THE UNIVERSITY OF ASTON IN BIRMINGHAM

IRON IN THE SUBSTRATES AND SPOROPHORES OF <u>AGARICUS BISPORUS</u>

(LANGE) PILAT DURING GROWTH AND DEVELOPMENT

BY

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SUMMARY

The changes of the concentration of iron in the growth substrates and the sporophores of Agaricus bisporus (Lange) Pilat that occurred during culture under standard commercial conditions, were observed using atomic absorption spectrophotometry and iron-59 radiotracing techniques.

The routes of translocation and sites of iron accumulation within the sporophore were shown to alter during development and by the use of novel, pelletised substrates the concentration of iron in the mycelium of the substrates and in developing sporophores was observed during culture. Findings indicated that the compost was the major source of iron and that the concentration of iron in the compost mycelium varied cyclically in relation to the periodic appearance of sporophores. In the casing layer the mycelium is organised into strands which are responsible for the movement of iron from the compost into developing sporophores.

A photographic technique for estimating sporophore growth rates showed that the accumulation of iron was not concomitant with sporophore growth and this was attributable to a declining quantity of available iron in the compost mycelium during sporophore growth. Variations in the quantity of iron in sporophores resulted primarily from differences in the quantity of water soluble iron in the compost but, the productivity of the crop, the type of casing layer and differences in watering also influenced sporophore composition.

Changes in the concentration of extractable iron in the compost and casing layer throughout culture were related to mycelial activity and to a lesser extent were influenced by watering and the bacterial populations of the casing layer.

Thus, the findings of this study give some indication of the relative importance that different cultural conditions exert over sporophore composition together with demonstrations of the movement of a single material within the sporophores and substrates during the cultivation of Agaricus bisporus.

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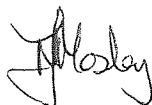
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DECLARATION

Work described in this thesis is the result of my own investigations except where reference is made to published material or where assistance is acknowledged. This work has not been submitted for any other award.

Martin J. Mosley

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INTRODUCTION

Edible mushrooms are usually considered in the U.K. for their value
as a condiment and as such, their potential nutritive value may be
of little significance. With increasing consumption and the trend
towards more vegetarian nutrition, however, it is increasingly
important to consider their value from a nutritional standpoint.

Historically, varying opinions have been expressed regarding
the true nutritive value of edible mushrooms, ranging from the
'vegetable beef steak' by some authors to of minor nutritional
significance in the estimation of others. Since few definitive
studies conducted by competent nutritionists are available, little
is known about the true nutritive value of mushrooms. Most of the
data on the nutritional contribution of mushrooms to the human diet
have been derived from empirical determinations of mushroom composition,
and that is based almost exclusively on protein content. The information
on mushroom composition is at best, difficult to evaluate in terms of
nutrition and minimal with respect to metals.

It is often impossible to compare results obtained by different
investigators working with the same species of mushroom since the
composition of a given species is affected by many variables. These
include; basic differences in strain characteristics, the complex and
variable nature of the growth substrates, the method of cultivation and
the inherent inaccuracies of analytical methods. Additionally significant
compositional changes can occur during the growth and development of the
organism. It was on this premise and on the basis that great compositional
variability exists in the published data, that a thorough evaluation of
the elemental composition of Agaricus bisporus should be undertaken.

Pure culture vegetative growth studies have been used to define the

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elemental requirement of this stage of growth. However, several investigations have indicated the different nutritional requirements of vegetative and reproductive growth. Thus, it is probably incorrect to extrapolate such pure culture nutritional requirement studies to the production of the sporophore crop. Additionally it has been suggested that inorganic and trace elements are unlikely to be limiting factors in Agaricus bisporus nutrition, due to the relatively large amounts of these elements present in the composts used in commercial cultivation. It is, therefore, apparent that a full understanding of the nutrition of Agaricus bisporus may only be gained through experimental systems utilising substrates used in commercial cultivation. Such studies have been restricted primarily because of the inherent complexity and variability of the substrates, which are based on unsterile composted plant residues. Determinations of carbohydrate and protein composition have generally failed to demonstrate clear trends of compositional changes related either to sporophore development or changes of cultural conditions. It was decided that the use of iron as a tracing substance would permit investigations of the changes of concentration of a single material in sporophores and substrates during cultivation of the A. bisporus. Iron was chosen because it was known to be present in sporophores at high concentrations in comparison with other plant foods and mushrooms may, therefore, represent an important source of iron in the vegetarian diet. The quantity of iron both in the substrates and sporophores was of a sufficient level to facilitate accurate determinations. In addition the radioactive isotope, Iron-59, has the necessary particle emission characteristics, particularly highly active gamma radiation and a sufficiently long half-life to permit experiments to be carried out over the long time-scales required for the cultivation of Agaricus bisporus.

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It was proposed that through the study of the movement of iron within the substrates and the uptake of iron into the sporophore crop, considerable information pertaining to the general _in situ_ nutrition of _Agaricus bisporus_ would be obtained. Particular emphasis would be placed on the determination of the relative importance that various environmental factors contribute to the compositional variability of the sporophores and the changes of crop composition associated with the growth and development of _A. bisporus_.

SECTION 1

LITERATURE REVIEW

1.1   Background to the cultivation of <u>Agaricus bisporus</u> (Lange) Pilat.

The nutrients required for the growth and development of <u>Agaricus bisporus</u> sporophores are provided in composts prepared from a mixture of cereal straw and animal manure. During composting the ingredients are subjected to a controlled thermophilic fermentation which includes a pasteurisation procedure. It is a nutritionally balanced substrate designed to favour the growth of <u>Agaricus bisporus</u> at the expense of competing micro-organisms. All aspects of composting have recently been reviewed by Hayes (1978).

In commercial practice, sufficient inoculum of <u>Agaricus bisporus</u> is provided to ensure the complete colonisation of the compost, at an optimum temperature of $24^{\circ}C$, within 14 days.

A casing layer of moist peat made alkaline with chalk or limestone is applied to the compost surface to a depth of four to six centimetres. Comprehensive reviews of all aspects of casing materials are by Hayes (1978), Steane (1979) and Yeo (1980). Mycelial growth into the nutritionally poor casing layer is rapid and the surface is reached after about seven days. The induction of sporophore formation in <u>Agaricus bisporus</u> is brought about by lowering the temperature to about $17^{\circ}C$ and aerating in order to maintain a carbon dioxide concentration in the air of less than 0.15 percent. Under normal circumstances, the transition from the vegetative to the reproductive stage of growth is completed within four days of the temperature and ventilation adjustment.

Apart from the addition of water to maintain a moist casing layer, the physical environment is not altered during cropping. The crop is produced in "breaks" - a rhythmical appearance of sporophores at intervals of seven to ten days. The number and total weight of sporophores produced from a given culture declines in

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successive breaks and in commerce only four to six breaks are harvested. This decline has been associated with the depletion of available nutrients in the compost and the accumulation of soluble salts in the casing layer.

1.2 Growth Characteristics of Agaricus bisporus.

1.2.1. Vegetative Growth.

An extensive mycelial network in the compost is a prerequisite for the production of Agaricus bisporus sporophores. The ramification of hyphae throughout the nutrient rich substrate ensures the efficient absorption of the nutrients made available by the activity of extracellular enzymes released from the mycelium.

The mycelial strand, has been described by Garrett (1954) as a loose federation of hyphae increasing in thickness by accretion of hyphae from the base. Hein (1930a) observed that the strands of Agaricus campestris began to develop from the inoculation point, but only after most of the substrate had become colonised. Butler (1957) studied the development of strands of Merulius lacrymans on non-nutrient substrates. She observed that the growth of strands was influenced by variations in the distance from the food base and that strand development arose from a narrow region behind the advancing mycelial margin. Watkinson (1971 and 1975) demonstrated that strand induction in Serpula lacrimans was favoured by lower concentrations of nutrients in the food base and observed the leakage of Carbon-14 containing substances from intact hyphae, which led to her suggestion that these substances may play a part in strand initiation. Weinhold (1963) showed that rhizomorph production in Armillaria mellea was induced by ethanol and related volatile compounds.

Mathew (1961) found that in _Agaricus bisporus_ the development of mycelial strands was inversely correlated with the concentration of free nutrients in the food base and directly correlated with the moisture content. Hein (1930a) andFlegg (1962) also observed that strand growth is more pronounced in composts and casings with a high water content.

Long and Jacobs (1968) showed that the growth of hyphal strands of _Agaricus bisporus_ into casing material was a process requiring carbon dioxide, the growth rate being proportional to the carbon dioxide concentration of the surrounding air. Flegg (1962 and 1967) showed that the development of mycelial strands in the casing layer was not dependent upon a well-established strand network in the compost and that when the mycelial growth in casing layer was disturbed a less dense network of strands formed. The earlier study of Garrett (1954) had shown that the formation of new strands was inhibited by the presence of older strands in the casing layer. Rasmussen (1959) stated that the tendency of the mycelium in the casing layer to re-anastomose was reduced once vegetative growth had given way to reproductive growth. Thus, the conditions governing strand formation have been clarified by a number of studies.

In 1954 Garrett suggested that the strands of the cultivated mushroom were an obvious means whereby nutrients are translocated, from an old substrate, in the necessary concentration to provide adequate inoculum potential. The pooling of nutrients from individual hyphae enables the colonisation of more distant substrates. Butler (1958) showed that the diameter of originally wide strands diminished as growth proceeded, possibly as a result of the utilization of storage materials within the hyphae. Lambert (1962) noted that a bundle of thick strands developed through an aperture between two

separated sections of compost and concluded that the networks of strands that developed were adapted to translocating nutrients.

The strands that develop in the substrates apparently function as translocating structures between the mycelial network of the compost and the developing reproductive structures at the surface of the casing layer.

1.2.2. Reproductive Growth.

Schwalb (1971) suggests that fructification in Schizophyllum commune is a response to a slowdown in vegetative growth. However, Tschierpe (1973) suggested that the slight inhibition of mycelial growth observed in Agaricus bisporus at the time of fructification, was not the cause but, a consequence of the production of sporophores. Flegg (1980) provided evidence to support this view because at higher temperatures, and therefore, at a higher growth rate, he found that greater numbers of 'initials' were produced.

The initial appearance of the reproductive structures of Agaricus bisporus is the formation of small nodules at or near the casing surface. These 'initials', as identified by Hein (1930a), occur usually by the thickening of the lateral branches of mycelial strands. The 'primordium' represents a more advanced stage of development. It is characterised by a constriction around the equator, which gives a visible distinction between the parts that are destined to become the stipe and the pileus of the mature sporophore. Hein observed that more primordia form than actually develop to maturity. Flegg (1980) reported that prior to the first harvest, the number of 'initials' was at a maximum. The number declined more or less steadily throughout cropping, and Flegg inferred that the sporophores of successive breaks develop from

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'initials' formed prior to the harvest of the first flush.

Following a short pause, the phase of expansion and maturation proceeds. Bonner et al. (1956) reviewed the early studies of morphogenesis in Agaricus bisporus. As a result of their studies they concluded that "All major problems of mapping out the overall shape of the mushroom and the laying down of the majority of the cells is done at a very early stage and that the subsequent increase in size is merely the expansion of specific parts of this preformed bud, which involves the sucking up of nutrient materials from the soil mycelium."

There is virtually no knowledge of the mechanisms controlling the periodic production of sporophores, which appear successively after increasing intervals of time. The decline in productivity of cultures with time is thought to be the result of nutrient depletion in the compost. In addition, the changes observed in the casing layer, particularly the build up of salts and the reduction of water retention capacity, may contribute to the decline in sporophore formation.

1.3   The Nutrition of Agaricus bisporus.

Considering the vast amount of knowledge concerning the production of the cultivated mushroom Agaricus bisporus it is surprising that so little is known of this organisms' requirement for and utilization of nutrients. The numerous approaches that have been adopted by researchers studying Agaricus bisporus nutrition were reviewed by Hayes (1978)

1.3.1.   Pure Culture Studies

The complex nature of the growing media used in commerce imposes

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major difficulties in defining the minimal nutritional requirements for the growth and development of the sporophore. The most important and comprehensive nutritional requirement studies are those of Treschow (1944) and Bohus (1959). However, these relate only to the production of the mycelium in pure, submerged culture. In a limited study of the trace element requirements Treschow found that iron was essential for mycelial growth. It may be misleading to extrapolate the results of such studies even to the establishment of the vegetative phase of growth quite apart from the production of sporophores. A Petri-plate study by Hayes (1972) confirmed that the requirements for vegetative growth and primordium formation are different.

1.3.2. Partially Sterile Cultures.

Bretzloff (1963) and Smith and Hayes (1972) devised similar techniques in which nutrient media of known composition were applied to inert carriers. After colonisation with Agaricus bisporus mycelium an appropriate non-sterile casing material was applied. Because of the requirement for complex nutrients and the nature of the substrates, problems in defining precise nutrient requirements were encountered. However, Smith and Hayes demonstrated that the yield of sporophores produced was related to the concentration of nutrients in the liquid medium. Bretzloff found that a trace element mixture gave slight increases in the yield and number of sporophores produced.

1.3.3. Changes of Compost Constituents.

The earlier works of Waksman and Burrows together with others were reviewed by Gerrits et al. (1967). In most of these studies

the changes of carbon and nitrogen containing materials were analysed.
However, Bretzloff and Fleugel (1962) observed the changes of total
and ammonium acetate extractable P, K, Ca, Mg and Na. Gerrits et al.
(1967) showed that lignin is utilized during the mycelial colonization
of the compost, while during the production of sporophores cellulose
and pentosan were utilized. The enzyme studies of Turner (1974),
Wood (1977), Wood and Goodenough (1977) and Giovannozzi-Sermanni
et al.(1979 showed that there was a steady increase in extracellular
laccase (a lignin degrading enzyme) during mycelial colonization of
the compost. After sporophore initiation the level of laccase fell
and remained low throughout fruiting. Decline in the activity at
fruiting might be due to a cessation of laccase synthesis and Woods'
(1980) findings indicate that inactivation of the enzyme occurs,
primarily by irreversible inhibition followed by protein degradation.
Turner (1974) observed increased tyrosinase activity at the time of
the appearance of the reproductive phase, particularly in the casing
soil. Tyrosinase (also a lignin degrading enzyme) may be inhibited
in the compost by the presence of phenolic products of laccase
activity, so that the decrease of laccase activity will allow the
observed increase of tyrosinase activity. Turner et al. (1975) and
Wood and Goodenough (1977) demonstrated that cellulose activity
increases at the time of fruiting, but Giovannozzi-Sermanni et al.
(1979) found no such increase. Wood (1979) observed no marked changes
in the activity of a number of other enzymes.

The physiological roles of enzymes produced during mycelial
growth and sporophore production are difficult to interpret, but the
suggestion made by Gerrits et al. in 1967 is not contradicted by the
enzyme studies that have been done since then. He proposed that the
nutritional pattern of the mycelium undergoes a shift from the

preferential utilization of lignin and protein polymers (which frees

high molecular weight carbohydrates) during mycelial growth to

cellulose and hemicellulose (required in the construction of large

sporophores) utilization when fruiting is initiated.

1.3.4. Nutrient Supplementation of Composts.

Applying nutrients to composts subsequent to spawning has been

used as a means of studying the nutrition of the crop, notably by

Schisler and co-workers at Pennsylvania State University. They and

other researchers observed the stimulating effect on yield given by

the addition of a range of vegetable materials and products. Adding

nutrients to the compost may benefit the mushroom either directly or

by stimulating the growth and activity of the microflora in the

substrate. Gerrits (1970) suggested that because of their relative

abundance in the compost, inorganic elements are unlikely to be

limiting in the nutrition of Agaricus bisporus. However, Stoller

(1954) suggested that a mixture of trace elements should be added

to synthetic composts.

1.3.5. Composition of the crop

A number of researchers have attempted to relate the composition

of the sporophore to its nutritional requirement. This is considered

an acceptable method in defining the elemental requirement of the

crop. However, the substances detected in the sporophore are not

necessarily required, but may be the result of passive uptake of

other materials into the sporophore. In addition the majority of

researchers have studied organic substances that are metabolized

in the organism. These studies provide no information on the

essentiality of these nutrients or whether they aremerely metabolic

by-products.

1.3.6. Mathematical Modelling.

Chanter and Thornley (1978) devised a mathematical model for the mycelial growth and the initiation and growth of sporophores of a mushroom crop. Several assumptions had to be made in order to construct the model, the main assumption being that the initiation and growth of sporophores are regulated by the density of a single substance in the mycelium. They found that in its qualitative behaviour, the model agreed reasonably well with real crop behaviour.

The nutrition of Agaricus bisporus is of fundamental importance in the production of the crop. In his review, Flegg (1961a) concluded that, "It is clear that further research on all of the major aspects of the compost is required. The greatest bar to progress is our ignorance of the nutritional requirements of the mushroom for fruiting." In another review of Agaricus bisporus nutrition, Hayes (1975) concluded that, "despite our existing knowledge of nutritional aspects concerning carbon and nitrogen nutrition, there still remains a serious gap in our knowledge relating to the mineral nutrition of Agaricus bisporus."

1.4 Fructification of Agaricus bisporus.

The application of a casing layer is obligatory to the production of sporophores in the commercial cultivation of Agaricus bisporus. In 1959 Tschierpe postulated that the function of the casing was to produce a carbon dioxide gradient between the compost and the air. Tschierpe and Sinden (1964) and Long and Jacobs (1968) found that in normal casing layers maximum sporophore initiation occurs between 0.03 and 0.1 percent carbon dioxide in the air. Mader

(1943) concluded from his studies that an unsaturated hydrocarbon and not carbon dioxide was responsible for the stimulation of fructification when present in small amounts, yet at higher concentrations this volatile arrested sporophore growth. In the early 1950's Stoller (reiterated in Stoller, 1979) hypothesised the existence of volatile substances, later named as quinones which are produced from lignin breakdown in the compost, which inhibit fructification. He surmised that the function of the casing layer was to provide an alkaline oxygenated medium where these volatiles were destroyed. A similar view was expressed by Schisler (1957), who proposed the existence of a high mdecular weight hormone-like substance cf low volatility produced by the compost mycelium. The casing layer functions to inhibit the volatilisation and/or diffusion of this substance sufficiently as to allow fructification. Lockard (1962 and 1967), Tschierpe and Sinden (1965) and Turner et al. (1975) have identified several volatile metabolites produced by the mycelium. Turner et al. (1975) concluded that ethylene was the only gas showing a pattern of production corresponding to cropping. Tschierpe and Sinden (1965) could not detect any effect on fructification or strand formation when a number of the isolated volatiles were applied to mushroom cultures.

Eger (1961) using the 'Halbschalentest', in which axenic compost and sterile casing are placed in different halves of a divided Petri-dish, showed that only unsterilised casing or autoclaved casing inoculated with a suspension of a productive casing induced fructification. Eger (1963) showed that the presence of too many bacteria stop mycelial growth. She suggested that the initiation of fructification may be corrected with this growth stoppage. A number of studies (Urayama (1961 and 1967), Curto and Favelli (1972) and

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Margheri and Vassilacakis (1977)) have reported increases of yield and initiation when a range of micro-organisms are applied to the substrates.

Hayes et al. (1969) demonstrated that Pseudomonas putida and Group IV Pseudomonads in peat based casing soils stimulated sporophore initiation. Hume and Hayes (1972) and Arrold (1972) showed that on agar media, the production of sporophore primordia was facilitated by the interaction of the mycelium with the bacterium Pseudomonas putida. Park and Agnihotri (1969) showed that the initiation of sporophores was triggered by the metabolites of soil-inhabiting bacteria and that the ability to induce initiation varied considerably within species and isolates of bacteria. Hayes and Nair (1976) found that the numbers of Pseudomonads were greatly increased in atmospheres enriched with ethanol, one of the volatiles found to be emitted by the mycelium.

Eger (1972), Couvy (1974) and Cresswell and Hayes (1979) observed that the greatest bacterial activity in casing soils occurs at about the time of sporophore initiation. However, changes in the bacterial population could not be related to any particular stage of growth of Agaricus bisporus.

The studies of Mader (1943) Eger (1972, in a review of her earlier studies), Long and Jacobs (1974), Angeli-Couvy (1975), Peerally (1979) and Stoller (1979) showed that activated charcoal appears to exert a similar influence to that of bacteria and permit the formation of primordia. The results of these studies appear to support the original proposal of Mader (1943) that fructification is controlled by the continuous removal of inhibitory metabolites leaked or secreted from the hyphal tips.

Nair and Hayes (1975) established an interaction between the degree of aeration of a casing soil and the activity of Pseudomonads in the casing layer. In addition, higher yields of sporophores were produced when high levels of Pseudomonads were found in the casing soil. Nair et al. (1976) found that at lower levels of bicarbonate in the casing layer, the numbers of Pseudomonads were greater and there was a percentage increase in the numbers of bacteria capable of reducing ferric ion. Hayes (1972) found that primordia form sporadically on malt extract agar. He found that some metals, zinc, tin and manganese, were inhibitory to sporophore formation at one part per million, but several iron-containing compounds stimulated primordium formation at ten parts per million. When ferrous salts were applied to the casing layer Hayes (1972), Kaul and Kachroo (1976) and Anon (1974) found that the number of primordia and yields of sporophores were increased. Hayes suggested that iron was complexed by the natural chelating agents of the organic matter in the casing soil and that Pseudomonas putida or similar organisms function to release iron for the purposes of growth associated with fructification. Hayes (1974) further postulated that the zones of differing aeration in the casing layer may create conditions of oxygen stress under which Pseudomonads might be expected to reduce iron. This may be of significance as regards the relative location of sporophores within the casing layer.

Using a strain of Agaricus bisporus that produced primordia on malt agar Wood (1976) found that the number of primordia that developed was regulated by the agar source, the pH, the carbohydrate sources and the carbon dioxide concentration. He found that the formation of primordia was not stimulated by the addition of live suspensions or culture filtrates of Pseudomonas putida or other

bacteria, iron salts or iron binding compounds.

Eger (1965 and 6), Rusmin and Leonard (1978) and Urayama (1971) have all shown that extracts and pieces of tissue of Agaricus bisporus stimulate initiation of basidiocarp formation in several basidiomycetes. The stimulative factors are water soluble and heat stable, but show no stimulative effect on the mycelium of Agaricus bisporus.

Hussain (Personal Communication) has obtained increased primordium initiation of Agaricus bisporus on malt agar using a range of phytohormones. Gibberelic acid was the most effective single hormone. Both he and Giovannozzi-Sermanni et al. (1976) have shown a concomitant rise in phytohormones and an increased bacterial population of the casing layer and compost respectively at about the time of initiation of the sporophores. Stanek (1969, 1972 and 1974) showed that thermophilic actinomycetes and cellulose decomposing thermotolerant fungi and bacteria that grow in the vicinity of Agaricus bisporus mycelium in the compost, produce polysaccharides and vitamins which stimulate the growth of the mycelium.

It is a long held view that the initiation of sporophore formation is a response to the extremes of nutritional status presented by the compost and the casing layer but, there is surprisingly little evidence that either supports or refutes this view. Using agar media Couvy (1972) demonstrated the initiation of primordia on a nutritionally 'poor' medium connected by mycelial strands to a 'rich' medium Flegg (1958) added simple organic nutrients or compost to the casing soil and obtained no detrimental effect on the yield of sporophores produced, although initiation was delayed. He suggested that sporophore initiation was not related to a difference between the nutritional status of the two substrates. Flegg (1979) showed that when each half of a compost were cased at different times, the

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earlier-cased portion became more strongly colonised by mycelium and produced more sporophores than the other half. He suggested that this dominance was a result of the competition for available nutrients between the mycelium in each half. Using sporophore transplantation experiments Sinden et al. (1962) showed that maturing primordia suppress the development and formation of other primordia. They suggested that this inhibition is not primarily due to the monopolisation of nutritional reserves, but is possibly a hormonal action. They conclude that the initiation of fructification and the growth of sporophores are two totally different phenomena governed by different factors.

Taking into account all knowledge of the formation of the sporophore, we can construct an overall description of the processes involved in and governing the initiation and growth of sporophores.

The growth of the mycelium is enhanced by the optimum temperature and high carbon dioxide concentration. This allows the build up of nutrients within the mycelium. The change in environmental conditions, by lowering the temperature and carbon dioxide concentration and increasing the rate of evaporation allows initiation to occur. The removal of inhibiting substances and/or production of stimulants by the bacterial population of the casing layer is also critical in the initiation process. Initials in the most favourable positions preferentially receive nutrients accumulated in the compost mycelium and the crop develops at the expense of these accumulated nutrients. Once the sporophores are harvested the mycelium starts to accumulate materials in readiness for the next break of sporophores. The time taken for this build up of nutrients increases because the nutrients become scarcer as they are removed from the compost by the crop.

1.5 The Nutritional Basis of Fungal Morphogenesis

It is perhaps paradoxical that few morphogenic studies have been
on the basidiomycete sporophore even though it is the largest
regularly shaped fungus structure and many species have been induced
to fruit in the laboratory, although not all on synthetic media.
Extensive reviews of the literature have been reported by Plunkett
(1951), Taber (1966) and Volz & Beneke (1969). The latter emphasised
the importance of the nutritional regulation of sporophore formation
and they concluded that in the majority of investigations the use of
randomly selected, complex media has confined our knowledge of the
nutritional requirements for fructification to a few species only.

Generally, the optimum concentration of the medium is almost
always lower for fructification than for vegetative growth. The
perithecial initiation in Gnomonia fructicola has been shown by
McOnie et al. (1966) to be dependent on glucose exhaustion and
Robert (1977) found that primordial initiation in Coprinus congregatus
occurred when glucose in the medium became depleted. A recurrent
concept in the study of fruiting in fungi is to consider growth and
reproduction as opposing phenomena, one predominating at the expense
of the other. A speculation as to this role is as follows:
endogenous nutrients accumulate during primary growth this continues
until a critical exogenous nutrient becomes limiting, thereis then
a cessation of primary growth. Secondary growth is then initiated
and proceeds along the line dictated by the genetic constitution.
Endogenous nutrients, and perhaps some exogenous ones, are then
used in respiration and the synthesis of the secondary hyphae which
will make up the fruit body. If this hypothesis is correct, then
it should be possible to detect changes in the content and extent
of growth of the primary mycelium.

During the growth and ageing of the mycelium of <u>Agaricus bisporus</u> Tsai <u>et al.</u>(1974) observed decreases in the amounts of carbohydrate, amino acid, RNA, DNA and proteins. However, this may be representative only of vegetative mycelia grown on agar media. In fungi that do fruit in plate culture (<u>Rhizoctonia solani</u> and <u>Sclerotium bataticola</u>) Molitoris <u>et al.</u>(1969) found that the dry weight and content of storage material of the mycelium increased with age. In <u>Phallus impudicus</u>,Grainger (1962) showed that rhizomorphs of different diameter were relatively uniform in chemical composition and that rhizomorphs without a fructification attached, had appreciably higher contents of total carbohydrate, fat, nitrogenous substances, ash and solids than rhizomorphs of the same diameter attached to afructification. From his observations in the field, Grainger suggested that a major portion of the five grams dry weight involved in the average fructification is transferred from the rhizomorphs in about 24 hours. This represents a considerable feat in nutrient transport.

The construction of a large sporophore clearly requires a considerable reserve of material on which to draw during development. As a consequence sporophores do not usually arise until a fairly extensive vegetative mycelium has developed. Frequently sporophores are found to be attached to the appropriate food bases by well developed strands or rhizomorphs. In the plate culture of <u>Coprinus cinereus</u> Madelin (1956 and 1960) has shown that hyphae immersed in the agar swell and stain heavily with I/KI solution. Once sporophores begin to develop, the swollen cells of the mycelium vacuolate and lose their dense contents. He interprets this as suggestive evidence for the storage of glycogen followed by its transfer to the sporophore. He demonstrated a direct transfer of materials by following the changes in dry weight, of the mycelium and sporophore over a 17-day period,

during which a crop developed, deliquesced and a new crop began.
During days 12-13 the increase in the sporophore weight was almost
exactly equalled by a loss in the weight of vegetative mycelium.
This is in keeping with Bonner's observation that the linearity of
a dry weight/wet weight plot during the elongation of the _Agaricus_
bisporus sporophore, represents translocation both of water and
materials (Bonner _et al_., 1956).

Madelin also investigated the factors which governed distribution
of sporophore on a plate colony. He adopted a suggestion of Hein's
(1930b) concerning the abortion of sporophores in commercial mushroom
beds. Namely that some primordia would be more favourably placed
than others, through slight differences in their times of initiation
for instance, to initiate a flow of nutrients in their direction and,
therefore, away from the other primordia. His results show clearly
that material is drawn uniformly from all parts of the mycelium but,
only transported to certain sporophores. The distribution of the
maturing sporophores was affected by an unknown internal mechanism.

A study by Wessels (1965) of _Schizophyllum commune_ is still the
most detailed biochemical investigation of sporophore morphogenesis
available. He found that the aggregation of hyphae to form initials
requires the availability of exogenous nutrients and that environ-
mental factors, particularly elevated temperatures and CO_2 concen-
trations, have a profound influence on the formative process. The
growth of primordia is characterised by a thickening of the hyphal
walls by polysaccharide accumulation and is strictly dependent on
an external carbon source. The vegetative mycelium supplies the
nitrogenous compounds necessary for the growth of primordia. The
expanding pilei draw completely upon the stunted sporophores and
stroma for the supply of nutrients. Hydrolysis of cell wall R-glucans

in these structures provides a low but continuous supply of glucose.
Wessels concluded that the whole morphogenetic system may be regarded
as a system in which particular structures areproduced in sequence,
each structure providing the substrate for the following. Thus, the
distinction between reserve and structural constituents has now
become rather meaningless.

Support for the results obtained by Wessels has been provided by
Kitamoto et al. (1974) with the organism Favolus arcularius. They
show that the sequence of nutrient utilisation was the same in both
organisms. In addition, they observed that the feeding of exogenous
nitrogen sources into the medium, prior to initiation, was inhibitory
to the initiation and development of the sporophores.

Wakita (1958) found that trehalose disappeared from the mycelium
of Flammulina velutipes during fruit body growth. In Coprinus
congregatus, Robert (1977 a and b) found that the primordia developed
and the stipe expanded at the expense of carbohydrates accumulated
during vegetative growth. Pileus maturation then utilises carbohydrates
accumulated in the stipe. Trehalose has been shown to accumulate in
the stipe of Coprimus lagopus by Rao and Niederpruem (1969) Kitamoto
and Gruen (1976) showed that glycogen was the main storage carbohydrate
in the mycelium and that this is broken down during the growth of
larger fruitbodies of Flammulina velutipes. Trehalose, arabitol and
mannitol were the low molecular weight carbohydrates translocated
into and stored in the basidiocarps. McLaughlin (1974) states that
it seems probable that much of the glycogen transported to the primordia
of Coprinus is not finally utilized until the differentiation of the
hymenium. Gruen and Wu (1972 a and b) with Flammulina velutipes
and Bret (1977) with Coprinus congregatus have shown that elongation
of the stipe is dependent on the presence of both the pileus and

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the vegetative mycelium throughout sporophore development.

Studies of changes in the composition, during morphogenesis, of the cultivated mushroom have been hampered primarily because of the inability to produce mature sporophores using agar culture methods. The majority of reports concern the changes of carbon and nitrogen containing substances during the growth of a mushroom crop; but several researchers have compared vegetative mycelium grown on agar with compost grown sporophores.

Hammond and Nichols (1977) suggested that glucose, fructose, trehalose and mannitol are all mobile in the sporophore. Glycogen (Hammond & Nichols, 1976b) and Mannitol (Rast, 1965, McConnell and Esselen, 1947 and Hammond and Nichols, 1976a) accumulate in the sporophore, particularly the pileus, during growth. 40% of the dry weight of the mature sporophores is mannitol (Dutsch and Rast 1972) formed almost exclusively from fructose (Dutsch and Rast, 1969 and Hammond and Nichols, 1977). Trehalose (Rast, 1965) and Fructose (Lin et al., 1975) are found at higher concentrations in small mushrooms than in later stages. No major qualitative differences were found by Byrne and Brennan (1975) between the lipids of vegetative and reproductive growth; although Holtz and Schisler (1971) observed the accumulation of free sterol in sporophores. Turchetto et al. (1977) found no differences of water and lipids either amongst the different components of a mushroom sporophore or successive stages of development. Paranjpe and Chen (1972) and Paranjpe (1979) have shown that primordia contained greater amounts of soluble proteins and phenolic substances than more mature sporophores and mycelium and that more of these substances are situated in the pileus than in the stipe. Haddad (1977), Maggioni et al. (1968), Latche et al. (1975), Kissmeyer-Nielsen et al. (1966)

and Hughes and Rhodes (1959) have studied the changes of a number of substances, particularly nitrogenous substances and amino acids in sporophores with respect to successive crops. A comparison between the results show many inconsistences in the amino acid profiles and generally no conclusions can be drawn about the changes observed. Parrish et al.(1976) observed a direct relationship between fluctuation in yield and mannitol content of the sporophores. They attribute this to changes in the metabolic activity of the organism.

Once formed the basidiomycete sporophore exhibits certain growth phenomena indicative of a hormone (or some form of endogenous growth regulator) - mediated response. The direction of sporophore growth in Agaricus bisporus is influenced by a growth-promoting substance according to Hagimoto and Konishi (1959) and Gruen (1963). They have confirmed that the hymenium is the site of production of the growth regulating substance(s) and from here they are translocated to the pileus and stipe. Pegg (1973) has isolated Gibberelin - like substances in the sporophore. Turner et al. (1975) observed that in contrast to other volatiles produced from mushroom beds, ethylene (a phytohormone) production appeared to be related to the reproductive phase of the crop. Bursts of ethylene production have been shown by Wood and Hammond (1977) and Ward et al.(1978) to be produced by the mycelium. This production coincided with the change of hymenial colour from pink to brown at each break. However, there is no evidence of a regulatory role of ethylene in the growth or development of Agaricus bisporus. Tschierpe (1959) and Turner (1977) have shown that high concentrations of carbon dioxide allow elongation of the stipe, whereas the expansion of the pileus and ripening of the hymenium are favoured by the removal of carbon dioxide.

Thus, a variety of changes in carbohydrates, sterols, lipids and nitrogenous compounds may be associated with sporophore development, but in the majority of cases they have not been related to any specific morphogenetic process. That external physical forces play a role in determining basidiomycete form is unquestionable but, just how they interact with metabolic processes within the cell is completely unknown.

1.6 Translocation in the vegetative mycelium.

The most recent and comprehensive reviews of translocation in fungi are those by Jennings (1976), Hill (1965), Burnett (1976) and Wilcoxson and Sudia (1968). The latter list a large number of studies which have demonstrated translocation in fungi, mostly using radioactive substances. Almost without exception, the researchers merely report that the fungi they have used, have the ability to translocate. Petri-plate techniques of growing fungi have been used in the majority of studies and because of the conditions of the environment, structure of the substrate and the nutrient availability, it is questionable if this system represents the conditions encountered in nature. Read and Stribley (1975) suggest that of the many experimental methods used, most prove to be reliable, although some earlier works may have been demonstrating diffusion through the medium and capillary movement between hyphae rather than translocation within hyphae.

Remarkably, the first study of any significance on fungal translocation was only reported by Schütte in 1956. He divided fungi into two groups, those capable of translocation and those incapable, on the basis of their ability or inability to grow onto a deficient medium. This pioneering study has been followed by many others,

using essentially the same technique but, with radioactive materials to detect translocation. Thrower and Thrower (1961), reported both translocating and non-translocating fungi as well as species with an indeterminate ability to translocate. When this work was repeated using labelled nutrients they (Thrower and Thrower, 1968a) found that translocation could occur through the mycelium of both colonizing and non-colonizing fungi. It is suggested that the movement of nutrients is adaptive to some extent, because Bokhary and Cooke (1974) have shown that labelled nutrients were moved more rapidly when the mycelium of _Phytophthora cactorum_ colonized a deficient medium, than when it colonized a nutrient medium.

The translocation of labelled glucose in the mycelium of _Rhizoctonia solani_ has been studied by Milne & Cooke (1969). They demonstrated that the translocation occurs both towards and away from the hyphal tips, although it was less effective in the latter direction. Within 24 hours of application most of the Carbon-14 labelled glucose became metabolized to trehalose and then into a soluble glucan. In their turn, these compounds became increasingly converted to ethasol-insoluble compounds which were presumably storage or structural polysaccharides. The apical margin, the younger part of the mycelium, was a persistent site of progressive accumulation of these compounds. The studies by Smith (1966 and 1967) and Lewis and Harley (1965) of carbohydrate translocation in the mycelium of _Rhizopus repens_ and an ectotrophic beech mycorrhiza, respectively, provided suggestive evidence that trehalose was the form in which carbohydrates are translocated within the mycelium. However, Bokhary and Cooke (1974) showed that glucose was the major translocatory carbohydrate in _Phytophthora cactorum_.

Monson and Sudia (1963) demonstrated that bidirectional translocation of Zn-65, S-35 and Sr-89 between the young and old parts of an established mycelium occurs in Rhizoctonia solani. In more extensive studies using Phosphorus-32, they showed clearly that young hyphal tips absorb and translocate more phosphorus than do the older hyphae. In 1960 Lucas found that Phosphorus was not translocated through an established mycelium of Rhizopus stolonifer. As the result of a later study, Lyon and Lucas (1969) proposed that the translocation of phosphorus is the result of the mycelial metabolism. They also showed that phosphorus is translocated in the form of orthophosphate. However, translocation and growth are not always necessarily directly linked. Both Milne and Cooke (1969) and Bokhary and Cooke (1974) have shown that the maximum translocation of carbohydrates occurs at a lower temperature than is the optimum for mycelial growth.

From their studies of Rhizoctonia solani, Littlefield et al. (1965a) suggested that the amount of phosphorus - 32 translocated was inversely proportional to the distance travelled from the source and thus implying that transport is by diffusion. Support for the view that translocation is a diffusive process has been claimed by Cowan et al. (1972 a and b) who investigated the movement of Potassium - 42 in the sporangiophores and mycelium of Phycomyces blakesleeanus. In the mycelium, translocation towards the apex was almost three times more rapid than in the opposite direction, whether or not there was a difference in the external Potassium concentration between the ends. They showed that their calculated values for the inherent electrical potential gradients were plausible and hence saw no reason to contemplate any motive force other than diffusion. However, Jennings (1976) argued that very high concentration gradients

would have to exist, to account for the movement of some materials through the mycelium. Using data from Butler's (1957) experiments and his own unpublished results of studies on Serpula lacrymans strands. Jennings suggested that the existence of a pressure driven bulk-flow system seems likely. Fruiting requires an obligatory large volume of mycelium in which the build-up of high concentrations of materials could create sufficient pressure loading to drive the system. The appearance of drops of liquid at the hyphal tips may indicate the operation of this system.

Garrett (1956) proposed that hyphal aggregations, in the forms of rhizomorphs and strands, are an advantage when attacking a new substrate by permitting the fungus to translocate nutrients from a food base on which it is established, to the new substrate. Bokhary and Cooke (1974) showed that the growth rate of the fungus across a non-nutrient substrate is proportional to the size of the food base. Garrett further suggested that the movement of substances through rhizomorphs must be efficient because some have found to be extremely long and to extend through environments not likely to support fungus growth. Weigl and Ziegler (1960) found little or no evidence of the uptake of Phosphorus - 32 by the strand hyphae of Serpula lacrymans and from this they concluded that these organs seem to be especially specialized for translocation. The translocation of both Phosphorus - 32 and Carbon - 14 labelled compounds was inhibited by low temperature and oxygen tension, thus providing evidence that metabolic processes are involved. Watkinson (1971) also using Serpula lacrymans, was able to demonstrate the translocation of Carbon -14 containing substances through strands between old and new food bases. She also proposed that Carbon -14 containing substances lost from intact hyphae were involved in the induction

of strand formation. Both Lucas (1960) and Grossbard and Stranks (1959) found that there was no appreciable exchange of Phosphorus-32 and Cobalt-60, respectively, between the mycelium and the medium. Schütte (1956) using fluorescein dye, demonstrated translocation in the subterranean mycelia of fairy rings both towards and into the sporophores of several species.

The ability of the mycelium of Agaricus bisporus to transport materials large distances through a commercial bed has been demonstrated by Lambert (1963) and Nielsen & Rasmussen (1963). The latter showed that sporophore yield could be correlated with the dry weight of compost up to 1.8 metres in depth. These results indicate that nutrients are transported through this depth of substrate.

1.7 Translocation into microscopic fruiting structures.

In 1959 Grossbard and Stranks and Abbot and Grove found that Cobalt -60 and 2:3:5 - Triphenyl tetrazolium chloride respectively, were translocated to and accumulated in sporangia. Later, Thrower and Thrower (1968b), showed that markedly greater quantities of labelled nutrients were present in reproductive structures than in the vegetative mycelium of all fungi that they investigated. Melin and Nilsson (1958) discovered that the rate of transferrence of nutritive elements from a mycorrhizal fungus to its' host was affected by the rate of host transpiration. In an investigation of the potassium translocation in sporangiophores of Phycomyces blakesleeanus Cowan et al.(1972c) found that the translocation of Potassium -42 was against an internal potassium concentration gradient and that the rate of accumulation was about ten times greater than would be expected by diffusion alone. They discovered that the concentration of potassium in the sporangiophores increased

with evapotranspiration and suggest that the evaporation pull caused
by these aerial structures can be a powerful factor in determining
the rates of translocation.

1.8 Translocation in Sporophores.

Both Roberts (1950) and Schütte (1956) used dyes to show that
some sporophores had distinct translocating zones. They showed that
the rate of translocation varied with the size and age of the
sporophores and the humidity conditions of the atmosphere. In young
fructifications·translocation took place in saturated air. Studies
of Lentinus tigrinus and Collybia velutipes by Littlefield (1966)
and Littlefield et al. (1963 and 1965) showed that as the Lentinus
tigrinus sporophore expands, the path of translocation becomes
restricted to the morphologically specialized, central, translocating
zone. This zone is also characterised by possessing a greater oxygen
consumption than the cells of the cortex. Translocation was shown
to be sensitive to relative humidity, but not impeded by high relative
humidity. When the sporophores were killed, translocation ceased.

In Agaricus bisporus Konishi (1967) autoradiographically, using
Carbon -14 labelled glycine and serine, showed that translocation
occurred both upwards and downwards in the sporophore, depending
where abouts the label was applied. Rast (1966) demonstrated that
the mannitol transport of the Agaricus bisporus sporophore proceeds
through two distinct routes. Two similar routes of phosphate
transport were observed by Kulaev et al. (1960) and Kritsky et al.
(1965a and 1965b). One route, characterized by a higher rate of
phosphate flow and mainly of acid soluble phosphorus compounds,
led from the stipe via the annulus to the pellicle of the pileus.

The other route, of lower intensity and effected through orthophosphate, is from the stipe immediately to the hymenium.

Viral diseases of <u>Agaricus bisporus</u> appear to spread most rapidly when the mycelium is actively growing and stop when the substrate is fully colonized or when fruiting begins. The infectious principle for the disease is in the living hyphae of the fungus and can readily be transferred to healthy hyphae. Van Zaayen (1972) observed virus particles in all tissues of the <u>Agaricus bisporus</u> sporophore and the presence of these particles in the dolipores suggests cell to cell translocation. Last <u>et al.</u> (1967) demonstrated that diseases caused by smaller virus particles spread more rapidly than diseases due to larger particles.

1.9 <u>Transpiration in Sporophores</u>.

Plunkett (1958) revealed a striking relationship between translocation and growth in <u>Polyporus brumalis</u>. The promotion of pileus formation by heightened evaporating conditions is shown to be associated with enhanced translocation rates into the sporophore. He was able to demonstrate a reasonably close correlation between the observed rate of transpiration and the movement of dyes and dry matter into the developing sporophore. Schütte (1956) had also noticed the effect of relative humidity on the translocation rates of dyes. By applying Vaseline to the surface of the sporophores he calculated that the hymenial surfaces are responsible for about two thirds of the water loss from the whole sporophore. Schütte (1961) also showed that the water losses from agaric fructifications were considerable, even when the atmosphere was saturated. Littlefield <u>et al.</u> (1965b) found that up to ten times more phosphorus -32 was translocated by sporophores of <u>Lentinus tigrinus</u> in dry air than in

water saturated air. Zoberi (1972) demonstrated that in a number of

agaric sporophores, transpiration was shown to decrease with increasing

atmospheric humidity, so that in nearly saturated air there is

virtually no transpiration. Using detached sporophores both Schütte

(1961) and Zoberi (1972) found that uptake into the sporophore

proceeds as long as the sporophore is young and healthy. San Antonio

and Flegg (1964) obtained no evidence of a progressive decrease of

transpiration from rapidly growing and developing sporophores of

Agaricus bisporus. They found that under different conditions of

temperature and relative humidity, the transpiration per unit area

of the sporophore surface was equivalent to evaporation from a free-

water surface. There was no indication of a marked increase in

water loss at veil break, and they estimated that during development

up to the open-veil stage, the quantity of water transpired was equal

to half of the fresh weight of the sporophore. Moser (1962) observed

that increased transpiration rates were the result of increases in

the velocity of air currents to which the sporophores were subjected.

Although translocation does occur when transpiration is unlikely,

conditions that favour transpiration also favour translocation.

The transpiration stream may therefore, be an important translocatory

mechanism. Russell and Shorrocks (1959) have shown that in intact

plants the rate of ion transfer correlates with the rate of trans-

piration.

1.10 Metals in The Wild Basidiomycete Sporophore

Relatively few studies have been made of the metallic composition

of basidiomycete sporophore tissue. In 1965 Sawada produced what is

likely to remain the most extensive study of the composition of wild

mushrooms. His analysis included fourteen mineral elements in a

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vast number of samples covering a wide range of fungal species.

During the last decade Stark (1972), Hinneri (1975), Allen and Steinnes (1978), Vogt and Edmonds (1980) and Tyler (1980) have studied the role of macrofungi in mineral element nutrient cycling. The mycelia of these fungi are in extensive contact with the substrate, this allows the nutrients released during the decomposition process, to be efficiently absorbed by the fungal tissue. However, owing to the difficulty of collecting vegetative mycelia from plant litter, the relative importance of macrofungi in concentrating certain nutrients can only be approximated by examining the nutrient immobilization patterns in the sporophores that are produced. It cannot be presupposed that the metal concentration of the sporophore is similar to that of the mycelium but, it is likely to indicate the capacity of the mycelium to absorb metals from the substrate.

Stark (1972) showed that both rhizomorph and sporophore tissue contain high concentrations of certain biologically important elements and that these tissues possess a high degree of efficiency at holding these elements against leaching. He also attributed great significance to the fact that greatly differing tissues from grossly different ecosystems, although extremely variable in elemental composition, tended to have the same general concentrating and holding abilities.

Because of the variability observed in different studies few general conclusions can be drawn for the accumulation of specific metals in sporophores. However, it does appear that the bioconcentration of one metal is often accompanied by a rather high level of one or several more metals. Tyler (1980) in an analysis of 130 species of basidiomycete sporophores found that iron was neither excluded nor concentrated in any of these species, but members of the genus Agaricus were frequently observed to contain higher than average

concentrations of heavy metals. Hinneri (1975) did not establish
any general trends in the mineral composition of sporophores when
comparing between juvenile and mature specimens, but in the genus
Agaricus young sporophores were found to contain more iron than older
ones. Allen and Steinnes (1978) noted that the concentration of
trace elements were two to three fold greater in the pileus than in
the stipe whereas, alkali metals and other elements were more evenly
distributed between the two.

Recently, particular concern and attention has been given to the
concentrations of potentially toxic elements in the sporophores of
wild, edible fungi. Some fungi appear to be such efficient accumulators
of heavy metals (particularly mercury, selenium, lead and cadmium)
that Stegnar et al. (1973), Rauter (1975) and Enke et al. (1977)
have suggested that basidiomycete sporophores may be used as indicators
of environmental pollution. The concentration of radioactive isotopes
from the soil by wild, basidiomycete sporophores has been shown in
the studies of Pesek (1964), Rohleder (1967), Grueter (1971) and
Johnson et al. (1970). They found that isotopic accumulation depended
upon the isotope itself, the fungal species and soil type that the
organism was inhabiting. It is evident, from the reported studies
on the heavy metal content of many fungal species, that both the
composition and accumulating abilities of sporophores are extremely
variable. Seeger (1976a) obtained no clear demonstrations of any
geographic influence over the mercury composition of sporophore
tissue. Several studies (Seeger, 1976a, Stijve 1977, Seeger et al.
1976 and Seeger 1978a) provide evidence that species dependent
accumulation of Mercury, Selenium, Lead and Cadmium occurs in the
wild. Members of the Family Agaricaceae contain a higher than
average accumulation of these elements. Mercury (Seeger, 1976B and

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Stijve and Roschnik, 1977), selenium (Stijve, 1977) and cadmium
(Seeger, 1977) were found at higher concentrations in the hymenium and
pileus than in the tissues of the stipe. Seeger (1977) showed that
young sporophores contain a greater concentration of mercury than did
more mature specimens. A positive correlation between the mercury
concentration and protein content of sixteen species of edible mushrooms
was demonstrated by Aichberger (1977).

