Relationship between Sporophore Morphology and Mushroom Quality

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Table of contents

PRACTICAL SECTION FOR GROWERS

Objectives and background

Mushroom texture is important both as a quality factor recognised by consumers and because the texture of mushrooms determines its resistance/susceptibility to bruising caused by handling during picking and damage during transport.

In this project we had a number of key objectives:

(i) Devise methods to measure texture

(ii) Find how texture can be improved by cultural treatment eg agronomic or environmental factors.

(iii) Determine what is the basis of texture from a point of view of cell structure inside mushrooms.

Summary of Results

1. How to measure texture

Texture is measured in the laboratory by compressing pieces of mushroom tissue using a sophisticated instrument called Instron Universal Testing Machine.
This instrument displays the data as stress-strain curve and by mathematical manipulations we can determine factors such as a Energy Requirement for compression, Energy Absorbed, Elasticity and amount of Plastic Deformation on mushroom caps, and stiffness (firmness). It has been found in some cases that high tissue stiffness is related to high dry weight. It is hoped to develop a formula to determine firmness from easily obtained measurements, so growers could monitor mushroom firmness in their own farms.

2. Cultural factors which influence mushroom texture

A number of agronomic and environmental factors have been examined to determine

which have major effects on mushroom texture.

The purpose of this work is for industry to utilize this information firstly to optimize the mushroom quality in specific farm conditions and secondly to identify possible reasons why some mushroom crops may be of poorer quality.

The agronomic, environmental and biological factors tested were as follows:

1 Type of casing
2 Humidity level
3 Compost supplements
4 Water potential of casing
5 Mushroom strain
6 Compost depth
7 Casing depth
8 CO_2 level

Factors which had major affect on mushroom texture are:

- Compost depth
- Casing depth
- CO_2 level
- Water potential of casing
- Casing type

These affects can be summarized as follows:

- Shallow casing on top of deep compost produces firm mushrooms whereas the reverse, deep casing and shallow compost produce soft mushrooms. In our experiment, shallow casing had a depth of 25 mm and deep compost represent trays (600x400 mm) filled with 19 Kg of compost.
- Low CO_2 levels result in firmer mushrooms.
- Water potential of casing does influence mushroom firmness but the extreme conditions of very wet or very dry casing which produced firmer mushrooms, resulted in loss of yield.
- The use of bulk extracted peat casing resulted in an increase of mushroom firmness compared with milled peat casing at the extremes of water potential.

The lack of major effects on texture by the other factors should not be treated as a negative result. For instance, it has been suggested that adding supplements to compost affects texture. These results suggest this is not so. The humidity of growing rooms did not affect the firmness of mushrooms.

3. Structural basis of mushroom texture

To determine the structural basis for mushroom texture, we have had to use the scientific discipline of biomechanics which examines the mushroom as a "material" or a "structure". For this reason we have been describing how the cells of a mushroom (hyphae) are packed and arranged. The cells on the surface of the mushroom cap are stretched over the top. They are arranged in a network of hyphae. Beneath this layer is a region containing small diameter cells with major air spaces in between. When the mushroom is mishandled or compressed the upper layer of cells are pushed into these air spaces leading to permanent deformation and cell damage. When we analysed the cell diameter of soft mushrooms and firm mushrooms from the compost depth - casing depth experiment, we found that the firmest mushroom had much smaller cell diameters. This might account for some of the differences between a firm and a soft texture. We have also found that the alignment or orientation of the cap cells plays a crucial role in how firm the tissue is. We are now looking at other structural differences between soft and firm mushrooms, to find out what other factors influence firmness.

I Introduction

I.1 Industrial, economic and biotechnological importance of mushrooms

The mushroom *Agaricus bisporus* is the largest horticultural crop grown in the UK, with a farmgate value of around £167 million in 1997 and a total retail sale of £250 million. The annual value of mushrooms is equal to the combined values of apples and tomatoes in the UK. In Europe, the major mushroom producing countries are the Netherlands, France, Italy and the UK. The UK mushroom industry which produced about 120,000 tonnes for the year 1995, have been facing large imports from the Netherlands and Ireland. A real growth in production is possible but depends on producing higher quality mushrooms, increasing *per capita* consumption and increasing added value through new products.

I.2 Mushroom cultivation and harvest

Originally, mushrooms were found growing in fields but today all the steps to produce mushrooms are managed. The processes involved in growing mushrooms are divided in several steps: preparation of spawn, preparation of compost, spawning, casing, growing and harvest.

- Preparation of spawn: the mycelium is mixed with a sterile substrate (wheat, rye or millet grain) and incubated for 2-3 weeks at 25°C until the mycelium has colonised the grain. Spawn is usually produced by commercial companies (Sylvan-Hauser, Le Lion, Amycel).
- Preparation of compost: compost is the substrate for growing mushrooms. Straw is mixed with chicken litter, gypsum, compost activator and water and composted in an open stack system with periodic mixing and watering. After two weeks the compost in moved into a tunnel where humidity, temperature and ventilation are controlled. This process is to pasteurise the compost and to allow thermophilic microorganisms to develop and complete the composting process. The compost will stay 6-12 days in the tunnels and then, will be ready for mushroom cultivation.
- Spawning: the spawn is mixed with the compost at a rate of 0.5% by weight. At Horticulture Research International (HRI) mushroom unit, mushrooms are grown in

trays. They are usually filled with 50 kg of compost and left 17-18 days at 20°C under 96% relative humidity until the spawn has colonized the compost.

- Casing: it is necesasary to cover the colonised compost with a layer of casing to induce sporophore formation. The casing mixture used is a proprietary brand Nooyen ready mix (80% peat and 20% sugar beet lime) which has a dark colour and a consistency of fibrous mud. It is usually mixed with Dimlin (pesticide) and CACcing (Compost added at casing) to hasten the mycelium growth in the casing. The compost is covered with a 45-50 mm deep layer of casing and the trays are incubated one week at 18°C at a relative humidity of 86-87%, until mushrooms start pining.

- Growing and harvest: the growing rooms are held at 18°C, a relative humidity of 86-87% and the CO_2 level is maintained at 1,000 ppm. When mushrooms start fruiting it is called a flush. The main flush last for 2-3 days. Once the trays have been harvested and trimmed, the next flush will come after 5-7 days. Mushrooms growers usually allow 3 or 4 flushes and then clean the rooms.

I.3 Mushroom quality-factors and interrelationships

The quality of a product is determined by its organoleptic characteristics, hygienic, physical properties (storage, use etc.) and nutritional. McCanna *et al.* (1968) determined a spectrum of quality for fresh mushrooms. Consumers are increasingly concerned about what they eat and are calling for freshness and quality. According to consumers, the most important features of a high quality mushroom are whiteness, texture, maturity and flavour. The colour is probably the most important feature as the market price is determined according to it. Mushrooms are very susceptible to browning for several reasons: mechanical damage, senescence and disease. The mechanical damage is the greatest cause of browning (Burton *et al.*, 1993) and occurs by handling mushrooms when they are harvested and during transport.

Instron Universal Testing Instrument

II General Materials and Methods

II.1 Mushroom strain

Mushroom *Agaricus bisporus* strain A12 (Sylvan-Hauser, UK) was used in most of the experiments unless otherwise stated. They were grown at the mushroom unit in HRI Wellesbourne according to commercial practices. Only stage 2 mushrooms (Hammond and Nichols, 1976) were harvested for experiments, they were selected to have a diameter of 32-37 mm and a height of 15-20 mm.

II.2 Compression tests

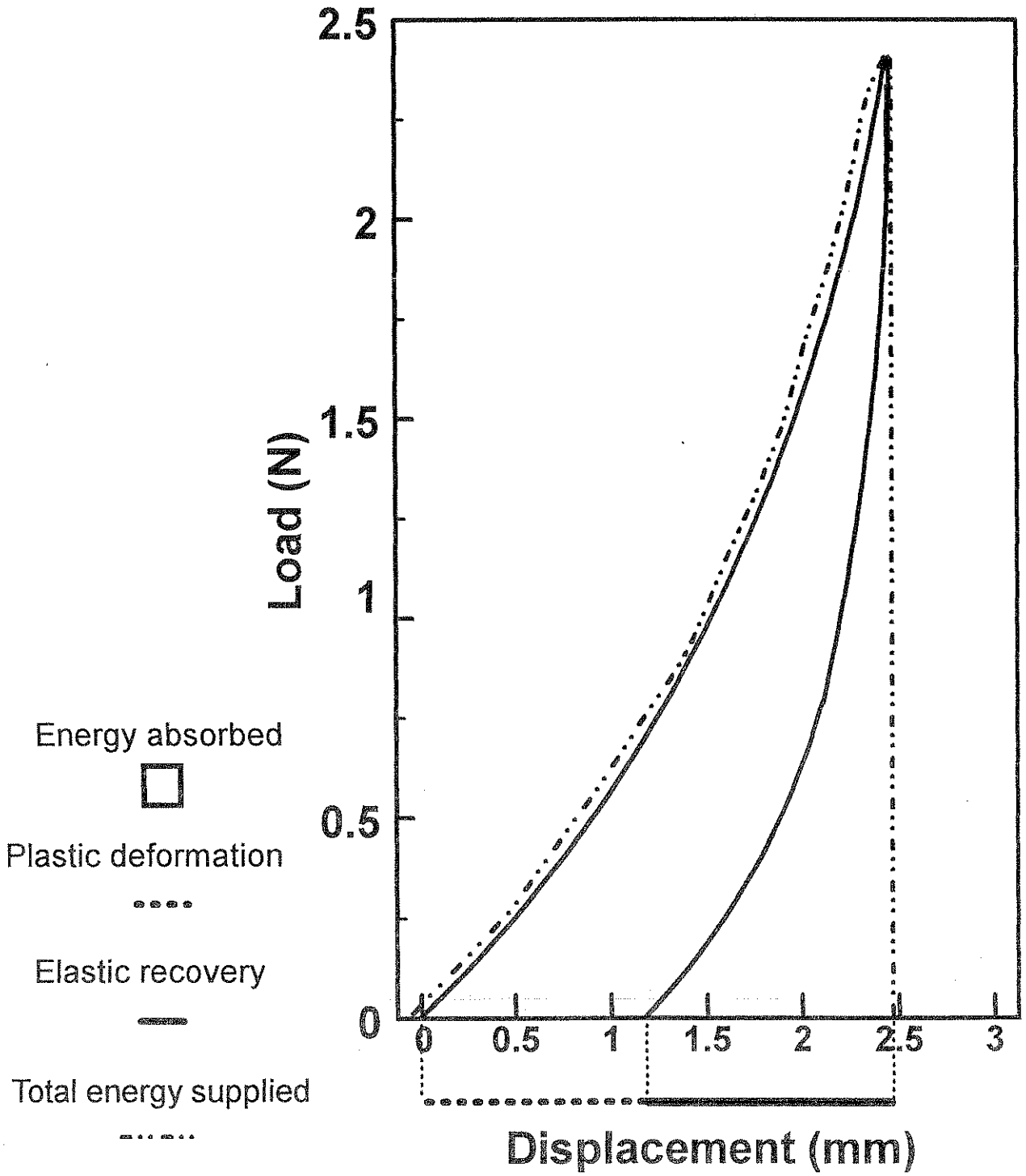
Compression tests were performed using an Instron Universal Testing Instrument (model 4301, High Wycombe, UK), equipped with the load cell of 100 N (Fig. 1). The compression was effected using a spherical steel probe (diameter = 8 mm) at a speed of 5 mm/min. Mushroom cubes of 19x19x10 mm (Length x width x Height) were taken from the top surface and compressed at 1 to 4 mm, then the probe was moved backwards at the same speed. During compression, the probe is pushed into the mushroom, causing displacement and an increase in load. When the required displacement is reached, the probe is moved backwards so the displacement and load decrease; this is the relaxation of the load. The relaxation curve is different to the compression one, this phenomenon is known as hysteresis (Fig. 1).

II.3 Total energy supplied and energy absorbed

The total energy supplied during compression is calculated from the hysteresis graph; it represents the area under the compression curve (Fig. 1). This area was integrated using the software supplied with the Instron Universal Testing Instrument. Energy is expressed in Joules (Newtons per square metre).

The energy absorbed represents the area between the compression curve and the relaxation curve (Fig. 1). This calculation is not available in the software so when needed, the load-displacement curve was plotted on a chart and the energy absorbed was calculated by image analysis.

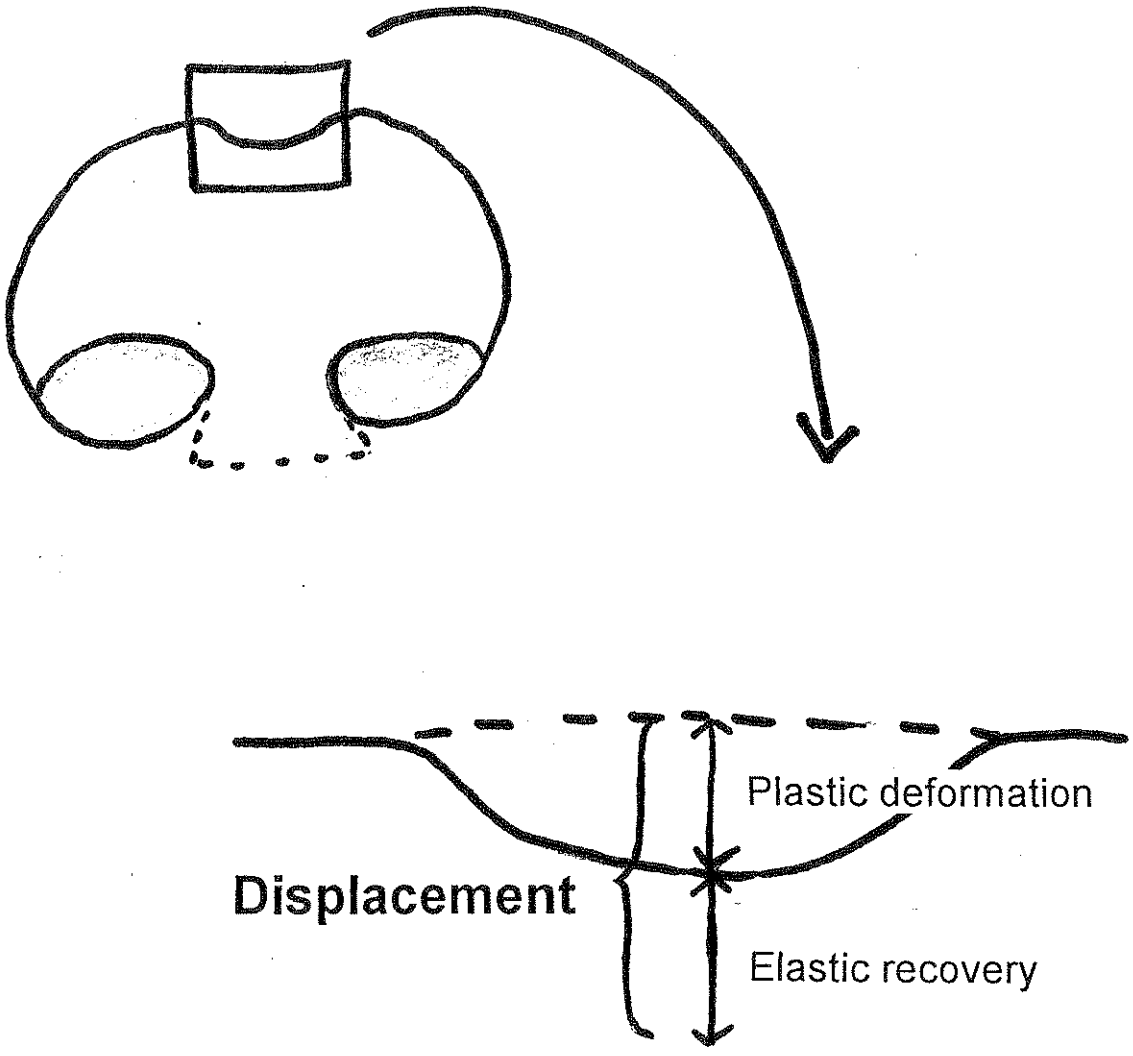
Figure 1: Load-Displacement curve



II.4 Plastic deformation

Compression produces an indentation at the surface of the mushroom. This indentation represents the plastic deformation of the compression (Fig. 2). The amount of plastic deformation is the displacement at which the relaxation curve comes back to a zero load. This value is obtained from the software attached to the Instron. The elastic recovery is the difference between plastic deformation and maximum displacement. Plastic deformation is expressed as a percentage of deformation against maximum displacement.

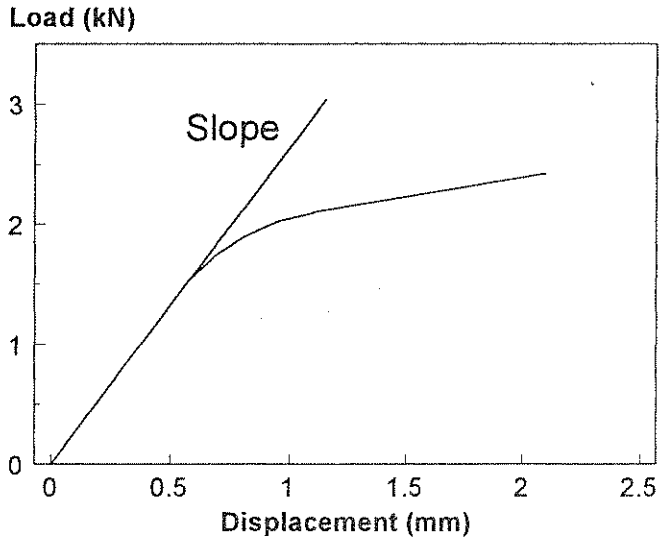
Figure 2: Scheme showing plastic deformation and elastic recovery after compression.



II.5 Stiffness (Young's modulus)

A cork-borer was used to remove 5 mm diameter cylinders from the sporophore. Cylinders were then cut at a length of 5 mm using parallel razor blades. These samples were then tested shortly after preparation (to prevent dehydration) on the Instron Universal Testing Instrument. The compression was done at a speed of 5 mm/min at a displacement of 2 mm using a flat probe. The stiffness (in Pascals) was calculated by the software attached to the Instron from the first slope of the curve.

Figure 3: Typical Load-Displacement curve when measuring mushroom tissue stiffness and calculation leading to the stiffness data.



$$\text{Stiffness} = \frac{\Delta \text{ Stress}}{\Delta \text{ Strain}} = \frac{\Delta \text{ Load x Gauge lenght}}{\Delta \text{ Displacement x Cross sectional area}} = \text{Slope } x \frac{\text{Gauge lenght}}{\text{Cross sectional area}}$$

Stress = Load/Cross sectional area

Strain = Displacement/ Gauge lenght

III Sporophore morphology

III.1 Introduction

Mushrooms have always intrigued people because of their characteristics and unusual shape. However, how the distintive mushroom shape is formed has not been yet identified. To create such a specific and consistent shape, the hyphal arrangement of the mushroom must be programmed and organised. Sporophores consist of several distinct tissues, the hyphal arrangement of which have been studied. The stipe has been well studied because it provides a simple model to study cell growth and elongation (Bonner *et al.*, 1956, Kamada *et al.*, 1977, Moore *et al.*, 1978, 1993, Reijnders, 1979, Kamada, 1994). It has been shown by several authors (Manocha, 1965, Craig *et al.*, 1977, 1979, Wood *et al.*, 1985) that hyphae in the stipe are parallel to the stipe axis. In the cap, hyphae were mainly studied for their structure and growth (Keresztes and Kovacs, 1987, Manocha, 1965, Pelok *et al.*, 1984). However, Bonner *et al.* (1956) who studied the growth of *Agaricus campestris* reported that the fruit body initial (pinhead) consists of a disorganised mass of hyphae but as mushrooms develop hyphae become radially orientated in the cap, however authors described difficulty in repeating results therefore there is uncertainty into that finding. There is insufficient published information on sporophore morphology to describe, or even speculate, on the cellular basis of texture, mechanism of bruising and resistance to bruising damage.

The aim of the study is to investigate the hyphal arrangement within the cap and to relate this to the measurements of its mechanical properties and to understand mushroom texture and the browning mechanism on the cap surface. During the investigation of the hyphal arrangement, it has also been possible to measure the hyphal diameter and the hyphal volume fraction at and below the surface of the cap of mushrooms.

Two different approaches to look at the cap morphology were used: light microscopy and Scanning Electron Microscopy (SEM).

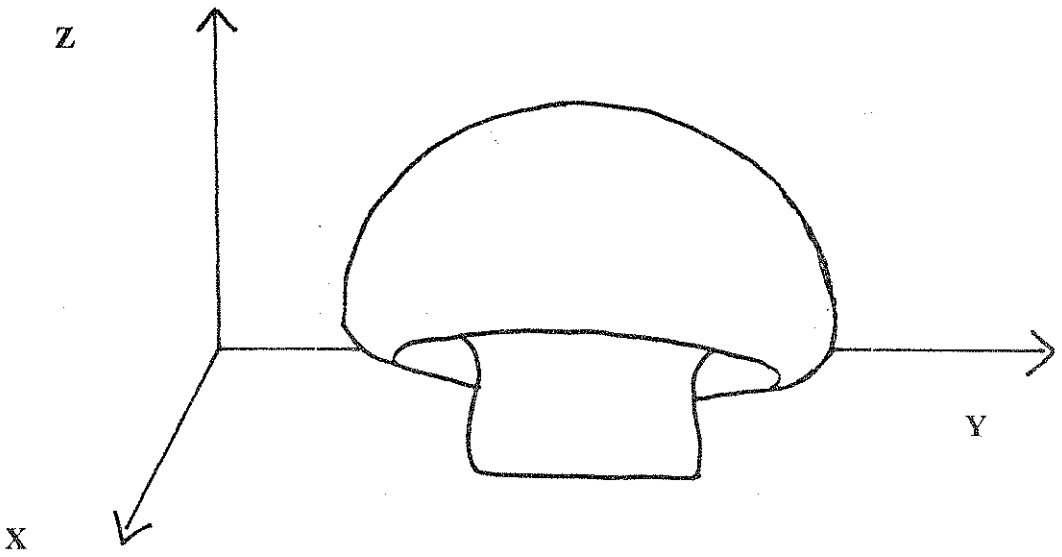
During the course of experiments investigating the environmental and agronomic

effects on mushroom texture, a number of treatments were found greatly affecting mushroom biomechanical properties. Most notably, the casing depth (Deep, Medium and Shallow)-compost depth (25, 40 and 55 mm) experiment produced a range of mushroom texture from firm to very soft (see V.2. for more details). Three treatments producing firm (25D), medium (40M) and soft (55S) mushrooms were chosen to examine and analyze any anatomical differences (hyphal diameter, volume fraction and dry matter content) which may account for their differences in biomechanical characteristics.

III.2 Materials and methods

III.2.1 Definition

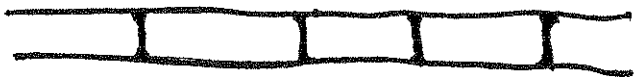
As mushroom caps are hemispherical, it is necessary to define words relating the orientation of sections taken of the mushroom to describe the hyphal arrangement.



