Final report:	April 1998
Project title:	Relationship between sporophore morphology and mushroom quality
Reports:	Annual Reports April 1996 & April 1997
Project number:	M 19
Project leader:	Dr Kerry Burton
Ph.D. student:	Tanouja Rama
Location:	Horticulture Research International, Wellesbourne, Warwick CV35 9EF
Project co-ordinator:	Jim Rothwell
Date commenced:	February 1995
Date completed:	January 1998
Keywords:	Quality, Texture, Bruising, Browning Reaction, Mushrooms, Biomechanics

CONTENTS Page No

PRACTICAL SECTION FOR GROWERS

Mushroom quality is an important factor for the UK mushroom industry as it has been shown to be one of the major components affecting competitiveness. Good mushroom quality will optimise consumption and sales. Mushroom quality can be defined as white colour, firm texture, uniform maturity and good flavour. This HDC project (M 19) addressed two of the quality factors, white colour and firm texture. This project examined texture and how the cell packing, ie. the morphology, contributed to texture.

Texture is important to quality for two reasons. Firstly texture is a quality attribute. Secondly, one of the reasons that mushrooms show brown discolouration is due to mechanical damage leading to bruising. The texture of a mushroom affects its resistance or susceptibility to bruising by its ability or inability to withstand mechanical forces. To undertake this study of texture, we have had to use a scientific discipline called "Biomechanics". This discipline treats the mushroom as a 'structure' or a 'material' and examines how the cells in the structure contribute to its strength and mechanical properties, similar to how the girders of a bridge contribute to its strength.

This project was funded by HDC, Chesswood Produce Ltd, Monaghan-Middlebrook Mushrooms Ltd and MAFF. The MAFF matching funding was provided under the Agro-Food Quality LINK Scheme. There is very little previous work on the subject area of this project, therefore, some theoretical work was a necessary part of the project. The involvement of grower organisations (HDC, Chesswood and Monaghan-Middlebrook) and MAFF allowed the project to examine both the practical aspects of texture and the more theoretical aspects of texture. This summary will emphasise the more practical aspects.

Project Results

(a) Cultural factors which influence mushroom texture

Texture and bruisability are known to vary widely between mushrooms of the same crop and between different crops. Some of this variation could be attributed to flush number. Second flush mushrooms were found to be the firmest, followed by third and then first flush mushrooms.

Experiments were performed to find if the remaining variation could be attributed to agronomic or environmental factors. If this was found to be the case then this knowledge would have practical implications.

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

1. Humidity
2. Watering
3. Compost supplements
4. Strain
5. Compost depth
6. Casing depth
7. CO_2 level
8. Water potential of casing
9. Casing type

Factors, which had a major effect on mushroom texture are:-

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

These effects can be summarised as follows:-

- Deeper compost leads to firmer mushrooms and the shallower the casing also leads to firmer mushrooms. If these factors are combined, then the effects are combined, so very firm mushrooms are produced from deep compost and shallow casing. Shallow compost covered in deep casing leads to the production of soft mushrooms.

- A clear relationship was found between CO_2 level in the growing room and firmness. Low CO_2 levels lead to firm mushrooms, higher levels lead to softer mushrooms.

- Varying the water potential of casing leads to firmer mushrooms but this occurred only at the extremes of very wet or very dry casing. These extreme conditions caused some loss of yield and it is possible that the increased firmness was caused by the yield loss, ie. more resources going into fewer mushrooms. (The converse of this is that mass pinning (ie. large yield) leads to large numbers of soft mushrooms).

- At the extremes of casing water potential, the use of bulk extracted peat produced firmer mushrooms than milled peat.

(b) Measurement of mushroom texture

(1) In the laboratory

The textural properties of the mushroom were determined in the laboratory using the Inston Universal Testing Machine. A number of different biomechanical measurements could be made using this machine, but this approach is not practical for use by a grower on a farm.

(2) 'On-farm' measurement

Two techniques were developed to assess textural properties for use 'on-farm':-

(i) Formula to estimate firmness

Mushroom firmness has been shown to correlate well with density. A procedure and a formula were developed to estimate firmness. A number of mushrooms are taken, plugs of tissue removed from the cap and weighed, the tissue plugs are oven dried and then re-weighed. When the fresh weights and dry weights are fitted into the formula, a good estimation can be made of the firmness of the mushrooms.

(ii) Bruisometer

The mechanical process of 'slip-shear' was identified as being the most damaging to mushroom quality. Two design teams from the Mechanical Engineering Department of Coventry University have been working with Kerry Burton and Tanouja Rama of HRI to design and build a device to inflict a controlled amount of slip-shear force to a mushroom. Further validation is required and will be done in HDC project M 19a 'Validation of the Mushroom Bruisometer'.

(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.

We have used the results of the cultural factors on mushroom texture to develop a model system (casing depth-compost depth) to produce soft and firm mushrooms. Then we have examined these mushrooms and tried to answer the question of what makes a firm mushroom to be firm. We have

found that firm mushrooms contain smaller diameter cells, more tightly packed and with a greater turgor pressure (internal water pressure). We have also found that the alignment or orientation of the cells plays a crucial role in how firm the tissue is.