The accumulation of metals into fungal sporophore tissue from the
soil varies over a wide range. Between 7 and 107 times the soil
concentration of mercury and selenium were found by Pallotti et al.
(1976), Stijve and Roschnik (1977) and Stijve and Cardinale (1977).
Seeger (1978b) obtained a 20 to 40 fold accumulation of potassium.
However, from these numerous studies, the concensus of opinion is
that the consumption of wild, edible mushrooms would not contribute
much to the heavy metal intake of the average person.

1.11 Uptake of Toxic Materials into Agaricus bisporus.

In common with wild edible mushrooms, recent fears over the
accumulation of potentially harmful or toxic substances in Agaricus
bisporus has prompted a number of investigations into this area of
study. Contamination of commercially produced sporophores in from
two potential sources; substances that are applied as crop protectants,
particularly insecticides and fungicides and substances that are
present in the substrates, particularly heavy metals.

Dutsch (1975) and Dutsch and Tschierpe (1974) found that the
systemic fungicide Benomyl is inactivated, mainly by absorption,
in casing soils with a high organic matter content. They also
demonstrated that there is a direct correlation between the bioactivity
of the fungicide and the concentration of the free active ingredient

in the water phase of the casing material. Jalali and Anderson (1976) obtained no definite trends towards increasing or decreasing Benomyl contamination of sporophores from harvest to harvest. However, they did find that drenching with Benomyl solution resulted in higher residues in the sporophores than when the Benomyl was mixed with spawning materials. Kalberer and Vogel (1978) obtained a correlation between the rate of the insecticide Carbofuran mixed in casing soils and the quantity of residue detected in and on the sporophores. The residues of a number of pesticides were observed to decrease rapidly in successive sporophore 'breaks' by Dabrowski and Czarnik (1976) and William and Martens (1967).

Several German researchers have recently been experimenting with the addition of municipal waste composts to the convential horse manure compost used in Agaricus bisporus cultivation. The addition of increasing quantities of municipal waste compost resulted in a proportional increase in the heavy metal (Cu, Zn, Pb, Cd and Hg) content of the mixture and as shown by Hasuk (1975) and Domsch et al. (1976), a demonstrable increase in the content of these metals in harvested sporophores. A small percentage of Agaricus bisporus sporophores analysed by Frank et al. (1974) were found to exceed the actionable level for mercury. Loughton and Frank (1974) showed that the compost was the source of the contaminant. When they applied mercury salts to the compost the mercury concentrations of the sporophores were in direct relationship to the amount of mercury applied to the compost. Aichberger and Horak (1975) and Laub et al. (1977) repeated this study using mercury and cadmium added to the compost. They obtained similar results and in addition they found that the concentration of both elements in pileus tissue is double that of the stipe. Lai (1977) using Mercury -203 labelled compounds

showed that the mycelium could absorb and accumulate these compounds. The appearance of Mercury -203 in sporophores away from where the isotope was applied to the casing, indicates that the mercuric compounds could be transferred by the mycelium and/or move from the casing soils to the non-treated area by watering. Autoradiographs of whole sporophores showed that most of the radioactivity was distributed in the pileus and hymenia.

The concentrations of lead (Leh, 1975) and mercury (Schelenz and Diehl, 1974) in the sporophore were shown to increase as it enlarged and matured.

1.12 The Nutritionally important mineral elements contained in Agaricus bisporus.

The nutritional value of mushrooms has long been a subject of contention and debate. In their recent review of the nutritional value of edible mushrooms, Crisan and Sands (1978) highlight a major problem as the comparability of results obtained in numerous studies. The production of the cultivated mushroom involves the control of many environmental factors, but the composition of the sporophore is primarily governed by two factors which are largely beyond accurate control; the substrates and the strain of Agaricus bisporus used.

Table 1. is a summary of the published evaluations of mineral elements in Agaricus bisporus sporophores. The earliest studies of Hebert and Hein (1909), Ramage (1930) are not included. The concentrations of the elements are all expressed as milligrams per kilogram dry weight. It is evident that a considerable variation in the sporophore mineral composition exists. However, generally the researchers concluded that Agaricus bisporus, when compared with other plant materials, is a good source of many trace elements in

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addition to the major mineral elements. They also found that higher

concentrations of the mineral elements occur in immature than mature

sporophores and that these metals are more abundant in the pileus

than in the stipe tissue.

Table 1.1.

Concentrations of Mineral Elements in the Sporophores of Agaricus
bisporus, from published data.   (Milligrams per Kilogram Dry Weight).

Aluminium   66(19) 3070(1)

Calcium   230(3) 630(26) 700(11) 4360(1) 2903(6) 2750(18) 990(21)
                880(12) 325(19) 4000(8) 2750(14)

Cobalt   0.1(16) 1(8)

Cadmium   0.25(24) 0.15(7) 0.45(15)

Mercury   1(24) 0.13(7) 1.36(2) 0.61(17) 1(25)

Iron   2(3) 83(26) 88(11) 596(10) 19.5(5) 23.5(27) 10(18) 220(21)
                110(12) 2920(19) 150(8) 110(14)

Potassium   47620(3) 43130(26) 28500(11) 7732(6) 40500(22) 50000(5)
                44000(18) 44000(14) 53460(21) 35420(12) 47500(19) 44000(8)

Magnesium   1350(26) 400(1) 1540(12) 1050(8)

Manganese   70.4(10) 66(18) 8.8(21) 11(12) 29.7(19) 7(8)

Molybdenum   3.52(12) 0.7(8)

Sodium   1560(26) 1060(11) 24470(1) 6891(6) 2200(18) 2200(14)
                660(21) 1320(12) 1070(19) 1500(8)

Phosphorus   14290(3) 12080(26) 7900(11) 15280(1) 1500(5) 14300(18)
                14300(14) 12650(21) 11330(12) 21420(19) 12300(8)

Lead   9.2(24) 5.5(13) 0.7(7)

Zinc   66.7(15) 30.8(18) 220(14) 30.8(21) 33(12) 64.5(19) 60(8)

Copper   0.13(3) 30(7) 27(15) 44.3(9) 1.45(5) 71.5(18) 220(14)
                197(12) 128(19) 45(8)

Traces of Titanium, Rubidium, Vanadium, Silver, Bromine, Nickel.

Source in Brackets.

1	Adriano and Cruz	(1933)	15	Meisch et al.	(1977)
2	Aichberger	(1977)	16	Mlodecki et al.	(1965)
3	Altamura et al.	(1967)	17	Pallotti et al.	(1976)
4	Amer and Brisson	(1973)	18	Randoin and Billaud	(1956)
5	Anderson and Fellers	(1942)	19	Sawada	(1965)
6	Charlampowicz et al.	(1973)	20	Schelenz and Diehl	(1974)
7	Collet	(1977)	21	Secchi	(1966)
8	Delmas	(1978)	22	Seeger	
9	Drbal et al.	(1975a)	23	Stijve	(1977)
10	Drbal et al.	(1975b)	24	Stijve and Besson	(1976)
11	F.A.O.	(1972)	25	Stijve and Roschnik	(1977)
12	Khor	(1975)	26	Watt and Merril	(1963)
13	Leh	(1975)	27	Zohm and Langenbein	(1976)
14	Mallet	(1959)			

SECTION 2

METHODS AND MATERIALS

2.1   Production of Agaricus bisporus in the Laboratory.

2.1.1   Growth Cabinets.

         Two types of controlled environment growth cabinets were
used in this study, Plate 2.1 shows three of the larger cabinets
connected to a thermostatically controlled ventilation unit.
The humidity in these cabinets was controlled by heating cables
submerged in moist sand in the base. Thermostatically controlled
heating cables maintained the air temperature at the required
level. At full capacity eighteen square (17 x 17 x 13 centimetres)
polypropylene growing units could be accommodated in any one
experiment.

         Experiments in which radioactive materials were used were
done in smaller cabinets. Four Humex 'Propatray' propagators
were joined together to form a single chamber with a capacity
of sixty smaller (11 x 11 x 9.5 centimetres) polypropylene
growing units. These propagators, like the larger cabinets,
were thermostatically controlled with moist sand in the base
to maintain a high humidity. Ventilation was achieved by
opening the side doors of the propagators.

2.1.2   Compost.

         Pasteurised compost was obtained from W. Darlington and
Sons Ltd., Angmering, Sussex. In this study a single white
strain (Darlington 649) of Agaricus bisporus was used. Grain
spawn was added to the compost at a rate of one percent by
weight. After mixing with spawn the compost was compressed
into the steam sterilised polypropylene growing units: one
kilogram in each of the large growing units and 500 grams in
each of the smaller units.

PLATE 2.1. CONTROLLED ENVIRONMENT GROWTH CABINETS.

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2.1.3   Casing.

The casing soil was prepared from: medium grade sphagnum
peat moss, Morden 'R' powdered chalk and water in the ratio of
8:1:8 by weight respectively. This casing material has a pH
of 7.4.

2.1.4   Conditions of Growth.

The pots of spawned compost were placed in the appropriate
cabinets, which were maintained at $24^{\circ}C$ and 90 percent relative
humidity until the compost was completely colonized or "run
through", usually after about fourteen days. Freshly prepared
casing material was then applied to give an even layer 3.5
centimetres deep on the compost surface. The incubating conditions
were maintained for about another seven to ten days, by which time
the mycelium will just be visible at the casing layer surface.

The induction of sporophore formation was initiated by
decreasing the temperature to $17^{\circ}C$ and the relative humidity to
75 percent and aerating.

These conditions were then maintained throughout the period
of cropping, usually from 21 days to about 60 days after the
application of the casing layer.

2.2   Analysis using Atomic Absorption Spectrophotometry.

2.2.1   Experimental Treatments.

Specific experimental details are outlined in later sections
but, generally the different treatments were restricted to the
addition of iron containing solutions to the compost. These iron
supplements were added on the day prior to the addition of the
casing layer. 25 millilitres of the appropriate iron containing
solution were applied to each kilogram of compost. Supplements

were also added to the surface of the compost to obtain a "plus compost" treatment or, injected half-way down the compost using a six centimetre hypodermic needle to obtain a "minus compost" treatment.

2.2.2   Collection and Preparation of Samples for Analysis.

The use of Atomic Absorption Spectrophotometry for the determination of the concentration of iron in materials requires that the sample under investigation should be brought down to a solution either by extraction or digestion. It is important that these solutions are prepared and stored in extremely clean equipment and containers. Prior to use, all equipment was washed with a phosphate-free detergent followed by several hot water rinses. The containers were then filled with ten percent sulphuric acid and left to stand for at least one hour. Finally they were rinsed three times with glass distilled and deionized water and allowed to dry at room temperature.

2.2.2.1   Harvesting of Sporophores.

Sporophores appear at approximately weekly intervals. About four of these "breaks" are produced in the culture systems used in this study whereas, six or more breaks may be expected in the commercial situation. It is important, if direct comparisons between yields are to be made, that all sporophores are harvested at the same state of development. In common with commercial practice the sporophores were harvested at the open cup stage of development. A detailed description of the phases of development is given in Appendix 3.3.2.1 and illustrated on Plate 3.3.2.1. Sporophores were harvested firstly by twisting to break connection of the mycelial strands and then pulling the sporophore away from

the casing layer. Any adhering casing material was gently
brushed off and the base of the stipe to which casing material
is attached was cut off or "chogged". The individual fresh
weight and numbers of sporophores harvested from each growing
units were recorded daily during cropping.

2.2.2.2 Drying and Grinding of Sporophore Samples.

For storage and subsequent analysis the sporophores were
dried. Because of their high water content the sporophores were
initially dried at 55°C for 48 hours to prevent the exudation of
water droplets and hence the loss of water soluble materials from
the sporophore. The drying was then completed by increasing the
oven temperature to 105°C for a further 24 hours. The individual
dry weights were then measured and the sporophores were immediately
ground and stored in a desiccator.

Dried sporophore tissue is brittle and non-fibrous and the
samples were easily ground to a fine powder using a pestle and
mortar. The sporophores from each box and each break were ground
separately.

2.2.2.3 Digestion of Sporophore Samples.

Allen et al. (1974) recommend mixed acid digestion for bringing
the metals of foodstuffs into solution, by complete destruction of
the organic materials. 2.5 grams of the dried sporophore material
was introduced into each 100 millilitre capacity Kjeldahl flask.
10 millilitres of concentrated Analar Nitric acid was added to each
flask. The flasks were gently warmed until the frothing and evolu-
tion of brown fumes ceased. One millilitre of concentrated Analar
Sulphuric acid and two millilitres of 60 percent Analar Perchloric
acid were then added and the heat of the digestion racks increased.

The digestion was continued for ten minutes following the evolution of white fumes. 20 millilitres of distilled water were then added to each flask and boiled gently for ten minutes, so that all iron was brought into solution. When cool the digests were diluted to 50 millilitres with distilled water. The Kjeldahl flasks were rinsed three times with the water used to make up the volume. The solutions were stored in 60 millilitre capacity polythene bottles.

2.2.2.4 Collection of Substrate Samples.

At least three growing pots were sampled for each treatment at each sampling time. The samples were taken by inverting the growing units onto a clean surface. The substrates separate from the polypropylene pots and could then be divided into three layers, namely: the casing layer, the upper half of the compost and the lower half of the compost. These entire substrate layers were then placed in different beakers.

2.2.2.5 Drying and Grinding of Substrate Samples.

According to Allen et al. (1974) it is preferable to extract some soils in the fresh state and that mineral soils should be dried at no more than 40°C and then passed through a two millimetre sieve. Because of the nature of the substrates used in the cultivation of Agaricus bisporus the conditions of sample preparation and extraction were altered in order to obtain consistent results. Both the compost and the casing are extremely variable in composition and it is difficult to obtain small, homogenous fresh samples. Homogenous samples were prepared by drying and grinding the substrates. Because of the high water and microbial content of the substrates it was necessary to dry the materials at 55°C to prevent degradation which may have occurred at lower temperatures.

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The dried samples were allowed to cool and then each sample was ground in a hammer-mill. The ground samples were stored in a desiccator prior to extraction.

2.2.2.6   Extraction of Substrate Samples.

In this study two extraction techniques were used to determine the quantity of "soluble" and "available" iron in the substrates. The method of extraction was the same in each case, only the extractants were different.

A.   Potassium pyrophosphate extraction.

'Available' iron was extracted using the method of Bascomb (1968). 0.1 molar tetra-potassium pyrophosphate is a mild extractant that displaces iron bound to the adsorption sites of organic complexes by supplying an excess of replacing ions.

2.5 grams of dry, ground compost or casing were added to each 100 millilitre capacity Erlenmeyer Flask. 50 millilitres of 0.1 molar tetra-potassium pyrophosphate and 10 glass balls were also added. The flasks were shaken for 6 hours at 100 revolutions per minute and $20^{o}C$ in an humidified orbital incubator. The substrate suspensions were then filtered (Whatman Number One) into 60 millilitre capacity polythene bottles, in which the samples were stored.

B.   Water Extraction.

'Soluble' iron was extracted using distilled water in place of the potassium pyrophosphate.

2.2.3   Analysis of Samples Using Atomic Absorption Spectrophotometry.

The quantity of iron in the replicated sample solutions and appropriate reagent controls was determined using a Perkin Elmer model 306 Atomic Absorption Spectrophotometer. This has a working

range, for iron, of 0-10 parts per million. Iron is measured at an absorbance wavelength of 248.3 nanometers and is not subject to interference by other elements at this wavelength.

The spectrophotometer was set up for use by adjusting the lamp alignment, burner height and flame temperature to obtain maximum sensitivity. The machine was then calibrated relative to a range of standard solutions. Once calibrated, the machine would display a digital value of the concentration of iron in any solution aspirated through the spectrophotometer flame, provided that it was within the range of measurement. Pyrophosphate extracts of the substrates required 25 fold dilution prior to measurement but, the sporophore digests and water extracts of the substrates required no dilution.

The concentrations of iron in the original dry samples were calculated by subtracting the concentration of the reagent blank from the determined sample solution concentration and then multiplying this result by the dilution factor:

$$\frac{50}{\text{Dry Sample Weight (G)}} \text{ X The number of times diluted}$$

The results were expressed as the concentration of iron in parts per million or milligrams per kilogram dry weight.

2.3   Studies using Iron-59 Isotope.

The isotope was obtained from The Radiochemical Centre, Amersham. One millicurie of Iron-59 was supplied in one millilitre of 0.1 molar hydrochloric acid. This quantity of isotope was found to be sufficient for the treatment of 50 small growing units.

Iron-59 is a powerful beta and gamma emitter and the appropriate safety procedures were, therefore, strictly adhered to during the handling of the isotope. Protective clothing was worn at all times

and all operations involving the handling of the concentrated and diluted isotope were performed in a fume-cupboard.

Glassware that was used in the drying and counting of samples was thoroughly washed and then left to soak in a decontaminating detergent solution for one week prior to re-washing and re-use. Forceps used in the transference of samples were carefully washed between samples and frequent checks for the contamination of equipment were made. All contaminated substances and materials were disposed of in accordance with legal requirements.

2.3.2   Experimental Treatments.

The isotope was diluted to 500 millilitres with distilled water. The precise details of the experimental treatments are given in Section 3 but, in each study the same isotope concentration was applied. 10 millilitres of the isotope solution were added to each growing unit using a syringe and needle.

2.3.3   Collection and Preparation of Samples for Analysis.

Iron-59 emits highly penetrating gamma radiation. The emanations from the isotope contained in a solid sample can be detected and measured using a gamma counter. Using the I.C.N. Gammaset 500 gamma counter in the Department of Biological Sciences at Aston University it was possible to determine the isotope concentration in solid samples to which the Iron-59 had been applied. Two basic requirements had to be met in sample preparation. Firstly the samples were dried and accurately weighed so that a precise isotope concentration could be calculated. Secondly the detection well of the Gammaset 500 was 4.8 centimetres deep, thus the sample in each measuring tube did not exceed 3 centimetres in size.

2.3.3.1 Sporophores.

The sporophores, which were individually numbered, were harvested and dried by the same methods detailed in Section 2.2.2. Individual sporophores were inserted into separate, numbered, glass sample tubes supplied with the gamma counter.

2.3.3.2 Substrates.

Entire growing units were sampled by inverting the pot onto a clean surface. Four replicate samples were taken from each of the four substrate layers: the casing layer, the top, middle and bottom of the compost. The samples were removed from the substrate layers using forceps. Each sample was inserted into a labelled, weighed, glass sample tube, in which the sample was dried at $105^{\circ}C$ for 48 hours prior to counting.

2.3.4 Measurement of the isotope concentration using The Gamma Counter.

The Gammaset 500 counter was equipped with plug-in modules which automatically set the required measuring parameters for the desired isotope. The gamma counter also had automatic sample change and result print out.

The sample tubes were loaded into cassettes which were loaded into the autochanger. The appropriate mode (isotope) and a counting time of one hour were selected and the couting was initiated. Empty tubes were included frequently to monitor background radiation. Control samples, to which no isotope had been applied, were also counted in order to ascertain the presence of natural radioisotope or contamination of samples.

The printed results gave the number of counts per hour of each sample but, because of radioactive decay any comparison of results

required that they should be expressed at a single standard time.
Each batch of isotope had an activity date at which time the iso-
tope had an activity of one millicurie. This activity date was
designated 'Zero' time. For every sample the number of hours
that had elapsed from zero time to when the sample was measured
were recorded. Using a modification of the radioactive decay
formula $N_+ = N_0 e^{-\lambda t}$ each sample activity was expressed at the
standard 'Zero' time. The correction used was:

$$\text{Corrected Count/Hour} = \frac{(\text{Sample Count Per Hour}) - \text{Control Count Per Hour})}{2.71828^{(-6.376692 \times 10^{-4} \times T)}}$$

where: T = The Elapsed time in hours from 'Zero' time.

-6.376692×10^{-4} = The Decay constant of Iron-59 (Hours).

The results were then expressed as the quantity of isotope
(Counts per hour per gram dry weight) in each sample.

2.4   Statistical Analysis of Results.

2.4.1   Replication of Samples.

The number of replicate samples used in these studies depended
on the type of study under investigation. The replication of the
different studies is given in Section 3. The mean values and the
standard deviations of these means were calculated for the
replicated samples.

2.4.2   T-test.

The significance of any difference between the means of
different treatments was calculated using the t-test, where:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\dfrac{\sigma_1^2}{n_1} + \dfrac{\sigma_2^2}{n_2}}}$$

$\bar{x}$ = The Mean

σ = The Standard Deviation

n = The Number of Replicates

1 and 2 are the treatments to be compared.

The significance of a calculated value of t was gauged by comparing it with values from a table of t for $n_1 + n_2 - 2$ degrees of Freedom.

A probability level of 0.05 is generally considered the minimum probability at which two means can be considered significantly different. Unless otherwise stated in the text, the significance of the results was given at this level of probability.

2.4.3   Confidence Limits.

95% Confidence Limits together with the mean were plotted in the Figures in Section 3 to give a visual impression of the variability of the results. The confidence limits of the means were calculated using:

$$t \times \frac{\sigma}{\sqrt{n}}$$ where t = Value of t for n-1 Degrees of Freedom and a Probability of 0.05 (= 95%)

n = The number of replicates

σ = The Standard Deviation.

The confidence limit value was then added to and subtracted from the mean to give the upper and lower limits. 95% of samples taken from that population would then lie within these limits. Normally only the confidence limit value was included in the tables. However, in radiotracer studies the logarithms of the upper and lower limits were included in the tables.

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2.4.4   Correlation and Linear Regression.

The relationship between two variables was determined by calculating the Correlation Coefficient between the two variables using:

The Correlation Coefficient $r = \dfrac{\sum((x - \bar{x})(y - \bar{y})}{\sqrt{(\sum(x - \bar{x})^2 \sum(y - \bar{y})^2)}}$

The significance of the calculated values of r were gauged by comparing them with values from a table of r using n-1 Degrees of Freedom.

When two variables were connected in the form of a linear relationship, the straight line best fitting this relationship was calculated using a Linear Regression procedure. A linear equation is given by y = mx + b, the slope (m) and y intercept (b) of this line can be calculated using:

$$m = \frac{\dfrac{\sum x \sum y}{N} - \sum xy}{\dfrac{(\sum x)^2}{n} - \sum x^2}$$

$$b = \bar{y} - m\bar{x}$$

The values of m and b then allow the plotting of a straight line best fitting the data.

All statistical analysis in this study was done using a Texas TI 51 III programmable calculator.

SECTION 3

EXPERIMENTS AND RESULTS

3.1   FACTORS INFLUENCING THE CONCENTRATION OF IRON IN THE

SPOROPHORES OF AGARICUS BISPORUS.

3.1.1   Introduction.

There have been many studies which have demonstrated the extreme variability of the composition of Agaric sporophores. Many factors have been suggested which may contribute to the observed variation but, the influence that different growing conditions exert over the composition has received little attention. However, from the information available it can be expected that sporophore composition is dependent on the size and number of individuals produced from a given substrate.

The objective of this section of the study was, therefore, to quantify the iron composition of the sporophore and to determine the effect of strain, alternative casing media, watering and supplementation of cultures with iron salts on this composition.

3.1.2   Strains.

Many different strains of Agaricus bisporus are used in the commercial cultivation of mushrooms. Four of the more commonly used white strains 621, 735, 649 and LX10 were grown in accordance with the methods outlined in Section 2.1. The iron composition of the sporophores produced were determined according to the methods given in Section 2.2 and are shown in Table 3.2.

Generally there were no significant differences between the iron composition of the different strains (Table 3.1). However, the sporophores from the second break of strain 735 contained a significantly higher concentration of iron than did those of strains 649 and LX10. In all strains the concentration of iron

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increased in successive breaks.

In the three strains; 735, 649 and LX10, similar yields were obtained and in all three there were negative correlations (-0.9996, -0.8351 and -0.843 respectively) between the dry weight yields and sporophore iron composition in successive breaks. This was only significant in strain 735. In strain 621, which produced only half the yield of the other strains, there was a slightly significant positive correlation of 0.9163 between the yield and sporophore composition in successive breaks.

The quantity of iron taken up into the sporophores (Table 3.3) of strain 621 increased in successive breaks but, in the other strains the trend was towards decreasing iron uptake. The total quantity of iron taken up was approximately equal in each strain, including 621 which had a considerably lower total yield than the other three strains. In all strains there were positive correlations between the dry weight yields and the quantity of iron taken up into the sporophores of different breaks. Strains 621 (0.991) and LX10 (0.993) were significant at a probability of 0.01 and Strains 735 (0.946) and 649 (0.940) were significant at a probability level of 0.1.

These results show that the quantity of iron contained in the sporophores of different strains in different breaks was proportional to the yield produced in those breaks. There was, however, no consistent relationship between the concentration of iron and the yields of different sporophore strains during culture. In each of the strains the concentration of iron increased in successive breaks, probably indicating the increased availability of iron during culture.

TABLE 3.1. DRY WEIGHT YIELDS OF DIFFERENT STRAINS.
(GRAMS PER KILOGRAM FRESH COMPOST.)

STRAIN	FIRST BREAK	SECOND BREAK	THIRD BREAK	TOTAL
621	3.841	4.050	6.814	14.71
735	14.36	6.424	1.948	22.73
649	12.10	6.846	2.893	21.84
LX 10	7.092	11.00	3.505	21.60

TABLE 3.2. CONCENTRATION OF IRON IN SPOROPHORES OF DIFFERENT
STRAINS. (MILLIGRAMS PER KILOGRAM DRY WEIGHT.)

STRAIN	FIRST BREAK	SECOND BREAK	THIRD BREAK
621	30.0 (5.783)	43.28 (13.07)	59.0 (20.08)
735	28.77 (17.21)	47.81 (7.338)	57.4 (9.180)
649	20.47 (7.939)	21.13 (9.51)	45.97 (8.79)
LX 10	27.85 (8.960)	28.06 (4.79)	37.23 (17.07)

Mean (Standard Deviation) of 3 Replicates.

TABLE 3.3. IRON CONTENT PER BREAK AND TOTAL IRON CONTENT IN
SPOROPHORES OF DIFFERENT STRAINS. (MICROGRAMS.)

STRAIN	FIRST BREAK	SECOND BREAK	THIRD BREAK	TOTAL
621	115.2	175.3	402.0	692.5
735	413.2	307.1	111.8	832.1
649	247.8	144.7	133.0	525.5
LX 10	197.5	308.7	130.5	636.7

3.1.3   Different Casing Materials.

In 1949 Courtieu suggested that the casing layer has a role
in sporophore nutrition because, he obtained slight correlations
between the levels of Calcium, Potassium and Phosphorus in the
casing and in the mushrooms derived from them. Bels-Koning (1950)
however, suggested that the casing layer provides no nutritive
elements. Ganney and Richardson (1973) observed extreme varia-
bility of the minerals contained in twelve commercially used
casing mixtures and questioned if any importance could be attached
to the chemical make-up of the casing layer.

Hayes et al. (1979) describe the use of a waste product from
a pulp and paper mill as a casing material. The success of this
material, designated PMB, as a casing medium provided the basis
of a comparison between the iron composition of sporophores
cultured on different casing soils. Wooden trays each containing
75 kilograms (dry weight) of compost were cased with the following
casing materials:

1.   Sphagnum Moss Peat Casing.

2.   PMB Casing - 100% Paper Mill By-product.

3.   PMB and Horticultural Chipped Bark in a 1:1 mixture.

Ten open cup sporophores were harvested from each of four replicate
trays in the first, second and fifth breaks of the three different
casing treatments. Also forty large and forty small sporophores
were taken from the different casing treatments in the second
break.

There were no significant differences between the yields
produced from the three different casing materials (Table 3.4).

The concentrations of iron in sporophores cultured on
different casing layers are shown in Table 3.5. In the first

TABLE 3.4. FRESH WEIGHT YIELDS PRODUCED FROM DIFFERENT CASING MATERIALS. (KILOGRAMS PER TRAY).

CASING MATERIAL	FIRST BREAK	SECOND BREAK	FIFTH BREAK
PEAT	21.94 (5.921)	11.11 (4.153)	1.554 (0.769)
PMB	22.38 (2.90)	11.69 (1.239)	2.166 (0.988)
PMB+BARK	25.62 (1.707)	9.639 (1.438)	1.542 (1.134)

Means and (Standard Deviations) of 4 Replicates.

TABLE 3.5. CONCENTRATIONS OF IRON IN SPOROPHORES HARVESTED FROM DIFFERENT CASING MATERIALS. (MILLIGRAMS PER KILOGRAM).

CASING MATERIAL	FIRST BREAK			SECOND BREAK			FIFTH BREAK		
	Mean	S.D	n	Mean	S.D	n	Mean	S.D	n
PEAT	41.38	2.654	5	41.20	10.45	30	34.80	4.324	4
PMB	31.01	5.492	5	40.44	11.42	31	44.81	3.60	6
BARK + PMB	34.50	4.567	5	28.88	7.448	28	53.73	13.48	6

TABLE 3.6. CONCENTRATIONS OF IRON IN LARGE AND SMALL SECOND BREAK SPOROPHORES HARVESTED FROM DIFFERENT CASING MATERIALS. (MILLIGRAMS PER KILOGRAM DRY WEIGHT).

CASING MATERIAL	SMALL SPOROPHORES			LARGE SPOROPHORES		
	Mean	S.D	n	Mean	S.D	n
PEAT	45.06	5.449	12	38.50	12.20	18
PMB	50.24	10.26	13	37.60	8.709	18
PMB+BARK	32.52	7.222	14	25.24	5.878	14

n = Number of Samples.
Mean = Mean of those samples.
S.D = Standard Deviation of those samples.

break, sporophores harvested from the peat cased beds contained a significantly greater concentration of iron than sporophores derived from PMB cased beds. In the second break, the sporophores produced from beds cased with the mixture of PMB and Bark contained a significantly lower concentration of iron than sporophores from the other treatments. Sporophores in the fifth break from the peat casing contained significantly less iron than sporophores from the casing materials containing PMB.

The earlier stages of sporophore development in each treatment contained higher concentrations of iron (significant in the casing mixtures containing PMB) than did more mature sporophores.

Although analysis of iron was only made on sporophores in three out of the five breaks harvested, it would appear that similar total quantities of iron were taken up into the sporophores of the different casing treatments. It is clear, however, that the type of casing layer does influence the iron composition of the sporophore.

3.1.4 Different Watering Regimes.

Numerous studies have shown the effect of watering on the production of the crop and it might be expected that different rates of water application to the substrates would result in changes of sporophore composition. However, Haddad (1977) found that the carbohydrate, protein and lipid composition of sporophores varied inconsistently with different watering regimes.

It was the intention of this study to determine if any relation-ship exists between; the rate of applying water, the yield and the iron composition of the sporophores. Three watering regimes were used and in each the water was applied daily to the six replicate

growing boxes of each treatment. In the Low Watering Regime 10 millilitres of water were applied to each box daily. This quantity of water was sufficient to prevent the casing surface from drying out. In the High Watering Regime 80 millilitres were added to each box daily. In this treatment the casing layer remained waterlogged for several hours following each application. Forty millilitres per day were added to the boxes of the Medium Watering Regime. The Low and High treatments represented the extremes of watering likely to be encountered in the commercial situation.

The mean daily fresh weight yields and the pattern of cropping of the three treatments are given in Table 3.7 and Figure 3.1. Low watering produced a large (22.5) total number of sporophores each of small (5.6g) average fresh weight. Whereas, in the high watering regime, fewer sporophores (8.85) each with a much larger average fresh weight (19.6g) were produced. The medium watering regime represents an intermediate state between the extremes and produced a mean of 14.67 sporophores each averaging 14.41 grams fresh weight. The total fresh weight yield was adversely affected by the addition of either too much or too little water to the casing layer, the greatest yield was obtained in the medium watering regime.

Different watering regimes considerably altered the cropping behaviour of the cultures. In the low watering regimes sporophores were produced in definite breaks. However, with increasing addition of water the sporophores were harvested more frequently.

The percentage dry weight of the sporophores from the treatments are shown in Table 3.9. The sporophores produced from the low watering regime contained significantly more dry matter than

FIGURE 3.1. DAILY FRESH WEIGHT YIELDS OF SPOROPHORES HARVESTED FROM GROWING UNITS TREATED WITH LOW, MEDIUM AND HIGH WATERING REGIMES.
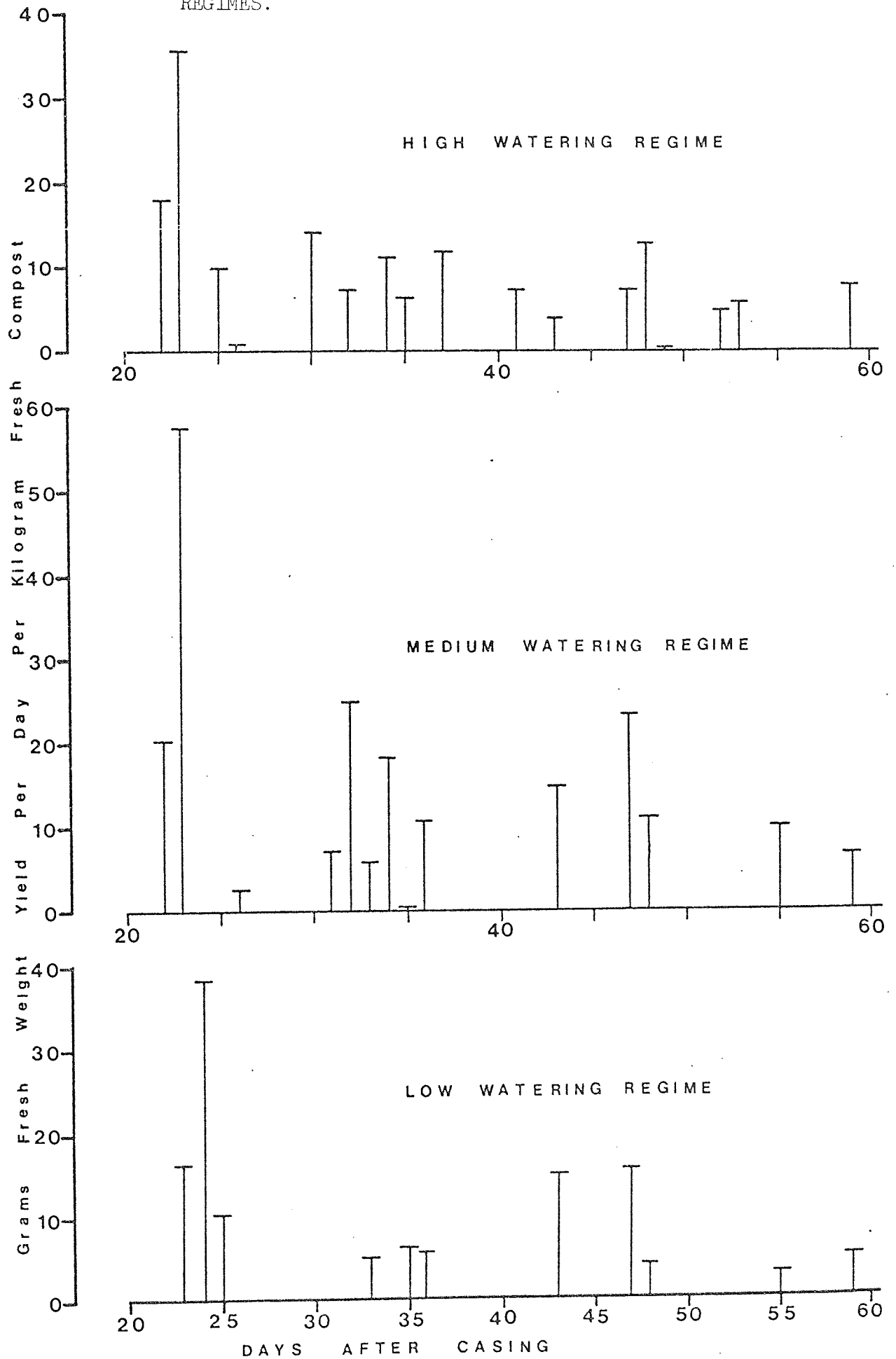
TABLE 3.7.   NUMBERS AND FRESH WEIGHT YIELDS OF SPOROPHORES HARVESTED
DAILY FROM GROWING UNITS TREATED WITH LOW, MEDIUM, AND
HIGH WATERING REGIMES.

DAYS AFTER CASING	LOW WATERING		MEDIUM WATERING		HIGH WATERING	
	No.	Wt.	No.	Wt.	No.	Wt.
22			1.33	20.43	1.00	27.90
23	1.17	16.57	3.33	57.49	1.67	35.56
24	3.83	38.40				
25	1.00	10.47			0.67	9.85
26			0.17	1.32	0.17	0.78
30					0.50	14.11
31			0.17	7.18		
32			2.5	24.87	0.33	7.29
33	0.67	5.14	0.33	5.82		
34			1.17	18.46	0.67	11.04
35	1.00	6.40	0.17	0.30	0.17	6.23
36	0.50	5.70	0.67	10.61		
37					0.83	11.78
41					0.67	7.20
43	3.50	15.13	0.83	14.66	0.50	3.78
47	5.00	15.74	2.17	23.29	0.33	7.21
48	3.33	4.05	1.00	10.94	0.50	12.69
49					0.17	0.35
52					0.17	4.64
53					0.33	5.49
55	0.83	3.15	0.33	9.77		
59	1.17	5.24	0.50	6.29	0.17	7.68
TOTAL	22.5	126.0	14.67	211.4	8.85	173.6

No.= Mean number of sporophores per growing unit(6 replicates).
Wt.= Mean fresh weight(grams) per growing unit (6 replicates).

TABLE 3.8. DRY WEIGHT YIELDS OF SPOROPHORES PER BREAK FROM
GROWING UNITS TREATED WITH LOW,MEDIUM,AND HIGH
WATERING REGIMES. (GRAMS).

	LOW WATERING			MEDIUM WATERING			HIGH WATERING		
BREAK	Mean	S.D	n	Mean	S.D	n	Mean	S.D.	n
FIRST	5.43	2.72	6	5.22	1.60	6	5.69	2.61	6
SECOND	3.68	0.87	4	4.42	1.78	6	4.23	1.74	6
THIRD	3.52	0.80	6	3.83	1.01	6	2.60	1.66	6
FOURTH	2.35	1.03	2	1.79	0.47	4	3.32	0.17	2
TOTAL	12.19	3.91	6	14.66	4.27	6	13.63	5.05	6

TABLE 3.9. PERCENTAGE DRY WEIGHTS OF SPOROPHORES HARVESTED
FROMGROWING UNITS TREATED WITH LOW,MEDIUM,AND HIGH
WATERING REGIMES.

	LOW WATERING			MEDIUM WATERING			HIGH WATERING		
BREAK	Mean	S.D	n	Mean	S.D.	n	Mean	S.D	n
FIRST	8.56	2.46	8	6.83	1.00	10	7.09	1.16	8
SECOND	8.59	2.11	6	6.98	0.93	10	7.06	0.93	8
THIRD	11.14	4.25	7	7.69	2.00	9	7.62	1.23	9
FOURTH	9.20	0.87	2	7.61	0.88	4	8.70	2.48	2
TOTAL	9.41	3.05	23	7.10	1.43	33	7.38	1.23	27

n   = Number of samples.

Mean = Mean of those samples.

S.D = Standard Deviation of those samples.

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those from the two other regimes, which were not significantly different from each other.

The dry weight yields, both total and for separate breaks, (Table 3.8) were very similar in each treatment. The differences in fresh weight yield of the treatments can be attributed to the water content of the sporophores.

The iron composition of sporophores are shown in Table 3.10. In the first break, sporophores from the low watering treatment contained a significantly lower concentration of iron than the sporophores from the two other treatments. Sporophores from the low watering regime contained significantly lower concentrations of iron than those from the high watering treatment in the second break and than those from the medium watering regime in the third break. The quantity of iron taken up into the sporophores of each break in the three treatments is given in Table 3.11. There was a significant ($P = 0.1$) correlation between the total amount of iron taken up and the total yields, both fresh (0.926) and dry (0.937). However, there was a highly significant ($P = 0.001$) negative correlation (-0.999) between the total amount of iron taken up in the three treatments and the overall percentage dry weight of the sporophores in those treatments. This suggests that the amount of iron contained in the sporophores was directly proportional to the water content. Thus, probably indicating that iron was translocated into the sporophore in a water soluble form. The total quantity of iron contained in the sporophores was similar in the high and medium watering regimes but, appreciably lower in the low watering treatment. Possibly a smaller quantity of water (and substances dissolved in it) was available for sporophore

TABLE 3.10. CONCENTRATIONS OF IRON IN SPOROPHORES HARVESTED

FROM GROWING UNITS TREATED WITH LOW,MEDIUM,AND

HIGH WATERING REGIMES. (MG PER KG DRY WEIGHT).

BREAK	LOW WATERING			MEDIUM WATERING			HIGH WATERING		
	Mean	S.D	n	Mean	S.D	n	Mean	S.D	n
FIRST	15.77	2.15	6	23.11	3.82	5	21.01	3.67	6
SECOND	21.35	2.56	2	20.59	8.02	4	29.63	3.91	5
THIRD	16.68	3.01	3	28.19	4.96	3	25.76	5.98	2

TABLE 3.11. IRON CONTENT PER BREAK AND TOTAL IRON CONTENT OF

OF SPOROPHORES HARVESTED FROM GROWING UNITS TREATED

WITH LOW,MEDIUM,AND HIGH WATERING REGIMES. (MICROGRAMS)

BREAK	LOW WATERING	MEDIUM WATERING	HIGH WATERING
FIRST	85.60	120.49	119.55
SECOND	78.64	90.94	125.41
THIRD	58.78	107.96	66.96
TOTAL	223.02	319.39	311.92

n   = Number of samples.

Mean = Mean of those samples.

S.D = Standard·Deviation of those samples.

construction in the low watering treatment and therefore, the
sporophores contained less iron and other water soluble
substances.

The results of this study show that the number of sporophores
produced and the concentration of iron that they contain were
influenced by the rate at which water was applied to the casing
layer. The total amount of dry matter used in sporophore construc-
tion was independent of the quantity of water applied to the casing
layer but, the production of a maximum fresh yield required the
addition of some water to the culture. However, when applied in
excess, the water caused a slightly decreased fresh weight yield.
The quantity of iron in the sporophores was proportional to the
amount of water they contained.

3.1.5   Supplementation of the Compost with Inorganic Iron Salts.

Hayes (1972) and Kaul and Kachroo (1976) reported that in
some instances the addition of ferrous salts to the casing soil
increased the numbers of primordia and yields of A. bisporus.
Increased yields have frequently been observed when many different
organic materials are added to the compost but, there is no evidence
that these supplements directly cause changes in sporophore composi-
tion. However, the heavy metal content of sporophores has been
shown by several researchers to be directly proportional to the
quantity of heavy metal contaminants added to the compost.

The objective of this section of the study was, therefore,
to determine if the addition of iron containing salts to the
compost could significantly increase the quantity of iron
contained in the sporophores.

3.1.5.1   Different Concentrations of Ferrous Sulphate.

Ferrous sulphate solutions were added to the compost in accordance with the methods outlined in Section 2.2. Four replicates were prepared for each of the 100, 200 and 300 parts per million treatments and six replicates for the control. The harvested sporophores were carefully dissected and divided into; the stipe, the pileus and the hymenia. The results of iron analysis in the pooled, replicate samples are given in Table 3.13. There were no significant differences in the sporophore iron composition, either between the different treatments or between the different tissues. Overall the concentration of iron in the different tissues was very similar. There were no significant differences between the yields of the different treatments shown in Table 3.12.

3.1.5.2   Ferrous Sulphate Applied to the Upper and Lower Compost.

25 ml of ferrous sulphate solution containing 200 milligrams of iron were added to the compost surface or injected into the compost to give the appropriate upper and lower supplementation treatments. Eighteen replicate boxes were prepared in accordance with the methods in sections 2.1 and 2.2 for each treatment and a control.

Boxes were removed for substrate sampling 4, 13, 27 and 39 days after casing and thus the number of replicates decreased in successive breaks but, sporophore samples were taken from a minimum of six replicate boxes in the third break.

There were no significant differences between yields of the three treatments in any of the breaks (Table 3.14). There were no significant differences of the concentration of iron in the

sporophores, either between breaks or between treatments (Table 3.15). The concentration of iron remained approximately constant in all four breaks. The quantity of iron taken up into the sporophores, shown in Table 3.16, was proportional to the yields and, therefore, the overall quantity of iron taken up decreased in successive breaks. The correlations between the yield and quantity of iron taken up in the successive breaks were significant in each of the treatments and overall. (Control 0.992, Plus Compost 0.994, Minus Compost 0.958 and Overall 0.983).

Thus, supplementation of the upper or lower compost with ferrous sulphate did not cause an increased yield or concentration of iron in the sporophores.

3.1.5.3   Different Inorganic Iron Salts.

In this study different iron containing salts were applied at a rate of 200 parts per million to the compost surface of three replicates boxes per treatment. Growing procedures and analytical techniques were done in accordance with the methods outlined in Sections 2.1 and 2.2.

There were no significant differences of either the dry weight yields (Table 3.17) or the sporophore iron composition (Table 3.18) between any of the treatments.

There were variable negative correlations between the sporophore iron composition and the dry weight yields in the different treatments (Table 3.20). Overall the negative correlation between the two was significant at a probability of 0.01. This may indicate that a limited quantity of iron was available for uptake into the sporophores and thus, the concentration of iron in those sporophores was related to the yield produced. High yields resulted

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in low iron concentrations in the sporophore and <u>vice versa</u>.

In Table 3.19 it can be seen that overall, the amount of iron taken up into the sporophores of each break decreased in successive breaks. In three of the treatments there was a significant correlation between the yields and iron content and overall there was a significant relationship between yield of sporophores and the quantity of iron they contained.

In common with 3.1.5.1 and 3.1.5.2, supplementation of the compost with iron salts failed to cause significant changes in sporophore iron composition.

TABLE 3.12. FRESH WEIGHT YIELDS OF SPOROPHORES HARVESTED FROM
COPOSTS SUPPLEMENTED WITH DIFFERENT CONCENTRATIONS
OF FERROUS SULPHATE. (GRAMS PER KILOGRAM OF COMPOST).

TREATMENT	FIRST BREAK			SECOND BREAK			TOTAL		
	Mean	S.D	n	Mean	S.D	n	Mean	S.D	n
CONTROL	73.57	8.96	6	132.10	24.39	6	205.67	17.13	6
100 ppm	63.39	18.90	4	110.29	44.96	4	173.68	51.33	4
200 ppm	84.89	28.12	4	108.55	21.86	4	193.44	31.92	4
300 ppm	71.82	20.05	4	157.17	27.14	4	228.99	32.34	4

TABLE 3.13. CONCENTRATIONS OF IRON IN DIFFERENT TISSUES OF
SPOROPHORES HARVESTED FROM COMPOSTS SUPPLEMENTED WITH
DIFFERENT CONCENTRATIONS OF FERROUS SULPHATE.
(MILLIGRAMS PER KILOGRAM DRY WEIGHT).

TREATMENT	STIPE			PILEUS			HYMENIUM			
	Mean	S.D	n	Mean	S.D	n	Mean	S.D	n	
CONTROL	46.5	4.4	6	51.5	14.4	5	63.8	27.3	5	
100 ppm	57.6	11.7	2	84.1	30.0	3	45.2	6.8	4	FIRST BREAK
200 ppm	40.1	3.1	4	36.6	2.8	4	50.8	12.1	4	
300ppm	73.8	22.2	2	58.8	24.4	4	66.5	26.4	3	
OVERALL	54.5	14.7	4	57.8	19.9	4	56.6	10.2	4	
CONTROL	46.8	12.9	6	76.4	16.6	6	57.4	8.9	6	
100 ppm	59.2	25.0	3	61.9	21.0	3	63.9	6.5	2	SECOND BREAK
200 ppm	90.8	27.2	4	67.2	12.5	4	65.3	14.5	4	
300 ppm	70.7	17.0	4	55.8	27.4	4	71.6	45.2	4	
OVERALL	66.9	18.7	4	65.3	8.7	4	64.6	5.8	4	

n   = Number of samples.
Mean = Mean of those samples.
S.D = Standard Deviation of those samples.

