - oven dry weight}}{\text{oven dry weight}} \times 100\%$

b) pH

Frequently pH determination were taken on the casing sample before it was placed in the oven. This was normally performed on a 1:1 w/w slurry made up with distilled water, and taken by means of a standard Pye pH probe with internal reference electrode, connected to a Pye Unicam series 92 pH meter.

c) Pore space and water holding capacity

Normal samples of casing, not having been processed in any way were filled loosely in weighed glass cylinders 12" x 1" in diameter, or 6" x $2\frac{1}{2}$" in diameter, the latter being preferable, covered at one end

with a piece of nylon gauze, and reweighed. The cylinders were then immersed in water up to their rims so that the water displaced the air in the spaces between the peat particles. A cap was then fitted, under water, to the bottom of the cylinder and the cylinder plus saturated contents reweighed, subtracting the weight of the cap. The casing was then allowed to drain freely until dripping out of free water had ceased, usually about one hour, and reweighed. Then the cylinders plus casing sample were dried in the oven for dry weight determination. From these readings, the following estimates could be derived:

(1) moisture contents, on fresh and dry oven weights, as above.

(2) pore space from the volume of air displaced by water expressed as a percentage of the overall casing volume: Pore space of fresh sample $= \frac{\text{saturated weight - fresh weight}}{\text{volume of cylinder}} \times 100\%$

Maximum pore space $= \frac{\text{saturated weight - drained weight}}{\text{volume of cylinder}} \times 100\%$

Minimum pore space $= \frac{\text{saturated weight - oven dry weight}}{\text{volume of cylinder}} \times 100\%$

(3) maximum water holding capacity on a dry or a fresh weight basis.

$\frac{\text{drained weight}}{\text{oven dry weight}} \times 100\%$ and $\frac{\text{drained weight}}{\text{fresh weight}} \times 100\%$

and also the water content of the fresh casing sample as a percentage of the maximum water holding capacity:

$$\frac{\text{fresh weight - oven dry weight}}{\text{drained weight - oven dry weight}}$$

d) Cation exchange capacity

This was determined by the standard A.D.A.S. method used for soils (M.A.F.F., 1972).

A 5 g. oven dry sample of each casing mixture or component to be tested was ground to pass a 0.5 mm mesh sieve, then left for 24 hours in a beaker with 20 ml 1.0 M ammonium acetate solution at pH 7.0. It was then filtered and leached with further 25 ml volumes of ammonium acetate solution until 250 ml had been collected. This solution could be used

for the determination of the quantities of exchangeable cations present.

The substance in the filter funnel was then treated with five 25 ml volumes of absolute alcohol to remove excess ammonium acetate. The exchanged ammonium ions, corresponding to the cation exchange capacity, were then extracted with 25 ml volumes of 10% potassium chloride solution at pH 2.5. The solution collected was made up to 100 ml, then its ammonium content was determined by the Kjeldahl method. 10 ml samples were steam distilled with 4 ml of 1.0 M sodium hydroxide and the resulting solution of ammonia in 5 ml 1% boric acid titrated to methyl-red methylene blue endpoint with 0.005 M sulphuric acid.

Titration results in ml (less blank) multiplied by 2 gave the cation exchange capacity of the sample in milliequivalents per 100 g.

4. RESULTS

A. Colonisation of casing and fruitbody formation

In order to observe and describe the events involved in the transition from the vegetative to the reproductive phase of A.bisporus, it was necessary to concentrate on individual sections of certain cultures. Observation chambers were used and the results recorded photographically. The following morphological changes were observed during the growth of mycelium into the casing, through the phases of the initiation of fruiting to the maturation and picking of the first flush.

Plate 1 shows a transect across the whole width of the casing layer on the observation chamber, taken at 8 days after casing. Although the compost is fully permeated with mycelium it shows as a darker area than the casing because its mycelium is very fine. The extent of mycelial penetration into the casing can be seen. Although fairly uniform, this follows quite closely the contour of the compost, and it shows that an even thickness of casing is necessary for even mycelial colonisation.

Plates 2 - 6 represent magnified views of the same chamber, also taken at 8 days after casing.

At this stage, the mycelium can only be considered to be vegetative in character, but its vigour can be seen to vary, and in Plate 2, some differentiation into thicker strands is evident. Plate 3 shows that these strands are virtually confined to the bottom of the casing, and oriented more or less vertically, but growing together to avoid cavities and irregularities.

Plate 4 shows the hyphal tips, which at this stage display practically no signs of differentiation, and fan outwards more. About 1 cm. of uncolonised casing can be seen above the mycelial margin.

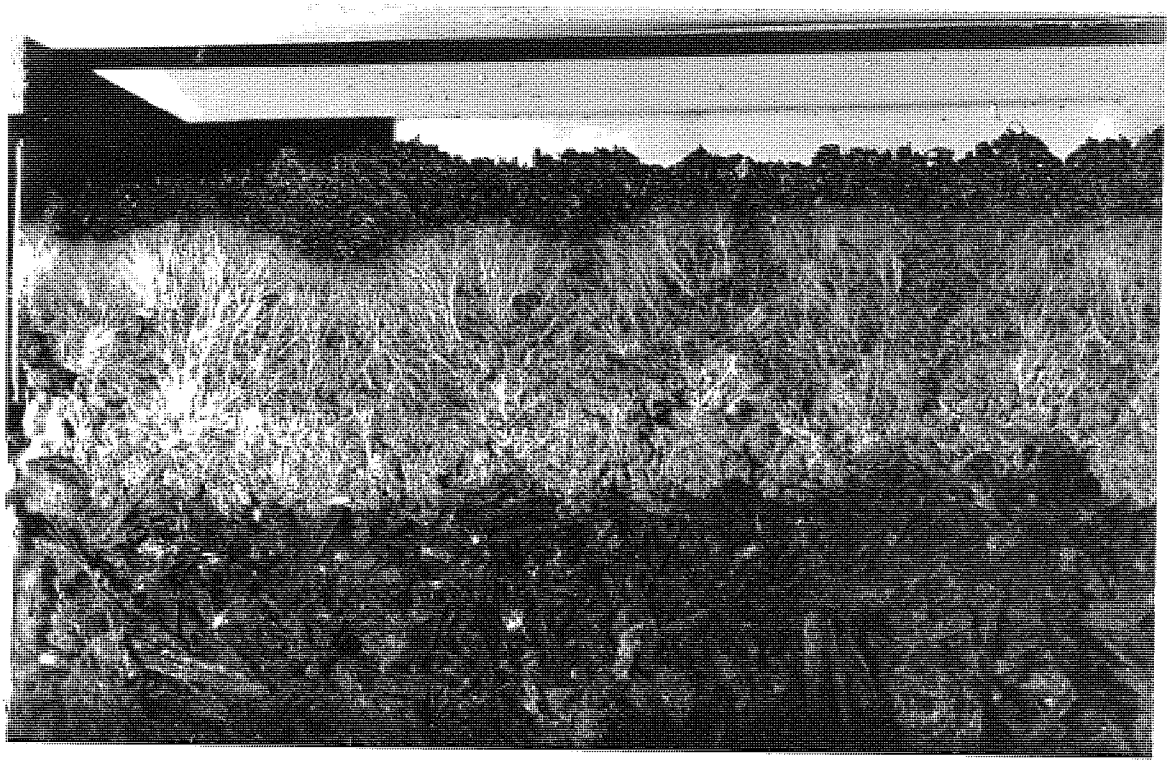
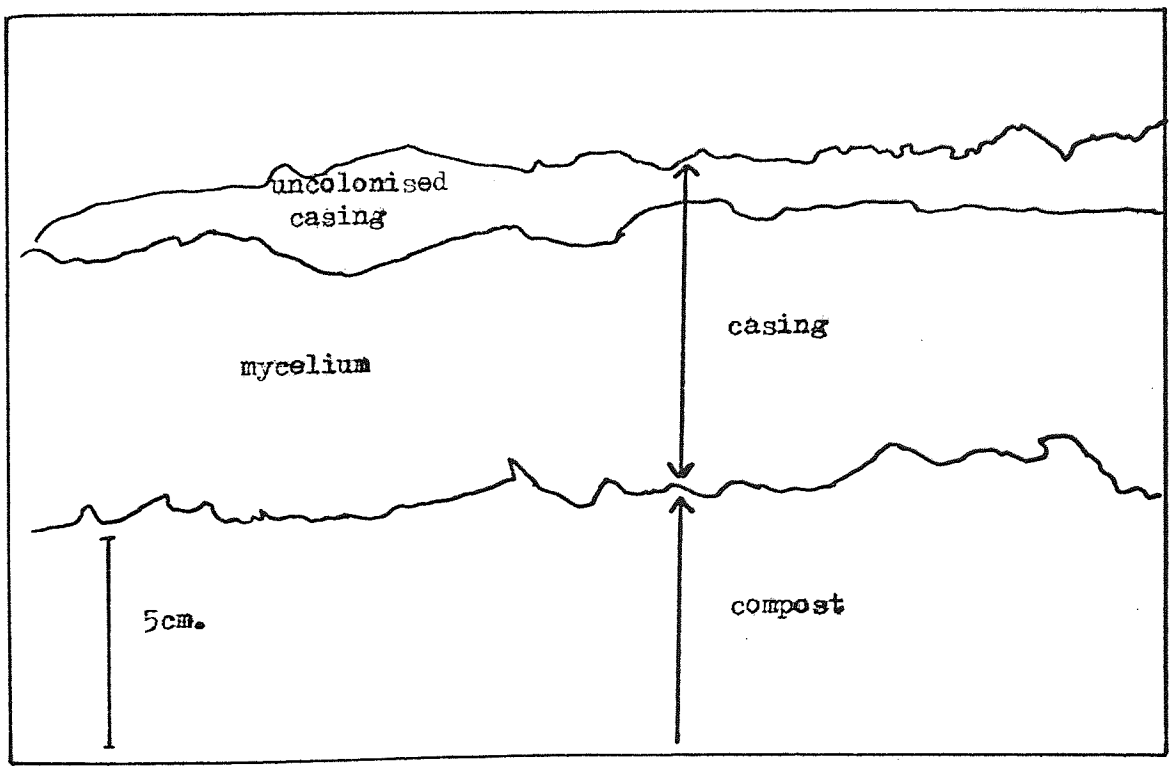


Plate 1. Compost and casing layer, showing mycelial penetration.

(8 days after casing.)



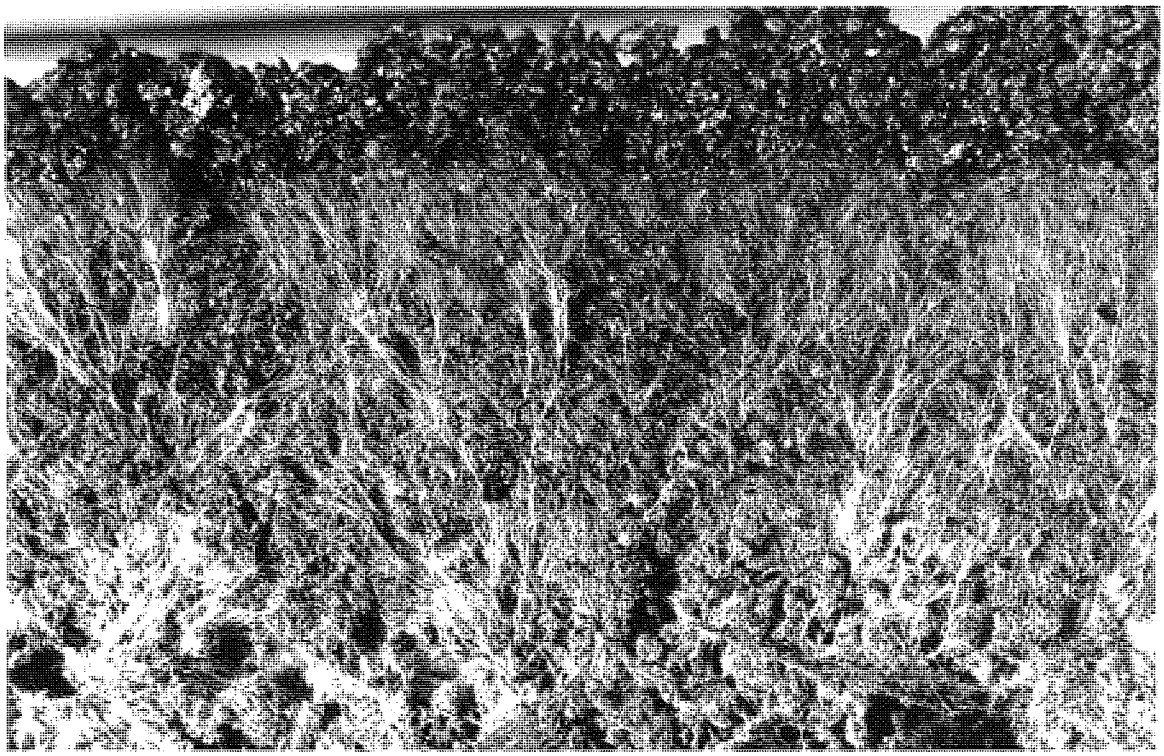
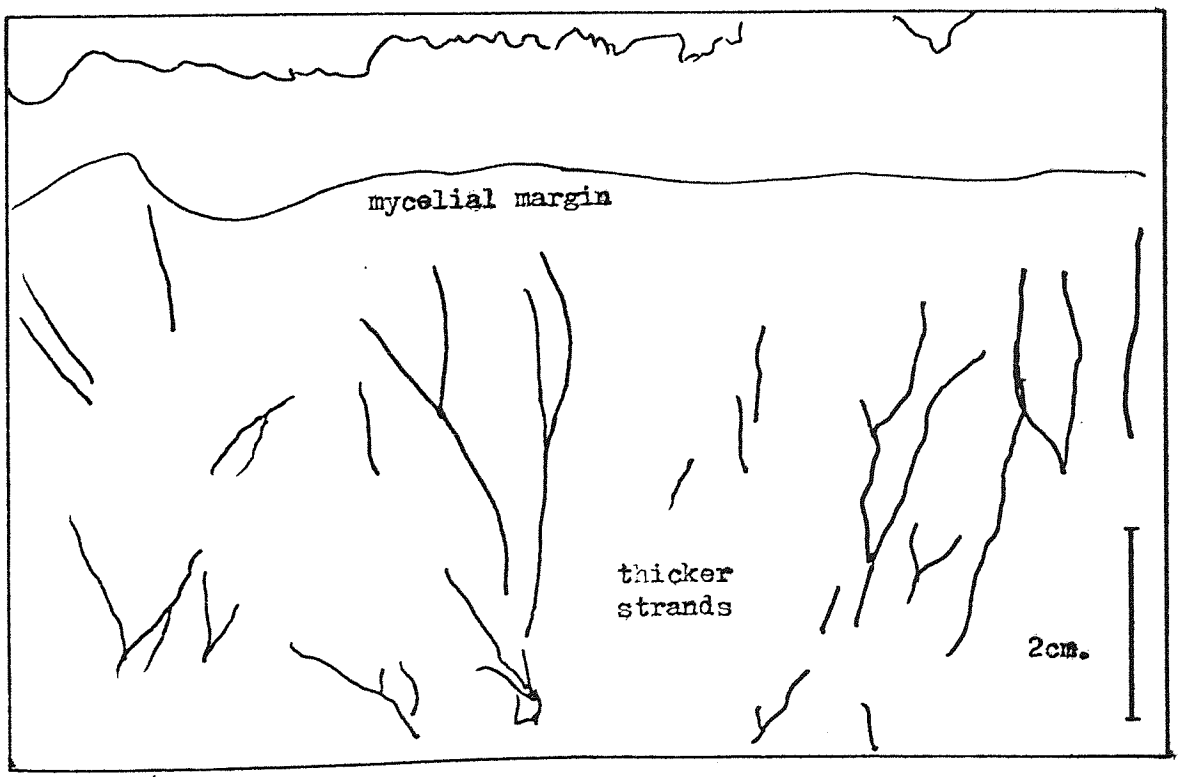


Plate 2. Casing layer, showing mycelial forms.
(8 days after casing)



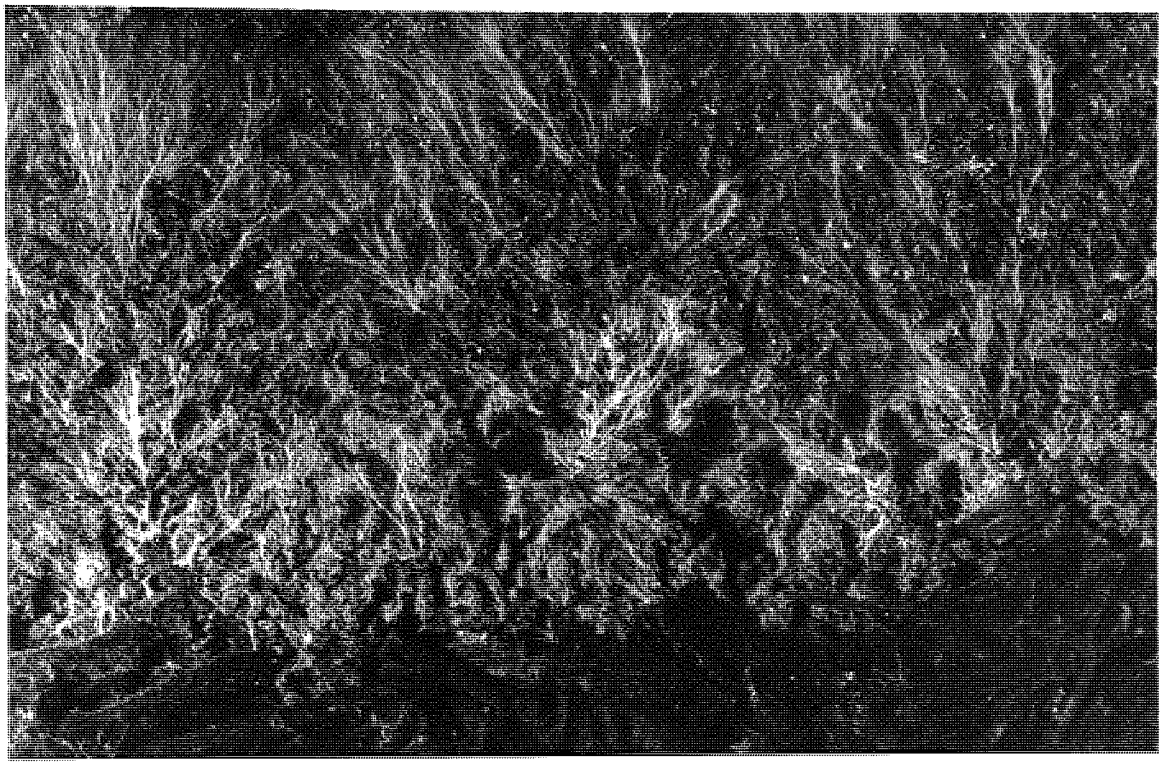
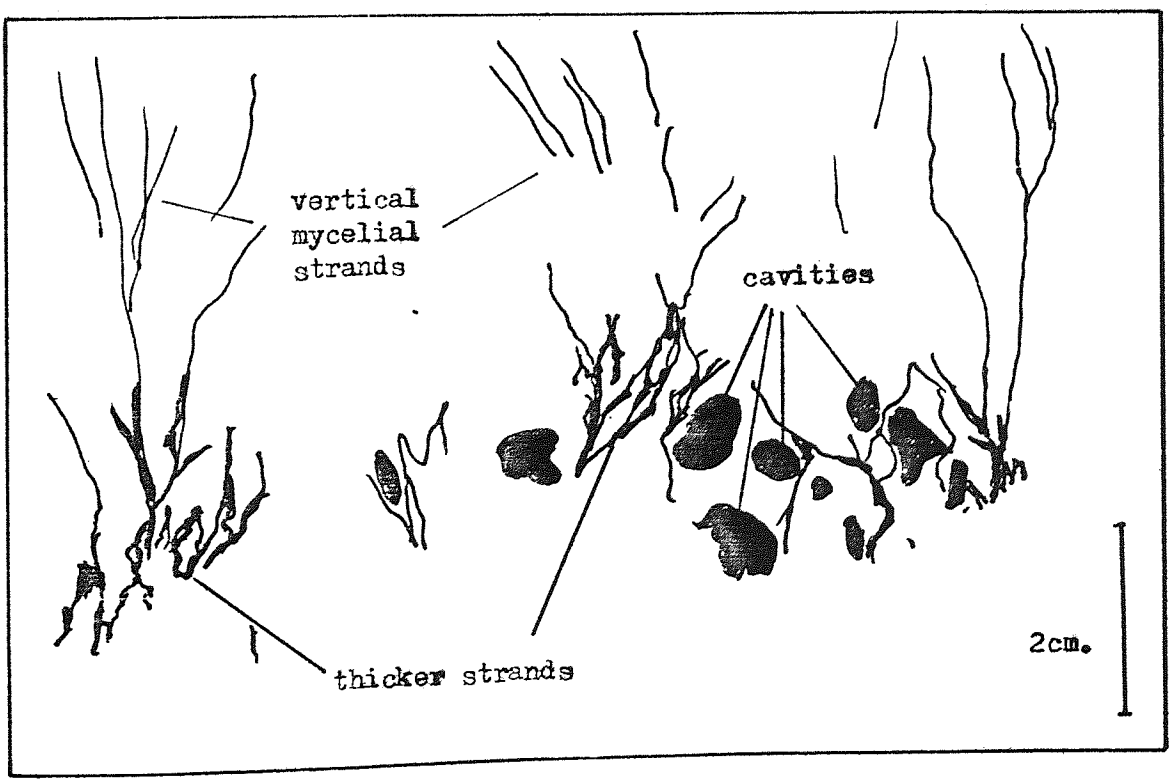


Plate 3. Bottom of casing, showing strand orientation.

(8 days after casing.)