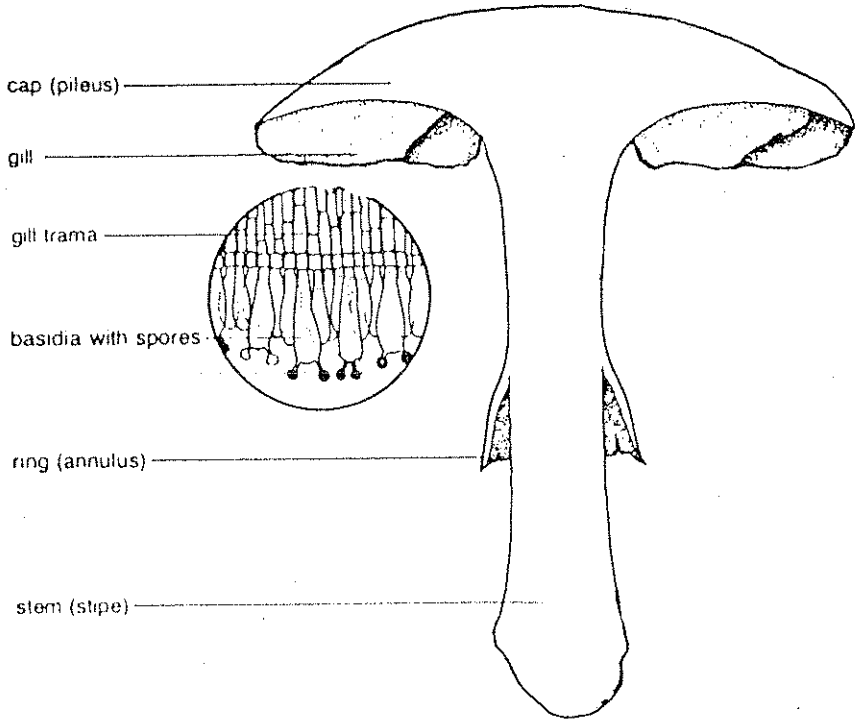
EXPERIMENTAL SECTION

A. GENERAL INTRODUCTION

1.1 The mushroom

In the multicellular eukaryotes group, it has been accepted that Fungi are a kingdom by itself along with Plants and Animals. Fungi parasitise living organisms, live upon dead organic material or live in symbiosis with living organisms. They differ from plants by their lack of chlorophyll, true root, stem and leaves and their life cycle is based on spore production. Mushroom is a fungus, typically having a stalk capped with an umbrella-like top (Figure 1.1).

Figure 1.1: The structure of a mushroom. *Reproduced by permission of Equinox (Oxford) Ltd.*



1.2 Definition and classification

The Fungi kingdom is divided in two groups slime moulds and true fungi which contains the zygomycetes, ascomycetes, deuteromycetes and the basidiomycetes based on the type of spores they produce. The basidiomycetes group includes genii like *Amanita, Coprinus, Lentinus, Pleurotus, Agaricus.* The *Agaricus* species commonly found in markets or fields are *Agaricus bisporus, A. bitorquis, A. harvensis, A. campestris,* however *A. bisporus* is the most commonly cultivated in Europe and America. It is often found written as *A. bisporus* (Lange) Imbach after the two people who named and classified the mushroom.

1.2 Biological cycle

One of the steps in the mushroom life is to produce and spread its spore to propagate the next generation. Spores are produced in large quantity by the basidiospores, about 40 millions spores produced per hour was estimated by Buller (1909) for *A. campestris*. The life cycle of *A. bisporus* taking in account nuclei migration is represented in Figure 1.2. Spore production starts by nuclear fusion in the basidium and after meiosis four nuclei are present. In *A. bisporus* the basidia will produce, in 95% cases, two spores each containing two nuclei. The 5% left are basidia of three spores (1 and 2 nuclei per spore) and four spores (1 nucleus per spore). The spores will be able to germinate only if they have received two different nuclei. The sporophore formation from the primordium to a mature cap is described in Figure 1.3.

Figure 1.2: The life-cycle of *A. bisporus* taking into account both random migration of nuclei and typical frequencies of aberrant basidia. *Reproduced by permission of the Glasshouse Crops Research Institute.*

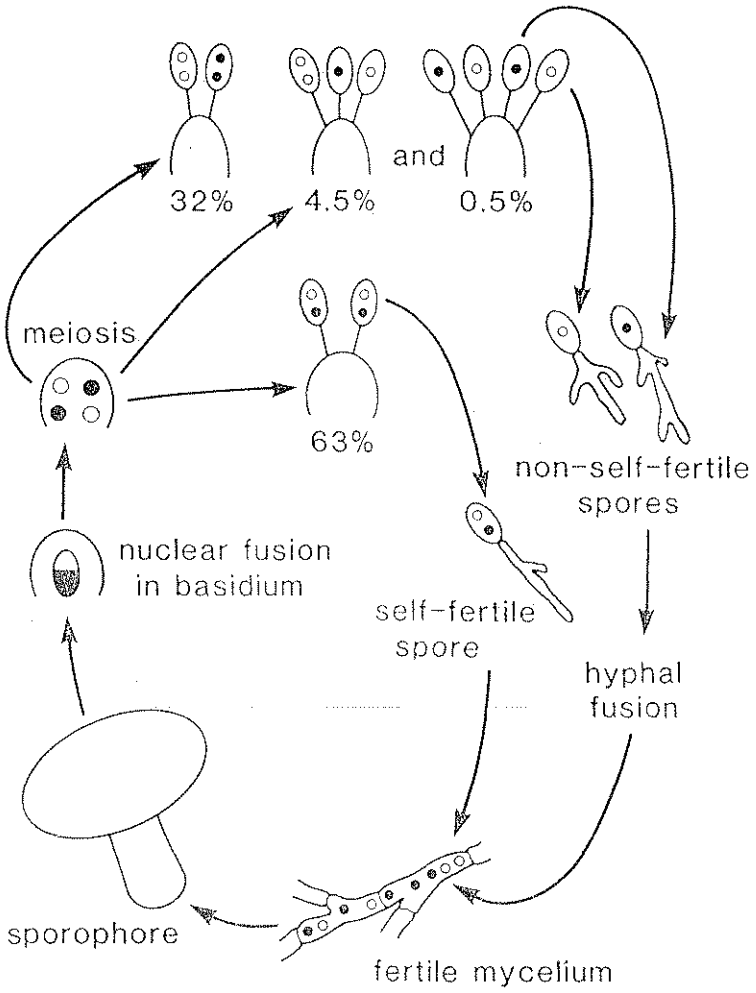
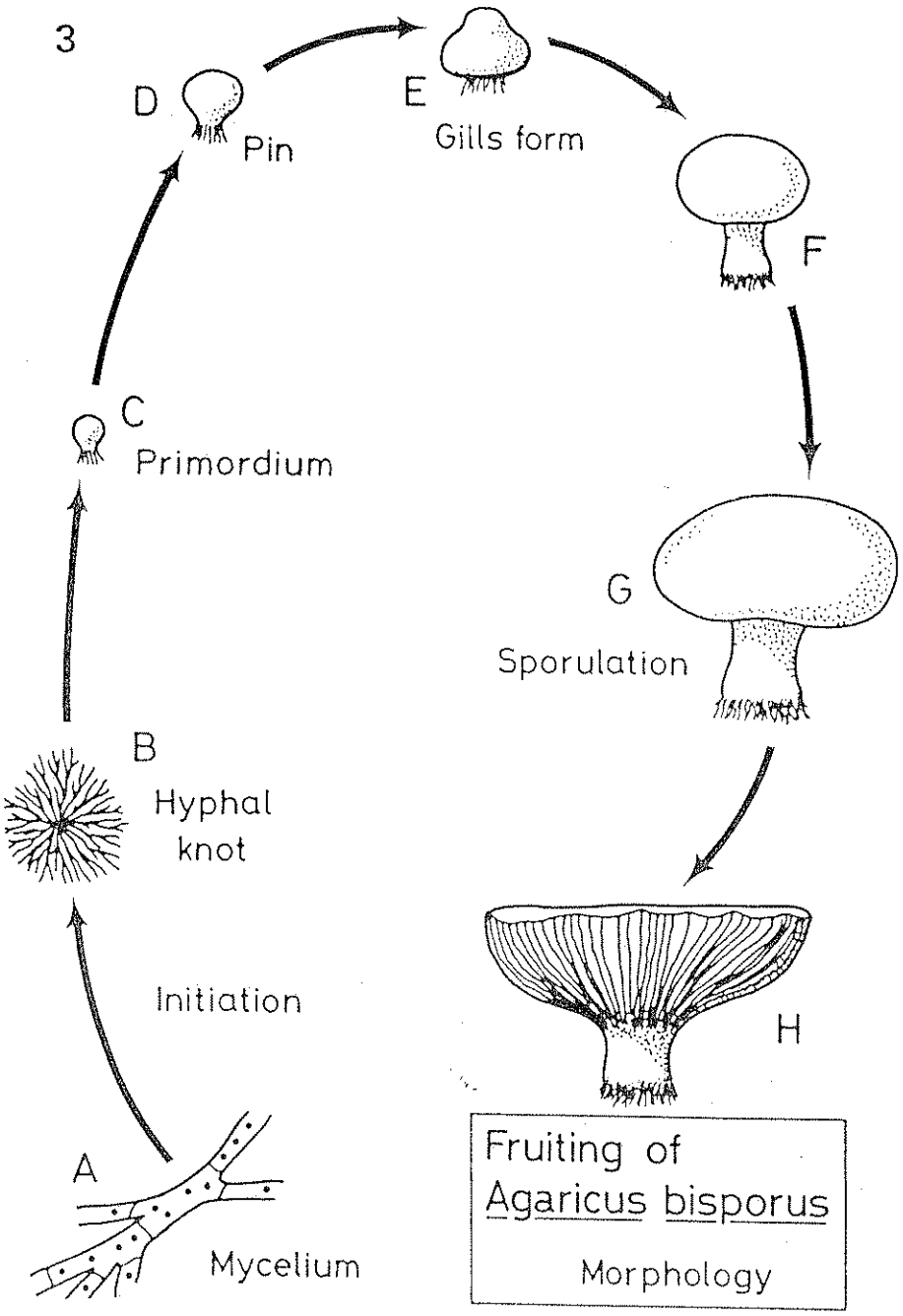