TABLE 3.14. DRY WEIGHT YIELDS OF SPOROPHORES HARVESTED FROM
COMPOSTS SUPPLEMENTED WITH FERROUS SULPHATE IN THE
UPPER OR LOWER LAYERS. (GRAMS PER KILOGRAM FRESH
WEIGHT OF COMPOST).

	FIRST BREAK			SECOND BREAK			THIRD BREAK			FOURTH BREAK		
TREATMENT	Mean	S.D	n	Mean	S.D	n	Mean	S.D	n	Mean	S.D	n
CONTROL	9.21	3.03	12	9.95	4.35	10	2.69	1.00	8	1.19	0.88	2
+ COMPOST	6.98	1.45	12	8.77	3.98	9	2.61	1.18	7	1.97		1
− COMPOST	7.96	1.18	12	10.6	2.69	10	3.25	1.07	6	1.46		1
OVERALL	8.05	2.33	36	9.81	3.67	29	2.82	1.06	21	1.81	1.35	4

TABLE 3.15. CONCENTRATIONS OF IRON IN SPOROPHORES HARVESTED
FROM COMPOSTS SUPPLEMENTED WITH FERROUS SULPHATE
IN THE UPPER OR LOWER LAYERS. (MILLIGRAMS PER
KILOGRAM DRY WEIGHT).

	FIRST BREAK			SECOND BREAK			THIRD BREAK			FOURTH BREAK		
TREATMENT	Mean	S.D	n	Mean	S.D	n	Mean	S.D	n	Mean	S.D	n
CONTROL	52.31	25.25	12	52.66	35.83	10	64.00	41.52	8	53.41	3.75	2
+ COMPOST	49.58	23.17	12	47.38	31.24	11	48.59	8.29	7	63.45		1
− COMPOST	66.92	68.87	12	50.32	34.67	10	43.74	6.50	7	45.79		1
OVERALL	56.27	43.81	36	50.03	32.80	31	51.81	25.86	22	54.02	7.56	4

TABLE 3.16. IRON CONTENT PER BREAK AND TOTAL IRON CONTENT OF
SPOROPHORES HARVESTED FROM COMPOSTS SUPPLEMENTED
WITH FERROUS SULPHATE IN THE UPPER OR LOWER
LAYERS. (MICROGRAMS).

TREATMENT	FIRST BREAK	SECOND BREAK	THIRD BREAK	FOURTH BREAK	TOTAL BREAK
CONTROL	480.9	454.2	148.2	100.6	1183.9
+ COMPOST	346.4	390.5	130.2	125.0	992.1
− COMPOST	532.9	515.9	145.8	66.9	1261.5
OVERALL	453.4	453.5	141.4	97.5	1145.8

        n   = Number of samples.
     Mean = Mean of those samples.
       S.D = Standard Deviation of those samples.
  + COMPOST = Ferrous sulphate applied to compost surface.
  − COMPOST = Ferrous sulphate injected into compost.

TABLE 3.17. DRY WEIGHT YIELDS OF SPOROPHORES HARVESTED FROM
COMPOSTS SUPPLEMENTED WITH DIFFERENT INORGANIC
IRON SALTS. (GRAMS PER KILOGRAM FRESH WEIGHT OF
COMPOST).

TREATMENTS	FIRST BREAK	SECOND BREAK	THIRD BREAK	TOTAL
FERROUS CHLORIDE	5.36 (2.82)	12.76 (1.51)	2.98 (1.00)	21.10 (2.24)
FERRIC CHLORIDE	8.73 (1.85)	11.07 (1.05)	3.76 (0.76)	23.56 (1.96)
FERRIC SULPHATE	8.71 (2.78)	11.61 (1.99)	3.54 (0.39)	23.86 (1.00)
FERRIC NITRATE	8.46 (1.48)	11.27 (0.73)	3.88 (0.34)	23.54 (0.50)
FERROUS SULPHATE	6.70 (2.93)	13.41 (0.51)	3.59 (0.61)	23.70 (2.06)
CONTROL	9.93 (1.29)	9.75 (1.59)	4.44 (1.71)	24.12 (1.93)

Means and (Standard Deviation) of 3 Replicates.

TABLE 3.18. CONCENTRATIONS OF IRON IN SPOROPHORES HARVESTED FROM
COMPOSTS SUPPLEMENTED WITH DIFFERENT INORGANIC IRON
SALTS. (MILLIGRAMS PER KILOGRAM DRY WEIGHT).

TREATMENTS	FIRST BREAK	SECOND BREAK	THIRD BREAK
FERROUS CHLORIDE	76.22 (41.38)	38.64 (7.61)	136.0 (113.2)
FERRIC CHLORIDE	107.3 (78.77)	50.15 (30.39)	75.62 (36.27)
FERRIC SULPHATE	85.06 (88.54)	96.04 (113.3)	124.8 (99.60)
FERRIC NITRATE	111.3 (75.05)	41.95 (6.39)	75.84 (27.79)
FERROUS SULPHATE	99.74 (106.1)	42.93 (23.40)	67.50 (19.98)
CONTROL	42.77 (23.40)	43.03 (10.62)	163.8 (123.5)
OVERALL	87.07 (71.67)	52.44 (49.77)	103.6 (78.98)

Means and (Standard Deviation) of 5 Replicates.

TABLE 3.19. IRON CONTENT PER BREAK AND TOTAL IRON CONTENT OF
            SPOROPHORES HARVESTED FROM COMPOSTS SUPPLEMENTED
            WITH DIFFERENT INORGANIC IRON SALTS. (MICROGRAMS).

TREATMENT	FIRST BREAK	SECOND BREAK	THIRD BREAK	TOTAL
FERROUS CHLORIDE	408.4	493.0	405.6	1307.0
FERRIC CHLORIDE	937.2	555.0	284.9	1777.1
FERRIC SULPHATE	741.1	1114.6	442.0	2297.7
FERRIC NITRATE	942.1	472.8	288.4	1703.3
FERROUS SULPHATE	668.4	575.6	242.2	1486.2
CONTROL	424.9	419.6	726.8	1571.3
OVERALL	687.0 (235.5)	605.1 (255.9)	398.3 (178.4)	1690 (340.4)

Means and (Standard Deviations) of 6 replicates.

TABLE 3.20. CORRELATIONS BETWEEN DRY WEIGHT YIELDS AND:
            A.CONCENTRATION OF IRON IN SPOROPHORES AND
            B.CONTENT OF IRON IN SPOROPHORES.

TREATMENT	A	B
FERROUS CHLORIDE	- 0.913	0.979 *
FERRIC CHLORIDE	- 0.253	0.590
FERRIC SULPHATE	- 0.806	0.975 *
FERRIC NITRATE	- 0.360	0.403
FERROUS SULPATE	- 0.608	0.389
CONTROL	- 0.999 ***	- 0.999 ***
OVERALL	- 0.611 **	0.478 *

*   Significant at probability of 0.05.
**   Significant at probability of 0.01.
*** Significant at probability of 0.001.

SECTION 3.2   FACTORS INFLUENCING THE CONCENTRATION OF IRON IN THE

SUBSTRATES DURING THE CULTURE OF AGARICUS BISPORUS.

3.2.1   Introduction.

During the growth and production of the Agaricus bisporus
crop, a loss of organic material occurs in the compost and results
in the accumulation of mineral elements. In addition the upward
diffusion of soluble salts from the compost results in the
accumulation of these salts in the casing layer.

The experiments in this section consider the quantitative
changes of iron that occur in the compost and casing substrates
during the culture of Agaricus bisporus and considers the influence
of a number of factors which may contribute to these changes.

3.2.2   Extractable Iron in the Substrates during Culture.

In this initial study the quantities of extractable iron in
the compost and casing were observed during the induction of
sporophore formation and cropping. Extraction with distilled
water was used to determine the quantity of freely available
iron. The extractant; tetra-potassium pyrophosphate was used
to estimate the amount of iron bound to the organic materials
of the substrates. Both extraction procedures were used in
accordance with the methods outlined in Section 2.2. The entire
casing layers, upper halves and lower halves of the compost were
sampled from six replicated growing units at 7, 14, 21, 28, 35
and 48 days after casing. The results of the extractions of
these substrate samples are given in Appendix 3.1 and are shown
in Figures 3.2 and 3.3.

3.2.2.1   <u>Water Extractable Iron</u> (Figure 3.2).

Overall there was a slightly increased concentration of water extractable iron in the casing layer with time. However, there was a sharply increased quantity of water extractable iron in the casing layer following the harvesting of the first break sporophores. This then declined throughout cropping. It is likely that harvesting of the sporophores caused considerable damage to the mycelial strands in the casing layer and thus allowed the leakage of materials into that substrate. Possibly the observed increase of iron was not purely the result of iron leaked from the disrupted mycelium but, was also caused by the enhanced activity of bacteria utilising other substances leaked into the casing layer.

In both the upper and lower compost samples, the quantity of water extractable iron decreased 14 days after casing, approximately coinciding with the time of sporophore initiation. This decrease may be associated with either, the increased uptake of iron and other materials into the mycelium, in readiness for sporophore production, or, a decreased mycelial activity in the compost during the initiation of sporophores in the casing layer. The concentration of water extractable iron then increased steadily with time until day 35. Following this time water extractable iron in the compost declined to the end of the experiment. These changes may be the result of the mycelial activity in the compost. During cropping this activity was high but, between 35 and 48 days after casing the culture ceased to produce sporophores probably indicating a decline in mycelial activity.

FIGURE 3.2.   CONCENTRATIONS OF WATER EXTRACTABLE IRON IN THE
SUBSTRATES DURING CULTURE.
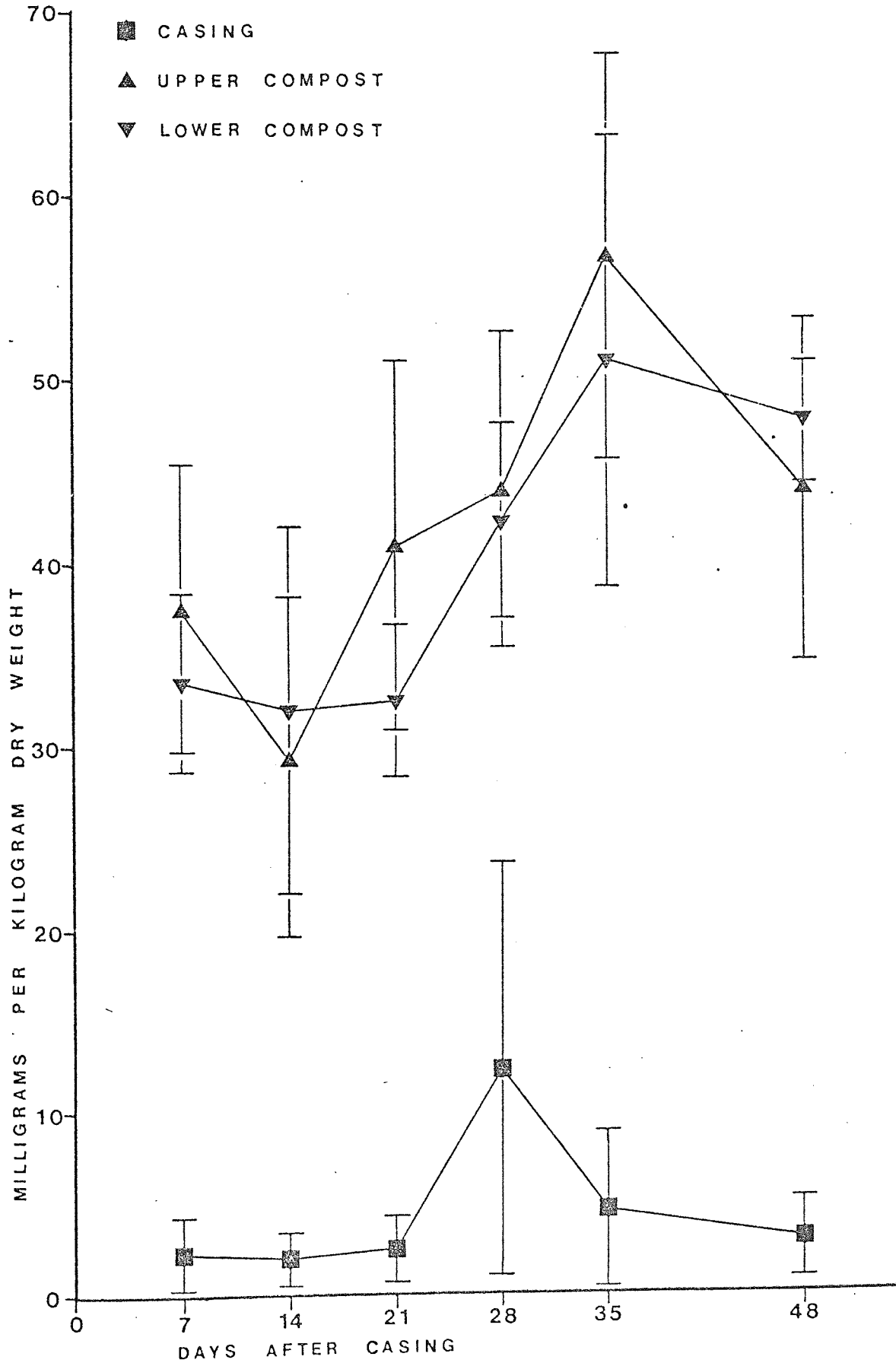
3.2.2.2 <u>Pyrophosphate Extractable Iron</u> (Figure 3.3).

The concentration of pyrophosphate extractable iron in the casing layer increased at an approximately constant rate until 35 days after casing. There was then a decline to the final sample taken 48 days after the application of the casing layer. The increase is possibly due to the upward diffusion of water soluble iron which becomes bound to humic substances in the casing layer, from which they are extracted by the pyrophosphate. However, by day 48 a decreased mycelial activity in the compost resulted in a smaller quantity of water soluble iron (Figure 3.2) diffusing upward into the casing. The iron present in the casing may have become more firmly bound and, therefore, not extractable be pyrophosphate, resulting in the observed decline of pyrophosphate extractable iron.

The pyrophosphate extracts of the compost samples contained very variable concentrations of iron. In the lower half of the compost pyrophosphate extractable iron increased linearly with time until day 35 after which there was a considerable increase. However, in the upper compost the concentration of pyrophosphate extractable iron declined until day 28. There then followed a dramatic increase to the final sample, 48 days after casing.

The changes in the concentration of water extractable iron in the substrates that occurred in this study appear to be related to the mycelial activity in the compost and casing layer. The concentration of water extractable iron in the compost is of the same order as the concentration of iron in the sporophores determined in Section 3.1. These observations may be indicative of the importance that soluble materials play in the nutrition of the sporophore.

FIGURE 3.3. CONCENTRATIONS OF POTASSIUM PYROPHOSPHATE EXTRACTABLE
IRON IN THE SUBSTRATES DURING CULTURE.
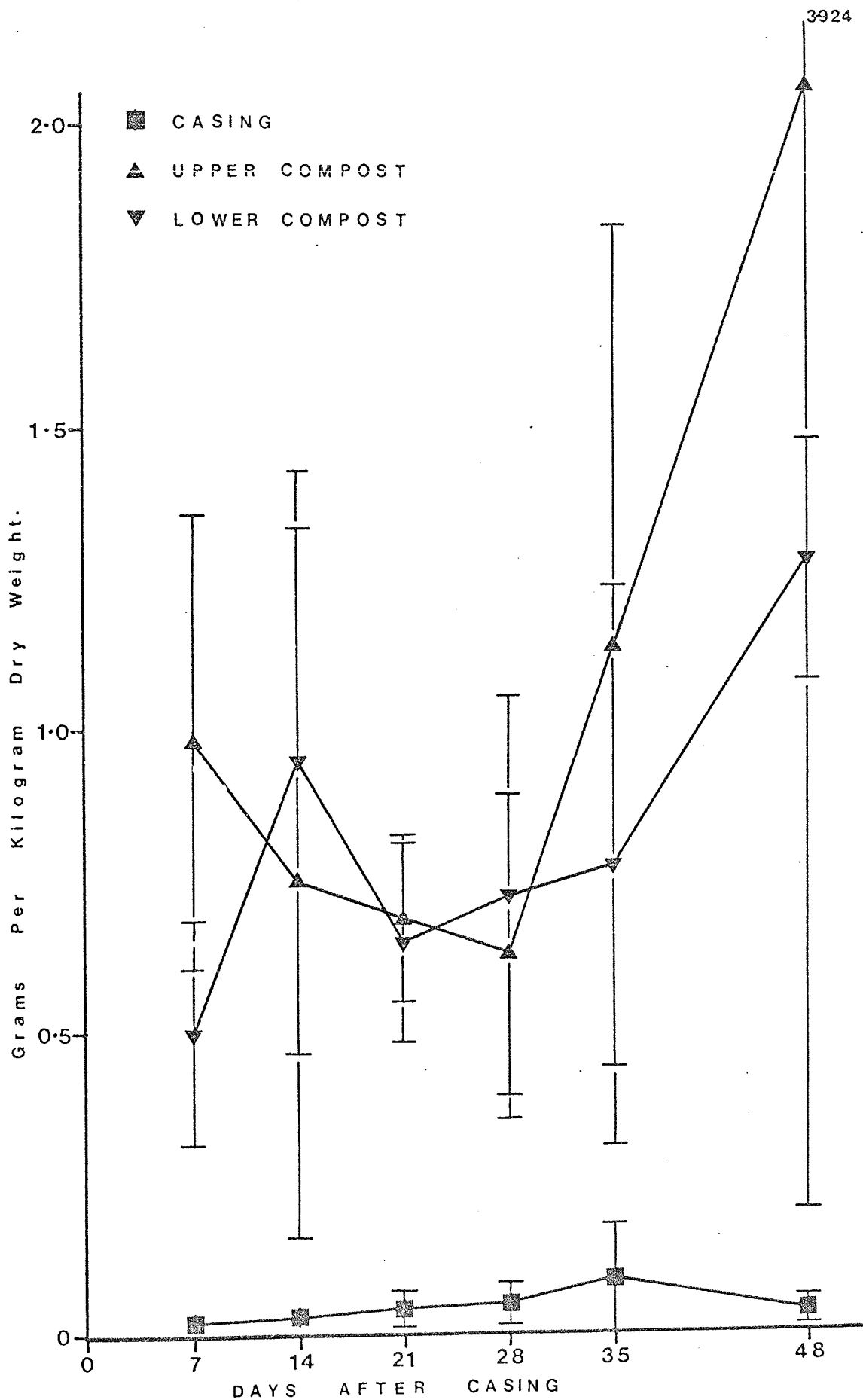
3.2.3   Water Extractable Iron in Substrates Supplemented with

Ferrous Sulphate.

In an experiment described in Section 3.1.5.2, ferrous
sulphate was added to the upper and lower layers of the compost
to determine the effect on the concentration of iron in the
sporophore. In that same experiment, described here, the changes
of water extractable iron that occurred in the substrates during
culture were monitored.

Samples were taken from the casing layer, the upper half and
the lower half of the compost 4, 13, 27, 39 and 55 days after
casing. Water extracts were prepared in accordance with the
methods given in Section 2.2 for the four replicates of each
sample. The concentrations of water extractable iron in the
samples were determined using Atomic Absorption Spectrophoto-
metry. The results are given in Appendix 3.2 and shown in
Figures 3.4 and 3.5.

In the casing layer (Figure 3.4) similar changes in the
concentration of water extractable iron occurred in each of the
three treatments. There was a gradual increase between days 4
and 27, following which there was a greater increase up to day
39 and a decrease to day 55. The concentration of water extract-
able iron in the casing layer of the control and the treatment in
which iron was injected into the compost were very similar through-
out. However, when iron was applied to the compost surface there
was initially more water extractable in the casing layer of this
treatment than the other two. There was also slightly more water
extractable iron present in the casing layer of this treatment on
days 27 and 39 after casing.

FIGURE 3.4. CONCENTRATIONS OF WATER EXTRACTABLE IRON IN CASING LAYERS OF GROWING UNITS, SUPPLEMENTED WITH FERROUS SULPHATE IN THE UPPER OR LOWER LAYERS OF THE COMPOST, DURING CULTURE.

CONTROL

IRON ADDED TO COMPOST SURFACE

IRON INJECTED INTO COMPOST
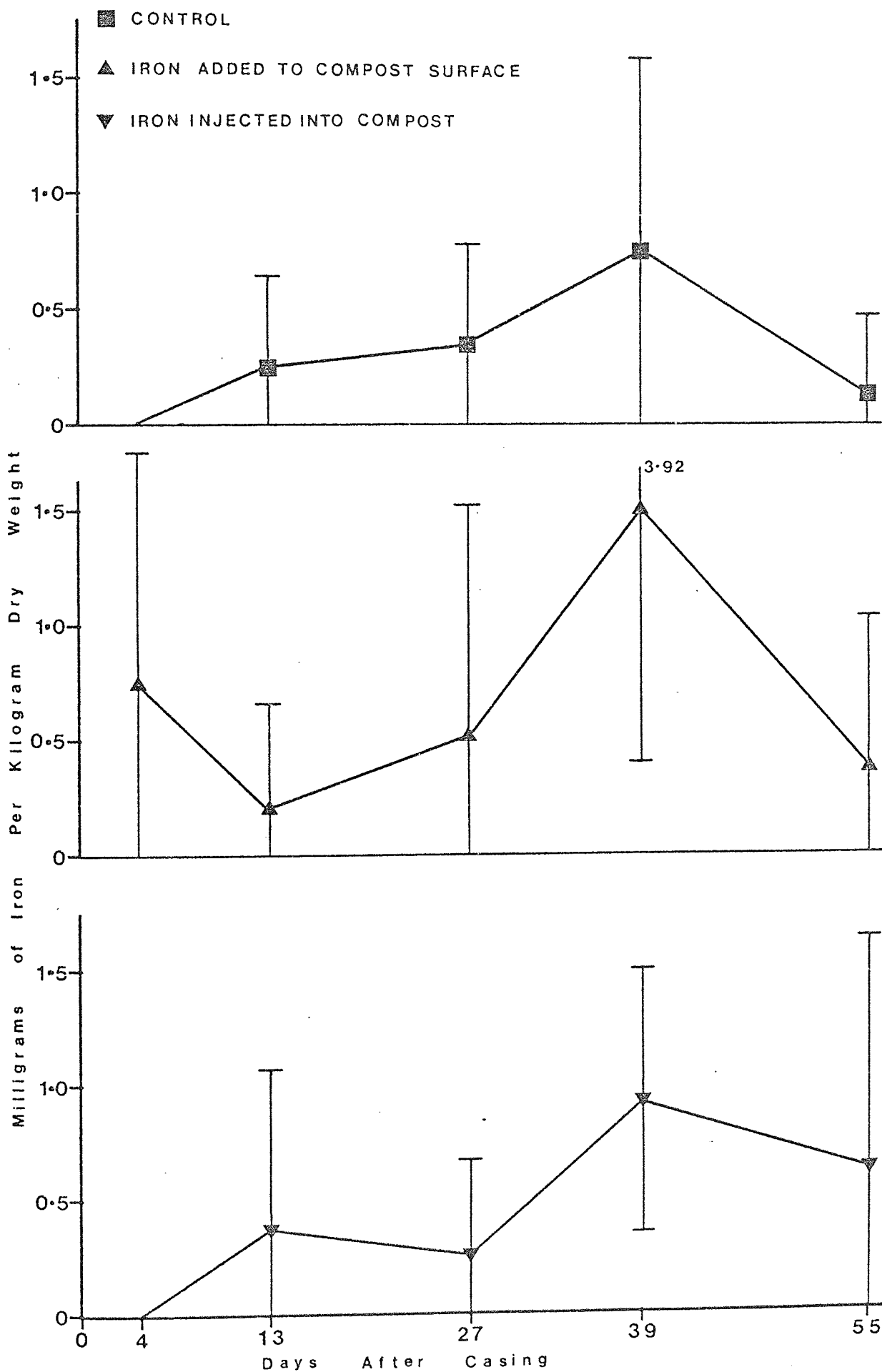
The concentrations of water extractable iron in the compost are shown in Figure 3.5. In the control, the lower half of the compost contained a consistently higher concentration of extractable iron than the upper half throughout the period of the study. However, the addition of ferrous sulphate solution to the compost surface increased the concentration of water extractable iron in the upper half of the compost to the level where it was equivalent to that in the lower half. Both halves then contained a similar concentration of water extractable iron to that found in the lower half of compost of the control.

When ferrous sulphate was injected into the compost, the upper half of the compost was unaffected and the changes of water extractable iron were the same as the upper compost of the control. The concentration of water extractable iron in the lower half of the compost increased linearly with time and at a greater rate than in the upper compost until day 39 after which it decreased slightly.

The overall pattern of changes of the water extractable iron in the substrates of this study are similar to those observed in the previous study (Section 3.2.2). The addition of ferrous sulphate solutions to the compost resulted in a small increased quantity of water extractable iron in the vicinity of the application of the supplement. This increase was approximately 5% of the soluble iron added to the compost. Thus, a large proportion of the soluble iron added to the compost was bound to the compost and made insoluble.

FIGURE 3.5. CONCENTRATIONS OF WATER EXTRACTABLE ·IRON IN COMPOSTS OF
GROWING UNITS,SUPPLEMENTED WITH FERROUS SULPHATE IN THE
UPPER OR LOWER LAYERS OF THE COMPOST,DURING CULTURE.
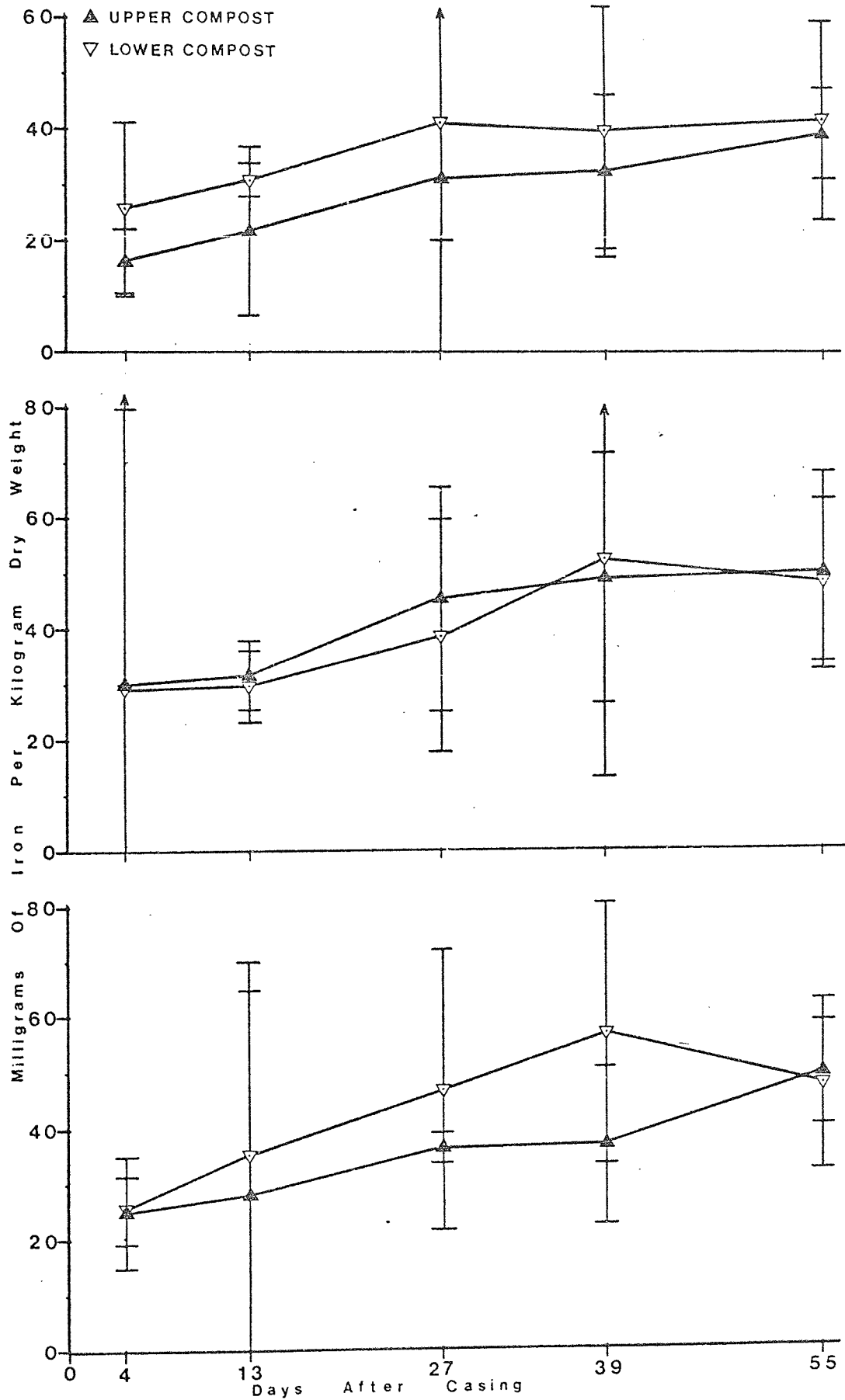
3.2.4   The Effect of Different Watering Regimes on the Concentration
         of Water Extractable Iron in the Substrates.

To determine the effect of water applied to the casing layer
on the quantity and movement of water extractable iron in the
casing and compost, substrate samples were taken from the growing
units used in the study of the effect of different watering regimes
on the iron composition of the sporophore. The details of the
experimental treatments and extraction procedures are given in
Sections 3.1.4 and 2.2. Six replicated samples were taken from
the casing layer and the upper and lower halves of the compost
in each of the three treatments at the conclusion of the experi-
ment, 65 days after casing. The percentage dry weight and the
concentration of water extractable iron are given in Table 3.2.1.

There was a negative correlation (-0.9254 which is significant
at a probability of 0.1) between the amount of water added to the
casing layer and the percentage dry weight of that substrate.
There were significant differences (at a probability of 0.05)
between the percentage dry weights of the casing layers from
the three different treatments.

In all three treatments, the upper half of the compost was
drier than the lower half but, this difference was only significant
in the lower watering regime. There were significant differences
of percentage dry weight in both the upper and lower halves of the
compost between the three treatments. Also, there were strong
negative correlations between the dry matter composition of both
the upper half (-0.9987, significant at a probability of 0.01) and
the lower half (-0.9878, significant at a probability of 0.02) of
the compost and the quantity of water added per day to the casing
layer.

TABLE 3.21. A. PERCENTAGE DRY WEIGHTS AND B. CONCENTRATIONS
OF WATER EXTRACTABLE IRON (MILLIGRAMS PER
KILOGRAM DRY WEIGHT) IN THE SUBSTRATES SUBJECTED
TO LOW,MEDIUM AND HIGH WATERING REGIMES.

	SUBSTRATE SAMPLE	LOW WATERING REGIME	MEDIUM WATERING REGIME	HIGH WATERING REGIME
A. PERCENTAGE DRY WEIGHT	CASING	63.30 (9.546)	32.14 (8.939)	23.04 (1.421)
	UPPER COMPOST	45.62 (2.524)	37.32 (6.622)	28.06 (5.295)
	LOWER COMPOST	41.13 (1.977)	32.82 (4.143)	26.41 (4.154)
B. IRON CONCENTRATION	CASING	0.698 (0.278)	0.745 (0.240)	0.659 (0.236)
	UPPER COMPOST	19.126 (2.964)	23.45 (3.494)	20.30 (3.353)
	LOWER	18.190 (4.346)	17.78 (3.115)	22.56 (3.566)

Means and (Standard Deviations) of 6 replicate samples.

There were no significant differences of the concentration of water extractable iron in the casing layers of the three watering treatments although, the medium regime casing contained slightly more than the low regime, which in turn contained more than the high watering regime.

More water extractable iron was found in the upper half of the compost than in the lower half in both the low and medium watering regimes but, this difference was significant only in the medium treatment. At a higher rate of water application, slightly more water extractable iron was present in the lower half of the compost.

Significantly more water extractable iron was found in the upper half of the medium treatment compost than in that of the low watering regime. The lower half of the compost in the high watering treatment contained significantly more water extractable iron than the lower compost of the medium treatment.

These results show that increasing water application to the casing layer increased the quantity of water extractable iron in the compost, which with heavy watering, was washed down into the lower layers of the substrate.

3.2.5   The Effect of Different Bacterial Isolates on the Concentration of Water Extractable Iron in the Substrates.

The presence of bacteria in the casing layer is obligatory for the initiation of sporophore formation. The involvement of bacteria in this phenomenon has received much attention but, their precise role has so far escaped definition. It seems likely that they are associated with the production of localised conditions in the casing layer essential to the initiation process. Hayes (1972)

suggested that bacteria associated with fructification might act by solubilizing iron, and other materials, in the casing layer making them available. The objective of this study was, therefore, to determine the iron solubilizing ability of bacteria isolated from the casing layer.

In an experiment by Cresswell, at the same time as these studies, different bacterial isolates were being assessed for their ability to initiate sporophore formation. A glass flask culture system was used to prevent contamination of the substrates. 250 grams of sterilised compost in each flask were inoculated with Agaricus bisporus. Following complete colonisation of the compost, 100 gram layers of sterilised PMB casing were added. The casing layers of three replicate flasks were each inoculated with cultures of a single specie of bacteria. Twelve different bacterial isolates were used and in addition three different controls were prepared. The controls were: compost and casing containing normal bacterial populations, normal compost with a sterilised casing, and sterilised compost and casing. Agaricus bisporus was present in each of these controls.

Forty days after the application of the casing layers, at the conclusion of the experiment, the compost and casing were removed from each flask. Water extracts of samples were prepared in accordance with the methods detailed in Section 2.2. The quantity of iron in the extracts was determined using atomic absorption spectrophotometry, and the results of this analysis are shown in Table 3.22.

In the compost extracts there were no significant differences in the concentration of water extractable iron in any of the treat-

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TABLE 3.22. CONCENTRATIONS OF WATER EXTRACTABLE IRON IN SUBTRATES
FOLLOWING THE ADDITION OF DIFFERENT BACTERIAL
ISOLATES TO THE CASING LAYER. (MILLIGRAMS PER
KILOGRAM DRY WEIGHT).

BACTERIAL ISOLATE TREATMENT		COMPOST	CASING
FLAVOBACTERIUM	7	17.66 (4.585)	42.64 (2.896)
SERRATIA	51	18.62 (1.407)	42.31 (8.623)
BACILLUS	64	19.51 (3.490)	60.27 (8.048)
ENTEROBACTER	10	20.39 (2.433)	37.38 (2.901)
ALCALIGENES	21	17.62 (8.508)	36.06 (2.034)
ALCALIGENES	62	16.56 (7.103)	46.48 (3.350)
ALCALIGENES	81	17.47 (3.378)	59.51 (5.829)
PSEUDOMONAS	19	18.03 (4.212)	46.24 (7.291)
PSEUDOMONAS	27	14.19 (3.651)	47.89 (0.595)
PSEUDOMONAS	28	15.16 (3.283)	37.42 (7.626)
PSEUDOMONAS	74	18.29 (2.840)	27.69 (5.386)
PSEUDOMONAS	84	18.77 (0.441)	32.14 (2.788)

CONTROLS

	COMPOST	CASING
STERILE CASING NORMAL COMPOST	16.01 (5.127)	39.46 (2.276)
NORMAL CASING AND COMPOST	18.99 (2.112)	48.47 (0.530)
STERILE CASING AND COMPOST	15.34 (6.647)	34.06 (3.615)

Means and (Standard Deviations) of 3 replicates.

ments or controls. However, the concentration of water extractable iron in the compost of the controls increased slightly with increasing bacterial presence in the culture. The axenic control contained less than the sterile casing control which, in turn, contained less than the 'normal' control.

In the casing layer the concentration of water extractable iron was considerably more variable between the different treatments. In the controls, the casing layer from the 'normal' culture control contained significantly more water extractable iron than the casing layers of the two other controls. The casing layer overlying the normal compost contained slightly more water extractable iron than the casing layer above the axenic compost.

Of the casing layers inoculated with different bacterial cultures, six (isolate numbers; 7, 19, 27, 62, 64 and 81) contained significantly more water extractable iron than the casing layer of the axenic control. The casing layers of only five cultures (7, 10, 21, 74 and 84) contained significantly less iron than the casing from the 'normal' control.

It is clear that a range of different bacteria, isolated from normal casing layers are capable of increasing the quantity of water soluble iron in the casing layer and that the normal bacterial populations of the casing layer are performing this function.

3.2.6   The Concentration of Iron-59 in the Substrates and Sporophores Following the Addition of Isotope to the Casing Layer, the Upper or the Lower Compost.

The mobility of ions in soils has frequently been studied using tracing techniques. Only one study of this type has been directed towards the substrates used in mushroom cultivation. Corin et al.

(1954) found that 28 days after the addition of Phosphorus-32 labelled mono-sodium phosphate to the compost, 90 percent of the Phosphorus-32 was recovered in the form of mineral phosphate of which 70 percent was soluble in cold water. Thus, the phosphate was not substantially bound to the organic colloids of the compost and was free to move in the substrates. Flegg (1961b) and Yeo (1980) have shown the passive accumulation of soluble salts in the casing layer and Fleckenstein and Grabbe (1979) have shown the increased mobility of heavy metals with percolation of the substrates. In this study, Iron-59 was applied at different levels in the substrate in order to observe the movement of this isotope within the substrates. Also the isotope content of the sporophore was measured, so that the source of isotope uptake could be determined.

The boxes were prepared in accordance with the methods detailed in Section 2.1. The isotope solution was applied to 16 replicate boxes for each treatment and the treatments were: isotope applied to the casing surface, to the compost surface and injected into the compost. Three replicate growing units were sampled from each treatment 4, 13, 26, 34 and 52 days after casing. Four samples were taken from each of four substrate levels in each growing unit. Statistical analysis showed that the variability of samples taken from one unit was as great as the variability of samples taken from different growing units within each treatment. The results given in Appendix 3.3 and Figure 3.6, 3.7 and 3.8 are the means of twelve replicate samples.

3.2.6.1   Isotope in the Substrates.

Isotope applied to the Casing Surface (Figure 3.6)

Despite variability the concentration of isotope in the casing layer remained approximately constant throughout the duration of the study.

In the compost the isotope concentration appears to increase slowly with time, probably as the result of the downward movement of the isotope. The application of water may enhance the downward percolation of isotope, because in the top of the compost an increased isotope concentration coincided with the watering of the casing layer which was required during the aeration of the boxes. This increase of isotope concentration was observed to occur later in the middle and bottom of the compost. This may be linked to the time required for downward movement of water.

It would appear that the isotope becomes bound to the casing layer because, the concentration of isotope in the top of the compost was only about 0.05 percent of that in the casing layer. Thus, indicating the small downward percolation of what was originally soluble iron-59.

Isotope Applied to the Compost Surface (Figure 3.7).

Again because of the variation of samples it is difficult to interpret any changes of isotope concentration. However, in each level of the substrate there was a gradual increased isotope concentration with time. This may be the result of: loss of dry matter, diffusion or percolation. The isotope does appear to be highly localised near to the point of application.

Isotope Injected into the Compost (Figure 3.8).

In common with when the isotope was applied to the upper layers of the substrate, the isotope was highly localised when injected into the compost and again because of the variability it is difficult to interpret any of the observed changes.

3.2.6.2   Isotope in the Sporophores (Table 3.23 and Figure 3.9).

It is likely that the content of iron in the sporophore is dependent upon; the size of the sporophore, the yield of sporophores produced from a particular set of cultural conditions and the concentration of iron in the substrates.   Using the data obtained in this study the significance of various relationships were tested using correlation coefficient calculations.

Table 3.24 shows the correlations in different treatments and breaks between the dry weights and Iron-59 concentrations of individual sporophores.   When the isotope was applied to the casing layer there were negative correlations in each break but, only the second break was significant.   The decreasing isotope concentration in sporophores of increasing size may indicate that a limited quantity of isotope was available for uptake into each sporophore particularly when large numbers of sporophores were produced.   There were no significant correlations when the isotope was applied to the compost surface.   However, when the isotope was injected into the compost, there were increasingly significant correlations in the later breaks, between the size and isotope content of sporophores.   The increasing correlation with decreasing sporophore number probably demonstrates that the quantity of isotope available for uptake into the sporophore did not decrease with the yield and thus, when few sporophores were produced the concentration

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of Iron-59 that each contained was proportional to its size.

The correlations between the dry weight yield and Iron-59 content of sporophores in each break are given in Table 3.25. When the isotope was applied to the casing layer there was a slight (but, not significant) negative correlation. Thus, the content of iron in the sporophores increased despite the decreasing yield in successive breaks. Possibly the increasing quantity of available iron was the result of downward movement into the compost, the substrate which supplies nutrients to the sporophore. The application of Iron-59 to the compost resulted in significant correlations between the decreasing dry weight yield per break and the decreasing quantity of isotope contained in those sporophores. This may show that Iron-59 is taken up from the compost in direct proportion to other materials and therefore, that the compost is the major source of nutrient supply, particularly the upper half of the compost.

To determine the existence of any correlations between the concentrations of isotope in the sporophores and in the substrates it was, firstly, necessary to estimate the concentrations of isotope in different levels of the substrate at the times corresponding to the harvest of the sporophores. These concentrations, together with the correlation coefficients are given in Table 3.26. There were no significant correlations between the concentration of isotope in the casing layer and in the sporophores of any of the treatments. However, when the isotope was applied to the casing layer the increasing concentration of Iron-59 in the sporophores was positively correlated with the increasing concentration of isotope in the compost (significantly so in the middle compost). This shows that the compost is the

source of nutrient supply. As more Iron-59 moves down into the compost from the casing layer it becomes available for uptake into the sporophores. When the isotope was added to the compost there were negative correlations between the concentration of iron in the upper compost and the sporophores. This may indicate that the iron in the sporophores was removed from the upper compost and, therefore, decreased concentrations of Iron-59 in the upper compost are associated with the increased concentration of isotope in the sporophores.

The results of this study show that Iron-59 added to the substrates became highly localised where it was applied. Very little movement of the isotope occurred between different levels of the substrate, that which did was probably by percolation and diffusion. The quantity of isotope contained in the sporophores was related to the yield and number of sporophores produced. The findings also show that the isotope in sporophores was obtained from the compost. The findings show that the majority of isotope in the sporophores was taken up from the compost, although a limited quantity was derived from the casing.