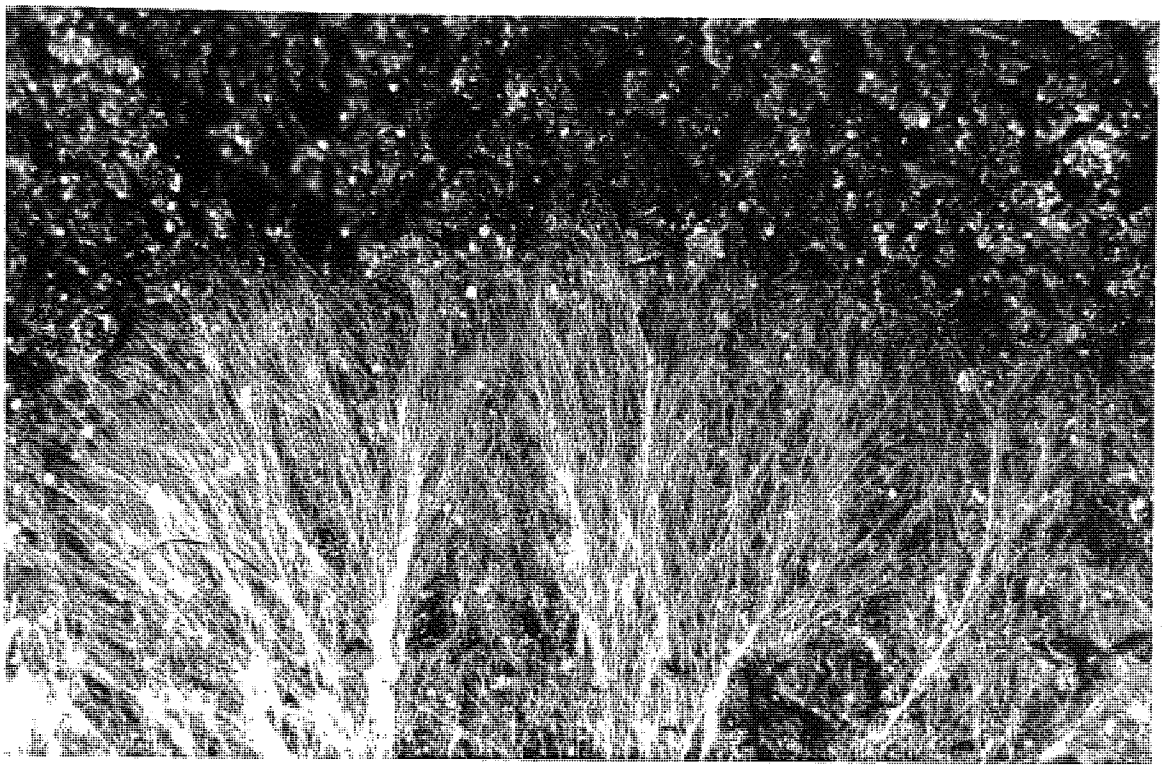
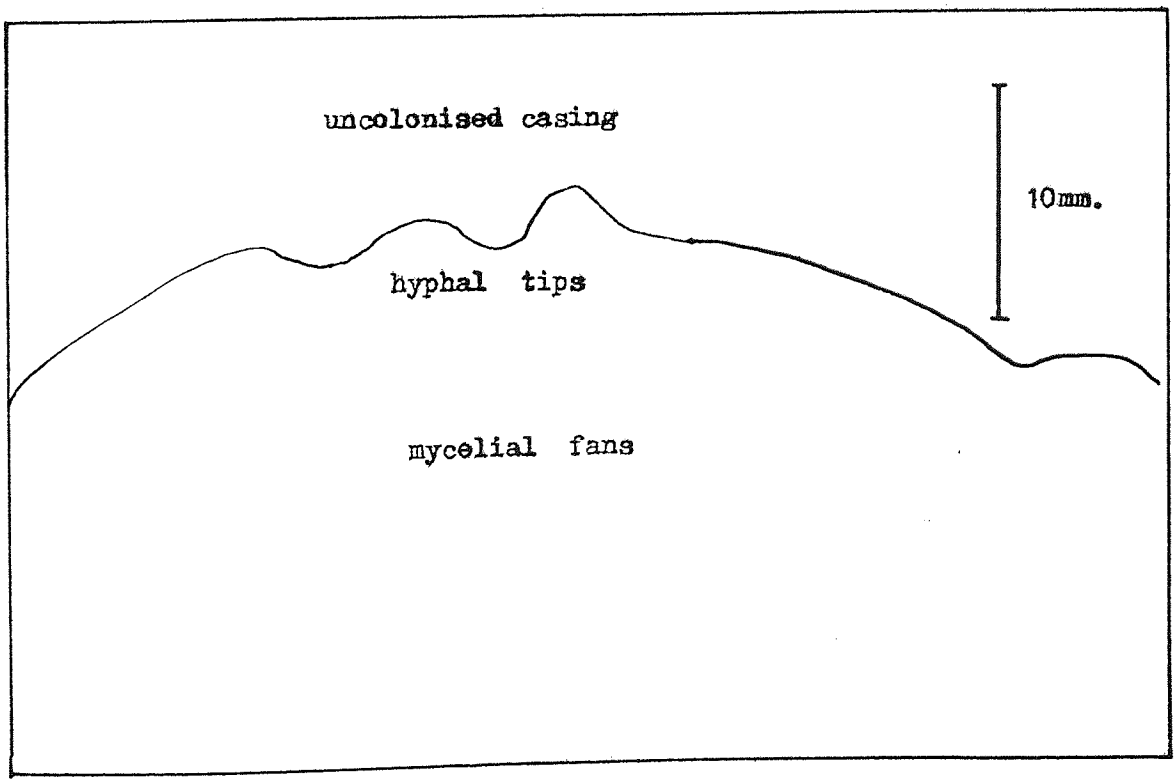


Plate 4. Top layer of casing, partly colonised by mycelium.
(8 days after casing.)



Odd strands of straw projecting upwards from the compost act as foci for the invasion of the casing by mycelium (Plate 5) and these also influence the density and orientation of mycelial strands in the casing. In the compost below, mycelial strands extend back down these straws for 1 - 2 cm. (Plate 6).

Ventilation and cooling for the initiation of fruiting was commenced at 10 days after casing, and the air temperature was lowered from 24°C to 17°C.

Plate 7, taken 12 days after casing, is a closeup of the same mycelial stranding as Plate 6, shows the strengthening of strand formation in the compost and casing. Most of the mycelium in the compost has a "woolly" appearance, due to its finer texture and its random spatial orientation. The strands which are present, however, run more or less vertically, towards the casing. Mycelium in the bottom of the casing can be seen to have become more dense.
Plate 8, also taken at 12 days after casing, shows a section of the mycelial margin at the top of the casing. Here the thickening of certain areas of mycelium into short strands can be seen. This is taking place about 6 mm. from the hyphal growing tips. However, the thickening is confined to the uppermost areas of these aggregations, which is in contrast to the strands in the bottom of the casing which become thinner as they extend upwards.

Plates 9 - 13 represent a study of a single section of casing, taken over a period of time to show the various stages of development involved in the fruiting process.

Plate 9, at 14 days after casing, shows the further thickening of mycelial strands in the top of the casing. These fluffy aggregations can be distinguished from the fine hyphae immediately above them, the well-formed strands below them and the dense undifferentiated mycelium below that.

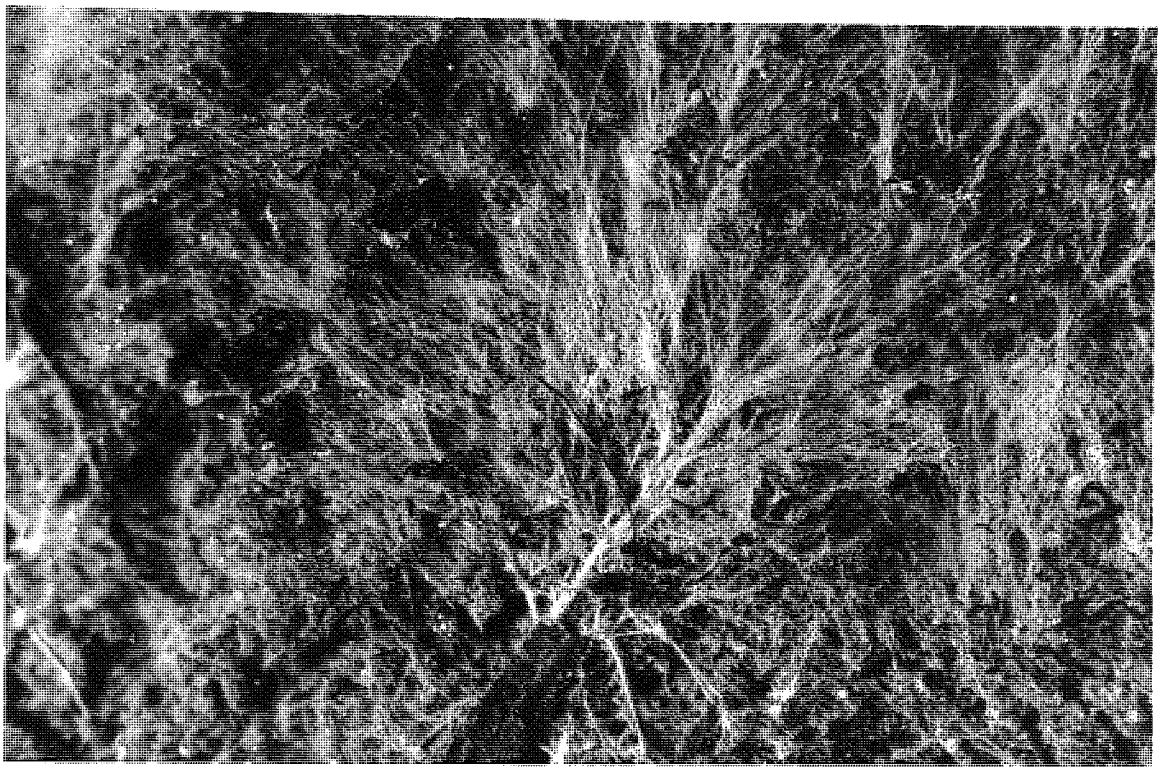
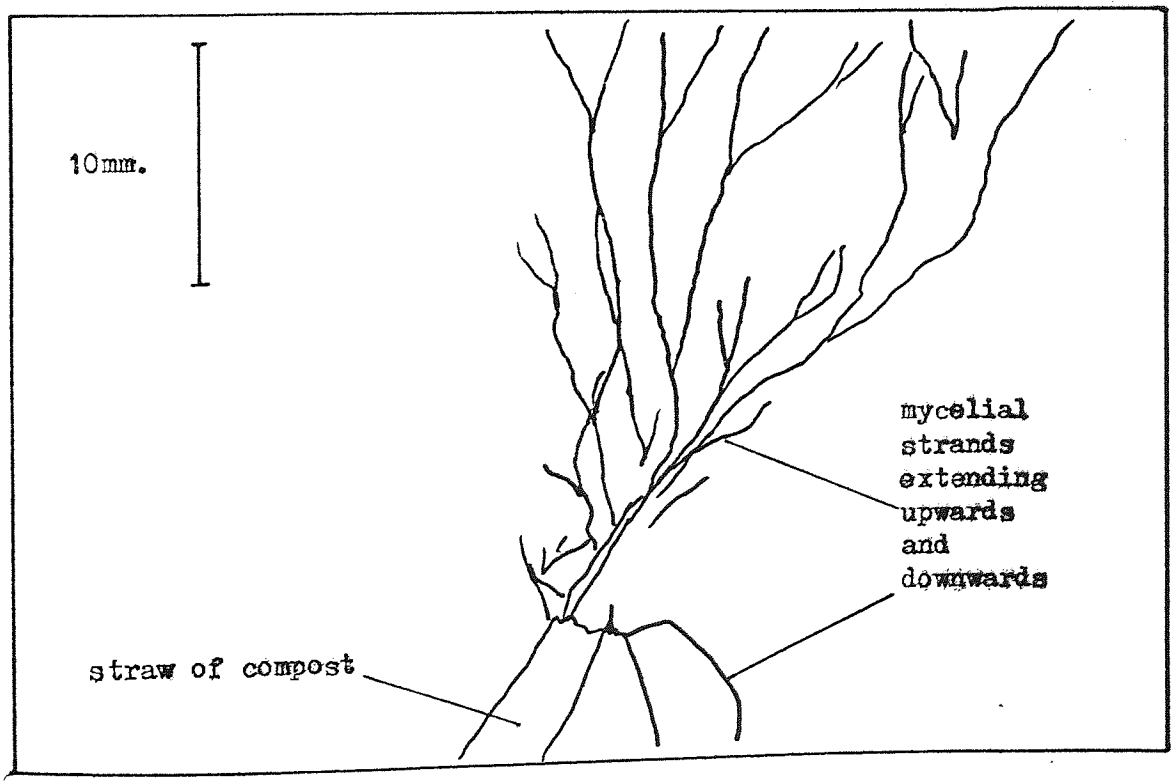


Plate 5. Bottom layer of casing, well colonised by mycelium.
(8 days after casing.)



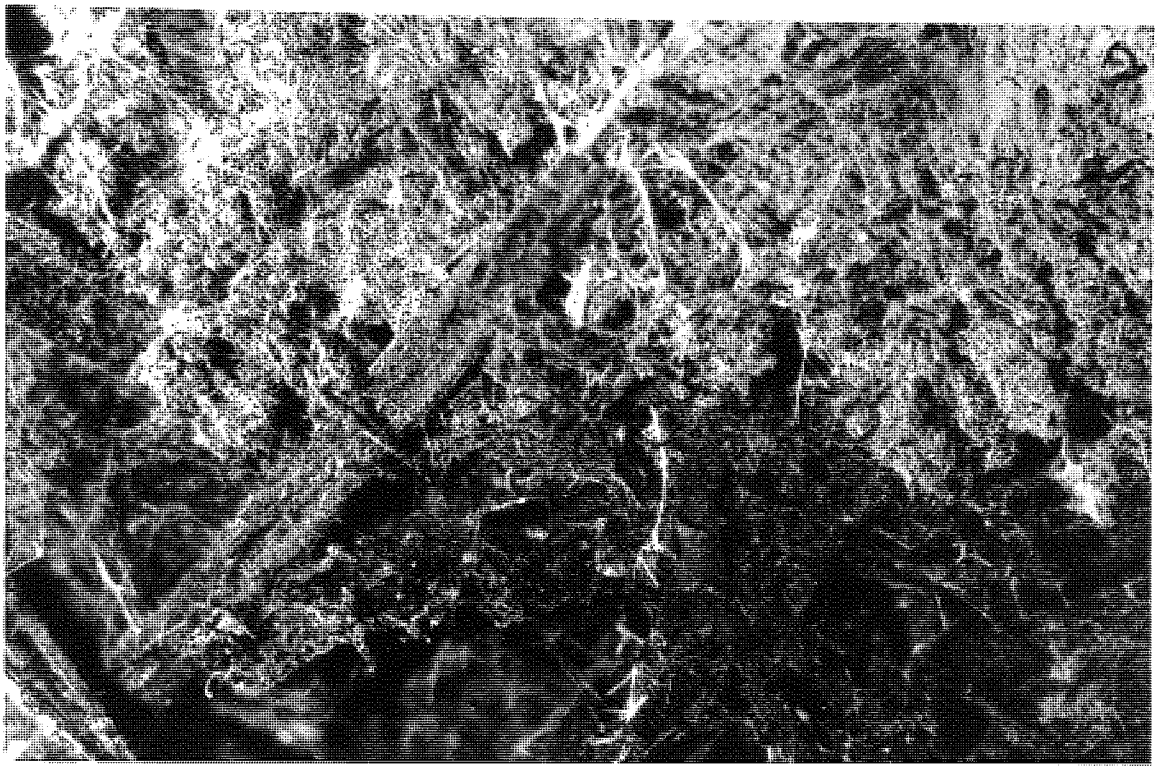
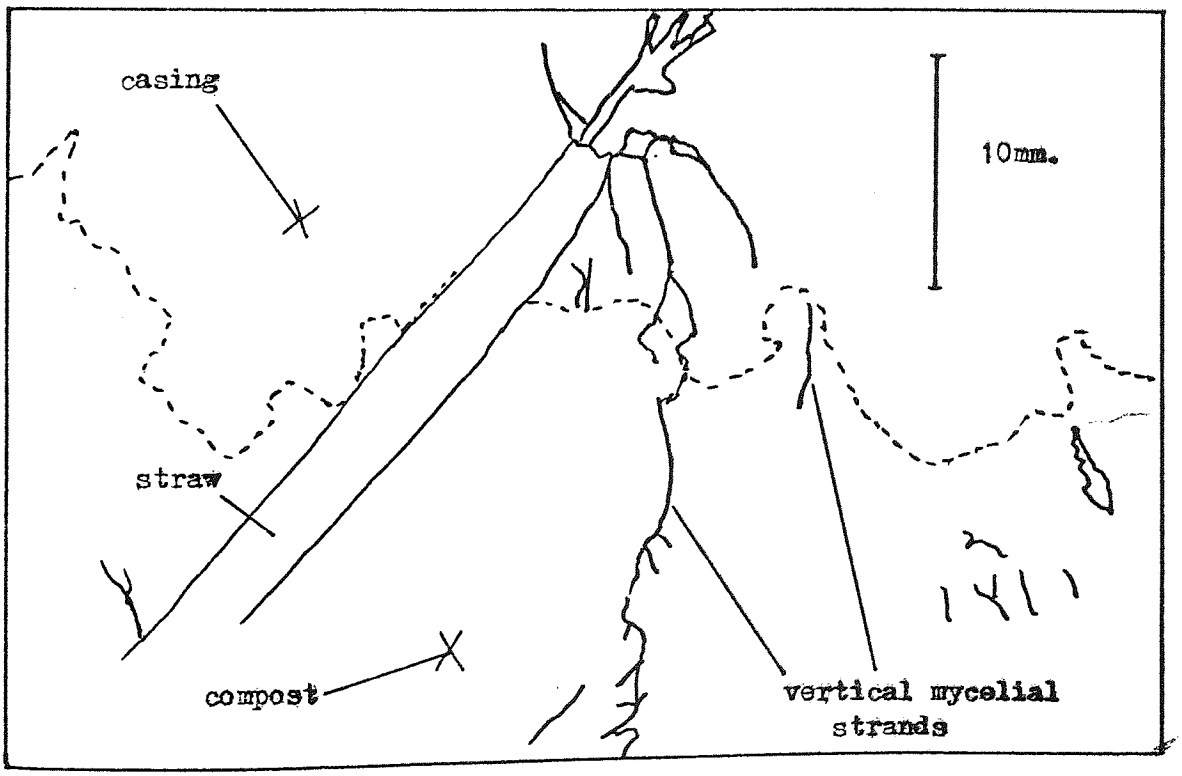


Plate 6. Compost-casing interface, and mycelial stranding.
(8 days after casing.)



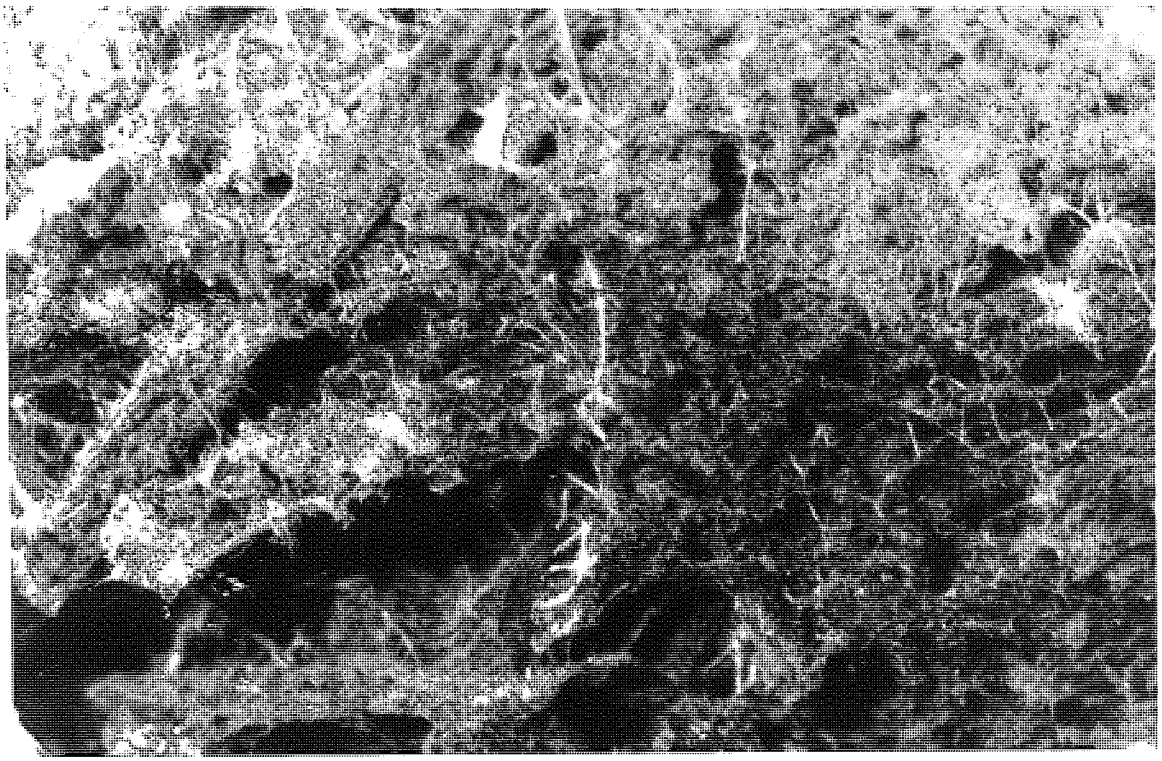
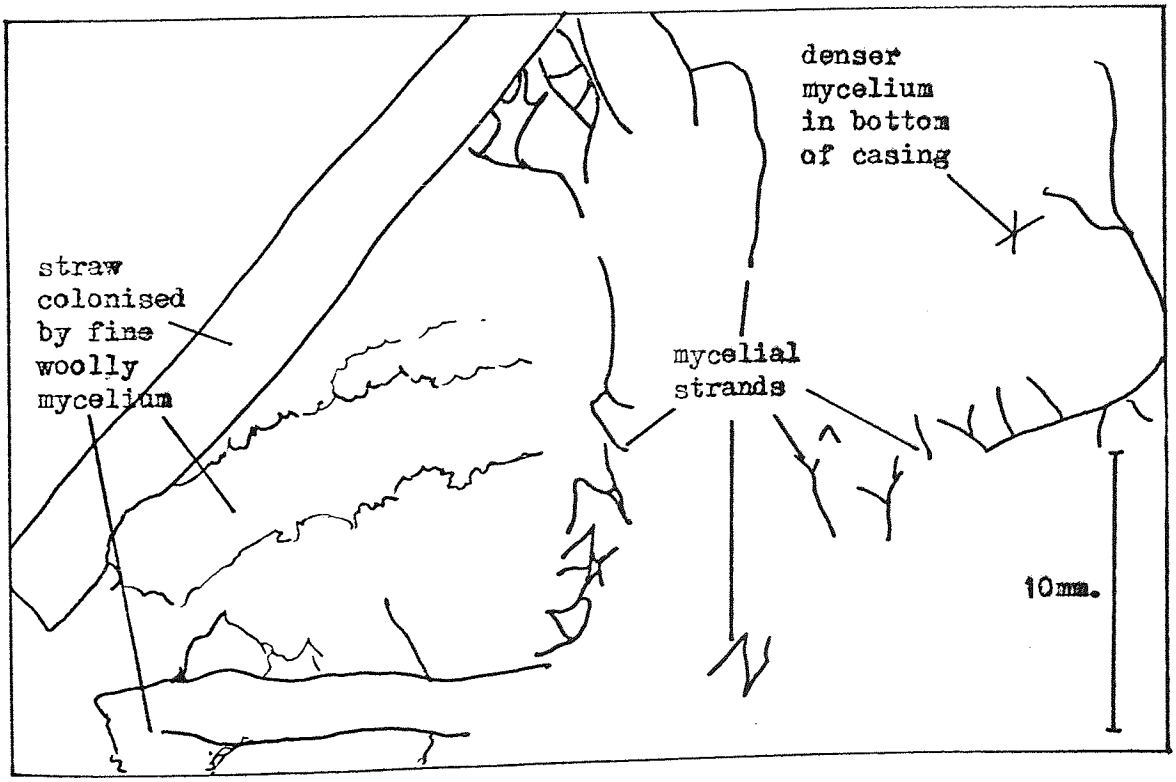


Plate 7. Compost-casing interface.