Figure 1.3: Diagram of the morphogenesis of the mushroom *A. bisporus* (Wood *et al.*, 1985).



1.3 Industrial, economic and biotechnological importance

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 produced in the UK. In Europe, the major mushroom producing countries are the Netherlands, France, Italy and the UK. The UK mushroom industry which had produced about 120,000 tonnes for the year 1995, has 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.

1.4 Mushroom cultivation, its history

Records about former habits, around the XVIIth century, suggested that wild mushrooms were collected in fields. The earliest comprehensive description of mushroom cultivation was done in 1707 by a French botanist J. De Tournefort. At that time horsemanure was used in which it was thought that mushroom "seeds" were present. The manure was arranged in ridge beds and were covered with rotted leaf mould (Gaze, 1985). Mushrooms were commercially produced in the open for the Paris market. In 1810 that Chambry made the first mushroom culture indoor in quarries located in Paris area. This is why mushrooms are commonly called in France "champignons de Paris". Later, in 1813 in England, Callow designed the first cropping houses, which were able to produce mushrooms all year round. The cultivation was still done in ridge beds of 1.2-1.5 m height, 1.2-1.5 m wide and 15 m long. It is then that the mushroom cultivation had spread and established in many countries in Europe and in the USA. As the mushroom cultivation settled in USA a new growing system in wooden shelves, running nearly the length of the houses, took place. The American shelf system has then evolved to trays, racks, bags and Dutch shelves. All of these systems including the American shelves are still currently in use.

1.5 Quality factors and interrelationships

In that last century, in Britain and in many other developed countries, there has been a

revolution in the food consumption. A survey conducted by the Food Policy Research, Bradford, on people's attitude to food (Fallows *et al.*, 1985), showed that 95% of the people agreed that they were more conscious of what they eat nowadays. A consequence of that is the decline in potato and grain consumption and an increase in fruit and vegetable consumption. Consumer choice of food is influenced by many factors, circumstances and it varies from individuals. Taste, appearance, texture and smell all help to influence a person's choice of food. Food has to be functional as well, since we spend less time preparing and eating our meals.
The most important factors in fruit and vegetable quality for consumers are:

1. Appearance (size, colour, shape)
2. Condition and absence of defects
3. Texture
4. Flavour
5. Nutritional value

McCanna *et al.* (1968) determined a spectrum of quality for fresh mushrooms which includes whiteness, disease free, maturity, flavour, firmness, cleanness, size and shape. But a recent survey (Berendse, 1984) reported that the most important quality attributes for mushrooms are, by importance order:

1. Colour
2. Firm texture
3. Uniform maturity
4. Flavour

It is detailed below how each of these parameters contributes to the mushroom quality.

1.5.1 Colour

Colour is often related to the maturity of fruit or vegetable products. For instance red tomatoes, yellow bananas or red apples... are signs of mature fruit. For mushrooms it is different as they are white from the early stages and throughout their growth. They are still white when harvested and should stay white until they reach the consumer. This is however, not what is happening in real conditions. A mushroom is a very fragile product and can be easily bruised by mechanical damage. So after they have been picked and transported, mushrooms often show some brown

patches on the cap surface. These patches affect the quality of the product as, white colour is associated with fresh and pure whereas, brown is more associated with old and damaged produce. The discoloration contributes also to financial losses as the market price is often based on the mushroom appearance.