FIGURE 3.6. CONCENTRATIONS OF IRON-59 IN DIFFERENT LEVELS OF THE SUBSTRATES FOLLOWING THE ADDITION OF ISOTOPE TO THE CASING LAYER.
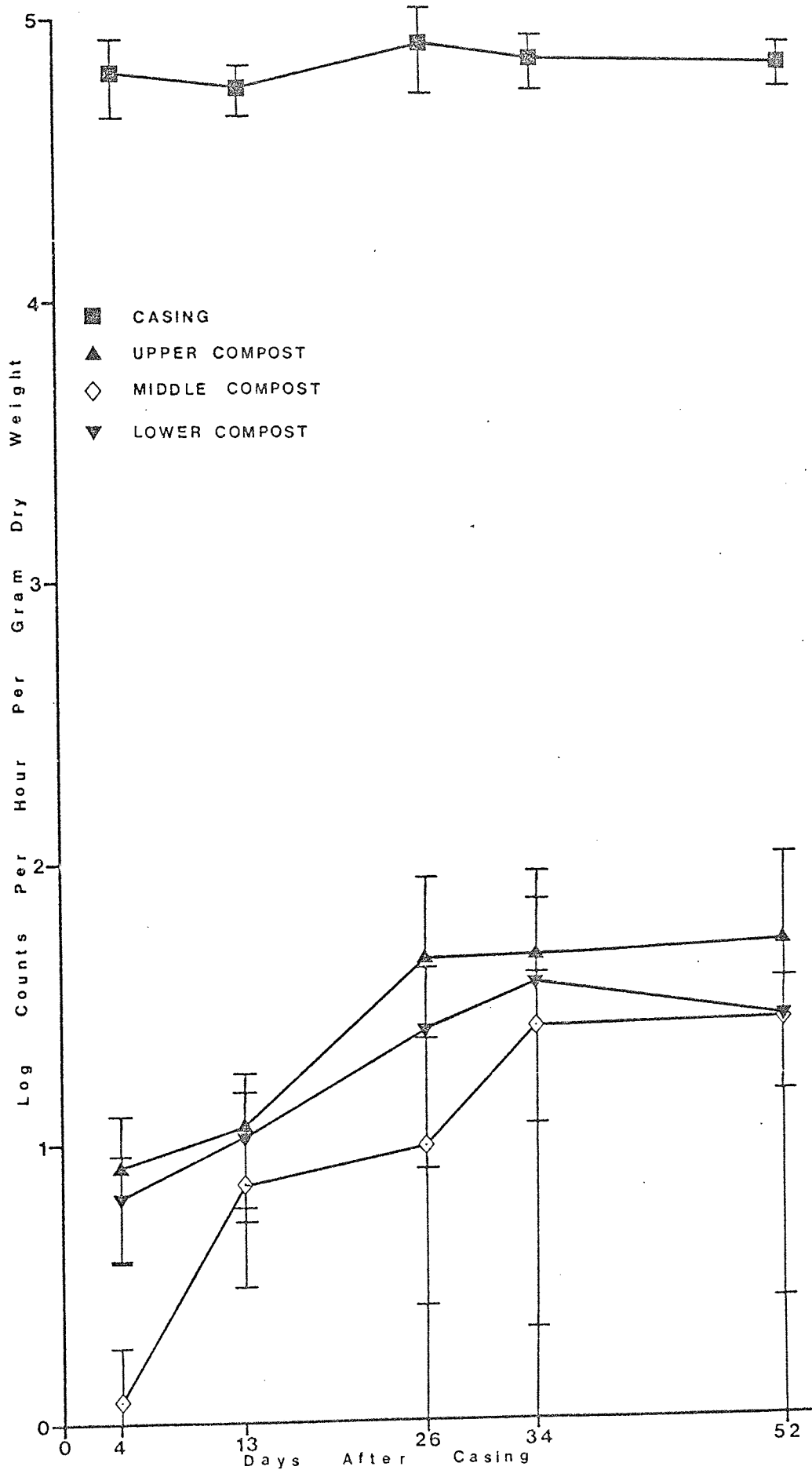
FIGURE 3.7. CONCENTRATIONS OF IRON-59 IN DIFFERENT LEVELS OF THE SUBSTRATES FOLLOWING THE ADDITION OF ISOTOPE TO THE UPPER COMPOST.
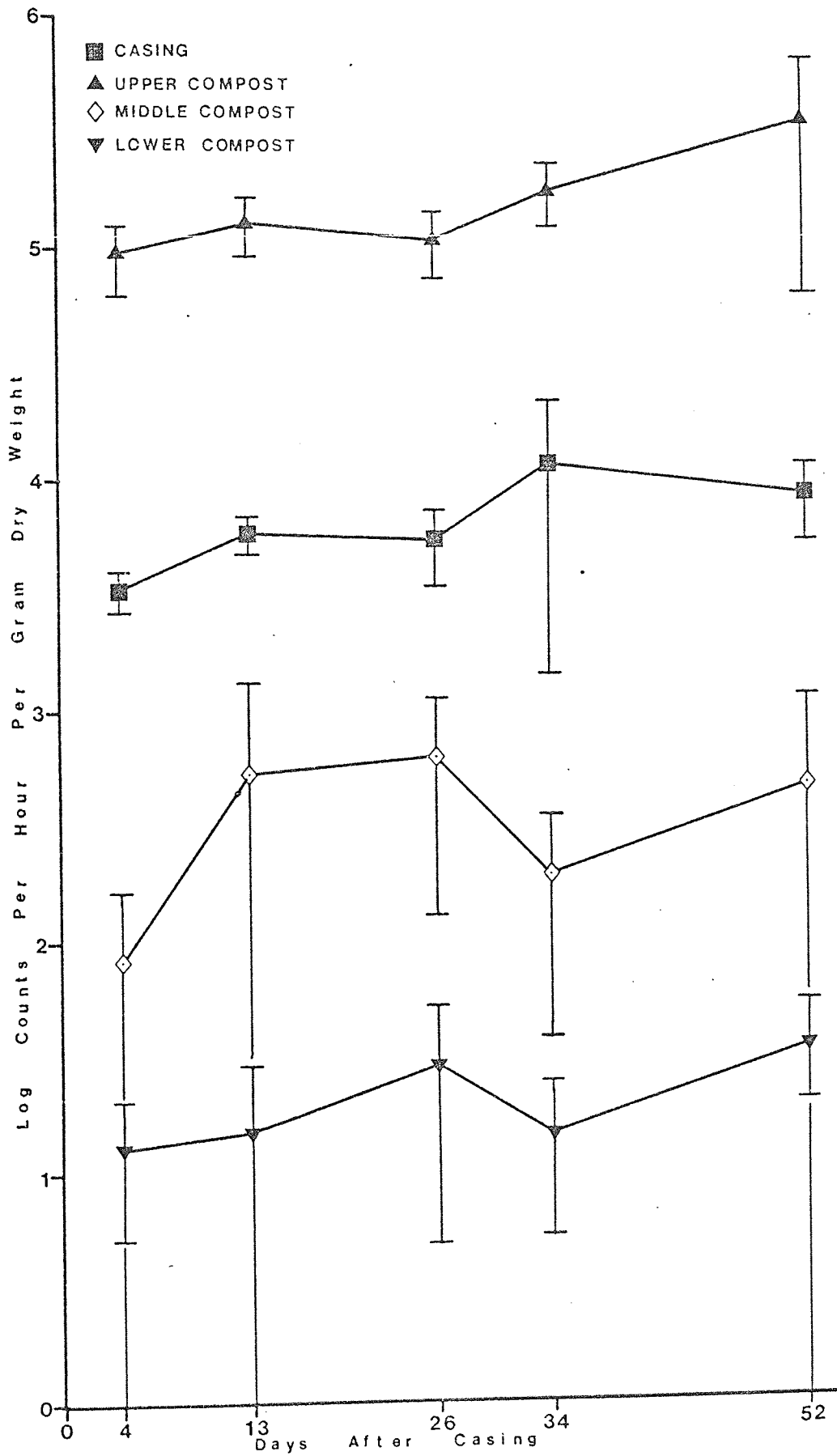
FIGURE 3.8. CONCENTRATIONS OF IRON-59 IN DIFFERENT LEVELS OF THE
SUBSTRATES FOLLOWING THE ADDITION OF ISOTOPE TO THE
LOWER COMPOST.
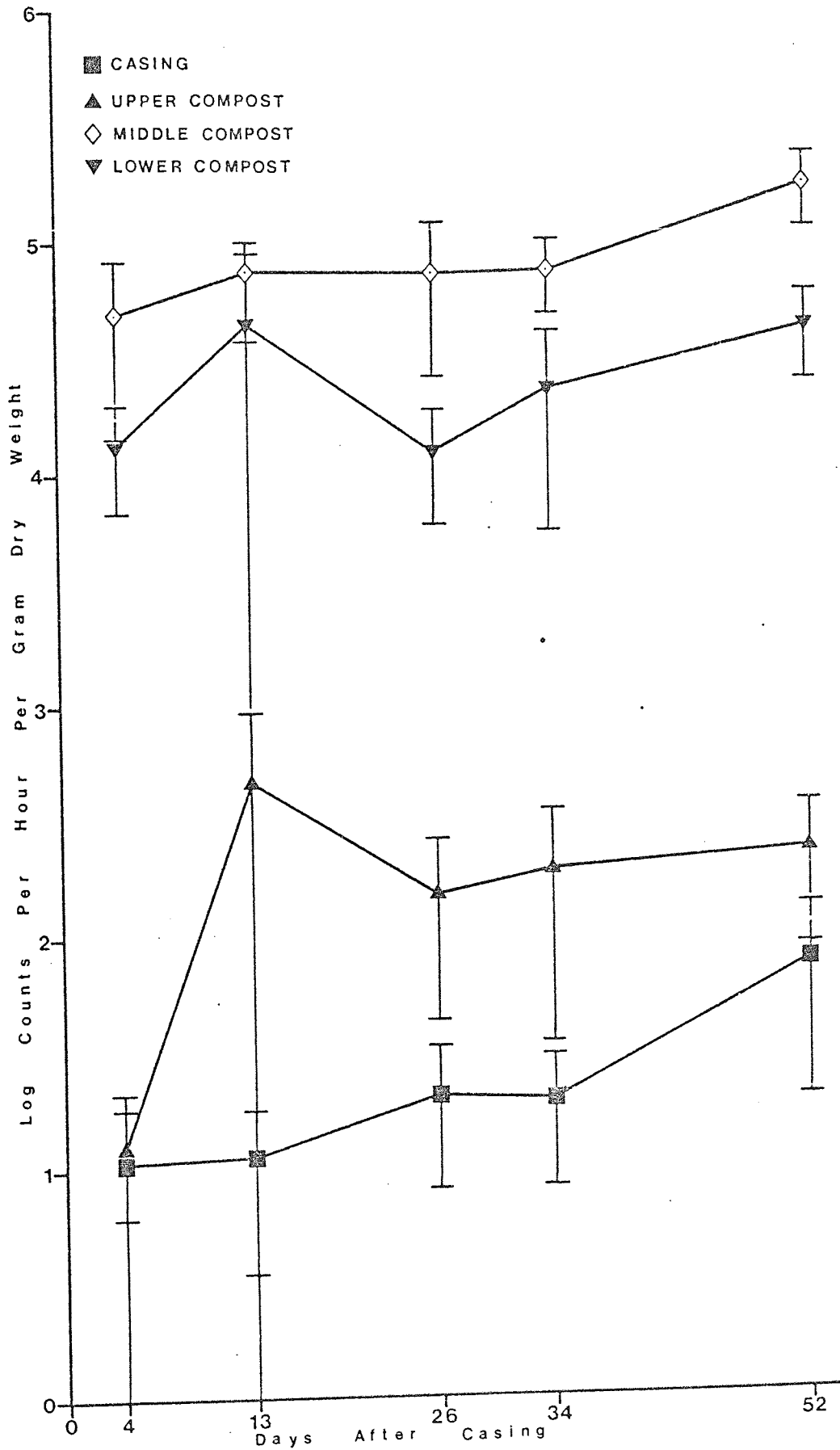
TABLE 3.23. DRY WEIGHT YIELDS AND QUANTITY OF IRON – 59 IN
SPOROPHORES HARVESTED FROM SUBSTRATES FOLLOWING
THE ADDITION OF ISOTOPE TO:

A. THE CASING LAYER.
B. THE UPPER COMPOST.
C. THE LOWER COMPOST.

	BREAK	NUMBER OF SPOROPHORES	IRON – 59 CONCENTRATION Log Count/Hour/Gram Dry Weight			MEAN DRY WEIGHT YIELD. Grams/ Growing Unit	TOTAL IRON – 59 CONTENT. Log Counts /Hour/ Growing Unit
			MEAN	95% CONFIDENCE LIMITS			
				UPPER	LOWER		
A	1	46	1.993	2.179	1.661	2.632	2.413
	2	77	2.050	2.168	1.888	3.259	2.564
	3	10	2.348	2.734	0	1.928	2.633
	4	9	2.584	2.944	0	1.496	2.759
B	1	42	2.680	2.747	2.600	2.876	3.139
	2	49	2.774	2.912	2.570	3.014	3.253
	3	9	2.613	2.749	2.415	1.304	2.728
	4	3	2.558	2.943	0	0.678	2.389
C	1	44	2.689	2.773	2.584	2.300	3.050
	2	15	2.878	2.991	2.725	1.937	3.165
	3	8	2.620	2.795	2.320	0.914	2.581
	4	1	2.828	–	–	0.488	2.516

FIGURE 3.9. CONCENTRATIONS OF IRON-59 IN SPOROPHORES OF DIFFERENT
BREAKS FROM SUBSTRATES WHICH HAD ISOTOPE ADDED TO
EITHER, THE CASING LAYER, THE UPPER, OR THE LOWER COMPOST.
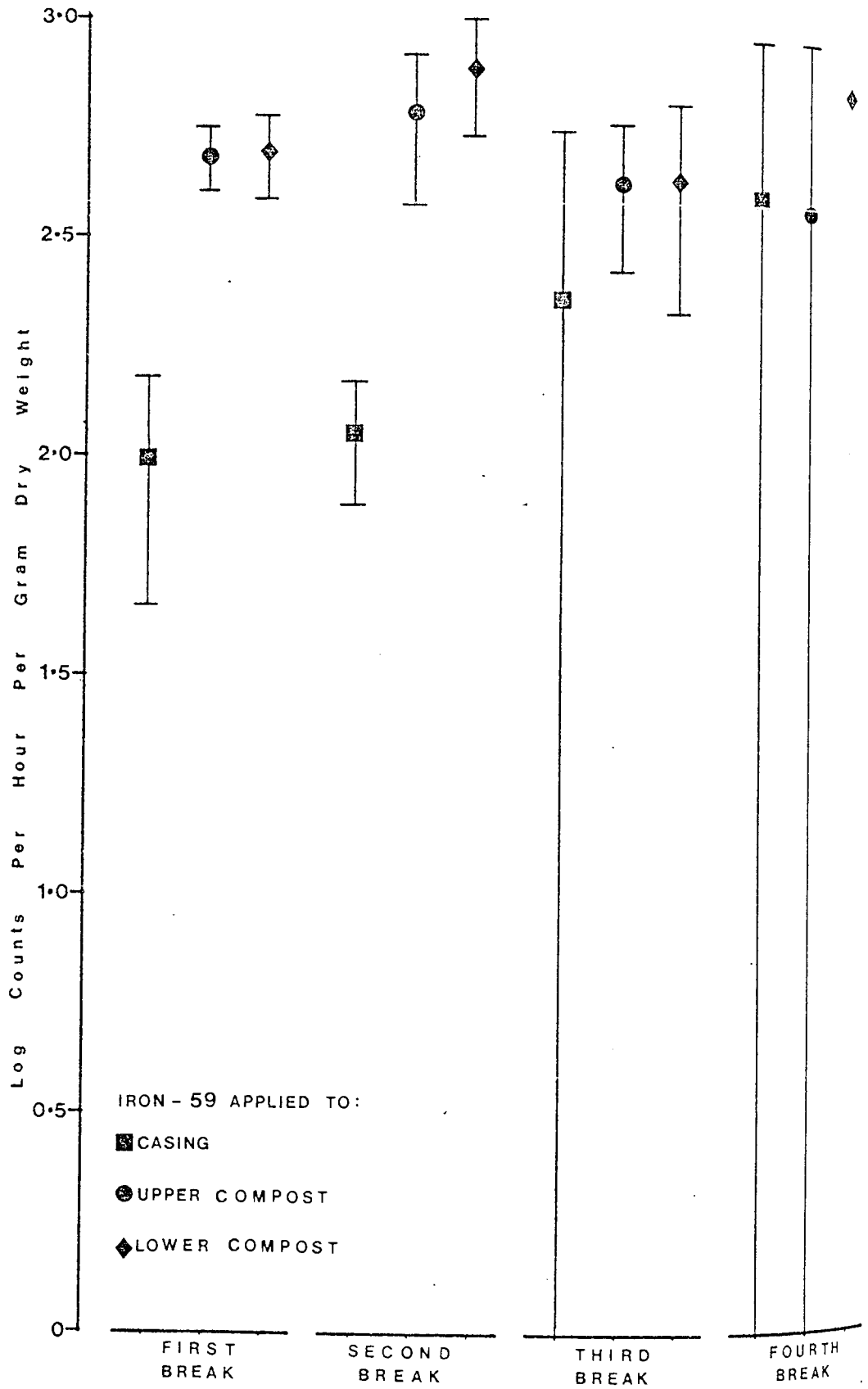
TABLE 3.24. CORRELATIONS BETWEEN INDIVIDUAL SPOROPHORE DRY WEIGHT AND IRON – 59 CONCENTRATION.

BREAK	ISOTOPE TO CASING		ISOTOPE TO COMPOST SURFACE		ISOTOPE INJECTED INTO COMPOST	
	r	n	r	n	r	n
FIRST	– 0.194	46	– 0.040	38	0.229	43
SECOND	– 0.338	77	0.270	44	0.610	15
THIRD + FOURTH	– 0.269	16	– 0.387	12	0.836	9

r = Correlation Coefficient.
n = Number of samples.

TABLE 3.25. CORRELATIONS BETWEEN DRY WEIGHT YIELD AND TOTAL IRON – 59 CONTENT OF SPOROPHORES PER BREAK.

ISOTOPE APPLIED TO CASING	ISOTOPE APPLIED TO COMPOST	ISOTOPE INJECTED INTO COMPOST
– 0.703	0.988	0.942

TABLE 3.26. CORRELATIONS BETWEEN THE CONCENTRATION OF IRON – 59 IN SPOROPHORES AND IN DIFFERENT LEVELS OF THE SUBSTRATES.

TREATMENT	SAMPLE	ISOTOPE CONCENTRATION Log counts/Hour/Gram Dry Wt.				CORRELATION COEFFICIENT
		FIRST BREAK	SECOND BREAK	THIRD BREAK	FOURTH BREAK	
	SPOROPHORE	1.993	2.050	2.348	2.584	
ISOTOPE APPLIED TO CASING SURFACE	CASING	4.857	4.908	4.924	4.863	– 0.031
	UPPER COMPOST	1.544	1.663	1.681	1.699	0.761
	MIDDLE COMPOST	1.0	1.146	1.415	1.415	0.918
	LOWER COMPOST	1.301	1.415	1.568	1.519	0.818
	SPOROPHORE	2.680	2.774	2.613	2.559	
ISOTOPE APPLIED TO COMPOST SURFACE	CASING	3.763	3.903	4.041	4.000	– 0.546
	UPPER COMPOST	5.114	5.164	5.380	5.423	– 0.863
	MIDDLE COMPOST	2.756	2.602	2.447	2.477	0.577
	LOWER COMPOST	1.362	1.362	1.342	1.415	0.143
	SPOROPHORE	2.689	2.873	2.620	2.828	
ISOTOPE INJECTED INTO COMPOST	CASING	1.204	1.301	1.544	1.845	0.138
	UPPER COMPOST	2.447	2.279	2.342	2.342	– 0.924
	MIDDLE COMPOST	4.857	4.863	5.061	5.255	0.045
	LOWER COMPOST	4.362	4.204	4.447	4.623	0.157

3.3   THE UPTAKE OF IRON INTO THE SPOROPHORE DURING GROWTH AND
        DEVELOPMENT.

3.3.1   Introduction.

        The growth of the Agaricus bisporus sporophore was shown by

Bonner et al. (1956) to be totally dependent on materials trans-

located from the subterranean mycelium. They also observed that

throughout development, the percentage dry weight of the sporophore

remained constant and from this concluded that water is taken up

only in proportion to these materials.

        Many studies since have shown that a large variety of substances

are translocated into the sporophore. However, the majority of these

materials were complex organic molecules and because of their involve-

ment in metabolic processes it was difficult to interpret the signifi-

cance of the uptake of these substances. In addition these studies

showed that the metabolism of Agaricus bisporus changes during

development of the sporophores but, neither these changes of

metabolism nor the uptake of materials have been specifically

linked to growth or any developmental process.

        The objective of this part of the study was, therefore, to

quantify growth of the sporophore and then to observe the changes

of iron that occur in the sporophore in assocation with growth

and development.

3.3.2   Growth of the Sporophore of Agaricus bisporus.

3.3.2.1   Introduction.

        There is little published information on the growth rate of

the Agaric sporophore. In 1956 Flegg and Gandy and Bonner et al.

quantified the expansion and extension of the stipe and pileus

during the development of the sporophore. In 1960 Cooke determined sporophore growth over a period of eight days. He found that sporophore growth followed a sigmoidal curve and that from day 2 to day 5 the growth was linear at about 8.66 grams fresh weight per day. Chanter and Cooke (1978) substantiated this growth pattern and demonstrated a number of factors which may influence the sporophore growth rate.

Flegg and Gandy (1956) observed a relationship between the pileus diameter and the fresh weight of the sporophore. They determined that the weight was proportional to the pileus diameter cubed and that this relationship differs slightly for cups and flats. (Flats: $Diameter^3 = 12.5$ x Weight and Cups: $Diameter^3 = 7.5$ x weight).

Growth rate is defined as the change in weight with time, thus it was proposed that by using the relationship observed by Flegg and Gandy it would be possible to calculate the weight and thereby the growth rate of the sporophore as it increased in size.

3.3.2.2   The Relationship Between the Pileus Diameter and Weight of the Sporophore.

Before any estimation of the growth rate could be made the precise relationship between the pileus diameter and weight of the sporophore was defined. This was done by harvesting sporophores of different sizes which were produced from 36 growing pots prepared in accordance with the methods detailed in Section 2.1.

A total of 360 sporophores were harvested. The dry weight, fresh weight, stage of maturity and pileus diameter were determined for each sporophore. The pileus diameter was measured using

calipers, taking the mean of two readings at right angles to each other. The stages of maturity are shown in Plate 3.1 and the descriptive characteristics of each stage are given in Appendix 3.4.

The pileus diameter and dry weight of all sporophores belonging to each stage of maturity were plotted to give a curve for each of the stages. The curves for each stage are shown in Figure 3.10. These curves correspond very closely to those obtained by Flegg and Gandy. In Table 3.27 it can be seen that the correlation coefficients of pileus diameter and sporophore dry weight were highly significant (at a probability of 0.001) for each of the developmental stages. There was, therefore, a very close relationship between the two parameters for each stage. It can also be seen from this table that the percentage dry weights of sporophores in the different stages were all similar. This observation is in accordance with the findings of Bonner et al. (1956).

The mean results for each stage given in Table 3.27 are plotted in Figure 3.11. The confidence limits demonstrate that there was a considerable variation in both dry weight and pileus diameter of the sporophores contained in each stage.

Table 3.28 contains the mean dry weights and pileus diameters of sporophores divided arbitrarily into 13 groups containing sporophores of similar weights. These results are also plotted in Figure 3.11 and show considerably less variation than the developmental stage plots. Also shown in Figure 3.11 is the curve of $y^{1.75} = 17x$, which most closely approximates to the two sets of data.

PLATE 3.1. DEFINED DEVELOPMENTAL STAGES OF THE SPOROPHORE.

PLATE 3.1. DEFINED DEVELOPMENTAL STAGES OF THE SPOROPHORE.
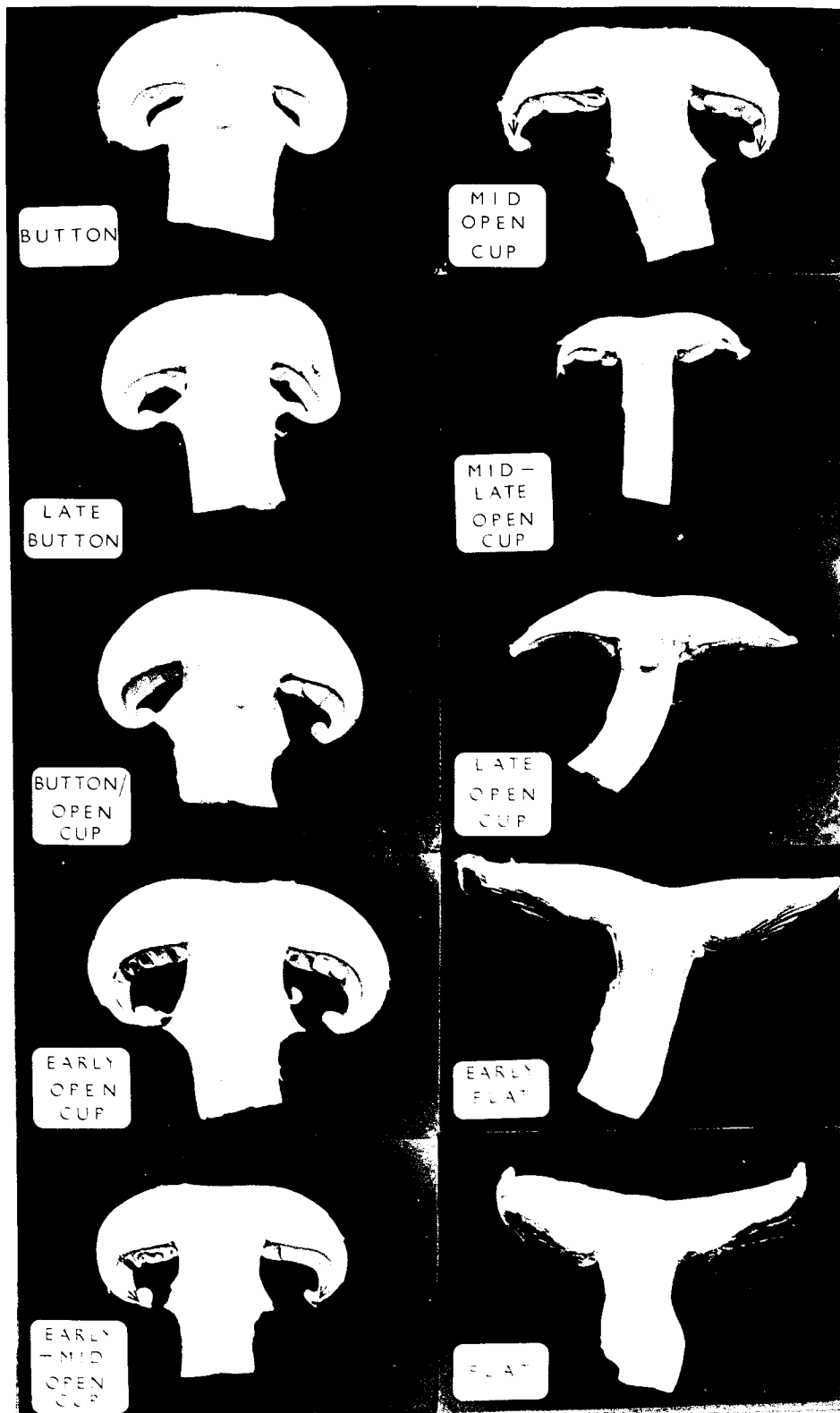
FIGURE 3.10. PILEUS DIAMETER AGAINST SPOROPHORE DRY WEIGHT FOR EACH DEVELOPMENTAL STAGE.

1 Flat & Early Flat
2 Late Open Cup
3 Mid-Late Open Cup
4 Mid & Early-Mid Open Cup
5 Early Open Cup
6 Late Button
7 Button/Open Cup
8 Button
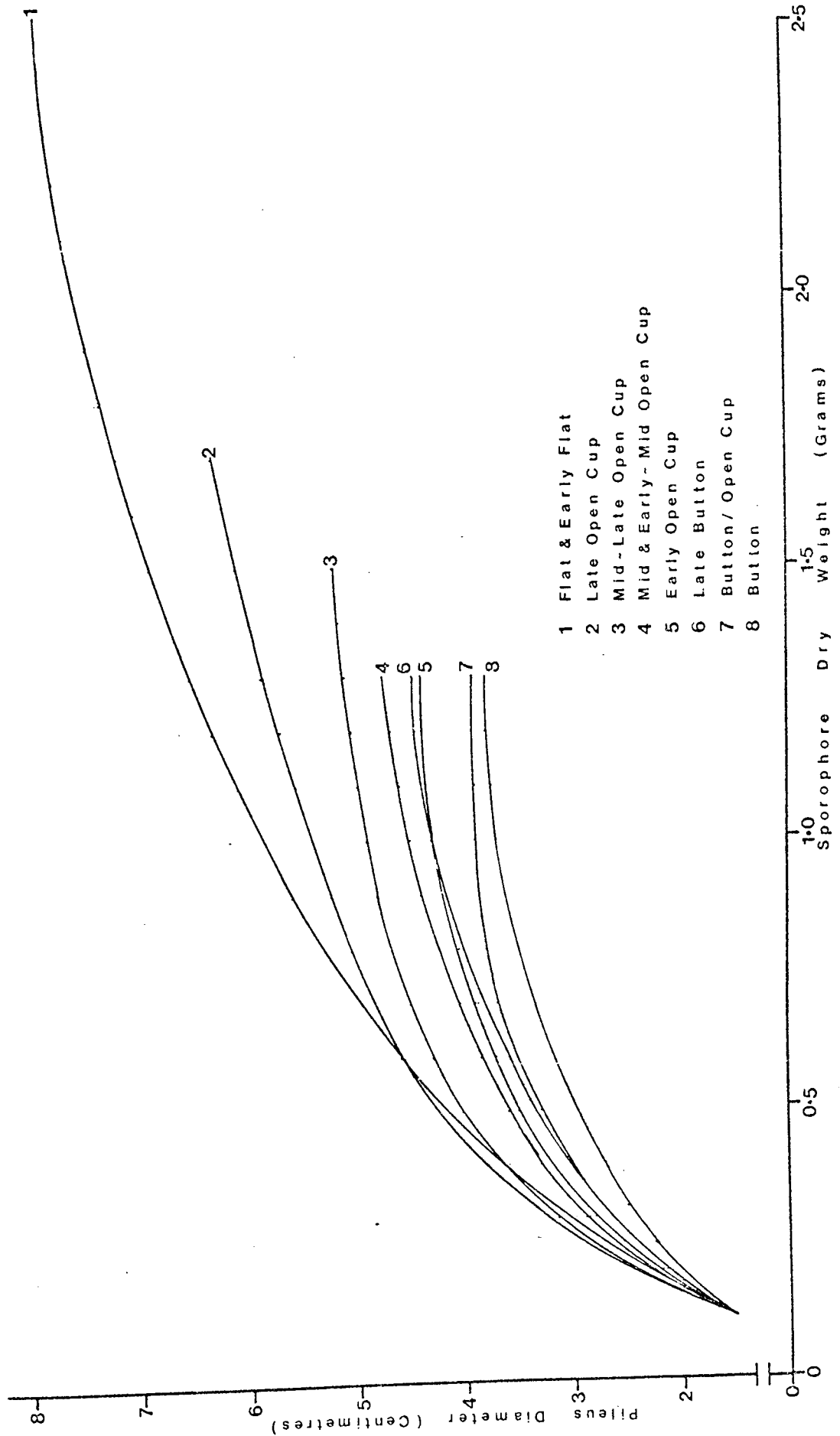
FIGURE 3.11. PILEUS DIAMETER AGAINST SPOROPHORE DRY WEIGHT OF
SPOROPHORES GROUPED ACCORDING TO :
1. SIMILARITY OF DRY WEIGHT.
2. DEVELOPMENTAL STAGE.

1   Button
2   Late Button
3   Button/Open Cup
4   Early Open Cup
5   Early-Mid Open Cup
6   Mid Open Cup
7   Mid-Late Open Cup
8   Late Open Cup
9   Early Flat
10   Flat

□ WEIGHT GROUPINGS

⬤ DEVELOPMENTAL STAGES

Pileus Diameter (Centimetres)

Sporophore Dry Weight (Grams)

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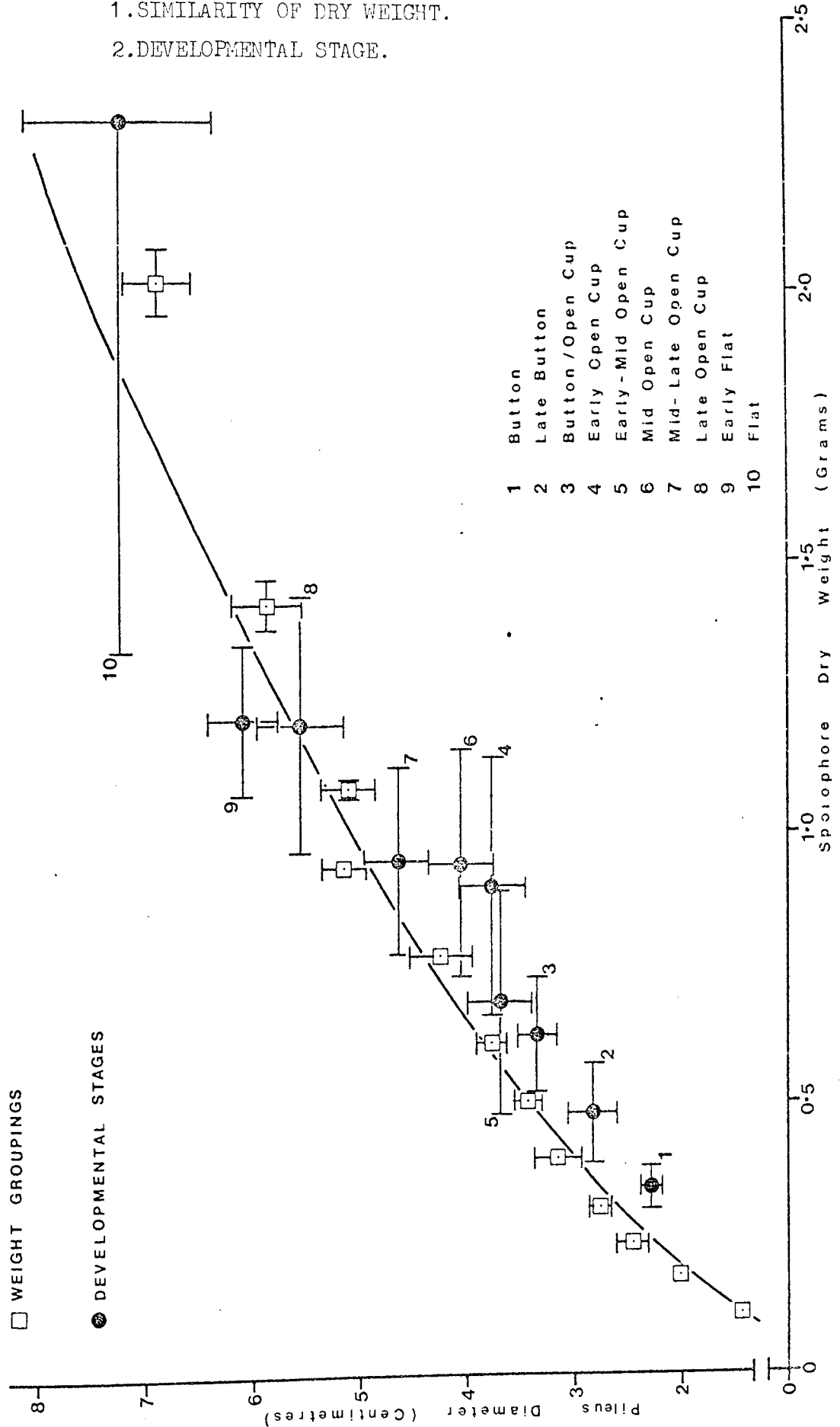
TABLE 3.27. PILEUS DIAMETERS AND DRY WEIGHTS OF DIFFERENT
SPOROPHORE DEVELOPMENTAL STAGES.

STAGE	SAMPLE SIZE	PILEUS DIAMETER(CM)		DRY WEIGHT (G)		CORRELATION COEFFICIENT	% DRY WEIGHT
		Mean	C.L	Mean	C.L.		
BUTTON	104	2.28	0.10	0.345	0.04	0.563	8.071
LATE BUTTON	27	2.82	0.233	0.483	0.093	0.931	8.511
BUTTON/OPEN CUP	20	3.32	0.185	0.628	0.106	0.880	8.511
EARLY OPEN CUP	20	3.77	0.307	0.903	0.237	0.899	8.171
EARLY-MID OPEN CUP	12	3.68	0.292	0.688	0.206	0.918	7.840
MID OPEN CUP	23	4.06	0.302	0.945	0.209	0.941	8.305
MID-LATE OPEN CUP	30	4.63	0.324	0.949	0.171	0.952	7.780
LATE OPEN CUP	39	5.55	0.399	1.199	0.233	0.921	8.064
EARLY CUP	35	6.09	0.318	1.207	0.139	0.733	8.446
FLAT	13	7.21	0.851	2.301	0.973	0.956	8.491

TABLE 3.28. PILEUS DIAMETERS AND DRY WEIGHTS OF SPOROPHORES DIVIDED
INTO GROUPS OF SIMILAR DRY WEIGHTS.

NUMBER OF SPOROPHORES	DRY WEIGHT (G)		PILEUS DIAMETER (CM)	
	Mean	C.L.	Mean	C.L.
27	0.108	0.0040	1.443	0.0657
25	0.180	0.0051	2.008	0.0784
22	0.240	0.0054	2.441	0.1457
35	0.307	0.0065	2.750	0.1151
26	0.400	0.0082	3.141	0.2234
27	0.503	0.0088	3.422	0.1274
38	0.614	0.0118	3.774	0.1455
19	0.776	0.0133	4.237	0.2940
25	0.938	0.0107	5.136	0.2129
23	1.082	0.0166	5.096	0.2477
22	1.419	0.0464	5.864	0.3218
21	2.009	0.0612	6.871	0.3160
12	3.575	0.8343	8.333	0.8923

Correlation Coefficient 0.9432 $12^{\circ}F$

Significant at Probability of 0.001.

C.L. = 95% Confidence Limits.

3.3.2.3   The Calculation of Sporophore Growth Rate.

Chanter and Cooke (1978) have shown that the removal of a
proportion of sporophores from a crop affects the further growth
of the remaining sporophores.   The in situ measurement of sporo-
phore growth will not disturb sporophore growth and hence offers
an advantage over the previously used method of growth estimation.

It was shown in Section 3.3.2.2 that there was a significant
correlation between the pileus diameter and the dry weight of the
sporophore.   Thus, by observing the pileus diameter of an individual
it would be possible to estimate the dry weight of that sporophore.
Hence a time-lapse study would demonstrate the increasing weight
of the sporophore.

Photographic techniques of recording pileus diameter thus,
allowed a large number of individual sporophores to be observed
at frequent intervals.   Eight growing units were photographed at
eight hour intervals for five days.   The photographs were taken
using a Fujica ST801 single lens reflex camera fitted with a 55 mm
focal length lens and using Ilford FP4 black and white print film.

For each growing pot a sequence of photographic prints were
produced.   Plate 3.2 shows one such series of prints.   The pileus
diameter of sporophores on the photographic prints were individually
measured and by comparison with a reference scale included on the
print, a value for the actual sporophore pileus diameter was
calculated.   The pileus diameter of sporophores that had become
obscured by other sporophores were measured, at the times when
photographs were taken, using calipers.   Using a dentist's observation
mirror the developmental stage of each sporophore was observed and
recorded at the time of each photograph.

Using the relationship established in Section 3.3.2.2 the

PLATE 3.2.   A PHOTOGRAPHIC TIME-LAPSE SEQUENCE OF A SINGLE GROWING
UNIT SHOWING THE INCREASING PILEUS DIAMETER OF
SPOROPHORES.
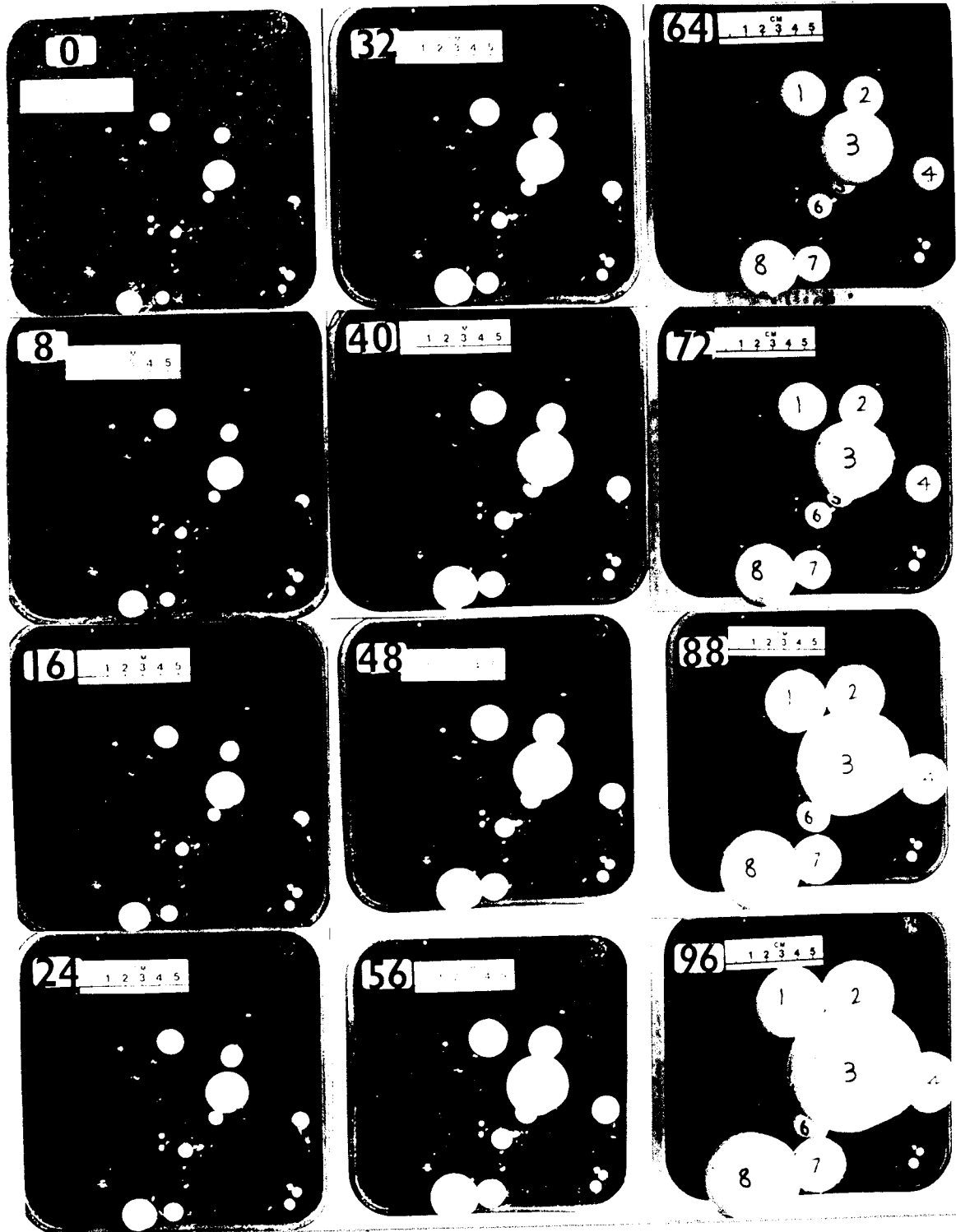
sporophore dry weights were calculated from the pileus diameter.

It is possible to calculate the dry weight using two methods:

1. Using the slope of the curve which best fits the data in
    Figure 3.11

    $$y^{1.75} = 17 x \quad \text{therefore} \quad \text{The Dry Weight (G)} = \frac{\text{Diameter (cm)}^{1.75}}{17}$$

2. Using Figure 3.10. The pileus diameter is followed across
    until the appropriate developmental stage line is met. The
    dry weight may then be read off on the x axis.

Table 3.29 compares the two methods of calculating the final
dry weight with the actual final dry weight of sporophores harvested
from a single growing unit. There was a highly significant correla-
tion between the actual and calculated dry weights, but the values
calculated graphically were closer to the actual value than those
values calculated using the formula. This is probably because the
graphical method allows for the latitude which is observed to exist
between the different stages. The correlation between the actual
and graphically calculated final dry weights of all sporophores is
extremely highly significant and it can thus be assumed that this
method provides an accurate technique for estimating sporophore
dry weight.

The dry weight of each of the 76 sporophores was calculated
at the time of each photograph. The dry weight values of a single
sporophore are given in Table 3.30. The changes in dry weight per
hour (= Growth Rate) were calculated between successive photographs.
These growth rates were expressed at the mean weight of the sporo-
phore over which time the growth rate values were calculated.

3.3.2.4   Methods of Expressing Growth Rate of Sporophores.

Usually the growth rate of an organism is expressed relative

TABLE 3.29. COMPARISON OF ACTUAL DRY WEIGHTS AND DRY WEIGHTS CALCULATED FROM THE PILEUS DIAMETERS OF SPOROPHORES HARVESTED FROM A SINGLE GROWING UNIT.

PILEUS DIAMETER (CM)	ACTUAL FINAL DRY WEIGHT (G)	FINAL DRY WEIGHT(G) CALCULATED GRAPHICALLY	FINAL DRY WEIGHT(G) CALCULATED USING $\frac{Y^{1.75}}{17} = X$
2.35	0.23	0.23	0.262
3.81	0.55	0.593	0.611
4.70	0.88	0.825	0.882
3.96	0.62	0.655	0.654
3.02	0.32	0.328	0.407
5.94	1.12	1.155	1.329
3.61	0.50	0.513	0.556
4.32	0.62	0.690	0.761
4.93	0.71	0.755	0.959
8.29	1.63	1.630	2.382

Correlation between the Actual and Graphically calculated

Dry Weight = 0.9965 9°F.

Correlation between the Actual and Formula calculated

Dry Weight = 0.9787 9°F.

The Overall Correlation between the Actual and Graphically calculated

Dry Weight = 0.994 74°F.

TABLE 3.30. PILEUS DIAMETERS, ESTIMATED DRY WEIGHTS AND GROWTH RATES FOR A SINGLE SPOROPHORE FROM A SEQUENCE OF PHOTOGRAPHS.

TIME OF PHOTOGRAPH(HOURS)	0	8	16	24	32	40	48	56	64	72	88	96
PILEUS DIAMETER (CM)	1.4	1.5	1.75	1.96	2.15	2.52	2.83	3.1	3.48	4.06	6.62	6.74
ESTIMATED DRY WEIGHT (G)	0.104	0.115	0.149	0.179	0.221	0.284	0.364	0.401	0.489	0.626	0.940	1.460

MEAN TIME (HOURS)	4	12	20	28	36	44	52	60	68	80	92
MEAN WEIGHT (G)	0.110	0.132	0.164	0.200	0.254	0.315	0.374	0.445	0.558	0.783	1.200
GROWTH RATE (MG/HOUR)	1.38	4.25	3.75	5.25	7.88	7.75	6.88	11.0	17.13	39.25	65.00

to time but, the primary objective of this study was to determine the relationship between the growth rate and size of the sporophore. All growth rate values and their associated mean sporophore dry weight values were distributed into 13 different groups on the basis of similarity of dry weight. The mean dry weight and mean growth rate values of each of these groups are given in Appendix 3.5 and Figure 3.12. The correlation between the growth rate and dry weight of the 598 calculated values (0.996) was significant at a probability of 0.001. The relationship between these two parameters was linear and thus, the growth rate of the sporophore increased in direct proportion to increasing sporophore size.

The methods used in this study also allowed the growth rate of the sporophore to be expressed relative to time. The growth rate of both the population and an individual sporophore can be expressed in this way.

The growth rate of the population over each eight hour period, between consecutive photographs, was taken as the mean of the individual growth rates in that period. The sequence of population growth rates is shown in Appendix 3.6 and Figure 3.13. It can be seen in Plate 3.2 that even in a single growing unit there was a considerable difference of size between individuals sporophores. It is probable that the range of sizes was contributing to the variability of the population growth rate values observed in Figure 3.13.

By combining the growth rate values from the 76 individual sporophores, a composite growth curve was constructed for the 'average' individual sporophore. This was done as shown in Table 3.31A. The data was positioned so that similar dry weight values of individual sporophores were aligned in vertical columns.

FIGURE 3.12. GROWTH RATES OF GROUPS OF SPOROPHORES OF SIMILAR DRY WEIGHT.
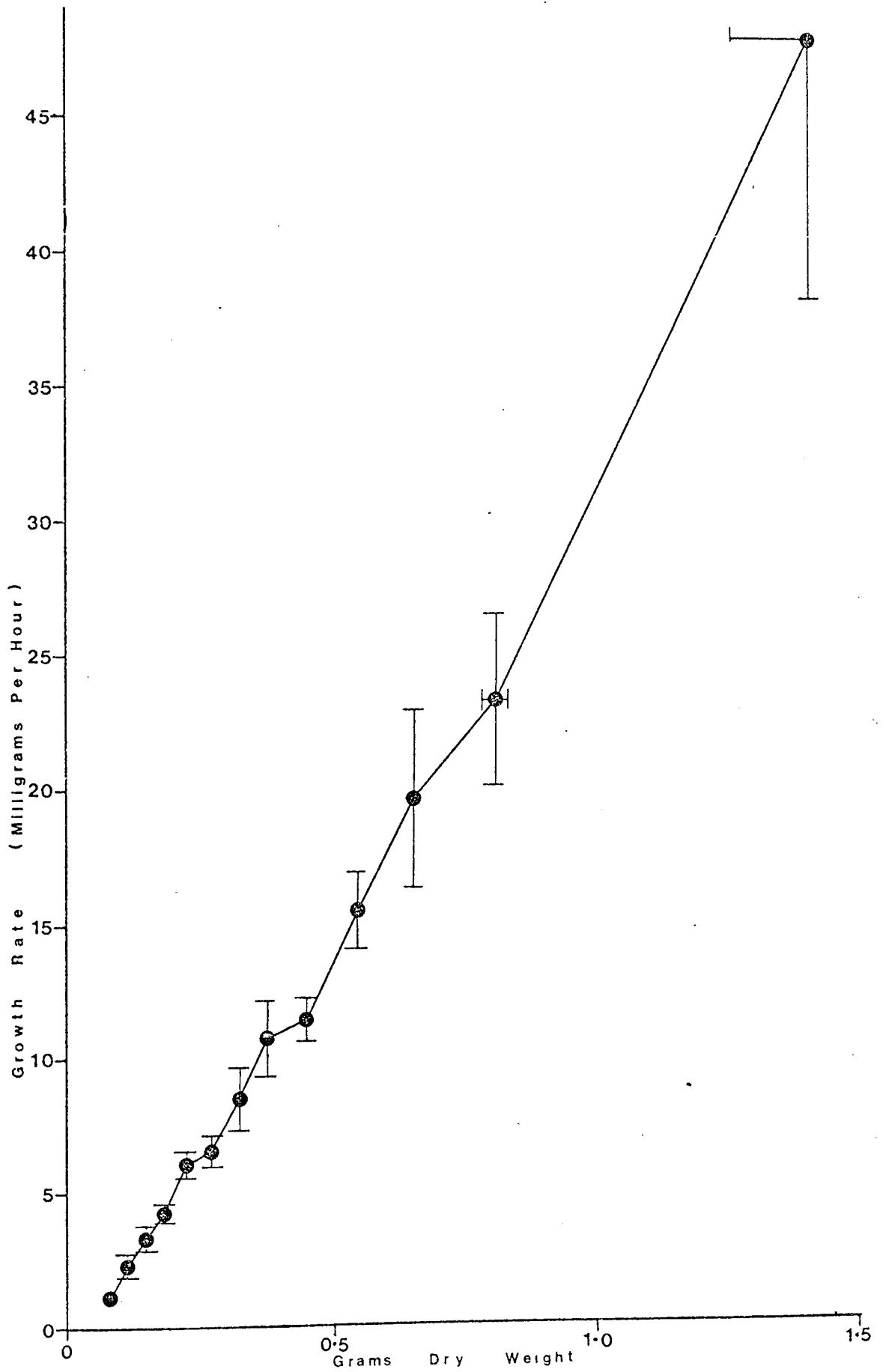
FIGURE 3.13. POPULATION GROWTH RATES CALCULATED FROM SUCCESSIVE PHOTOGRAPHS.
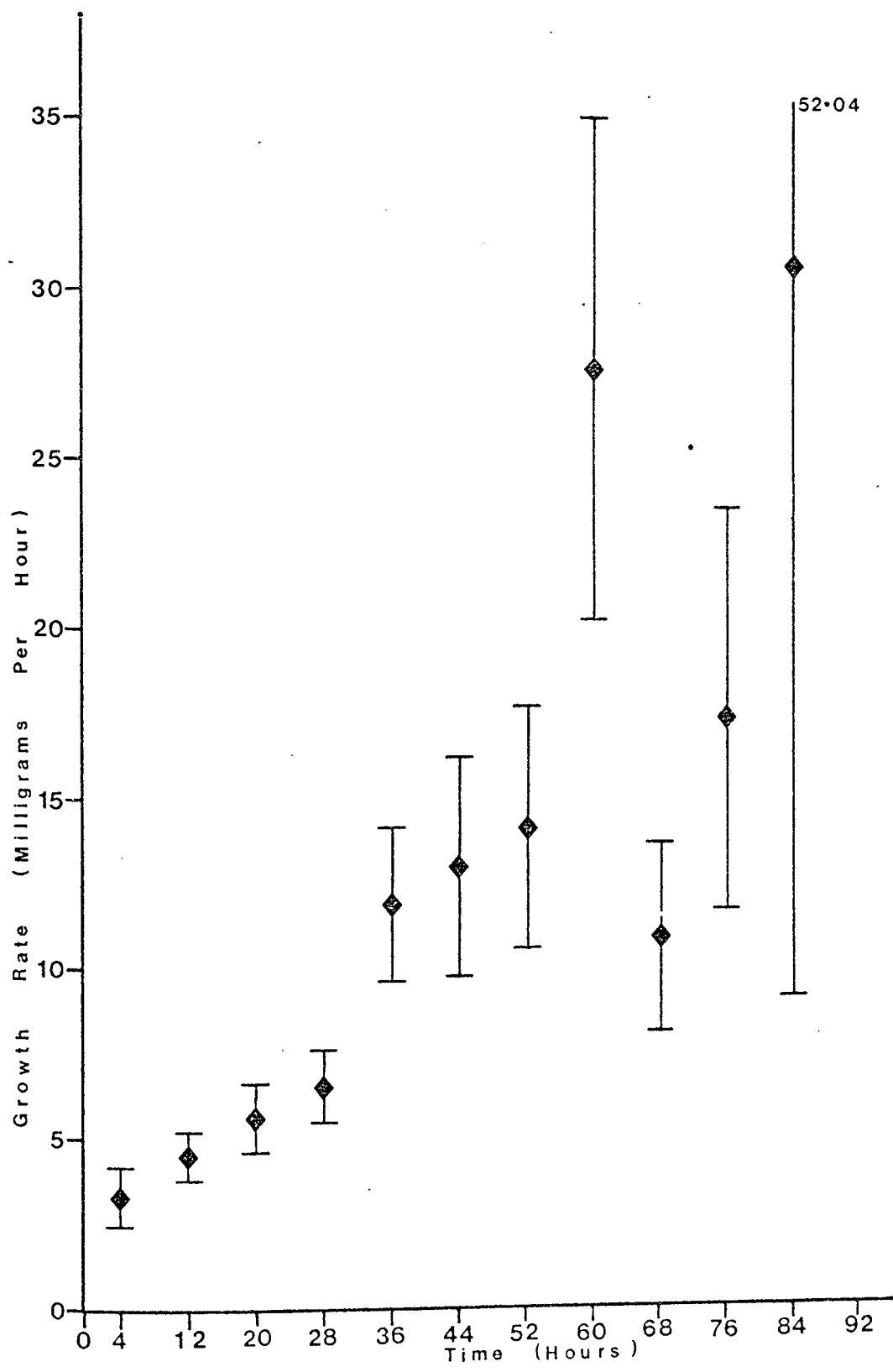
TABLE 3.31. ALIGNMENT OF GROWTH RATE SEQUENCES OF INDIVIDUAL SPOROPHORES TO OBTAIN A GROWTH RATE CURVE FOR THE 'AVERAGE' SPOROPHORE

TO SHOW: A. ALIGNMENT OF 4 (OUT OF 76) INDIVIDUAL SPOROPHORE GROWTH RATE SERIES RELATIVE TO ONE ANOTHER.
B. MEAN OF ALIGNED COLUMNS.