(12 days after casing.)



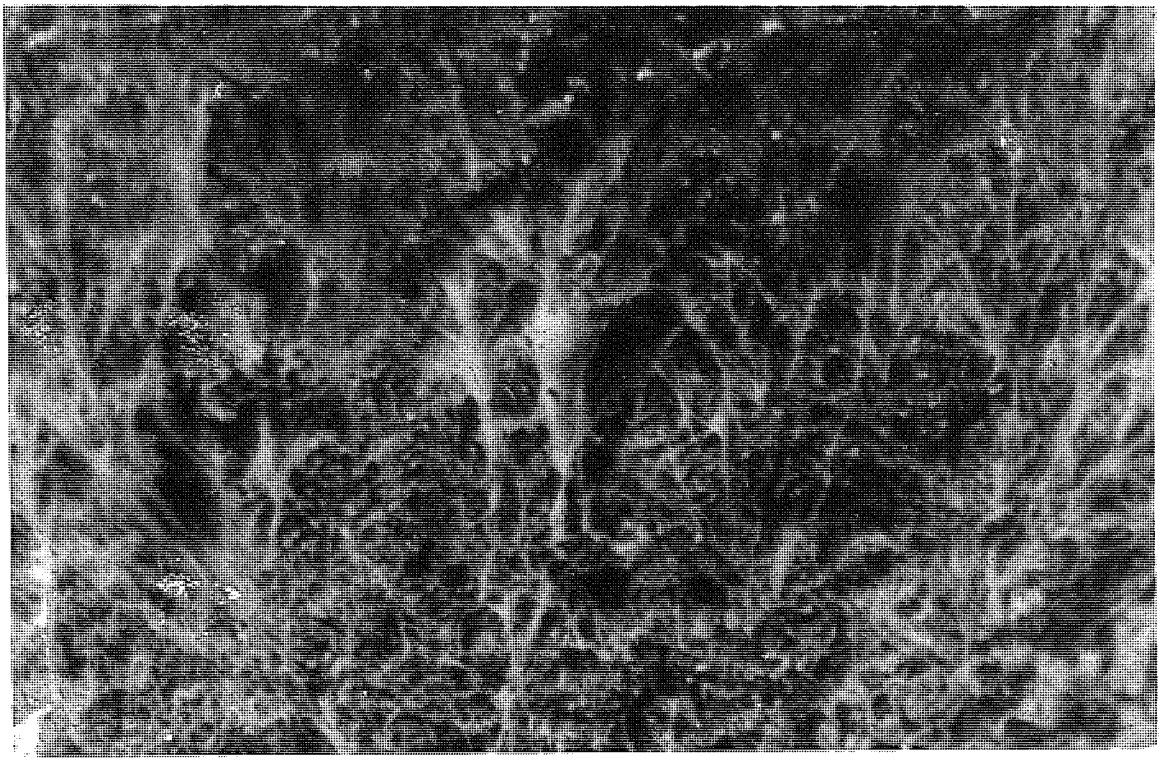
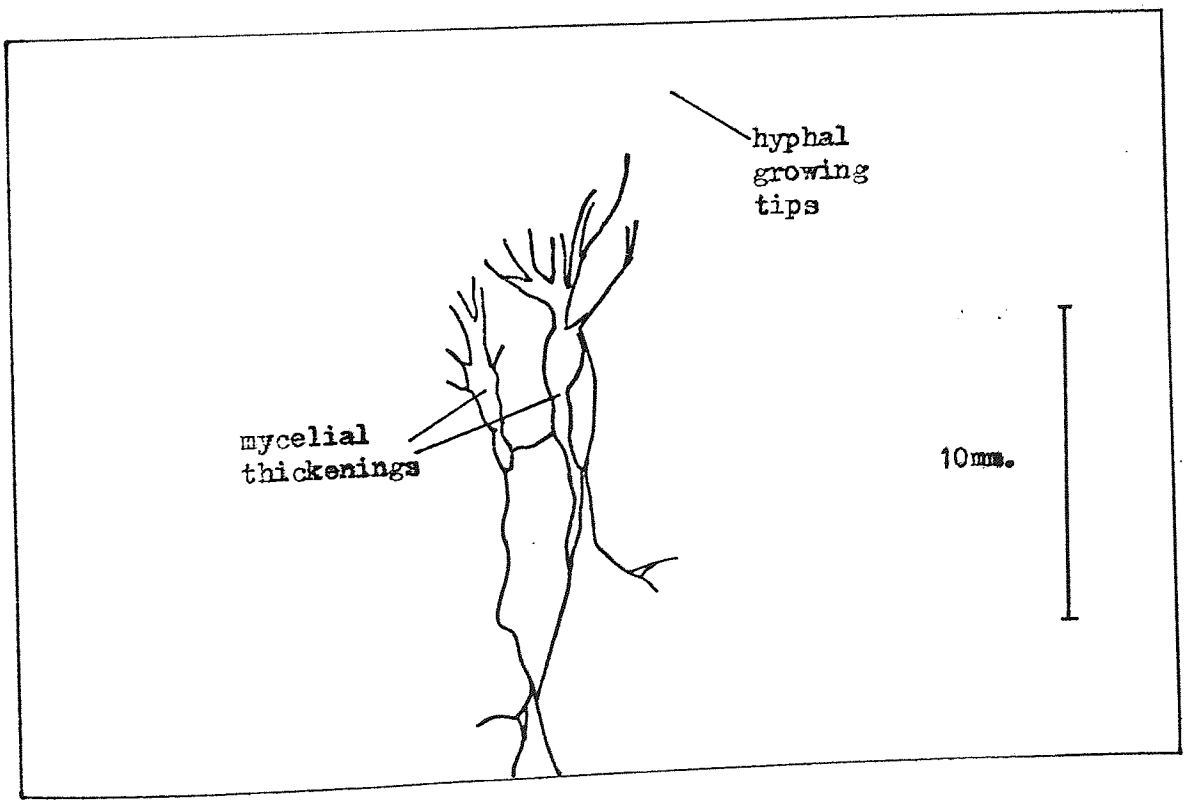


Plate 8. Thickening of mycelial strands.

(12 days after casing.)



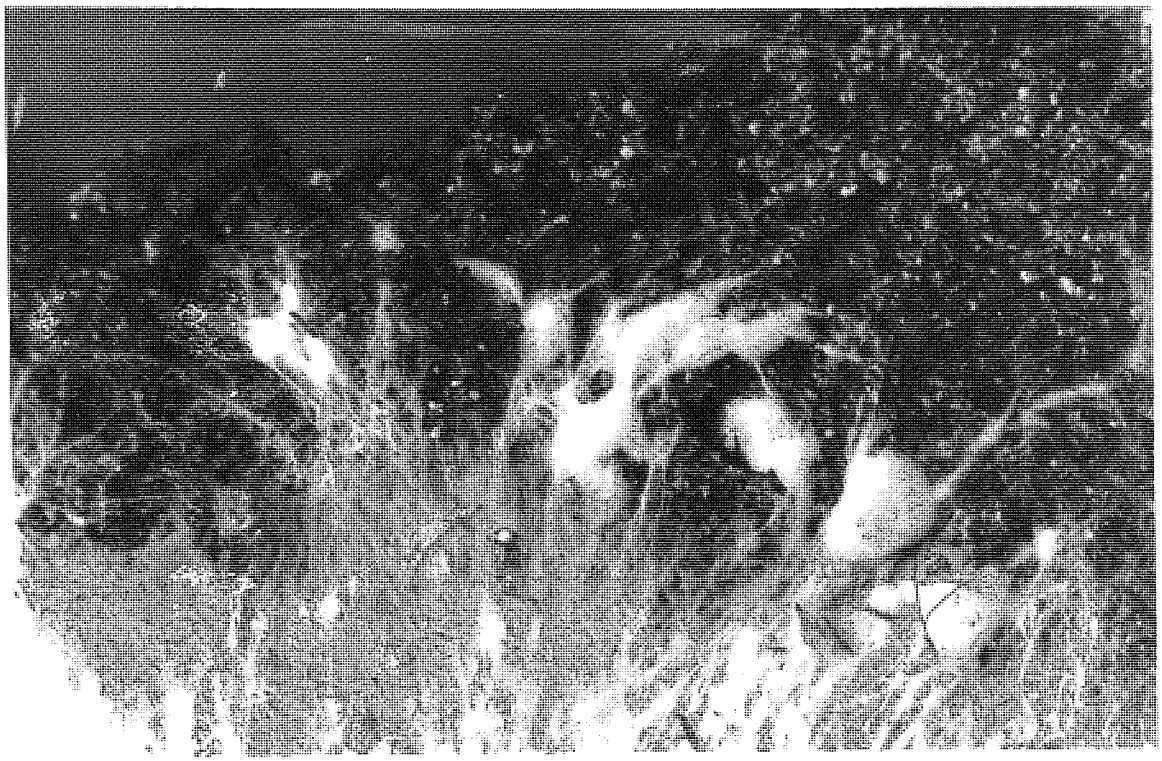


Plate 9. Hyphal aggregations.

(14 days after casing.)

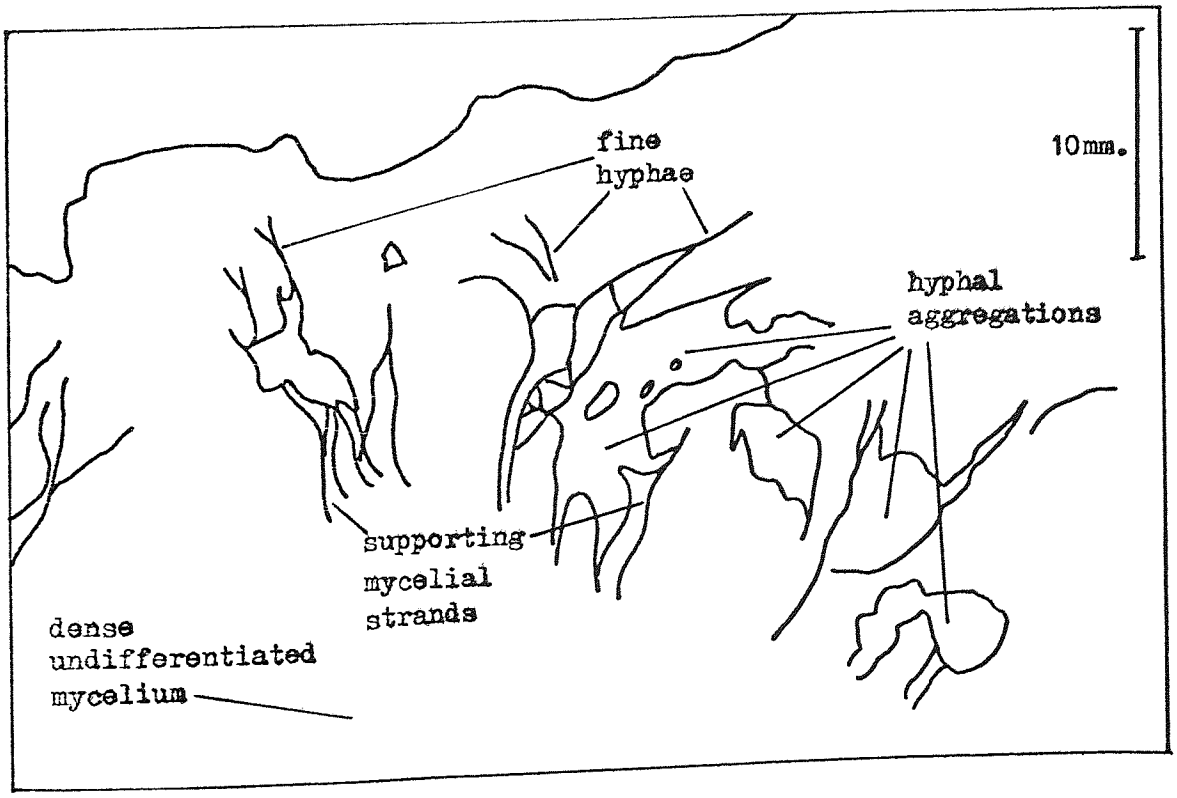


Plate 10, at 18 days after casing, shows the same aggregates, having increased in size, together with their well-developed network of mycelial strands beneath them.

Plate 11, at 21 days after casing, shows that these aggregates have progressed to the primordium stage, in which the stipe and pileus can be distinguished, and a proportion of these primordia are becoming enlarged. Fine mycelium is no longer visible above the primordia, possibly due to the action of watering, from above, or due to the disruption of the surface of the casing layer caused by their enlargement.

Although in general it seems impossible to forecast which primordia will develop further to produce mature fruitbodies, the superficial fissures on three of these primordia may be relevant in suggesting that further development is already taking place. They may represent either stresses of development or the associated requirement for water.

Plate 12, at 25 days after casing, shows the outcome of this process of maturation of primordia, the front three fruitbodies originating from the primordia identified in the previous plate. The fissures cannot be traced on the mature fruitbodies, although one is slightly malformed due to the constraints on growth caused by the glass on the front of the chamber, and the wooden strut across the top. Most of the primordia shown in the previous plate have developed no further. The whole depth of the casing can be seen, together with a section of compost, with the straw and mycelial strands shown in plates 5, 6 and 7, which have participated in supplying nutrients to the developing fruitbodies.

Plate 13, taken on day 27 after casing, after the picking of some of these maturing fruitbodies, shows that some of these primordia are becoming moribund, with the degeneration of mycelial stranding and reversion to a fluffy condition within the crater left by picking.

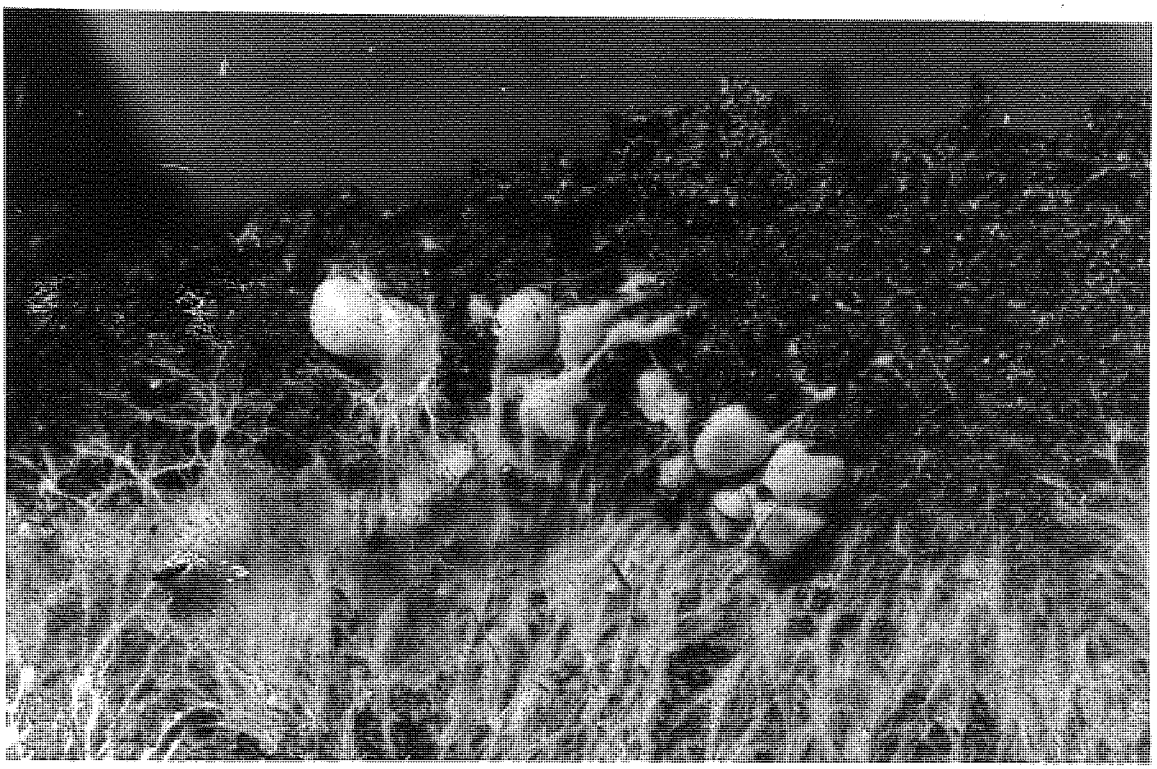
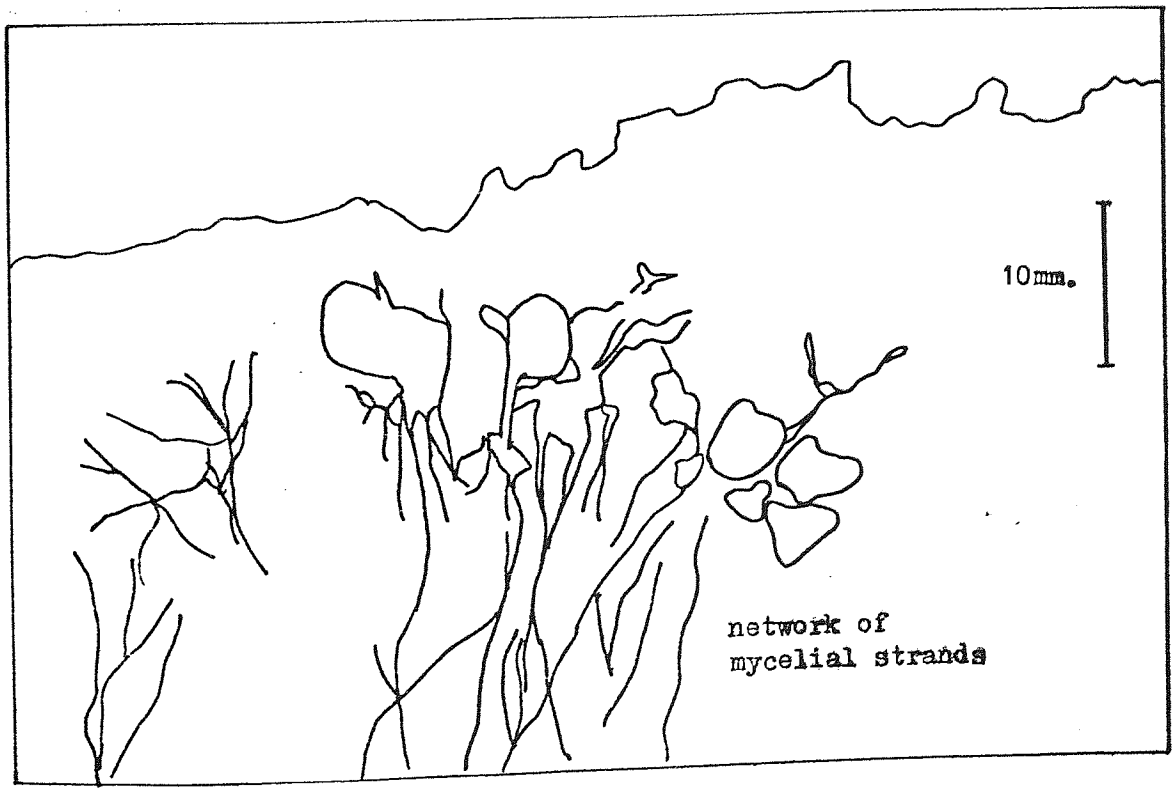


Plate 10. Hyphal aggregations.
(18 days after casing.)



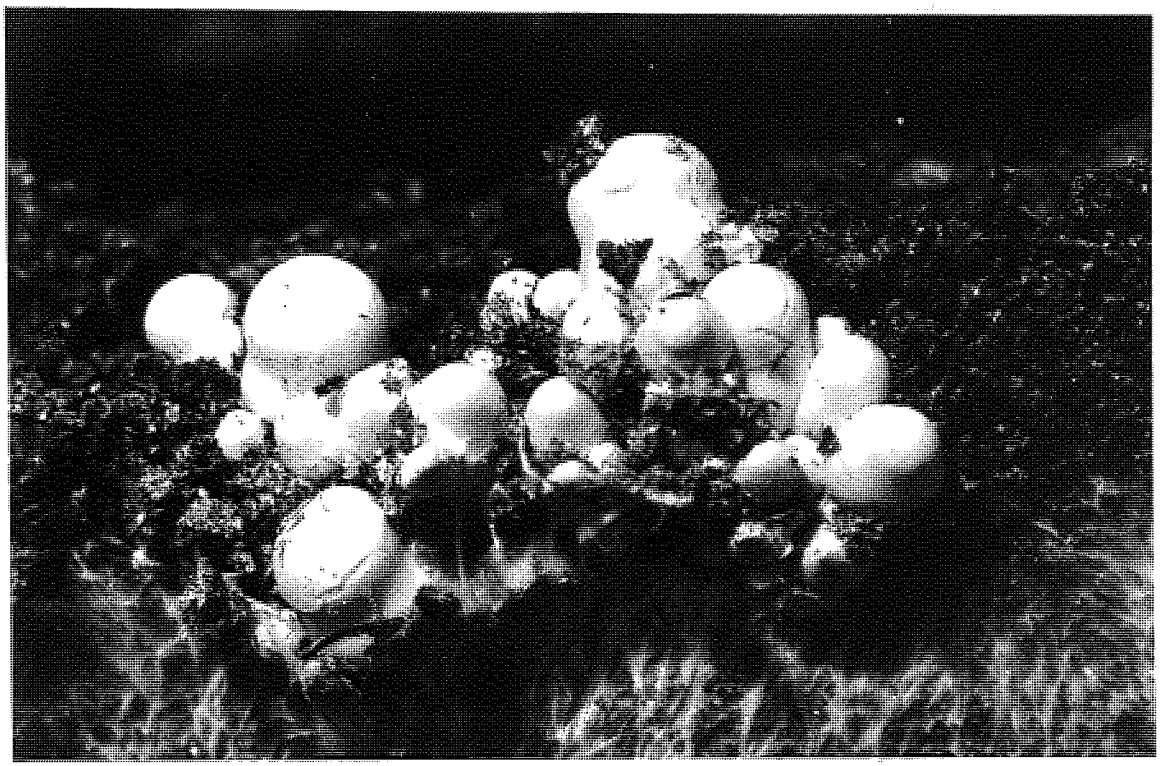
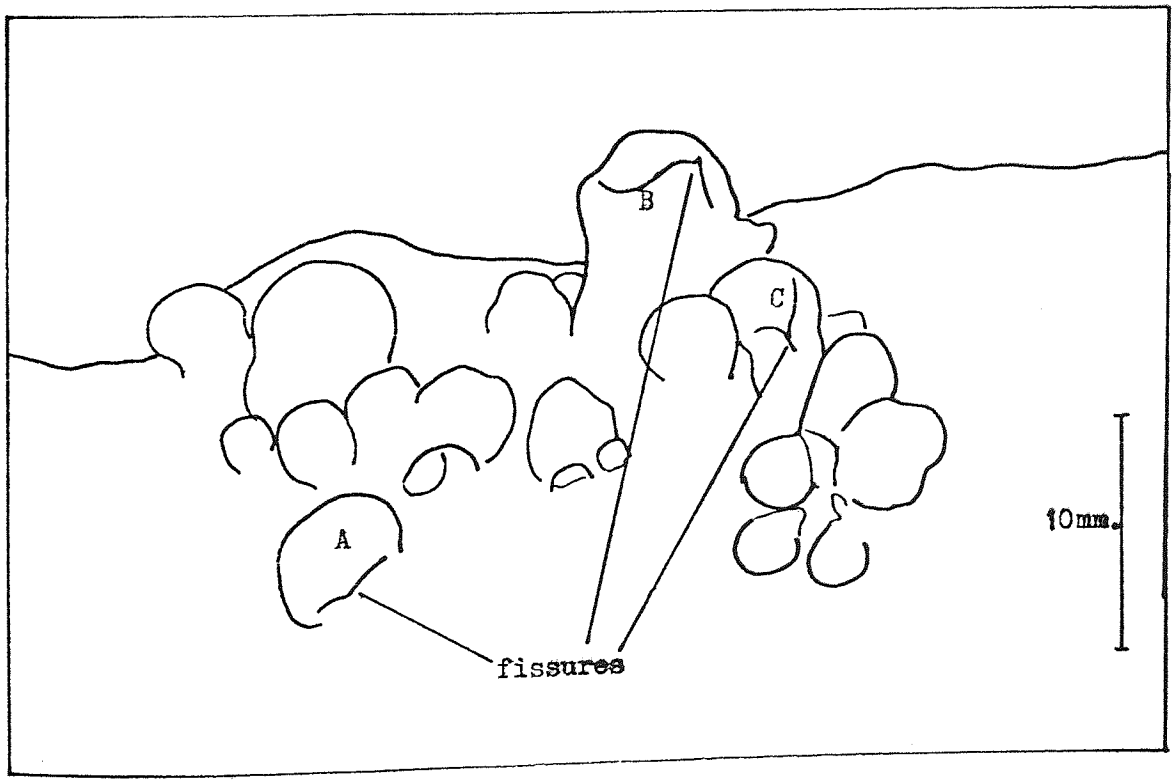


Plate 11. Fruitbody primordia.