- Longitudinal or cross-section of mushroom: when mushrooms are cut in a longitudinal or cross-section, they are cut in the middle of the cap along the Z axis and through the stipe. If one looks at the cut area, we should see the mushroom shape. - Transverse section of mushroom: when mushrooms are cut in a transverse section, they are cut along the X axis. The cap will be cut at the area where the

length of the cap in the Y axis is maximum. If one looks at the cut area, we should see a circle.

- Longitudinal section of hyphae:



- Cross-section of hyphae:

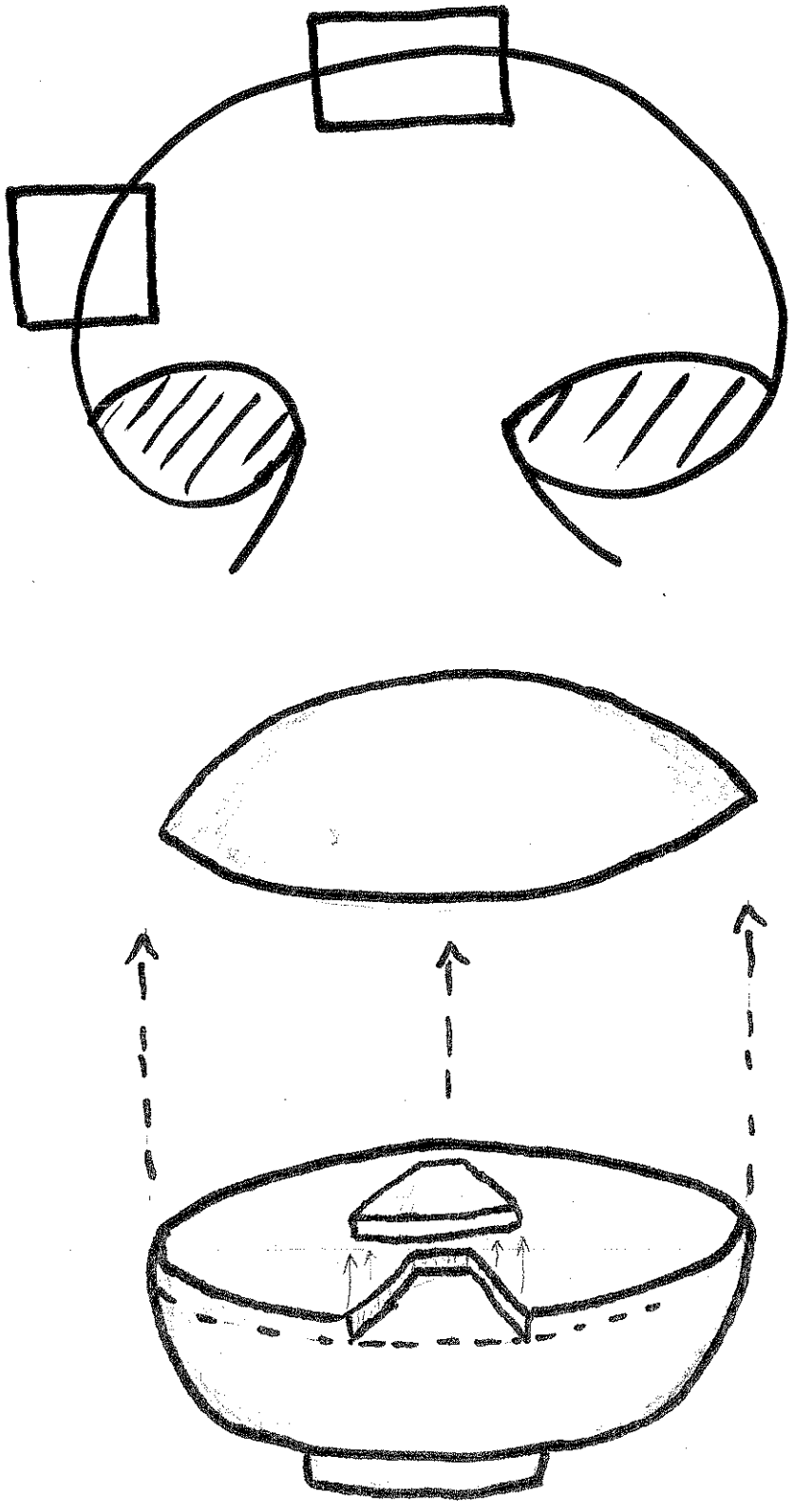
III.2.2 Preparation of tissues for light microscopy

Fresh samples of mushrooms (about 0.5 cm^3) were taken from the top and side and, in the transverse section of mushrooms (Fig. 4) and fixed 2 days in Bouin's fluid (71% (v/v) aqueous picric acid, 24% (v/v) formaldehyde, 5% (v/v) glacial acetic acid). They were then dehydrated in alcohol from 70% to 100% and placed in Histoclear (National diagnostics, Georgia, USA) before being embedded in wax. Sections of 8 µm thick were cut with a rotary microtome and mounted onto slides. Prior to staining, wax from the slides was removed in histoclear then the sections were hydrated in a series of alcohol to distilled water. The microsections were stained in 1% toluidene blue. The sections were then dehydrated in a series of alcohol and placed in histoclear before being covered by DPX (mountant for microscopy) and the coverslip.

III.2.3 Preparation of tissue for Scanning Electron Microscopy

Samples were taken from both the top and the side of the mushroom. A cube of about 0.5 cm^3 taken from the surface, was rapidly frozen in liquid nitrogen and transferred into the cryo-preparation chamber of the microscope where it was fractured using the cooled knife assembly. Samples were fractured in the longitudinal way so that the cross section became exposed to observation. Samples were analyzed under a Cambridge S200 Scanning Electron Microscope (Cambridge Instruments Ltd, Cambridge, England).

Figure 4: Samples taken in the cross-section of mushrooms at the top and the side area, and samples taken in the transverse section.

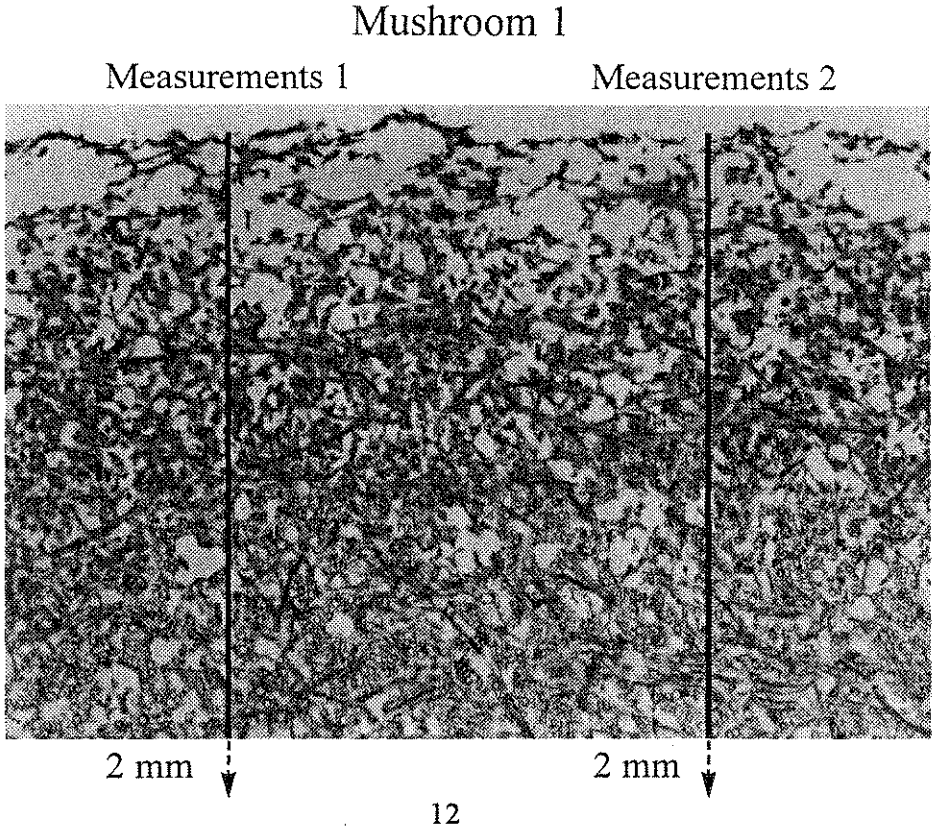


III.2.4 Hyphal diameter

Measurements were taken on mushrooms grown in the casing-compost depth experiment (see V.2.). The hyphal diameter was determined from slides obtained from the preparation of tissue for light microscopy. The slides were observed under a Leitz Dialux 20 microscope connected to a video camera (Model KP-M1E/K, Hitachi Denshi, Japan) which in turn was connected to a PC. The image was analyzed using an image analysis software (Image-Pro Plus, version 1.3 for windows). The diameter was measured either on a circular cross-section of hyphae or on a width of a longitudinal hyphae. The image seen on the computer screen, transferred from the microscope, is called a frame. This frame represents an area of 0.14 mm (depth) x 0.085 mm (width) of the mushroom tissue. In each frame, 5 hyphal diameters were measured.

Two mushrooms were examined per compost-casing depth treatment and on each of them two sets of hyphal diameter measurements were made from the surface to a depth of 2 mm (Fig. 5).

Figure 5: Measurements taken at the top area on a mushroom cross-section. The measurements were made from the surface, up to 2 mm in the flesh.



III.2.5 Volume fraction

Measurements of volume fraction were taken from mushrooms grown in from the casing-compost depth experiment (see V.2.). The volume fraction is the proportion occupied in a unit volume by cells. The volume fraction can be related to density although density represent the weight per volume. The volume fraction was determined from slides obtained from the preparation of tissue for light microscopy. Image were analyzed by the image analysis software cited in III.2.3. Black and white images were transformed to a 5 colours image (purple, blue, green, yellow, red) based on the light intensity (*i.e.* purple represents the brightest areas and red the darkest areas). For each slide examined a preliminary calibration is necessary to take into account any variation in the staining intensity between samples. The calibration was carried out by adjusting the microscope light intensity, so only the void (brightest) areas are coloured in purple. The calculation obtained by the software is the percentage of each colour in the frame selected. The volume fraction was calculated according to the equation:

Volume Fraction = 100 - % Purple

The purple colour represents the brightest areas, *i.e.* those not containing cellular contents. Two mushrooms were examined per compost-casing depth and each of the volume fraction measurements were taken from the surface to a depth of 2 mm (Fig. 5).

III.3 Results

III.3.1 Morphology under light microscopy

III.3.1.1 Side of the cap

Samples taken from side of caps showed that hyphae are parallel to each other and orientated in a direction from the centre of the cap to the side and the velum (Fig. 6)

Note the intercellular spaces in between the hyphae.

In the transverse sections of the side (Fig. 7) groups or bundles of hyphae can be observed in their cross section. This supports the observations made from Fig. 6 that hyphae are parallel to each other. In the transverse section Fig. 8, the change in diameter of hyphae in relation to depth from the surface can be easily observed. Near the surface hyphae have a small diameter but deeper in the flesh, the hyphal diameter is larger.

III.3.1.2 Top of the cap

In the top region of the cap, most of the hyphae are parallel to the surface and were observed in both cross-section and longitudinal section (Fig. 9). At the surface (Fig. 10), there are some detached hyphae which suggest that they are not well supported by and connected to the underneath trama.

The light micrograph (Fig. 10) shows the cross section of the cap top region from the surface to approximately 1.2 mm in the flesh. There are two distinct layers which can be distinguished by the intensity of the staining. The first layer, more intensely stained, is made of organised and orientated hypahe as observed in Fig. 9. This layer represents a depth of about 0.3 mm in the mushroom section selected but this depth may vary from mushroom to mushroom. In the second layer, where the staining is less intense, it is noticeable that hyphal diameter is larger. There are cross-section and longitudinal section of hyphae, some of these latter are parallel to the surface but others are to be going from deep flesh towards the skin.

III.3.2 Anatomy under electron microscopy

III.3.2.1 Surface of the cap

The surface of the mushroom, either on the side or on top of the cap, was observed under the scanning electron microscope to be a network of hyphae (Fig. 11), observation also described by Atkey and Nichols (1983).

III.3.2.2 Side of the cap

The electron micrographs, taken from the side of the mushroom (Fig. 12 & 13), show hyphae running in the same direction and parallel to the surface. The two electron micrographs show cross-section of hyphae (Fig. 12) and longitudinal section of hyphae (Fig. 13) depending on the orientation of the fracture in the tissues.

III.3.2.3 Top of the cap

Samples taken from the top region (Fig. 14) show hyphae parallel to the surface that do not run in a single predominant direction. Note that there are many intercellular spaces in between the hyphae.
A wider view of the cross-fracture section of cap (Fig. 15) shows that at and just underneath the surface there is a low hyphal density layer. Deeper in the flesh, hyphae are more densely packed and there are no or few intercellular spaces present (Fig. 16).

III.3.3 Hyphal diameter

The overall trend shows that at the surface, hyphae have a small diameter of about 4 μm. The hyphal diameter was found to be greater when measured into the tissue below the surface up to a maximum of 8-12 μm (Fig. 17). When examining mushrooms from different treatments, mushrooms from treatment 25D, who were found to be the firmest, have on average a lower hyphal diameter (although the standard deviation is large) than mushrooms from treatment 40M and 55S. Not much difference was found in hyphal diameters between treatment 40M and 55S.

III.3.4 Volume fraction

The relationship between volume fraction to depth in the tissue shows there is a clear trend (Fig. 18). The volume fraction in the first 0.14 mm deep layer of the surface is low at 60 %, then increases to reach about 93 % at 2 mm depth below the surface. It was not possible to detect any difference between the 3 treatments (25D, 40M, 55S) because the standard deviations were too large.

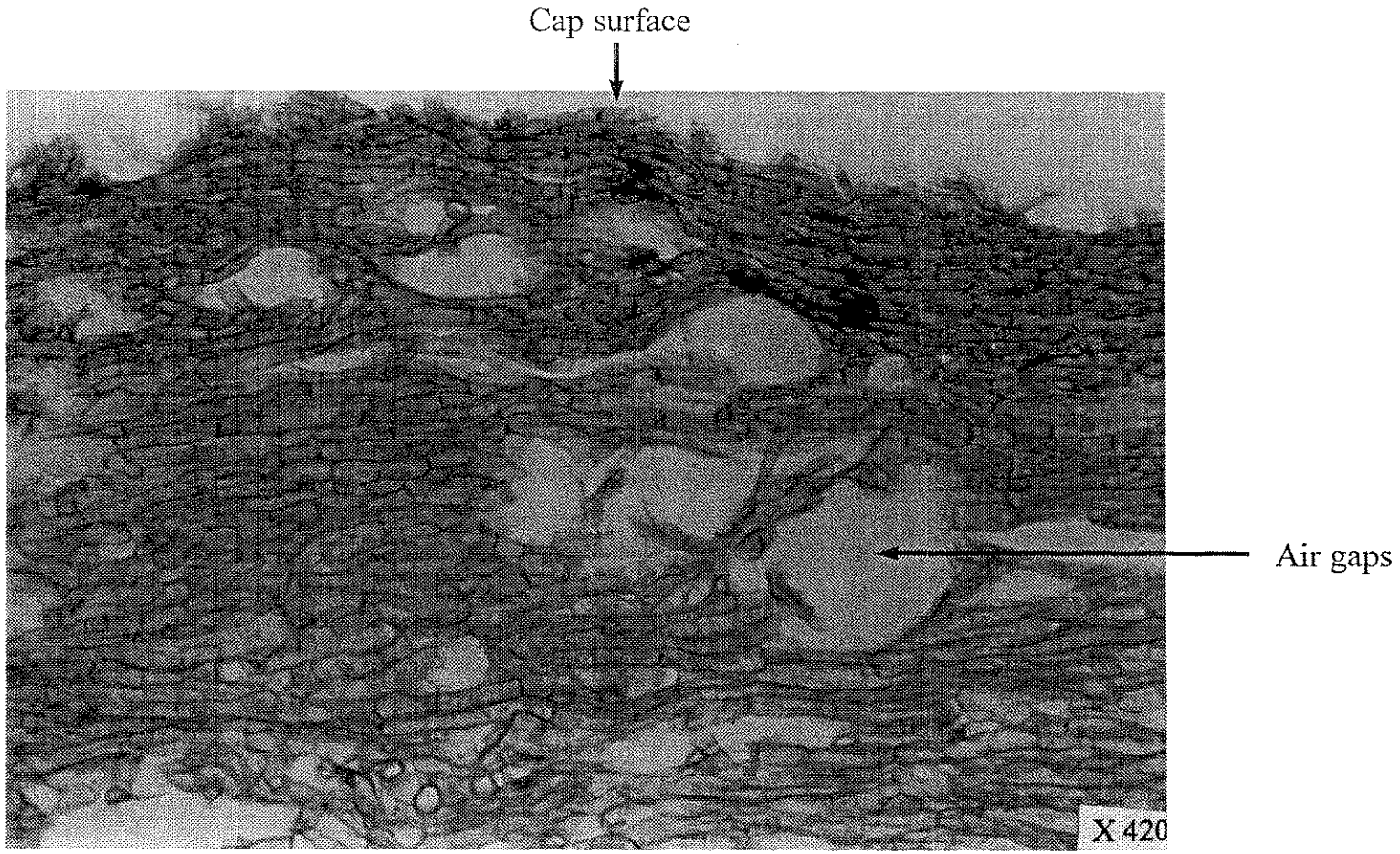


Figure 6 - Light microscopy section of side of mushroom cap showing longitudinal sections of hyphae.

Figure 7 - Light microscopy of transverse section into mushroom cap showing most hyphae as cross-sections and organised as bundles on the side of the cap.

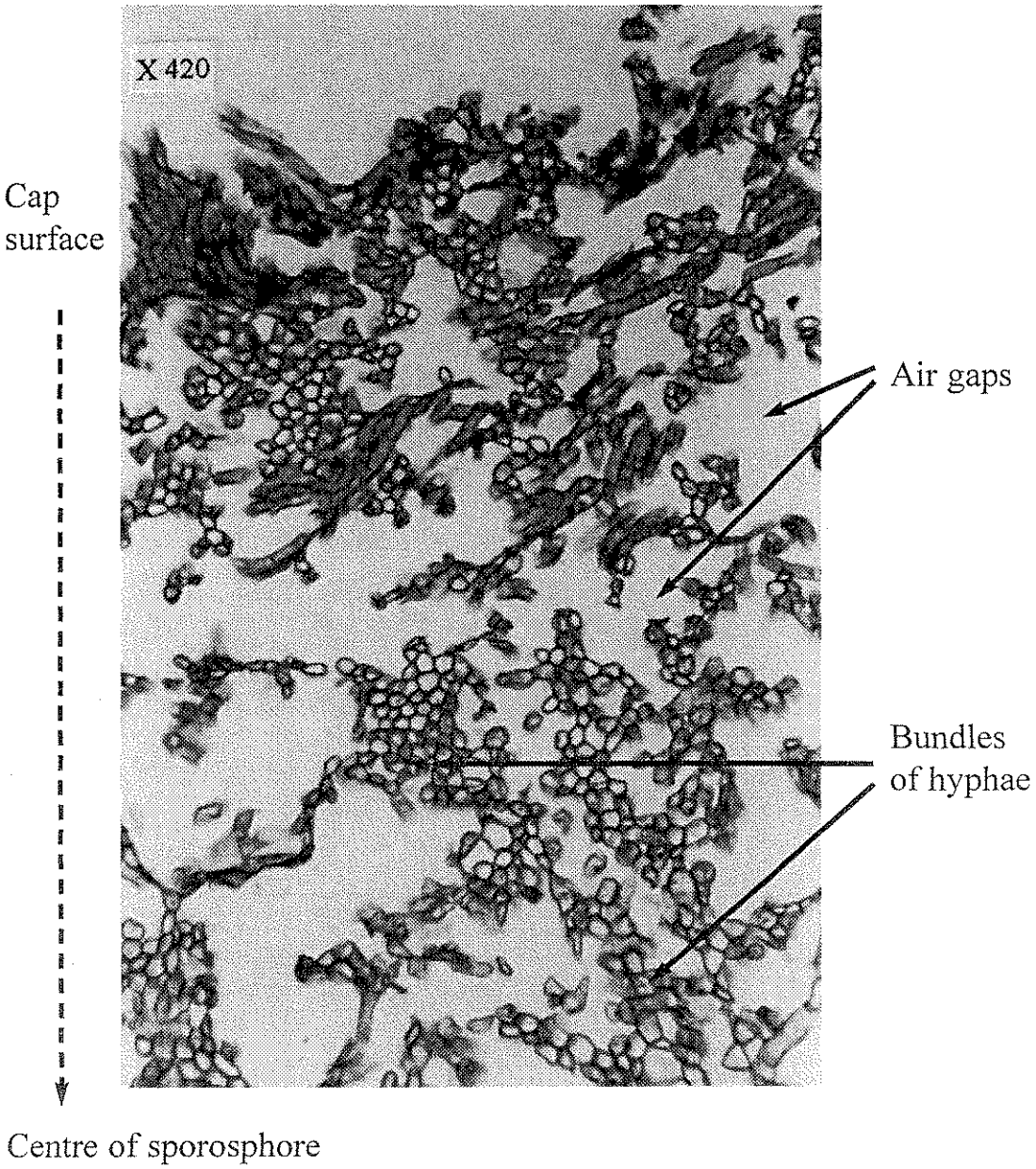
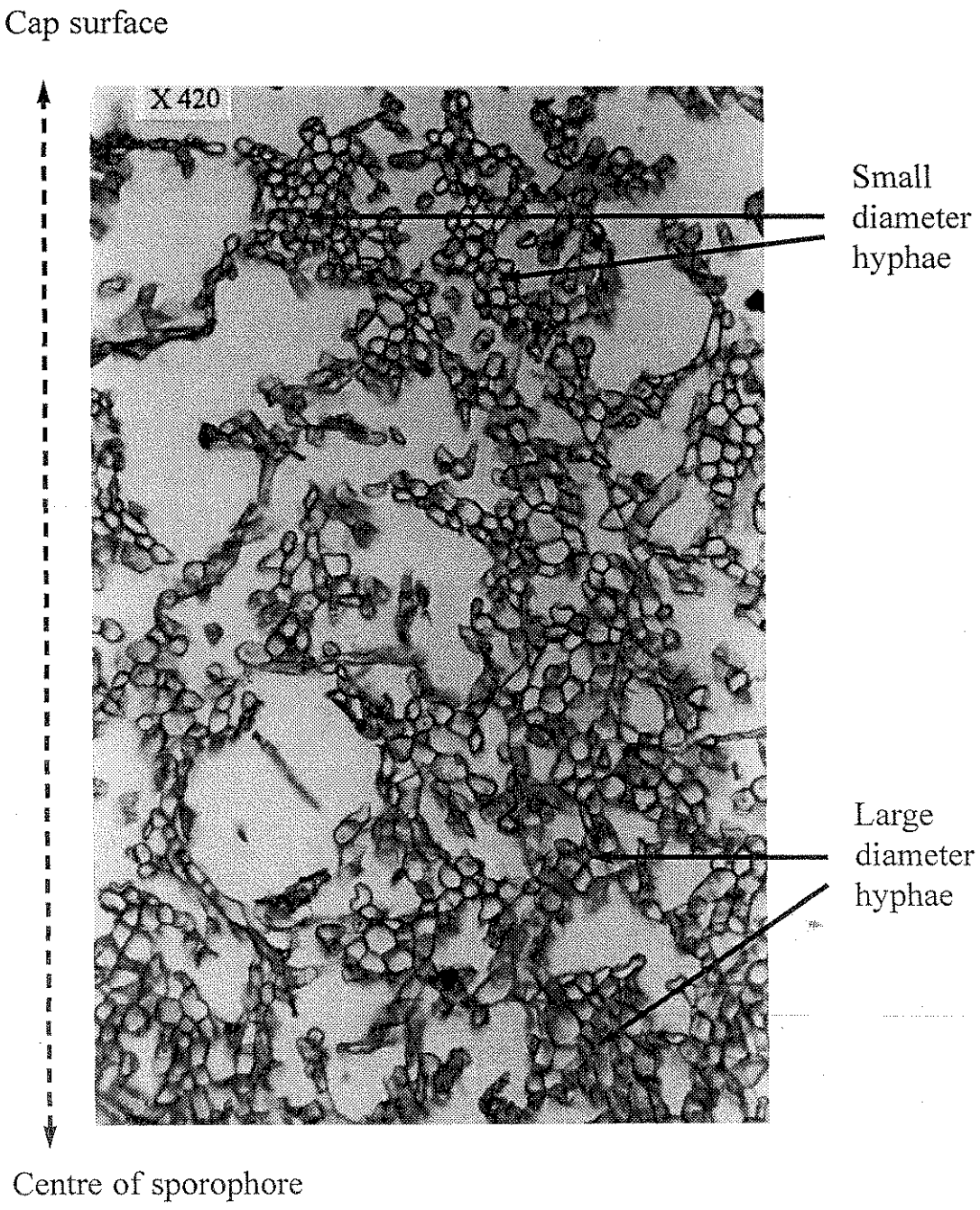


Figure 8 - Light microscopy of transverse section into mushroom cap (showing small diameter hyphae at side cap surface and larger diameter hyphae in centre of cap).