GROUPS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
ARBITRARY TIME SCALE (HOURS)	0	8	16	24	32	40	48	56	64	72	80	88	96	104	112	120	128	136	144	152
WEIGHT(G)	0.064	0.078	0.090	0.103	0.132	0.170	0.207	0.255												
1. GROWTH RATE	2.29	1.50	1.88	5.38	5.38	3.88	5.38	6.75												
WEIGHT(G)							0.207	0.245	0.300	0.365	0.462	0.592	0.754	1.001						
2. GROWTH RATE							3.86	6.13	7.50	8.75	15.63	16.88	23.50	38.38						
A. WEIGHT(G)									0.416	0.495	0.610	0.751	1.012	1.292	1.470	1.59				
3. GROWTH RATE									5.57	14.88	15.0	2	45.63	24.28	2	10				
WEIGHT(G)											0.106	0.130	0.172	0.215	0.266	0.314	0.397			
4. GROWTH RATE											0.860	2.570	4	6.880	5.75	6.38	14.13			
SAMPLE SIZE	8	8	30	26	47	53	46	52	43	44	45	48	42	32	29	17	11	6	2	1
B. GROWTH RATE	1.11	1.289	1.181	1.589	2.838	3.757	4.676	6.525	6.695	8.865	10.25	12.92	15.87	19.51	28.13	35.76	42.10	87.86	66.57	81.25
95% C.L.	0.506	0.353	0.232	0.302	0.354	0.446	0.441	0.466	0.565	1.163	1.274	1.124	1.951	2.753	3.670	9.276	16.32	46.49	218.4	-

GROWTH RATES IN MILLIGRAMS PER HOUR.   95% C.L. = 95% CONFIDENCE LIMITS.

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FIGURE 3.14. GROWTH RATE CURVE OF THE 'AVERAGE' SPOROPHORE.
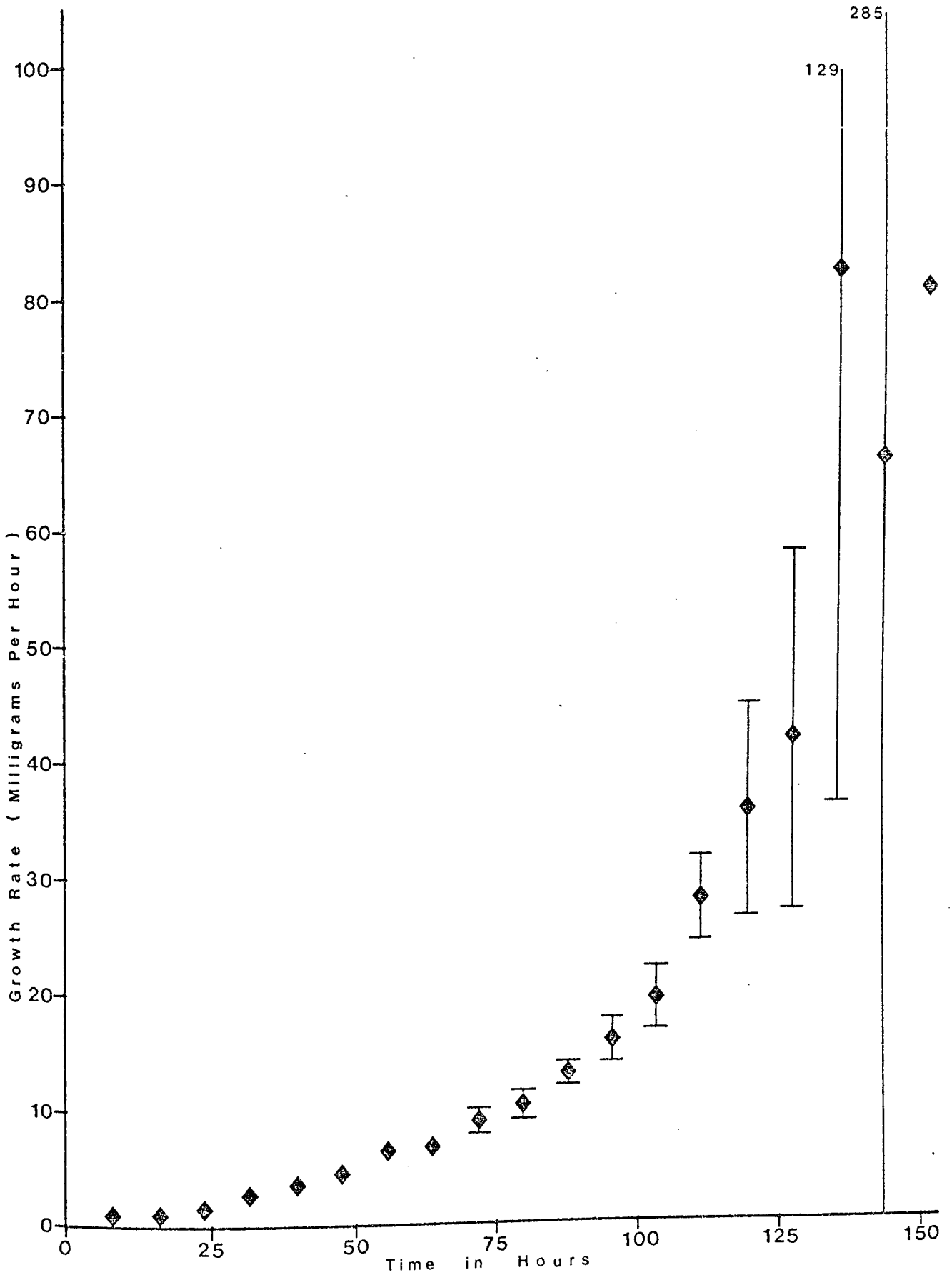
The series of data for individual sporophores must occupy consecutive columns in order to retain the eight hour periodicity of the growth rate results. Table 3.31B shows the mean values of the vertical columns when all 76 sporophores were aligned in such a manner. These growth rates were plotted against the arbitrary time scale and are shown in Figure 3.14. The curve represents the growth rate of an average sporophore during its development from a small primordium to a large flat. This development took place over a period of about six days during which time the growth rate increased exponentially. It does not appear that there was a curtailment of growth rate in the later sporophore stages studied here.

The results of this study demonstrate that the growth rate of individual sporophores and the crop can be successfully estimated in situ.

3.3.3 Concentrations of Iron in Different Developmental Stages of the Sporophore.

It has been frequently observed that the concentration of certain materials, particularly metals, is greater in smaller sporophores than in larger, mature sporophores. The results of Section 3.1.3 indicate that the concentration of iron in Agaricus bisporus sporophores follows this pattern.

Using sporophore material that was produced in Section 3.3.2.2 the concentration of iron was analysed in sporophores that had been divided into the ten defined developmental stages. The results of these analyses for first and second break sporophores are given in Table 3.32.

In the first break there were no significant differences of iron composition between the different sporophore developmental

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TABLE 3.32.   CONCENTRATIONS OF IRON IN DIFFERENT DEVELOPMENTAL
STAGES OF THE SPOROPHORE .(MILLIGRAMS PER KILOGRAM
DRY WEIGHT).

STAGE	FIRST BREAK			SECOND BREAK		
	Mean	S.D.	N	Mean	S.D.	N
BUTTON	28.40	0.676	2	34.75	1.411	3
LATE BUTTON	23.05	8.031	4	23.80	1.855	3
BUTTON - OPEN CUP	34.37	12.39	3	27.19	5.480	3
EARLY OPEN CUP	26.18	0.817	4	21.66	3.782	2
EARLY-MID OPEN CUP	33.19	6.367	3	24.84	1.434	3
MID OPEN CUP	27.29	6.625	4	24.35	0.782	3
MID-LATE OPEN CUP	24.80	1.536	5	24.84	1.038	3
LATE OPEN CUP	37.41	14.12	4	22.04	0.931	3
EARLY FLAT	29.45	4.789	4	27.75	6.377	4
FLAT	27.23	2.223	3	18.04	4.880	3

N = Number of samples.

Mean = Mean of those samples.

S.D. = Standard Deviation of those samples.

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stages. In the second break sporophores the concentration of iron in the button stage was significantly greater than in all but two other stages. Despite the considerable variation within each developmental stage there was an overall, gradual decrease in the concentration of iron in the more mature stages of development, shown by a negative slope (-0.845) for the best fitting straight line.

3.3.4   Concentrations of Iron-59 in Sporophores of Different Sizes.

The study of the uptake of iron into sporophores of increasing size was facilitated by the use of iron-59 and the automated gamma counter in the Department of Biology at the University of Aston in Birmingham. The quantity of iron-59 contained in sporophores of different sizes would then show if the uptake of iron-59 was concomitant with the growth of the sporophore.

A description of the methods used is given in Sections 2.1 and 2.3. The isotope solution distributed evenly throughout the compost of fifty growing units using a syringe and hypodermic needle. The first break sporophores, 290 in total, were harvested 21 days after the application of the isotope and the casing layer. The dry weight, radioactive count per hour and stage of development were determined for each sporophore.

The 290 sporophores were ranked in order of increasing dry weight and then divided into groups, each containing ten sporophores of similar dry weight. The mean dry weights and mean isotope concentrations (given by Log counts/hour/gram dry weight) of the sporophores in these groups are given in Appendix 3.7 and shown in Figure 3.15. The concentration of isotope decreased with increasing sporophore size. The correlation coefficient (-0.823)

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between weight and concentration was significant at a probability of 0.001. The negative nature of this correlation shows that there was a close relationship between increasing sporophore size and decreasing isotope concentration.

The mean dry weights and mean isotope contents (Log counts/hour/sporophore) of the 29 groupings are given in Appendix 3.7 and Figure 3.16. The total amount of isotope contained in each sporophore can be seen to be approximately similar in sporophores of widely different size. The correlation (-0.178) between weight and content was not significant but, linear regression analysis showed the constancy of isotope content in sporophores of increasing dry weight.

124 of the sporophores harvested were separated into pileus and stipe. The quantity of isotope in these tissues was determined and the data obtained were grouped according to similarity of sporophore size. The relative distribution of dry weight and isotope between the two tissues in the eight groups is shown in Appendix 3.8 and Figure 3.17. Figure 3.17A shows that with increasing sporophore size, up to about 0.7 grams dry weight, the pileus contributed an increasingly larger proportion of the sporophore. Beyond this size, which approximates to the mid open cup stage, the relative proportions of stipe to pileus remained constant. Figure 3.17B shows that with increasing sporophore size, the proportion of isotope contained in the pileus decreased.

The concentrations of isotope in the eight groups of sporophores and in the pilei and stipes of those sporophores are given in Appendix 3.9 and Figure 3.18. Figures 3.18A and C show that the decreasing isotope concentration in sporophores of increasing

size was due to the declining concentration of isotope in the pileus. The concentration of isotope in the stipe (Figure 3.18B) remained approximately constant throughout. The negative correlation (-0.857) between the pileus as a percentage of the total sporophore size (Figure 3.17A) and the concentration of isotope in the pileus (Figure 3.18C) was significant at a probability of 0.01. This shows that as the sporophore grew, little or no more isotope entered the pileus. The declining isotope concentration of the pileus was directly related to the increasing uptake of other materials used in sporophore construction.

All of the sporophores harvested in the first break were also grouped according to their stage of development. The mean dry weights and isotope concentrations of these groups are given in Appendix 3.10 and Figure 3.19. These groups show considerable variations of both dry weight and isotope concentration, when compared with groupings made on the basis of dry weight. There was, however, a definite trend of decreasing isotope concentration with increasing sporophore maturity.

Sporophores from the second and third breaks were analysed in the same manner as those from the first break. In all three breaks, similar changes of isotope concentration and isotope content occurred with increasing size although the results increased in variability with the decreasing sporophore numbers harvested from the second and third breaks.

The results show that the quantity of iron-59 contained in sporophores covering a wide range of individual sizes, from 0.1 to 2.0 grams dry weight was approximately constant. It is probable that the limited quantity of the isotope either available to or contained in the mycelia of the substrates, moves into the sporophore

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FIGURE 3.15. CONCENTRATIONS OF IRON-59 IN GROUPS OF SPOROPHORES OF SIMILAR DRY WEIGHT.
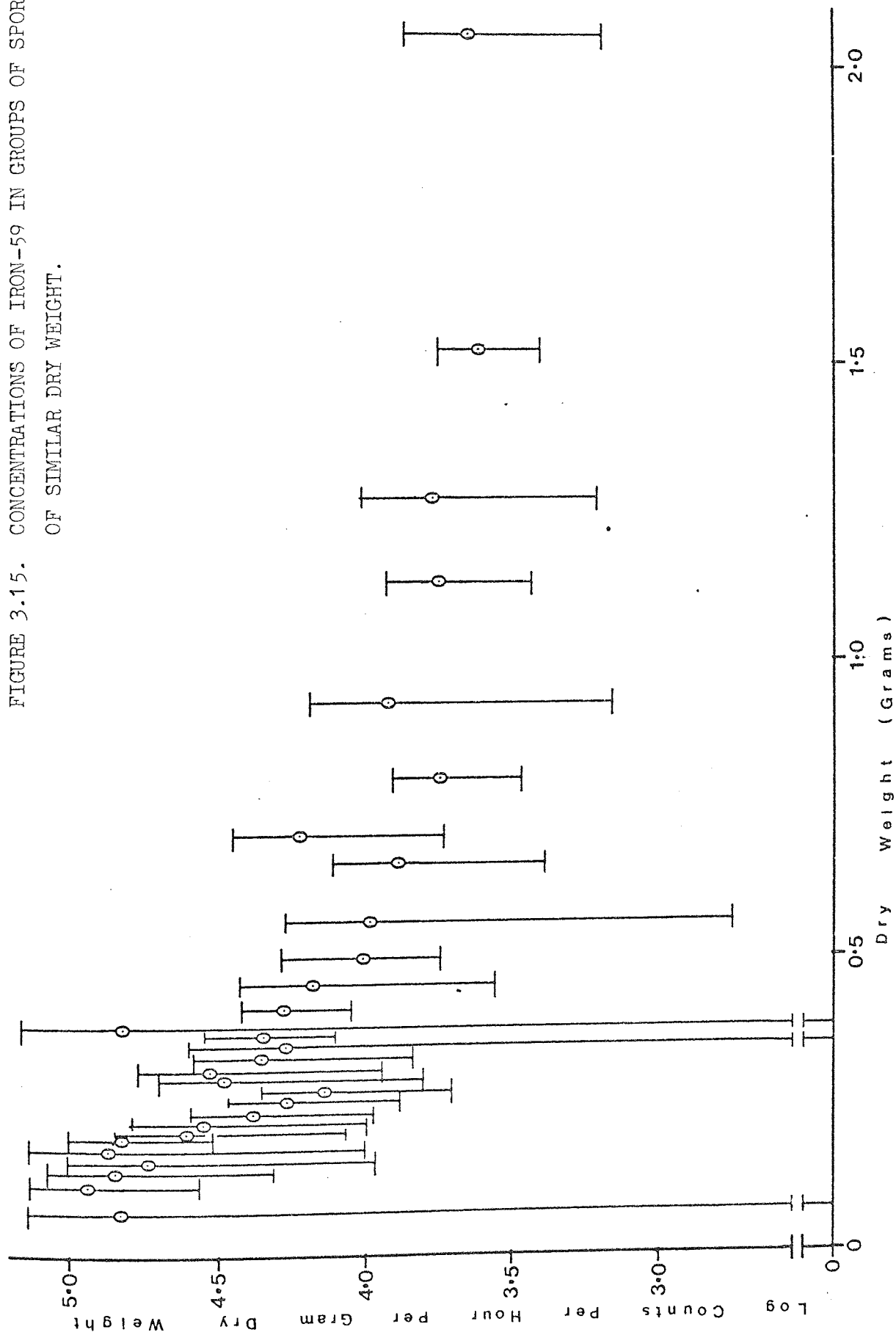
FIGURE 3.16. IRON-59 CONTENT IN GROUPS OF SPOROPHORES OF SIMILAR DRY WEIGHT.
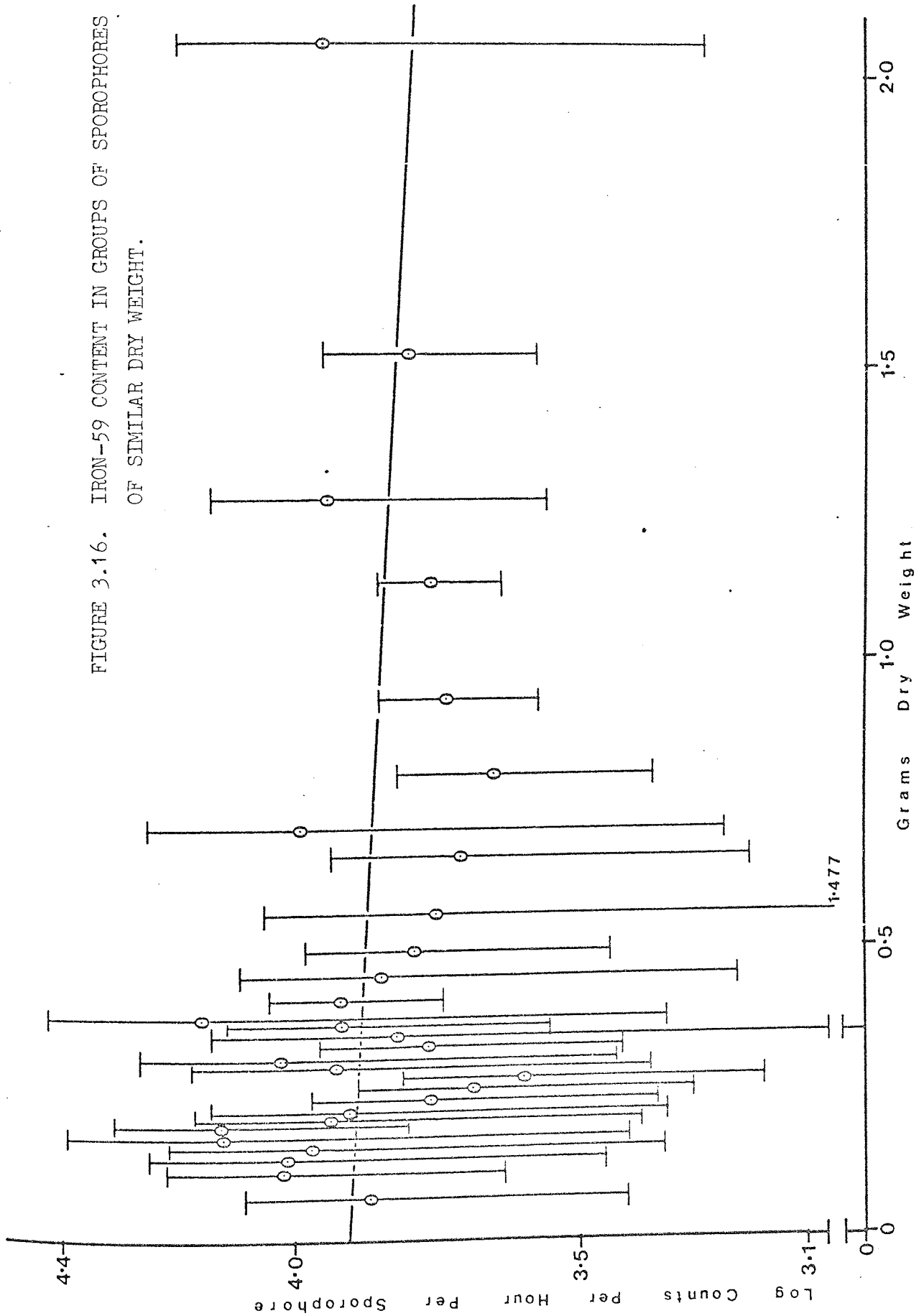
FIGURE 3.17. A.DRY WEIGHT AND B.IRON-59 CONTENT OF THE PILEUS, AS PERCENTAGES OF THE ENTIRE SPOROPHORE, IN GROUPS OF SPOROPHORES OF SIMILAR DRY WEIGHT.
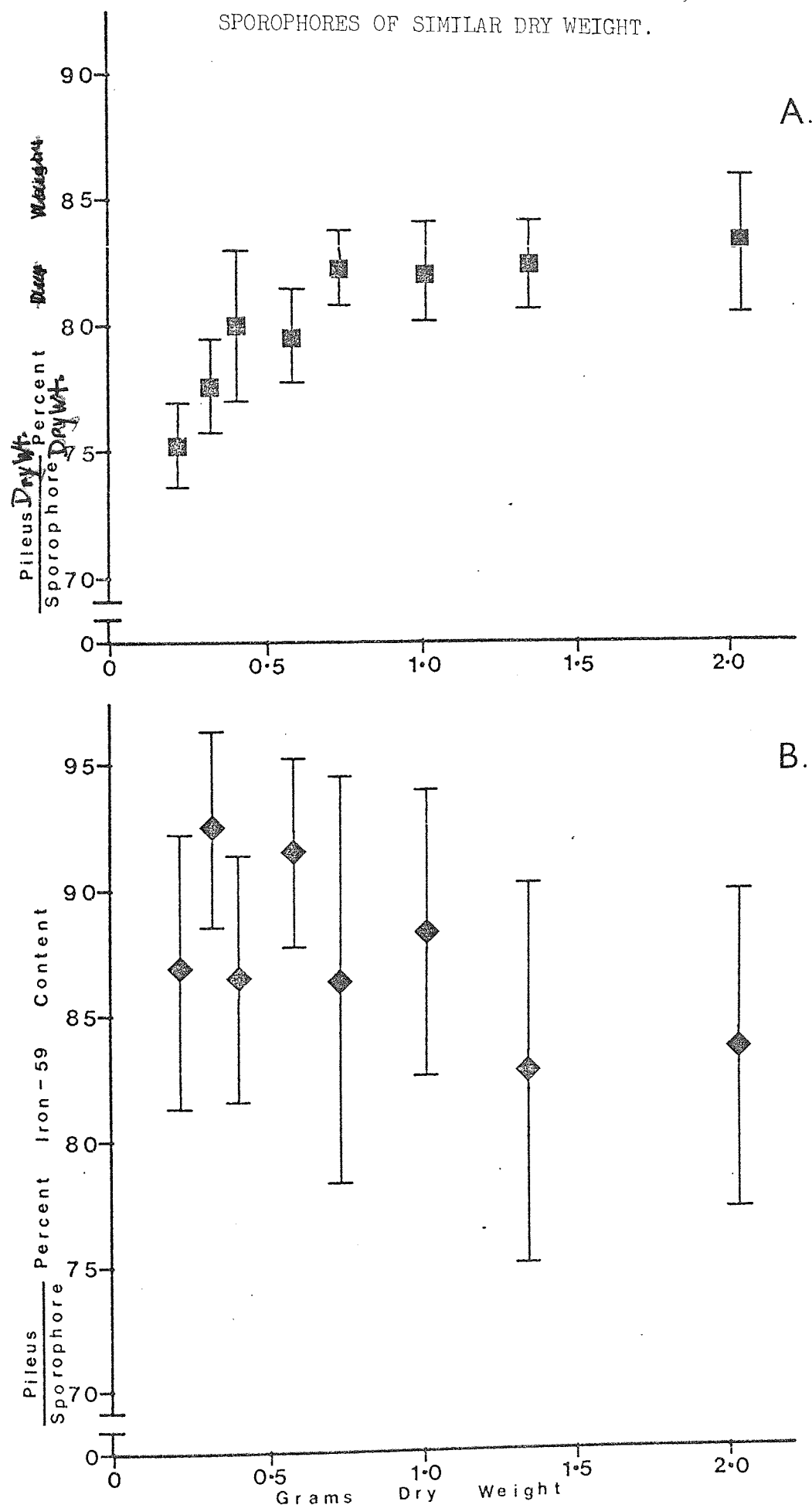
FIGURE 3.18. CONCENTRATIONS OF IRON-59 IN THE PILEUS, STIPE AND ENTIRE SPOROPHORE, IN GROUPS OF SPOROPHORES OF SIMILAR DRY WEIGHT.
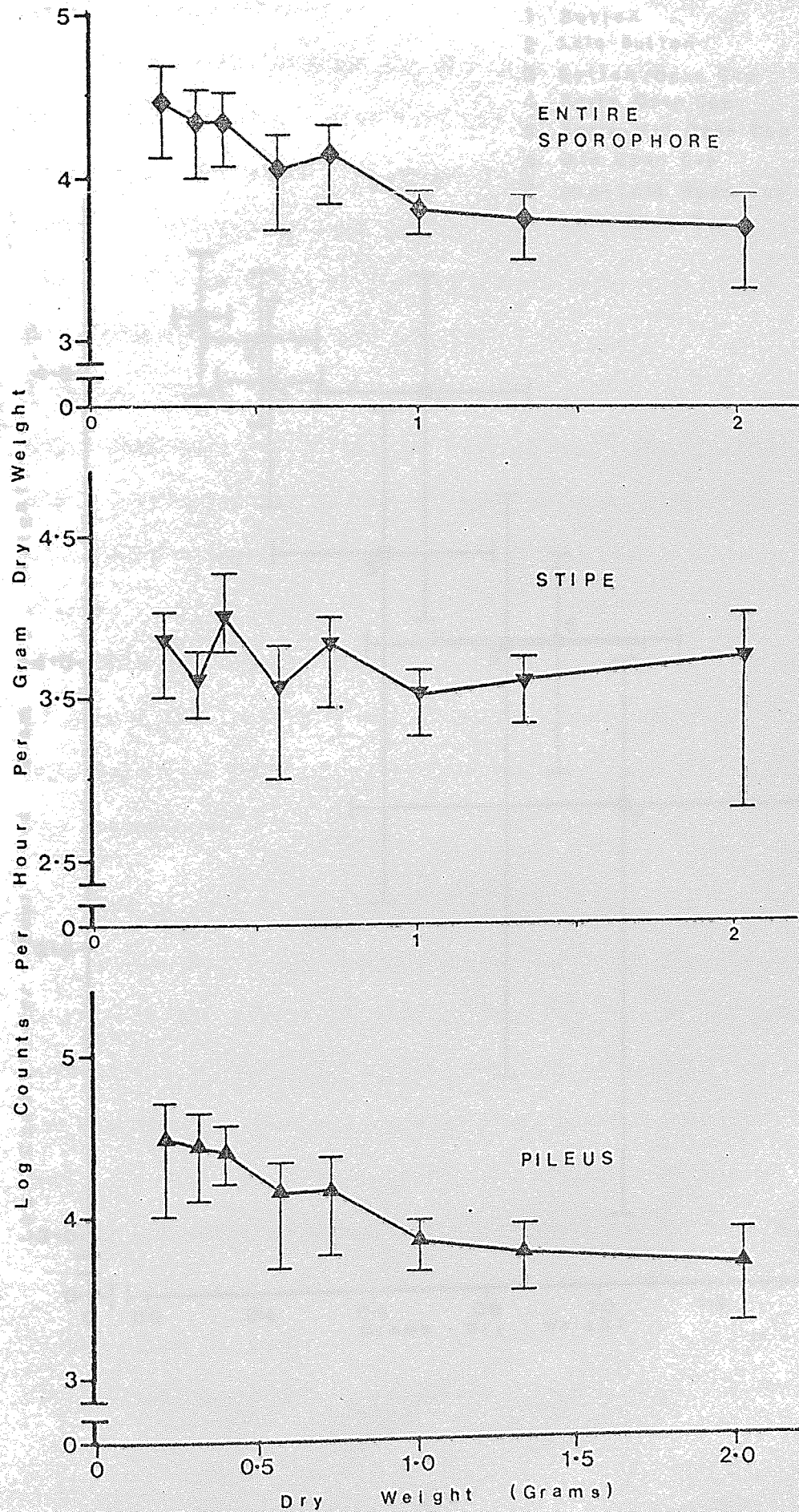
FIGURE 3.19. DRY WEIGHTS AND CONCENTRATIONS OF IRON-59 IN
SPOROPHORES GROUPED ACCORDING TO DEVELOPMENTAL
STAGE.

1 Button
2 Late Button
3 Button / Open Cup
4 Early Open Cup
5 Early-Mid Open Cup
6 Mid Open Cup
7 Mid-Late Open Cup
8 Late Open Cup
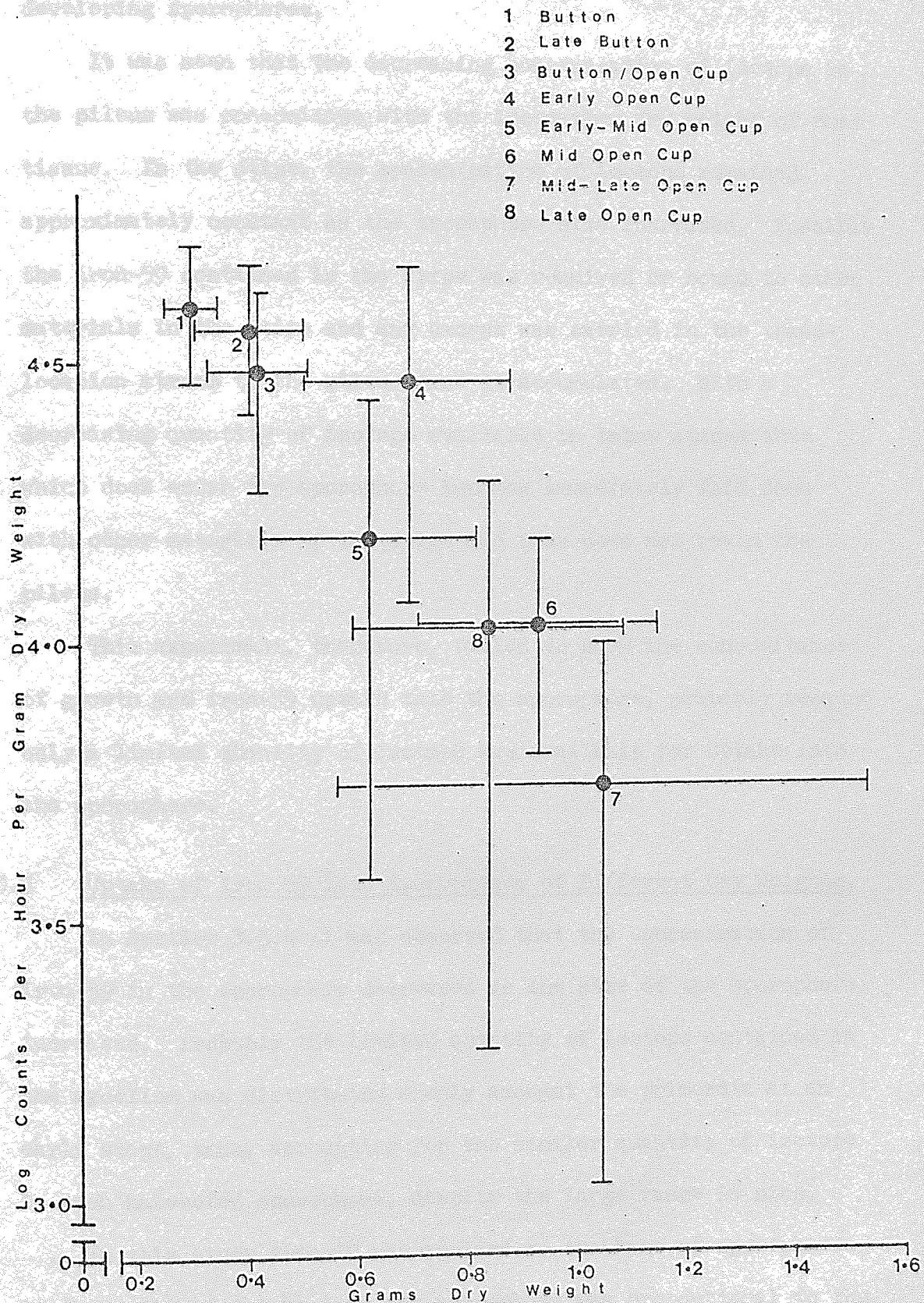
at an early stage of development. It appears that the available quantity of isotope is also evenly distributed amongst the developing sporophores.

It was seen that the decreasing concentration of isotope in the pileus was concomitant with the increasing dry weight of that tissue. In the stipe, the concentration of isotope remained approximately constant as the sporophore size increased. Possibly the iron-59 contained in the stipe was required or bound to other materials in the stipe and any excess was carried in the translocation stream to the pileus where it accumulated. With a decreasing quantity of isotope available in later stages that which does enter the sporophore becomes immediately laid down with other materials of the stipe and thus does not reach the pileus.

This experiment, therefore, failed to show the concomitance of growth and iron-59 uptake into the sporophore, probably because only a limited quantity of isotope was available for uptake into the sporophore.

3.3.5   Uptake of Iron-59 into Sporophores of Different Dry Weights.

In Section 3.3.4 it was observed that the concentration of iron-59 in the sporophore decreased as the size of the sporophore increased. Probably the limited quantity of isotope contained in the mycelium was distributed evenly amongst the primordia at an early stage, thus, accounting for the similar quantity of isotope in each harvested sporophore, despite the large range in size.

In this study iron-59 was applied to the base of sporophores to determine if uptake into the sporophore was proportional to the size of the sporophore. One millilitre of isotope solution (pre-

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FIGURE 3.20. CONCENTRATIONS OF IRON-59 IN GROUPS OF SPOROPHORES OF SIMILAR DRY WEIGHT.
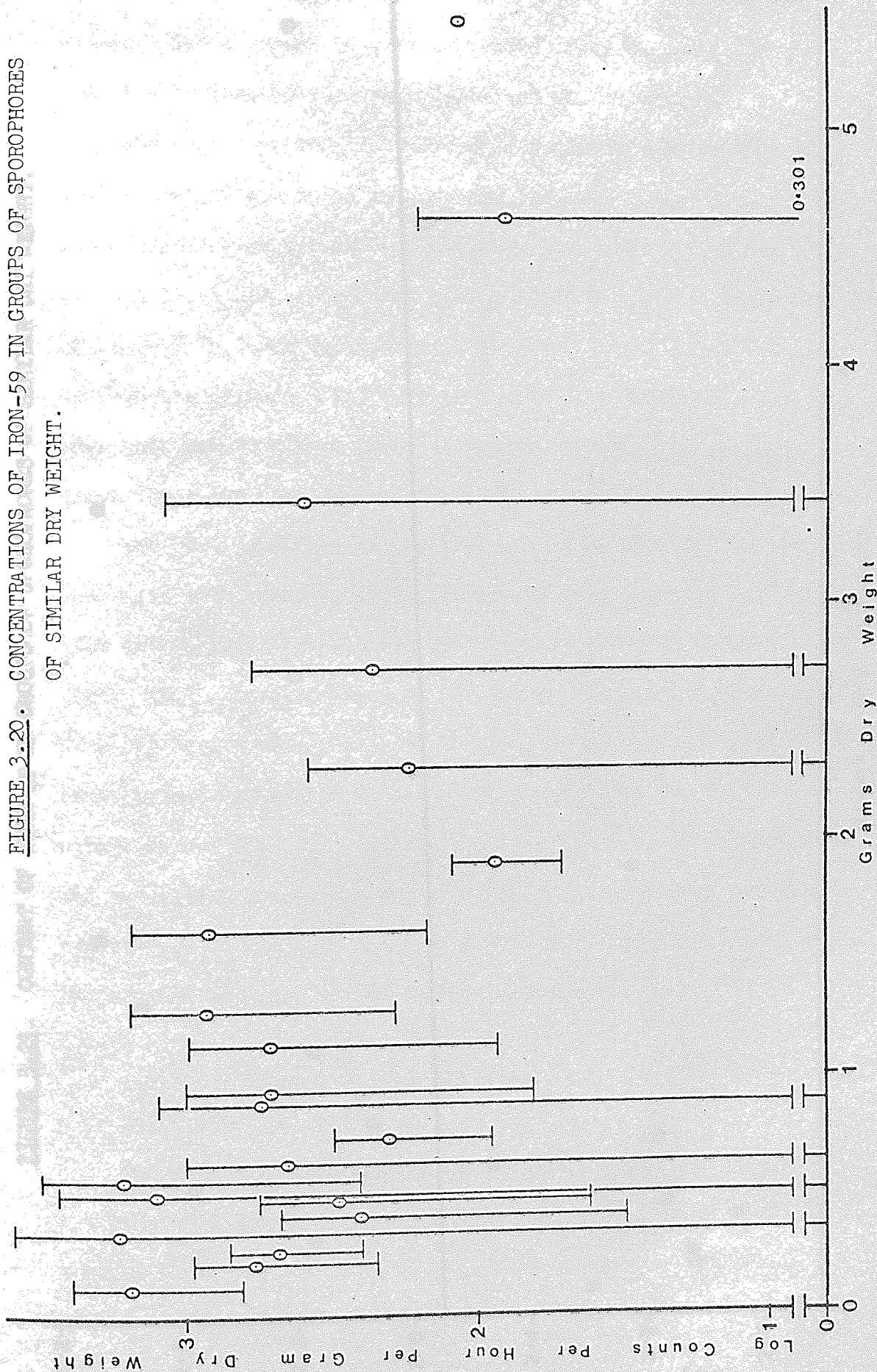
FIGURE 3.21. CONTENT OF IRON-59 IN GROUPS OF SPOROPHORES OF SIMILAR DRY WEIGHT.
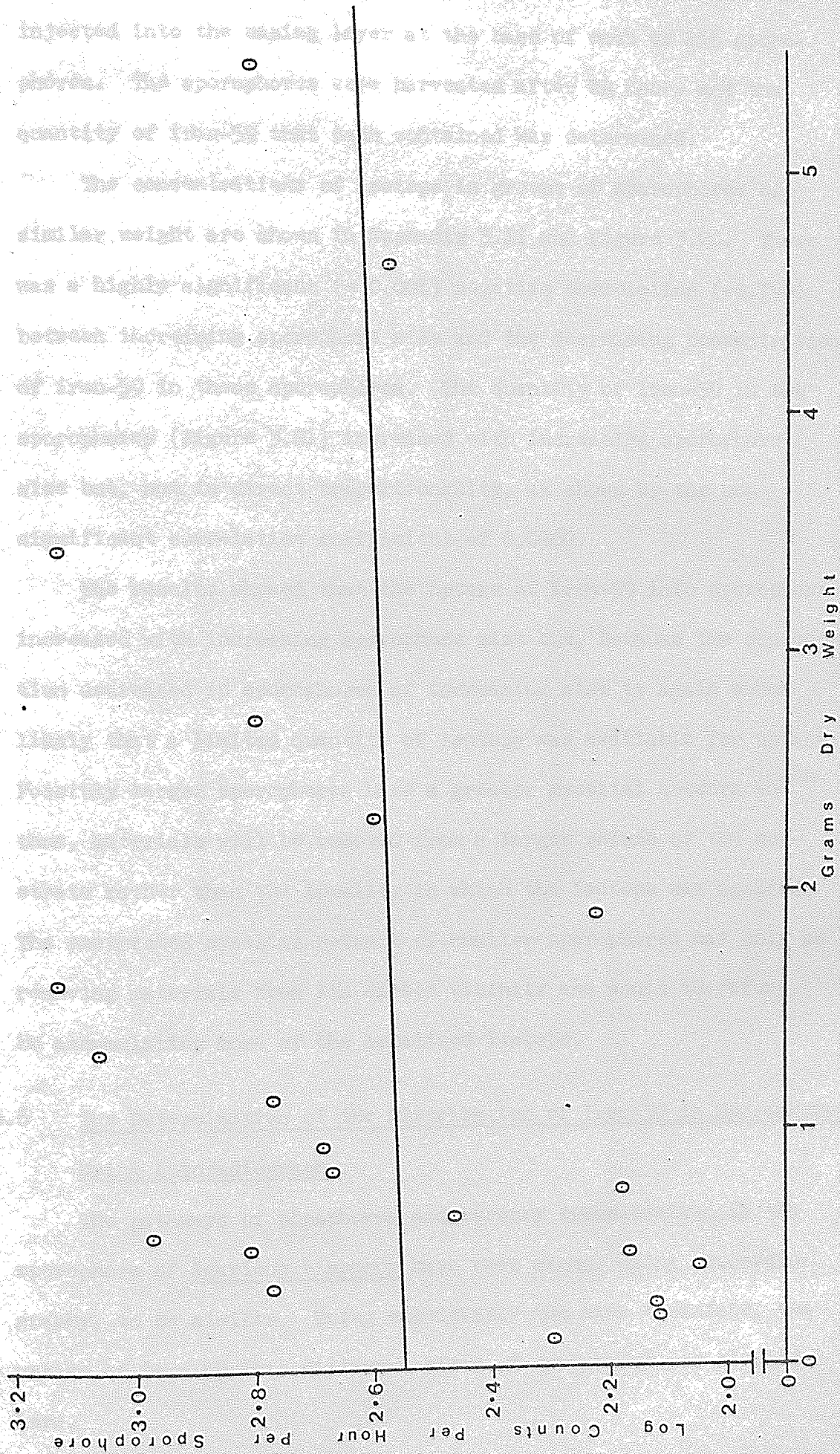
pared in accordance with the methods given in Section 2.3) was injected into the casing layer at the base of each of 216 sporophores. The sporophores were harvested after 24 hours and the quantity of iron-59 that each contained was determined.

The concentrations of isotope in groups of sporophores of similar weight are shown in Appendix 3.11 and Figure 3.20. There was a highly significant (= 0.001) negative correlation (-0.794) between increasing sporophore size and the decreasing concentration of iron-59 in those sporophores. The quantity of iron-59 in the sporophores (Figure 3.21) increased with increasing sporophore size but, not in direct proportionality, as shown by the non-significant correlation coefficient of 0.0969.

The results showed that the uptake of iron-59 into sporophores increased with increasing sporophore size but, because the concentration decreased in sporophores of increasing size it again seems likely that a limited quantity of isotope was available for uptake. Possibly larger sporophores have a greater mycelial network and thus, materials will be removed from a larger volume of the substrate rather than the locality in which the isotope was applied. The restricted mycelial network of smaller sporophores may only be removing materials from its direct vicinity and would therefore, be accumulating more of the localised isotope.

3.3.6   The Determination of the Distribution of Iron-59 in Sporophores

Using Autoradiography.

The pathways of phosphorus and mercury translocation in the sporophore of Agaricus bisporus have been shown, using autoradiography, to be similar. Using essentially the same technique, the uptake of iron-59 into different sizes of sporophore was studied here.

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X-ray film was used to prepare autoradiographs of entire sporophore sections. Different sizes of sporophore were carefully plucked from the casing soil so that mycelial strands remained attached to the stipe base. Each sporophore was then placed in a plastic universal which had been trimmed to enable the base of the stipe to be just immersed in 5 millilitres of 2 microcuries per millilitre iron-59 solution. After 12 hours the sporophores were removed from the isotope solution. The bases of the stipes were removed and each sporophore was bisected longitudinally. Longitudinal slices, two millimetres thick, were taken from each sporophore. Twenty of these slices were placed in direct contact with each sheet of Kodak 'Kodirex' X-ray film. The film sheets were handled and developed in accordance with the methods given on the appropriate Kodak Data Sheet (see Bibliography). The film and sporophore slices were firmly held together and placed in light-proof protective envelopes. A light pressure was applied to ensure contact between the samples and film and then liquid nitrogen was introduced into the envelope to facilitate freezing. The films were then stored in a deep freeze throughout the six week period of exposure.

Plate 3.3 shows autoradiographs of different sizes of sporophore. A darker image shows that more iron-59 was contained in the larger sporophores. In the smaller sporophores (A) the isotope was localised in two areas of the stipe; the centre and the periphery. Some isotope was found in hymenia. In the more mature sporophore stages most of the isotope was contained in the centre of the stipe. In the mid open cup (B) the isotope had entered the pileus and moved outwards in the pileus tissue adjacent to the hymenia. In the late open cup (C) some isotope had moved to the tissue adjacent to the

PLATE 3.3.   AUTORADIOGRAMS OF SPOROPHORES CONTAINING IRON-59.

(ACTUAL SIZE.)
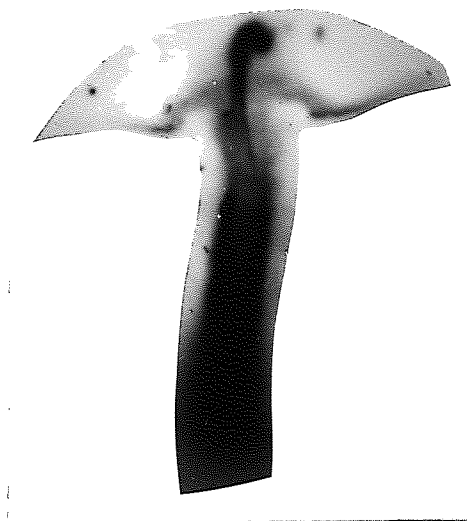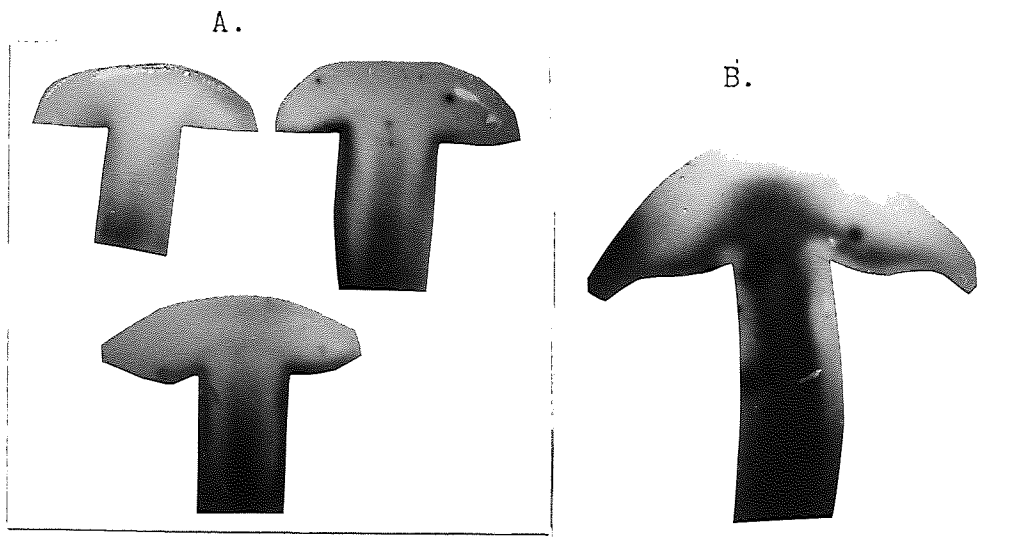
hymenia but, there was a greater flow towards the upper surface of the pileus. In the flat (D) virtually all isotope movement in the pileus was towards the tissues of the upper surface of the pileus underlying the pellicle.