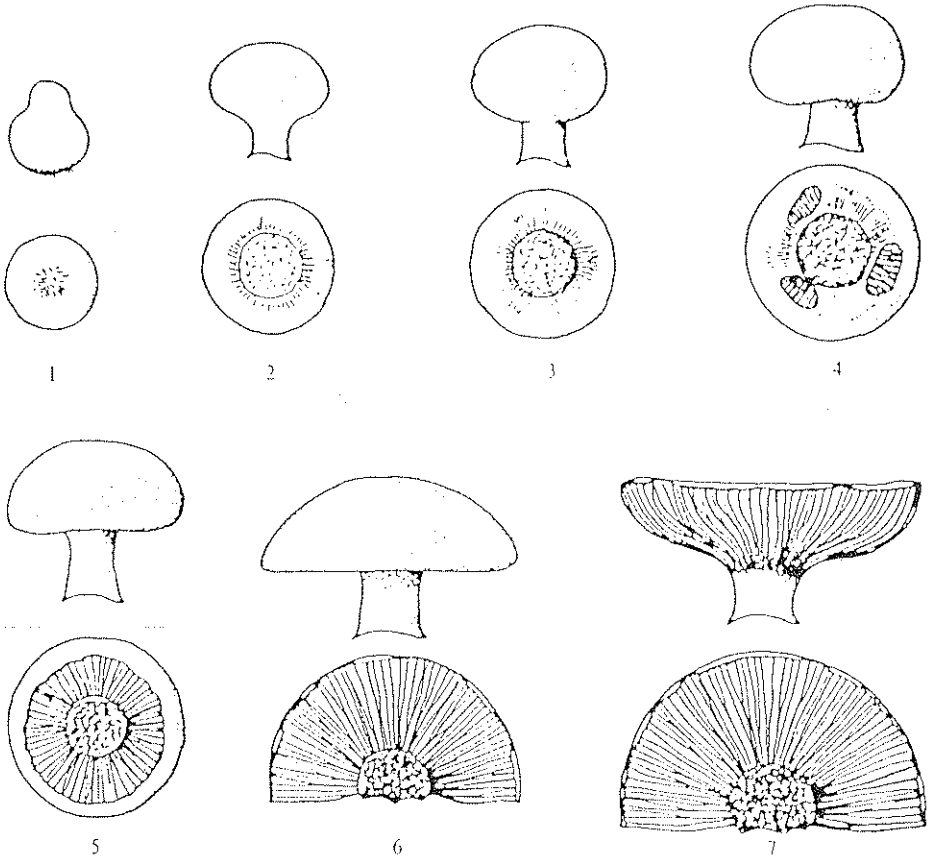
1.5.2 Texture

Texture is important for most fruit and vegetables, it is perceived by touching the product and in the mouth. When people buy mushrooms they usually squeeze them gently between two fingers to appreciate their firmness. The particularity in England is that about 95% of the mushroom production are sold as fresh mushrooms and consumers can easily appreciate the quality of the product. There is a social and cultural tradition in England for cooked mushrooms at breakfast so fresh mushrooms with a good texture are very important. In other countries like France, texture is not so important as most mushrooms are bought in cans.

1.5.3 Maturity

The maturity of mushrooms is not the same as maturity of other fruit or vegetables where maturity usually means ripening stage and therefore, ready to eat. Mushrooms do not ripen they change size and shape as they grow. The mushroom growth has been divided into several stages by Hammond *et al.* (1976a), Figure 1.4. Mushrooms are edible at any stage, but some of the stages are preferred by the consumers. For instance in France, mushrooms are sold fresh at stage 2-3 and the older stages or large mushrooms will be discarded. In England stage 4-5 mushrooms are normally sold in supermarkets and the large open mushrooms also have a good response from the consumers. Even after harvest, mushrooms keep on growing but to reduce the speed of the growth it is important to maintain them at low temperature.

Figure 1.4: The seven different stages of sporophore development in *A. bisporus* (Hammond and Nichols, 1975)



1.5.4 Flavour

The organoleptic properties of mushrooms are important but there is not much variation between one lot of *Agaricus bisporus* and another. The main compounds for mushroom flavour are 1-octen-3-ol responsible in general for the mushroom-like aroma (Mau J.-L *et al.*, 1992, Picardi SM & Issenberg P., 1973) and 1-octen-3-one which seems to be responsible for the cooked mushroom aroma (Pyysalo H. & Suihko M., 1976).

1.6 Aims and objectives

This project is aimed at improving the quality of mushrooms grown in UK to reduce imports and to provide a springboard for export. To achieve this goal, the texture and the discolouration of mushrooms was investigated throughout the steps detailed below:

- Investigate the mushroom cap morphology with the use of light microscopy and scanning electron microscopy. Identify the hyphal fringe on the cap surface which is more prone to damage than deeper-seated hyphae.
- Investigate mechanical properties of the mushroom cap and devise methods to measure texture.
- Develop a technique to measure mushroom firmness on farm and evaluate the performance of this technique.
- Determine the effects of environmental and agronomic factors on mushroom texture.
- Determine the intracellular location of the browning reaction on the cap surface.

B. PART I

General Materials and Methods

Instron Universal Testing Instrument

I.1 Mushroom strains

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 were harvested for experiments, they were selected to have a diameter of 32-37 mm and a height of 15-20 mm.

I.2 Mushroom cultivation

The processes involved in growing mushrooms indoors are divided into several steps: preparation of spawn, preparation of compost, spawning, casing, growing and harvest.

I.2.1 Preparation of spawn

The mushroom 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. This is called spawn and it is usually produced by commercial companies (Sylvan-Hauser, Le Lion, Amycel).

I.2.2 Preparation of compost

Compost is the substrate for growing mushrooms. The compost preparation is a long and difficult step so most growers prefer buying already made compost. Compost is a mixture of wheat straw and horse manure or sometimes, chicken manure. In our mushroom unit chicken litter is used, mixed with some gypsum, compost activator and water to allow a good moisture degree of the straw. During this phase I, the compost is shaken up every two, three days. In between turns, the temperature can reach 80°C because of the microbiological activity to degrade the cellulose of straw. After two weeks the compost in moved into tunnels where humidity, temperature and ventilation are controlled. This process, phase I, is to pasteurise the compost and to allow thermophilic micro-organisms 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.

I.2.3 Spawning

The mushroom spawn is mixed with compost at a rate of 0.5% by weight and put into trays (91.5 x 61 cm). 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 colonised the compost.