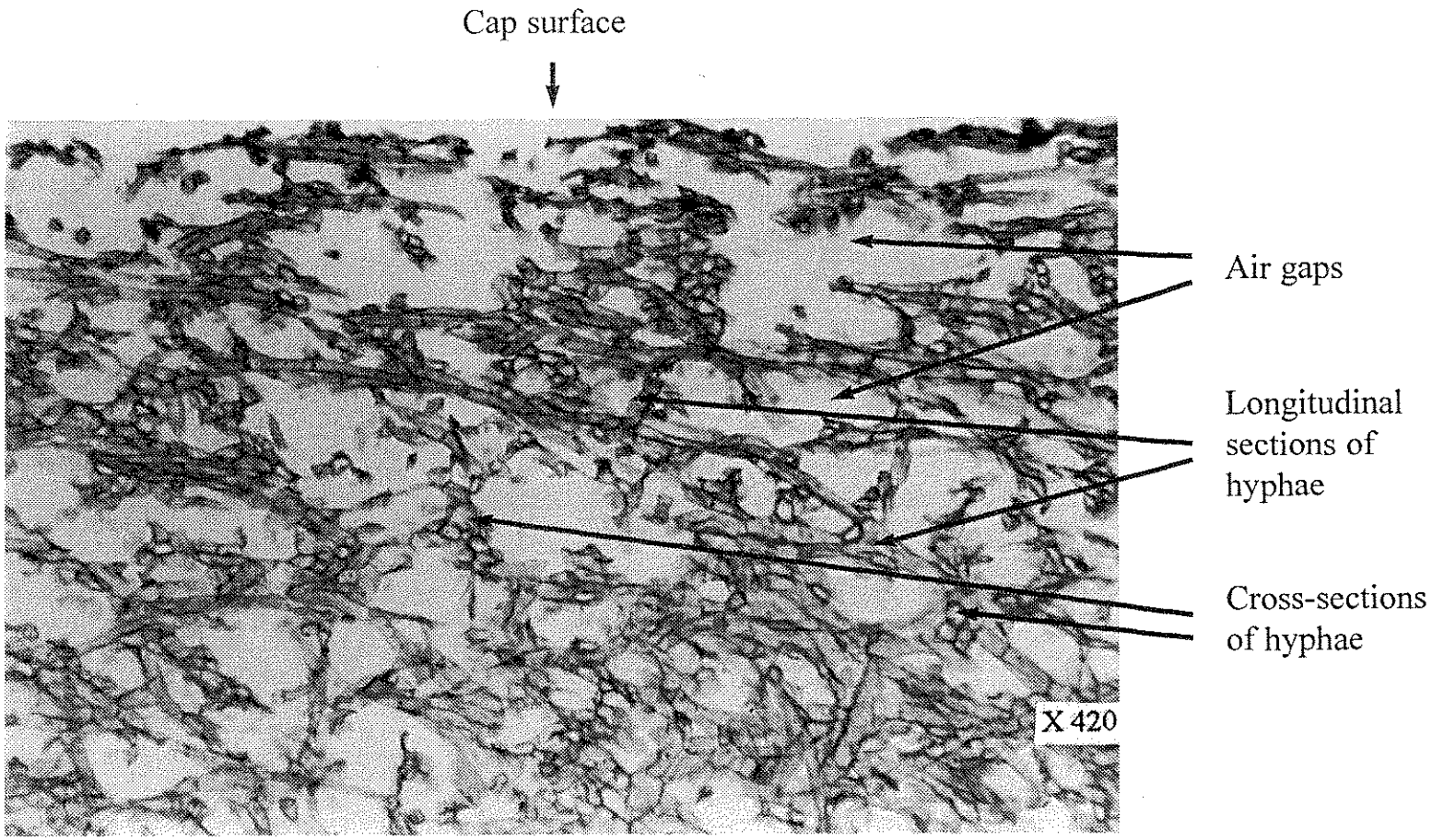


Figure 9 - Light microscopy section from the top showing a mixture of longitudinal and cross-sections of hyphae.

Figure 10 - Light micrograph of the cross-section of the top of mushroom showing a high intensity staining layer near the surface and a low intensity staining layer in the flesh.

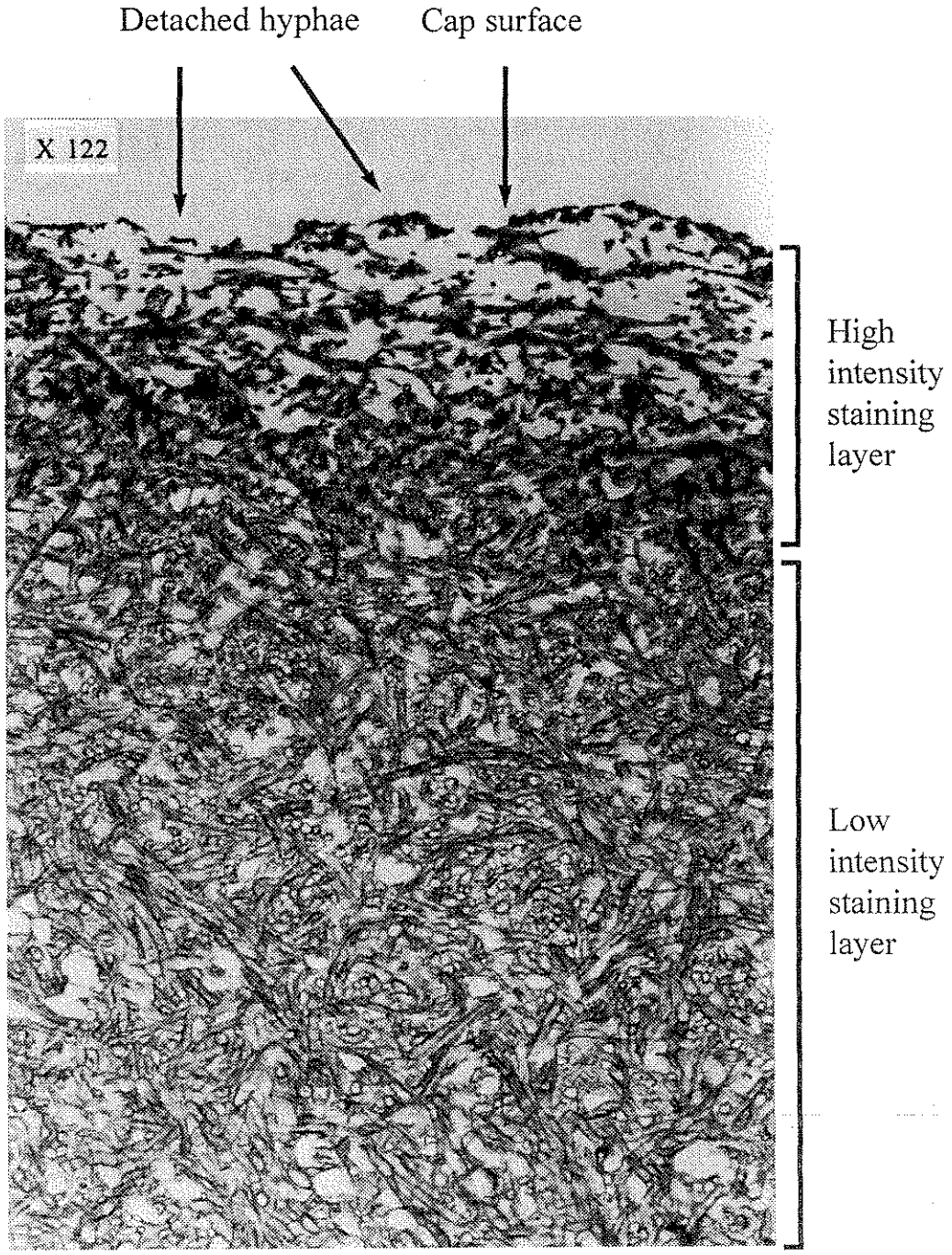
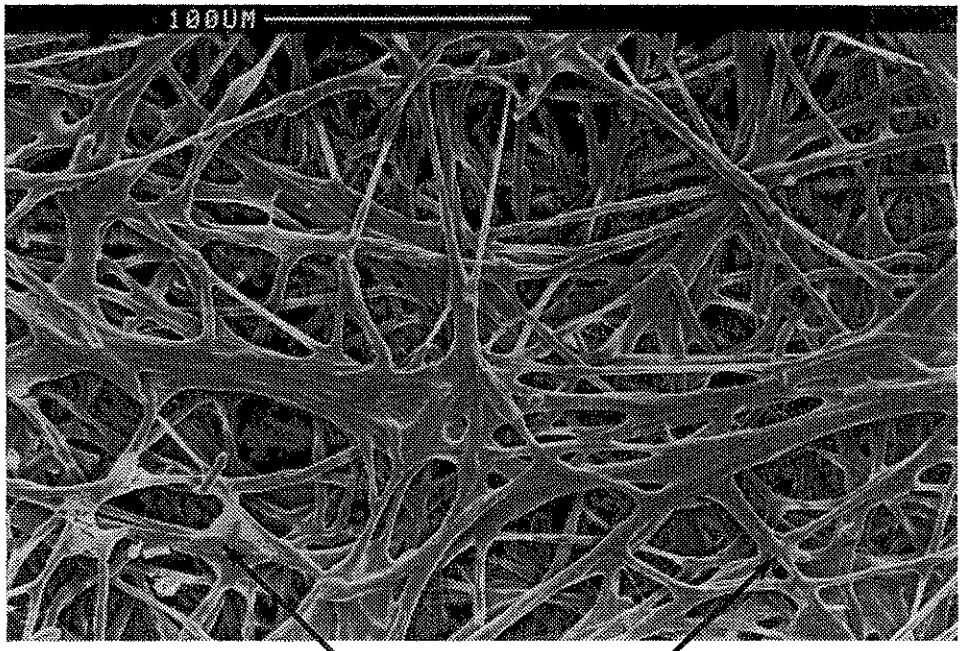


Figure 11 - Electron micrograph of the mushroom cap surface on outermost layer showing a network of hyphae.



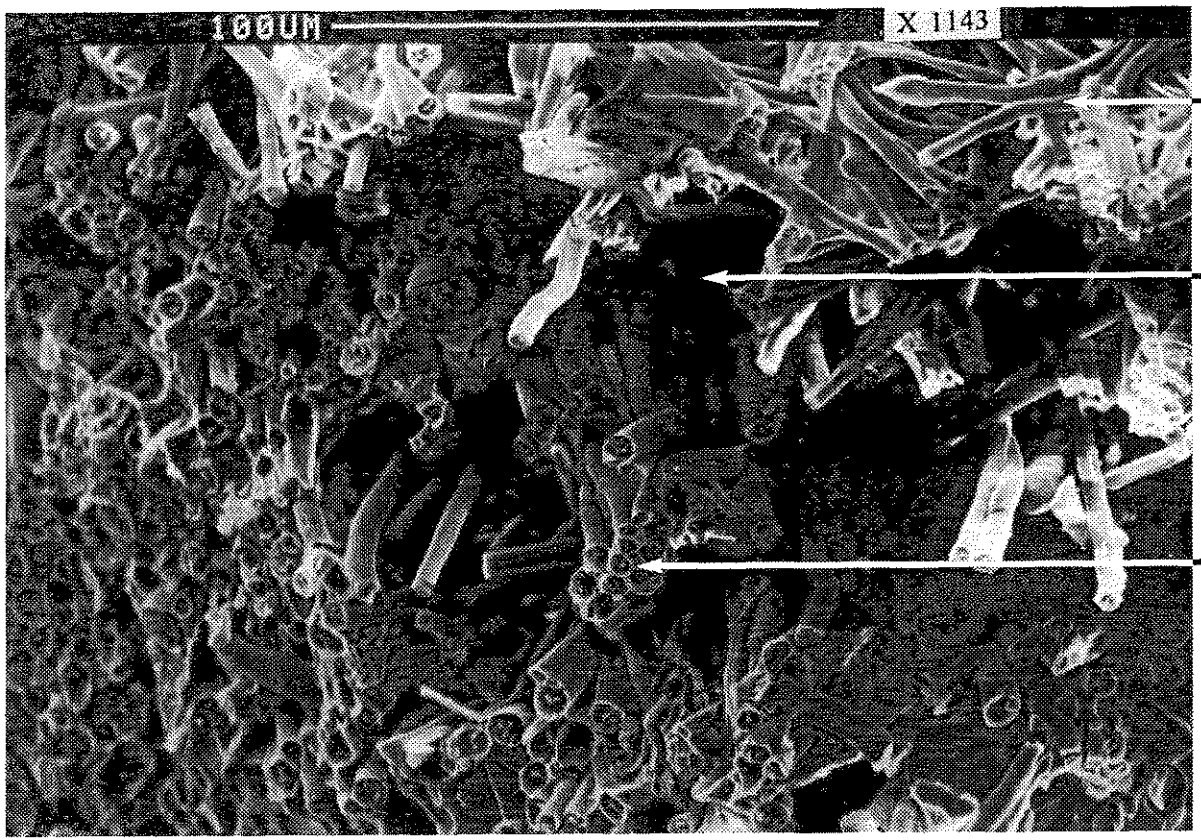


Figure 12 - Scanning Electron micrograph showing the surface and cross-fracture section of the side of mushroom cap.

Note hyphae in fracture are mainly in cross-section therefore with orientation parallel to surface.

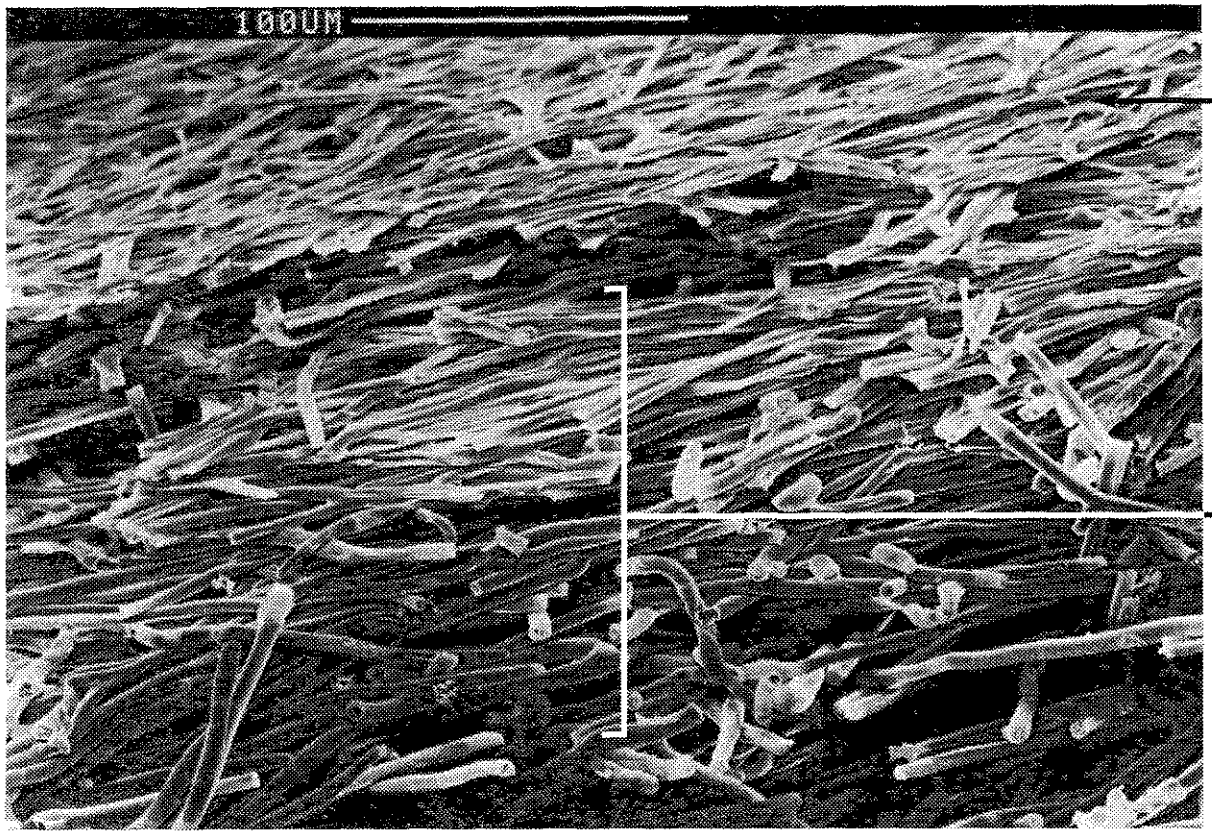


Figure 13 - Electron micrograph of a cross-fracture section from the mushroom side.

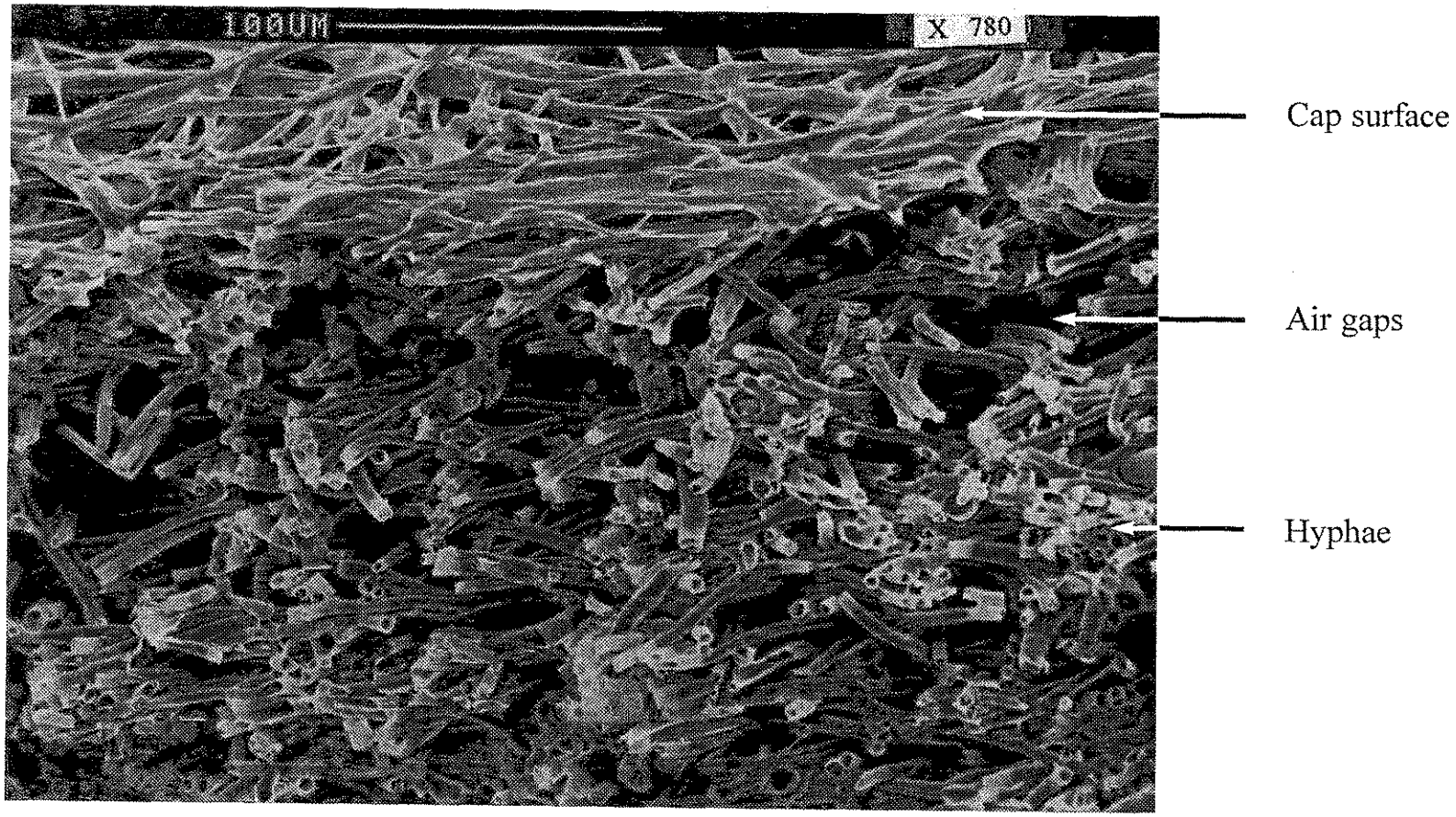


Figure 14 - Scanning Electron micrograph showing the top cross-fracture section of mushroom cap. Note hyphae are in random directions but in plane parallel to surface.