The autoradiographs show that iron was translocated into the sporophore throughout development and the quantity of iron-59 taken up was proportional to the size of the sporophore. The soluble Ferric-59 Chloride was probably demonstrating the general translocatory streams within the sporophore.

The results show that the movement of materials in the sporophore are not purely the result of passive transpiration flow, there appears to be some control of the direction of the translocatory stream. Movement of iron-59 towards the hymenia occurred at the time of maturation of these tissues. Whereas, in the later stages, the major movement of iron-59 was towards the tissues of the upper layers of the pileus - an area involved in the expansion of the sporophore.

3.3.7   The Concentration of Iron-59 in Primordia and Small Sporophores.

In Section 3.3.4 it was found that the concentration of iron-59 decreased in sporophores of increasing size. It was decided to extend this study further and to determine the concentration of isotope in primordia and small sporophores. The methods used were as in Section 3.3.4. 18 days after casing the primordia were carefully dissected from the casing material (Plate 3.4) of twelve growing units. Following drying the primordia were sorted into ten groups of similar sized individuals. Approximately 100 milligrams of primordium tissue were placed into each sample

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PLATE 3.4. REMOVAL OF PRIMORDIA FROM THE CASING LAYER.

FIGURE 3.22. CONCENTRATIONS OF IRON-59 IN GROUPS OF SPOROPHORES
AND PRIMORDIA OF SIMILAR DRY WEIGHT.
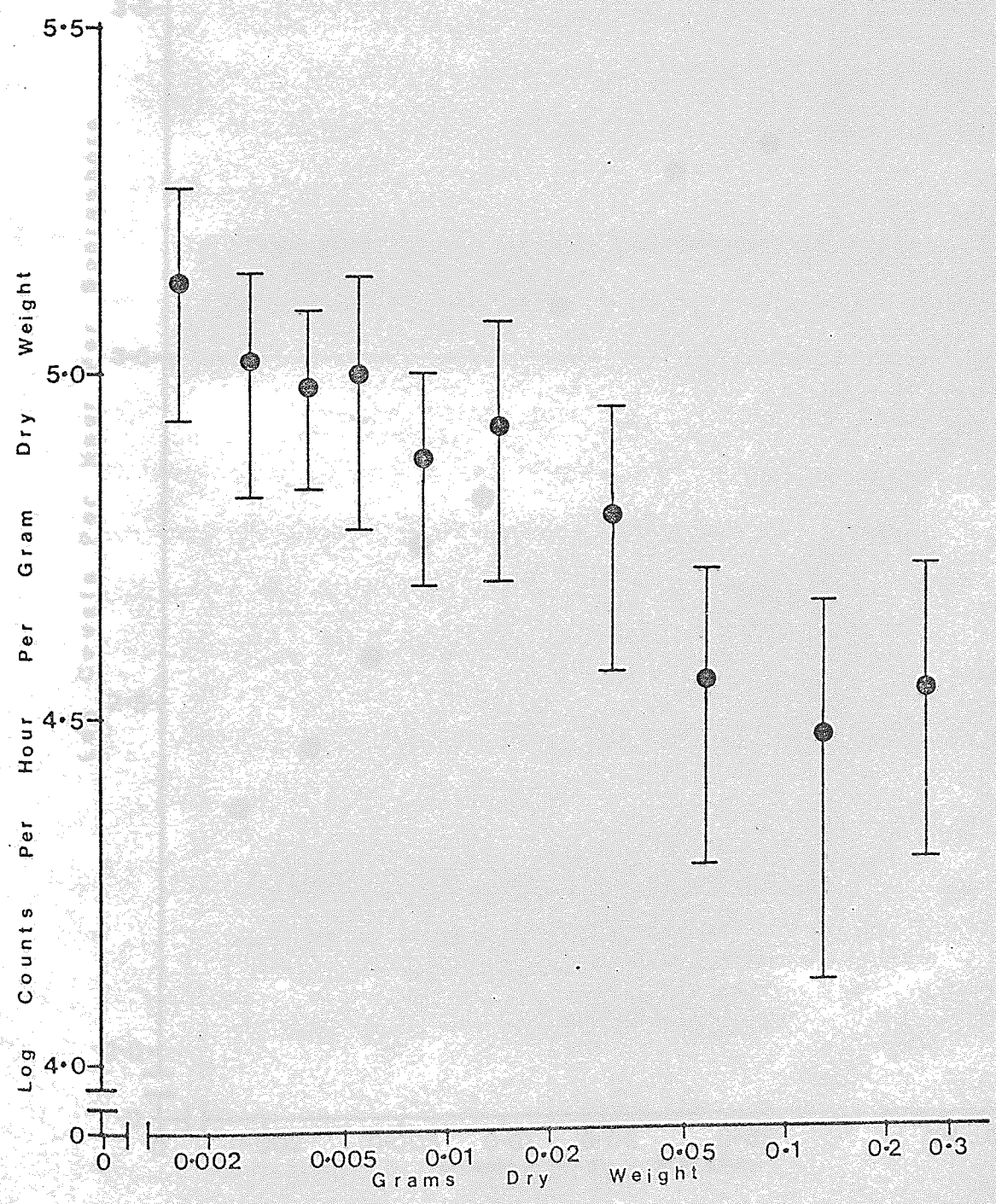
FIGURE 3.23. IRON-59 CONTENT IN GROUPS OF PRIMORDIA AND SMALL SPORPHORES OF SIMILAR DRY WEIGHT.
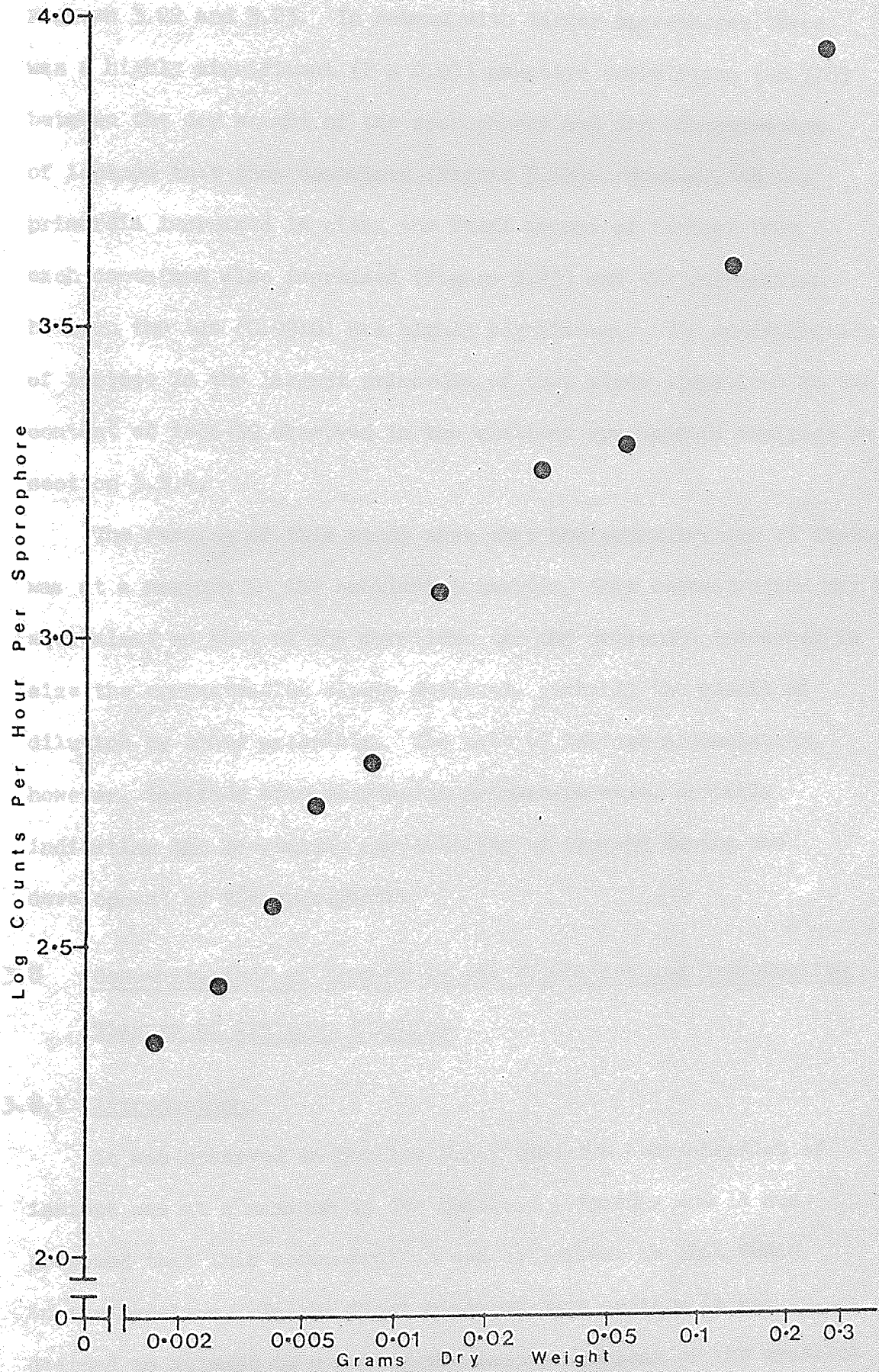
tube, about 80 of the smallest primordia equalled this weight.

The results of the analysis are shown in Appendix 3.12 and Figures 3.22 and 3.23. In common with larger sporophores there was a highly significant (P = 0.01) negative correlation (-0.778) between the dry weight of the sporophores and the concentration of isotope that they contained (Figure 3.22). However, as the primordia increased in size, the total amount of isotope that each contained also increased (Figure 3.23) and the correlation between the two (0.9916) was highly significant. The concentration of isotope in the largest primordia of this study equivalent to the content of iron-59 observed in the smallest sporophores analysed in section 3.3.4.

The results of this study show that the concentration of isotope was at a maximum in the smallest primordia, this concentration may be equivalent to that of the mycelium. As the primordia increased in size the concentration slowly declined, probably the result of dilution by other materials. The rate of isotope accumulation, however, declined with increasing primordium size, probably indicating the decreasing availability of iron-59 during the development of the sporophore.

3.3.8   Concentrations of Iron-59 in the Vegetative and Reproductive Tissues of Agaricus bisporus.

3.3.8.1 Introduction.

It was observed in Section 3.3.7 that the concentration of isotope was at a maximum in the smallest primordia and it was proposed that this concentration was equivalent to that found in the mycelium. In the final study of this section it was decided to attempt to quantify the isotope content of the mycelium

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and observe any changes that occur during the production of the crop.

A large number of researchers have studied the mycelium of Agaricus bisporus on synthetic media. It is highly likely that this method of study is totally unrepresentative of the natural situation, primarily because A. bisporus does not fructify on definable media.

It was decided that the changes that occur in the mycelium during fructification could only be studied using substrates and conditions that closely mimic those of normal growing practises. It was, therefore, necessary to develop substrates that filled these criteria but, in addition would allow the mycelium to be sampled. It was considered that normal substrates would be ideal for the growth and development of the organism and that the alteration of the physical structure would facilitate sample removal. In an exploratory study the pelletisation of the substrates yielded satisfactory results and thus this technique was adopted.

3.3.8.2   Preparation of Pelletised Compost.

Pasteurised compost was air dried and then ground in a hammer mill to pass through a two millimetre sieve. High viscosity Carboxymethylcellulose was added to the ground compost at a rate of five percent, to act as a binding agent. Six parts of water were added to four parts of the dried compost and mixed very thoroughly. The thick compost paste was then forced through a Hobart Commercial Mincing Machine with a five millimetre aperture grid. The extruded strands of compost naturally broke into lengths of between two and three centimetres as they emerged from the apertures. Plate 3.5

PLATE 3.5. PELLETISED COMPOST

PLATE 3.6. PELLETISED CASING.
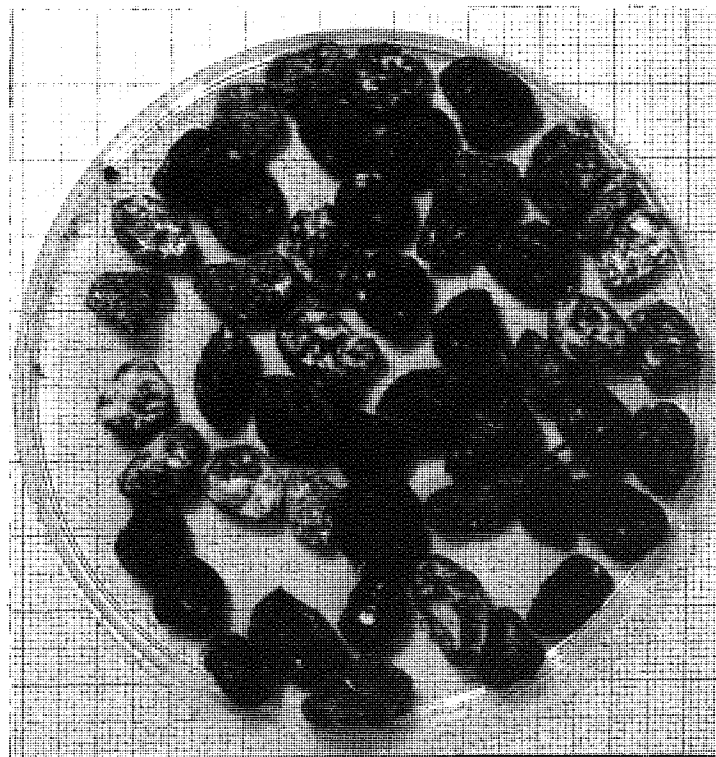
PLATE 3.7. MYCELIAL GROWTH IN PELLETISED COMPOST.
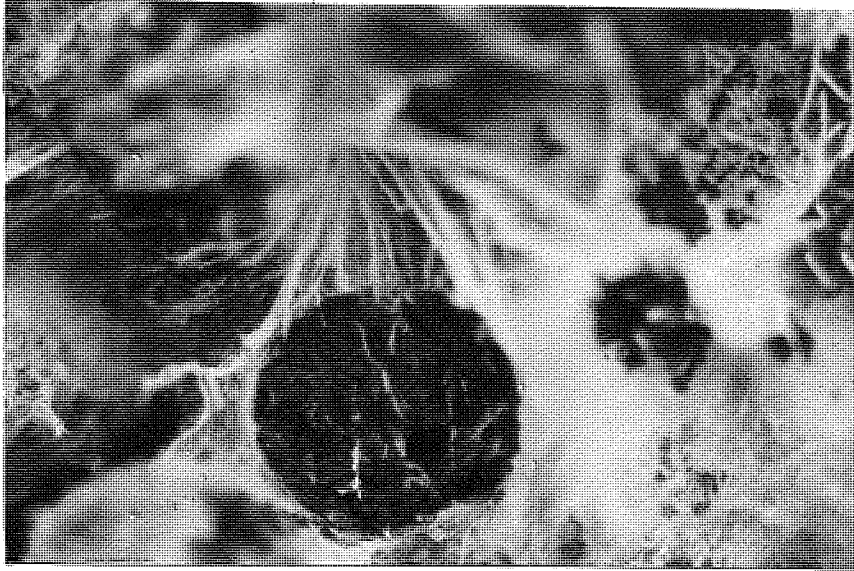
PLATE 3.8. MYCELIAL STRANDS AND SPOROPHORE PRIMORDIA IN

PELLETISED CASING.

PLATE 3.9. FRUCTIFICATION OF AGARICUS BISPORUS ON PELLETISED SUBSTRATES

IN FLASK CULTURE.
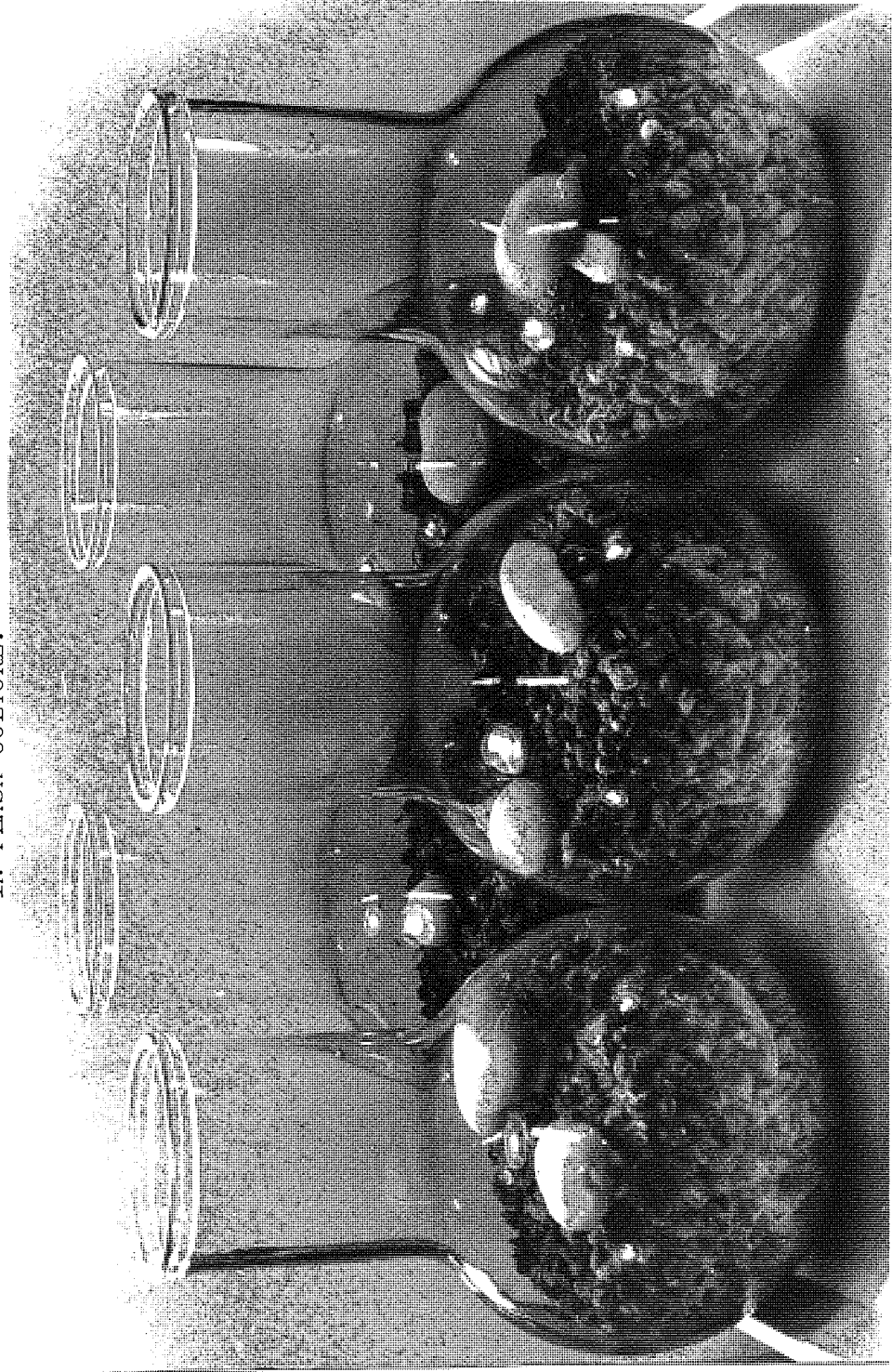
shows freshly prepared compost pellets. 300 grams of the pellet-
ised compost were put into each one litre Pyrex glass flask.
The flasks were sealed using cotton wool bungs and after auto-
claving for one four at 15 pounds per square inch on three
successive days, were inoculated with six grams of grain spawn.
The flasks were incubated at 25°C for fourteen days and then the
isotope solution and casing layer were applied to the compost
surface. The isotope solution was prepared and applied by the
methods given in Section 2.3.

3.3.8.3   Preparation of Pelletised Casing.

Hayes et al. (1979) describes the preparation of a casing
material using a waste product from the pulp and paper making
process. Because of the amount of clay contained in this waste
product, when mechanically treated and mixed with neutralising
chalk the material tends to aggregate into particles of varying
sizes. Particles of between five and ten millimetres diameter
were separated out and used in this study. Plate 3.6 shows
pellets of this casing material. Prior to application to the
compost, the casing material was steam-air pasteurised at 65°C
for one hour. 100 grams of the casing material was then added
to each flask. Incubation of the flasks was then continued at
25°C for a further seven days, until the mycelium had colonised
the casing layer. The cotton wool bungs were then removed and
the flasks placed in an aerated growth cabinet at 17°C. Plate
3.9 shows fructification in flasks on these pelletised substrates.

3.3.8.4   The concentration of iron-59 in different tissues removed
from the pelletised substrates during culture.

The mycelia, primordia and sporophores were carefully dissected from the substrates in six replicate flasks 15, 18, 21, 24 and 27 days after casing. Plate 3.7 shows mycelial growth in the pelletised compost substrate 20 days after casing. There was a limited amount of mycelial growth within the compost pellets but, the majority of the fungal tissue adhered loosely to the surface of the pellets or was in the form of mycelial strands between the pellets.

In the casing layer also 20 days after the casing of the cultures (Plate 3.8) the mycelium was in the form of mycelial strands, anchoring the developing primordia to the casing particles and connecting the primordia to the mycelium in the nutrient rich compost.

The concentration of iron-59 contained in the different tissues removed from the substrates are given in Appendix 3.13 and shown in Figure 3.24.

The concentration of iron-59 in the compost mycelia was subject to considerable variability and thus, there were no significantly different changes in isotope concentration between any of the five sampling times. The variation of the results probably reflects the uneven distribution of isotope within the compost pellets which would be expected when 10 milli-litres of the solution was added to 300 grams of compost. Figure 3.24 does, however, show that small changes of isotope concentration in the compost mycelium occurred. The concentration was at a maximum 18 days after casing, the isotope concentration then declined slightly over the period in which the sporophores were developing. This may show the movement of iron-59 out of the

FIGURE 3.24. CONCENTRATIONS OF IRON-59 IN MYCELIA, PRIMORDIA AND SPOROPHORES DURING CULTURE.

▽ COMPOST MYCELIUM
▲ CASING MYCELIUM
◈ PRIMORDIUM
● SPOROPHORE
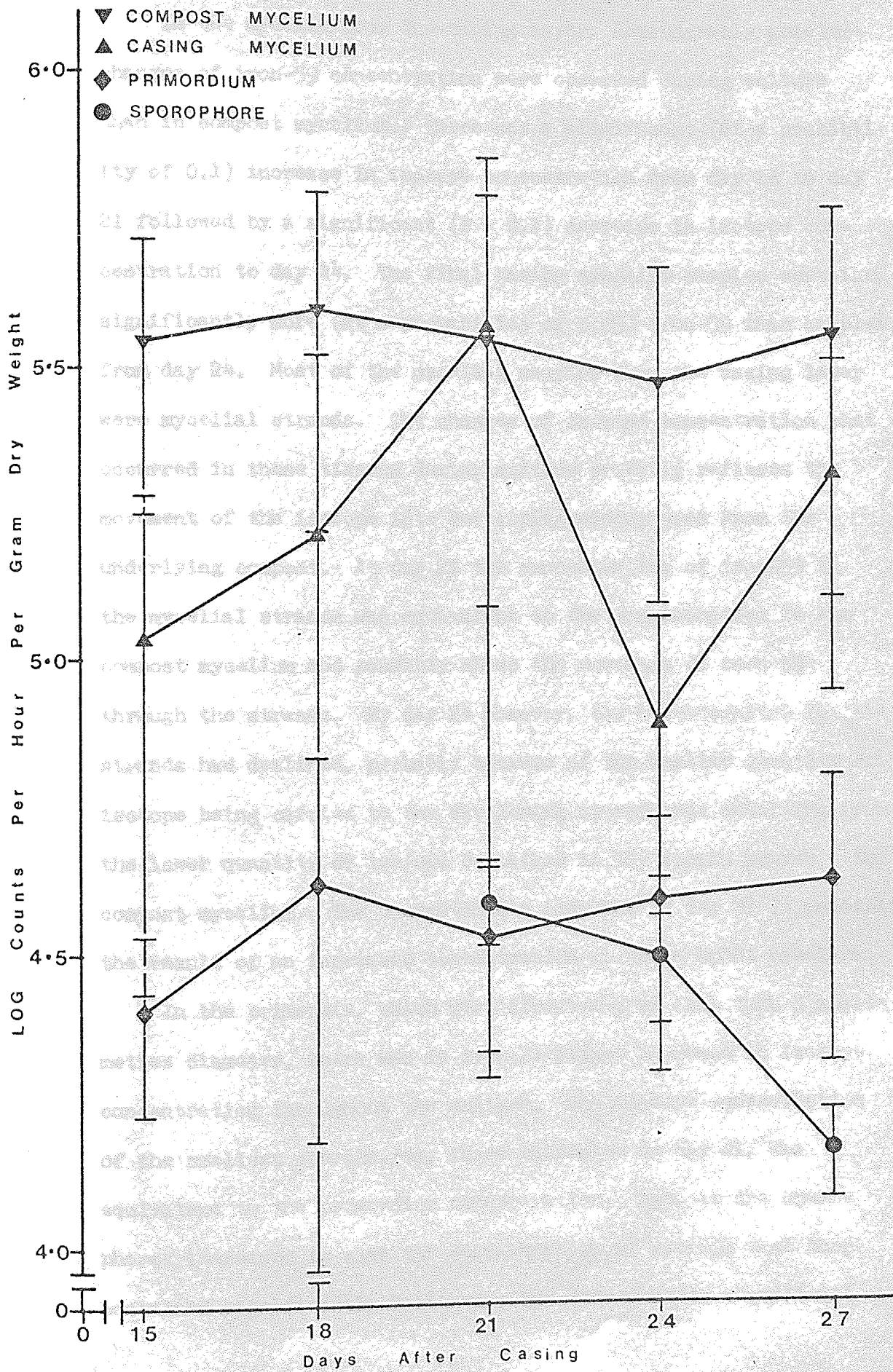
compost and into the developing sporophores.

In the mycelia from the casing layer, considerably greater changes of iron-59 concentration were observed during culture than in compost mycelium. There was a significant (at a probability of 0.1) increase in isotope concentration from day 15 to day 21 followed by a significant (P = 0.1) decrease in isotope concentration to day 24. The final casing mycelium samples contained significantly more (at a probability of 0.05) iron-59 than samples from day 24. Most of the mycelial samples from the casing layer were mycelial strands. The changes of isotope concentration that occurred in these tissues during culture probably reflects the movement of the isotope into developing sporophores from the underlying compost. At day 21 the concentration of iron-59 in the mycelial strands was equivalent to the concentration in the compost mycelium and possibly shows the movement of iron-59 through the strands. By day 24 however, the concentration in the strands had declined, probably because of the smaller quantity of isotope being carried to the developing sporophores resulting from the lower quantity of isotope contained in the supply source - the compost mycelium. The concentration increase to day 27 is possibly the result of an increased concentration in the compost mycelium.

In the primordia, which were structures of less than 5 millimetres diameter, there was an overall slight increase in isotope concentration throughout the culture. The isotope concentration of the smallest sporophores, those harvested on day 21, was equivalent to the primordium concentration. But, as the sporophores increased in size the concentration of iron-59 that they contained significantly declined. The decrease was significant

at a probability of 0.01 between days 24 and 27.

Thus, despite the variability of results, changes of iron-59 concentration were observed to occur in the different tissues of _Agaricus_ _bisporus_ during culture.

3.4 CONCENTRATIONS OF MINERAL ELEMENTS IN THE SPOROPHORES OF AGARICUS BISPORUS.

There has been no definitive study of the elemental composition of the cultivated mushroom Agaricus bisporus. The results of a large number of restricted investigations are shown in Table 1.1. In the main, these studies were of small samples of unspecified source, break, strain and size of sporophores it is, therefore, not surprising that a large variation between different analyses is evident. In this study sporophores of a single strain (Darlington 649), all of open cup stage, were harvested from a large number of studies over a three year period. The different batches of compost were all obtained from W. Darlington and Sons Ltd. and peat based casing layers were used throughout.

In total three kilograms of dry sporophore material was collected. This was ground and thoroughly mixed in a hammer mill. 100 gram samples of the dry homogenised sporophore material was put into each of three large crucibles. Following the accurate determination of sample dry weight the crucibles were placed in a cool muffle furnace.

Ashing is an effective method of reducing the bulk of a sample and is suitable for the analysis of the majority of metallic elements but, some are volatilized at high temperatures. Allen et al. (1972) recommend that ashing is not used for lead and arsenic, but, they conclude that reasonable results have been produced when low temperatures of about $430^{\circ}C$ have been used. The samples were, therefore, slowly heated to $430^{\circ}C$ and then ashed at that temperature for five days. Approximately ten percent of the sporophore tissue remained after ashing. Five 5 gram

samples and three blanks were digested in accordance with the methods given in Section 2.2. A number of metal elements were analysed in the sporophore samples using a Perkin-Elmer 306 Atomic Absorption Spectrophotometer. The results of the analyses are given in Table 3.33.

The concentrations of the different elements in the sporophore samples determined in this study usually fall within the range of published results shown in Table 1.1.

TABLE 3.33.   CONCENTRATIONS OF SOME MINERAL ELEMENTS IN THE
SPOROPHORES OF AGARICUS BISPORUS.
(MILLIGRAMS PER KILOGRAM DRY WEIGHT).

ALUMINIUM	(Al)	3.967	(1.315)
ARSENIC	(As)	3.204	(0.295)
CALCIUM	(Ca)	3165.22	(87.66)
COBALT	(Co)	0.935	(0.045)
CHROMIUM	(Cr)	BELOW DETECTION	
COPPER	(Cu)	45.262	(1.319)
IRON	(Fe)	28.073	(1.889)
POTASSIUM	(K)	50157.6	(1028.6)
MAGNESIUM	(Mg)	802.02	(26.28)
MANGANESE	(Mn)	3.43	(0.127)
MOLYBDENUM	(Mo)	BELOW DETECTION	
SODIUM	(Na)	637.7	(37.87)
NICKEL	(Ni)	1.514	(0.218)
PHOSPHORUS	(P)	11220.8	(494.6)
LEAD	(Pb)	7.038	(0.463)
ZINC	(Zn)	49.705	(2.022)

Means and (Standard Deviations) of 5 samples.

SECTION 4

DISCUSSION AND

CONCLUSIONS

4.1   FACTORS INFLUENCING SPOROPHORE COMPOSITION.

Despite the relative ease with which <u>Agaricus bisporus</u> can be brought to fruition under laboratory conditions, the majority of research into the cultivated mushroom has been directed towards the increase of crop productivity through the improvement of the substrates and growing techniques used.   In comparative terms very little emphasis has been placed upon the product composition and the factors which may influence this composition.   The application of numerous supplementary materials and even different quantities of water to the culture media are known to influence the yield of sporophores produced but, there is virtually no knowledge of the extent that these supplements affect sporophore composition. Similarly, several cultural practices are known to affect crop productivity but, any resultant compositional changes are unknown.

The experiments described in Section 3.1 were conducted with the aim of quantifying the sporophore iron content and elucidating the influence that changing cultural conditions may exert over this composition.   Different strains of <u>Agaricus bisporus</u>, different casings materials and watering regimes, and different iron containing supplements were considered as the factors most likely to cause changes of sporophore iron composition.   In addition, different batches of compost were used in each of the separate studies and thus differences between composts were considered as the fifth and probably most important factor contributing to the variability of sporophore composition.

It was found that despite the considerable differences of

sporophore yields between different strains of *Agaricus* *bisporus* there were generally no significant differences of iron concentration in the sporophores harvested. In high yielding strains, the increasing iron content of the sporophores of successive breaks was correlated with decreasing yields but, in a lower yielding strain there was a positive correlation between increasing yield and increasing iron composition of the sporophores. It seems likely that the increasing iron content of sporophores harvested at later stages of the culture were related to the increasing availability of iron in the substrates, rather than as a direct function of the metabolic activity of the organism. Parrish *et* *al*. (1976) in a study of the mannitol content of different strains of *Agaricus* *bisporus*, proposed that the positive correlations between the fluctuations of mannitol content and sporophore yield encountered during the crop cycle of the different strains was a direct function of the metabolic rate of the culture. The conflict between results of this study and those of Parrish *et* *al*. (1976) probably demonstrates the difference between the two materials determined. Mannitol is a product of metabolic activity which accumulates in sporophores during growth (Hammond and Nichols, 1976a) whereas, iron is not a product and its appearance in the sporophore may well be a consequence of the uptake of water and/or other materials into the sporophore. The quantity of iron detected in the sporophore tissue of *Agaricus* *bisporus* is certainly greatly in excess of the optimum vegetative mycelial requirement of 0.1 ppm determined by Treschow (1944) and may, therefore, be accumulated to excess from the compost or casing during sporophore growth.

Despite considerable knowledge of the required structure

and environmental conditions the casing layer must provide
to ensure maximum crop productivity, the precise nature of
the interaction between Agaricus bisporus and the casing
layer is far from fully understood. It is generally consid-
ered that the casing layer provides little in the way of
nutrients required for sporophore production, the compost
being the major source. There is, however, little experimental
evidence to either support or refute this assumption. Courtieu
(1949) obtained slight correlations between the levels of
calcium, potassium and phosphorus in the casing soil and in
the mushrooms derived from them.

The importance of bacterial populations to the induction
of sporophore initiation is unquestionable. The creation of
optimum localised conditions, either by the removal of inhibiting
substances or, the production of stimulatory substances from the
bacteria are suggested as the cause of the sporophore initiation
phenomenon. Hayes (1972) suggested that the bacteria present
might act by solubilizing iron in the casing layer, thus making
it available to Agaricus bisporus. His postulation was based
on the observation that ferrous sulphate applied to the casing
layer resulted in enhanced sporophore initiation.

In this study it was found that sporophores harvested from
growing units cased with different casing materials contained
significantly different concentrations of iron. The total
quantity of iron contained in the sporophores of five breaks
from different treatments was approximately equal but, with
peat casing the sporophore iron concentration decreased in
successive breaks, whereas, with PMB based casings the iron

concentration increased. In peat casings less than 1 milligram per kilogram dry weight of water extractable iron is present throughout culture (Section 3.2.2) and in PMB casings about 30 mg per kilogram dry weight of water extractable iron are present throughout (Yeo, 1980). Thus, it is unlikely that differing quantities of available iron in the different types of casing material are contributing to the observed sporophore compositional changes. Possibly the greater upward diffusion of iron into the highly absorptive peat casing, together with the greater water holding capacity of peat (Yeo, 1980) may induce the flow of an initially greater quantity of iron from the compost to the peat casing where it becomes incorporated into the developing sporophore initials. In the PMB casing materials lower upward fluxes would result in a more even distribution of iron throughout cropping rather than the initial burst observed for the peat casing layer.

The principal role of watering is to replace water lost from the casing layer by evaporation. From his studies on the composition of composts Gerrits (1968) concluded that the compost supplies all of the water required by the mushroom crop. However, Reeve et al. (1959) demonstrated that the moisture content of harvested sporophores was associated with casing soil moisture. Also, Flegg (1974b and 1975) showed that in the later stages of cropping there was a positive correlation between the amount of water applied and the weight of the crop produced. The sporophores of Agaricus bisporus contain in excess of 90% water therefore, the availability of water to the sporophore is not only important in terms of productivity but, also in the overall

composition of the crop.

A number of researchers have quantified the optimum rate and pattern of water application to the casing layer. Above and below this optimum the fresh weight yields are depressed. Reeve et al. (1959) and Kindt (1965) observed that the number of sporophores produced declines with increased addition of water. The same cropping patterns were observed in this study with respect to both fresh weight yields and the number of sporophores produced. In addition the results given in Section 3.1.4 show that with low rates of water application there was a decreased tendency to produce distinct breaks, probably because the smaller sporophores produced, matured and were harvested sooner and more frequently. Another observation was that the total dry weight yields were similar in the different watering treatments and thus the differences in fresh weight were directly attributable to the water content of the sporophores. The quantity of other materials (apart from water) used in sporophore construction and derived from the substrates is therefore, independent of the water added to the casing layer. However, watering of the casing layer may significantly affect the way in which these materials are distributed into sporophores. Flegg (1975) had shown that stranding of the mycelium is more pronounced in wetter casing layers and Yeo (1980) observed decreasing primordial numbers with increasing water application to the casing layer. Both of these findings were also observed in this study. Thus, with higher rates of water application a suppression of sporophore formation occurs. This may be the result of disruption of the casing layer and mycelium caused by heavier watering or the creation of conditions not favourable

to initiation. Plunkett (1956) has implicated evaporation in the initiation process of an agaric and a polypore. Excessive quantities of water in the casing may create conditions of high humidity in the microclimate of the casing layer and thereby reduce the rate of evaporation and development of primordia. It is also possible that the greater quantities of water in the casing are suppressing the activity of bacterial populations associated with the initiation process.

An approximately constant quantity of dry matter was available for sporophore construction, therefore, when more sporophores were successfully initiated they reached a smaller maximum size than when few were initiated. Obviously water is important in sporophore construction but, sufficient is contained in the compost to allow the development and maturation of sporophores. However, the increased water made available by addition to the casing layer was taken up into the sporophore. Additionally the total amount of iron contained in the sporophores was directly related to the quantity of water the sporophores contained. Iron and probably many other materials are, therefore, entering the sporophore in a soluble form and thus, their accumulation in the sporophore would be dependent both upon the quantity of soluble material present in the substrates and the amount of water moving into the sporophore.

Numerous studies (reviewed by Haddad, 1977) have failed to show any relationship between the addition of organic materials to the compost and changes in sporophore composition. Using heavy metal salts, however, Loughton and Frank (1974), Aichberger and Horak (1975) and Laub et al. (1977) have shown that the

accumulation of the heavy metals in sporophores was proportional to the quantity of heavy metal contaminants added to the compost.

In this study iron was added to the compost at a rate which had been shown by Hayes (1972) to stimulate sporophore formation when added to the casing layer. In three separate experiments the compost was supplemented with iron at different concentrations, at different levels of the compost and as different inorganic salts. None of these treatments resulted in a significant increase in the quantity of iron in sporophores. It is probable that the quantity of iron added to the compost was insignificant compared with the quantity of iron already present in the compost. In the other studies mentioned above however, the quantity of heavy metal supplements were equivalent to the normal compost heavy metal content and hence gave a significantly increased quantity available for uptake into the sporophore.

In Section 3.1.5.1 it was found that there were no significant differences of the concentration of iron in the pileus, stipe or hymenia. The heavy metals, selenium (Stijve, 1977), cadmium (Seeger, 1978a) and mercury (Seeger, 1975b) were all shown to occur at the highest concentrations in the hymenia and at the lowest in the stipe but, Seeger (1978b) showed that the pileus and stipe contained more potassium than the hymenia. It would therefore, appear that the accumulation of mineral elements within the sporophore is dependent upon the element involved.

Many reports show that younger sporophores are richer in content of a range of materials, including heavy metals, than more mature sporophores. This is also the case with iron as shown in Table 3.6. The cause of this declining concentration with growth may be due to decreasing iron availability as the

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sporophore increases in size or changes in the physiology of
the culture during development. A discussion of these points
at greater length is given in Section 4.3.

A different batch of compost was used in each of the
experiments of Section 3.1. The concentrations of iron in
sporophores harvested from these experiments cover different
ranges:

Section 3.1.2	20 - 60	Milligrams Iron/Kilogram Dry Weight
3.1.3	30 - 50	"
3.1.4	15 - 30	"
3.1.5.1	55 - 65	"
3.1.5.2	40 - 65	"
3.1.5.3	25 - 160	"

These different concentrations show considerably more variation
than the differences that occurred between treatments within an
experiment. This indicates that the major determining factor
over sporophore iron composition is; the quantity of iron that
is available for uptake into the sporophore from the substrate
which supplies the nutrients - the compost.

In each experiment there were considerable variations of
iron concentration in the sporophores of different breaks but,
no clear trend was apparent. In some cases the concentrations
increased in successive breaks, in others they decreased. There
were frequently negative correlations, usually not significant
however, between the yields and concentrations of iron in sporo-
phores of different breaks. This may indicate that in some cases
a limited quantity of iron was available for sporophore construc-
tion. This available quantity of iron was distributed amongst

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those sporophores which developed. The concentration or iron in the sporophores, thus appears to be governed primarily by the quantity of iron available in the compost.

Of the factors investigated in Section 3.1 it is clear that the concentration of iron in the sporophore is primarily governed by the substrates, particularly the compost but, also to a lesser degree, the casing layer. However, the individual size and yield of the sporophores produced, together with watering do result in small changes of composition.

4.2 FACTORS INFLUENCING CHANGES IN SUBSTRATE COMPOSITION.

The concentrations of iron in the sporophores is clearly dependent upon the availability of iron in the substrates. The availability and transport of micronutrient cations in soil systems is well documented and extensively reviewed by Stewart (1963) and Stevenson (1972). The quantity of any given micronutrient cation occurring in the solution phase of the soil is small in comparison with the total amount held by humus colloids, or as precipitates. It is suspected that metals are transported in water as stable organic matter complexes, probably with simple biochemical compounds. These constituents have only a transitory existence, being both synthesised and destroyed by micro-organisms. High percentages of micronutrient cations in displaced soil solutions occur in chelate form with these low molecular weight organic constituents. Organic acids have been the subject of special interest because of their ubiquitous nature and effective solubilising ability. The translocation of iron in plants has been shown to be associated with citric and other organic acids.

The high proportion of metals occurring in organic soils in insoluble forms are bound mainly to the more highly humified substances, particularly fulvic and humic acids, which are encountered in both composts and casing materials. Unlike mineral soils, the substrates used in mushroom cultivation are likely to contain a very small proportion of metal elements in the forms of insoluble oxides and clay complexes. The ramification of the mycelium of Agaricus bisporus throughout the essentially organic substrates used in cultivation, together with the release of

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extracellular enzymes, in all probability enables <u>Agaricus</u> <u>bisporus</u> to obtain a high proportion of the metals contained in the growth substrates.

In Section 3.2.2 an experiment was described in which the concentrations of available iron were monitored during the culture of <u>Agaricus</u> <u>bisporus</u> sporophores. Two methods of iron extraction were used. It was seen in Section 3.1.4 that the quantity of iron taken up into the sporophore was related to the water content of the sporophore, the inference being that; water soluble iron was being translocated. Therefore, extraction using water was chosen as a method of determining the quantity of soluble iron in the substrates. Another extractant 0.1 Molar tetra-potassium pyrophosphate was chosen because of its ability to displace iron from the organic colloids of the substrate and hence, give an indication of the total quantity of iron available to the mycelium during substrate degradation.

Observations showed that in the casing layer there were slight overall increases in the concentration of water soluble iron during culture, probably demonstrating the upward diffusion of soluble salts previously shown by Flegg (1961b) and Yeo and Hayes (1979). A great increase of soluble iron in the casing layer was observed following the harvest of the first break sporophores. It is proposed that the harvesting of sporophores causes extensive rupturing of the mycelial strands in the casing layer and results in a considerable leakage of materials into the casing layer. Possibly either soluble iron is lost into the casing layer directly, or the leakage of other materials results in the proliferation of bacteria in the casing layer observed by Cresswell and Hayes (1979) to occur during cropping. The increased

bacterial activity may result in the enhanced solubilization of iron in the casing layer.

In the compost, changes of water extractable iron concentration appear to be linked to the mycelial activity in that substrate during culture. An initial decline of water soluble iron coincides with the initiation of sporophore formation and may be the result of either, declining mycelial activity during sporophore initiation or, possibly enhanced nutrient uptake in readiness for sporophore formation. During cropping, the quantity of water soluble iron increased steadily, probably in relation to the degradation and solubilization of the compost. Forty eight days after casing, by which time crop productivity is very much reduced, the concentration of water extractable iron in the compost again decreased, possibly as a result of declining mycelial activity and the re-adsorption of soluble iron into the organic colloids of the compost.

The quantity of potassium pyrophosphate extractable iron in the casing layer showed a continual increase during active cropping of the culture. A continual, upward diffusive flow of soluble iron into the casing layer is known to occur and it is likely that much of this iron becomes bound to the organic colloids of the peat from which it is extracted by pyrophosphate. In the final sample, 48 days after casing, the concentration of pyrophosphate extractable iron had declined. This may have been due to the decreased upward diffusion of water soluble iron from the compost following declining mycelial activity and the subsequent firmer binding of iron to the casing materials.

Pyrophosphate extracts of the compost contained variable quantities of iron but, overall there were considerable increases

of pyrophosphate extractable iron in the compost, particularly in the later stages of the culture. These changes may be associated with the declining productivity and staling of the culture system.

The changes of the concentration of water extractable iron in substrates supplemented with ferrous sulphate showed similar trends to those seen in Section 3.2.2. The supplements increased the concentrations of water soluble iron in the substrate where they were applied but, by considerably less than the total amount of iron supplied. The observations that the sporophore iron content was not increased by these supplements probably supports the earlier proposal that water soluble compounds are the form in which iron is translocated into the sporophore.

The results of Section 3.2.4 demonstrate that the amount of water contained in the substrates at the end of culture are directly related to the quantity of water applied to the casing layer during culture. Probably because of loss by evaporation and downward percolation the correlation between water contained and water added was least significant in the casing layer. In the more densely packed compost however, percolation is probably restricted and thus, water tended to accumulate in proportion to the quantity applied to the casing layer.

Increasing the quantity of water applied to the casing layer resulted in increased quantities of water extractable iron detected in the compost. Fleckenstein and Grabbe (1979) have also shown that the percolation of substrates containing a natural compost microflora and mushroom mycelium, with water, resulted in an increased mobility of heavy metals within the substrates. The

results of this study show that an excessive application of water washed some water extractable iron down into the lower half of the compost, or alternatively created conditions where more became available by water extraction.

In casing layers subjected to different watering regimes, there were no significant differences of the concentration of water extractable iron between the three treatments used although slight differences were observed. In the medium watering casing, the greater concentration of iron compared with the casing of low watering regime may be the result of greater upward diffusion or evaporation enhanced movement of soluble iron from the compost. When applied in excess, as in the high watering regime, water carried some of the water extractable iron down out of the casing layer or possibly the waterlogged conditions of the casing material prevented solubilisation of the iron present.

Bacteria are known to be associated with the initiation of sporophore formation but, their precise role has been the subject of considerable speculation. Some evidence suggests that the removal of inhibitors is the primary function of the bacteria, while other studies have implicated the production of stimulatory substances effective in the initiation process. Cresswell and Hayes (1979) have shown an increased bacterial population associated with sporophore initiation and Hussain (Pers. Comm.) has found that the concentrations of several phytohormones in the casing layer increased concomitantly with the bacterial population, possibly indicating the importance of stimulating agents. In 1972 Hayes postulated that bacteria

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associated with fructification might act by solubilizing iron in the casing layer. Yeo (1980) however, found that increases in the concentrations of soluble iron in the casing layer could be attributed to changes of mycelial activity during culture. In an earlier section of this study it was suggested that an increase in the quantity of water extractable iron in the casing layer during cropping, was the result of enhanced bacterial activity stimulated by nutrient leakage from the mycelium. At the same time as the research described here, Cresswell was studying the bacterial populations of the casing layer also at the University of Aston. In one experiment she determined the effect of different bacteria on the stimulation of sporophore formation. At the termination of that experiment the quantity of water extractable iron contained in the substrates was determined. There were no significant differences of water extractable iron concentrations in the composts of the different treatments. In the casing layers however, the concentrations of water extractable iron varied considerably between the different treatments. In the three different controls the quantity of soluble iron was clearly related to presence of bacteria in the substrates. The lowest concentration was found in the axenic control (<u>Agaricus bisporus</u> only) and the highest concentration was found where the normal bacterial flora was present in both the compost and the casing layer. The casing layers to which different single bacterial isolates were added contained a wide range of concentrations of water extractable iron. Six isolates produced casing layers containing significantly more water extractable iron than the casing layer of the axenic control. In the two genera represented by

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more than one isolate there were no consistent trends of iron
solubilising activity in the casing layer. It is, however,
clear that a number of bacteria isolated from the casing layer
can significantly alter the concentration of water extractable
iron in the casing layer and the results show that the indigenous
bacterial populations of the casing layer do increase the avail-
ability of iron in the casing layer.

The fact that bacteria in the casing layer may significantly
alter the gross composition of that substrate probably indicates
that these bacteria are also capable of producing localised condi-
tions within the casing layer of very different characteristics
to the general structure of the casing material. This may be
indicative of the crucial involvement that bacteria play in the
initiation process, particularly with reference to the selective
induction and positioning of sporophores within the casing layer.