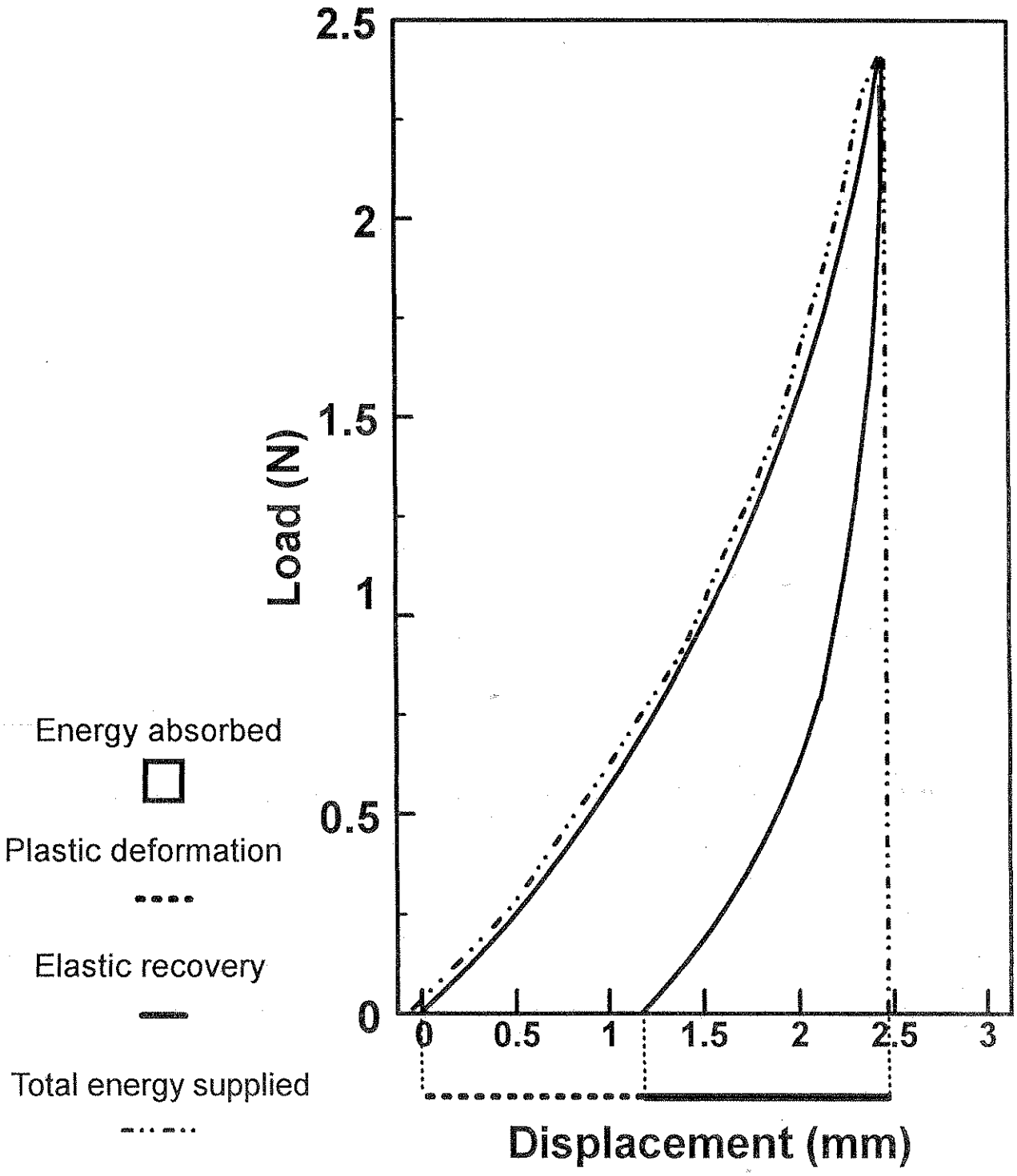
I.2.4 Casing

It is necessary to cover the colonised compost with a layer of casing (soil) 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 (about 19 kg) and the trays are incubated one week at 18°C at a relative humidity of 86-87%, until mushrooms start pinning.

I.2.5 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 peak of a flush, where most mushrooms are harvested, lasts 2-3 days. Once the trays have been harvested and trimmed, the next flush will come after 5-7 days. Mushroom growers usually allow 3 or 4 flushes and then clean the rooms. Mushrooms are traditionally picked by hand but a robot harvester has been designed and is under experimental testing (Reed *et al.*, 1994 and 1995, Noble *et al.*, 1997, Stewart-Wood, 1998).

Figure I.1: Load-Displacement curve



I.3 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 (Figure I.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 (Figure I.1).

I.3 Total energy supplied and energy absorbed

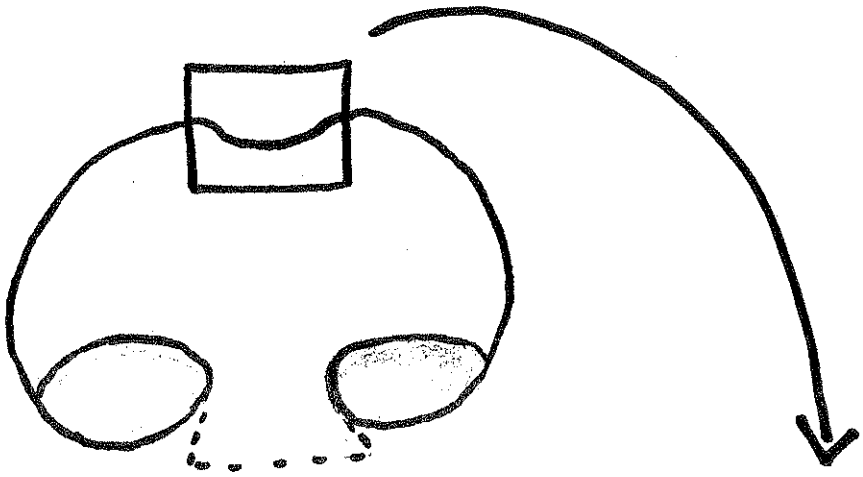
The total energy supplied during compression is calculated from the hysteresis graph; it represents the area under the compression curve (Figure I.1). This area was integrated using the software supplied with the Instron Universal Testing Instrument. Energy is expressed in Joules (Newtons per square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

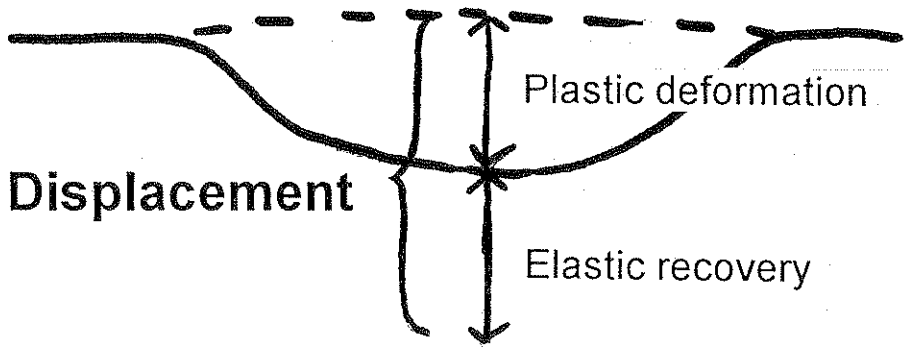
The energy absorbed represents the area between the compression curve and the relaxation curve (Figure I.1). This calculation is not available in the software so when needed, the load-displacement curve was plotted on a chart and the energy absorbed was calculated by image analysis.

I.4 Plastic deformation

Compression produces an indentation at the surface of the mushroom. This indentation represents the plastic deformation of the compression (Figure I.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 I.2: Scheme showing plastic deformation and elastic recovery after compression.