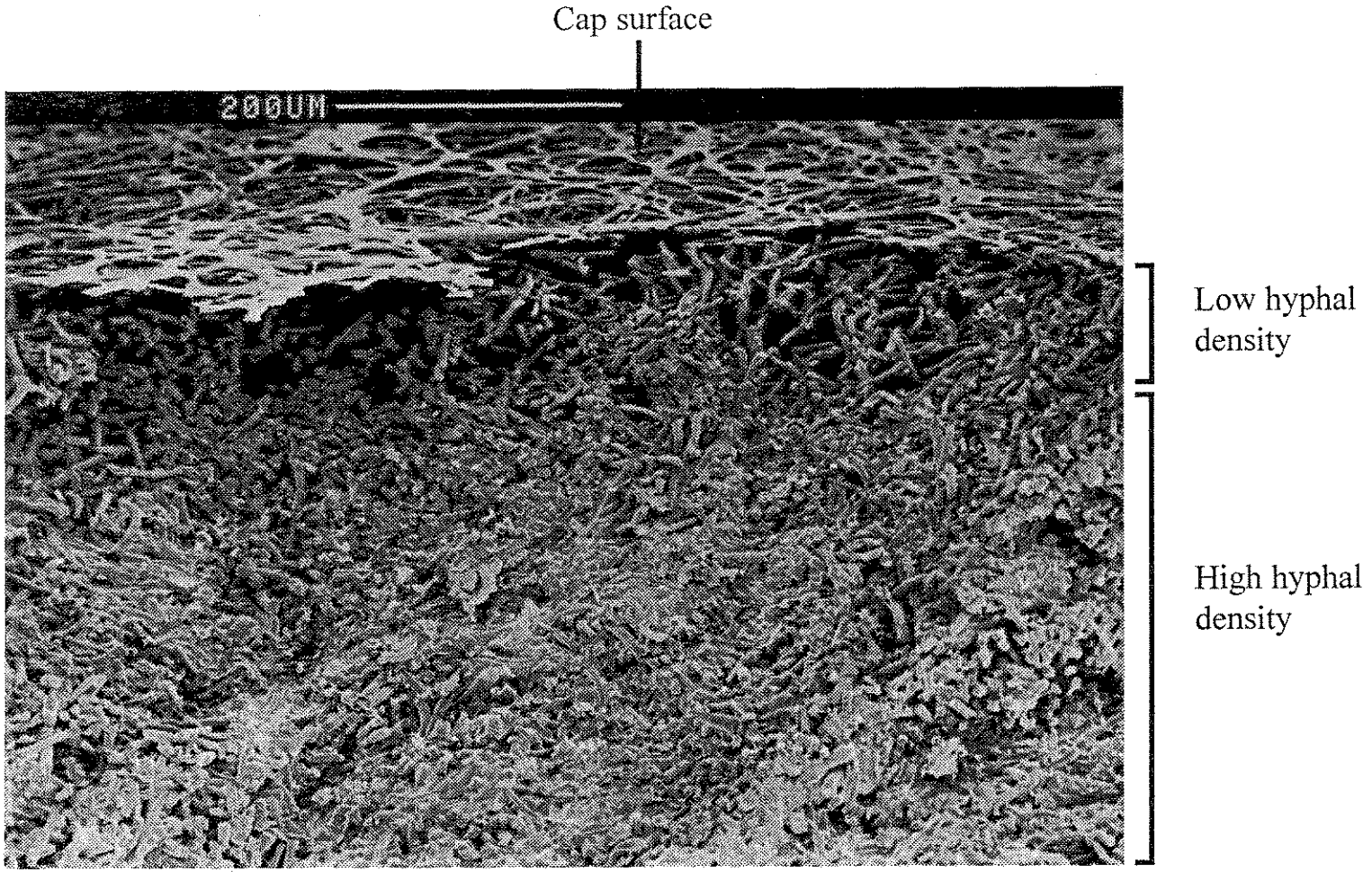


Figure 15 - Scanning electron micrograph showing fracture at the top of the mushroom cap.
Note the change in density, a low hyphal density layer near the surface and a higher hyphal density layer in the flesh

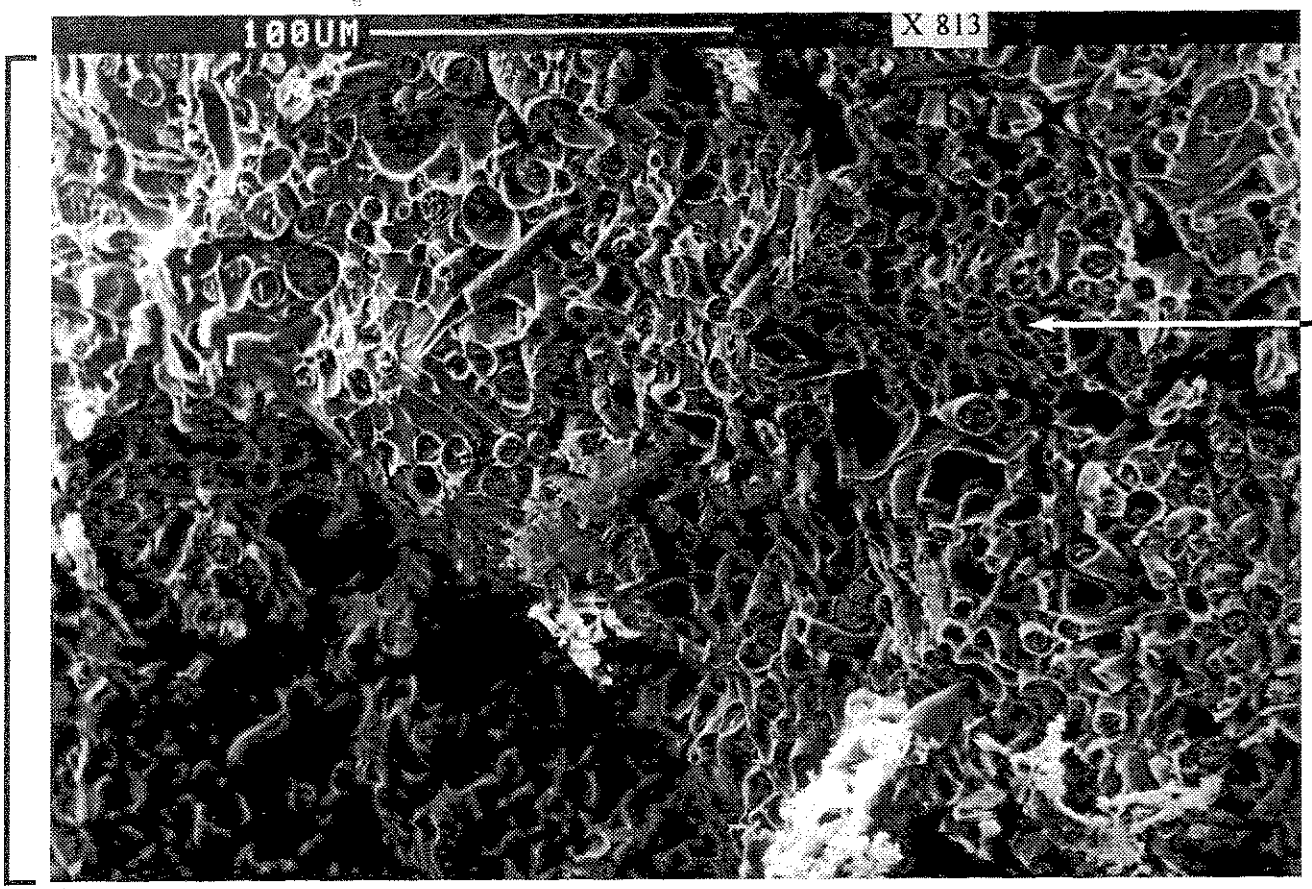


Figure 16 - Scanning electron micrograph of a cross fractured section in the deep flesh of mushroom cap showing densely packed hyphae.

Figure 17: Hyphal diameter from the surface to 2 mm in the flesh of mushrooms from treatment 25D, 40M and 55S.

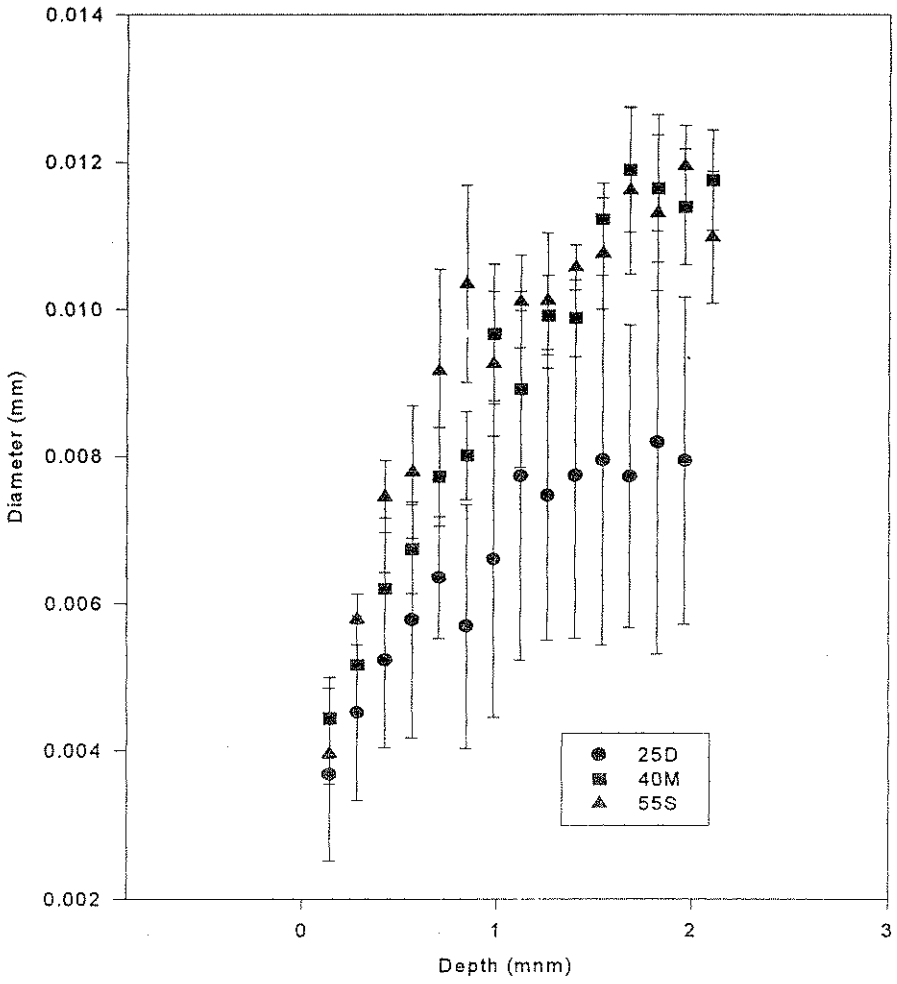
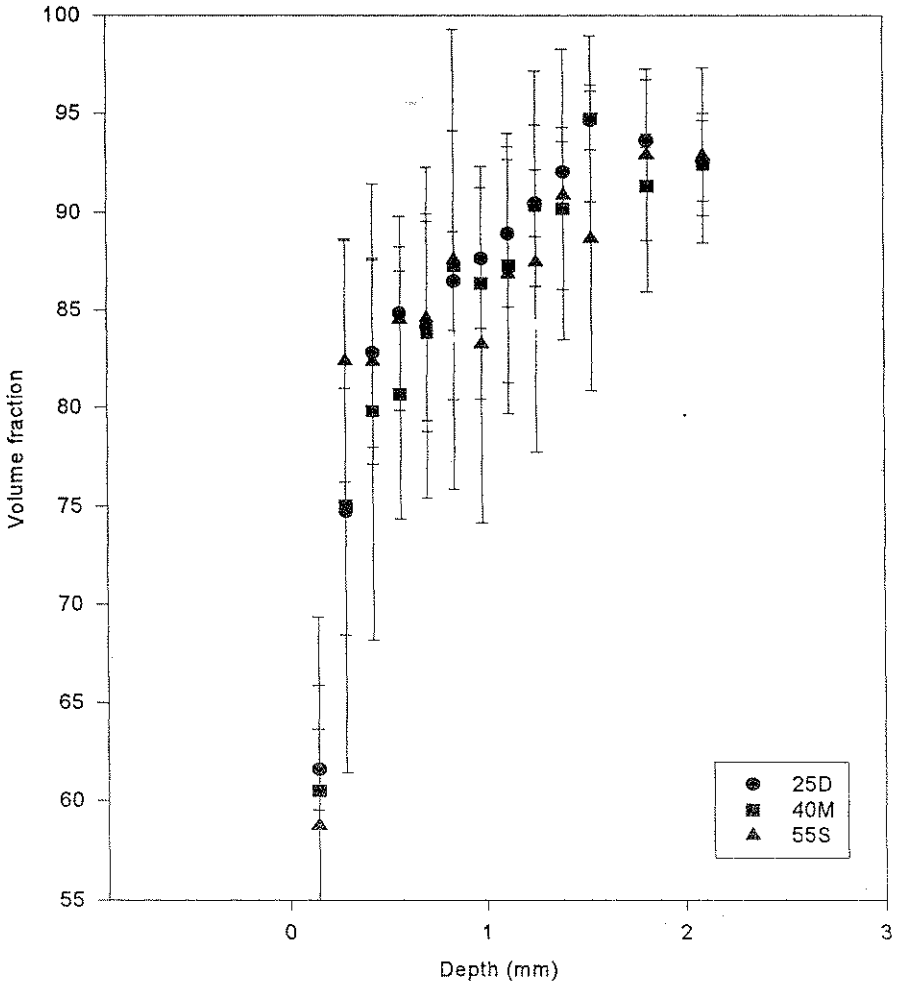


Figure 18: Volume fraction from the surface to 2 mm in the flesh of mushrooms from treatment 25D, 40M and 55S.



III.4 Discussion

III.4.1 Arrangement of hyphae on the surface

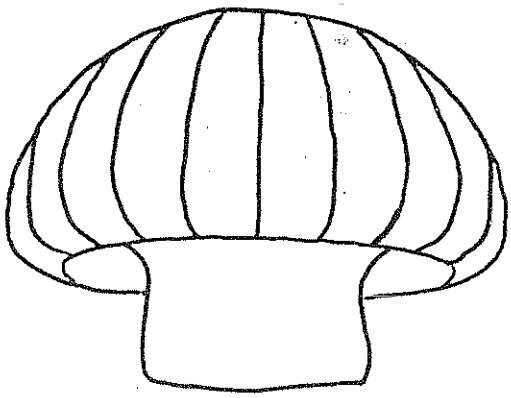
The outermost surface layer was observed to be a network of hyphae (Fig. 11). With the naked eye it was very difficult to differentiate this outer layer from the rest of the cap as it is a thin layer of about 15 μm with is no clear delineation with the underlying tissues. There is speculation about the role and function of the skin, it could be a defensive layer protecting mushrooms from insects and pests but it is also possible that the skin has a mechanical role as well. The network of hyphae in the skin could make the surface smoother to help mushrooms piercing through the casing soil which can be heavy and compacted. This function is analogous to the root cap cells of a plant root. These cells, located at the root tip, are closely held on to the root and become scraped off as the root grows into new regions of soil thereby reducing the friction of root extension. When mushrooms grow, hyphal expansion is located at the margin of the cap (Bonner *et al.*, 1956), there is therefore an increase of area in that region. To cover that increase, either there is some branching in the radially orientated hyphae or the bundles of hyphae split to cover the increase of area. There is no evidence that there is sufficient branching in hyphae from the margin, so it is probable that the bundles of hyphae split apart when mushroom cap grows. The splitting does not propagate to the surface because of a covering by the network of hyphae at the outer surface. In some *A. bitorquis* strains, stage 4-5 mushroom caps often split in their radial orientation (personal observations) probably because the network is not strong enough to maintain the underneath hyphae together.

III.4.2 Orientation of hyphae under the surface layer

The light micrographs (Fig. 6 & 9) and electron micrograph (Fig. 13 & 14) have shown that under the cap surface, hyphae are parallel to the surface and radially orientated as shown in Figure 19. These observations support the idea of Bonner *et al.* (1956) who said that it was occasionally possible to see a considerable amount of radially orientated hyphae in longitudinal sections. The origin of the high degree of orientation of skin hyphae has raised some questions: do the hyphae grow already

orientated from the pinhead or do they become orientated when they are stretched by the growth of the cap below? There were many hypotheses for the hyphal cap arrangement, for instance a complete disorganised mass shaped as a mushroom, hyphae coming from the stipe towards the top and spreading on the edge of the cap or hyphae coming from the stipe and growing in the cap like a bunch of flowers. The radial arrangement of hyphae, however, explains how mushroom caps can grow by expanding the cap area from the elongation of cells at the margin (Bonner *et al.*, 1956) and why mature mushrooms are flat and not like a bunch of flowers if hyphae were longitudinally orientated.

Figure 19: Scheme representing hyphae radially orientated underneath the cap surface



III.4.3 Identification of layers in the cap

Observation of the anatomy of the mushroom sporophore has revealed a number of different layers, or zones, at the surface of the mushroom cap. These zones are not clearly delineated, nevertheless they can be distinguished from one another by a number of featers e.g. staining intensity, hyphal density and orientation (Fig. 20).

III.4.3.1 Layer A

The outermost layer, about 15 µm thick, consists of a network of hyphae orientated in various directions but always parallel to the surface. There is considerable hyphal contact within this layer but apparently small contact between this layer and the

hyphae below. Because of the presence of so many air gaps in the layer below, there is no real support for the outer surface hyphae and therefore they are vere easily to move or be removed; these hyphae can be seen in some light microscopic sections. It is common that when touching mushrooms, residues of hyphae are removed from the cap and adhere on your fingers. The electron micrograph of freeze fractured mushroom (Fig.15) reveals a "weakness" below the surface and the skin forming a shelter. The profile of the fracture might be due because of the low volume fraction underneath the skin and because of the network of hyphae on the surface. This outermost surface layer may be acting as an extendable net, delineating the outer surface of the mushroom cap and confining the hyphae within.

Below the outermost layer are zones distinguished by differences of two features, hyphal density and intensity of staining. The separation below the low and high hyphal density regions does not coincide with position separating the zones of high and low intensity of staining. Therefore three zones can be identified:

- low hyphal density and high intensity of staining
- high hyphal density and high intensity of staining
- high hyphal density and low intensity of staining

III.4.3.2 Layer **B**

The zone immediately below the outermost layer has low hyphal density and high intensity of staining. This layer is approximately 80 μm thick. Hyphae are radially orientated in the cap, the hyphal diameter is small.

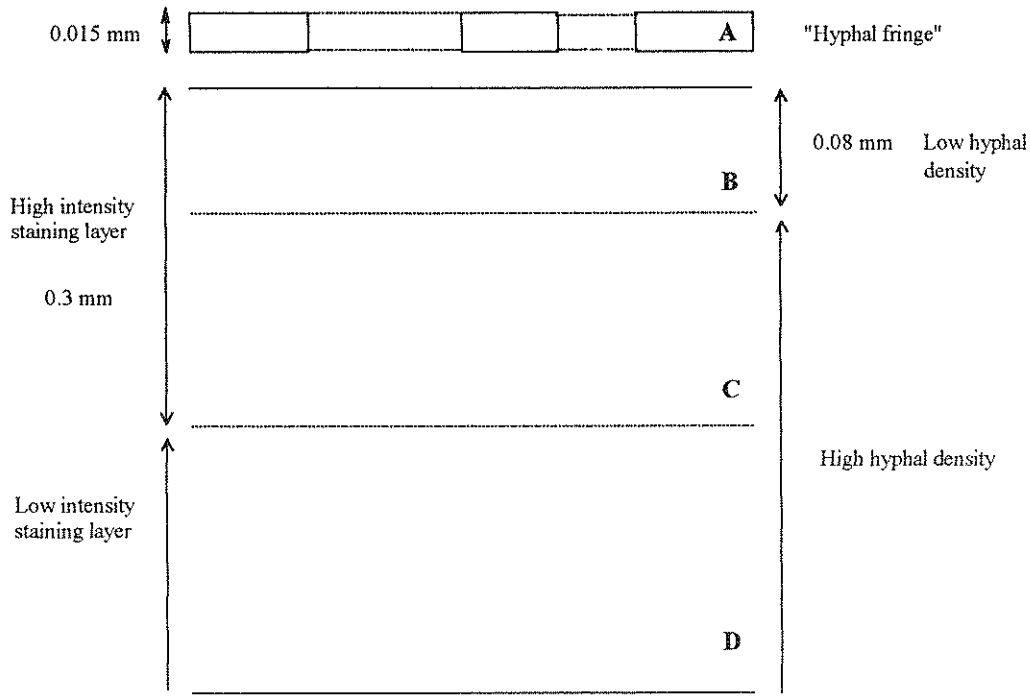
III.4.3.3 Layer **C**

This 22 mm thick layer has high intensity staining and high hyphal density. Hyphae are radially orientated in the cap, the hyphal diameter is small.

III.4.3.4 Layer **D**

This layer has low intensity staining and high hyphal density. At the moment it is not known how deep is that layer. Hyphae have a larger diameter than hyphae of the previous layers. Most of the hyphae are parallel to the surface but there are also others which are to be going from deep flesh towards the skin.

Figure 20: Upper area of the mushroom cap illustrated by several layers according to staining intensity, hyphal density and orientation.



IV Mechanical properties of the sporophore

IV.1 Introduction

There is every reason to believe that biological structures are as carefully designed in Nature through the process of evolution, as structure designed by humans e.g. complex bridges, houses or even a simple boxing ring. Why the Golden Gate Bridge can sustain hundreds of cars, why a house will not collapse when it is windy and why the structure of a boxing ring protect the boxers from bruises? The answers are that each structure is built for a particular purpose using appropriate (*i.e.* soft, strong, elastic) materials. As seen in Chapter III, mushrooms have also a complex structure which gives a mushroom the ability to support its own weight, develop and grow, disperse spores above above ground level and force the growing structure through compacted soil. The mechanical properties of mushroom tissue are a support in the understanding of mushroom texture and quality.

Mushrooms are very susceptible to bruising caused by mechanical damage during handling and transport. Other fruits and vegetables like potatoes, apples, peaches, pears etc, are also very susceptible to bruise and it has be shown that the mechanical properties of their tissues influence the bruise development. By applying a controlled mechanical damage on mushroom caps, it is possible to reproduce a bruise whose volume can be measured and by using microscopy the inner damage can be analyzed. Controlled fast and slow compressions were applied to mushrooms to simulate forces commonly occurring during harvest and handling.

Tests were carried out on *A. bisporus*, as this is the most commomly cultivated mushroom species in the UK and also *A. bitorquis* as it was found to have a different texture to *A. bisporus*. Tests were carried out on both species *A. bisporus and A. bitorquis* to understand what components contribute to the difference in texture.

IV.2 Materials and methods

IV.2.1 Relationship of stiffness to hyphal orientation

Cylindrical cores of mushroom tissue were taken at different position and orientation (Fig. 21) in the cap and tested for their stiffness. Ten replicates for each orientation over the first three flushes were tested. Two mushroom strains, A12 and X25, were tested for this experiment.

IV.2.2 Plastic deformation related to displacement

Cubes of mushrooms were compressed by 0.5 to 4 mm. The plastic deformation and the total energy supplied were recorded on the Instron. Ten replicates were tested at each displacement.

IV.2.3 Rapid compression (drop test)

During the rapid compression (commonly called drop test) a 10 g steel ball (diameter=13.5 mm) was dropped from a height of 50, 125 or 200 mm on top of mushrooms. The experiment was recorded by video camera so the ball rebound could be measured by image analysis from which the energy released for the rebound could be determined. Four mushrooms replicates per each level of height were tested.