(21 days after casing.)



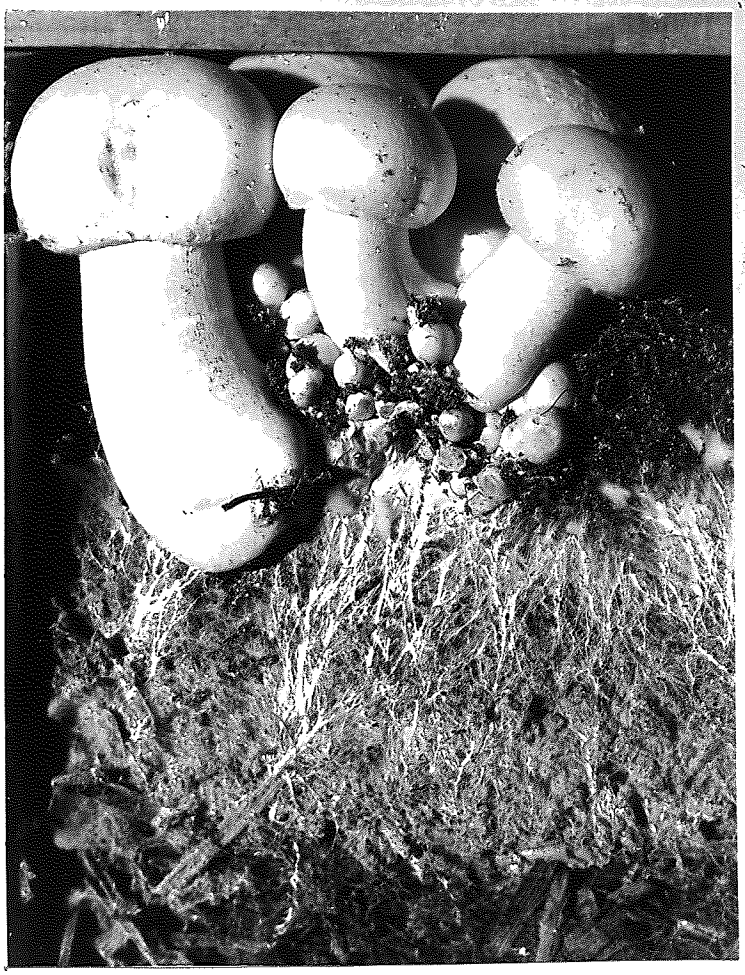
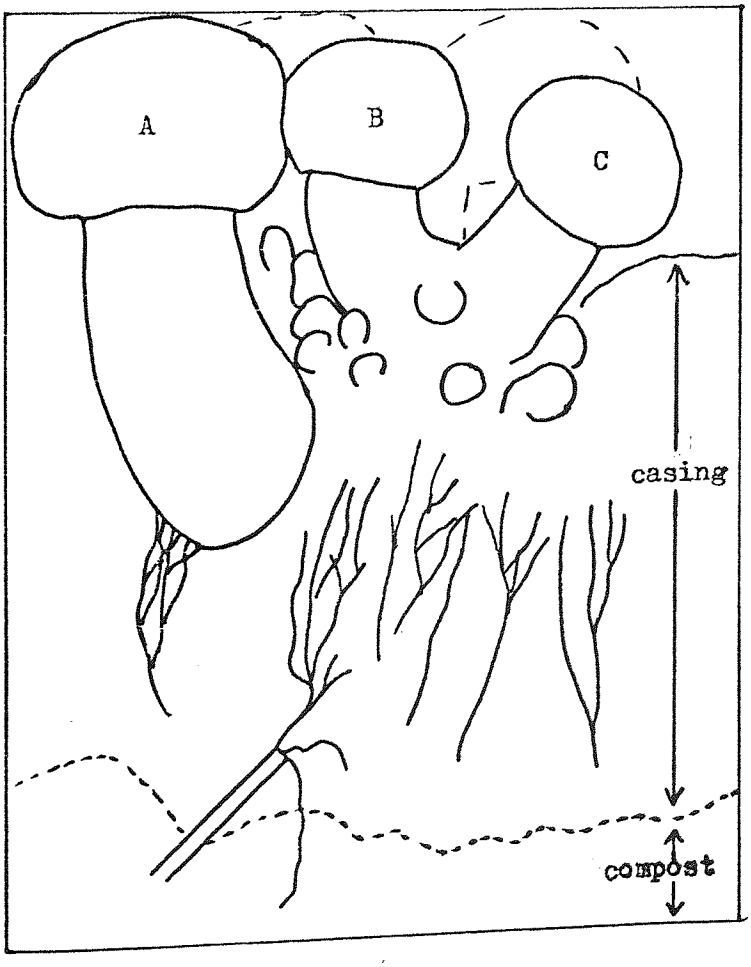


Plate 12. Fruitbodies at harvesting stage.

(25 days after casing.)



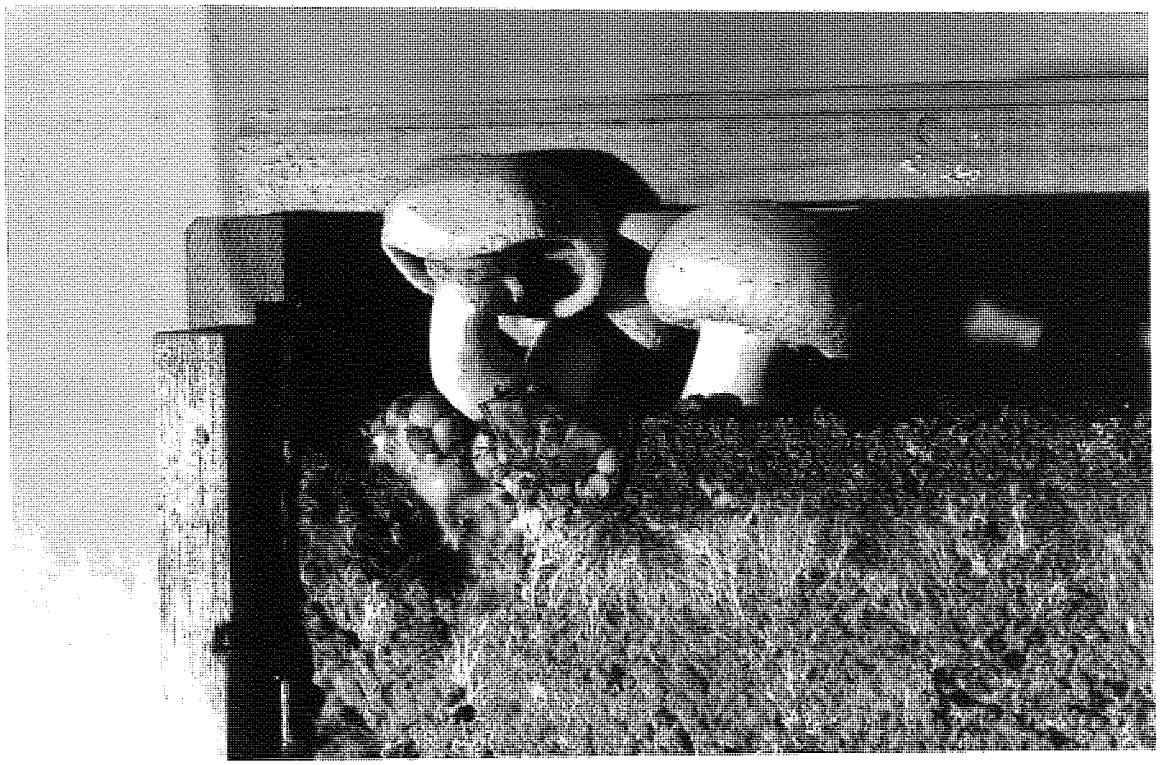
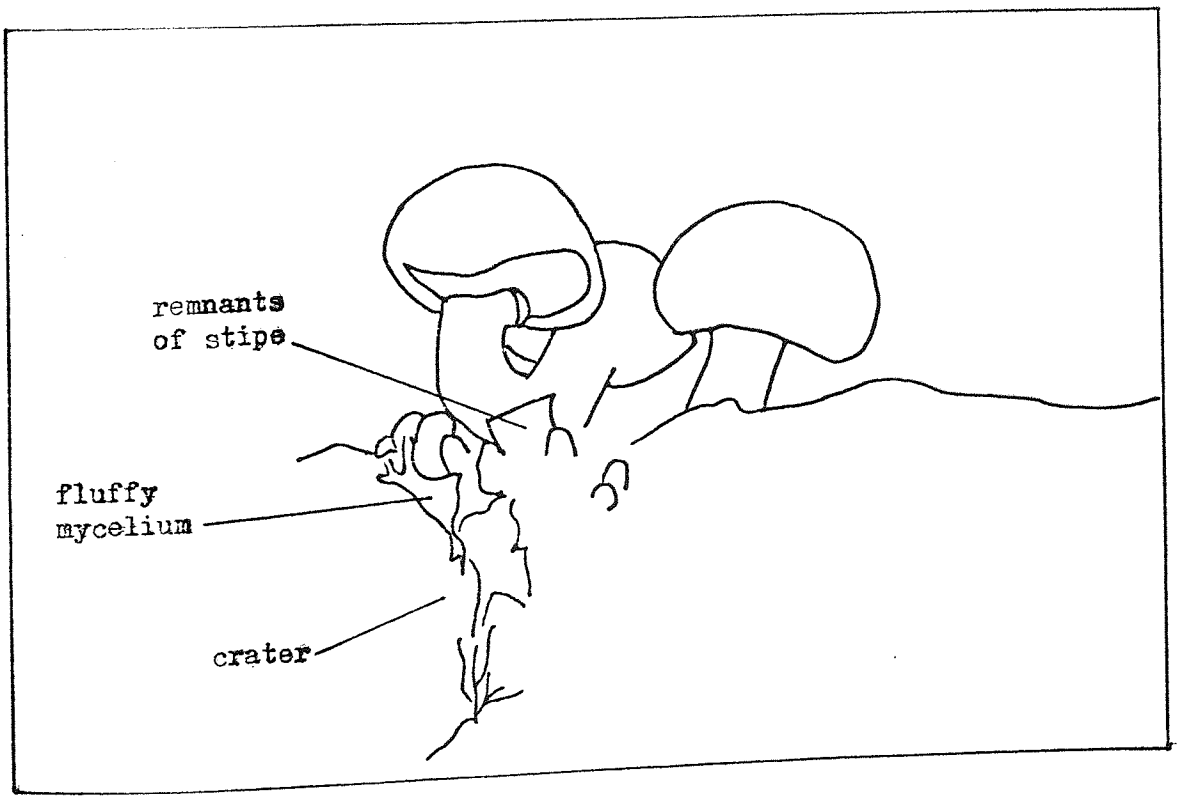


Plate 13. The situation after picking.

(27 days after casing.)



These plates illustrate the changes in morphology associated with the various phases of the fruiting process. They also indicate the relationship between the compost and the casing layer. Initiation can be seen to have taken place below the hyphal tips, so that primordia formed within the casing, below the surface. By visual detection at least, the formation and distribution of primordia appeared to take place at random.

Only a proportion of these primordia matured to become fruitbodies, and this process also seemed to be at random.

B. Formation and maturation of primordia

The major variable likely to affect the processes of initiation and development of primordia is temperature. For this reason, an experiment was set up in which cultures were subjected to different temperature treatments during the cropping phase. Prior to the initiation of fruiting, during the period of mycelial colonisation of compost and casing, both sets of cultures were given the normal treatment - 24°C with no ventilation. Ten days after casing, ventilation was commenced, but the cultures in one cabinet were given supplementary heating to keep the air temperature at 24 - 26°C, whereas the other cultures were treated normally and cooled to 17°C, then maintained at 16 - 18°C. Numbers of primordia observed, also numbers and weight of, fruitbodies harvested, were recorded for the first two flushes, and totals of each are presented in Table 1.

Table 1. The effect of temperature on the formation and development of sporophore primordia

Temperature	Number of primordia per m2	Number of mature fruitbodies per m2	Percentage maturing	Fresh weight of fruitbodies kg. per m2
16 - 18°C	1895	555	29.3	8.24
24 - 26°C	3292	153	4.6	3.58

This shows that more primordia formed at the higher temperature, but substantially fewer matured, resulting in a marked limitation in overall productivity.

Later experiments involved the counting of mycelial aggregates on small trays sacrificed at intervals, the casing of which was removed and its top layers washed away down to the level of the hyphal tips. Table 2 shows the number of these aggregates found at different stages after casing, in a series of cultures given the normal management regime, with ventilation commencing 10 days after casing.

Table 2. Aggregate formation in the casing at different time intervals

Time from casing (days)	0	7	14	21	28	35	42	49
Aggregates per tray (3 replicates)	0	0	26	172	168	123	151	83

Their numbers can be seen to rise to a maximum at the time of maximum production of the first flush, then they more or less level off, thereafter declining somewhat.

The effect of temperature and aeration on aggregate formation was also studied further. Trays were cased as usual, and then subjected to 4 management regimes:

A. "Normal" - Temperature maintained at 24°C for 10 days after casing, then lowered to 16°C with the introduction of fresh air;

B. "Extended Incubation" - 20 days at 24°C, followed by cooldown to 16°C;

C. 24°C, with ventilation, from casing onwards;

D. 16°C, with ventilation from casing onwards.

Two samples from each treatment were removed at 10, 15, 17, 20, 25, 30 and 35 days after casing.

Figure 3 is a graphical representation of the estimated number of aggregates counted on each treatment at the different times.

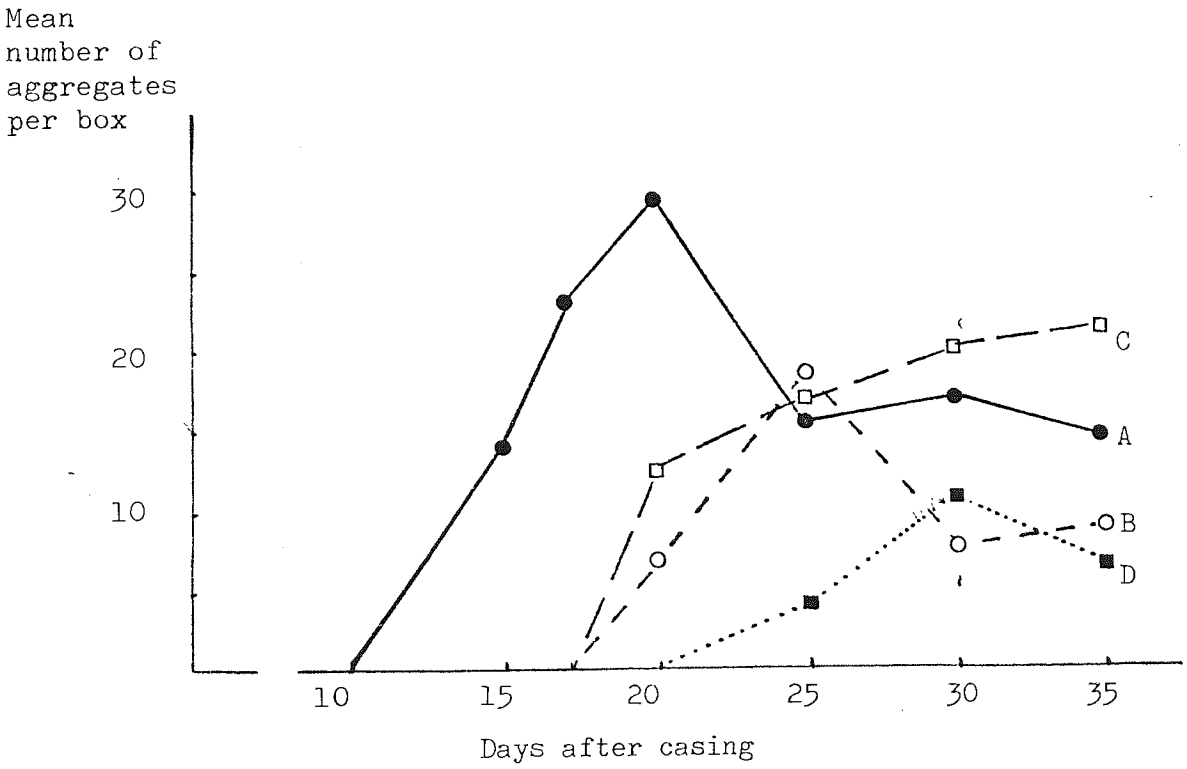


Figure 3 Numbers of aggregates forming at different temperature regimes

A ("normal") 10 days at 24°C, then 16°C

B 20 days at 24°C, then 16°C

C 24°C

D 16°C

The degree of aggregate formation was not so marked as in the previous experiment, presumably because the spawn strain used was unsuited to summer conditions.

The normal treatment, A, again showed a rise in the number of these aggregates up to 20 days after casing, falling off thereafter.

Extended incubation, B, showed a similar pattern, the rise being delayed by about 7 days, which might be explained as due to the 10 days extra incubation at higher temperature. However, at the peak number of aggregates B did not reach the same as treatment A, indicating that the overall efficiency of the system was decreased by the extra incubation time, possible due to excessive mycelial growth forming areas of non-productivity overlay on the casing surface.

Keeping temperature at 24°C after casing, C, also delayed the rise in the numbers of aggregates visible, but there was no tendency to peak noticed in the duration of these observations.

Treatment D, also delayed the rise in aggregate formation, but this was seen to peak normally.

These experiments showed that elevated temperatures during the cropping phase had marked effects on the production and maturation of fruitbody primordia, but taken overall these effects suppressed production. Normal temperature treatments were found to give the highest productivity, which justifies the temperature regime adopted commercially.

C. Normal pattern of fruitbody formation

Usually the first fruitbodies are harvested 17 to 20 days after casing. More mushrooms are pickable a day or two later, and most mushrooms from the first flush are picked by 23 days after casing, depending on the spawn strain and picking stage. There follows a break in production before the next flush becomes pickable, about 6-10 days

after the first. The first two flushes give the greatest yield. Subsequent flushes usually diminish gradually, and the intervening periods between flushes increase slightly.

Consequently the production of mushrooms on a single box does not proceed in a truly linear fashion with respect to time, since the first production is offset by 17-20 days, then rises quickly to a maximum and falls off again, is at zero for 3-4 days before rising again to another maximum and falling again, repeated on a decreasing scale with lower frequency.

This means that mushroom production cannot be readily compared with other microbial growth curves, or with production of other crop plants.

If plotted as a cumulative yield, the typical yield pattern of Figure 4 is obtained. The contribution to yield of each flush is thus clearly demonstrated, together with the period between flushes.

The pattern of production can be "smoothed" to fit a sigmoid curve implying that a given system is capable of a fixed overall production.

This also shows that, once fruiting has been initiated, the production of fruitbodies becomes an ongoing process and flushing is therefore of real commercial significance in that cropping continues for an appreciable period of time.

D. Amendments to peat-based casings

(i) Addition of inactive materials

Peat-based casings as normally used consist of a mixture of peat and chalk or limestone of some form, in quantities greater than may be necessary merely to neutralise the acidity of the peat. The chalk or limestone clearly modifies the physical structure of the resulting mixture as well as having a chemical action. It was decided to initiate experimentation on the physical structure of casing through the addition of materials which would not directly participate in any chemical reactions. Gravel was used because of its properties of

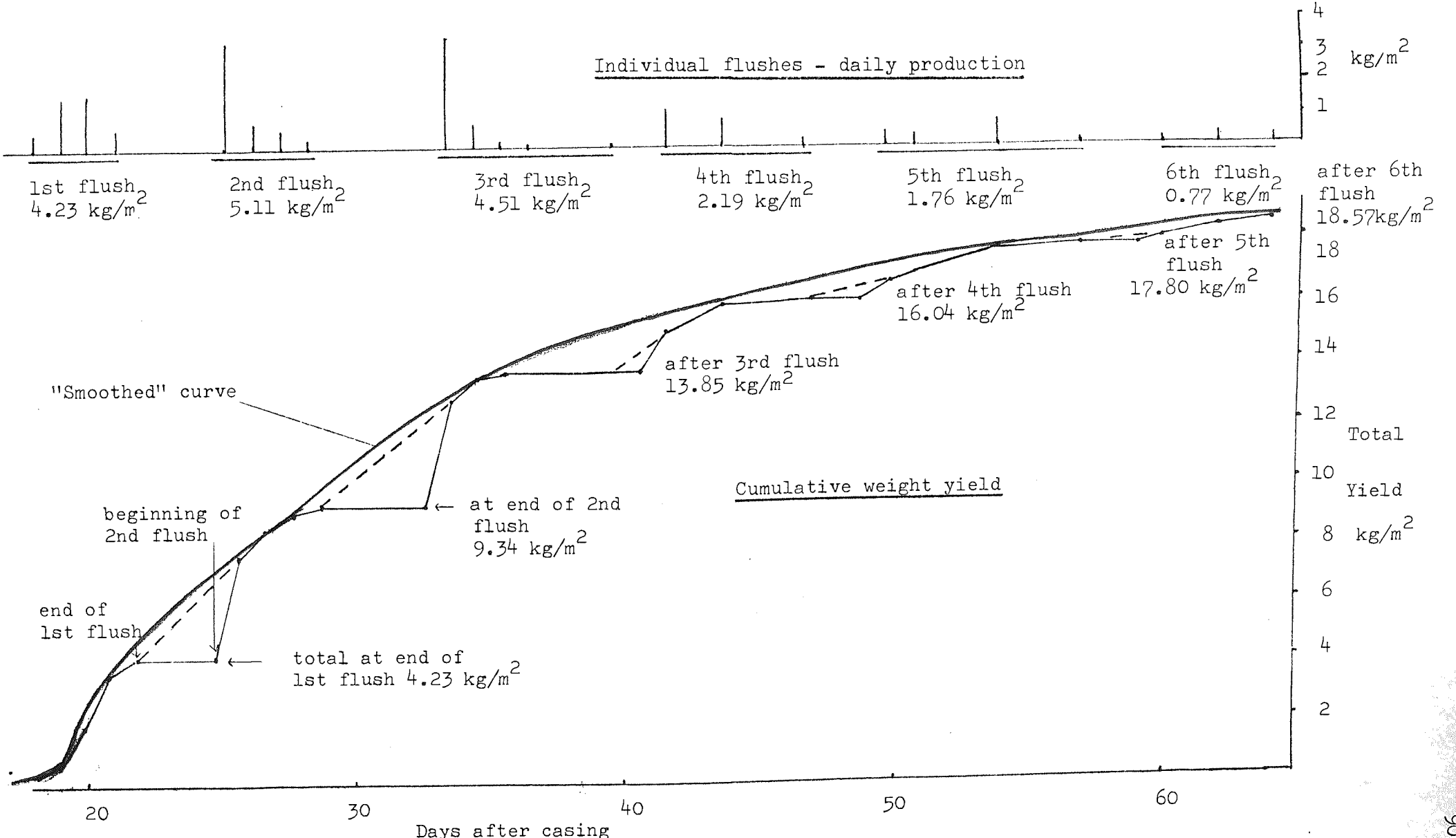


Figure 4 Typical production pattern for the mushroom crop

assisting aeration and drainage of soils.