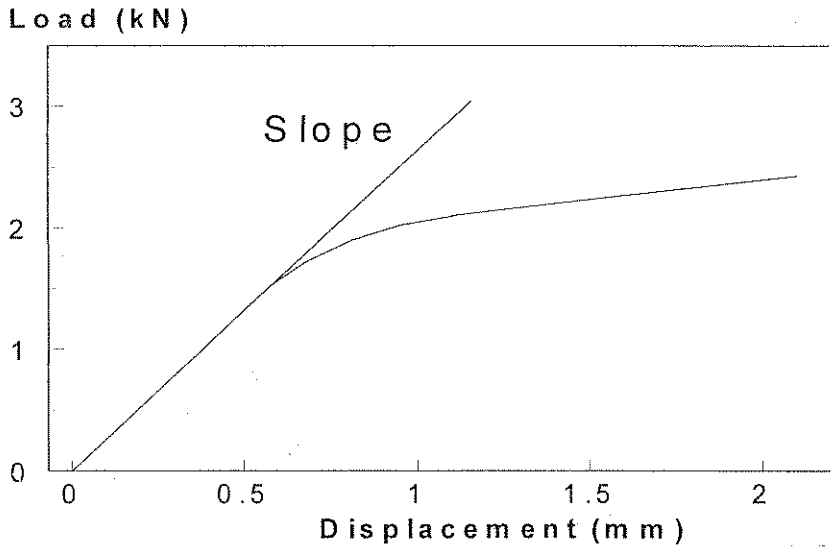




I.5 Stiffness (Young's modulus)

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 I.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

B. PART II

Sporophore morphology

II.1 Introduction

Mushrooms have always intrigued people because of their characteristic and unusual shape. However, how the distinctive mushroom shape is formed has not been yet determined. 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 in 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 mushroom cap.

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

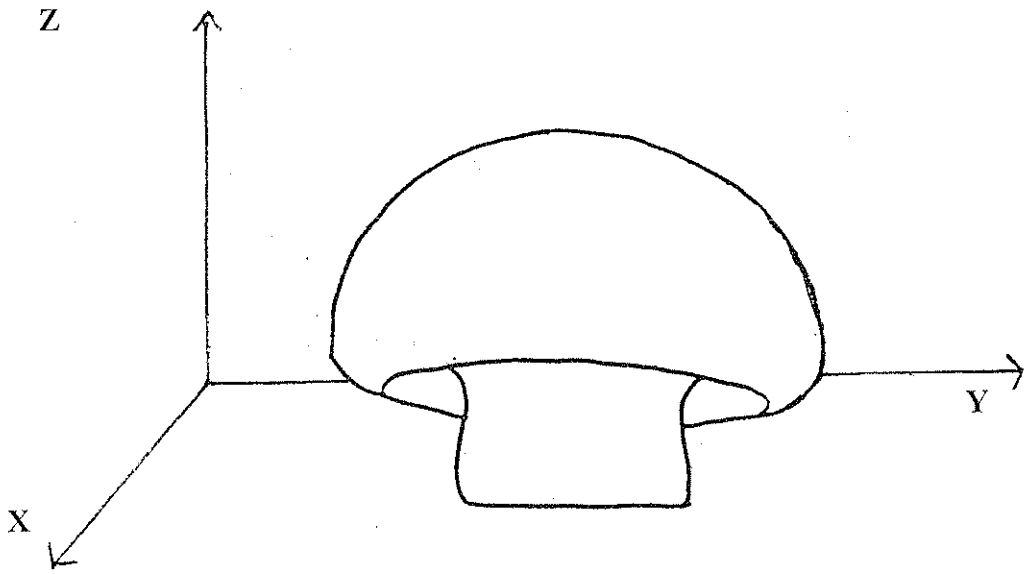
During the course of experiments investigating the environmental and agronomic effects on mushroom texture, a number of treatments were found greatly affecting mushroom biomechanical properties. Most notably, the casing depth (Deep, Medium and Shallow)-compost depth (25, 40 and 55 mm) experiment produced 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.

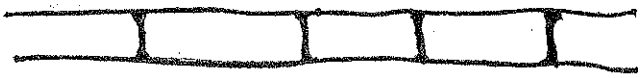
II.2 Materials and methods

II.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:

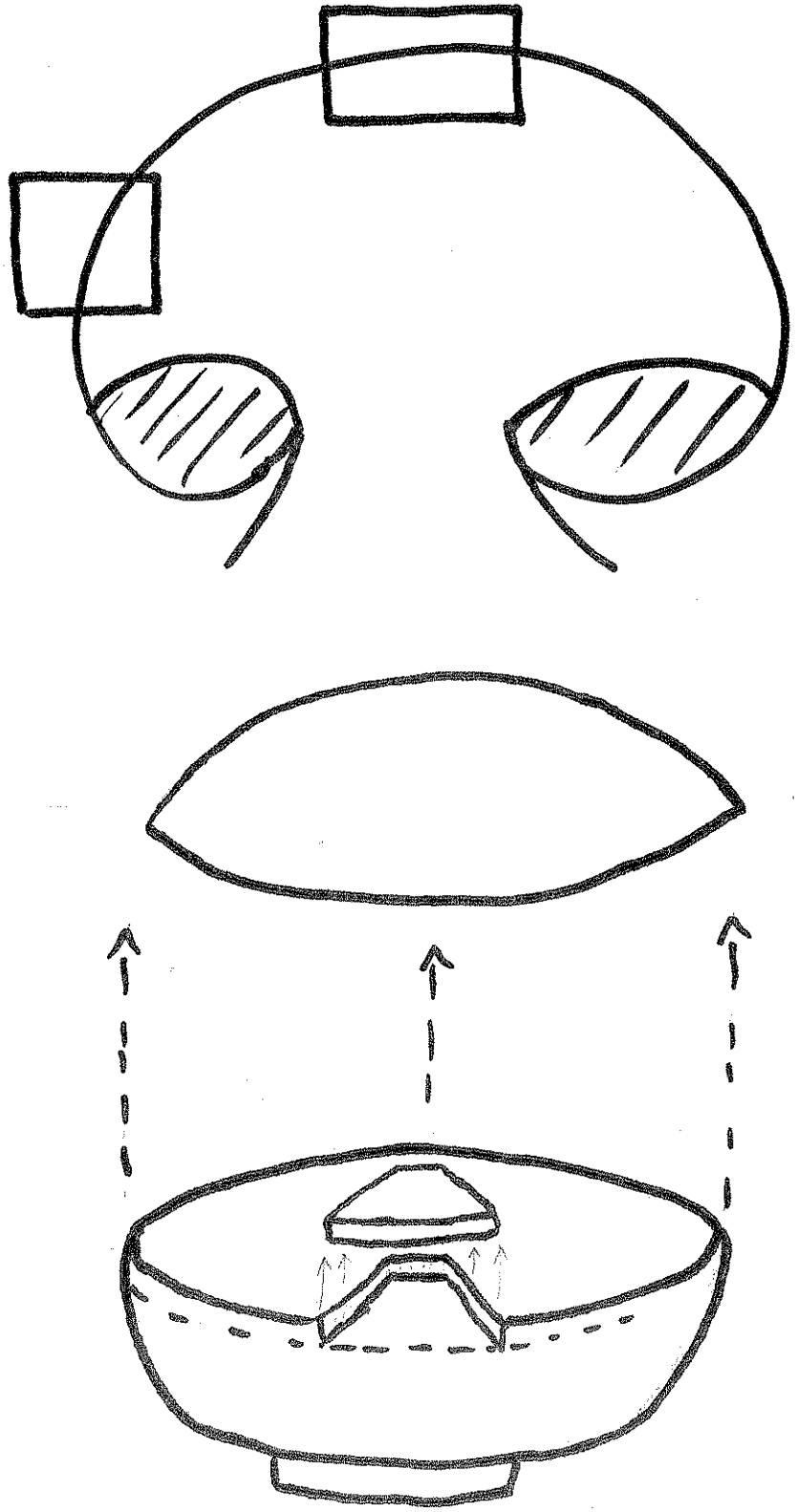
II.2.2 Preparation of tissues for light microscopy