The total energy supplied to mushrooms is E=m x g x h.
m=Ball weight, $10x10^{-3}kg$
g=Acceleration, 9.8 $m.s^{-2}$
h=Drop height, 50, 125, 200 mm

The energy absorbed (Eabs) is the difference between the total energy supplied and the energy released for the rebound.
Eabs=m x g x (Drop height - Rebound height)

IV.2.4 Slow compression

The compression was performed on top of whole mushrooms at a displacement range of 2/2.5/3/3.5/4 mm and a speed of 5 mm/min. The total energy supplied and the

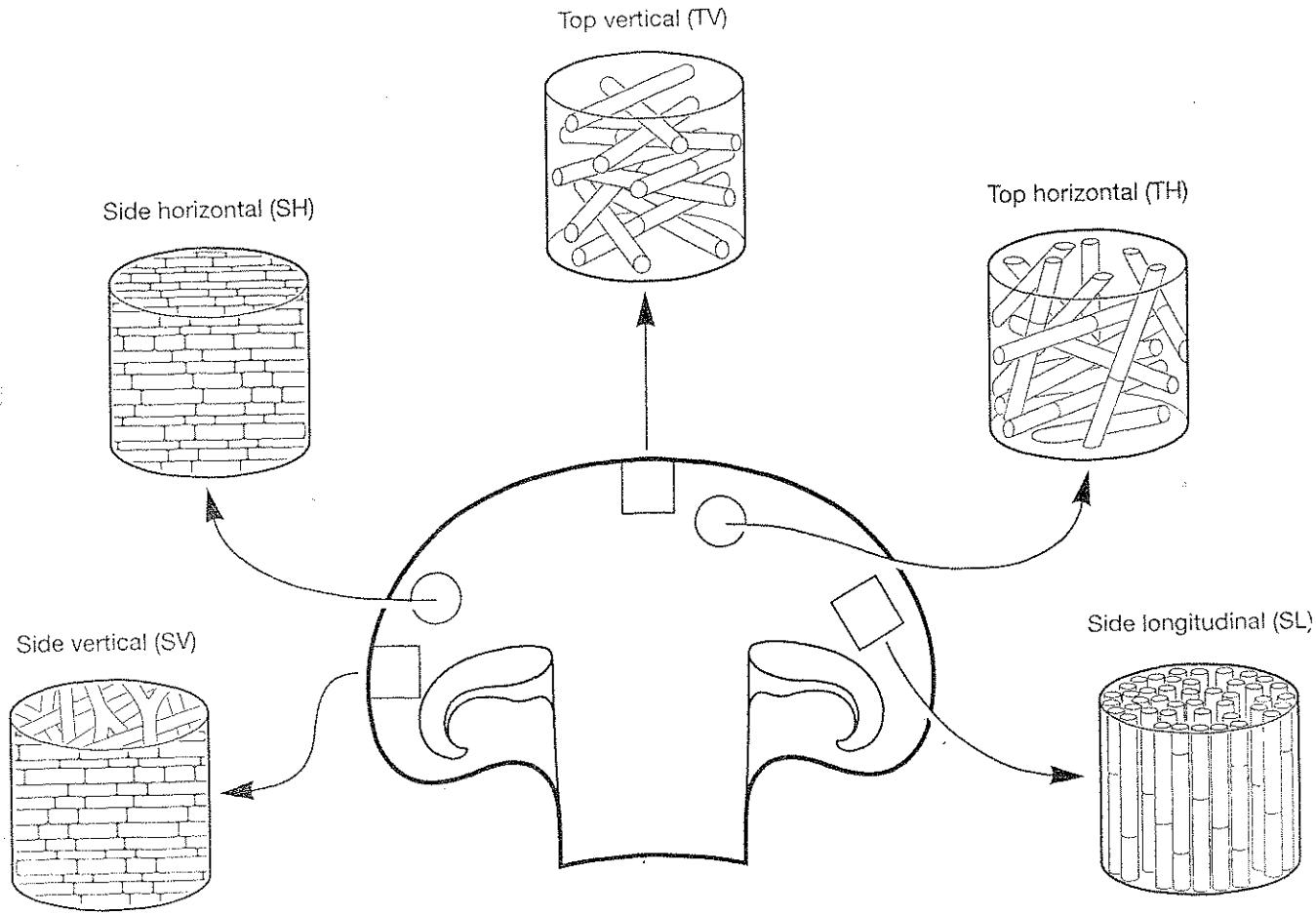


Figure 21: Cylinders position and orientation in the cap

energy absorbed was measured from the load-displacement graph. Five mushrooms replicates were tested per each displacement distance.

IV.2.5 Bruise volume calculation

After compression (slow or rapid), mushrooms were stored on the bench at room temperature for 2 days to allow the bruise reaction to develop. They were then cut in half across the impact area and the maximum width and depth of the bruise volume underneath the surface was measured according to the formula of Holt and Schoorl, 1977 (Fig. 22).

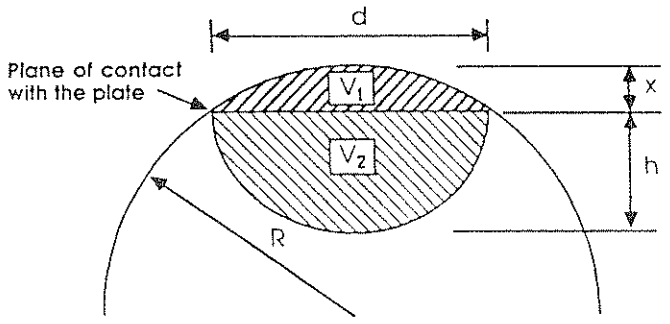
Figure 22: Calculation of bruise volume (Holt and Schoorl, 1977)

$$V = V_1 + V_2$$

$$V = \frac{\Pi h}{24}(3d^2 + 4h^2) + \frac{\Pi x}{24}(3d^2 + 4x^2)$$

$$x = R\sqrt{R^2 - \frac{d^2}{4}}$$

h = height below contact
x = height above contact
d = diameter of the bruise
R = radius



IV.2.6 *Agaricus bitorquis* strains

Four strains of *A. bitorquis* were tested for their stiffness: the strains W20 and W2F, and hybrids of those strains BC1 and BC4 (courtesy to J. Smith for providing them). Cylinders were taken in the cap in the top vertical (TV) and side vertical (SV) position (see Fig. 21). Ten mushrooms from flush 3 were tested.

IV.3 Results

IV.3.1 Relationship of stiffness to hyphal orientation

The analysis of variance has shown that there is a significant difference ($p<0.001$) in the tissues stiffness depending on the orientation and where the tissues were cut from the sporophore (Fig. 23). The stiffest tissues were found on the top of the cap in the horizontal orientation (TH) and on the side of the cap in the longitudinal orientation (SL). The tissues in the top of the cap in the vertical orientation was found less stiff than the previous ones, then the tissues with the lowest stiffness were the tissues taken on the side of the cap in a vertical or horizontal orientation (SV and SH). The stiffness of these two last tissues was not significantly different. The tissues taken from the top of the cap in either a vertical or horizontal orientation (TV and TH) were stiffer than the tissues taken from the side of the cap in a vertical or horizontal orientation (SV and SH). The two mushroom strains A12 and X25 were significantly different ($p<0.001$) for only the tissues taken on the side of the caps in the longitudinal orientation. In this experiment no significant difference between the flushes was found.

IV.3.2 Comparison of tissue stiffness in *A. bitorquis* and *bisporus*

The comparison of tissue between *A. bisporus* and *bitorquis* showed that *A. bisporus* tissue was found stiffer than *A. bitorquis* tissue ($p<0.001$). The tissue from the top of the cap showed that it was significantly stiffer ($p<0.001$) than the tissue from the side of the cap. Among the *A. bitorquis*, strains W2F was found significantly ($p<0.001$) less stiff than the other strains W20, BC1, BC4 (Fig. 24).

IV.3.3 Plastic deformation related to displacement

The percentage of plastic deformation was found to increase both with increased experimental displacement treatments and with the energy requirement (Fig. 25 & 26). There is a linear regression between the percentage of plastic deformation and the displacement, up to 4 mm compression (Fig. 25). For a slight puncture

(compression of 0.5 mm) the indentation (plastic deformation) was about 35% of the applied compression but under a compression of 4 mm the indentation was more than 50%. The plastic deformation plotted against the energy requirement has shown that it is a non linear regression curve (Fig. 26). In proportion, it requires a lot more energy to create and indentation of 55% than to create an indentation of 35%. To make sure that the results obtained were consistent, the energy requirement was plotted against the displacement and it showed that the curve obtain is a typical load (or energy)-displacement curve (Fig. 27).

IV.3.4 Comparison between a rapid and a slow compression

The results have shown that mushrooms damaged by a rapid compression absorbed more energy than mushrooms subjected to a slow compression using the same amount of energy supplied (Fig. 28). The energy absorbed increases linearly when the total energy supplied to mushrooms during rapid or slow compressions increases. The bruise volume resulting from both the rapid and slow compressions, increases with the increase in energy absorbed. The bruise volume resulting from slow compression tests have shown to be greater that bruise volume resulting from rapid compression test for the same amount of energy absorbed (Fig. 29).

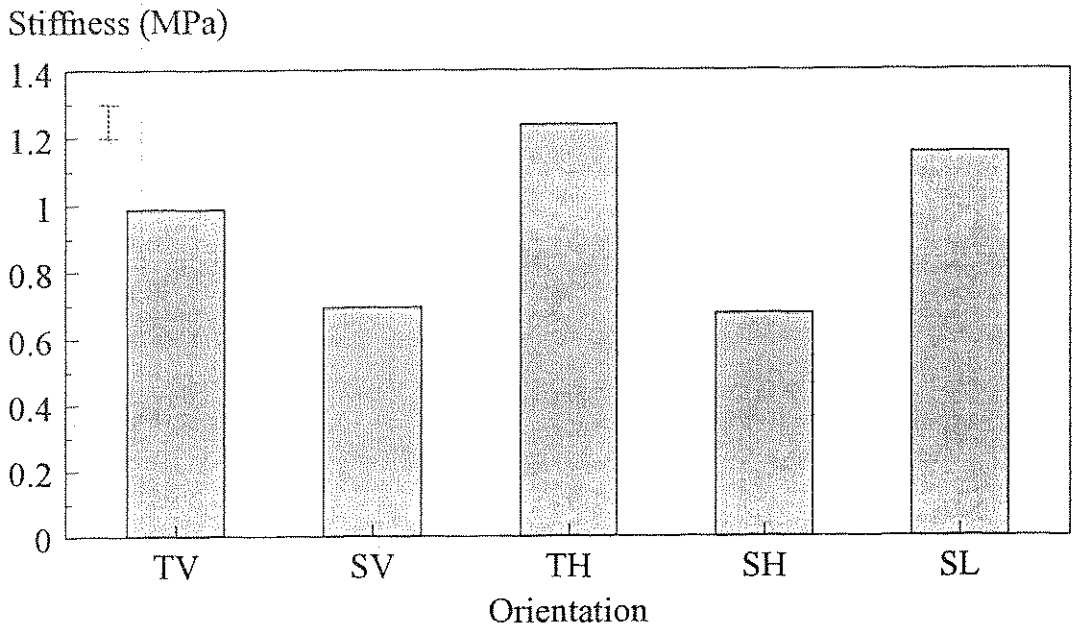


Figure 23: Stiffness of mushroom tissue taken in different position and orientation in the cap (see Fig.)
TV: Top Vertical, TH: Top Horizontal, SV: Side Vertical, SH: Side Horizontal, SL: Side Longitudinal.
Bar indicates Least Significant Difference (0.05)

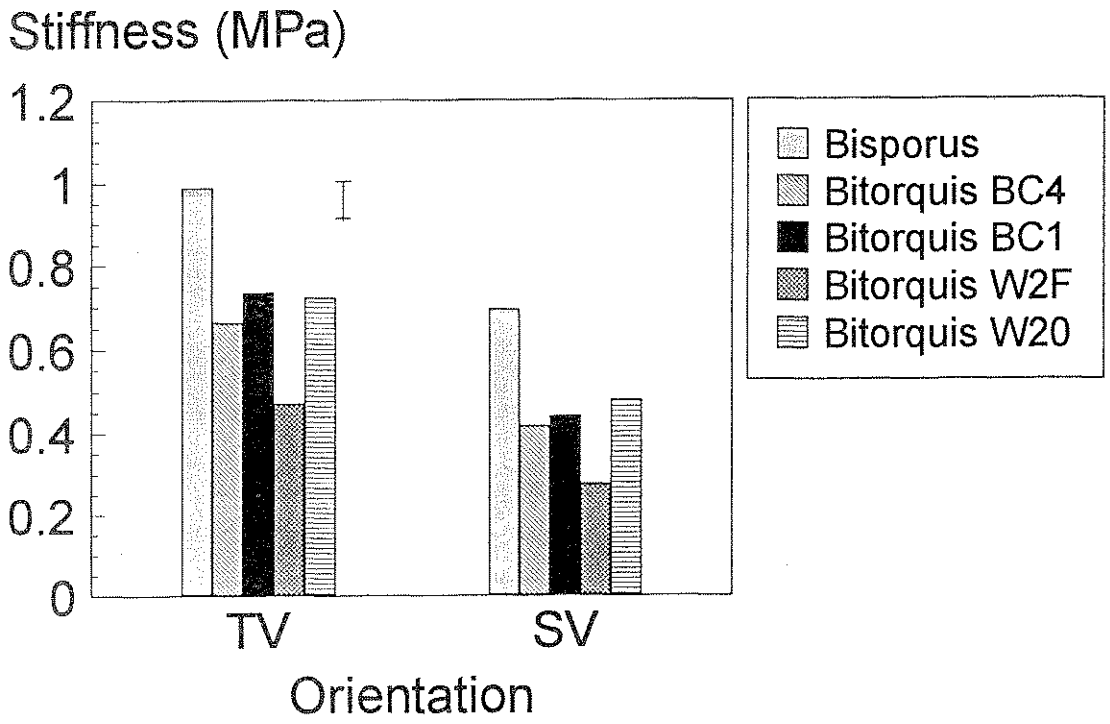


Figure 24: Stiffness of the mushroom tissue in species *A. bitorquis and bisporus*

TV: Top Vertical, SV: Side Vertical. Bar indicates Least Significant Difference (0.05)

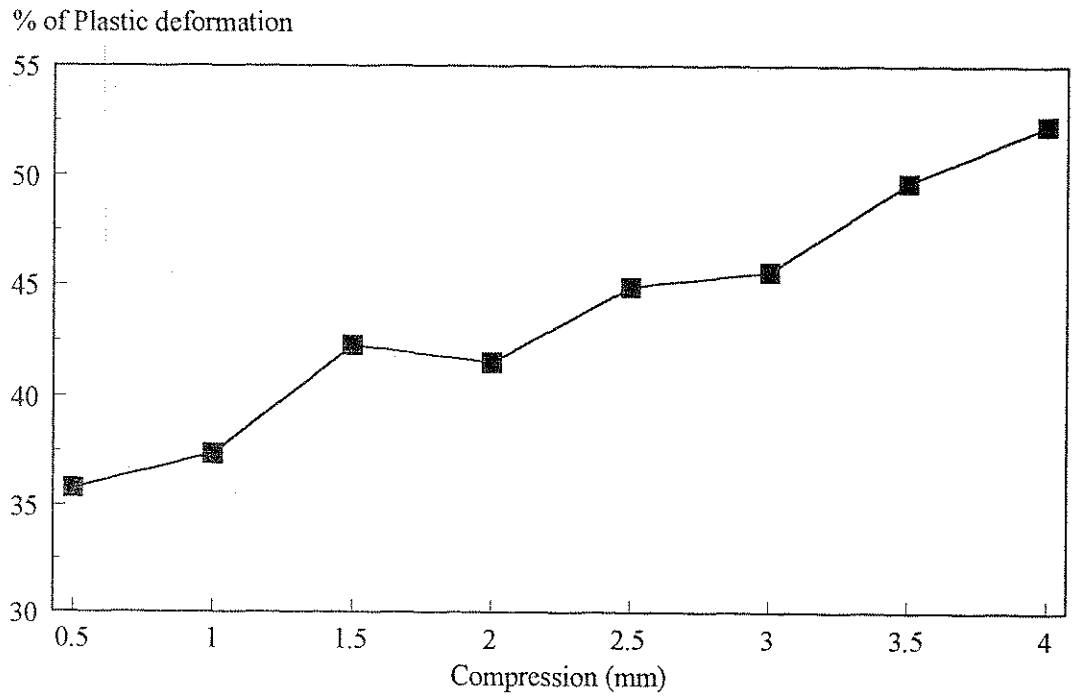


Figure 25: Percentage of plastic deformation produced on mushrooms cap, under compression at increasing displacement.

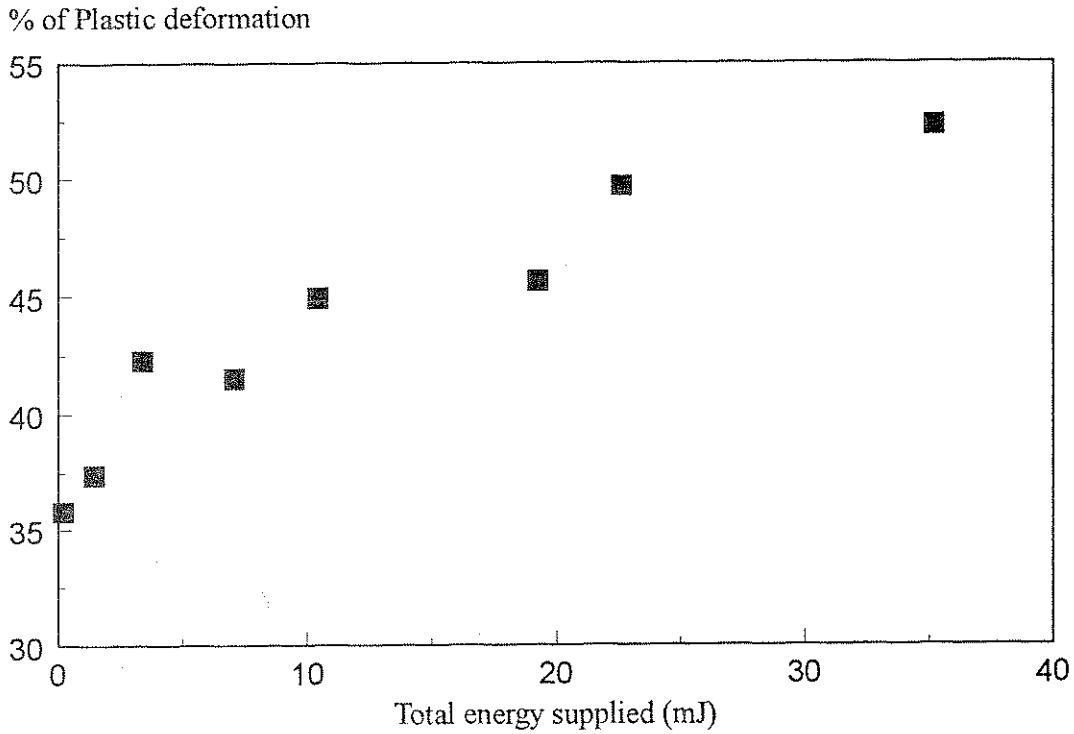


Figure 26: Total energy supplied to mushroom cap to produce a plastic deformation.

Figure 27: Total energy supplied to mushroom cap during compression at increasing displacement.

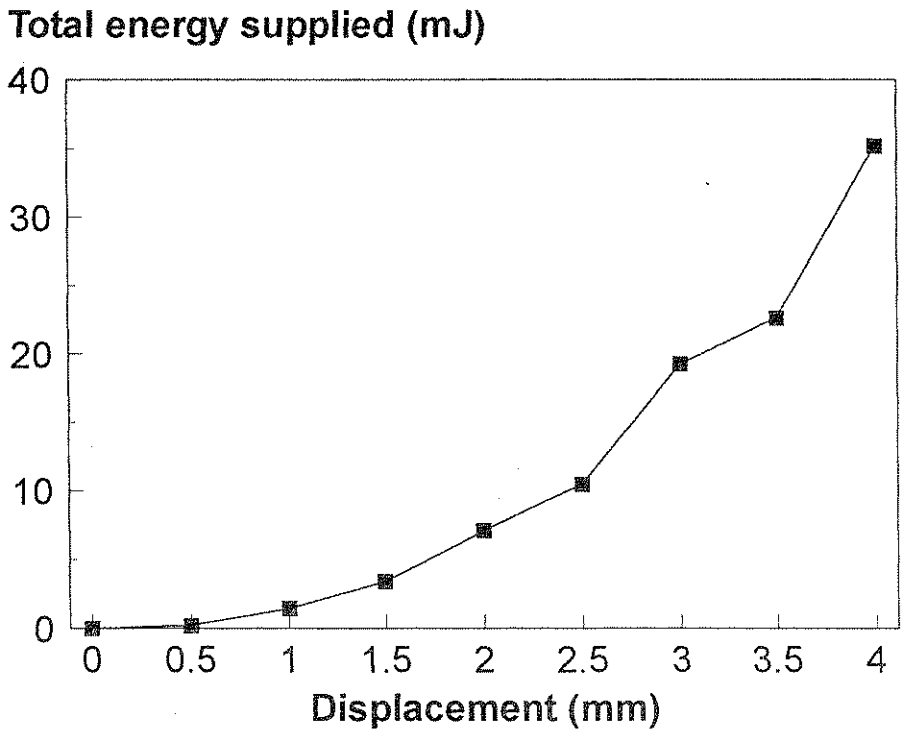


Figure 28: Energy absorbed by mushroom caps when subjected to slow and rapid compressions.