In a preliminary experiment, a comparison was made between standard peat-chalk casings and those amended by the admixture of two grades of gravel. Cropping experiments were performed in conjunction with determinations of the physical characteristics of the mixtures.

Table 3 shows that the water holding capacity of the mixtures diminished due to the addition of approximately 6% by weight of each gravel. This is not surprising since gravel does not hold water in the same way as peat.

Thus the gravel amended mixtures, applied at the same moisture content with respect to their peat/chalk content, were in fact at application not only lower in actual percentage moisture content per unit weight, but also higher in their percentage of maximum water-holding capacity i.e. could take up and hold less water on subsequent watering than the control.

The maximum pore space of the mixture appeared to be virtually unaffected by the addition of 6 mm. gravel. This may be a reflection of the problems of determining the pore space of peat-based casings.

However, the figures for bulk densities of the resulting mixtures suggest that 12 mm. gravel had a greater effect on the compaction of the peat matrix.

The yield results from cropping on these casings are shown in Table 4. In the first flush, both gravels gave an apparent increase over the unamended peat-chalk casing, which were reflected in both weight yield and in numbers of fruitbodies harvested.

The 6 mm. gravel showed a 57.3% increase on weight and a 41.2% increase in numbers, with 12 mm. gravel giving 44.5% and 50.0% increases respectively.

After the second flush the amended casings were not so far in advance of the control, the 6 mm. treatment was only 8.9% higher on

Table 3 Physical characteristics of casing mixtures amended by addition of gravel (1)

Constituent ratios	Standard peat/ chalk casing. % by volume	% by weight	Peat casing+ 6 mm. gravel % by volume	% by weight	Peat casing+ 12 mm. gravel % by volume	% by weight
peat	66.7	10.3				
chalk	3.7	7.1	SCM 96.4	94.1	96.4	93.7
water	29.6	82.6				
gravel	--	--	3.6	5.9	3.6	6.3
Water content						
as % of wet weight		73.3		67.4		59.5
(as applied)						
as % of maximum water		53		67		68
holding capacity						
Pore space total %		85.1		85.3		74.8
Bulk density (wet) g/cm^3		0.46		0.50		0.53

Table 4 The effect of additions of gravel to peat-based casings on mushroom production (1)

Casing medium	First Flush		Second Flush		Overall		
	Weight Yield kg/m^2	Number of mushrooms per m^2	Yield	Number	Yield (+ standard error)	Number	Mean weight of individual fruitbodies
Standard peat chalk casing (SCM)	1.44	54.2	2.68	71.6	4.12 (0.56)	125.8	32.75g.
SCM + 5.9% w/w 6 mm gravel	2.26	76.5	2.22	67.0	4.48 (0.73)	143.5	31.22g.
SCM + 6.3% w/w 12 mm gravel	2.08	81.3	2.14	73.2	4.22 (0.25)	154.5	27.31g.

Least significant difference between overall weight yields @ 5% probability level 3.10 kg/m^2
@10% probability level 2.37 kg/m^2

Results expressed as mean of 3 replicate boxes 45 cm by 45 cm

14 kg compost per box 67.0 kg/m^2 = 13.7 lb/sq.ft.

Spawn strain: Le Lion.

Influences on production: mean cropping temperature 22°C.

3 by 3 (Vertical) Latin Square Arrangement.

weight yield and 11.4% higher on numbers of mushrooms produced, with 12 mm. gravel showing 2.6% and 22.8% increases. In other words there was a tendency for the standard casing to improve its performance compared with the amended casings. Statistically no significance can be attributed to the results due to large variations between replicates of each treatment, as indicated by the standard errors of the means.

The cumulative mean production graph (Figure 5) shows that eventually yield from the control actually exceeded that from the amended casings, although clearly there was little difference between the three treatments.

In a subsequent experiment, the 6 mm. gravel was used at two higher rates, and again compared with a standard peat-chalk casing. The effects on physical characteristics of adding gravel, at 20% and 80% by weight, are shown in Table 5. Visually, these rates appeared to have marked effects on casing structure, which is borne out by these figures. For instance, the higher rate of addition of gravel caused a great increase in bulk density and an appreciable decrease in total pore space, whereas the lower rate caused much smaller changes. The actual water content at application and also the maximum water-holding capacity of the casing were greatly reduced by the addition of gravel, and this reduction was disproportionate to the rate of replacement of peat-chalk casing with gravel, because the gravel contributed so much to the weight of the mixture.

This experiment suffered somewhat due to the positioning of the boxes on the shelves in the basement laboratory. Also, a certain amount of damage due to larvae of mushroom flies occurred in this experiment.

Nevertheless, Table 6 shows that yields were notably higher than the previous experiment, and also larger numbers of mushrooms were produced, hence mean weight of individual mushrooms was 2-3 times lower than in the previous experiment.

Both gravel-amended casings gave greater yields than the control

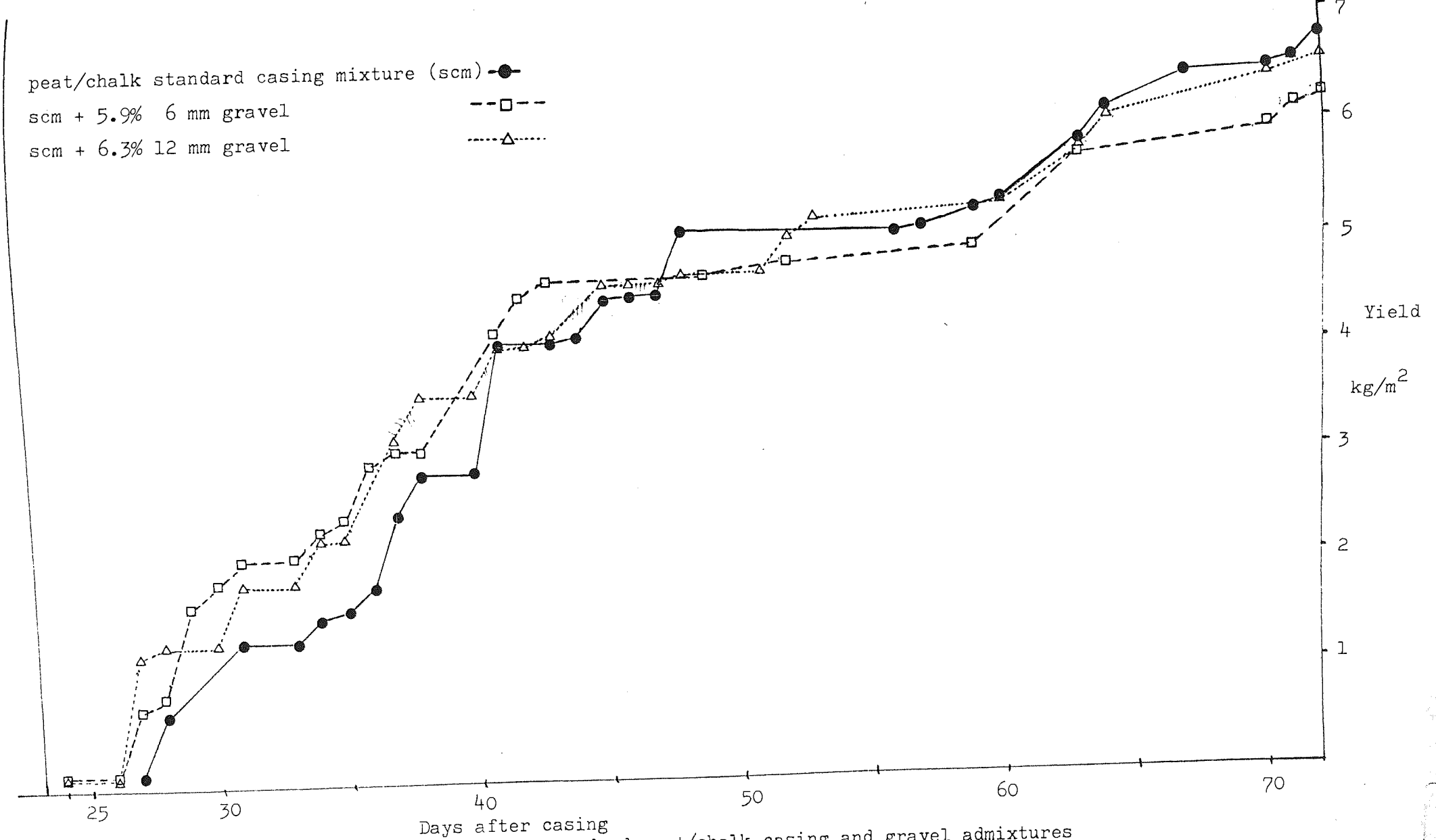


Figure 5 Cumulative production pattern of standard peat/chalk casing and gravel admixtures

Table 5 Physical characteristics of casing mixtures amended by the addition of gravel (2)

Constituent ratios (%w/w)	Standard peat/ chalk casing	Peat casing + high gravel	Peat casing + low gravel
	Peat 27.4% Chalk 17.8% Water 54.8% } SCM	SCM 20% 6 mm gravel 80%	SCM 80% 6 mm gravel 20%
Gravel admixture rate by weight	---	80%	20%
Amount of casing applied per box (to 38 mm depth)	4 kg.	10 kg.	5 kg.
Equivalent amount of standard peat/chalk casing per box	4 kg.	2 kg.	4 kg.
Bulk density	0.45g/cm^3	1.12g/cm^3	0.56g/cm^3
Water content as applied on dry weight	290.6%	29.2%	144.9%
Water content as applied on wet weight	74.4%	22.6%	59.2%
Pore space total	85.8%	64.57%	86.30%
Maximum water holding capacity on dry weight	606.1%	72.2%	354.0%

Table 6 The effect of addition of gravel to peat-based casing on mushroom production (2)

Casing medium	First flush		Second flush		Third flush		Overall		
	Mean wt. yield kg/m^2	Nos. of mushrooms m^2	Mean wt. yield kg/m^2	Nos. of mushrooms m^2	Mean wt. yield kg/m^2	Nos. of mushrooms $m/^2$	Weight yield (+ standard error)	Nos.	Mean weight of individual fruitbodies
1) Standard peat/chalk casing	2.76	274	2.70	228	3.20	88	8.65 (0.85)	590	14.66g.
2) Peat casing+ 20%w/w 6 mm gravel	3.34	385	2.98	306	3.46	133	9.78 (0.42)	824	11.87g.
3) Peat casing+ 80%w/w 6 mm gravel	3.52	361	2.76	310	2.33	85	8.61 (0.29)	756	11.39g.

Least significant difference between overall weight yields @ 5% probability level 2.17 kg/m^2

@10% probability level 1.76 kg/m^2

4 boxes 46 cm. square per treatment

14kg. compost per box

Spawn strain: Darlington 621. Spawn run period 16 days

Influences on production: 1) Mushroom fly infestation

2) Unsatisfactory positioning of boxes (lower yields in bottom boxes)

3) Open cellar experiment

in the first two flushes, although they differed in ranking, but in the third flush the control exceeded the casing containing 80% gravel, and, taken overall, its yield was marginally better. The lower 20% amendment rate gave the best total yield, and was more consistent at each flush. Again, the results could not be taken to be statistically significant, however.

Nevertheless, it is noteworthy that in each case greater numbers of mushrooms were produced on gravel-amended casings, so that the mean size of individual mushrooms was reduced. This suggests that fruiting was stimulated by the addition of gravel to peat-chalk casings, but this effect tended to be lost in later flushes.

(ii) Biologically active materials and inert simulations

The technique of "spawned casing" - the addition to the casing of a small proportion of compost colonised by mushroom mycelium - may be described as the addition of biologically active material. The compost furnishes not only an inoculum of mushroom mycelium, causing more even and rapid colonisation of casing, but it also contains a diverse bacterial population and provides nutrition for both mycelium and bacteria. The straws in the compost may affect certain physical characteristics of the casing, such as aeration and drying rate, which Nair and Hayes (1974) showed could be simulated by the addition of glass fibre strands which can only be considered to be biologically inert.

Accordingly, an experiment was set up in which standard peat-chalk casing was compared with casings to which had been added 5% by weight of either spawn-run compost, or two forms of glass fibre - strands or staples. Strands were 5 cm. lengths of woven glass fibre sleeving, 4 mm. in diameter, which resembled straws in size and structure, and staples were short (8 mm.) lengths of simple extruded glass fibre, as used in glass fibre laminations.

Table 7 compares the physical characteristics of spawned casing at

Table 7 Physical characteristics of "spawned casing" mixture

	Control standard peat/chalk casing	"Spawned casing"
Constituent ratio % w/w	Peat:Chalk:Water 27:18:55%	SCM: "spawn-run compost" 95:5%
	SCM	
Amount of casing applied per box	4.0kg.	4.2kg.
Bulk density	0.45g/cm^3	0.43g/cm^3
Water content as applied on dry weight	290.6%	262.2%
on wet weight	74.4%	72.4%
Pore space (total)	85.8%	87.9%
Maximum water holding capacity on dry weight	606.1%	600.2%
on wet weight	155.2%	165.7%
% of maximum water holding capacity as applied	47.95%	43.7%

makeup with standard peat-chalk casing mixture, from which it can be seen that the addition of compost to casing had only minor effects but it did increase the total pore space by 2%.

The yield results presented in Table 8 show that spawned casing gave a slightly lower first flush than the control, but somewhat higher second and third flushes. The glass fibre amended casings both gave higher yields than the control, especially in the case of the staples. Taken overall, the strand-amended casing performed better than the others. None of these differences can be regarded as statistically significant, however.

A mushroom fly infestation which occurred during this experiment seriously affected all the replicates of the spawned casing treatment, the sciarid larvae consuming a large number of primordia. This treatment also became colonised with mycelium well before the others and may have suffered from the delay before fruiting was initiated. Undoubtedly, without these restraints the spawned casing would have performed better.

Primordium formation on the spawned casing was the most superficial, and most evenly spread across the whole area, and mushrooms developed mostly individually. A similar effect was obtained with glass fibre strand-amended casing, and in many cases mushrooms were found to have formed very close to the strands and even to have incorporated them into the bases of the stalks.

Table 9 shows the number of days after casing taken for the first primordia to appear, and the timing of flushes, expressed in terms of mid-flush dates. It had previously been noted that the beginning and end of a flush could not be located with accuracy because in some cases one or two mushrooms matured more quickly, giving a false impression of timing. Hence the "mid flush date", being the date on which half of the total weight of mushrooms for a particular flush were picked, was used for comparisons.

First primordia were seen on the spawned casing about 3 days before

Table 8 The effect of "spawned casing" and glass fibre-based simulations on mushroom production

Casing medium	First Flush		Second Flush		Third Flush				
	Weight yield kg/m^2	Numbers per m^2	Weight yield	Numbers	Weight yield	Numbers	Weight yield (+ standard error)	Numbers	Mean weight of individual fruitbodies
1) Standard peat/chalk casing (S.C.M.)	2.76	274	2.70	228	3.20	88	8.65 (0.85)	591	14.6g.
2) "Spawned casing" (S.C.M. + 5% spawn-run compost)	2.50	201	2.82	208	3.41	85	8.74 (0.55)	494	17.7g.
3) S.C.M. + 5% glass fibre strands	2.96	264	3.81	132	2.51	71	9.28 (1.49)	467	19.9g.
4) S.C.M. + 5% glass fibre staples	3.12	285	2.91	102	0.64	20	6.67 (1.79)	407	16.4g.

No significant difference between overall yields at the 10% probability level.

Results expressed as mean of 4 replicate boxes per treatment.

Table 9 Timing of production of mushrooms on "spawned casing" and glass fibre-based simulations compared with standard peat/chalk and gravel amended casings

Casing medium	Days after casing			
	First primordia seen	Mid First Flush	Mid Second Flush	Mid Third Flush
1) Standard peat/chalk casing (S.C.M.)	20.7	28.3	41.0	48.5
2) "Spawned casing"	17.7	27.0	39.3	47.5
3) S.C.M.+ glass fibre strands	18.0	28.0	40.3	48.0
4) S.C.M.+ glass fibre staples	21.7	31.0	45.7	52.0

the control, but during cropping this lead was reduced to 1-2 days, and the strand-amended casing gave an intermediate result. By comparison, primordium formation and especially production on staple-amended casing were delayed.

Spawned casing had the following effects, compared with the control standard peat-chalk casing:

1) earlier fruiting, leading to slightly earlier harvesting.
2) slightly increased yields, due presumably to longer cropping period.
3) depression of fruiting, causing increased mean weight of individual fruitbodies.
4) more even distribution of fruitbodies, and reduced clumping.

Glass fibre strand-amended casing gave results comparable in all respects, but staples were greatly inferior.

(iii) Casing disturbance

Raking or scuffling of the casing layer when fully or partly colonised by mycelium might be considered in relation to "spawned casing". It shares with that technique the purpose of spreading the mycelium evenly throughout the casing, hence leading to more even production of mushrooms, but additionally it allows the breaking up of panning or overlay, and evens up the depth of the casing.

In one experiment, one half of each box cased with standard peat-chalk casing was subjected to a disturbance, nine days after casing. The entire surface of this part of the tray was dug over, using a 2 cm. wide spatula, so that the even surface of the casing was reduced to a series of aggregates of this approximate diameter, an action which partially inverted the casing layer. Mycelial penetration into the casing layer at this stage was quite advanced, and served to maintain this structure to some extent, against the action of watering.

Prior to this treatment the surface of the casing had become somewhat panned as a result of watering, and heavy condensation from the cabinet roof, so that there was a considerable contrast in appearance between the disturbed and undisturbed sections of the casing.

Ventilation for initial fruiting was applied one day after this treatment, by which time mycelial re-anastomosis was visible on the disturbed casings.

The effect of this treatment on primordium formation, weight yields and numbers of mushrooms developing on the 2 sections of each of the boxes is shown in Table 10.

A comparison between the top two lines of this table demonstrates that productivity of the sections of casing disturbed immediately before initiation of fruiting was higher than those sections left undisturbed. This is shown by the number of primordia visible before the first and second flushes, also the number of these maturing, and the total weight of mushrooms picked. There was no second flush production at all on the undisturbed casing.

Table 10 also includes results from a box theoretically identical to the above, but having been used as a source of casing samples for other investigations, the distinction became less marked in the second flush.

In a subsequent experiment, a single box was subjected to a similar process of disturbance in 3 zones, at 6, 9 and 12 days after casing. Ventilation wasagain commenced at 10 days.

The productivities of these 2 zones are expressed in Table 11, compared with a whole tray left undisturbed.

In this case, the casing section disturbed 12 days after casing, 2 days after the start of ventilation, initially produced over double the weight yield of the other 2 disturbed sections, and the undisturbed control. Cropping on the section disturbed 6 days after casing then increased markedly, whereas the other disturbed sections both dropped

Table 10 The effect of casing disturbance on yield patterns (1)

	Casing treatment	First flush			Second flush			Overall		
		Number of primordia	Yield Number	Weight	Number of primordia	Yield Number	Weight	Yield Number	Weight	Mean weight of individual fruitbodies
Box 1	Disturbed	198	31	581	130	50	728	81	1309	16.2
	Undisturbed	19	2	46	9	--	---	2	46	23.0
Box 2	Disturbed	215	52	781	57	36	469	88	1250	14.2
	Undisturbed but sampling caused disturbance	117	30	499	47	32	338.6	62	837.6	13.5

Number of primordia expressed as number visible on day of first picking of each flush.

Yields expressed as g. and numbers of fruitbodies per section of box 23 cm. x 46 cm.

Table 11 The effect of casing disturbance on yield pattern (2)

	Section	Time of disturbance (days after casing)	Cumulative weight and number yield at the following days after casing							
			30		40		50		60	
			Weight	Number	Weight	Number	Weight	Number	Weight	Number
Box 1	Front	6	172.1	10	956.6	69	1440.4	120	1788	139
	Middle	9	170.5	10	609.9	40	1054.6	70	1206.1	76
	Rear	12	367.3	19	677.5	37	1085.2	71	1250.5	82
	Total		709.9	39	2244	146	3880.2	261	4244.6	297
Box 2	Total	Not disturbed	523.7	20	1969.5	64	3365.4	130	4362	185

Yields expressed as g. and numbers of fruitbodies per section 15 cm. x 46 cm. or per whole box 46 cm. x 46 cm.

below par with the control as the experiment progressed.

Numbers of mushrooms developing on the disturbance treatments represented increases over the control in all cases. Any interpretation of this sort of treatment applied to different sections of casing of an individual box is open to criticism, since each experimental section of bed does not operate as an independent unit, and might have an influence on the production of an adjacent section, which it clearly would not have if the casing treatment had been applied to whole casings of separate boxes.

Conceivably, the practice used might have the effect of magnifying such differences as are likely to occur in such a situation, if the more productive sections of the casing could draw upon the nutritional reserves in the compost below themselves, and also below other sections. Accordingly it would appear that despite its poor initial showing, the section disturbed at 6 days after casing managed to gain cropping superiority after the first flush by monopolising the supply of nutrition for the second flush, possibly as a result of reaching a pickable stage for this flush approximately one day before the other two treatments.

This result is somewhat anomalous, as it represents an example of a casing treatment which yielded poorly in its first flush, but which went on to outcrop others.

From the relative numbers of mushrooms forming on the disturbed and undisturbed sections, it can be seen that the practice of disturbing the casing results in a stimulation of fruiting which is important if the surface structure of the casing has deteriorated.