Fresh samples of mushrooms (about 0.5 cm^3) were taken from the top and side and, in the transverse section of mushrooms (Figure 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.

II.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.

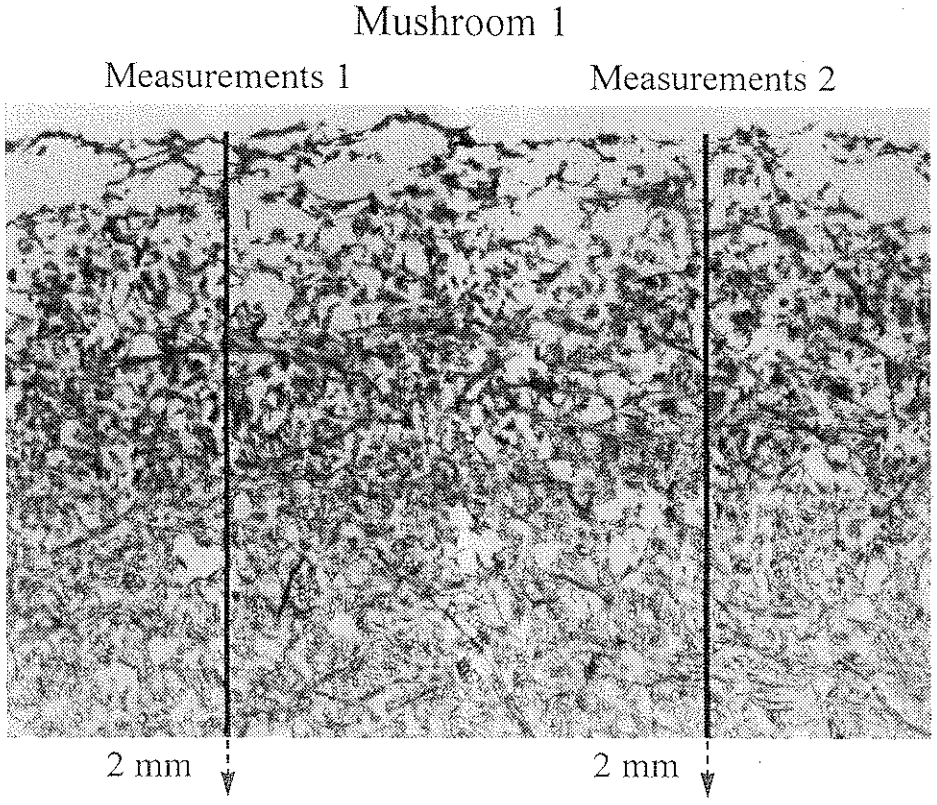


II.2.4 Hyphal diameter

Measurements were taken on mushrooms grown in the casing-compost depth experiment (see Chapter V). 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.



II.2.5 Volume fraction

Measurements of volume fraction were taken from mushrooms grown in the casing-compost depth experiment (see Chapter VI). The volume fraction is the proportion occupied in a unit volume by cells. The volume fraction can be related to density although density represents the weight per volume. The volume fraction was determined from slides obtained from the preparation of tissue for light microscopy. Images were analyzed by the image analysis software cited in II.2.3. Black and white images were transformed to a 5 colour 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 (Figure 5).

II.3 Results

II.3.1 Morphology under light microscopy

II.3.1.1 Side of the cap

Samples taken from the 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.

II.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 that can be distinguished by the intensity of the staining. The first layer, more intensely stained, is made of organised and orientated hyphae 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 seen going from deep flesh towards the skin.

II.3.2 Anatomy under scanning electron microscopy

II.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).

II.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.

II.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).

II.3.3 Hyphal diameter

The overall trend shows that at the surface, hyphae have a small diameter of about 4 □m. The hyphal diameter was found to be greater when measured into the tissue below the surface up to a maximum of 8-12 □m (Fig. 17). When examining mushrooms from different treatments, mushrooms from treatment 25D, that 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.

II.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.