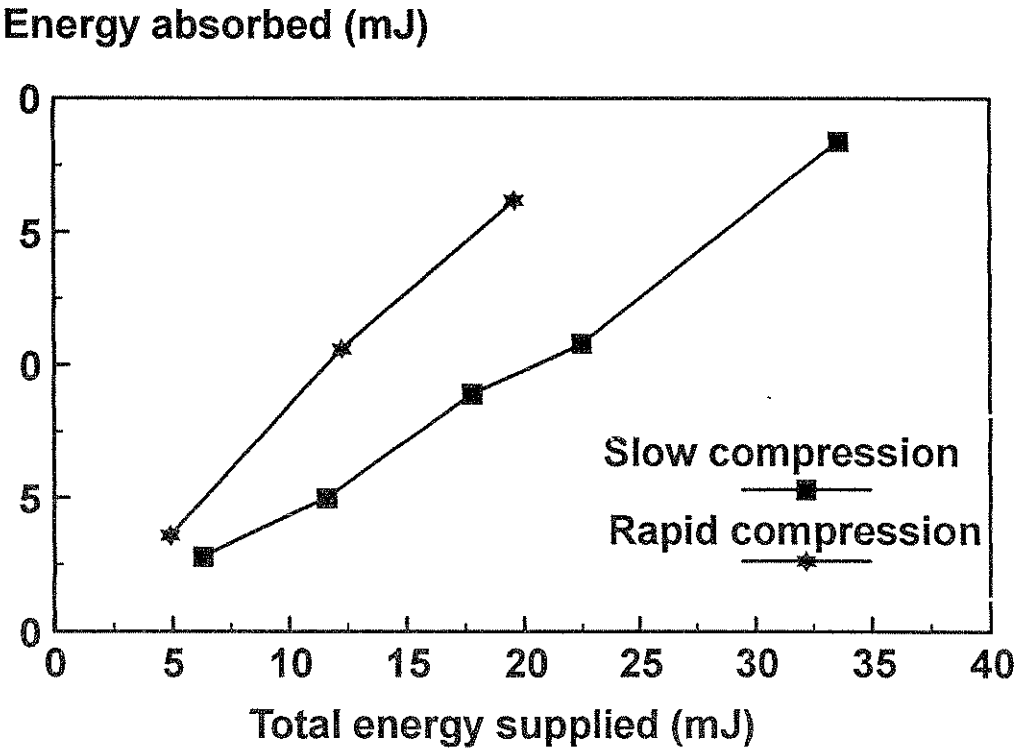
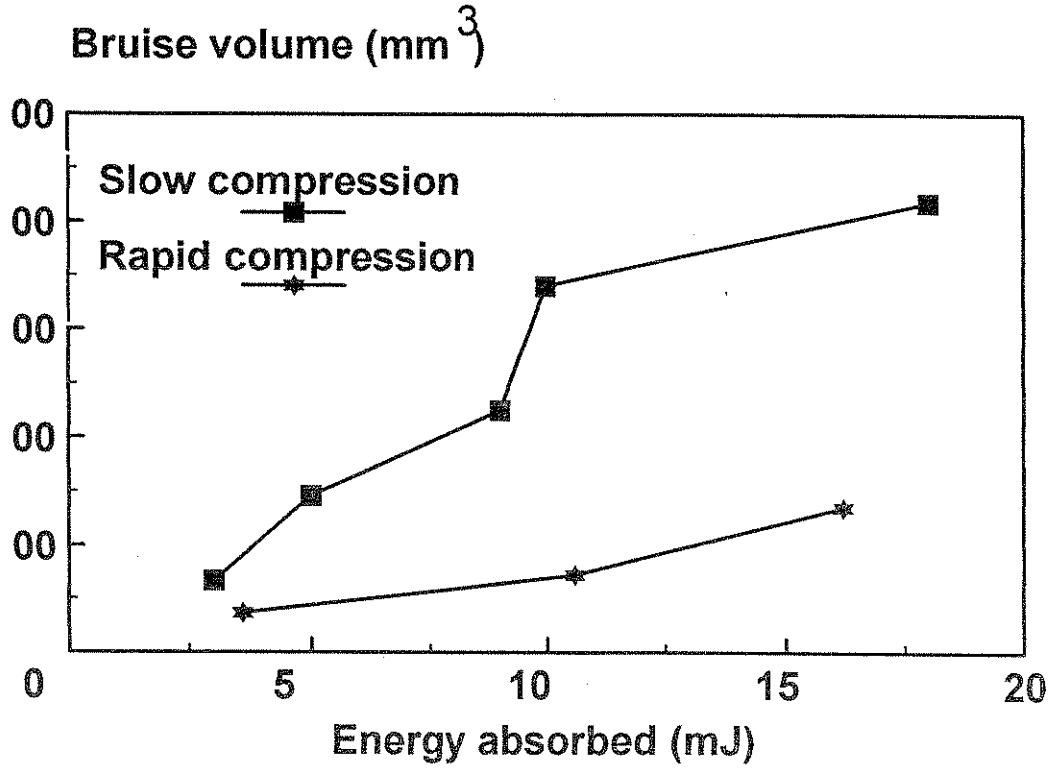


Figure 29: Bruise volume developed in mushroom caps after either a slow or a rapid compression.



IV.4 Discussion

IV.4.1 Relationship of stiffness to hyphal orientation

The measurements have shown that the position and orientation of hyphae affect the stiffness of mushroom tissue. Tissue taken from the top was found to be stiffer than the tissue taken from the side (vertical and horizontal orientation). As reported previously for *A. bisporus* (see IV.3.2) during mushroom growth, primordium need to pierce through heavy casing which this can explain the requirement for the top of the cap to be stronger than the side as it needs to resist the compression forces occurring between the stipe and the casing.

No difference in stiffness was found between cylinders taken from the side in the vertical or horizontal orientation. Although the hyphal orientation was the same in both cylinders from the tops and sides (see Fig. 21), the difference between the two cylinders is that the cylinder taken in the vertical orientation still has the skin on one face. Thus, it seems that the skin does not affect the stiffness of the cap tissue. This was already suspected in a previous experiment (see V. for more details) where the total energy supplied to compress 1 mm whole peeled mushrooms and whole unpeeled mushrooms was the same. Unlike bananas, kiwis, apples... where the skin has for role to resist applied forces, mushroom skin does not seem to have that role. Results found here for the stiffness of mushroom tissue are in the range of the results previously found by McGarry *et al.* (1993) and Hiller (1994). This technique for measuring mushroom stiffness seems reliable provided that all the characteristics concerning the tissue (maturity, storage conditions, position and orientation) are recorded.

IV.4.2 Mechanical characteristics of the upper part of the mushroom cap

The percentage plastic deformation increases with increasing compression displacement (Fig. 25). This reveals the heterogeneity in mushroom tissue previously observed in light microscopy (see Chapter III). Near the surface where the volume fraction is low, the tissue will show little plasticity because air spaces are displaced when a compression force is applied. Deeper into the flesh tissue, fewer air spaces

means compressive forces damage the tissue leaving permanent deformations. It is also possible that, because the hyphal diameter in the flesh are larger than near the surface, these hyphae might be more brittle and would burst under the compressive forces.

IV.4.3 Effects of a rapid and a slow compression on mushroom tissue

Two types of compression, slow and rapid, were performed on mushrooms to reproduce bruises which normally occurred by handling or during transport. The results showed that for the same amount of energy applied, during a rapid compression mushroom caps absorbed more energy than in a slow compression but produced less bruise volume at equal energy absorbed (Fig. 28 & 29). The linearity between energy supplied, energy absorbed and bruise volume can be found with other biological products like potatoes (Noble, 1985), apples (Schoorl *et al.*, 1980) and peaches (Schulte, 1994). The energy absorbed by mushrooms is dissipated by shearing, breaking and compressing hyphae and their contents. Burton *et al.* (1986) hypothesized that discoloration at mushroom surface occurs when the enzyme tyrosinase,and the phenolic substrate, originally in two different compartments, are mixed after a mechanical damage or during senescence. It is probable that rapid compression does not damage mushroom hyphae the same way a slow compression does. During a slow compression more hyphal contents are probably disturbed than in a rapid compression therefore more of a bruise was developed.

V Environment and agronomic effects

V.1 Introduction

There is anecdotal evidence that the same strain when cultivated in different farms can have different textural properties, suggesting that texture can be influenced by growing techniques. As variations in texture can not come from their genotype (considering the same strain), texture might be influenced by external factors like humidity, atmospheric gas, growing conditions and techniques. The importance of these experiments is for the mushroom growers who will be able to experiment the results on their farms. Although the growers try to optimize the growing conditions to produce high quality mushrooms, they can still loose their expected quality. By the design of these experiments, it is hoped to identify how environment and agronomic effects influence mushroom texture and quality and how growers could adjust the growing conditions during a crop if needed.

The agronomic and environment effects on mushroom texture and quality were tested in a serie of factorial experiments:

- Storage
- Casing type x Humidity level
- Casing depth x Compost depth
- CO_2 level x Strain-cultivars
- Supplements
- Water potential x Casing type

V.1.1 Storage

Mushrooms often arrive a couple of days after harvesting on the supermarket shelves. During this time, the mushrooms have had time to continue their development. The aim of this experiment was to look at the effects of storage on mushroom texture and to see if mushroom skin, from fresh and stored mushrooms, affects mushroom texture.

V.1.2 Casing type x Humidity level

At the HRI mushroom unit, mushrooms are grown using casing based on sugar beet lime and peat. Usually, the choice of casing for growers is based on the availability of the products, often waste from industries or local products are used. For that reason, the formulation of casing varies from areas and countries. The optimum relative humidity of the growing rooms is about 85 %, at high humidity diseases could prolifere (Brown blotch, Trichoderma, verticillium...) and at low humidity the mushroom yield is reduced and mushrooms are dry out. Nevertheless, mushrooms were grown at these 3 humidity levels and tested for the quality. The aim of the experiment was to see if casing mixture and the growing room relative humidy could affect mushroom texture and quality.

V.1.3 Casing depth x Compost depth

Mushrooms are grown on compost and casing depth which vary from farm to farm because it depends on which casing and compost are used. The compost and casing amount used for growing mushrooms has an effect on the amount of nutrients available, the water availability and the fruit body induction. Experiments were designed to test how the compost and casing depth can affect mushrooms texture and quality.

V.1.4 CO_2 level x Strain-cultivars

There are many *A. bisporus* strains available from the spawn company. Each strain has it own characteristic and mushrooms produced have different quality and textures. The quality properties of four strains were tested in this experiment. Mushrooms usually grow at HRI in rooms were the CO_2 level is around 1000 ppm. A lower level of 800 ppm and a higher level of 1200 ppm were set up in the growing rooms to look at the effect of CO_2 level on mushroom texture and quality.

V.1.5 Supplements

It is claimed by manufacturers that adding supplements during the preparation of compost, can increase the mushroom yield. In this experiment three commercial supplements, added at two different rate, were tested to determine their effect on texture and quality.

V.1.6 Water potentials

The major compound in mushrooms is water, which represent 85 to 92 % of the weight. Mushrooms can loose up to 10 % water after one day storage at 15-21°C (Gormley *et al.*, 1967) and this can affect their texture. Water has also a great influence on discoloration. It is well known that wet mushrooms, when harvested, are more susceptible to discoloration. An experiment was designed to study mushroom texture and discoloration on mushrooms grown on casing of variable water potentials.

V.2 Materials and methods

V.2.1 Growing conditions and agronomic variations

V.2.1.1 Storage

Mushrooms were harvested and stored 1, 2, 3 and 4 days at 18°C under a relative humidity of about 90% before being tested.

V.2.1.2 Casing types

- French style: based on 25% peat and 75% of oolitic limestone from Smiths limestone, Broadway, Gloucester. The limestone is a mixture of 2/3 of larger ground size plus 1/3 of smaller ground size limestone. The final preparation has a yellow-beige colour and has the consistency of moist ready-mix concrete.
- Peat based casing: consists of Irish Spagnum peat supplemented with chalk (1 vol of chalk for 12 vol of peat). It has the consistency of porridge with a light brown colour. This casing is used in Ireland.

- English style: consists of Nooyen ready mix (80% peat, 20% sugar beet lime) which has a dark colour and a consistency of fibrous mud, for more details see II.
- Bulk peat based casing: consists of a mixture of sugar beet lime (25 %) and bulk peat (75 %)
- Mill peat based casing: consists of a mixture of sugar beet lime (25 %) and mill peat (75 %)

V.2.1.3 Humidity of the growing chambers

Mushrooms were grown at a standard relative humidity of 85 %. An experiment was designed to determine the effects of a lower and a higher relative humidity (75 and 95 %) on mushroom texture.

V.2.1.4 Casing and compost depths

Mushroom were grown under standard conditions on compost and casing of three different depths. Trays were filled with 3 different amounts of compost: shallow, medium and deep which represent 4.5, 10.5, 19 kg of compost respectively. The trays were then covered with a sugar beet lime casing of a depth of: 25, 40 or 55 mm.

V.2.1.5 Compost supplements

During the preparation of compost for mushroom cultivation, the supplements Betamyl, Springboard or Promycel were added at a rate of 0.5% and 1% (w/w).

V.2.1.6 Water potential in casing

First, the moisture content of the casing was measured on a weight basis. Then the water potential was determined from a calibration curve for water potential and moisture content. The water potentials of each casing is summurized in the table below. The potential is expressed in mm of water. A low water potential is equivalent to a wet casing and a high potential to a dry casing.

Water Potential	P1	P2	P3	P4	P5	P6
Bulk peat	1.17	250	478	569	963	1107
Mill peat	215	263	381	747	1251	1551

V.2.2 Mechanical tests performed on in each growing condition and agronomic effect experiment

V.2.2.1 Storage experiment

Whole mushrooms with the stipe removed were compressed at a displacement of 1 mm. Tests were performed on top and side of the sporophore with the skin retained or removed. Skin was dissected from the cap by peeling from the edge to the centre. The compression tests were performed on freshly harvested mushrooms (day 0) and on mushrooms stored for 1, 2, 3 and 4 days at 18°C.

The experiment had a factorial design with the following number of treatments: 2 positions (tops or sides) x 2 tissues (skin retained or removed) x 5 storage days (day 0, 1, 2, 3 and 4). For each treatment combination, 15 mushrooms were examined. The data were statistically analyzed by analysis of variance.

V.2.2.2 Casing type x humidity experiment

Mushrooms were grown in chambers under 3 different relative humidity: 79%, 85% and 95%. Mushrooms from the first three flushes of each treatment were harvested and cubes of mushrooms were compressed at a displacement of 1 mm. The total energy supplied and the plastic deformation were recorded.

The experiment had a factorial design with the following number of treatments: 3 casing type x 3 humidity. For each treatment combination, 10 mushrooms were tested. The data were statistically analyzed by analysis of variance.

V.2.2.3 Casing depth x compost depth experiment

Stage 2 mushrooms were harvested and cubes were taken of for compression at a displacement of 4 mm. The total energy supplied and the plastic deformation were recorded.

The experiment had a factorial design: 3 compost depth levels (Shallow, Medium, Deep) x 3 casing depth levels (25, 40, 55). For each treatment combination, 10 mushrooms were tested. The data were statistically analyzed by analysis of variance.

V.2.2.4 Mushroom strain x CO_2 Level experiment

Mushrooms, strain A12, U3, S130 and U1 were grown under 3 different levels of $C0_2$: 800, 1000 (usual level) and 1200 ppm. Cubes of mushrooms were taken for compression at a displacement of 4 mm. The total energy supplied and the plastic deformation were recorded.

The experiment had a factorial design: 4 strain types x 3 CO_2 levels. For each treatment combination, 15 mushrooms were tested. The data were statistically analyzed by analysis of variance.

V.2.2.5 Supplements experiment

The stiffness of the mushrooms was measured on cylinders removed from the top in the vertical orientation, 2 mm underneath the surface (see IV.).

The experiment had a factorial design: 3 supplement types x 2 rates. For each treatment combination, 10 mushrooms were tested. The data were statistically analyzed by analysis of variance.

V.2.2.6 Water potential x casing type

The stiffness of the mushrooms was measured on cylinders removed from the top in the vertical orientation, 2 mm underneath the surface (see IV.).

The experiment had a factorial design: 6 water potential levels x 2 casing types. For each treatment combination, 10 mushrooms were tested. The data were statistically analyzed by analysis of variance.

V.2.3 Bruise area calculation

Cubes of mushrooms were compressed according to the method described in III. The cubes were stored 2 days at room temperature and then, slices of about 2 mm thick was cut across the compression. The slices were placed on a U.V. transilluminator (UVP, Inc., San Gabriel, USA) to reveal the bruise area caused by compression, and photographic records were taken with a Mitsubishi video copy processor. The bruise area was then measured by image analysis.

V.2.4 Dry weight

Cylinders taken from the mushroom caps for stiffness measurements were allow to dry one week at room temperature.

V.2.5 Tyrosinase activity

The skin of the sporophore was obtained by peeling the mushroom from the side to the centre. The skin was rapidly frozen in liquid nitrogen and stored at -18°C. Samples were ground and homogenised in 7 ml 100mM sodium phosphate buffer, pH 8.0. The extracts were centrifuged 20 min at 35,000 *g* and the supernatant was tested spectrophotometrically for tyrosinase activity in 10 mM catechol, 10mM proline in 100mM sodium phosphate buffer pH 6.0. The activity was measured at 525 nm at 30°C by measuring the initial slope of increase in absorbance. The activity was also measured in the presence and absence of SDS in a final concentration of 0.1% (w/v).

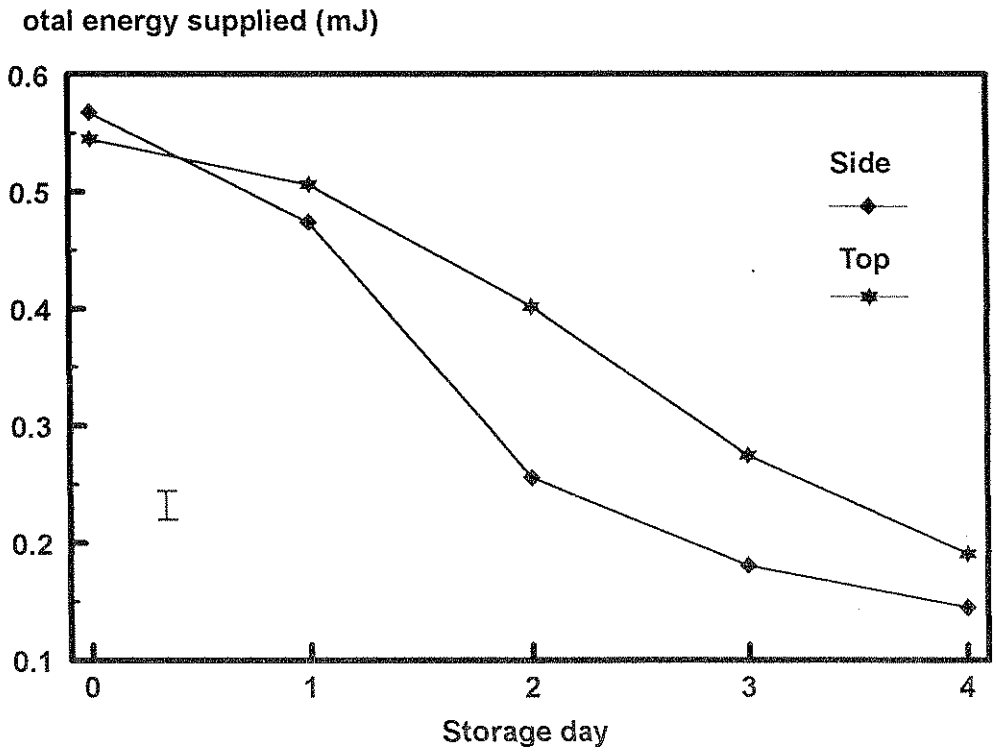
V.3 Results

V.3.1 Storage

The analysis of variance has shown that there is no significant difference in the energy absorbed to compress the mushroom tissue with or without the skin. However a significant difference ($p = 0.001$) was identified between the top and the side of the

mushroom under compression (Fig. 30). When the results over the storage period are combined, the top of the mushroom cap was found to require 18% more energy (ie firmer) to be compressed by 1 mm than the sides ($p = 0.001$). However, the analysis of variance reveals that the energy to compress either tops or sides of fresh mushrooms (day 0) is approximately the same. During storage, the energy required decreased for both tissues, but the decline was significantly more rapid in the sides than the tops ($p = 0.001$). It has been found a decrease of 55% for the side and 26%

Figure 30: Total energy supplied on fresh and stored mushrooms



for the top after 2 storage days.

V.3.2 Casing x Humidity

Mushrooms from flush 1 were the most plastic (i.e. most liable to permanent deformation) followed by flush 3 and then flush 2 (the mushrooms most resistant to deformation). The sides of the mushrooms were found to have significant ($p < 0.001$) higher plasticity (i.e. softer) than the tops. When french style casing was used the difference between tops and sides was more pronounced (Fig. 31).

Similarly, the analysis of variance of the energy required for the probe to compress the tissue by 1 mm, found no significant differences between humidity or casing treatments (Fig. 32). Flush 2 mushrooms were found to require significantly ($p < 0.001$) more energy (i.e. therefore firmer) than mushrooms in flush 3 follow by flush 1. Also analysis of the energy requirement showed that sporophore tops required more energy (i.e. firmer) than the sides (Fig. 33).

The analysis of variance showed that there is no significant differences in tyrosinase activity between the humidity treatments and the casing type treatments in presence or absence of SDS.

V.3.3 Casing depth x Compost depth

The analysis of variance of plasticity data showed that the main treatment effect of plasticity come from casing depth ($p<0.001$). The 25 mm casing treatment had the lowest plasticity (most resistant to permanent deformation) followed by 40 mm treatment and then 55 mm treatment (Fig. 34). This trend was more prominent for flush 1 mushrooms than flush 2.

The analysis of variance of the energy requirement to make the compression revealed that both casing depth and compost depth were highly significant ($p < 0.001$), Fig. 35. When examining the overall treatments, deep compost produced the highest energy requirement (i.e. firmest) followed by medium and then shallow compost. The casing treatment followed the opposite trend, the highest energy requirement (firmest) being observed with the 25 mm casing depth. Therefore, of the treatments combinations, the highest energy requirement was with mushrooms grown in deep

Figure 31: Percentage of plastic deformation on top and side of mushroom caps for the three casing types.