(iv) Action of particle size of casing constituents

The addition of various substances to standard peat-chalk casings and their effects on physical structure were dealt with in sections

(i) and (ii). The effects of different physical formulations of the main constituents of casing were also tested. This is especially relevant considering the different types, grades and proportions of materials used in casing media, as described in the literature survey.

a) Chalk

Three different types of chalk were used, of differing particle size.

The control treatment was the chalk used previously, and also used by many Sussex growers including W. Darlington & Sons Ltd.: Duncton chalk, screened to include all particles below 6 mm. -"$\frac{1}{4}$ inch to dust" grade.

A much finer product was Morden R, a whiting produced by heat treatment and classification by centrifugation into a well-defined particle size range. This substance resembled flour in texture, having a mean particle size of 3.5 µm.

For an example of a coarser chalk, Mere lump chalk was used. This originated from a quarry in Wiltshire, and the size of particles was 12-6 mm., that is to say $\frac{1}{2}$ to $\frac{1}{4}$ inch, with virtually no "dust" fraction.

In view of the differing surface areas of the particles, and the various rates of use of these substances customary with different mushroom growers, these chalks were tested at two rates: equal parts by volume and by weight of peat.

Each of the three types of chalk was mixed with peat, at the two different levels, and the effectiveness of the resulting casings compared. The resulting cumulative yield patterns of weight, and numbers of mushrooms produced are given in Figures 6 and 7 respectively.

Mean yields, together with standard errors, are presented in Table 12 along with indications of the significance of their differences from the control Duncton chalk (equal volume) casing.

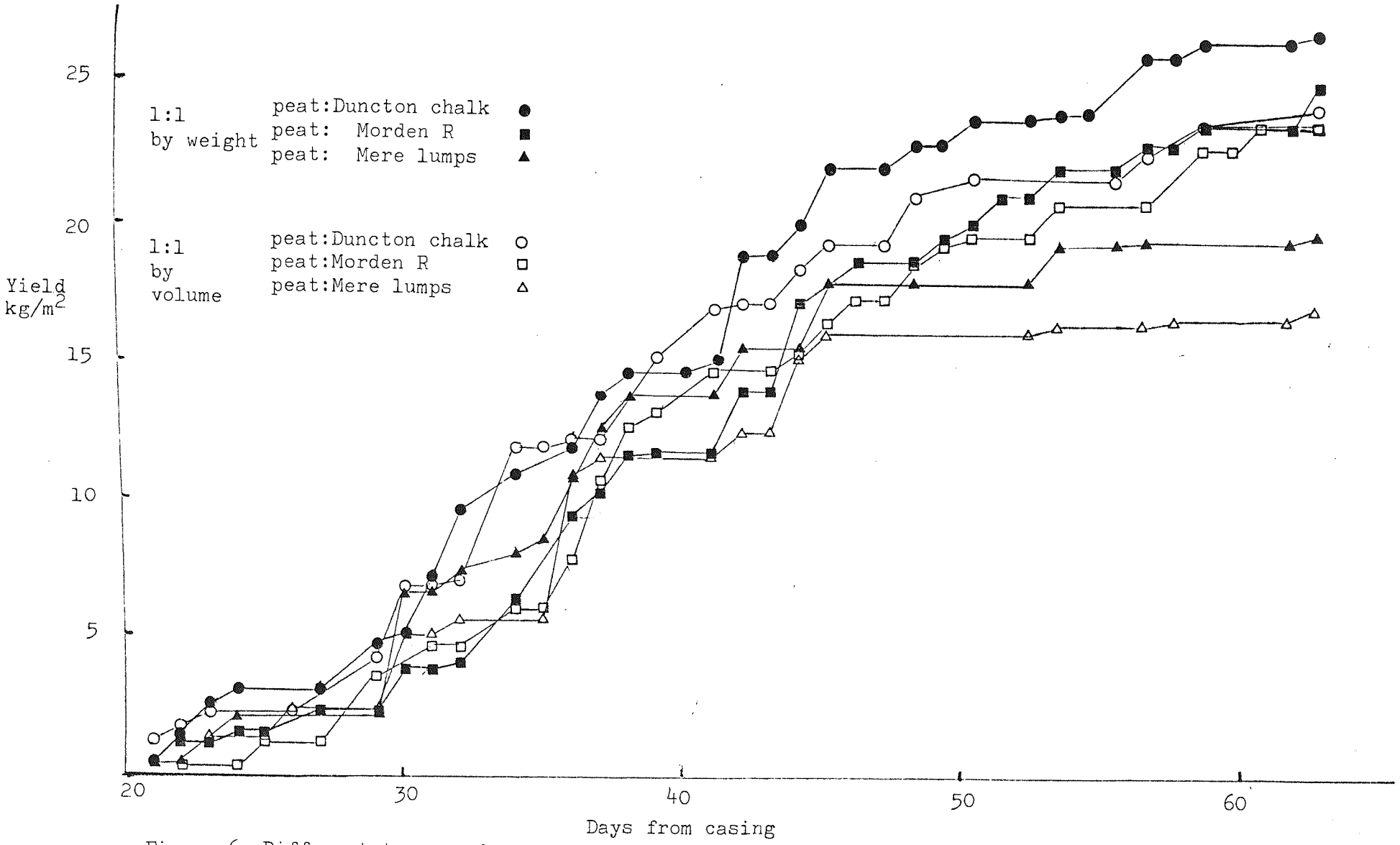


Figure 6 Different types and proportions of chalk in peat casings - effect on weight yield

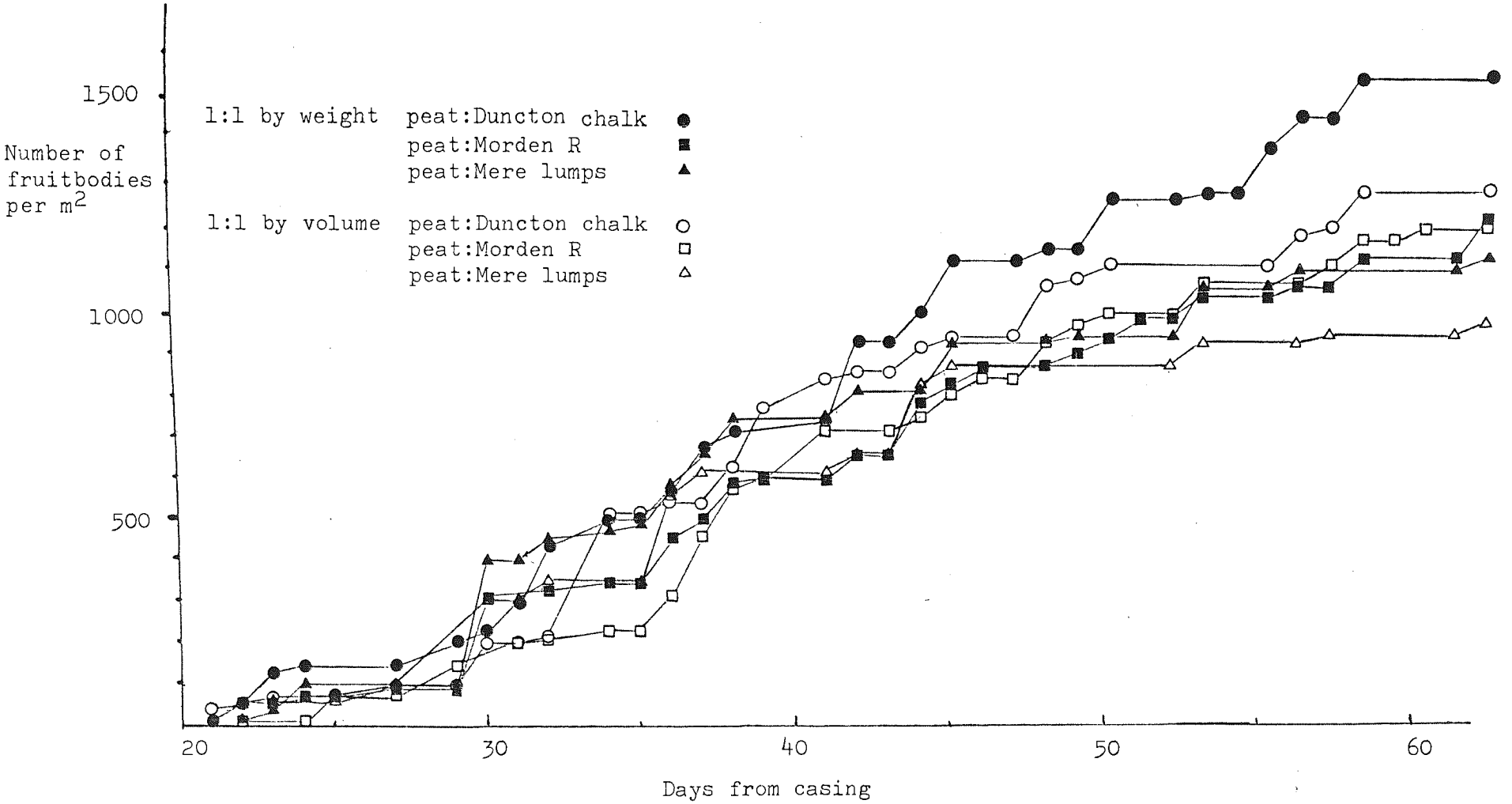


Figure 7 Different types and proportions of chalk in peat casings - effect on number yield

Table 12 The effect on yields of different types - and proportion of chalk in casing

Casing treatment		Mean cumulative yields - weights in kg/m^2 (+standard errors) and numbers of mushrooms per m^2, at the following intervals after casing								
		to 30 days		to 40 days		to 50 days		to 60 days		Mean weight of of individual fruitbodies
		Weight	Nos.	Weight	Nos.	Weight	Nos.	Weight	Nos.	
Equal proportions of peat and chalk by volume	Duncton	5.22 (1.96)	229	14.72 (1.79)	718	23.69 (0.77)	1277	26.38 (0.64)	1550	17.0g.
	Morden R.	5.84 (1.64)	316	11.83 (2.35)	603	20.13 (1.70)	947	24.37* (0.56)	1134	20.7g.
	Mere lumps	6.62 (0.67)	402	13.85 (1.48)	746	18.03 (1.61)	947	19.52 (2.07)	1105	17.7g.
Equal proportions of peat and chalk by weight	Duncton	6.86 (1.07)	201	15.33 (0.76)	775	21.81 (0.28)	1119	23.95* (0.02)	1272	18.8g.
	Morden R.	4.18 (1.20)	187	13.38 (1.27)	603	19.67 (1.65)	1005	23.40 (1.21)	1206	19.5g.
	Mere lumps	5.09 (1.41)	301	11.68 (0.94)	617	16.25* (1.51)	875	16.67** (1.51)	947	17.6g.

3 replicate boxes per treatment, each 0.25 ft^2 = 0.0232 m^2 in area.
2 kg. compost per box = 86.1 kg/m^2.
Spawn strain : Darlington 621.

* t test shows significant difference from Duncton (equal volumes) at 10% probability level.

** t test shows significant difference from Duncton (equal volumes) at 5% probability level.

For statistical treatment, yields were assessed in terms of cumulative totals at 30, 40, 50 and 60 days after casing.

The significance of the results is affected by variation in results from individual boxes. This variation would be even more marked if expressed in terms of increment in yield over the respective 10 day periods.

Although all of the mixtures initially yielded roughly the same amount, by the end of the experiment differences could be clearly discerned. Duncton chalk outyielded the fine chalk Morden R which was in turn superior to Mere lump chalk. In each case, equal parts by volume of peat and chalk were preferable to equal parts by weight.

In terms of numbers of mushrooms produced, a roughly similar, if slightly less distinct, picture was obtained.

The proportions of peat to chalk used, indicated in Table 13, were in each case much lower than those used in the preceeding experiments.

Previously casings consisted of peat and chalk in the approximate proportions 1.5:1 by air-dry weight, whereas in this experiment, the casings of equal proportions by volume worked out to approximately 0.15:1 by weight, and for"equal weight" casings this ratio was 1:1.

From the proportions of ingredients and the quantity of mixture used to case each experimental box, can be derived the weights of peat and chalk of the variuos types applied per box. The two rates of addition resulted in marked differences in amounts of peat and of chalk applied per box in each case.

It was also interesting that the initial pH of all six mixtures was the same, especially since the peat mixed with an equal weight of lump chalk seemed visually to be virtually unaffected by this addition, lacking any fine particles. The fall in pH during the course of the experiment was greater in the case of "equal volume" than the "equal weight" mixtures.

Table 13 Casing mixtures used in experiments on chalks

Casing treatment: Mixture proportions	Chalk type	Constituent ratio % on air dry materials: peat	chalk	water	Average amount applied per box	pH of mixture: initial	final
1:1 by volume	Duncton	10.1	67.4	22.5		8.0	7.4
	Morden R	10.8	60.2	29.0	1000g. mixture (104g. peat 638g. chalk)	8.0	7.45
	Mere lumps	10.4	63.8	25.8		8.0	7.3
1:1 by weight	Duncton	32.1	32.1	35.8		8.0	7.7
	Morden R	32.6	32.6	34.8	500g. (164g. peat 164g. chalk)	8.0	7.6
	Mere lumps	32.6	32.6	34.8		8.0	7.6

The type of chalk used in peat-based casings thus had a major influence on their productivity. Duncton chalk, with particle size ranging from 6 mm. downwards, was the most productive, and Morden R, which was almost as good, contained particles of the order of 3.5 µm. The worst, Mere lump chalk, did not contain a fine component, 6 mm. being its smallest particle size.

From these results, it would appear that chalk used in casing should contain a balanced spectrum of particle sizes, with a majority of fine particles but some larger particles ranging up to 6 mm.

Casing containing greater quantities of chalk (over 6 times the weight of peat) were slightly more productive than casings based on equal weights of peat and chalk.

b) Peat

Peats are not supplied in a wide range of physical formulations, as chalks are, and the different grades provided by some manufacturers are merely milled to different extents before being compressed into bales. The structure of the peat may to some extent be influenced by the treatment given to it by the mushroom grower in breaking down the bales and mixing the casing constituents together.

An experiment was thus carried out to test the effect of varying the particle size of the peat, whilst keeping constant the size of the chalk particles.

Peat from a single representative bale was crumbled up in the usual manner, and then subjected to a sieving process. The various fractions of peat thus obtained were then used as casings, after the addition of chalk on an equal weight basis, and water. For this experiment it was thought best to use the fine Morden R chalk, which would be expected to have a minimal effect on the physical characteristics of the peat particles. At application each casing was brought practically to its maximum moisture holding capacity, by saturating it with water and allowing it to drain for 24 hours.

Table 14 shows the water holding capacities of the individual fractions of peat, together with an estimate of their relative proportions in peat broken up from the bale for use directly in casing. The subjectively acceptable percentage of particles greater than 8 mm. seems to be fairly high. It was also observed that the chalk mixed more easily with the finer peat, and that with the larger peat particles it tended to separate out, due to the reduced surface area of the particles. The larger particles, being less compressible, tended to take up water less easily, so that chalk was more easily wasted off during watering.

The result presented in Table 15 shows that the different grades of peat had a marked effect on cropping. The finer grades gave the highest yields, both in terms of weight and numbers of mushrooms produced. However, these were not in direct proportion to one another, so that the mean weight of individual mushrooms produced was more or less proportional to the range of particle size of the casings on which they were produced.

In addition, it was noted that mushrooms forming on the finest peat casings were of various size, and on spindly stalks, reminiscent of the form of mushrooms grown with inadequate ventilation. However, they were in fact grown in close proximity to apparently normal mushrooms on the other casings. Evidently, the fine particle size of this casing was responsible for this effect, by hampering gaseous exchange and promoting a microclimate within the casing, characteristic of a stagnant air - i.e. with a high CO_2 level.

When the peat was separated into distinct classes of particle size, each component was seen to have a different effect on both the quantity and the quality of the resulting crops.

Table 14 Particle size distribution and physical characteristics of peat fractions

Particle size range (mm.)	Percentage in air dry peat	Bulk density of dry peat (g/cm^3)	Water holding capacity of 1:1 dry peat chalk mixture (ml. water per g. mixture)	
Above 8	25.7%			
8 - 4	17.5%	0.18	0.67	Peat and chalk separate easily in dry mix
4 - 2	16.7%	0.15	0.94	Separation in wet mixture - easily compressed
2 - 1	10.5%	0.14	1.58	
Below 1	29.6%	0.17	1.92	

Table 15 The effect of particle size range in peat casings on yield and size of mushrooms

Peat fraction	Mean yield - first flush only		Mean fresh weight of individual fruitbodies
	Weight kg/m^2 (+standard error)	Number/m^2	
Below 1 mm.	7.85 (0.97)	875	9.0g.
2 - 1 mm.	5.85 (0.65)	344	17.0g.
4 - 2 mm.	3.62 (0.43)	187	19.4g.
8 - 4 mm.	4.61 (1.07)	186	24.8g.

Least significant difference between weight yields
@ 5% probability level 1.76 kg/m^2
@ 1% probability level 2.66 kg/m^2

3 boxes per treatment.

Surface area 0.25 sq.ft. = 0.0232 sq. m.

Spawn strain - Somycel 53

E. "Synthetic" casings

Although peat represents a fairly satisfactory basis for a casing medium, it was decided to make preliminary trials on other materials, for the following reasons:

1) The high price of peat.

2) Peat deposits will eventually become exhausted (it was forecast in 1973 that Irish peat would last for at least 15-20 years). This is emphasized by the occasional unavailability of peat, due to supply difficulties.

3) Peat can cause dirty mushrooms.

4) If allowed to dry out it is very difficult to rewet without the risk of seepage of water into the compost below.

5) The undefined physical, biological and chemical constitution of peat makes investigation of its function difficult. Its particle size and shape is extremely variable, and its cation exchange capacity complicates the ionic reactions.

6) In order to learn something of the individual steps in normal mushroom fruiting, it was decided to start with a medium likely to be deficient, and upgrade it so as to observe the effect of individual amendments on mushroom fruiting. A short list of possible alternative casing materials was prepared but most were rejected on the grounds of not being easily available, of problems in storage or of not being adequately original.

Perlite was selected as a possible substance. This material, also known commercially as "Conlite", is produced by a high temperature expansion process, similar to that used in the exfoliation of vermiculite. It is a type of volcanic glass containing trapped air bubbles, so that it is very light. It is widely used in the building industry as an insulant component of plasters, and also agriculturally as a soil texture improver.

Its pH is approximately neutral and it is substantially inert, containing no organic matter. It is white in colour and somewhat gritty in texture.

(i) Preliminary experiments with perlite

An initial experiment was carried out to compare perlite with peat chalk casing, the perlite being used alone, or mixed with peat, or in a layer above or below peat. The results (Table 16) demonstrate that the perlite could function as a casing medium, but that in conjunction with peat based casings its effectiveness was greatly increased. Indeed only one of the trays cased with perlite alone came into crop, and its average yield was comparable with about 70% of that obtained from each of the peat-cased trays. However, the number of mushrooms formed on it was low, so that their mean individual weight was high, 33.7g, as against 23.99 for peat. This was taken to signify that perlite represented a deficent medium for the fruiting process, so that initiation and productivity were reduced.

The mixture of peat and perlite was the most promising both from the point of view of productivity and also because of the most uniform spacing of the mushrooms, possibly due to its fine open texture. It also tended to continue production of mushrooms after the second flush as did the perlite on top of peat. Table 16 also shows the timing of appearance of first primordia, and of the middle of the first flush picking on the various casings. It will be seen that both primordium formation and development were delayed on perlite casing, and peat layered on top of perlite. This may be due to lower speed of mycelial colonisation of perlite.

A further trial on the feasibility of perlite as a casing medium was included in another experiment in which chalk was added to perlite, in order to ensure that calcium was not lacking, and also to provide a pH buffering effect.

Table 16 Yields from Standard peat/chalk casing and Perlite, as single applications, layers and mixture

Casing medium	Days after casing		First flush		Second flush		Mean overall yield	
	First primordia seen	Middle first flush	Weight yield	Numbers of fruitbodies	Weight	Numbers	Weight	Numbers
1) Standard peat/ chalk casing	19	28.3	630 (37)	44.8	1077 (420)	26.2	1707 (392)	71.3
2) Peat/Perlite mixture (equal volumes)	19.3	28.3	807 (150)	54,3	1605 (237)	39.6	2412 (295)	93.9
3) Peat layered on top of Perlite (equal volumes)	23	28.7	723 (84)	43.6	1321 (297)	27.7	1978 (362)	71.3
4) Perlite layered on top of S.C.M. (equal volumes)	20.3	27.3	641 (118)	36.8	752 (254)	30.5	1392 (160)	67.4
5) Perlite alone*	25	33	180 (147)	9.1	220 (180)	2.8	400 (327)	11.9

Yields in g/m^2 (+standard error) and numbers of fruitbodies per m^2.

3 replicate polypropylene trays per treatment. 8kg. compost per tray. Spawn strain : 621.

* 1 tray only produced fruitbodies, hence multiply x 3 for yield on this tray.

In addition to 2 boxes of this medium at the normal temperature regime, one box was subjected to a different temperature regime, being held at 24°C after the commencement of ventilation.

No mushrooms at all were formed on the perlite/chalk casing held a "normal" temperature, whereas the same casing did produce a few mushrooms at the higher temperature, although this was much delayed with respect to the control standard peat/chalk casing mixture. The first mushroom on perlite/chalk casing was picked 29 days after casing, and by the termination of this experiment, 41 days after casing, 12 mushrooms had been picked, amounting to 368.7g. per box. A further 20 primordia remained undeveloped.

By comparison, a single box with standard casing under the same conditions had produced in 2 flushes, 32 fruitbodies, amounting to 747.5g., and 688 primordia had been counted.

The yield from the standard peat casing maintained at higher temperatures represented only 43% of the mean yield on the same casing at lower temperatures, but 73% more pins were formed at higher temperatures. The addition of chalk to perlite did not therefore significantly improve its effectiveness as a casing.

Perlite thus represented a substance which could function as a casing medium, but under normal growing conditions its productivity was poor because it was unfavourable to fructification. This could be partly overcome by stimulating fructification by higher temperatures.

(ii) Addition of clay minerals to synthetic casing

The next experiment was designed to check the possibility that perlite was deficient in cation exchange capacity.

Perlite was mixed with two different types of clay mineral and the effectiveness of the resulting mixtures as casings compared with peat/chalk casing. The two types of clay were a montmorillonite clay

(fuller's earth) and a kaolinite (china clay). The former was chosen for its higher cation exchange capacity, and was tested at three different levels of admixture with perlite:2:1, 1:1 and 1:2 by weight. The latter type of clay had a lower cation exchange capacity, and was included as a control, hopefully to mimic the physical effects of the other clay in binding particles together, altering pore space and holding water, and was added at only two levels: 2:1 and 1:2 by weight. The cation exchange capacities of these constituents and mixtures are set out in Tables 17 and 18. These mixtures thus represented a wide range of cation exchange capacity.

The cropping results of this experiment are presented in Table 19. The usefulness of the technique of presenting yields at different periods, here cumulative yields at 10 day intervals after casing, is clearly illustrated. The weight yield production patterns of the individual casings are portrayed graphically in Figure 8.