In Sections 3.2.2. and 3.2.3 the concentrations of extract-
able iron in the substrates were monitored throughout culture.
In agreement with the findings of Yeo and Hayes (1979) there was
an upward diffusion into the casing layer of soluble iron. An
excessive application of water to the casing layer was shown in
Section 3.2.4 to percolate downwards through the substrates
probably carrying water soluble iron with it. Thus, there is
a limited mobility of soluble materials within the substrates
during culture. Using Phosphorus-32 Corin et al. (1954) demon-
strated that 70% of a mineral phosphate added to the compost
remained in the compost, over a 4 week period, in a water soluble
form. Nielsen and Rasmussen (1963) had used Phosphorus-32 and
Calcium-45 to demonstrate the transportation capabilities of
mycelia in the substrates. In the experiment described in

Section 3.2.6, Iron-59 was applied to different levels in the substrates so that the movement within the substrates, and the uptake into the sporophores from different substrate depths could be studied.

When the isotope was applied to the substrates it became highly localised where applied. Some downward movement out of the casing layer occurred during culture but, otherwise there was little transfer of isotope between the different levels of the substrate. The overall slight increases in isotope concentrations are probably related to the loss of substrate dry matter through mycelial and bacterial respiration.

In Section 3.1 it was shown that sporophore composition was affected by the size of the sporophore and the yield to sporophores produced. Similarly in 3.2.6 the uptake of isotope into the sporophore was found to be related to size and yield. Iron-59 applied to the casing layer was only available in small quantities for uptake and therefore, there was a negative correlation between increasing sporophore size and decreasing isotope concentration. When isotope was injected into the compost significant positive correlations between the increasing sporophore size and increasing sporophore isotope content were found in later breaks. It is probable that as the yield and number of sporophores decreased in later breaks more isotope became available for uptake into each sporophore and thus, the concentration of Iron-59 in each sporophore was proportional to its size.

In Table 3.25 it was seen that the quantity of isotope contained in sporophores was significantly correlated with the dry weight yield when the isotope was added to the compost but, not

when it was added to the casing layer. Thus, assuming that the isotope is accumulated in direct proportion to other materials in the sporophores, it is extremely probable that the compost is the major source of iron and other nutrient supply.

To test the relationship between the isotope concentration in the substrates and isotope concentration in the sporophores, correlation coefficient calculations were made. Table 3.2.6 shows the estimated isotope concentration in various levels of the substrate at times corresponding to the harvesting of breaks. When the isotope was applied to the casing layer there was a significant correlation between the increasing isotope content of the sporophores and the increasing isotope concentration in the compost. This again indicates the probable importance of the compost as the source of supply of iron-59.

When the isotope was added to the compost, however, there were variable correlations between the sporophore and substrate iron-59 concentrations. It may be that yield and other effects such as the binding of the isotope to the substrates are affecting the uptake of the isotope. Whereas, in the casing layer treatment, the iron-59 moving down into the compost was probably in an available form and therefore, available for uptake into the sporophores.

In the substances therefore, the quantity of water extractable iron (the form in which iron is most likely translocated into the sporophore) changes throughout culture. Both watering and bacterial populations are capable of altering the concentration of water soluble iron in the substrates but, the greatest changes occurring in the compost appear to be related to mycelial activity.

4.3   COMPOSITIONAL CHANGES ASSOCIATED WITH SPOROPHORE DEVELOPMENT.

The experiments described in Section 3.3. were used to investigate the uptake of iron into the sporophore during its growth and development. Compositional changes during sporophore development generally show an increase in structural carbohydrates and a decrease in mineral elements and proteins. The lipid content and amino acid profiles vary inconsistently between small and large sporophores. Usually comparisons have been made between button, open cup and flat stages of development. It is probably the variability of sporophore size within these artificial group-ings which is contributing to the difficulty of demonstrating clear trends of compositional change within the sporophore.

In Section 3.3 the changes of iron concentration which occurred as the sporophores increased in size from small primordia to large flats were observed and this was related to the growth rate of the sporophore.

An in situ method of growth rate estimation was devised using the relationship between pileus diameter and sporophore weight observed by Flegg and Gandy (1956). In 3.3.2.2 it was shown there was a relationship between the pileus diameter and the sporophore dry weight and that the curves produced corres-ponded closely to those obtained by Flegg and Gandy. Further to this it was found that by using this relationship it was possible to closely approximate the dry weight of a sporophore from the knowledge of its pileus diameter.

Using a time-lapse photographical method of recording pileus diameters, the increasing size of a large number of sporophores was observed over four days. The calculated

increases in weight per unit time (growth rates) were plotted against the weight of the sporophore (Figure 3.12) and showed that the growth rate increased in direct proportion to the size of the sporophore over a wide range of sporophore sizes. Thus, at least up to the late open cup stage of development, the sporophore grows at an increasingly greater rate. This result may indicate that the growth rate of sporophores is under endogenous control and is related to the metabolic activity of each individual. Transpiration flow induced by surface evaporation has been shown to be involved in the movement of materials into the sporophore but, because the ratio of surface area to volume decreases with increasing size, the results, therefore, show that evapo-transpiration cannot be entirely responsible for the translocation of materials into the sporophore.

The method of growth rate estimation used here also allows sporophore growth rates to be related to time. The population growth rate varies inconsistently with time probably as a result of variation in size between individual sporophores making up the population. The results, therefore, cast doubt on the method of growth rate estimation used by Cooke (1960) in which 'representative' samples were removed from the population daily during the development of a culture. In addition Chanter and Cooke (1978) had shown that the removal of sporophores from a culture affected the subsequent development of the remaining sporophores. Bearing in mind the problems of obtaining a representative population sample and the consequences of sample removal on subsequent growth, the non-

disruptive method of growth rate estimation used in this study

offers certain advantages over the methods previously employed.

A composite growth curve for an average sporophore was

constructed by combining individual growth curves. The results

show that this growth curve is exponential in nature and again

indicates that the growth rate is proportional to sporophore

size. The time taken for sporophores to develop from primordia

to large, mature individuals was about six days. This time is

in accordance with the period that occurs between the harvest

of successive breaks.

The high growth rate of mature sporophores in all probabil-

ity indicates that the conclusions of Flegg (1980) and Cooke and

Flegg (1962 and 1965) concerning the monopolisation of nutrients

by mature sporophores in preference to primordia, are correct.

From the results of this study it would appear, at least in later

breaks, that an initial build up of nutrients is not the pre-

requisite for the initiation of sporophores as proposed by

Chanter (1978). The removal of sporophores of the preceding

break allows the transfer of materials to pre-formed primordia.

The initial slow growth of these primordia allows the accumulation

of materials in the mycelium. The accumulated materials are then

increasingly rapidly transferred to the sporophores as they increase

in size. Similarly with the sporophores harvested in the first

break: following the initiation process, which results from the

induced environmental changes, the initial slow growth of primordia

would allow a build up of the materials within the mycelium required

for further sporophore construction. Thus it is feasible that the

mycelium of the compost is responsible for the continual degradation

of the compost and the accumulation of both the materials necessary

for sporophore construction and other substances released during substrate degradation. By chance those primordia forming in the most advantageous situations, perhaps through metabolites released from bacterial activity or through evaporative induced flow of nutrients, gain an initial advantage over other primordia. This initial advantage becomes enhanced as the primordia increase in size by the preferential monopolization of nutrients supplied from the compost mycelium.

Assuming that the materials used in sporophore construction are stored within the mycelium of the substrates during the development of the sporophores, it is possible that soluble substances or materials in short supply will be translocated from the storage mycelium into the sporophores in declining quantities during development due to the declining concentration of that material contained within the mycelium. Thus, the frequent observations contained in the literature of higher concentrations of several mineral elements in younger sporophores may indicate the declining availability of these metals during later sporophore development. In Section 3.1.3 it was shown that small sporophores contained significantly higher concentrations of iron than did more mature specimens. In Section 3.3.3 however, there was considerably less difference of the iron concentration between a range of sporophore stages. The large sample size required for Atomic Absorption Spectrophotometric iron analysis precluded the use of this technique in determining the quantity of iron contained in small individual sporophores. The addition of minute quantities of radioactive iron-59 to the substrates, however, allowed observations of the movement of iron into the tissues of individual

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sporophores to be made. It is likely that the small quantities of soluble radioisotope added to the substrates were soon converted into the forms of iron usually present in the compost and casing layer. The movement of the isotope into the sporophore may, therefore, be representative of the overall movement of iron into the sporophore.

It was found that when iron-59 was added to the compost at the time of casing layer application, the concentration of isotope in the sporophores that developed, decreased with increasing sporophore size. The quantity of isotope contained in each sporophore was approximately equal, despite considerable differences in the sizes of sporophores. The relationship of declining sporophore isotope concentration with increasing sporophore size took the form of a decay curve. From this it may be inferred that the decline results from a diminishing quantity of isotope contained within the mycelium. Despite the dissimilarity of sporophore size the constancy of sporophore isotope content may indicate that sporophore initiation is a synchronous event, in which the isotope contained within the mycelium of the substrates is initially transposed during the induction of sporophore formation. Each aggregate receives an equal proportion of the available isotope during the initiation process. During subsequent sporophore enlargement, little isotope is available for uptake into the sporophores and, therefore, the content that the individual sporophore contains is independent of the size that each eventually attains.

Also during this study, the changes of isotope concentration that occurred in the stipe and pileus of sporophores of different size were observed. In the smallest sporophores a considerably

greater isotope concentration was found in the pileus than in the stipe. However, with increasing size the iron-59 concentration of the pileus declined, whereas that of the stipe remained constant. The decreasing isotope concentration is probably the result of dilution by materials involved in sporophore construction. It is possible that a small quantity of iron-59 is taken up from the substrates by the mycelium throughout culture. This isotope is carried in the translocatory stream to the maturing sporophores where it is possibly laid down in the stipe in direct proportion to the other structural materials thus, resulting in the maintenance of isotope concentration in the stipe. Any excess iron-59 will be carried into the pileus in the translocation stream.

In Section 3.3.5 a quantity of isotope was added to the casing layer at the base of many sporophores. The sporophores were harvested 24 hours later and it was found that the quantity of iron-59 they contained increased with increasing individual sporophore size. This quantity did not however, increase in direct proportion to the sporophore size. It is suggested that either a limited quantity of isotope was available or, the more extensive mycelial network of larger sporophores, not in contact with isotope is responsible for the declining rate of isotope uptake in larger sporophores.

When the bases of excised sporophores were immersed in isotope solution for 12 hours, the quantity of iron-59 taken up over that period was approximately proportional to the size of the sporophore as shown by the autoradiograms in Plate 3.3. In addition, those autoradiograms show the translocatory pathways of iron-59 in sporophores of different sizes. Kritsky et al.

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(1965a) suggested from their work on phosphate transport in
Agaricus bisporus that nutrient transport was via specialized
systems of morphologically indistinguishable hyphae. They
showed that P 32 moved at different rates to different parts
of the cap and demonstrated the existence of two separate
translocatory pathways. Rast (1966) also observed the trans-
location of mannitol through two distinct routes within the
sporophore of Agaricus bisporus. In both of these studies the
sporophores used were relatively immature, probably late button
to Button/Open cup stages according to the classification used
in this study. Littlefield et al. (1963) showed that during
the expansion of Lentinus tigrinus sporophores, translocation
becomes restricted to the central zone of the stipe. In this
study using iron-59 and in support of the findings of Kritsky
et al. (1965a) and Rast (1966) there appears to be two translocatory
pathways in the stipes of younger sporophores. In more mature
individuals, however, the major translocatory pathway is restricted
to the centre of the stipe. It was also observed that during
sporophore maturation, translocation switched from the original
route of supply to the hymenia to supply the tissues on the upper
surface of the pileus. Thus assuming that the soluble iron-59 is
revealing the major translocatory pathways within the sporophore,
it appears that the movement of materials within the sporophore
occurs in accordance with the specific demands of the different
tissues. This phenomenon appears to be related to the metabolic
activity of the tissues involved. It is probable that transloca-
tion towards the hymenia is the result of activity during the
maturation of those structures and the processes associated with
sporulation. The subsequent flow of isotope to the upper pileus

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is a reflection of the activity in that area associated with pileus expansion required for efficient spore dispersal. Thus, translocation of materials may be associated with transpiration from the sporophore surface, but more significantly, the metabolic activity of sporophore tissues appears to dictate the direction of flow within the sporophore.

In Sections 3.3.4, 3.3.5 and 3.3.6 the movement of iron-59 into sporophores of different sizes was observed. The experiment described in Section 3.3.7 was an extension of 3.3.4 and was a study of the changes of iron-59 concentration that occurred during the development of small mycelia aggregates through primordia to very small sporophores. In common with the findings of Section 3.3.4 the iron-59 concentration declined with increasing primordium size. The quantity of isotope in each primordium however, increased with enlargement of the primordia but, the rate of isotope accumulation declined with increasing primordium size. Thus, it appears that in the mycelial aggregates iron-59 concentration is at a maximum, probably equivalent to mycelial composition, and during subsequent growth the declining quantity of iron-59 in the primordia results from less isotope being available within the substrate mycelium.

Perhaps the greatest hindrance in any physiological, bio-chemical or developmental study of Agaricus bisporus has resulted from the inability to culture the organism to full reproductive maturity in defined media. In several physiological studies researchers have attempted to collate results from pure culture vegetative studies with those obtained from normal commercial cultivation studies. They usually concluded by questioning the validity of such comparisons. It is highly improbable that the

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nutritional requirements determined using vegetative growth techniques are in any way representative of the requirements in natural substrates, particularly appertaining to fructification.

In the experiment described in Section 3.3.8 Agaricus bisporus was cultured to reproductive maturity using two novel substrates in a flask culture system. These novel substrates were developed to fulfil two criteria, namely: to be of similar composition to substrates used in commercial cultivation and to enable the vegetative and reproductive phases of growth to be sampled during culture. The normal substrates were considered ideal for nutrient supply and the provision of factors necessary in the initiation process. The second criterion was met by modifying the physical structure of these substrates.

Grinding and homogenisation of the compost probably negates the variable nature of compost composition and therefore, makes this material suitable for small scale experimentation. When ground compost was formed into pellets the mycelium of Agaricus bisporus tended to grow between and on the surface of the pellets. The mycelial growth into the pelletised PMB casing layer was mostly in the form of mycelial strands. Thus, with careful dissection it was possible to remove the mycelium from the substrates. Iron-59 was added to the compost and the concentrations that occurred in the vegetative mycelium of the compost and casing, the primordia and sporophores during culture were measured. This novel culture system was therefore, used to demonstrate the changes of composition that took place within the mycelia of the substrates during the growth and development of the sporophore crop.

The isotope content of mycelial samples from compost varied considerably, possibly as a result of the uneven application of isotope to the substrate. There were no significant changes of isotope concentration in the compost mycelium throughout culture but, a slight decline of iron-59 concentration was concurrent with increasing primordium and sporophore size and probably reflects the upward movement of isotope into those developing structures from the compost mycelium. In the mycelium from the casing layer, greater and significant isotope concentration changes occurred during culture. It is likely that the changes of isotope concentration resulted from the movement of iron-59 to the developing sporophores from the compost mycelium via the mycelial strands of the casing layer. Thus, the concentration of iron-59 in the mycelial strands changed according to the flow of iron-59 into developing sporophores. In several sections of this study the concentration of iron in sporophores was found to decline as the sporophores matured and it was proposed that this resulted from a declining quantity of available iron in the mycelium. It is probable, therefore, that greater quantities of iron are in transit in the mycelial strands of the casing layer during the early stages of sporophore development. Subsequently a declining concentration of iron would be observed in the strands due to the reducing availability of material in the underlying compost mycelium. This was indeed the case, as was shown in the results of Section 3.3.8. Additionally a significantly reduced isotope concentration accompanied sporophore enlargement and thereby confirmed earlier observations.

The findings of Section 3.3. of this study show that the movement of iron into sporophores is not concomitant with growth.

The various subsections of this study have demonstrated the importance of the compost mycelium as a storage organ and the involvement of mycelial strands in the casing layer as organs of translocation. Declining concentrations of Iron-59 in developing sporophores are indicative of the declining specific nutrient availability during sporophore development. This may provide an explanation for the observations of the lower metal content of more mature sporophores frequently reported in the literature.

In the final section of results the mean concentrations of 16 metallic elements in sporophore material collected over a three year period are shown (Table 3.3.3). The results are usually within the range of previously published determinations shown in Table 1.1. It is evident that the sporophore composition of Agaricus bisporus is very variable with respect to all metallic elements. The findings of this study using iron may well be applicable to many of these metallic elements, the concentrations of which more than fully satisfy the basal nutritional requirements of the organism defined by submerged culture studies.

4.4   CONCLUSIONS.

The objective of this study was to elucidate some physio-
logical aspects of the nutrition of Agaricus bisporus by the
use of iron as a 'tracer' material. Three main areas of
research were conducted with the objective of demonstrating;
the contribution that various cultural factors make towards
sporophore composition, changes in the composition of the
organism associated with its growth and development, and those
changes in the substrate composition that occur during cultiva-
tion. The two major analytical procedures used (Atomic absorption
spectrophotometry and Iron-59 gamma radiotracing) were found to
be efficacious in these studies.

Investigations into the effect that the five cultural factors
considered to be most important in determining sporophore iron
composition showed that the compost was of the greatest significance
in dictating the quantity of iron in the sporophores. Different
casing layers resulted in significant compositional changes of
different breaks but, the total amount of iron in all sporophores
harvested from different casing materials was approximately equal.
It is suggested that the earlier uptake of iron with some casing
layers is related to the physical characteristics of the casing
layer material particularly; increased evaporation and diffusion
which result in an enhanced upward movement of iron from the
compost.

It was generally found that the concentration of iron increased
in successive breaks of sporophores. This may be associated with
the declining yield allowing a greater quantity of available iron
per unit weight of sporophore tissue and/or an increasing quantity
of available iron in the substrates, resulting from substrate

degradation. An inverse relationship between the sporophore yield and iron content of different strains probably indicates that a certain quantity of iron is available in the substrates and that this amount is distributed amongst those sporophores which form.

In support of several earlier works it was found that sporophore fresh weight yields are related to the quantity of water applied to the casing layer during culture. In addition it was shown the excessive watering suppresses sporophore formation whereas, reduced watering allows the development of greater sporophore numbers. These results may be indicative of the important rôle that evaporation plays in the initial formative stages of sporophore development. It was found that the total dry weight of sporophore material produced was similar irrespective of the quantity of water applied to the casing layer. However, the quantity of iron in sporophores increased in direct relation with the quantity of water they contained thus indicating that iron enters the sporophore in a water soluble form.

Supplementation of the substrates with iron containing compounds failed to significantly increase the concentration of iron in sporophores or the concentration of water extractable iron in the substrates. The quantity of iron in these supplements although great in comparison with sporophore iron was small in relation to the total amount of iron present in the substrates. Both extraction and iron-59 radiotracing techniques showed that added soluble iron becomes rapidly bound to the substrates, although limited downward percolation and upward diffusion of soluble iron do occur in the compost and casing.

Early work had shown the link between water and iron content of the sporophores and it was also found that the concentration of water extractable iron in the compost was similar to the total iron concentration of the sporophores. Thus, further studies of the changes of extractable iron in the substrates were made. An overall steady increase of the concentration of water extractable iron in the casing layer was associated with upward diffusion from the compost. A considerable increase of water soluble iron in the casing occurred at about the time of first break harvest and was thought to be the result of material lost from the mycelial strands following disruption during sporophore harvest. Additionally it is suggested that the reported burst of bacterial numbers at this time is resultant from this release. Further studies showed that different bacterial isolates could significantly increase the concentration of water extractable iron in the casing layer. The results thus provided evidence that indigenous casing layer bacteria may cause gross compositional changes of the casing material and thereby indicating the probable capability of bacteria to create conditions in the casing layer favourable to sporophore initiation.

In the compost, changes of the concentration of extractable iron may be linked with the mycelial activity of the culture. The concentration declined at the time of sporophore initiation and was followed by a continual increase throughout sporophore production and finally a decline following cropping.

Excessive application of water to the casing layer was shown to cause downward percolation of soluble iron through the substrates although tracer studies showed that under normal conditions iron

movement between horizontal strata of the substrates was very limited. It was also apparent from radiotracer studies that the compost was of considerably greater importance of iron supply than was the casing layer.

It was considered important to study iron uptake during sporophore development, particularly as a comparison with the changing growth rate of the organism and thus a photographic time-lapse method of estimating sporophore dry weights _in situ_ was devised, from which it was possible to accurately calculate sporophore growth rates relative to either time or to size. This method showed that sporophores increase in weight exponentially at least as far as the size at which they would normally be harvested. With further development this technique may provide a useful method for the prediction of yields and ideal harvest times of the mushroom crop several days in advance.

Using atomic absorption spectrophotometry it was found that in common with several reports for a number of elements in basidiomycete sporophores, the concentration of iron declined with increasing sporophore maturity. However, because of the relatively large sample size required for this type of analysis several experiments using iron-59 radiotracing, which allows the determination of concentrations in individual sporophores, were done. They showed that in small primordia the quantity of isotope increased with increasing size, although, not in direct proportion with dry weight. The total amount of iron-59 attained a maximum in very small buttons and thereafter remained approximately constant. The declining concentration of iron in developing sporophores was attributed to the limited quantity of available material in the substrate mycelium being progressively consumed during

sporophore formation.

The distribution and routes of iron transport within the sporophore were shown to change during its development. With increased maturity translocation became restricted to the stipe cortex. In the pileus, iron translocation was initially towards the maturing hymenia and subsequently into the upper pellicle probably in association with the expansion of the pileus. Thus, it appears that transport within the sporophore is metabolically regulated and that the accumulation of materials within the sporophore occurs in accordance with the demands of specific tissues during growth and development.

The development of novel pelletised substrates used in a flask culture system closely mimicked normal cultivation conditions thus allowing the development of sporophores to full reproductive maturity. This system also permitted, through careful dissection, the removal of mycelia and developing reproductive structures from the substrates and the subsequent analysis of their iron content. It was found that slight cyclical changes of isotope concentration occurred in the compost mycelium in conjunction with sporophore development and this may indicate the rôle of the compost mycelium as a storage depot which is drawn upon during sporophore enlargement. In the mycelial strands of the casing layer greater changes of isotope concentration were observed. These changes may be associated with the flow of isotope between the compost and developing sporophores. Initially large quantities of isotope would be moving into primordia but, the declining quantity of available material in the compost mycelium would result in the decreased quantity of isotope moving

through mycelial strands into more mature sporophores. This novel method of culture offers an effective system in which compositional changes of both the vegetative and reproductive phases of Agaricus bisporus growth may be studied. It, therefore, offers a basis for the further elucidation of the compositional, nutritional and physiological changes associated with fructification and morphogenesis in Agaricus bisporus.

Analysis of 16 metallic elements in sporophores harvested from various trials gave average results which fell within the range of previously published analyses. The more extensive studies of iron in the substrates and sporophores of A. bisporus described here show the relative importance of several cultural factors which contribute to compositional variations. These results may also indicate the causal factors leading to the compositional extremes of various materials frequently reported in basidiomycete sporophores.

BIBLIOGRAPHY.

BIBLIOGRAPHY

ABBOT, M.T.J. and GROVE, J.F. (1959).   Uptake and translocation of

organic compounds by Fungi.   I.   Microspectrophotometry

in the study of translocation.

EXPERIMENTAL CELL RESEARCH   17, 95-104.

ADRIANO, F.T. and CRUZ, R.A. (1933).   The chemical composition of

Philippine mushrooms.

PHILIPPINE JOURNAL OF AGRICULTURE   4, 1-11.

AICHBERGER, K. (1977).   Untersuchungen uber den Quecksilbergehalt

Osterreichischer Speisepilze und seine Bezeihungen zum

Rohproteingehalt der Pilze.   ZEITSCHRIFT FUR LEBENSMITTEL

UNTERSUCHUNG UND FORSCHUNG   163, 35-38.

AICHBERGER, K. and HORAK, O. (1975).   Quecksilberaufnahme von

Champignons (A.b) aus kunstlich angereichertem Substrat.

DIE BODENKULTUR   26 (1), 8-14.

ALLEN, R.O. and STEINNES, E. (1978).   Concentrations of some

potentially toxic metals and other trace elements in wild

mushrooms in Norway.   CHEMOSPHERE 4, 371-378.

ALLEN, S.E., GRIMSHAW, H.M., PARKINSON, J.A. and QUARMBY, C. (1974).

Chemical analysis of ecological materials,   BLACKWELL

SCIENTIFIC PUBLICATIONS, OXFORD.

ALTAMURA, M.R., ROBBINS, F.M., ANDREOTTI, R.E., LONG, L. and

HASSELSTROM, T. (1967).   Mushroom ninhydrin-positive

compounds.   Amino acids, related compounds, and other

nitrogenous substances found in cultivated mushroom,

A. campestris JOURNAL OF AGRICULTURE AND FOOD CHEMISTRY

15, 1040-1043.

AMER, M.A. and BRISSON, G.J. (1973). Selenium in human food stuffs
collected at the Ste-Foy (Quebec) food market. JOURNAL
OF THE CANADIAN INSTITUTE OF FOOD AND SCIENCE TECHNOLOGY
6, 184-187.

ANDERSON, E.E. and FELLERS, C.R. (1942). Food value of mushrooms.
PROCEEDINGS OF AMERICAN SOCIETY OF HORTICULTURAL SCIENCE
41, 301-304.

ANGELI-COUVY, J. (1975). La fruitification de l'Agaricus bisporus
(Lange) Sing. sur milieu aseptique on presence de charbon
actif. COMPTES RENDUS ACADEMIE DES SCIENCES, PARIS
SERIS D. 280, 2101-2103

ANON. (1974). Addition of ferrous iron to casing material.
NEW SOUTH WALES DEPARTMENT OF AGRICULTURE. SYDNEY.
ANNUAL REPORT 1973-1974, P.94.

ARROLD, N.P. (1972). Confirmation of the ability of Pseudomonas
putida to cause fruiting of the cultivated mushroom.
MUSHROOM GROWERS ASSOCIATION BULLETIN 269, 200.

BARBER, D.A. (1968). Micro-organisms and the inorganic nutrition
of higher plants. ANNUAL REVIEW OF PLANT PHYSIOLOGY 19,
77-88.

BASCOMB, G.L. (1968). Distribution of pyrophosphate-extractable
iron and organic carbon in soils of various groups.
JOURNAL SOIL SCIENCE 19 (2), 251-268.

BELS-KONING, H.C. (1950). Experiments with casing soils, water
Supply and climate. MUSHROOM SCIENCE 1, 78-84.

BISHOP, O.N. (1971)   Statistics for biology. Second Edition,
LONGMAN GROUP LIMITED, LONDON.

BOHUS, G. (1959)   Investigations concerning the life processes of
the cultivated mushroom.   MUSHROOM SCIENCE 4, 86-129.

BOKHARY, H.A. and COOKE, R.C. (1974)   Translocation of ^{14}C Glucose
by Phytophthora cactorum.   TRANSACTIONS OF THE BRITISH
MYCOLOGICAL SOCIETY, 63 (3), 535-540.

BONNER, J.T., KANE, K.K., and KEVEY, R.H. (1956). Studies on the
mechanics of growth in the common mushroom, Agaricus
campestris.   MYCOLOGIA 48 (1), 13-19.

BORRISS, H. (1934).   Beitrage zur Wachstums und Entwicklungs-
physiologie der Fruchtkorper van Coprinus lagopus. PLANTA
22, 28-69.

BRET, J.P. (1977). Respective role of cap and mycelium on stipe
elongation of Coprinus congretatus. TRANSACTIONS OF THE
BRITISH MYCOLOGICAL SOCIETY 68 (3), 363-369.

BRETZLLOFF, C.W. (1963). A quantitative method for the study
Mushroom initiation in the laboratory. MUSHROOM SCIENCE
5, 241-245.

BRETZLLOFF, C.W. and FLUEGEL, M.S. (1963).   Chemical composition of
mushroom compost during Composting and Cropping. MUSHROOM
SCIENCE 5, 46-60.

BUTLER, G.M. (1957).   The development and behaviour of Mycelial
Stands in Merulius lacrymans (Wulf) Fr. I. Strand develop-
ment during Growth from a Food-base through a Non-nutrient
medium. ANNALS OF BOTANY 21, 523-537.

BUTLER, G.M. (1958). The development and behaviour of mycelial
Strands in Merulius lacrymans (Wulf)Fr. II. Hyphal
Behaviour during Stand formation. ANNALS OF BOTANY 22,
219-236.

BYRNE, B.S.F. and BRENNAN, P.J. (1975). The lipids of Agaricus
bisporus. JOURNAL OF GENERAL MICROBIOLOGY, 89 (2) 245-55.

CHANTER, D.O. and COOKE, D. (1978). Some factors affecting the
growth rate of sporophores of the cultivated mushroom.
SCIENTIA HORTICULTURAE 8, 27-37.

CHANTER, D.O. and THORNLEY, J.H.M. (1978). Mycelial growth and
the initiation and growth of sporophores in the mushroom
crop: a mathematical model. JOURNAL OF GENERAL
MICROBIOLOGY 106 (1): 55-65.

CHARLAMPOWICZ, Z., KLAWITTER, M. and WOZNIAK, W. (1973). Content
of metals in some (10) mushroom species. I. Determination
of Na, K, and Ca in 10 kinds of edible mushrooms.
BROMATOLOGIA I CHEMIA TOKSYKOLOGICZNA 6 (4), 451-454.

COLLET, P. (1977). Determination of heavy metals in food by inverse
polarography.II. Content of Pb, Cd, and Cu in edible fungi.
DEUTSCHE LEBENSMITTEL RUNDSCHAU 73 (3), 75-82.

COOKE, D. (1960). The growth rate of mushroom. REPORT OF THE
GLASSHOUSE CROPS RESEARCH INSTITUTE 1959.

COOKE, D. and FLEGG, P.B. (1962). The relationship between yield
of the cultivated mushroom and stage of maturity at picking.
JOURNAL OF HORTICULTURAL SCIENCE 37, 167-174.

COOKE, D. AND FLEGG, P.B. (1965). The effect of stage of maturity at picking on the flushing of crops of the cultivated mushroom. JOURNAL OF HORTICULTURAL SCIENCE 40, 207-212.

CORIN, C., LAUDET, J. and PICARD, M. (1954). Etude de l'evolution des compostes mineraux incorpores au compost. MUSHROOM SCIENCE 2, 15-20.

CORTIEU, P. (1949) Le gobetage dans les cultures de champignon de couce (son influence sur le rendement et sur la composition chimique des carpophores). ANNALES AGRONOMIQUES DE PARIS 19, 770-781.

COUVY, J. (1972). Etude de l'introduction de la fruitification chez Agaricus bisporus (Lange) Sing: action du glucose. COMPTES RENDUS HEBDOMADAIRES DES SEANCES DE L'ACADEMIE DES SCIENCES, SERIES D 274, 2475-2477.

COUVY, J. (1974). La fructification du champignon de couche - Les facteurs microbiologicues. BULLETIN DE LA FERERATION NATIONALE DES SYNDICATS AGARICOLES DES CULTIVATEURS DE CHAMPIGNONS 3,734-739.

COWAN, M.C., LEWIS, B.G. And THAIN, J.F. (1972 b). The mechanism of translocation of potassium in mycelium of Phycomyces blakesleeanus in an aqueous environment. TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY 58(1), 103-112.

COWAN, M.C., LEWIS, B.G. and THAIN, J.F. (1972 C). Uptake of potassium by the developing sporangiophore of Phycomyces blakesleeanus. TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY 58 (1), 113-126.

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COWAN, M.C., THAIN, J.F. and LEWIS, B.G. (1972a).   Mechanism of
    translocation of potassium in sporangiophores of _Phycomyces_
    blakesleeanus in an aqueous environment.   TRANSACTIONS OF
    THE BRITISH MYCOLOGICAL SOCIETY 58 (1), 91-102.

CRESSWELL, P.A. (1980)   Personal Communication. UNIVERSITY OF ASTON
    IN BIRMINGHAM.

CRESSWELL, P.A. and HAYES, W.A. (1979). Further investigations on
    the bacterial ecology of the casing.   MUSHROOM SCIENCE
    10 (1),   347-359.

CRISAN, E.V. and SANDS, A. (1978).   Chapter 6: Nutritional
    Value.   THE BIOLOGY AND CULTIVATION OF EDIBLE MUSHROOMS.
    Ed. CHANG AND HAYES. ACADEMIC PRESS. LONDON AND NEW YORK.

CURTO, C. and FAVELLI, F. (1972). Stimulative effect of certain
    micro-organisms (Bacteria, yeasts, microalgae) upon fruit
    body formation of _Agaricus bisporus (Lange) Sing_. MUSHROOM
    SCIENCE 8,   67-74.

DABROWSKI, J. and DZARNIK, W. (1976).   Untersuchungen uber die
    Ruckstande von Benomyl in Champignons. NACHRICHTENBLATT
    FUR DEN PFLANZENSCHUTZ IN DER DDR 30 (12), 254-256.

DELMAS, J. (1974)   Valeur alimentaire du champignon de couche.
    BULLETIN DE LA FEDERATION NATIONALE DES SYNDICATS
    AGARICOLES DES CULTIVATORS DE CHAMPIGNONS 15, 783-799.

DLEMAS, J. (1978)   La composition du Champignon de couche.
    MEDECINE ET NUTRITION 14 (2). 99-109.

DOMSCH, K.H. GRABBE, K. and FLECKENSTEIN, J. (1976).

    Schwermetallgehalte in Kultursubstrat und Erntegut des

    Champignons, Agaricus bisporus bein Einsatz von Mullklar

    schlammkompost.   ZEITSCHRIFT FUR PFLANZENERNAHRUN UND

    BODENKUNDE 4, 487-501

DRBAL, K., KALAC, D., SEFLOVA, A. and SEFL, J. (1975a). Content of

    copper in some edible mushroom.   CESKA MYKOL 29 (3),

    184-186.

DRBAL, K., KALAC, P., SEFLOVA, A. and SEFL, J. (1975b).   Content of

    trace elements Fe and Mn in some edible mushrooms.

    CESKA MYKOL 29 (2), 110-114.

DUTSCH, G.A. (1975).   Reduktion der Bioktivitat von Benomyl durch

    verschiedene Deckerdetypen in der Champignonkultur.

    PHYTOPATHOLOGISCHE ZEITSCHRIFT 83, 14-22.

DUTSCH, G. and RAST, D. (1969)   Biosynthese von Mannitol in

    Agaricus bisporus. ARCHIV FUR MIKROBIOLOGIE 65, 195-207.

DUTSCH, G. and RAST, D. (1972).   Uber die rolle der Mannitbildung

    in Agaricus bisporus.   MUSHROOM SCIENCE 8, 387-397.

DUTSCH, G.A. and TSCHIERPE, H.J. (1976).   Uber der aktiuitaet

    von benomyl in der champignonkultur bei verwendung

    veischiedener Deckerdetypen. MUSHROOM SCIENCE 9 (1),

    293-303.

EDDY, B.P. and JACOBS, L. (1976). Mushroom compost as a source of

    food for Agaricus bisporus.   MUSHROOM JOURNAL 38, 56-59.

EGER, G. (1961). Untersuchungen uber die Funktion der Deckschicht bei der Fruchtkorperbildung des kulturchampignons, Psalliota bispora (Lange). ARCHIV FUR MIKROBIOLOGIE 39, 313-334.

EGER, G. (1963). Untersuchungen zur Fruchtkorperbildung des Kulturchampignons. MUSHROOM SCIENCE 5, 314-329.

EGER, G. (1965a). Untersuchungen uber die Bildung und Regeneration von Fruchtkorpern bei Hutpilzen. I. Pleurotus flórida. ARCHIV FUR MIKROBIOLOGIE 50, 343-356.

EGER, G. (1965b). Untersuchungen uber die Bildung und Regeneration von Fruchkorpern bei Hutpilzen. III. Flammulina velutipes Curt. ex Fr. und Agaricus bisporus (Lange) Sing. ARCHIV FUR MIKROBIOLOGIE 52, 282-290.

EGER, G. (1972) Experiments and comments on the action of bacteria on sporophore initiation in Agaricus bisporus. MUSHROOM SCIENCE 8, 719-725.

ENKE, M., MATSCHINER, H. and ACHTZCHN, M.K. (1977). Heavy metal enrichments in mushrooms. NAHRUNG 21 (4), 331-334.

FLECKENSTEIN, J. and GRABBE, K. (1979). Investigations concerning the behaviour of heavy metals during turnover of organic matter in the rotting process of mushroom cultivation. MUSHROOM SCIENCE 10 (2), 69-76.

FLEGG, P.B. (1958). Some aspect of the casing layer in relation to fruiting of the cultivated mushroom. REPORT OF THE GLASSHOUSE CROPS RESEARCH INSTITUTE 1956, 93-105.

FLEGG, P.B. (1959) The functions of compost and casing layer in
Relation to Fruiting and Growth of cultivated Mushroom.
Psalliotta (Agaricus) hortensis. MUSHROOM SCIENCE 4,
205-210.

FLEGG, P.B. (1961a) Mushroom composts and composting: A review of
the literature. REPORT OF GLASSHOUSE CROPS RESEARCH
INSTITUTE 1960, 125-134.

FLEGG, P.G. (1961b). The natural accumulation of soluble salts
in casing layer of mushroom beds. THE JOURNAL OF
HORTICULTURAL SCIENCE 36 (2), 139-144.

FLEGG, P.B. (1967). Effect of shaking up casing layer on growth of
mycelial strands and on yield. MUSHROOM SCIENCE 6,
359-370.

FLEGG, P.B. (1974a) The measurement of evaporative loss in relation
to water management during cropping of Agaricus bisporus.
MUSHROOM SCIENCE 9, 285-292.

FLEGG, P.B. (1974b). Water Requirement of the Mushroom Crop.
SCIENTIA HORTICULTURAE 2, 237-247.

FLEGG, P.B. (1975). The response of the mushroom crop to the pattern
of watering. SCIENTIA HORTICULTURAE 3,137-142.

FLEGG, P.B. (1979). Effects of competition on the Development of
mycelium, mycelial aggregates and sporophores of Agaricus
bisporus. SCIENTIA HORTICULTURAE 11, 141-149.

FLEGG, P.B. (1980). Effect of temperature on the mushroom Agaricus
bisporus: A brief Review of 20 years of Research.
THE MUSHROOM JOURNAL 86, 65-67.

FLEGG, P.B. and GANDY, D.G. (1956). Size and form in the mushroom sporophore. (Psalliota hortensis). I. Development of normal sporophores. MUSHROOM SCIENCE 3, 119-128.

FLEGG, P.B. and GANDY, D.G. (1962). Controlled environment cabinets for experiments with cultivated mushroom. JOURNAL OF HORTICULTURAL SCIENCE 37, 124-133.

FOOD AND AGRICULTURE ORGANISATION (1972). Food Composition Table for Use in East Asia. FOOD POLICY AND NUTRITION DIVISION, F.A.O. UNITED NATIONS, ROME.

FRANK, R., RAINFORTH, J.R. and SANGSTER, D. (1974). Mushroom production in respect of Mercury Content. CANADIAN JOURNAL OF PLANT SCIENCE 54, 529-534.

GANNEY, G.W. and RICHARDSON, S. (1974). Chemical and Physical analyses of 12 Commercial Mushroom Casing mixtures. THE CASING LAYER. ED. W.A. HAYES. W.S. MANEY AND SON LTD. LEEDS.

GARRETT, S.D. (1953). Rhizomorph Behaviour in Armillaria mellea (Vahl) Quel. Part 1. ANNALS OF BOTANY 17, 63-79.

GARRETT, S.D. (1954). Function of the mycelial strands in substrate colonisation by the cultivated mushroom, Psalliota hortensis. TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY 37, 51-57.

GERRITS, J.P.G. (1968). Organic compost constituents and water utilised by the cultivated mushroom during spawn run and cropping. MUSHROOM SCIENCE 7, 111-127.

GERRITS, J.P.G. (1970) Inorganic and Organic Supplementation of Mushroom Compost. MUSHROOM GROWERS ASSOCIATION BULLETIN 251, 489-508.

GERRITS, J.P.G. (1971) The Influence of water in the preparation of mushroom compost and its control. MUSHROOM SCIENCE 8, 43-57.

GERRITS, J.P.G., BELS-KONING, H.C. and MULLER, F.M. (1967). Changes in compost constituents during composting, pasteurisation and cropping. MUSHROOM SCIENCE 6, 225-243.

GIOVANNOZZI-SERMANNI, G., BASILE, G., and LUNA, M. (1979). Biochemical changes occuring in the compost during growth and reproduction of Pleurotus ostreatus and Agaricus bisporus. MUSHROOM SCIENCE 10 (2), 37-53.

GIOVANNOZZI-SERMANNI, G., GRAPELLI, A., CACCIARI, I., and PIETROSANTI, W. (1976). Influence of Arthrobacter sp. on Microflora, Phytohormones of the compost and on Sporophore Formation of Agaricus bisporus. ANNALI DI MICROBIOLOGIA 26, 129-138.

GRAINGER, J. (1962). Vegetative and fructifying growth in Phallus impudicus. TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY 45 (1), 147-155.

GROSSBARD, E. and STRANKS, D.R. (1959) Translocation of Co^{60} and Cs^{137} by fungi in Agar and Soil cultures. NATURE 184, 310-314.

GRUEN, H.E. (1963) Endogenous growth regulation in carpophores of Agaricus bisporus. PLANT PHYSIOLOGY 38, 652-667.

GRUEN, H.E. and WU, S.H. (1972a). Dependence of fruit body elongation on the mycelium of Flammulina velutipes. MYCOLOGIA 64, 995-1007.

GRUEN, H.E. and WU, S. (1972b)   Promotion of stipe elongation in
isolated _Flammulina velutipes_ fruit bodies by carbohydrates,
natural extracts, and amino acids. CANADIAN JOURNAL OF
BOTANY 50, 803-818.

GRUETER, H. (1971). Radioactive fission product Cs^{137} in mushrooms
in W. Germany during 1963-1970.   HEALTH PHYSICS 20,
655-656.

GUDE, W.D. (1968)   Localization of Radioisotopes in Biological
Material.   PRENTICE - HALL INC. NEW JERSEY.

HADDAD, N.A. (1977).   Studies on the composition and nutritional
value of the cultivated mushroom, _Agaricus bisporus_ (Lange)
Sing. PhD. THESIS. UNIVERSITY OF ASTON IN BIRMINGHAM.

HAGIMOTO, H. and KONISHI, M. (1959).   Studies on growth of fruit
body of fungi. I. Existence of a hormone active to growth
of fruit-body in _Agaricus bisporus_ (Lange) Sing.
BOTANICAL MAGAZINE (TOKYO) 72, 359-366.

HAMMOND, J.B.W. (1977). Carbohydrate metabolism in _Agaricus bisporus:_
oxidative pathways in mycelium and sporophore.
JOURNAL OF GENERAL MICROBIOLOGY 102, 245-248.

HAMMOND, J.B.W. (1979)   The role of non-structural carbohydrates in
the life cycle of _Agaricus bisporus._ MUSHROOM SCIENCE 10
(1), 391-400.

HAMMOND, J.B.W. and NICHOLS, R. (1976a). Carbohydrate metabolism in
Agaricus bisporus (Lange) Sing. : Changes in soluble
carbohydrates during growth of mycelium and sporophore.
JOURNAL OF GENERAL MICROBIOLOGY 93, 309-320.

HAMMOND, J.B.W. and NICHOLS, R. (1976b). Glycogen in _Agaricus bisporus_
TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY 66, 325-327.

HAMMOND, J.B.W. and NICHOLS, R. (1977). Carbohydrate metabolism in
Agaricus bisporus: metabolism of ^{14}C labelled sugars by
sporophore and mycelium. NEW PHYTOLOGIST 79, 315-325.

HASUK, A. (1975). Untersuchungen uber die Aufnahme von Cu, Zn,
Pb, Cd, und Hg durch den Champignon. MULL UND ABFALL
7 (6), 172-176.

HAYES, W.A. (1972). Nutritional factors in relation to mushroom
production. MUSHROOM SCIENCE 8, 663-674.

HAYES, W.A. (1974). Editor. THE CASING LAYER. W.S. MANEY AND SON
LTD. LEEDS.

HAYES, W.A. (1977). Editor. COMPOSTING. W.S. MANEY AND SON LTD.,
LEEDS.

HAYES, W.A. (1978). CHAPTER 8 and 9 IN BIOLOGY AND CULTIVATION OF
EDIBLE MUSHROOMS. Editors CHANG S.T. and HAYES W.A.
ACADEMIC PRESS, LONDON AND NEW YORK.

HAYES, W.A. and NAIR, N.G. (1976). Effects of volatile metabolic
by-products of mushroom mycelium on ecology of casing layer.
MUSHROOM SCIENCE 9 (1), 259-268.

HAYES, W.A., RANDLE, P.E. and LAST, F.T. (1969). The nature of
microbial stimulus affecting sporophore formation in
Agaricus bisporus. ANNALS OF APPLIED BIOLOGY 64, 177-187.

HAYES, W.A., YEO, S.G., CRESSWELL, P.A. and JAKEMAN, K.J. (1978).
Paper and pulp mill By-product as a casing medium for
mushroom culture. MUSHROOM JOURNAL 62, 38-44.

HERBERT, A. and HEIM, F. (1909) Sur la nutrition minerale du
champignon de couche. ANNALS DE LA SCIENCE AGRONOMIQUE,
FRANCAISE ET ETRANGERE 2 (3), 1-12.

HEIN, I. (1930a) Studies of the mycelium of Psalliota campestris.
AMERICAN JOURNAL OF BOTANY 17, 197-211.

HEIN, I. (1930b) Studies on morphogenesis in Agaricus campestris.
AMERICAN JOURNAL OF BOTANY 17, 882-915.

HILL, E.P. (1965). Uptake and Translocation in, THE FUNGI I.
457-463 Editors SUSSMAN, A.S. and AINSWORTH, G.C.
ACADEMIC PRESS, LONDON AND NEW YORK.

HINNERI, S. (1975). Mineral elements of macrofungi in oak-rich
forests on Lenholm Island, inner archipelago of SW Finland.
ANNALES BOTANICI FENNICI 12 (4), 135-140.

HOLTZ, R.B. and SCHISLER, L.C. (1971). Lipid metabolism of Agaricus
bisporus (Lange) Sing : I. Analysis of sporophores and
mycelial lipids. LIPIDS 6(3), 176-180.

HUGHES, D.H. and RHODES, Y.E. (1959). Changes in amino acid
composition of Agaricus campestris with respect to
successive crops. MUSHROOM SCIENCE 4, 176-182.

HUME, D.P. and HAYES, W.A. (1972). The production of Fruit-body
Primordia in Agaricus bisporus (Lange) Sing. on Agar media.
MUSHROOM SCIENCE 8, 527-532.

HUSSAIN, A.A.K. (1980)   Personnal communication. MUSHROOM SCIENCE
UNIT, UNIVERSITY OF ASTON IN BIRMINGHAM.

JALALI, I. and ANDERSON, J.P.E. (1976). Uptake of benomyl by the
cultivated mushroom, Agaricus bisporus. JOURNAL OF
AGRICULTURE AND FOOD CHEMISTRY 24 (2), 431-432.

JANDAIK, C.L., BHANDARI, A.R., ARORA, C.L. and RANGAD, C.O. (1979)
Chemical composition of some Edible Fungi. MUSHROOM SCIENCE
10(2), 685-688.

JENNINGS, D.H. (1976).   Transport and Translocation in Filamentous
Fungi. In THE FILAMENTOUS FUNGI III, 32-64. Editors
SMITH, J.E. and BERRY, D.R. E. ARNOLD. LONDON.

JOHNSON, W. and NAYFIELD, C.L. (1970). Elevated levels of Caesium-
137 in Common Mushrooms (Agaricaceae) with Possible
Relationship to high levels of Caesium -137 in Whitetail
Deer. RADIOLOGICAL HEALTH DATA AND REPORTS 11, 527-531.

KALBERER, P. and VOGEL, E. (1978). Residue of insecticides in
Agaricus bisporus, cultivated in soils treated with the
insecticides. PESTICIDE SCIENCE 9 (2), 146-150.

KAUL, T.N. and KACHROO, J.L. (1976).   Use of Ferrous Sulphate for
Stimulating fruit-body formation at mushroom farms in
Kashmir. MUSHROOM JOURNAL 43, 214.

KHOR, G.L. (1975). Mushrooms as food. UMAGA/FAUM FOOD CONFERENCE
1975.

KINDT, V. (1965).   Beziehungen zwischen Bewasserung und
Ertragsbildung im Champignonanbau. ARCHIV FUR GARTENBAU
13 (4), 313-328.