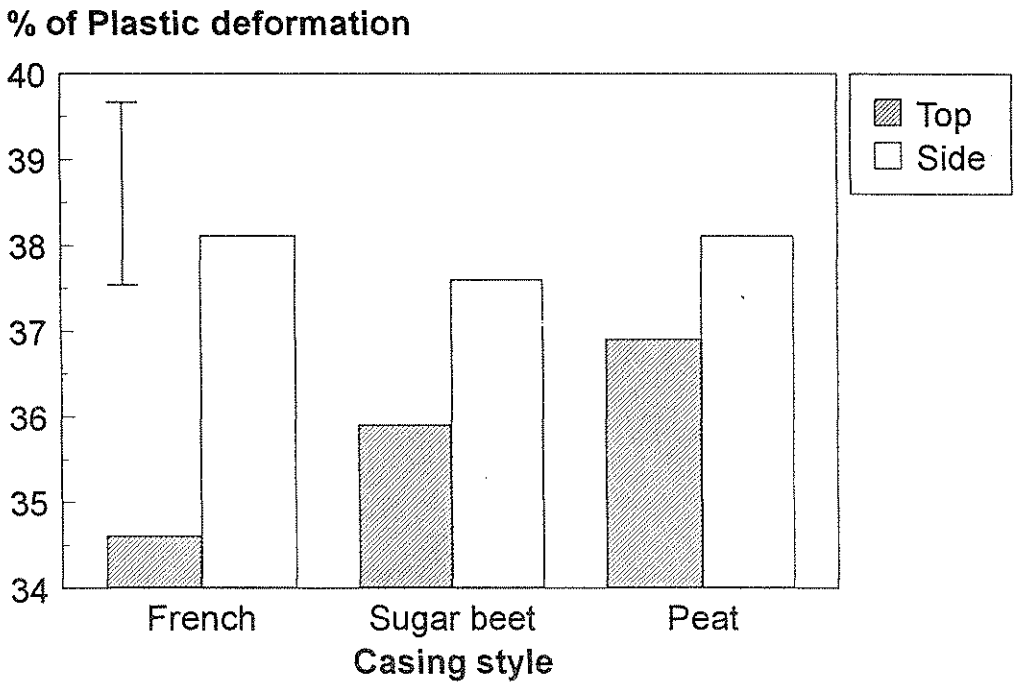


Figure 32: Total energy supplied on top and side of mushroom caps for the three casing types

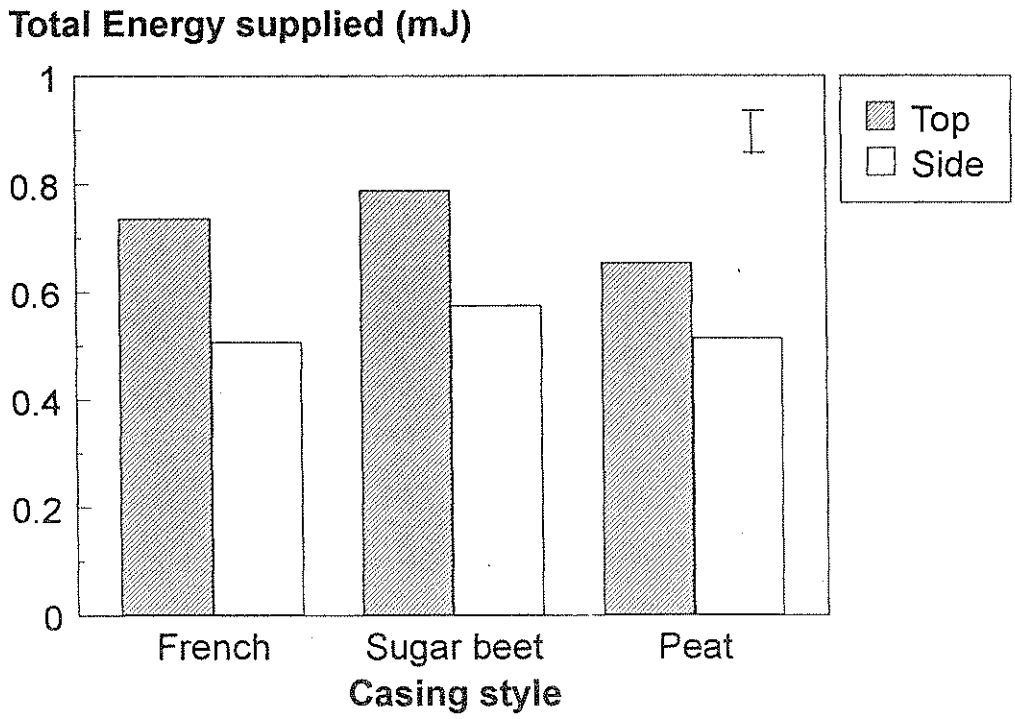
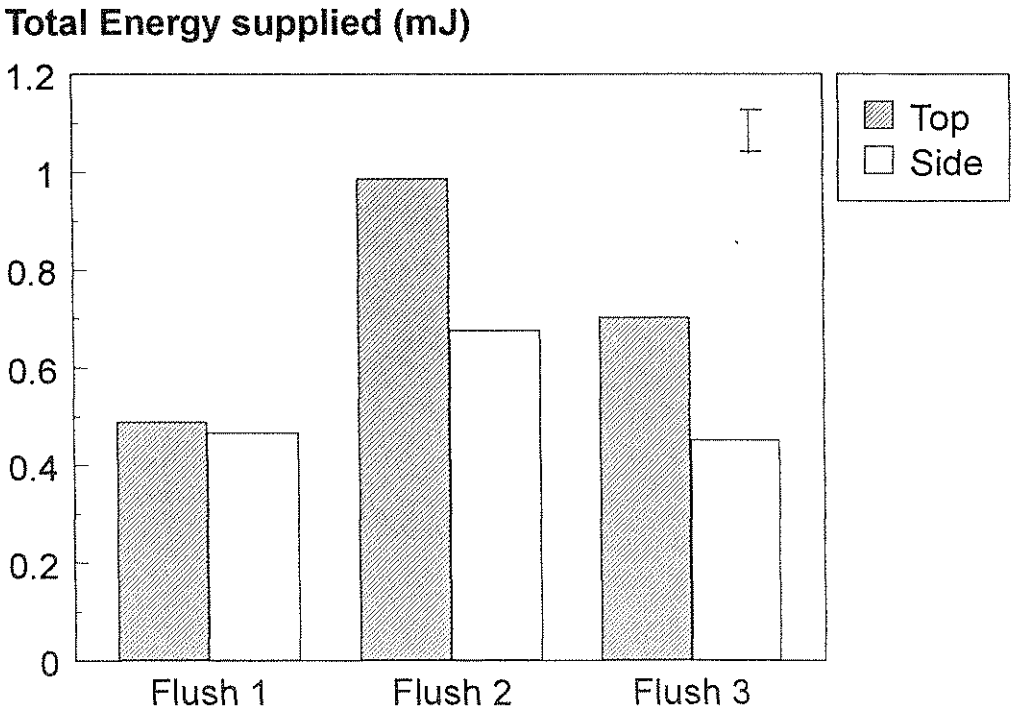


Figure 33: Total energy supplied on top and side of mushroom caps over the first three flushes.



shallow casing.

However, the analysis of the percentage of dry matter content showed that treatment 25D had a higher percentage of dry matter than 40M which in turn had a higher percentage of dry matter than 55S (Fig. 36).

V.3.4 CO_2 level x Mushroom strain

The statistical analysis has shown a significant difference ($p < 0.001$) in energy requirement between the CO_2 level treatment (Fig. 37). There is a trend in firmness for the three CO_2 levels. Mushrooms grown under low CO_2 level (800 ppm) had a higher energy requirement (firmer), than the mushrooms grown under medium CO_2 level (1000 ppm) which in turn were higher than the mushrooms grown at 1200 ppm which produced the softest mushrooms. Measurement of energy in the four strains has revealed a significant difference ($p < 0.1\%$). Strain U3 was found to be much firmer than strain U1, S130 and strain A12 which had approximately similar values (Fig. 39).

The analysis of variance on plasticity revealed that both strains and CO_2 levels are significant ($p < 0.1\%$). There is a trend for CO_2 level, mushrooms produced under a low CO_2 level were more plastic (less resistant to permanent deformation) than mushrooms produced under high or medium CO_2 level (Fig. 38). Strain U3 was found to be the more plastic over the first three flushes than S130, U1 and A12 which were similar to one another (Fig. 40). The statistical analyses appear to be contradictory as low CO_2 level grown mushrooms required more energy for compression (i.e. firmer) but that compression leads to greater plastic deformation.

Figure 34: Total energy supplied to compress mushroom caps grown on variable casing and compost depths.

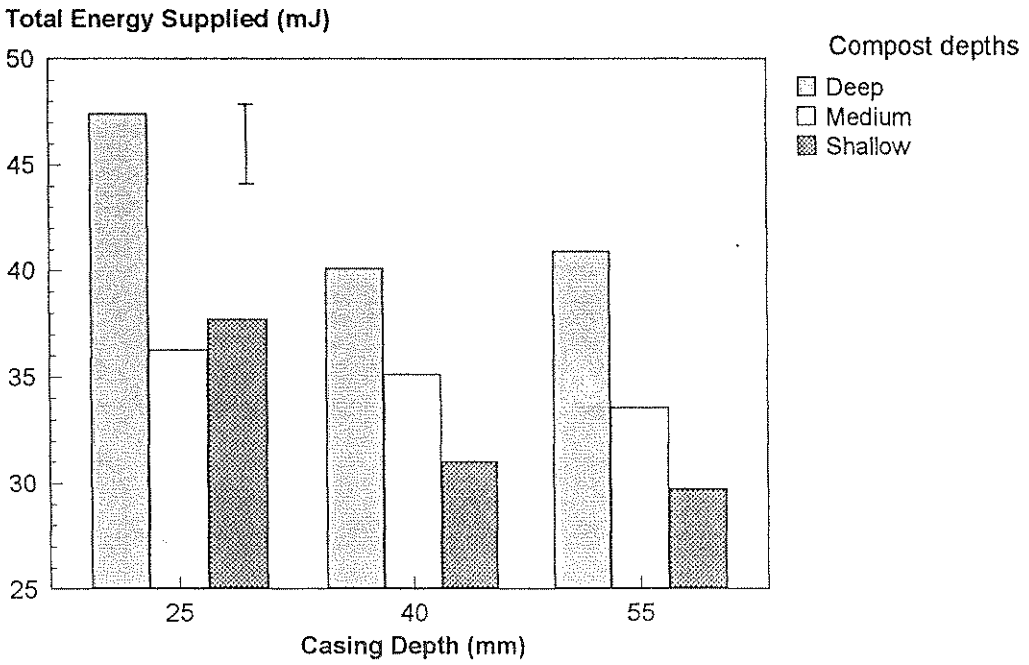


Figure 35: Percentage of plastic deformation after compressing mushrooms grown on variable casing and compost depths.

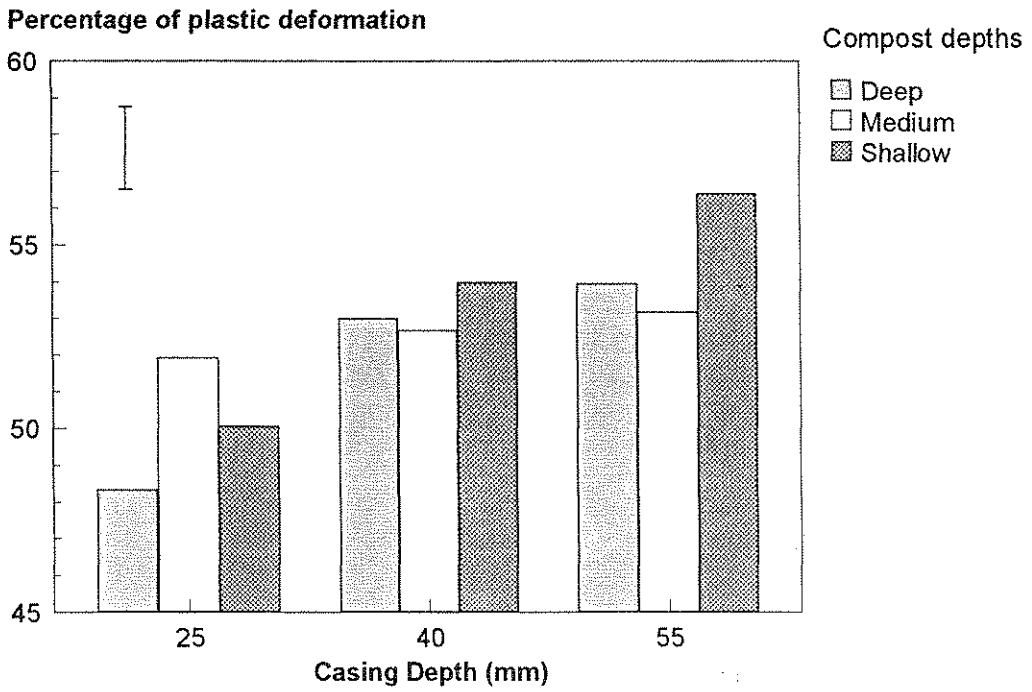


Figure 36: Percentage of dry matter content of mushrooms grown on variable casing and compost depths.

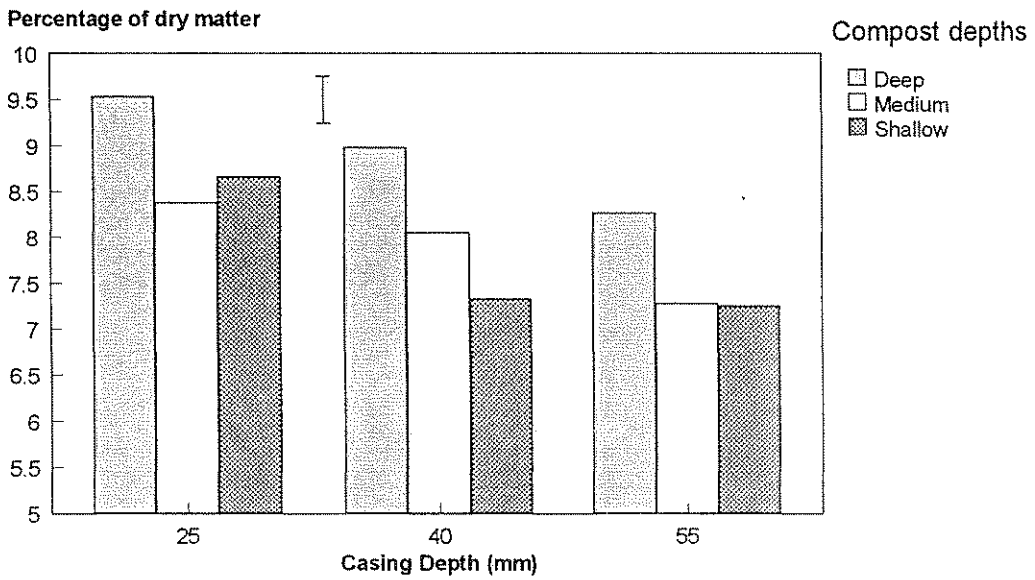


Figure 37: Total energy supplied to compressed mushroom caps grown at three atmospheric CO_2 levels.

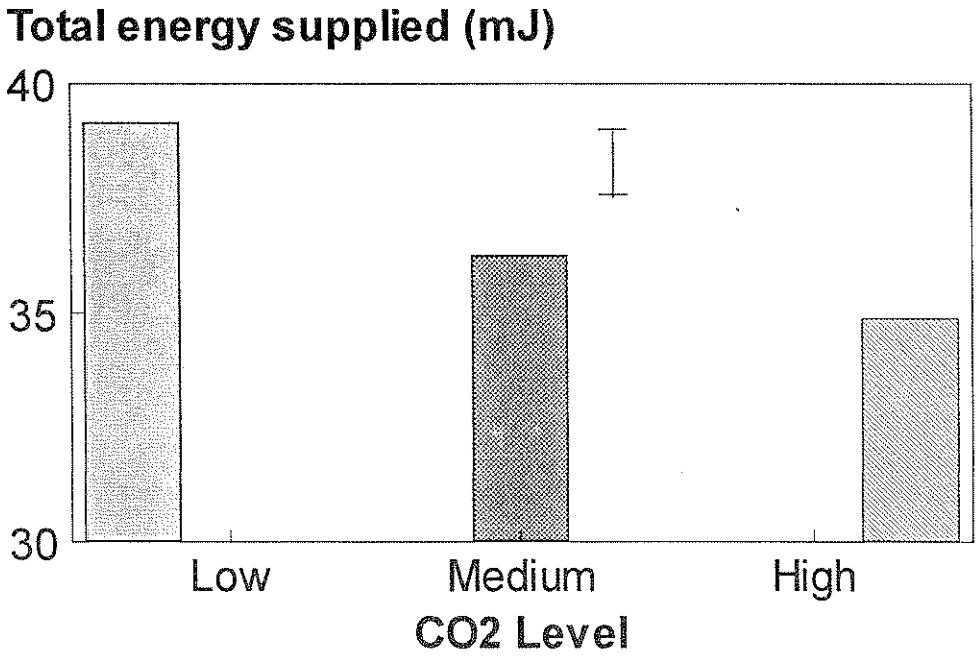


Figure 38: Percentage of plastic deformation after compression of mushrooms grown at three atmospheric CO_2 levels.

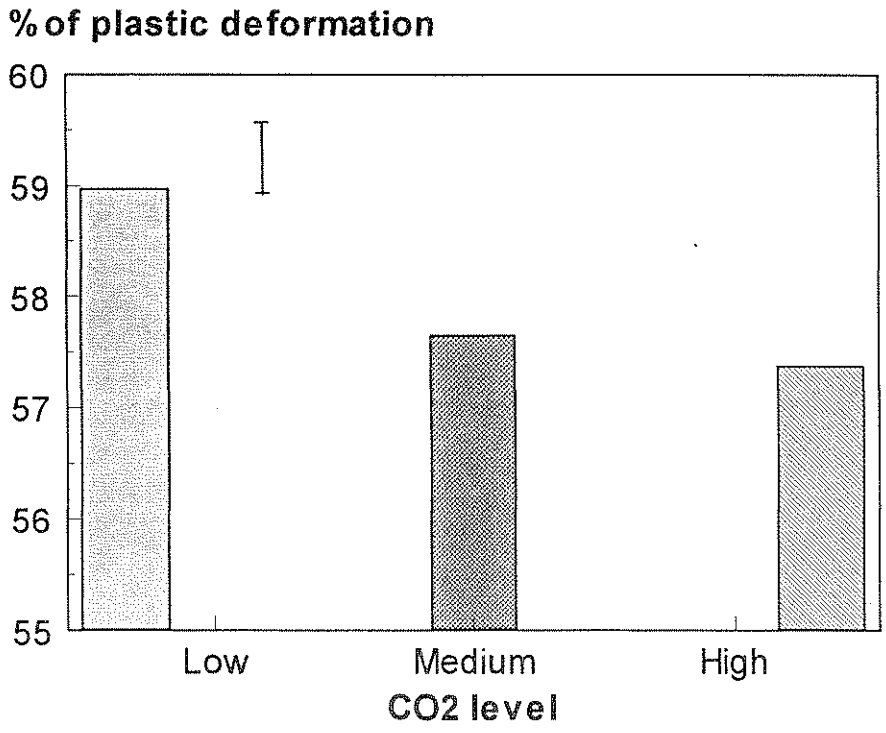


Figure 39: Total energy supplied to compressed mushroom caps of four commercial mushroom strains

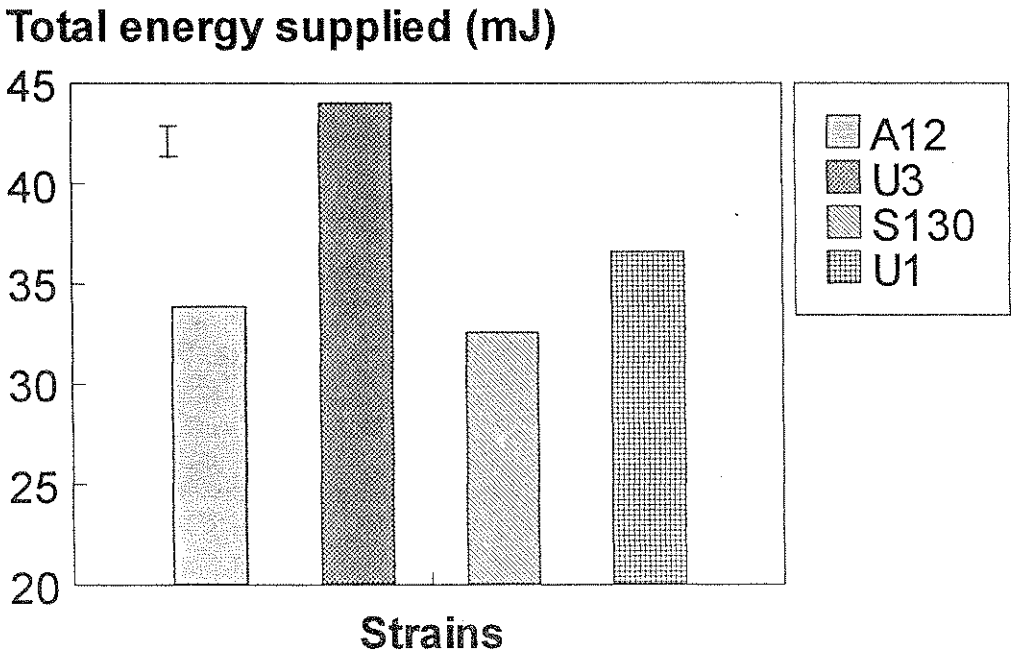
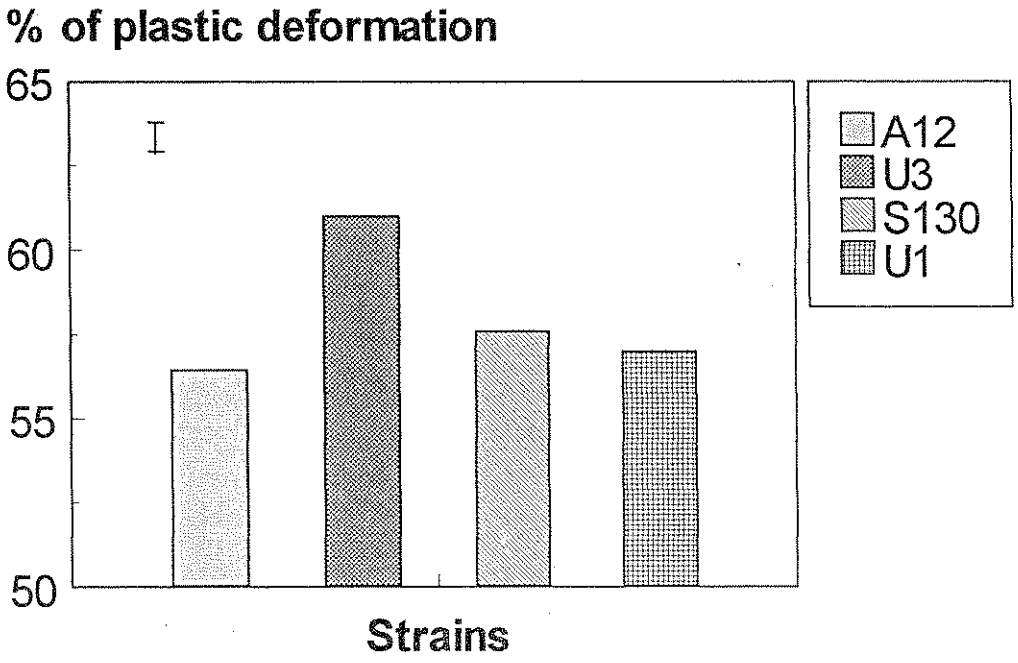


Figure 40: Percentage of plastic deformation after compression of four commercial mushroom strains



V.3.5 Compost supplements

The analysis of variance has shown that there is no significant difference, on average, in stiffness between mushrooms grown on supplemented compost or not (Fig. 41). A major effect was found in flush 3 which produced significantly less stiff mushrooms ($p<0.001$) for both supplemented and non-supplemented compost.

There is, however, a major effect ($p<0.001$), on average, of the dry weight of mushroom cores between the control (no supplement added to compost) and the supplemented compost (Fig. 42). The control compost had a higher dry weight of mushroom cores. By looking in detail at the supplemented compost, only Betamyl and Promycel added at a rate of 0.5% produced mushrooms with lower dry weight of mushroom cores ($p<0.05$). It was found that, on average, flush 3 mushrooms cores had a lower dry weight ($p<0.001$). During the first flush, there is no significant difference in mushroom core dry weight, between supplemented and non-supplemented compost. Then, during flush 2 and 3, the supplemented compost produced mushrooms with significantly ($p<0.05$) lower mushroom core dry weight.

A positive correlation was found between the dry weight of mushroom cores and their stiffness (Fig. 43). The average regression equation is $y = -0.2684 + 0.0237\,x$ ($r^2 = 0.95$). The yield produced in this crop is higher than the average yield produced in farms, however, the treatments with 0.5 % Betamyl and 1 % Sprinboard had a slightly lower yield than the other treatments (Fig. 44).