Thus it may readily be seen that by the addition of montmorillonite to perlite, greater productivity was achieved. Kaolinite, on the other hand performed only poorly. The peat casing maintained its superiority throughout the cropping period, but the perlite/montmorillonite casing tended to compensate during the later phase of production, so that the best of these casings eventually achieved 85% of the production on the standard peat control casing at the termination of the experiment.

The various levels of addition of each of the 2 clay minerals appeared to have little effect on the cropping pattern. Indeed the differences between the productivities of the different casings could not be related to differences in cation exchange capacity.

The ameliorative properties of montmorillonite, when used in conjunction with perlite, were checked in a further experiment which also included an investigation of three different physical formulations of perlite.

Table 17 Cation exchange capacities of casing constituents

Substance	Cation exchange capacity in oven-dry state	Percentage moisture in "air dry" state	Effective cation exchange capacity in air dry state (approximate)
Peat	125	40	75
Duncton Chalk	3	15	2.5
Perlite	5.5	0.1	5.5
Montmorillonite	115	15	97.7
Kaolinite	10	10	9

C.E.C. expressed in milli-equivalents per 100g.

Table 18 Cation exchange capacities of casing mixtures

Casing mixture (ratios of components by air-dry weight)		Cation exchange capacity (C.E.C.) in oven-dry weight	Water content at application (on wet weight)	Effective C.E.C. (on wet weight)	Amount of casing per box	Overall C.E.C. (Meq. per box)
Perlite/	2:1	30	67%	9.9	350g.	35
Montmorillonite	1:1	43	62%	16.3	360g.	59
	1:2	56.5	60%	22.6	550g.	124
Perlite/	2:1	7	60%	2.4	355g.	9
Kaolinite	1:2	12	57%	5.2	650g.	34
Standard peat/ chalk casing	1.5:1	35	50%	17.5	700g.	122

Table 19 A comparison of cropping on Perlite casings amended with clays, and with standard Peat/chalk casing

Casing medium		Mean cumulative yields after casing									
		30 days Weight	No.	40 days Weight	No.	50 days Weight	No.	60 days Weight	No.	70 days Weight	No.
Perlite/ Montmorillonite	2:1 by weight	1.75	100	5.70 (2.0)	359	10.50 (1.53)	517	18.77 (0.14)	962	21.64a (0.18)	1192
	1:1 by weight	1.63	158	4.05 (1.22)	230	14.53 (3.05)	674	20.48 (2.56)	976	23.35a (1.90)	1091
	1:2 by weight	-	-	6.95 (3.05)	459	13.02 (3.68)	775	18.46 (2.54)	1120	21.87a (2.4)	1304
Perlite/ Kaolinite	2:1 by weight	-	-	1.04 (0.43)	29	4.55 (0.65)	158	7.15 (1.75)	258	10.74b (0.81)	431
	1:2 by weight	-	-	1.03 (0.46)	72	1.87 (0.15)	86	6.58 (0.38)	172	12.93b (0.62)	330
Peat/Chalk casing mixture		10.36	775	20.40 (0.56)	1622	23.52 (0.81)	1852	25.72 (0.68)	2024	27.50c (0.35)	2124

Results marked with the same letter (a, b or c) are not significantly different at the 5% probability level, but differ from those with other letters.

Yields in kg/m^2 (+standard error) and numbers of fruitbodies per m^2. 3 Replicate polyethylene boxes per treatment

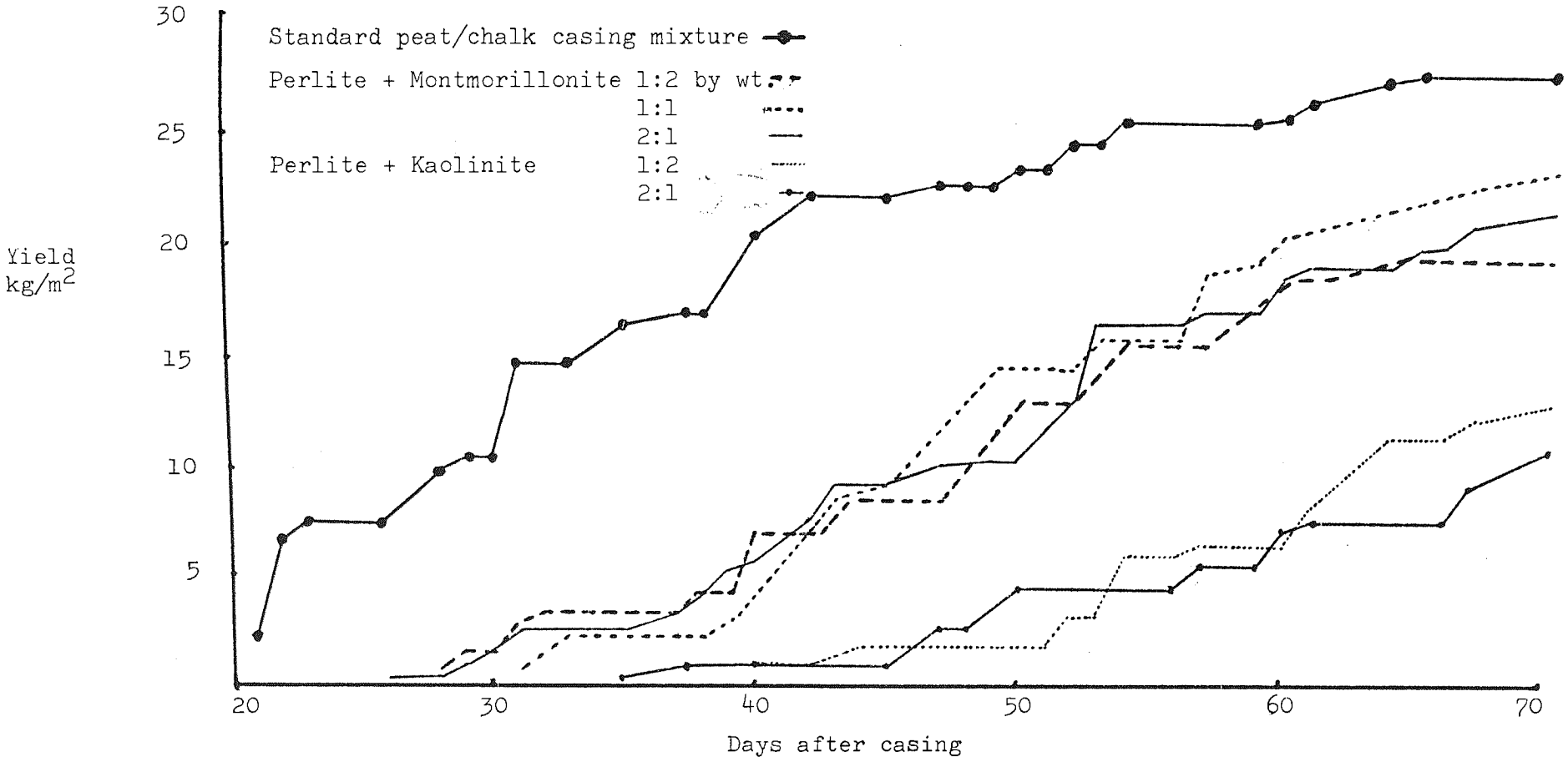


Figure 8 Cumulative weight yield curves for perlite/montmorillonite and perlite/kaolinite casings compared with standard peat/chalk casing

Here again an improvement in cropping performance was obtained by the addition of montmorillonite at a rate equivalent to the middle 1:1 ratio used previously, which was reflected not only in higher yields (Table 20) but also in earlier cropping (Figure 9). In this instance, the highest yielding montmorillonite amended treatment actually performed better than a standard peat-chalk casing in a parallel experiment carried out in a different cabinet, which could not really be regarded as a control treatment.

There was no significant difference in cropping between the grades of perlite, although initially it appeared that in each series (amended and unamended) the coarser 1/5 grade outyielded the finer 2/7 and 4/7 grades. However, at the end of the experiment, the 2/7 perlite seemed to have out-yielded the other two, and the ranking of productivity in the montmorillonite amended casings remained the same.

Analysis of physical structure (pore space, etc.) and particle size distribution could not be performed due to the light nature of perlite which caused a large proportion of it to float, and its crumbly texture which would not permit screening. The 1/5 grade seemed much coarser than the other two; these were not so distinct, but the 2/7 seemed a little coarser than the 4/7 grade. Each sample contained a substantial proportion of very fine, dusty material. The perlite used in previous experiments was the equivalent of 1/5 grade.

Although there was little difference between the three grades of perlite in each series the addition of montmorillonite to perlite clearly had a major effect on cropping in each case.

This effect was probably due to an improvement in the water-holding properties of the resulting mixtures and cation-exchange reactions, both brought about by the montmorillonite.

Table 20 The effect of adding montmorillonite to perlite casings of different grades

Casing medium	Mean cumulative yields at the following intervals after casing					
	30 days		40 days		50 days	
	Weight	Number	Weight	Number	Weight (+standard error)	Number
1) Perlite 1/5 grade	-	-	2.15	143	4.08 (1.16)	284
2) Perlite 2/7 grade	-	-	1.76	86	5.68 (1.17)	273
3) Perlite 4/7 grade	-	-	1.02	57	4.53 (0.69)	258
4) Perlite 1/5 plus montmorillonite 1:1 by weight	1.28	43	7.75	488	12.38 (0.59)	762
5) Perlite 2/7 plus montmorillonite 1:1 by weight	1.61	72	6.49	316	10.48 (1.08)	676
6) Perlite 4/7 plus montmorillonite 1:1 by weight	-	-	5.68	359	9.66 (0.67)	775

Yields in kg/m^2 and numbers of fruitbodies per m^2

3 replicates per treatment

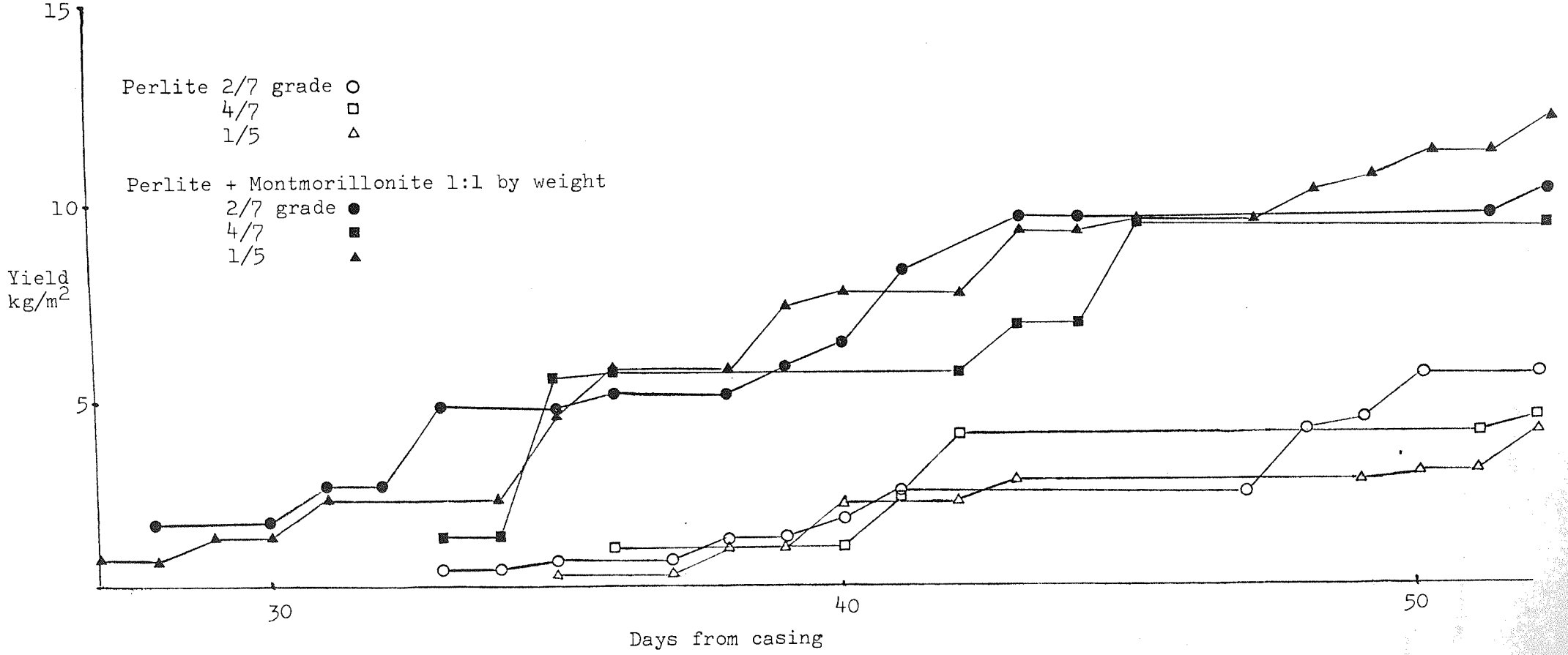


Figure 9 Production curves from casings composed of perlite of different grades with and without montmorillonite

(iii) Biological amendments of synthetic casing

It was decided to observe the effects of adding a series of carbon sources, on the premise that perlite/montmorillonite casing did not offer a satisfactory substrate for stimulatory bacteria and mushroom mycelium.

Purified alpha-cellulose powder, as used in chromatography, was incorporated into the perlite/montmorillonite casing in two experiments.

The first was a small-scale experiment in which cellulose was added at two different levels - 3% and 10% by weight.

At the lower level of addition, the casing formed a solid layer which almost "set" like concrete, forming a pan at casing, but it improved with watering. Although the higher level seemed to have a more "open" structure, it was extensively infected by the mould Stysanus sp.. Presumably the deterioration of physical structure of the perlite/montmorillonite casings due to mixing in the cellulose was responsible for the unpromising results shown in Table 21.

In the second experiment, the effect of adding cellulose, glucose and ethanol, alone and in combinations, was investigated. Cellulose was added at the level of 4% by weight, with glucose at 0.01% and ethanol at 0.1% of the water content of the mixture. The yield results are presented in Table 22.

The addition of cellulose was again inhibitory to production, and all cellulose-amended casings were overrun with the mould Chromelosporium ollare. Ethanol appeared to have no effect, but addition of glucose to perlite/montmorillonite casing without cellulose seemed to have a stimulatory effect. Again, this treatment came into cropping earlier and continued to be superior in weight of production for the entire duration of the experiment.

Further attempts were made to enhance the productive capacity of perlite/montmorillonite, by the addition of glucose at three levels, and by the administration of extracts of normal peat based casing and

Table 21 Comparison of cellulose amended Perlite-montmorillonite casing with standard peat/chalk casing

Casing medium	Yield at 50 days after casing (per small tray)	
	Weight g. mushrooms	Number of mushrooms
Perlite	57.1	4.75
Perlite + montmorillonite	93.6	6.25
Perlite + montmorillonite + 3% cellulose (by weight)	79.6	5.50
Perlite + montmorillonite + 10% cellulose (by weight)	46.4	2.75
Peat-chalk casing	112.1	19

Yield expressed as mean of 4 replicate trays

Table 22 Cropping on Perlite/montmorillonite casing amended with various organic compounds

Casing medium	Mean cumulative yield at the following intervals after casing (weights in kg/m^2, numbers per m^2)			
	30 days		40 days	
	Weight	Number	Weight	Number
1) Perlite/montmorillonite	-	-	2.14	158
2) Perlite + montmorillonite + 0.01% glucose	1.84	129	4.28	316
3) Perlite/montmorillonite + 0.1% ethanol	0.89	57	2.30	158
4) Perlite/montmorillonite + 4% cellulose	0.07	14	0.517	43
5) Perlite/montmorillonite + 4% cellulose + 0.01% glucose	0.58	57	1.05	86
6) Perlite/montmorillonite + 4% cellulose + 0.1% ethanol	0.60	43	2.26	158

Solution added at stated percentage in water added to casing at mixing, cellulose added at 4% of total wet weight of mixture. 3 replicate boxes per treatment.

compost. For this, moist peat/chalk casing, unused but kept in a sealed bag for 7 days after mixing, and unspawned compost of the same origin as the growing substrate in this experiment, were used. The extracts were made by suspending 200g. of casing or compost in 1000ml. of cold tap water leaving it for 24 hours, then filtering it through muslin. The murky liquid was then centrifuged and the clear supernatant used in place of water in the casing mixture.

The results in Table 23 indicate that the addition of glucose to perlite/montmorillonite casing did increase productivity, the middle concentration (0.01%) being the most effective, but extracts of standard peat chalk casing and compost performed better. Casing extract also gave a large increase in numbers of fruitbodies picked.

A different approach to the problem of obtaining better and earlier production from the perlite/montmorillonite casings was adopted in the next experiment.

It was attempted to duplicate the effect of spawned casing by mixing into the synthetic casing 5% of either spawn-run compost or similar materials. These included compost colonised by two different but compatible mushroom strains, and also two types of aseptically prepared mushroom spawn of the same two strains, as well as glass fibre strands of the type used previously.

Perlite spawn was prepared by the method of Lemke (1971).

The results (Table 24) indicate that cropping on perlite/ montmorillonite casings can be improved by the admixture of spawn-run compost. Lowest yields were obtained using in the casing spawned compost of the same origin as the compost used as growing medium, which underlines the poor quality of this substrate. Glass fibre strands gave the best results, as they did in Section D(ii).

Table 23 Cropping on perlite/montmorillonite casing amended with peat casing and compost extracts and glucose

Casing medium	Mean overall weight and number yields to 46 days after casing	
	Weight (kg/m^2)	Number of mushrooms (per m^2)
1) Perlite/montmorillonite control (P+M)	1.84	100
2) P + M + peat casing extract	5.23	445
3) P + M + compost extract	4.15	201
4) P + M + 0.1% glucose	2.63	129
5) P + M + 0.01% glucose	3.98	2.5
6) P + M + 0.001% glucose	3.34	157

3 replicate boxes per treatment

Table 24 The "spawned casing" technique applied to perlite/montmorillonite casings

Casing medium	Overall weight yield and number of mushrooms produced, to 47 days after casing	
	Weight (kg/m^2)	Number of mushrooms per m^2
1) Perlite/montmorillonite (P + M) - unamended control	2.02	115
2) P + M + 5% w/w 621 spawned compost - same as growing substrate	1.98	72
3) P + M + 5% w/w 649 spawned compost	4.64	287
4) P + M + 5% w/w 649 manure spawn - commercially prepared	3.33	230
5) P + M + 5% w/w 621 perlite spawn	2.99	129
6) P + M + 5% w/w glass fibre strands	4.81	359

Spawn strain used (in compost) Darlingtons 621

3 replicate boxes per treatment

The production of mushrooms on perlite/montmorillonite casings was thus hastened and stimulated by the addition of the following substances:

1) glucose solution
2) compost extract
3) normal peat/chalk casing extract (which also gave a marked stimulation of fruiting)
4) compost colonised by mushroom mycelium
5) glass fibre strands.

F. Amendments of peat/chalk casings with perlite and montmorillonite

As a link between the investigations on standard and synthetic casings, trials were made on the amendment of standard peat/chalk casing with perlite, and montmorillonite.

Peat/chalk casing was compared with an identical mixture to which had been added montmorillonite at the same amount as had been used in the perlite experiments. Also included in the comparison was a 1:1 mixture by volume of perlite and peat casing, which was mentioned in the section on synthetic casings.

The results in Table 25 show that both of these amended mixtures were superior to the control. Both cropped more heavily at the first flush and were consistently ahead of the standard peat casing. They also showed signs of continuing to be productive at the termination of the experiment, but the control casing appeared to have exhausted its potential by 60 days after casing. These results were not statistically significant, however.

In later experiments, three similar mixtures were again tested for effectiveness as casings, together with a standard casing amended with both perlite and montmorillonite. These experiments were duplicated: one set was carried out at Aston under laboratory conditions as were the experiments previously described; the second was a commercial scale

Table 25 The effect of amending standard peat casing with montmorillonite or perlite (1)

Casing medium	Mean yields (cumulative totals) at the following intervals after casing: (Weights in kg/m^2, numbers per m^2)									
	30 days		40 days		50 days		60 days		70 days	
	Weight	No.	Weight	No.	Weight	No.	Weight	No	Weight (+standard error)	No.
1) Peat/chalk standard casing mixture - S.C.M. (600g. wet casing per box)	3.44	215	9.74	861	11.34	1120	11.63	1191	11.63 (2.51)	1191
2) S.C.M. + montmorillonite, 12% by weight (600 + 70 = 670g. per box)	6.22	459	13.92	1650	15.79	1895	16.44	1981	16.57 (0.72)	2009
3) S.C.M. + perlite, 1:1 by volume, 3:2 by wet weight (300 + 200g. = 500g. per box)	6.49	531	12.14	1306	13.43	1521	13.92	1564	14.17 (0.43)	1578

No significant difference between overall yields at the 10% probability level

3 replicate polythene boxes per treatment

Spawn strain: Darlington 649

experiment performed in the Research and Development Unit of Messrs. W. Darlington and Sons Ltd. Both experiments used exactly the same casing materials based on a single batch of peat/chalk casing made up at Lucksfield Nurseries, in conjunction with a similar compost and the same spawn strain. The level of addition of montmorillonite was slightly higher than in the previous experiment.

At Aston, certain problems were encountered in the management of this experiment, resulting in "overlay" of mycelium on the casing surface, and it was decided to break up this crust in order to get water into the casings after the first flush was picked, 23 days after casing. The pattern of production was undoubtedly affected by this action, but this treatment was applied equally to all of the casing types.

In spite of this reservation, it appeared that the standard peat/chalk casing cropped better than any of the three amended casings (Table 26). This treatment was superior from the beginning of cropping onwards, and peat plus perlite gave comparable results at the start of the experiment, but fell behind towards the end. Both peat plus montmorillonite, and peat plus montmorillonite plus perlite, although initially poor, were tending towards the productivity of the control at the end of the experiment, 70 days after casing.

Perlite plus montmorillonite amended casings, and to a lesser extent montmorillonite-amended casings, gave proportionately fewer mushrooms than their corresponding weight yields, so that mean fruitbody weight was increased. In the case of peat plus perlite plus montmorillonite, although weight yield at 30 days after casing was only 75.5% of the control, numbers of mushrooms picked were only 47% of those on the control casings. This could represent an economy in picking problems and costs, which could partly compensate for the shortcomings in yield, if duplicated under commercial conditions, although it might be due to inhibition of fruiting due to the overlay

Table 26 The effect of amending standard peat casing with montmorillonite and/or perlite (2)

Casing medium	Mean yields (cumulative totals) at the following intervals after casing (weights in kg/m^2, numbers per m^2)									
	30 days		40 days		50 days		60 days		70 days	
	Weight	Number	Weight	Number	Weight	Number	Weight	Number	Weight (+standard error)	Number
1) Peat chalk standard Casing mixture - S.C.M. (600g. per box)	6.78	359	10.69	559	14.78	761	15.62	818	17.85 (0.81)	990
2) S.C.M. + perlite - 1:1 by volume (500g. per box)	6.23	272	10.84	373	12.87	445	13.88	531	15.63 (0.46)	660
3) S.C.M. + montmorillonite - 15% by weight (690g. per box)	4.73	330	8.17	402	10.16	474	14.84	789	17.04 (0.83)	990
4) S.C.M. + perlite + montmorillonite - rates as above (590g. per box)	5.12	172	7.35	215	10.98	301	14.53	761	17.19 (1.28)	990

No significant difference between overall weight yields at the 20% probability level
3 replicate polythene boxes 0.25ft^2 per treatment
Spawn strain - Darlingtons 649

and consequent disturbance of the casing.