KISSMEYER-NIELSEN, E., McCLENDON, J.H. and WOODMANSEE, C.W. (1966).
Changes in Amino acids and urea in the Cultivated Mushroom,
Agaricus bisporus, as influenced by Nutrient Supplementation
of Compost during the Growth Cycle. JOURNAL OF AGRICULTURE
AND FOOD CHEMISTRY 14 (6), 633-636.

KITAMOTO, Y. and GRUEN, H.E. (1976). Distribution of cellular
carbohydrates during development of the mycelium and fruit-
bodies of Flammulina velutipes. PLANT PHYSIOLOGY 58,
485-491.

KITAMOTO, Y., YAMANE, N., HOSOI, N., and ICHIKAWA, Y. (1974).
Nutritional aspects of fruitbody formation of Favolus
arcularis in replacement culture. TRANSACTIONS OF THE
MYCOLOGICAL SOCIETY OF JAPAN 15, 60-71.

KODAK DATA SHEET FM-17. 'KODIREX' X-RAY FILM, KODAK LIMITED, LONDON.

KONISHI, M. (1967). Growth promoting effect of certain amino acids
on the Agaricus Fruitbody. MUSHROOM SCIENCE 6, 121-132.

KRITSKY, M.S., KULAEV, I.S. KLEBANOVA, L.M. and BELOZERSKY, A.N.
(1965a). Two pathways of phosphate transport in the
sporophore of the field mushroom, Agaricus bisporus.
DOKLADY AKADEMII NAUK USSR 160, 949-952.

KRITSKY, M.S., KULAEV, I.E., MAYOROVA, I.P., FAIS, D.A. and
BELOZERSKY, A.N. (1965b). Translocation of phosphates in
sporophores of Agaricus bisporus. BIOKHIMIYA 30 (4),
778-789.

KULAEV, I.S., KRITSKII, M.S. and BELOZERSKII, A.N. (1960).
The metabolism of polyphosphates and of other phosphorus
compounds in the course of development of fruit-bodies of
the mushroom Agaricus bisporus (Lange). BIOKHIMIYA 25,
736-748.

LAI, H.H. (1977)   Evidence of mercury absorption in mushroom
mycelium and fruiting body.   TAIWAN MUSHROOMS 1 (3),
86-94.

LAMBERT, E.G. (1963)   Cropping experiments to test the efficiency
of nutrient translocation by cultivated mushrooms.
MUSHROOM SCIENCE 5, 340-347.

LAST, F.T., HOLLINGS, M. and STONE, O.M. (1967). Some effects of
cultural treatments on virus diseases of cultivated
mushroom, Agaricus bisporus. ANNALS OF APPLIED BIOLOGY
59, 451-462.

LATCHE, J.C. and PIQUEMAL, M. (1975).   Variations in the amino acid,
amide, and organic acid contents of composts for Agaricus
bisporus culture as a function of sporophore production.
ANNALES AGRONOMIQUES 26 (2), 199-213.

LAUB, E., WALIGORSKI, F. and WOLLER, R. (1977). Uber die
cadmiumanreicherung in Champignons. ZEITSCHRIFT FUR
LEBENSMITTEL-UNTERSUCHUNG UND FORSCHUNG 164, 269-271.

LEH, H.O. (1975).   Bleigehatte in Pilzen. ZEITSCHRIFT FUR
LEBENSMITTEL-UNTERSUCHUNG UND FORSCHUNG. 157. 141-142.

LENDENMANN, J. and RAST, D. (1978).   Glycogen in spores and
mycelium of Agaricus bisporus. TRANSACTIONS OF THE
BRITISH MYCOLOGICAL SOCIETY 71 (1), 146-148.

LEWIS, D.H. and HARLEY, J.L. (1965). Carbohydrate Physiology of
Mycorrhizal roots of beech. NEW PHYTOLOGIST 64, 224-269.

LITTLEFIELD, L.J. (1966). Translocation of P-32 in Sporophores of
Collybia velutipes. PHYSIOLOGIA PLANTARUM 19, 264-270.

LITTLEFIELD, L.J. WILCOXSON, R.D. and SUDIA, T.W. (1963). Trans-
location in sporophores of Lentinus tigrinus related to
specialized cells. PHYTOPATHOLOGY 53, 881-888.

LITTLEFIELD, L.J. WILCOXSON, R.D. and SUDIA, T.W. (1965).
Translocation of ^{32}P by Rhizoctonia solani. PHYTOPATHOLOGY
55, 536-542.

LOCKARD, J.D. (1962). An investigation of the metabolic gases
produced by the cultivated mushroom Agaricus bisporus
(Lange) Sing. PhD. THESIS. PENNSYLVANIA STATE UNIVERSITY.

LOCKARD, J.D. (1967). Metabolic gases of the Cultivated Mushroom.
MUSHROOM SCIENCE 6, 157-164.

LONG, P.E. and JACOBS, L. (1969). Some observations on CO_2 and
sporophore initiation in the cultivated mushroom.
MUSHROOM SCIENCE 7, 373-384.

LONG, P.E. and JACOBS, L. (1974). Aseptic fruiting of the cultivated
mushroom, Agaricus bisporus. TRANSACTIONS OF THE BRITISH
MYCOLOGICAL SOCIETY 63 (1), 99-107.

LOUGHTON, A. and FRANK, R. (1974). Mercury in Mushrooms (Agaricus
bisporus).MUSHROOM SCIENCE 9 (1), 347-355.

LUCAS, R.L. (1960) Transport of Phosphorus by Fungal Mycelium.
NATURE 188, 763-764.

LYON, A.J.E. and LUCAS, R.L. (1969). The effect of temperature on the translocation of phosphorus by Rhizopus stolonifer. NEW PHYTOLOGIST. 68, 963-969.

MADELIN, M.F. (1956). Studies on Nutrition of Co prinus lagopus Fr., especially as affecting fruiting. ANNALS OF BOTANY 20, 307-330.

MADELIN, M.F. (1960) Visible changes in the vegetative mycelium of Co prinus lagopus Fr. at the time of fruiting. TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY 43, 105-110.

MADER, E.O. (1943). Some factors inhibiting the fructification and production of cultivated mushroom, Agaricus campestris. PHYTOPATHOLOGY 33, 1134-1145.

MAGGIONI, A., PASSERA, C., RENOSTO, F. and BENETTI, E. (1968). Composition of cultivated mushrooms, Agaricus bisporus during the growing cycle as affected by the nitrogen source introduced in composting. JOURNAL OF AGRICULTURE AND FOOD CHEMISTRY 16 (3), 517-519.

MALLET, M.L. (1959). Les proprietes biotiques at alimentaires du champignon de Paris. MUSHROOM SCIENCE 4, 465-469.

MARGHERI, M.C. and VASSILACAKIS, B. (1977). Effetto stimolante di alaine microalghe sulla crescita ela differenziazione dei corpi fruitferi di Agaricus bisporus. MICOLOGIA ITALIANA 62(2), 23-30.

MATHEW, K.T. (1961). Morphogenesis of mycelial strands in the cultivated mushroom, Agaricus bisporus. TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY 44, 285-290.

MATTONI, R.H.T.,ACKER, D., MARX, P.F.B., BIANK, B.B. and NISHIDA, J.
(1966). Experimenta on aflatoxin translocation to the
commercial mushroom. MUSHROOM SCIENCE 9, 315-319.

McCONNELL, J.E.W. and ESSELEN, W.B. (1947). Carbohydrates in
cultivated mushrooms (Agaricus campestris). FOOD RESEARCH
12, 118-121.

McLAUGHLIN, D.J. (1974). PROTOPLASMA 80, 91-108.

McONIE, K.C. and SNYDER, W.C. (1966). Nutrient depletion and
production of perithecia by Gnomonia fructicola.
CANADIAN JOURNAL OF BOTANY 44, 149-154.

MEISCH, H.U., SCHMITT, J.A. and REINLE, W. (1977). Schwermetalle
in horeren Pilzen. Cd, Zn und Cu. ZEITSCHRIFT FUR
NATURFORSCHUNG 32 (C), 172-181.

MELIN, E. and NILSSON, H. (1958). Translocation of nutritive
elements through mycorrhizal mycelia to pine seedlings.
BOTANISKA NOTISER 111, 251-256.

MILNE, L. and COOKE, R.C. (1969). Translocation of ^{14}C glucose by
Rhizoctonia solani. TRANSACTIONS OF THE BRITISH MYCOLOGICAL
SOCIETY 53, 279-289.

MLODECKI, H., LASOTA, W. and TERSA, S. (1965). Grzyby jako zrodlo
kabaltu w zywnosci. FARMACJA POLSKA 21, 337-339.

MOLITORIS, H.P., VAN ETTEN, J.L. and GOTTLIEB, D. (1969). Age
dependent changes in composition and metabolism of fungi.
MUSHROOM SCIENCE 7, 59-67.

MONSON, A.M. and SUDIA, T.W. (1963).   Translocation in Rhizoctonia
        solani.   BOTANICAL GAZETTE   124, 440-443.

MOSER, M. (1962).   Die rolle des Wassers im Leben der horeren Pilze.
        SCHWEIZERISCHE ZEITSCHRIFT FUR PILZKUNKE 40, 129-141.

NAIR, N.G. and HAYES, W.A. (1975). Some effects of casing soil
        amendments on Mushroom Cropping.   AUSTRALIAN JOURNAL
        OF AGRICULTURAL RESEARCH 26, 181-188.

NAIR, N.G., SHORT, C.C. and HAYES, W.A. (1976).   Studies on gaseous
        environment of casing layer. MUSHROOM SCIENCE 9 (1),
        245-257.

NEHRING, P. (1977). Zum Zinn und Eisengehalf in Champignonkonserven.
        CHAMPIGNON   195, 20-23.

NEILSEN, G. and RASMUSSEN, C.R. (1963). Transportation capabilities
        of mushroom mycelium determined by means of radioactive
        isotopes.   MUSHROOM SCIENCE   5, 348-383.

O'DONOGHUE, D.C. (1963). New light on fruit-body initiation.
        MUSHROOM SCIENCE 5,   247-249.

PALLOTTI, G., BENCIVENGA, B. and VEGLIANTE, A. (1976).
        Contenuto in mercurio totale di funghi seluatici e
        coltivati.   INDUSTRIE ALIMENTARI 15 (12), 57-60.

PARANJPE, M.S. (1979)   Morphogenesis of Agaricus bisporus.
        Changes in protein and enzyme activity. MYCOLOGIA 71 (3),
        469-479.

PARANJPE, M.S. and CHEN, P.K. (1972).   Biochemical characteristics
        of growth stages of Agaricus bisporus. PLANT PHYSIOLOGY
        46 (Supplement 230), 43.

PARK, J.Y. and AGNIHOTRI, V.P. (1969). Sporophore production of
Agaricus bisporus in aseptic environments. ANTONIE VAN
LEEUWENHOEK 35, 523-528.

PARRISH, G.K., BEELMAN, R.B. and KNEEBONE, L.R. (1976).
Relationship between yield and Mannitol content during
crop cycle of Cultivated mushrooms. HORTICULTURAL
SCIENCE 11 (1), 32-33.

PEERALLY, A. (1979). Sporophore initiation in Agaricus bisporus
(Lange) Sing. and Agaricus bitorquis (Quel.) Sacc. in
relation to bacteria and activated charcoal. MUSHROOM
SCIENCE 10 (1) 611-639.

PEGG, G.F. (1973). Gibberelin-like substances in the sporophores of
Agaricus bisporus (Lange). JOURNAL OF EXPERIMENTAL
BOTANY 24(81), 675-688.

PESEK, F. (1964). Predbezne sdeleni o autoradiografickem dukazu
pritomnosti radioaktiunich pruku v plodnicich hub.
CESKA MYKOLOGIE 18, 232-233.

PLUNKETT, B.E. (1951) Some aspects of the Physiology of Fruit-
body production in the Hymenomycetes. PhD THESIS,
UNIVERSITY OF LONDON.

PLUNKETT, B.E. (1956). The influence of Factors of the Aeration
Complex and Light upon Fruit-body From in Pure Cultures
of an Agaric and a Polypore. ANNALS OF BOTANY 20 (80)
563-586.

PLUNKETT, B.E. (1958) Translocation and Pileus Formation in
Polyporus brumalis. ANNALS OF BOTANY 22 (26) 237-249.

RAMAGE, H. (1930)   Mushrooms - mineral content.   NATURE 126, 279.

RANDOIN, L. AND BILLAUD, S. (1956).   Composition chimique et
valeur nutritive du champignon de couche.   MUSHROOM
SCIENCE 3,   59-79.

RAO, P.S. and NIEDERPRUEM, D.J. (1969) Carbohydrate metabolism during
Morphogenesis of Coprinus lagopus (sensu Buller).
JOURNAL OF BACTERIOLOGY 100, 1222-1228.

RAST, D. (1965).   Zur stoffwechselphysiologischen Bedeutung von
Mannit und Trehalose in Agaricus bisporus.   PLANTA 64,
81-93.

RAST, D. (1966).   Translokation von Mannitol im Fruchtkorper von
Agaricus bisporus.   BERICHTE SCHWEIZERISCHE BOTANISCHE
GESELLSCHAFT   76,   176-184.

RAUTER, W. (1975). Mushrooms as indicators of Mercury Emissions.
ZEITSCHRIFT FUR LEBENSMITTEL UNTERSUCHUNGEN UND FORSCHUNG
159, 149-151.

READ, D.J. and STRIBLEY, D.P. (1975).   Diffusion and translocation
in some fungal culture systems.   TRANSACTIONS OF THE
BRITISH MYCOLOGICAL SOCIETY 64, 381-388.

REEVE, E., BACKES, R.W. and SCHRAMER, I.M. (1959) Casing Soil Moisture
Studies. MUSHROOM SCIENCE   4,   198-204.

ROBERT, J.C. (1977a).   Fruiting of Coprinus congregatus:
Biochemical changes in Fruit-bodies during morphogenesis.
TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY 68 (3),
379-387.

ROBERT, J.C. (1977b). Fruiting of Coprinus congregatus: relationship to Biochemical changes in the whole culture. TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY 68 (3) 389-395.

ROBERTS, O. (1950) Translocation in fungi. PROCEEDINGS OF THE 7th INTERNATIONAL BOTANICAL CONGRESS 7, 453-454.

ROHLEDER, K. (1967) Zur radioaktiven kontamination von speisepilzen. DEUTSCHE LEBENSMITTEL RUNDSCHAU 63, 135.

RUSMIN, S. and LEONARD, T.J. (1978). Biochemical induction of fruiting in Schizophyllum - isolation and preliminary purification of an inducing substance from Agaricus bisporus mushrooms. PLANT PHYSIOLOGY 61 (4), 538-543.

RUSSELL, R.S. and SHORROCKS, V.M. (1959). The relationship between transpiration and the absorption of inorganic ions by intact plants. JOURNAL OF EXPERIMENTAL BOTANY. 10, 301-316.

SAN ANTONIO, J.P. (1966). Effects of Injection of Nutrient Solutions into Compost on Yield of Mushrooms. PROCEEDINGS OF THE AMERICAN SOCIETY OF HORTICULTURAL SCIENCE 89, 415-422.

SAWADA, M. (1965). Studies of chemical components of wild mushrooms and toadstools in Japan. BULLETIN OF TOKYO UNIVERSITY. FORESTS No. 59, 33-162.

SCHELENZ, R. and DIEHL, J.F. (1974). Quecksilber in Pilzen. ZEITSCHRIFT FUR LEBENSMITTEL - UNTERSUCHUNGEN UND FORSCHUNG 154 (3), 160-161.

SCHISLER, L.C. (1957).   An investigation of sporophore initiation
in the cultivated mushroom Agaricus campestris L. ex Fr.
Ph.D THESIS, PENNSYLVANIA STATE UNIVERSITY.

SCHUTTE,K.H. (1955) Translocation in fungi.   NEW PHYTOLOGIST 55 (2),
164-182.

SCHUTTE, K.H. (1961)   Transpiration in agaric fruitifications.
PHYTON   17,   167-171.

SCHWALB, M.N. (1971)   Commitment to fruiting in synchronously
developing cultures of the basidiomycete Schizophyllum
commune.   ARCHIV FUR MIKROBIOLOGIE 79,   102-107.

SECCHI, G. (1966)   I Funghi Nell' Alimentazione.   ANNALI FACOLTA
DI ECONOMIA E COMMERCIO, UNIVERSITA MESSINA 4, 623-632.

SEEGER, R. (1976a) Quecksilbergehalt der Pilze.   ZEITSCHRIFT FUR
LEBENSMITTEL-UNTERSUCHUNG UND FORSCHUNG 160 (3), 303-312.

SEEGER, R. (1976b) Die Verteilung des Quecksilbers in den
Fruchtokorpen van Steinpilzen und Champignons.   ZEITSCHRIFT
FUR LEBENSMITTEL-UNTERSUCHUNG UND FORSCHUNG 161 (2), 115-117.

SEEGER, R. (1977)   Quecksilber in jungen und alten Pilzen und in
Pilzsporen.   DEUTSCHE LEBENSMITTEL-RUNDSCHAU 73 (5),
160-162.

SEEGER, R. (1978a). Cadmium in mushrooms.   ZEITSCHRIFT FUR
LEBENSMITTEL-UNTERSUCHUNG UND FORSCHUNG 166 (1), 23.

SEEGER, R. (1978b). Kaliumgehalt horerer Pilze.   ZEITSCHRIFT FUR
LEBENSMITTEL -UNTERSUCHUNG UND FORSCHUNG 167 (1), 23-31.

SEEGER, R., MAYER, E. and SCHONHUT, S. (1976).   Blei in Pilzen.
        ZEITSCHRIFT FUR LEBENSMITTEL - UNTERSUCHUNG UND FORSCHUNG
        162 (1), 7-10.

SINDEN, J.W., TSCHIERPE, H.J. and HAUSER, E. (1968).
        Transplantation of sporophores as a new method for studying
        growth and nutritional factors of mushrooms.
        MUSHROOM SCIENCE 5, 250-266.

SMITH, J.F. and HAYES, W.A. (1972).   Use of autoclaved substrates
        in nutritional investigations on the cultivated mushroom.
        MUSHROOM SCIENCE   8, 355.

SMITH, S.E. (1966) Physiology and ecology of orchid mycorrhizal
        fungi with reference to seedling nutrition. NEW PHYTOLOGIST
        65, 488-499.

SMITH, S.E. (1967). Carbohydrate Translocation in Orchid Mycorrhizas.
        NEW PHYTOLOGIST 66, 371-378.

STANEK, M. (1969)   Effects of cellulose-decomposing micro-organisms
        groups stimulated growth of mushrooms. MUSHROOM SCIENCE
        7, 161-172.

STANEK, M. (1972)   Micro organisms inhabiting mushroom compost
        during fermentation.   MUSHROOM SCIENCE 8, 797-811.

STANEK, M. (1976). Bacteria associated with the mushroom mycelium
        (Agaricus bisporus (Lange) Sing.) in Hyphosphere.
        MUSHROOM SCIENCE 9 (1), 197-207.

STARK, N. (1972)   Nutrient cycling pathways and littler fungi.
        BIOSCIENCE 22, 355-360.

STEANE, R.G. (1979) The casing layer in the culture of Agaricus

        bisporus, the cultivated mushroom.   PhD. THESIS. THE

        UNIVERSITY OF ASTON IN BIRMINGHAM.

STEGNAR, P., KOSTA, L., BYRNE, A.R. and RAUNIK, V. (1973).

        The accumulation of mercury by, and the occurrance of

        methyl mercury in, some fungi. CHEMOSPHERE 2, 57-63.

STEVENSON, F.J. (1972). Role and function of humus in soil with

        emphasis on absorption of herbicides and chelation of

        micro-nutrients.   BIO SCIENCE 22, 643-650.

STEWART, I. (1963). Chelation in the absorption and translocation

        of mineral elements.   ANNUAL REVIEW OF PLANT PHYSIOLOGY

        14, 295-310.

STIJVE, T. (1977). Selenium content of mushrooms.   ZEITSCHRIFT

        FUR LEBENSMITTEL-UNTERSUCHUNG UND FORSCHUNG 164 (3),

        201-204.

STIJVE, T. and BESSON, R. (1976). Hg, Cd, Pb, and Se Content of

        Mushroom Species belonging to Genus Agaricus.

        CHEMOSPHERE 2, 151-158.

STIJVE, T. and CARDINALE, E. (1977).   Selenium and mercury content

        of some edible mushrooms.   NESTLE RESEARCH NEWS 1976/7,

        128-129.

STIJVE, T. and ROSCHNIK, R. (1977).   Mercury and methyl mercury

        content of different species of fungi. NESTLE RESEARCH

        NEWS 1976/77 122-127.

STOLLER, B.B. (1954).   Principles and practice of Mushroom Culture
        ECONOMIC BOTANY   8,   48-95.

STOLLER, B.B. (1979)   Synthetic Casing For Mushroom Beds.
        MUSHROOM SCIENCE   10   (3), 187-216.

TABER, W.A. (1966) Morphogenesis in Basidiomycetes.   In THE FUNGI
        2, 387-412.   Editors AINSWORTH, G.C. and SUSSMAN, A.S.
        ACADEMIC PRESS, LONDON AND NEW YORK.

THOMAS, D.S., MULLINS, J.T. and BLOCK, S.S. (1964).   Involvement
        of volatiles in mushroom sporophore initiation. MUSHROOM
        GROWERS ASSOCIATION BULLETIN 177, 429-433.

THROWER, L.B. and THROWER, S.L. (1968a) Movement of nutrients in
        Fungi. I.   The Mycelium. AUSTRALIAN JOURNAL OF BOTANY
        16, 71-80.

THROWER, L.B. and THROWER, S.L. (1968b).   Movement of nutrients
        in Fungi. II. The Effect of Reproductive Structures.
        AUSTRALIAN JOURNAL OF BOTANY 16, 81-87.

THROWER, S.L. and THROWER, L.B. (1961). Transport of Carbon in Fungal
        Mycelium. NATURE 190,   823-824.

TOWNSEND, B.B. (1954).   Morphology and development of fungal
        rhizomorphs.   TRANSACTIONS OF BRITISH MYCOLOGICAL SOCIETY
        37, 222-233.

TRESCHOW, C. (1944) Nutrition of the cultivated Mushroom. DANSK
        BOTANISK ARKIV 11,   1-180.

TSAI, C.Y., CHEN, C.C. and WU, L.C. (1976). Biochemical changes
        in Agaricus bisporus with age. MUSHROOM SCIENCE 9, 357-370.

TSCHIERPE, H.J. (1959). Die Bedeutung des Kohlendioxyds fur den
Kulturchampignon. GARTENBAUWISSENSCHAFT. 24, 18-75.

TSCHIERPE, H.J. (1973). Environmental factors and mushroom growing.
MUSHROOM JOURNAL 1, 30-45 and 2, 77-93.

TSCHIERPE, H.J. and SINDEN, J.W. (1964). Weitere Untersuchungen
uber die Bedentung von Kohlendioxyd fur die Frucktifikation
des Kuffurchampignons. ARCHIV FUR MIKROBIOLOGIE 49,
405-425.

TURCHETTO, E., GOVI, G. DEFRANCESCO, C., BIAGI, P.L., BARZANTI, V.,
MARANESI, M. and BRANZANI, B. (1977). Composizione e
valore nutritivo di Psalliota bispora. MICOLOGIA ITALIANA
1, 35-41.

TURNER, E.M. (1974). Phenoloxidase activity in relation to
substrate and development stage in the mushroom, Agaricus
bisporus. TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY
63 (3), 541-547.

TURNER, E.M. (1977). Development of excised sporocarps of
Agaricus bisporus and its control by CO_2. TRANSACTIONS
OF THE BRITISH MYCOLOGICAL SOCIETY 69(2), 183-186.

TURNER, E.M., WRIGHT, M., WARD, T., OSBOURNE, D.J. AND SELF, R. (1975).
Production of Ethylene and Other Volatiles and Changes in
cellulose and Laccase Activities during the life cycle of
the cultivated mushroom, Agaricus bisporus. JOURNAL OF
GENERAL MICROBIOLOGY 91, 167-176.

TYLER, G. (1980). Metals in sporophores of Basidiomycetes.
TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY 74 (1),41-49.

URAYAMA, T. (1961) Stimulating effect of certain specific bacteria upon mycelial growth and fruit body formation of Agaricus bisporus (Lange) Sing. BOTANICAL MAGAZINE, TOKYO 74, 56-59.

URAYAMA, T. (1967). Initiation of pinheads in Psiolocybe panaeoliformis caused by certain bacteria. MUSHROOM SCIENCE 6, 141-156.

URAYAMA, T. (1972). Influence of extracts from fruit-bodies of Agaricus bisporus and some other Hymenomycetes upon pinhead initiation in Marasmius species. MUSHROOM SCIENCE 8, 647-655.

VAN ZAAYEN, A. (1972). Intracellular appearance of mushroom virus in fruiting bodies and basidiospores of Agaricus bisporus. VIROLOGY 47, 94-104.

VOGT, K.A. and EDMONDS, R.L. (1980). Patterns of nutrient concentration in basidiocarps in Western Washington. CANADIAN JOURNAL OF BOTANY 58, 694-698.

VOLZ, P.A. and BENEKE, E.S. (1969). Nutritional regulation of Basidiocarp formation and mycelial growth of Agaricales. MYCOPATHOLOGIA ET MYCOLOGIA APPLICATA 37, 225-253.

WATIKA, S. (1958). Biochemical studies on Collybia velutipes. IV. Relation between growth and the fructification of fungus. JOURNAL OF THE AGRICULTURAL CHEMISTRY SOCIETY OF JAPAN 32, 562-566.

WANG, H.H., CHIOU, C.M., MA, D.R., LING, M.C. and HUANG, S.Y. (1974). CO_2 levels inside composts and fruitification of Agaricus bisporus MUSHROOM SCIENCE 9 (1), 279-284.

WARD, T., TURNER, E.M. and OSBOURNE, C.J. (1978). Evidence for the production of ethylene by the mycelium of Agaricus bisporus and its relationship to sporocarp development. JOURNAL OF GENERAL MICROBIOLOGY   104(1),   23-30.

WATKINSON, S.C. (1971). The mechanism of mycelial strand induction in serpula lacrimans: A possible effect of nutrient distribution. NEW PHYTOLOGIST   70,   1079-1088.

WATKINSON, S.C. (1975). The relation between nitrogen nutrition and formation of mycelial strands in Serpula lacrimans. TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY 64 (2), 195-200.

WATT, B.K. and MERRILL, A.L. (1963).   Composition of foods. U.S. DEPARTMENT OF AGRICULTURE, AGRICULTURE HANDBOOK 8.

WEIGL, J. and ZIEGLER, H. (1960). Wasser haushalt und Stoffleitung bei Merulius lacrymans (Wulf.) Fr. ARCHIV FUR MICROBIOLOGIE 37, 124-133.

WEINHOLD, A.R. (1963).   Rhizomorph production by Armillaria mellea induced by Ethanol and related compounds. SCIENCE 142, 1065-1066.

WESSELS, J.G.H. (1965). Morphogenesis and Biochemical processes in Schizophyllum commune Fr.   WENTIA 13, 1-113.

WILCOXSON, R.D. and SUBBARAYUDU, S. (1968). Translocation to and accummulation of ^{32}P in sclerotia of Sclerotium rolfsii. CANADIAN JOURNAL OF BOTANY 46, 85-88.

WILCOXSON, R.D. and SUDIA, T.W. (1968). Translocation in Fungi.
BOTANICAL REVIEW 34, 32-50.

WILLIAMS, A. and MARTENS, P. (1967). Residues of Pesticides in
Cultivated Mushrooms. MUSHROOM SCIENCE 6, 507-514.

WOOD, D.A. (1976). Primordium formation in axenic cultures of
Agaricus bisporus (Lange) Sing. JOURNAL OF GENERAL
MICROBIOLOGY 95, 313-323.

WOOD, D.A. (1977). Fruit-body initiation and development of Agaricus
bisporus. SECOND INTERNATIONAL MYCOLOGICAL CONGRESS, TAMPA,
FLORIDA U.S.A. p.476.

WOOD, D.A. (1979) Biochemical changes during growth and development
of Agaricus bisporus. MUSHROOM SCIENCE 10 (1), 401-417.

WOOD, D.A. (1980). Inactivation of Extracellular Laccase during
Fruiting of Agaricus bisporus. JOURNAL OF GENERAL
MICROBIOLOGY 117, 339-345.

WOOD, D.A. and GOODENOUGH, P. (1977). Fruiting of Agaricus bisporus:
changes in extracellular enzyme activities during growth
and fruiting. ARCHIVES OF MICROBIOLOGY 114, 161-165.

WOOD, D.A. and HAMMOND, J.B.W. (1977). Ethylene production by anexic
fruiting cultures of Agaricus bisporus. APPLIED AND
ENVIRONMENTAL MICROBIOLOGY 34, 228-229.

YEO, S.G. (1980). The ecology of paper mill By-product and its
evaluation as a casing medium in the culture of Agaricus
bisporus (Lange) Pilat. PhD. THESIS, UNIVERSITY OF ASTON
IN BIRMINGHAM.

YEO, S.G. and HAYES, W.A. (1979). A new medium for casing mushroom
beds. MUSHROOM SCIENCE 10 (2), 217-229.

ZOHM, H. and LANGENBEIN, T. (1976). Veranderung des Eisen und
Zinngehaltes von Champignon konserven wahrend der Lagerung.
INDUSTRIELLE OBST-UND GEMUSEWERTUNG 61 (15) 408-9.

APPENDICES.

APPENDIX 3.1. CONCENTRATIONS OF WATER AND POTASSIUM PYROPHOSPHATE
EXTRACTABLE IRON IN THE SUBSTRATES DURING CULTURE.
(MILLIGRAMS PER KILOGRAM DRY WEIGHT).

DAYS AFTER CASING	SUBSTRATE SAMPLE	WATER EXTRACTABLE IRON	PYROPHOSPHATE EXTRACTABLE IRON
7	CASING	2.38 (1.98)	20.64 (5.54)
	UPPER COMPOST	37.43 (7.92)	980.9 (374.9)
	LOWER COMPOST	33.42 (4.82)	499.5 (185.4)
14	CASING	2.16 (1.32)	28.16 (11.44)
	UPPER COMPOST	28.92 (9.26)	747.6 (585.5)
	LOWER COMPOST	33.13 (8.97)	947.5 (481.1)
21	CASING	2.49 (1.73)	41.69 (29.20)
	UPPER COMPOST	40.82 (10.26)	687.4 (136.4)
	LOWER COMPOST	32.52 (4.06)	649.0 (164.7)
28	CASING	12.33 (11.40)	48.03 (34.67)
	UPPER COMPOST	44.07 (8.59)	629.1 (263.4)
	LOWER COMPOST	42.22 (5.39)	724.4 (330.3)
35	CASING	4.52 (4.47)	89.24 (91.10)
	UPPER COMPOST	56.60 (11.09)	1136 (695.7)
	LOWER COMPOST	51.00 (12.27)	774.0 (463.3)
48	CASING	3.07 (2.06)	33.39 (23.51)
	UPPER COMPOST	44.03 (9.26)	2063 (1860)
	LOWER COMPOST	47.60 (3.45)	1278 (199.3)

The Mean and (95% Confidence Limits) of 6 replicated samples.

APPENDIX 3.2. WATER EXTRACTABLE IRON CONCENTRATIONS IN THE SUBSTRATES OF GROWING UNITS SUPPLEMENTED WITH FERROUS SULPHATE IN THE UPPER OR LOWER LAYERS OF THE COMPOST. (MILLIGRAMS PER KILOGRAM DRY WEIGHT).

DAYS AFTER CASING.

SUBSTRATE SAMPLE	4	13	27	39	55
1.					
CASING	0 (0)	0.252 (1.082)	0.390 (0.956)	0.747 (1.850)	0.122 (0.313)
UPPER COMPOST	16.26 (5.75)	21.80 (15.11)	31.13 (11.00)	32.60 (13.84)	39.87 (8.315)
LOWER COMPOST	25.70 (15.49)	30.92 (3.02)	41.65 (53.83)	39.74 (22.76)	42.19 (18.18)
2.					
CASING	0.76 (4.86)	0.189 (0.60)	0.522 (2.24)	1.478 (2.438)	0.370 (0.645)
UPPER COMPOST	29.94 (49.82)	31.60 (6.27)	45.38 (20.26)	49.03 (22.69)	50.58 (18.07)
LOWER COMPOST	29.28 (44.93)	29.69 (6.55)	38.74 (21.03)	52.63 (39.53)	48.63 (14.87)
3.					
CASING	0 (0)	0.378 (0.693)	0.257 (0.552)	0.857 (1.385)	0.623 (1.039)
UPPER COMPOST	25.29 (6.051)	27.98 (36.70)	36.17 (2.77)	35.31 (14.38)	49.26 (9.54)
LOWER COMPOST	25.67 (10.05)	35.06 (35.05)	46.72 (25.36)	56.62 (23.70)	47.28 (15.58)

Mean and (95% Confidence Limits) of 4 replicate samples.

1. = Control
2. = Ferrous sulphate solution applied to compost surface.
3. = Ferrous sulphate solution injected into compost.

APPENDIX 3.3. CONCENTRATIONS OF IRON - 59 IN DIFFERENT LEVELS
OF THE SUBSTRATES FOLLOWING THE ADDITION OF
ISOTOPE TO:

1. THE CASING LAYER.
2. THE UPPER COMPOST.
3. THE LOWER COMPOST.

SUBSTRATE	DAYS AFTER CASING				
SAMPLE	4	13	26	34	52
CASING	4.811	4.762	4.920	4.867	4.859
UPPER COMPOST	0.916	1.062	1.659	1.665	1.712
1. MIDDLE COMPOST	0.084	0.857	0.988	1.414	1.433
LOWER COMPOST	0.810	1.026	1.403	1.578	1.441
CASING	3.520	3.766	3.726	4.053	3.917
UPPER COMPOST	4.968	5.104	5.025	5.237	5.544
2. MIDDLE COMPOST	1.906	2.720	2.793	2.279	2.654
LOWER COMPOST	1.105	1.175	1.455	1.165	1.503
CASING	1.032	1.066	1.320	1.295	1.893
UPPER COMPOST	1.092	2.670	2.187	2.288	2.369
3. MIDDLE COMPOST	4.689	4.877	4.872	4.883	5.255
LOWER COMPOST	4.127	4.650	4.099	4.372	4.643

APPENDIX 3.4.   ESSENTIAL CHARACTERISTICS OF DEVELOPMENTAL
                STAGES OF THE SPOROPHORE.

                See also Plate 3.1.

1.BUTTON:

        When viewed from underneath no trace of the pink colouration
        due to the hymenia can be seen through the veil.

2.LATE BUTTON:

        Pink colouration can be seen through a translucent veil.

3.BUTTON/OPEN CUP:

        The veil is partially broken.

4.EARLY OPEN CUP:

        The veil has just completely separated between the stipe
        and pileus.

5.EARLY-MID OPEN CUP:

        The aperture between the stipe and pileus has opened,but the
        hymenia curve back towards the stipe.(Arrowed in Plate 3.1).

6.MID OPEN CUP:

        The outer hymenia are pointing downwards and the veil remnants
        on the edge of the pileus point towards the veil remnants on
        the stipe.

7.MID-LATE OPEN CUP:

        The veil remnants on the outer edge of the pileus point
        downwards.

8.LATE OPEN CUP:

        The veil remnants point outwards and the upper pileus surface
        is convex.

9.EARLY FLAT:

        The top surface of the pileus is flat but,the veil remnants
        can still be observed from below.

10.FLAT:

        The upper surface of the pileus is concave,the veil remnants
        cannot be seen from below.

APPENDIX 3.5. GROWTH RATES OF SPOROPHORES IN GROUPINGS OF
SIMILAR DRY WEIGHT.

| NUMBER OF | DRY WEIGHTS (GRAMS) | | GROWTH RATE (MG/HOUR) | |
SAMPLES	Mean	C.L.	Mean	C.L.
56	0.081	0.0041	1.172	0.176
51	0.115	0.0026	2.296	0.444
52	0.146	0.0026	3.350	0.470
56	0.181	0.0032	4.294	0.337
57	0.226	0.0038	6.014	0.426
45	0.271	0.0045	6.574	0.586
48	0.324	0.0047	8.518	1.183
41	0.374	0.0045	10.76	1.415
46	0.447	0.0078	11.45	0.811
43	0.544	0.0088	15.53	1.415
23	0.648	0.0128	19.68	3.326
42	0.808	0.0249	23.34	3.188
38	1.405	0.1521	48.07	9.770

Correlation between Dry Weight and Growth Rate for 598 sporophores
=0.99624   597°F.

APPENDIX 3.6. A SEQUENCE OF POPULATION GROWTH RATES CALCULATED
FROM SUCESSIVE PHOTOGRAPHS.

| NUMBER OF | MEAN TIME OF GROWTH | GROWTH RATE (MG/HOUR) | |
SAMPLES	RATE CALCULATION.	Mean	C.L.
73	4	3.318	0.85
73	12	4.509	0.713
73	20	5.635	0.993
73	28	6.544	1.074
70	36	11.91	2.263
71	44	13.00	3.217
61	52	14.13	3.555
58	60	27.58	7.366
15	68	10.89	2.792
15	76	17.33	6.161
8	84	30.58	21.46

C.L. = 95% Confidence Limits.

APPENDIX 3.7.   IRON - 59 CONCENTRATION AND CONTENT IN GROUPS OF
SPOROPHORES OF SIMILAR DRY WEIGHTS.

DRY WEIGHT OF WEIGHT GROUPINGS		CONCENTRATION LOG COUNTS/HOUR/ GRAM DRY WEIGHT			CONTENT LOG COUNTS/HOUR/ SPOROPHORE		
MEAN	C.L.	MEAN	UPPER C.L.	LOWER C.L.	MEAN	UPPER C.L.	LOWER C.L.
0.0777	0.0129	4.8316	5.1394	0	3.8724	4.0893	3.4193
0.1207	0.0069	4.9401	5.1388	4.5633	4.0234	4.2246	3.6369
0.1459	0.0052	4.8460	5.0777	4.3157	4.0164	4.2529	3.4582
0.1621	0.0026	4.7385	5.0008	3.9701	3.9738	4.2195	3.3531
0.1807	0.0041	4.8731	5.1396	4.0570	4.1267	4.3928	3.3162
0.1993	0.0047	4.8250	5.0026	4.5194	4.1295	4.3129	3.8058
0.2107	0.0021	4.6124	4.8464	4.0689	3.9400	4.1745	3.3934
0.2257	0,0029	4.5535	4.7901	3.9941	3.9081	4.1447	3.3481
0.2431	0.0043	4.3848	4.5916	3.9761	3.7686	3.9736	3.3670
0.2640	0.0042	4.2686	4.4690	3.8850	3.6906	3.8923	3.3021
0.2816	0.0047	4.1414	4.3546	3.7050	3.6011	3.8130	3.1703
0.301	0.0045	4.4481	4.697	3.8029	3.9308	4.1808	3.2769
0.3155	0.0056	4.5276	4.7677	3.9453	4.0275	4.2684	3.4398
0.337	0.0045	4.3539	4.5834	3.8363	3.770	3.9591	3.4276
0.3574	0.0076	4.2737	4.5982	0	3.8249	4.1457	0
0.3734	0.0015	4.3498	4.5455	4.1051	3.9215	4.1171	3.5562
0.3843	0.0044	4.8234	5.1607	0	4.1621	4.4283	3.3489
0.4187	0.0099	4.2758	4.4225	4.0527	3.9209	4.0458	3.7448
0.4608	0.0095	4.1794	4.4251	3.5575	3.8516	4.0978	3.2266
0.5029	0.0091	4.0954	4.2869	3.7447	3.7937	3.9834	3.4492
0.5659	0.0249	3.9853	4.2736	2.7419	3.7551	4.0550	1.4771
0.665	0.0081	3.8870	4.1131	3.3879	3.7129	3.9407	3.2049
0.7126	0.0132	4.2232	4.4474	3.7342	3.9938	4.2540	3.2477
0.809	0.0194	3.7432	3.9089	3.4719	3.6541	3.8228	3.3744
0.935	0.0445	3.9250	4.1868	3.1617	3.7387	3.8556	3.5782
1.1375	0.0297	3.7508	3.9315	3.4358	3.7612	3.8555	3.6405
1.2779	0.0522	3.7747	4.0117	3.2127	3.9426	4.1420	3.5629
1.5286	0.0698	3.6134	3.7537	3.4048	3.8027	3.9497	3.5787
2.0588	0.2979	3.6502	3.8677	3.1942	3.9468	4.1976	3.2865

C.L.= 95% Confidence Limits.

APPENDIX 3.8.   DRY WEIGHT AND IRON - 59 CONTENT OF THE PILEUS AS A
                PERCENTAGE OF THE ENTIRE SPOROPHORE, FOR GROUPS OF
                SPOROPHORES OF SIMILAR DRY WEIGHTS.

NUMBER OF SPOROPHORES	DRY WEIGHT (G) OF GROUPINGS.	IRON - 59 CONTENT	DRY WEIGHT
17	0.219 (0.022)	86.76 (5.389)	75.20 (1.714)
16	0.322 (0.013)	92.43 (3.943)	77.66 (1.895)
16	0.403 (0.018)	86.53 (5.605)	79.99 (2.297)
16	0.575 (0.039)	91.50 (3.672)	79.55 (1.880)
16	0.732 (0.027)	86.39 (8.147)	82.30 (1.493)
16	1.010 (0.060)	88.19 (5.874)	82.03 (1.787)
17	1.339 (0.168)	82.60 (7.674)	82.45 (1.771)
10	2.038 (0.303)	83.56 (6.358)	83.48 (2.889)

The Mean and (95% Confidence Limit).

$$\text{IRON - 59 CONTENT} = \frac{\text{PILEUS COUNT PER HOUR}}{\text{SPOROPHORE COUNT PER HOUR}} \times 100 \qquad \text{DRY WEIGHT} = \frac{\text{PILEUS DRY WEIGHT}}{\text{TOTAL DRY WEIGHT}} \times 100$$

APPENDIX 3.9.   CONCENTRATIONS OF IRON - 59 IN THE PILEUS, STIPE AND
                ENTIRE SPOROPHORE, FOR GROUPS OF SPOROPHORES OF
                SIMILAR DRY WEIGHTS.

DRY WEIGHT (G)	PILEUS MEAN	UPPER C.L.	LOWER C.L.	STIPE MEAN	UPPER C.L.	LOWER C.L.	ENTIRE SPOROPHORE MEAN	UPPER C.L.	LOWER C.L.
0.219	4.492	4.710	4.032	3.868	4.061	3.510	4.457	4.644	4.122
0.322	4.441	4.631	4.095	3.612	3.771	3.358	4.343	4.530	4.006
0.403	4.392	4.559	4.120	4.089	4.270	3.773	4.343	4.513	4.060
0.575	4.110	4.322	3.681	3.560	3.785	3.066	4.058	4.263	3.655
0.732	4.156	4.358	3.767	3.821	4.021	3.440	4.141	4.324	3.818
1.010	3.839	3.964	3.665	3.516	3.675	3.264	3.804	3.919	3.648
1.339	3.761	3.918	3.511	3.589	3.752	3.323	3.744	3.900	3.499
2.038	3.683	3.885	3.295	3.759	4.039	2.719	3.693	3.891	3.318

C.L. = 95% Confidence Limit.

APPENDIX 3.10.   DRY WEIGHTS AND CONCENTRATIONS OF IRON - 59 IN
SPOROPHORES GROUPED ACCORDING TO DEVELOPMENTAL
STAGE.

STAGE	NUMBER OF SAMPLES.	GRAMS DRY WEIGHT. MEAN	GRAMS DRY WEIGHT. C.L.	LOG COUNTS/HOUR/ GRAM DRY WEIGHT. MEAN	LOG COUNTS/HOUR/ GRAM DRY WEIGHT. UPPER C.L.	LOG COUNTS/HOUR/ GRAM DRY WEIGHT. LOWER C.L.
BUTTON	98	0.300	0.048	4.6033	4.7094	4.4652
LATE BUTTON	46	0.404	0.098	4.561	4.6730	4.4072
BUTTON/OPEN CUP	43	0.420	0.089	4.4835	4.6261	4.2698
EARLY OPEN CUP	36	0.691	0.186	4.4677	4.6714	4.0715
EARLY-MID OPEN CUP	16	0.620	0.195	4.1875	4.4322	3.5730
MID OPEN CUP	39	0.92	0.220	4.0323	4.1834	3.7987
MID-LATE OPEN CUP	8	1.04	0.487	3.741	3.9984	3.0220
LATE OPEN CUP	17	0.835	0.248	4.0278	4.2892	3.2686

C.L. = 95% CONFIDENCE LIMIT.

APPENDIX 3.11. CONTENT AND CONCENTRATION OF IRON – 59 IN GROUPS
OF SPOROPHORES OF SIMILAR DRY WEIGHTS.

MEAN DRY WEIGHT OF GROUPS(G)	NUMBER OF SPOROPHORES	LOG COUNTS/HOUR/ GRAM DRY WEIGHT.			LOG COUNT /HOUR/ SPOROPHORE
		MEAN	UPPER C.L.	LOWER C.L.	
9.320	1	1.279	–	–	2.248
5.440	1	2.072	–	–	2.808
4.620	7	1.914	2.21	0.301	2.579
3.427	5	2.594	3.057	–	3.129
2.729	8	2.365	2.774	–	2.801
2.320	10	2.238	2.584	–	2.603
1.911	9	1.944	2.093	1.716	2.225
1.624	12	2.920	3.180	2.179	3.131
1.338	20	2.934	3.183	2.286	3.060
1.146	16	2.715	2.979	1.934	2.774
0.941	12	2.715	2.989	1.806	2.689
0.847	14	2.744	3.086	–	2.672
0.750	12	2.303	2.494	1.954	2.178
0.647	11	2.655	2.993	–	2.466
0.576	9	3.212	3.478	2.403	2.972
0.520	12	3.093	3.422	–	2.809
0.483	11	2.483	2.754	1.613	2.167
0.425	8	2.403	2.677	1.491	2.031
0.353	12	3.225	3.574	–	2.773
0.273	10	2.683	2.853	2.400	2.119
0.223	8	2.766	2.974	2.354	2.114
0.129	9	3.182	3.379	2.810	2.293

C.L. = Confidence Limit.

APPENDIX 3.12. CONTENT AND CONCENTRATION OF IRON-59 IN GROUPS OF PRIMORDIA AND
SMALL SPOROPHORES OF SIMILAR DRY WEIGHT.

NUMBER OF SAMPLES.	TOTAL NUMBER OF PRIMORDIA.	MEAN PRIMORDIUM DRY WEIGHT. (MILLIGRAMS).	LOG COUNTS/HOUR/GRAM DRY WEIGHT.			LOG COUNTS/HOUR/ SPOROPHORE.
			MEAN	UPPER C.L.	LOWER C.L.	
12	940	1.65	5.130	5.266	4.930	2.348
11	540	2.67	5.012	5.145	4.817	2.439
11	438	3.89	4.977	5.088	4.827	2.567
11	308	5.46	4.992	5.138	4.771	2.729
10	176	8.44	4.872	4.999	4.691	2.798
11	116	14.14	4.924	5.073	4.695	3.074
12	76	29.93	4.797	4.947	4.564	3.273
11	48	58.28	4.552	4.718	4.280	3.318
10	20	137.1	4.471	4.664	4.113	3.608
2	4	264.6	4.541	4.720	4.229	3.963

C.L. = Confidence Limit.

APPENDIX 3.13. CONCENTRATION OF IRON-59 IN THE VEGETATIVE AND REPRODUCTIVE TISSUES DURING CULTURE.

DAYS AFTER CASING	COMPOST MYCELIUM	CASING MYCELIUM	PRIMORDIA	SPOROPHORES
	5.7153	5.2813	4.5302	
15	5.5421	5.0378	4.4043	
	5.2497	4.4326	4.2263	
	5.7948	5.5193	4.8334	
18	5.5961	5.2140	4.6192	
	5.2193	0.0	4.1787	
	5.7924	5.8566	4.6572	4.6449
21	5.5466	5.5672	4.5234	4.5834
	5.0904	4.2840	4.3290	4.5118
	5.6712	5.0732	4.7275	4.626
24	5.4728	4.8882	4.5861	4.4899
	5.0970	4.5594	4.3750	4.2906
	5.7722	5.5134	4.7994	4.2284
27	5.5562	5.3159	4.6194	4.1584
	5.1074	4.9435	4.3064	4.0749

Upper 95% Confidence Limit..

Mean   of 6 samples.

Lower 95% Confidence Limit.

LOG COUNTS PER HOUR PER GRAM DRY WEIGHT.