V.3.6 Water potential x casing type

There is a significant ($p<0.001$) effect of casing on mushroom stiffness. Mushrooms grown on bulk peat were found on average stiffer than mushroom grown on mill peat. The water potential of the casing had also, on average, a significant ($p<0.005$) effect on mushroom stiffness. Potential P1 and P2 produced significantly stiffer mushrooms. When the interaction between casing and water potential was analyzed, mushrooms grown on bulk peat were the stiffest mushrooms at potential P1 and mushrooms grown on mill peat were the stiffest at P2 (Fig. 45). At all other potential, mushrooms grown on mill peat were not significantly different in stiffness. Flush 3 mushrooms

Figure 41: Stiffness of mushrooms grown on supplemented compost

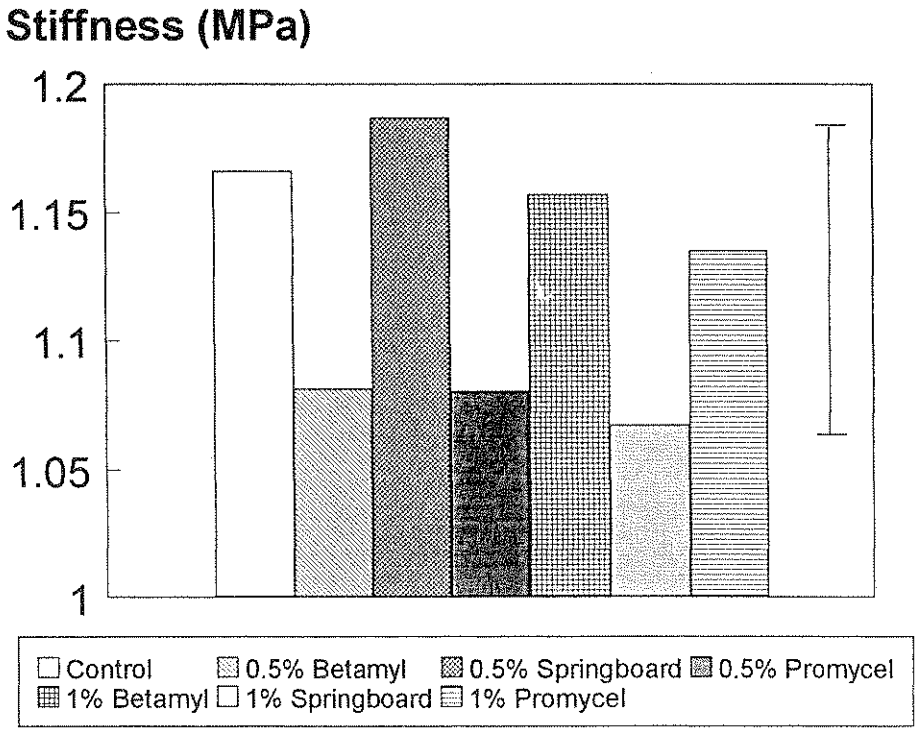


Figure 42: Dry weight of mushrooms grown on supplemented compost

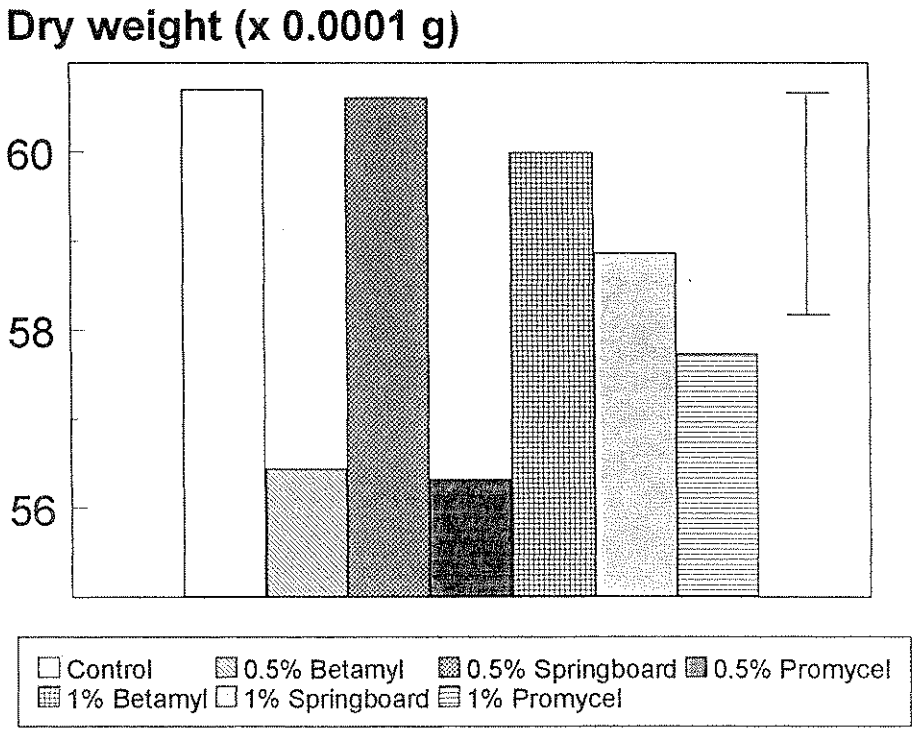


Figure 43: Relationships between stiffness and dry weight of mushrooms grown on supplemented compost

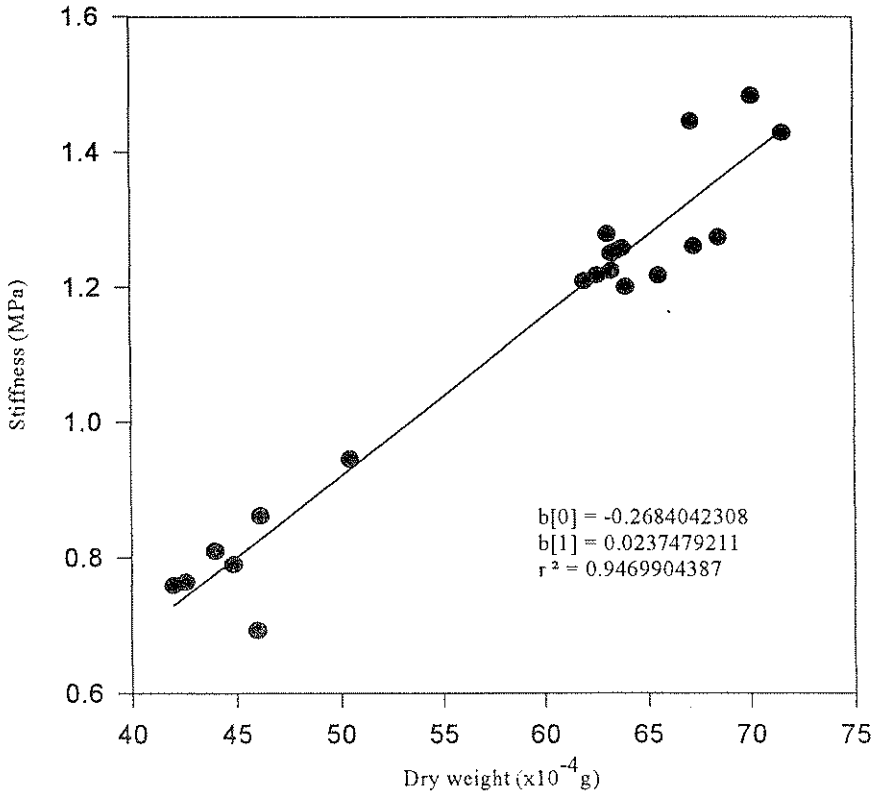
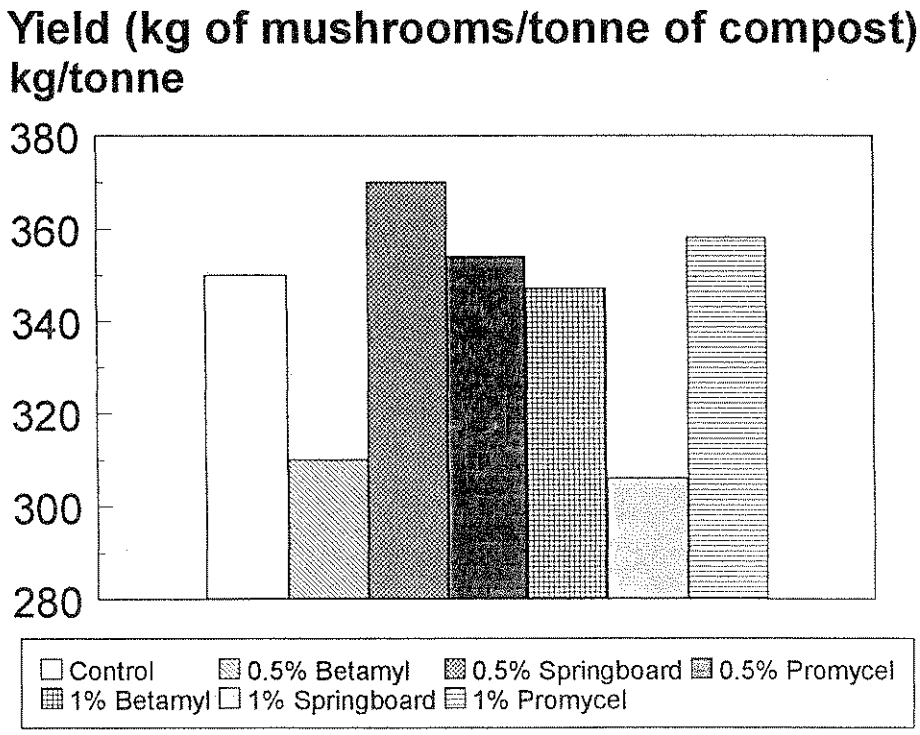


Figure 44: Yield of mushrooms grown on supplemented compost



were found to be significantly ($p<0.001$) the stiffest of the 3 flushes. Mushrooms grown on bulk peat based casing had significantly ($p<0.001$) higher dry weight of mushroom cores than mushrooms grown on mill peat based casing. There is a significant decreasing trend of the dry weight on mushroom cores between potential P1 and potential P5. This trend is mainly due to mushrooms grown on bulk peat. Mushrooms grown on mill peat had a significant higher dry weight only when grown on water potential P2 (Fig. 46). Flush 3 mushrooms were found to have the highest dry weight of mushroom cores of the 3 flushes.

A positive correlation was found between the dry weight of mushroom cores and their stiffness from mushrooms grown on mill peat based casing (Fig. 47). The average regression equation is $y = - 0.3749 + 0.0263\ x$ ($r^2 = 0.69$). No significant correlation ($r^2=0.20$) was found between the dry weight of mushroom cores and their stiffness from mushrooms grown on bulk peat based casing (Fig. 48).

V.4 Discussion

The agronomic and enviroment experiments have shown that firm or soft mushrooms can be produced by variation of growing treatments. A major effect observed was that the second or third flush consistently produced the firmest mushrooms. It was found that the mushroom top was on average firmer than the side. Although these tests were carried out on whole or half mushrooms, it has been found earlier (see chapter IV) by measuring the stiffness of mushroom tissue that the top was firmer than the side. The skin tissue either at the top or side of sporophore appears to offer no protective function against mechanical damage to the rest of the mushroom. This is in contrast with apples, bananas, kiwis etc., where the skin plays an important role in preventing cracking and damages.

Over a storage period of 5 days, it was found that mushroms keep their texture during the first storage day, but then, the mushroom firmness decreases.

When mushrooms where grown at high or low relative humidity, no affect on texture was observed. However, when mushrooms were grown on casing (wet to dry), a

Figure 45: Stiffness of mushrooms grown on 2 types of casing and at various water potentials.

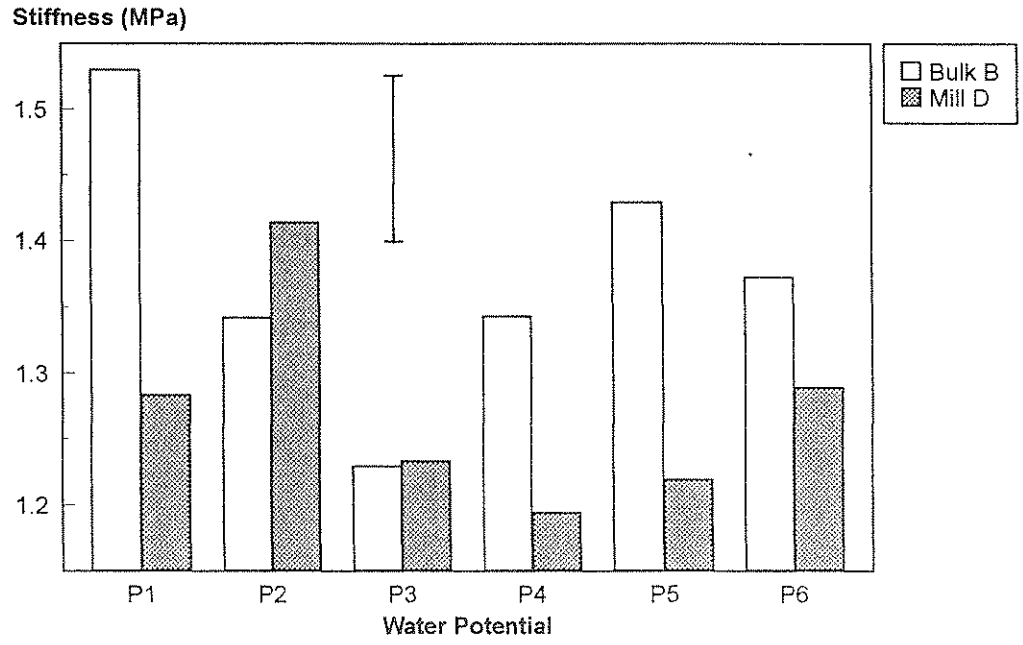


Figure 46: Dry weight of cores from mushrooms grown on 2 types of casing and at various water potentials.

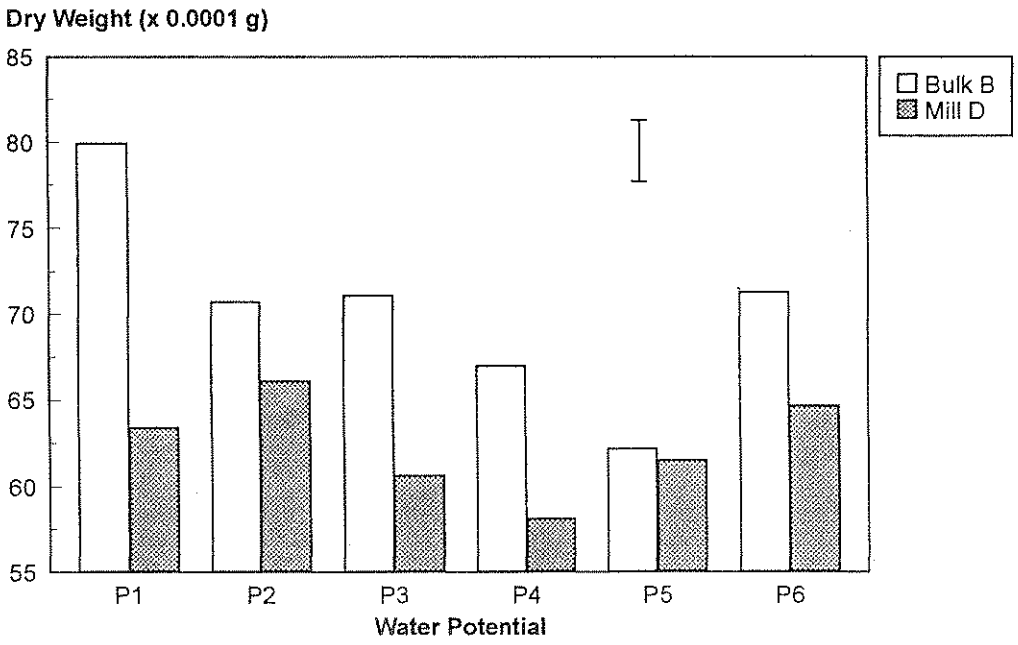


Figure 47: Relationship between stiffness and dry weight of mushrooms grown on mill peat.

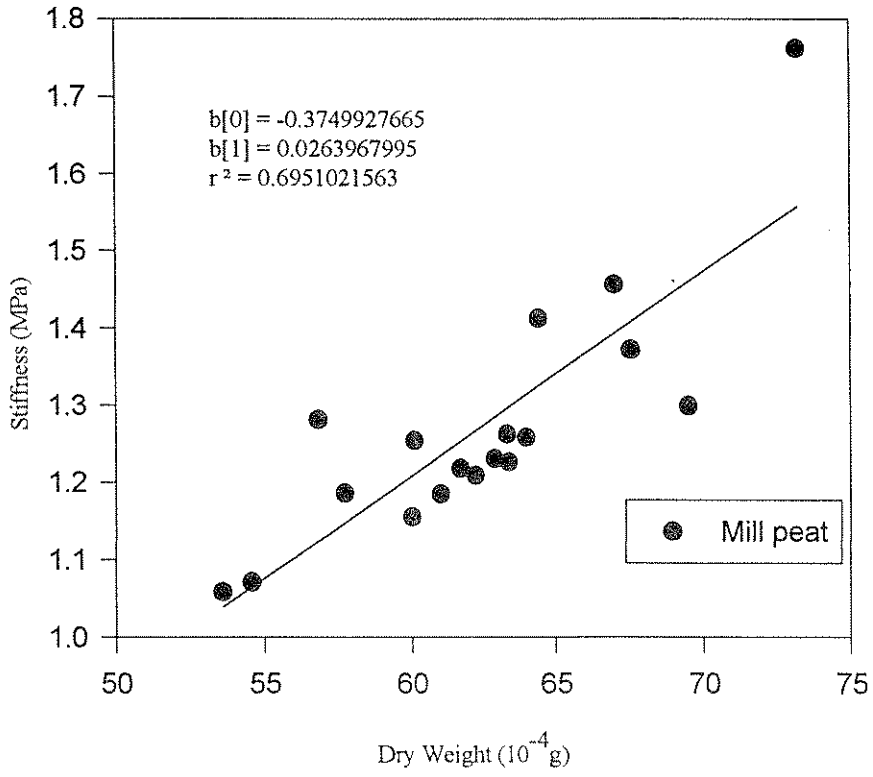
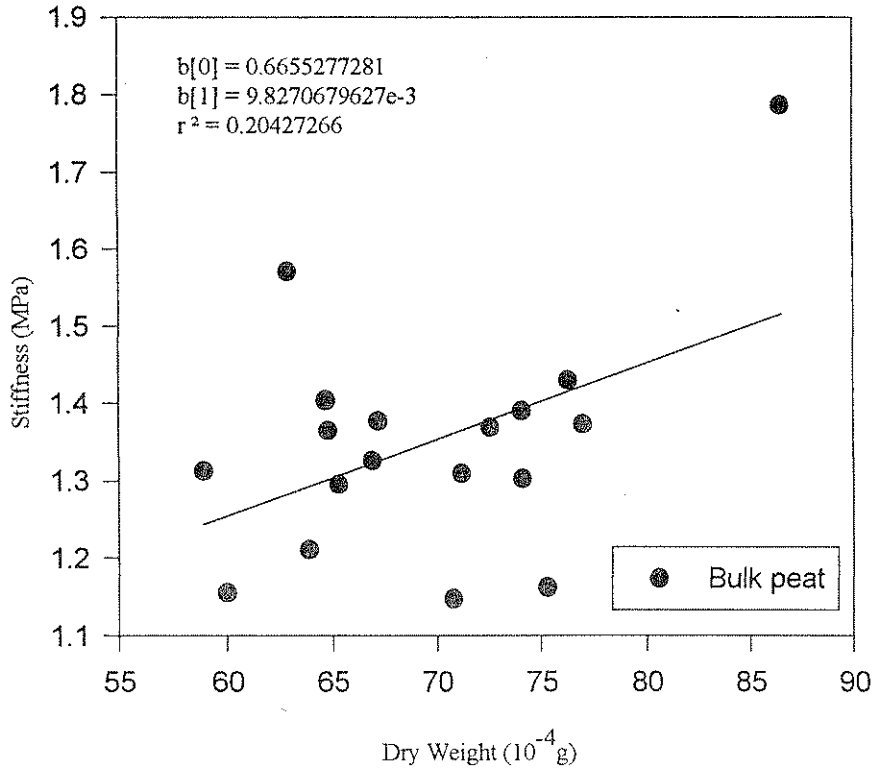


Figure 48: Relationship between stiffness and dry weight of mushrooms grown on bulk peat.



significant difference in stiffness was found. The main source of water for mushrooms is from casing and compost, so it is possible that mushrooms grown at low relative humidity counterbalance the lack of humidity by taking more water form the casing or compost. Mushrooms contained more water when grown on wet casing than on dry casing and were significantly firmer. It seems that mushrooms are more affected by the low water potential of the casing (wet casing) than the high relative humidity.

Texture of mushrooms grown on different casing types were measured either by the total energy supplied or the stiffness of mushroom tissue. No difference was found in firmness between the French style casing, the peat based casing and the English style casing. However, a significant difference was found between the mill peat based casing and the bulk peat based casing. Mushrooms were stiffer when grown on that later casing.

Mushrooms grown at low CO_2 level (800 ppm) were found to be firmer than mushrooms grown at high CO_2 level (1200 ppm). It is, therefore, important to maintain sufficient ventilation in growing rooms to produce good quality mushrooms.

The choice of cultivar depends on which mushrooms quality it is expected from them. Some cultivars might be resistant to discoloration, others may behave well under storage, others might be firm, others might have strong flavour and others may produce a good yield. There is no strain available which owes all the features cited above. In our study, four mushroom strains, U3, U1, S130 and A12 were tested for their quality regarding firmness. Strain U3 was found to be significantly firmer than the other strains.

Supplemented compost are often used to increase mushroom yield. There was a concern that, the increase in yield might affect (negatively) mushroom quality. No difference in stiffness has been found between mushrooms grown on supplemented and non-supplemented compost. Surprisingly, the mushroom yield with supplemented compost was not higher than the yield with non-supplemented compost but the overall yield of the crop was higher than the average yield found in farm.

A great influence on texture was found in the variation of the casing and the compost depth. Mushroom grown on deep compost and shallow casing were found to be the firmest (highest energy requirement for compression) and the more plastic. The shallow casing, on average, has also produced mushrooms with the lowest plasticity (more likely to spring back to original position after compression).

VI Overall conclusions

The techniques used to study mushrooms morphology have pointed out a difference in structure between the top and the side of the sporophore. Mechanical tests have revealed an anisotropy in the mushrooms. The outermost layer of hyphae has been shown to have a different structure from the inside flesh, the function of which is probably to help the mushroom to emerge from the heavy casing layer. It has been found that the upper surface layer has a very low volume fraction which is easely compressed when subjected to a load. Damage to reproduce discoloration on mushrooms has shown that a slow compression will produce more bruise volume even if the mushroom has absorbed less energy. The agronomic and environment effects on mushrooms have shown that a range of firm to soft mushrooms can be produced depending on compost depth, casing depth, CO_2 concentration and water potential of casing. Further examination of hyphal morphology or anatomy (hyphal diameter, volume fraction and dry matter) will be carried out identify the factors responsible for firmness and plastic deformation.

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