Table 27 shows the results of the parallel commercial trial. It should be stressed that in this experiment exactly the same casing and spawn strains were used.

These figures portray a different picture from those of the laboratory experiment. Yields were better generally, particularly in the earlier stages of cropping. Here, however, standard peat casing was consistently inferior to all of the amended casings. Perlite-amended casing again was more productive from the start. At 30 days after casing production on this casing was 26% more than on the control, at 40 days it was 15% greater and by 50 days it was only 7.5% greater. Both montmorillonite-amended and perlite plus montmorillonite amended casings showed signs of improvement at the termination of the experiment. Again, no statistical significance could be attached to differences between means in this experiment.

The most positive interpretation which can be placed on these results is therefore that the addition of equal parts by volume of perlite to a normal peat-chalk mixture produced a casing medium which was at least as good as, if not better than, the usual mixture.

The scale of the laboratory experiment was such that it was possible to express yield results in terms of both weight and numbers of mushrooms produced, but in the larger scale commercial trial this was not possible. However, in the latter case, records were kept of the proportion by weight of higher grade to lower grade mushrooms picked, that is to say the ratio of open to closed mushrooms. It is notable that clay amended casing gave the highest percentage of "higher grades" presumably due to its greater water holding capacity and ability to withstand dehydration which must be related to the ability of the mushroom crop to "hold" on the beds.

Table 27 The effect of amending standard peat casing with montmorillonite and/or perlite (3) under commercial conditions

Casing medium	Mean yields (cumulative totals) at the following intervals after casing (weights only, in kg/m^2)			
	30 days	40 days	50 days	53 days (+standard error)
1) Peat chalk standard casing mixture - S.C.M. (76 kg. per tray)	10.19	13.34	15.21	15.70 (0.72)
2) S.C.M. + perlite. 1:1 by volume 62% : 38% by wetted weight (61 kg. per tray)	12.86	15.35	16.35	16.70 (0.64)
3) S.C.M. + montmorillonite 15% by weight (87.5 kg. per tray)	10.69	13.81	15.98	16.43 (1.23)
4) S.C.M. + perlite + montmorillonite, compound of rates as above (72.5 kg. per tray)	11.50	14.76	15.50	16.12 (0.09)

No significant difference between overall weight yields at the 20% probability level
3 replicate wooden trays 25ft^2 per treatment, except S.C.M. - 9 replicates
Spawn strain - Darlingtons 649

The trials at Darlington's Research and Development Unit were carried out on trays 25 square feet in area; at Aston, polythene, boxes 0.25 square feet in area were used. Hence, the difference in scale of cropping amounted to a factor of one hundred times.

Mushroom cropping experiments can be notoriously affected by variation between individual experimental units, which can reduce the reliability of the results obtained. Experiments performed on a large scale frequently cannot take account of differences between individual experimental units, by replication of a treatment, and it is sometimes assumed that the greater scale of experimentation as such will counteract this uncertainty.

Conversely, smaller scale experiments are sometimes viewed with scepticism, even although they may provide better opportunities for more comprehensive comparisons based on differences between and within treatments.

Table 28 shows the coefficients of variation within treatments, which can be taken to represent the confidence which can be attached to experimental results, for the two experiments performed on a commercial and a laboratory scale. This shows that there was no loss in precision at the smaller scale of experimentation, as used throughout this investigation.

Table 28 Comparison of variability within similar mushroom cropping experiments under laboratory and commercial conditions

Experimental location	Co-efficient of variations = $\frac{\text{standard deviation of Replicate Observations}}{\text{mean}} \times 100\%$ of the following casing treatment yields				
	S.C.M.	S.C.M. +P	S.C.M. + M	S.C.M. + M + P	Overall
Darlington's R. & D. Unit, Lucksfield Nurseries	13.8%*	6.7%	13.0%	10.0%	11.6%
Aston Laboratory for the Cultivation of Edible Fungi	7.8%	5.1%	8.5%	12.9%	10.3%

All comparisons based on 3 replicates per treatment

except * - 9 replicates

5. DISCUSSION AND CONCLUSIONS

Basic processes in fruitbody production

The studies on the formation of fruitbodies showed that the process could be considered to consist of various component phases: preliminary colonisation of casing, initiation of fruiting with the formation of hyphal aggregates developing into primordia, followed by maturation of a proportion of these primordia with the cyclical production of fruitbodies.

All the steps in the fruiting process occurred within the casing layer, with initiation taking place in the surface layers of the casing. Neither the application of the casing nor the initiation process had a direct effect on the compost itself. The formation and distribution of primordia seemed to be at random, and their maturation to become fruitbodies was also apparently a random process.

The subdivision of this process into phases is in accord with the results of Couvy (1973a) who showed that in Agaricus arvensis each phase had a different environmental requirement.

Mushroom growing is one of the most controllable cultivation processes, and the major environmental variable is the growing temperature. This was seen to have marked effects on both initiation and maturation of fruitbodies, but temperatures optimal for mycelial growth resulted in increased initiation but reduced maturation, hence limiting overall productivity. This reinforces the regime used commercially, a higher incubation temperature followed by a fall for the cropping phase accompanied by ventilation (Flegg 1968, 1970b).

After initiation and fructification, the production of fruitbodies is a continuing process taking place over a period of time. The casing layer may have an effect on factors concerned with initiation which governs the pattern of production but it also provides a substrate, providing support and supplying water for developing fruitbodies throughout the cropping period.

Experimentation with peat-based casings.

The yield experiments were carried out on a small scale, so that both numbers and weights of fruitbodies forming on each experimental unit could be recorded and analysed at different stages during the cropping period, which was useful in assessing the effects of different casing treatments. In experiments reported by other workers it is common to find results expressed in weight yields only, at the termination of the experiment.

Adding inert substances to standard peat-chalk casings generally caused initial increases in production. Greater numbers of fruitbodies were produced on gravel amended casings than the unamended control, showing that amendment had its effect by stimulating fruiting, hence causing a reduction im mean weight of individual fruitbodies. The stimulatory effect was confined to the beginning of the productive phase.

Gravel greatly altered the physical structure of the casing; however this was not reflected in such a marked increase in productivity. The 6 mm. gravel promoted extra fruiting compared with the control when added at 6 and 20% (w/w) but at 80%, this effect was overcome. Conceivably a rate between 6 and 20% would have been better.

These results confirm the findings of Bewley (1950), Gardner and Davies (1962), O'Donoghue (1963) and Hayes and Nair (1976), in which the productivity of diverse casing materials - field soils, Sphagnum and Phragmites peats - benefitted from the admixture of gravel.

Mixing compost colonised with mycelium into the casing ("spawned casing"), had very small effects on the physical structure of the casing, but it resulted in earlier production. Although this casing did not retain this lead throughout the whole cropping period, yield from later flushes did not fall behind that of the control, and this improved yield was accompanied by an increase in the mean size of individual fruitbodies. In addition, this treatment resulted in a

more even distribution of fruitbody primordia.

Addition of glass fibre strands did to some extent mimic this effect. Again, earlier production of fruitbodies was brought about, although this effect also did not endure. The increase in yield over the control was presumably not a reflection of increased fruiting since a reduced overall mean weight of individual fruitbodies was obtained.

In their initial description of the spawned casing technique, MacCanna and Flanagan (1972) did not record any effect on fruiting or on mean size of mushroom fruitbodies resulting from this practice. However Nair and Hayes (1974) observed a marked stimulation in fruiting with spawned casing and a slightly less marked effect using woven glass fibre of the type used in this investigation, although the mean fruitbody size was in each case rather small.

Flegg (1954b) obtained increased weight yields and a proportionately greater increase in number of fruitbodies when he added uncolonized milled compost to the casing layer. In later experiments Flegg (1958) obtained by the same method greater weight yields, unaccompanied by a stimulation in fruiting. This might be due to the presence of salts in the compost which Flegg (1961b) also showed to have an inhibitory effect on fruiting.

In the experiments reported here, the glass fibre strands which had a comparable effect to compost colonised by mycelium were well washed and did not contain any salts at application; however since these amendments both appeared to result in a greater evaporation of water from the casing, they could have brought about an increased accumulation of salts from the compost below by simple evaporative processes, similar to that shown by Flegg (1958), with the glass fibre strands acting as wicks for this transpiration. This explanation would account for both the partial inhibition of fruiting and the faster colonisation of casing and earlier fruiting.

The practice of raking or scuffling the casing layer was shown in this investigation to have beneficial effects on fruiting and yield. Presumably this was due to the exposure of the mycelium within the casing to the action of ventilation required for fruiting. On the disturbed sections of casing mycelium could be seen at numerous sites on the surface, whereas the surface of the undisturbed casing was uniformly dark, with mycelial penetration from below stopping only a few millimetres short of the wet top layer.

Disturbance of the casing did not have a deleterious effect on the mycelium, reanastomosis had occurred within 24 hours, resulting in equivalent or better mycelial growth than before, throughout the whole casing depth.

Even when this disturbance took place 2 days after the beginning of ventilation for initiation, the process of fructification was not adversely affected, as casing sections receiving this treatment actually performed better than other sections disturbed previously, and the control. However, the section disturbed at the earliest stage, 6 days after casing, eventually established superiority as it recovered from the effects of the disturbance.

These findings bear out the assertions of Barnard (1974) that the technique of raking the casing may be worthwhile, especially if the physical structure of the casing has deteriorated due to the peat becoming panned, by the action of water.

It is also widely practised on the Continent, especially Holland. In some cases, this procedure is taken to the extent of dragging up straws from the compost below, effectively simulating the effect of "spawned casing". It may represent less of a disease hazard than admixing compost colonised with mycelium at the normal time of casing application. However, it is rather a laborious process, especially with small culture units. The Dutch system of growing on shelves employs a simple machine consisting of a rotating bar with times to produce this effect.

In these intensive culture systems, employing mushroom strains in which the bulk of their production is obtained from the first two flushes, it is important to bring the casing to maximum water-holding capacity before the start of fruiting, but this usually requires such frequent and heavy watering that a marked deterioration occurs in the casing surface structure. In this case it is practically mandatory to carry out a raking operation at ventilation for fruiting. The result is usually a marked stimulation in fruiting which enhances the production of the early flushes, hence justifying the extra attention.

These early experiments suffered from problems of crop management, variability due to arrangement of boxes, and pest infestation. Indeed, analyses of variance showed that variations due to positioning in these experiments were more significant than those due to the treatments under test. Consequently, later experiments were designed to minimise these problems, and smaller boxes, filled at greater substrate compression, were used in conjunction with the cabinet technique. Later results were much more reliable.

The next experiments involved investigations of the effects of different physical formulations of the main casing constituents.

In the first, chalk of different particle sizes was mixed with peat and the resulting mixtures tested for effectiveness as casings. These chalk samples were from different origins, two having been merely crushed and screened, whereas the third had been subjected to a sophisticated heating and centrifugation process.

The form of chalk used in peat-based casings was shown to have a major influence on the productivity of the mixture obtained. The divergence between the cumulative production curves of Figures 6 and 7 shows the differences which were found, and the standard errors of the means presented in table 12 show that more confidence can be placed in them. The differences found were taken to represent affects of physical structure, rather than physico-chemical differences, because the pH

values of the mixtures were so similar.

The chalk providing the most productive casing, Duncton chalk, contained particles varying from 6 mm "to dust", whereas Morden R, which was almost as good, was composed of fine particles within a narrow range, and the least productive, Mere lump chalk, was mainly composed of particles from 12 to 6 mm. in diameter.

Hence it may be concluded that a casing should contain chalk with a balanced spectrum of particle size, with mainly fine particles and a few larger particles ranging up to 6 mm. in diameter.

It was also particularly noteworthy that the chalks retained their ranking in effectiveness at both levels of addition. It may seem surprising that in each case the higher rate of addition of chalk was the more successful one. Commercially it is normal to use equal proportions by weight of peat and fine ground chalk, but equal parts by volume when using lump chalk. Equal volumes of peat and chalk seemed in this experiment to be the more satisfactory, despite the concrete-like appearance of the Duncton and Morden R casing mixtures. Conceivably water retention was better on these mixtures, but records of watering, (performed according to visual assessments) did not indicate any trend between the different casing types. Similarly, this phenomenon could not be attributed to pH, since the lower chalk addition rate clearly had an adequate buffering capacity.

High proportions of fine chalks in casings are sometimes disapproved of commercially because of problems of crop management mainly the formation of a hard pan after watering, although clearly there is some economic attractiveness in this because chalk is cheaper than peat.

In the preceding experiment, the chalk types were used in conjunction with peat of an undefined particle size range. As normally used for casing, peat contains a spectrum of various particle sizes, being simply crumbled from the compressed state as it occurs

in the bale. The effect of varying the particle size of the peat whilst keeping the chalk particle size constant was therefore examined.

Peat was well shredded and the resulting particles were screened into several non-overlapping size classes, each representing a halving in particle diameter. When used as casings in conjunction with very fine chalk (Morden R, as used in the experiment discussed previously), these fractions of peat not only gave a graded yield response in terms of weights of mushrooms produced, but also in terms of their numbers, resulting in a marked effect on mean weight of individual fruitbodies.

This may seem to be at variance with the results of O'Donoghue (1962b) who found that a mixture of coarse and fine peats gave a higher yield than either grade above. However, her results were obtained using overlapping ranges, and much less defined particle sizes.

The finest peat fraction, below 1 mm. gave the highest weight yield, but fructification was stimulated so that a disproportionately higher number of fruitbodies were produced, and consequently the mean size of fruitbodies was reduced. These fruitbodies resembled mushrooms formed in conditions of reduced ventilation although they were in fact grown in close proximity to apparently normal mushrooms on other casings. This was probably due to the fine particle size of this casing which, by hampering gaseous diffusion, promoting a microclimate within the casing characteristic of stagnant air, that is to say, high in carbon dioxide. A reduction in pore space would be in accordance with the increased water-holding capacity of this fraction. Consequently this accumulation of carbon dioxide within the casing could have favoured better mycelial colonisation of the casing and even trapping of volatile substances to select for stimulatory bacteria, leading to increased productivity. The stipe elongation of the fruitbodies on this casing could also be due to the effects of self-produced CO_2 resulting from more rigorous fruiting.

The implications of these results in commercial terms are an

expression of the classical "quantity or quality" paradox. At one end of the scale, large particle size casings gave low yields but fruitbodies were individually larger, separate and slow growing - characteristics representing mushrooms of high quality; whereas at the other extreme, small particle size casings resulted in greater overall yield in terms of weight, but even greater numbers of fruitbodies. These were of lower quality, being frail and tending to break at the veil to become "opens". Hence, to obtain an approximately twofold yield increase in production on a weight basis, an increase of more than four times in numbers of fruitbodies was necessary. Since a major proportion of the costs of mushroom production is taken up with labour, mainly picking costs, the mean size of individual fruitbodies is of interest to the grower, in that it can affect the picking rate of his picking staff. However, mean size of mushroom fruitbodies is only one aspect of this question because much of the time spent in the picking operation is taken up in "grading" the product - separating into different categories by size and development stage. In the experiment described, each peat fraction was characterised by a very even distribution of fruitbody size. Conceivably, the variation in size of individual mushrooms normally obtained could be attributed to the effects of the different particle sizes in the peat used for casing.

This experiment was carried out using only one spawn strain, and it would be necessary to repeat the experiment with other spawn strains before confidence could be based on it. Nevertheless it may be that a modification of this method could find commercial application, with several advantages.

Synthetic casing mixtures

The development of a synthetic casing medium was based on th inert heat-expanded glass-like mineral, perlite.

This material has been tested as a casing by various workers. Stoller (1969) thought that mushroom fruitbodies formed on it could

be picked without trimming the base of the stalk, since the perlite particles did not contaminate them, but he found that fruitbodies tended to form deep within the casing layer. Barnard (1974) experienced difficulty in judging the amount of watering required by perlite casings, resulting in waterlogged mushrooms. This problem was to some extent overcome when perlite was applied in a layer on top of normal peat casing.

Lemke (1971) developed a type of mushroom spawn in which perlite was used as an inert particulate carrier material.

Initial experiences with perlite as a casing showed that this medium on its own represented a medium unfavourable for the fructification of Agaricus bisporus. This was shown by lower weight yields, but proportionately even fewer fruitbodies and delayed production, compared with control peat casings. Productivity under normal growing conditions was extremely poor, but this could be improved by maintaining a higher temperature in order to stimulate fruiting.

The addition of the clay mineral montmorillonite to perlite resulted in a marked improvement. The rate of admixture did not appear to cause any significant difference between the ratios 2:1 and 1:2 by weight. However the addition of another clay mineral, kaolinite, to perlite did not result in any improvement.

Since montmorillonite had over ten times the cation exchange capacity of kaolinite, this result might at first sight be taken to indicate that the addition of montmorillonite to perlite improved its performance as a casing material by increasing its cation exchange capacity. However these determinations were carried out on oven dry material, and when allowance is made for the moisture contents of the constituents and mixtures, together with the actual quantities of mixtures used in casing, such an interpretation cannot be supported.

For example, the mixtures represented a wide range in overall cation exchange capacity per box, but this was not correlated with productivity. The cation exchange capacity of peat/chalk casing was practically the same as that of the highest perlite/montmorillonite casing, and the others were in approximate proportion to their montmorillonite content, but there was no significant difference in yield between the three perlite/montmorillonite casings. Although perlite/kaolinite casings had the lowest cation exchange capacities, the higher addition rate was almost equivalent to the lowest perlite/montmorillonite casing, yet neither perlite/kaolinite casing differed significantly from the other, nor did their productivity approach that of the perlite/montmorillonite casings.

It is unlikely that this improvement was due to the effects on physical structure of the perlite due to the addition of montmorillonite, since kaolinite had a somewhat similar effect, although there was a difference in that the kaolin mixtures felt chalky to the touch whereas montmorillonite mixtures were sticky. In fact at the highest levels of addition of each clay, the mixture was quite viscid.

Montmorillonite and kaolinite also differ in their affinity for water, as a consequence of their differences in structure. Montmorillonite will retain more water than kaolinite under drying conditions, especially at higher pF values (low water contents). In fact it was noted that perlite/kaolinite casings required more watering to keep their moisture contents constant. Resistance to desiccation could thus explain the effect of montmorillonite when added to perlite in that this would protect both mycelium of A.bisporus and its associated microflora from drying out, which is a major problem with perlite.

Alternatively, and perhaps most plausibly, it may be that the montmorillonite used either contained some stimulatory substance,

possibly at its cation exchange sites, or that it removed an inhibitory substance, presumably also by cation exchange reactions. Fuller's earth is well known for these properties.

The action of montmorillonite in improving the productivity of perlite casings was checked in later experiments. The cumulative yield curves show that the three grades of perlite used above as casings did not differ to any extent, nor did the same perlite grades amended with montmorillonite differ, but there was a marked difference between the two series, amended and unamended, which confirmed the effectiveness of montmorillonite although no effect was obtained due to differences in the physical structure of the perlite.

The basis of the action of montmorillonite could not be further elucidated in this investigation. A series of geologically similar minerals and commercial derivatives were screened for effectiveness, and ion-exchange resins treated to different extracts with a variety of ions and mixtures were also incorporated into both perlite and peat-based casings. However these experiments were inconclusive, for technical reasons.

In an endeavour to make these perlite/montmorillonite casings more hospitable to colonisation both by bacteria and A.bisporus, various carbon sources were added to the casing layer. Pure alpha-cellulose powder added to the synthetic casing mixture at 10% by weight merely made this casing more hospitable to the mould Stysanus stemonitis and at the 4% level casings were overrun with the brown mould Chromelosporium ollare. Mushroom yields were reduced in each case.

Ethanol at the concentration of 0.1%, added to the water used for making up the casing mixture at application appeared to have little effect, except possibly in conjunction with cellulose, possibly by repressing growth of Chromelosporium.

However, glucose, added at 0.01% of the casing water, was shown to increase the efficiency of the perlite/montmorillonite casing

which was reflected in earlier production, greater numbers of fruitbodies and increased weight yields. A second experiment showed that this level of addition gave superior results to 0.001% or 0.1% glucose. This medium was as good a casing medium as standard peat/chalk casing.

Nevertheless, this result was bettered by extracts of compost and peat casings. Whether this effect was due to a contribution to the nutrition of Agaricus bisporus, or of the stimulatory bacteria or to the provision of an inoculum of these organisms was not certain. An analysis of microbial numbers in the different casing treatments was inconclusive, but perlite/montmorillonite alone seemed to support fewer types of organism (judged on colony type) than the other casings, or peat/chalk casings. Perlite alone seemed to be represented by an even more restricted microflora.

Production from perlite/montmorillonite casing could also be increased through mixing into it a proportion (5% by weight) of compost colonised by mycelium of Agaricus bisporus.

The best synthetic formula, 1 part perlite: 1 part montmorillonite clay: 3 parts 0.01% glucose solution by weight, thus represented a casing medium the productivity of which was comparable with normal peat-based casings and which was in no way based on the substances currently in general use. Although it would probably be uneconomical in practice, the development of this medium therefore fulfilled one of the objectives of this investigation, namely to find a substitute for peat. However, this mixture retains some of the undefined nature of peat, especially in regard to its ionic reactions and water relations.

Amendment of peat casings with perlite and montmorillonite

The two lines of investigation into the action of casing overlapped somewhat, so that both the major constituents of the synthetic casing

were tried in conjunction with the normal peat/chalk casing.

It was thought that both substances might act by stabilizing the physical structure of the casing; although superficially different in texture, each had the effect of preventing the "panning" of the casing surface which occurred with the control. The montmorillonite clay achieved this function by binding the small particles of peat into water-stable aggregates, and perlite by an opening-out or dilution of the peat particles, which was borne out by visual examination.

Indeed, the addition of montmorillonite clay to a peat/chalk casing was in most cases beneficial. However it was not a significant improvement, especially taking account of the cost of this substance.

Nevertheless, these casings did produce mushrooms of better quality under commercial conditions, probably by maintaining a more even casing moisture content. This is confirmed by the observations of mushroom growers who obtained better quality crops on clay soils than on peat-based casings.

One of the objectives of the investigation was to attempt to discover a substance which could be used as an extender in conjunction with peat. This might be necessary if peat became uneconomical or unavailable, either in the short term as a result of supply difficulties or in the long term, as deposits became exhausted.

In several experiments, the result of adding an equal volume of perlite to a standard peat-chalk casing was shown to be as good a casing material as the peat-chalk casing alone; indeed it yielded more consistently, as shown by lowered standard errors of the means compared with unamended peat-chalk casings. This might be due to its better structure which resulted in increased fruiting in the early flushes. This structure was maintained against the action of watering.

Thus, perlite can be suggested for use as an extender or diluent of peat-chalk casings added at the ratio of 1:1 by volume.

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