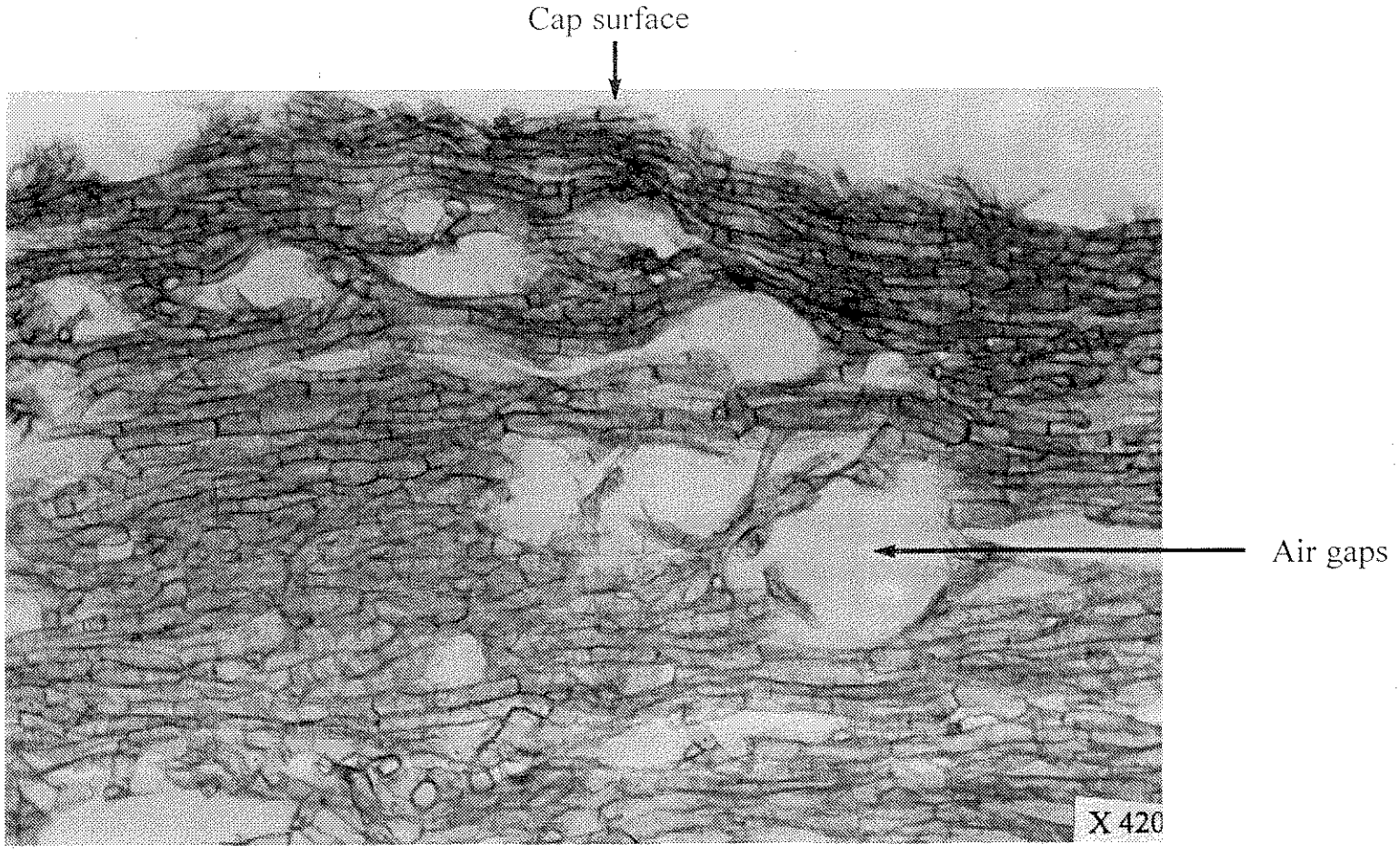


Figure 6 - Light microscopy section of side of mushroom cap showing longitudinal sections of hyphae.

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.

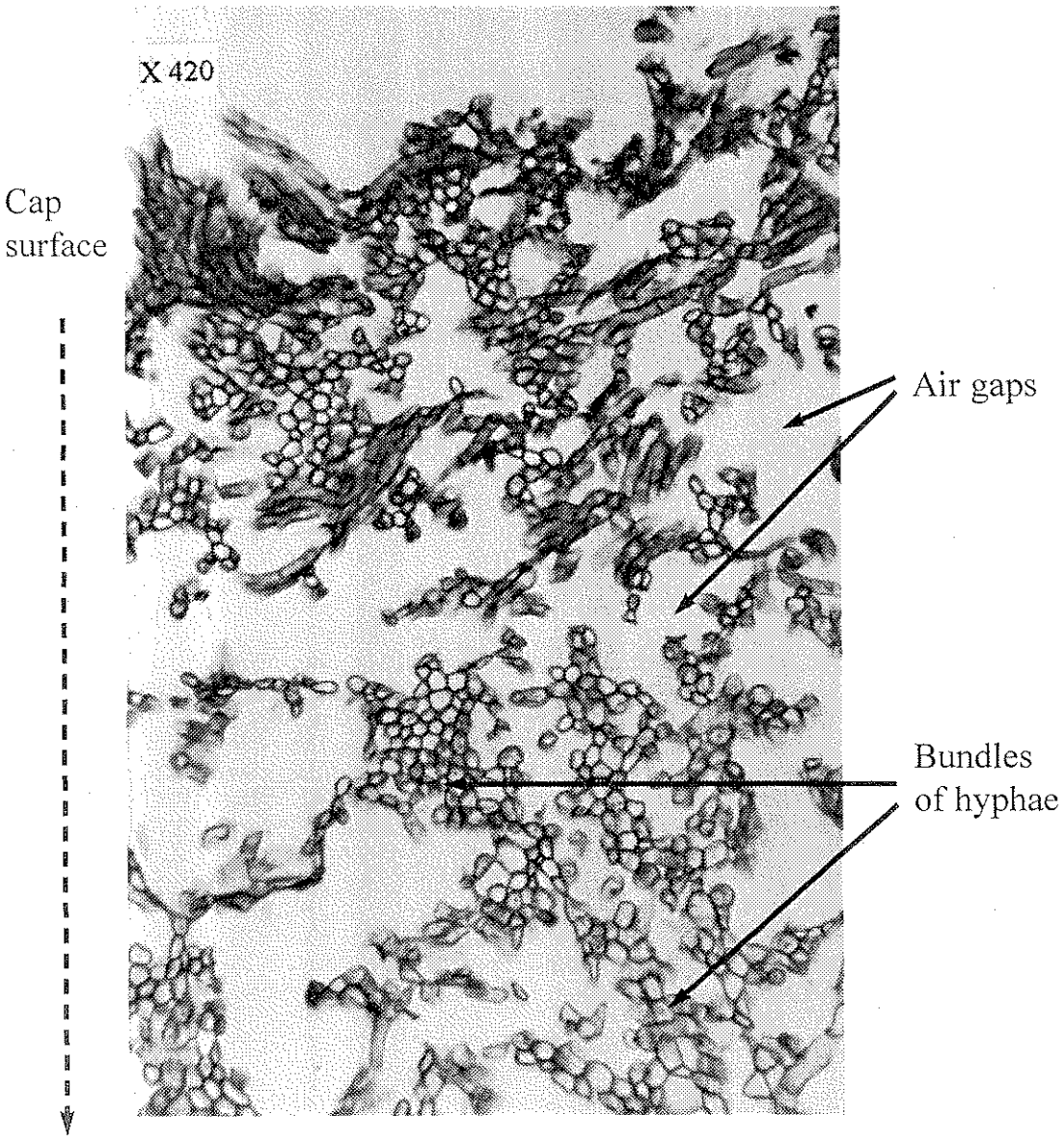
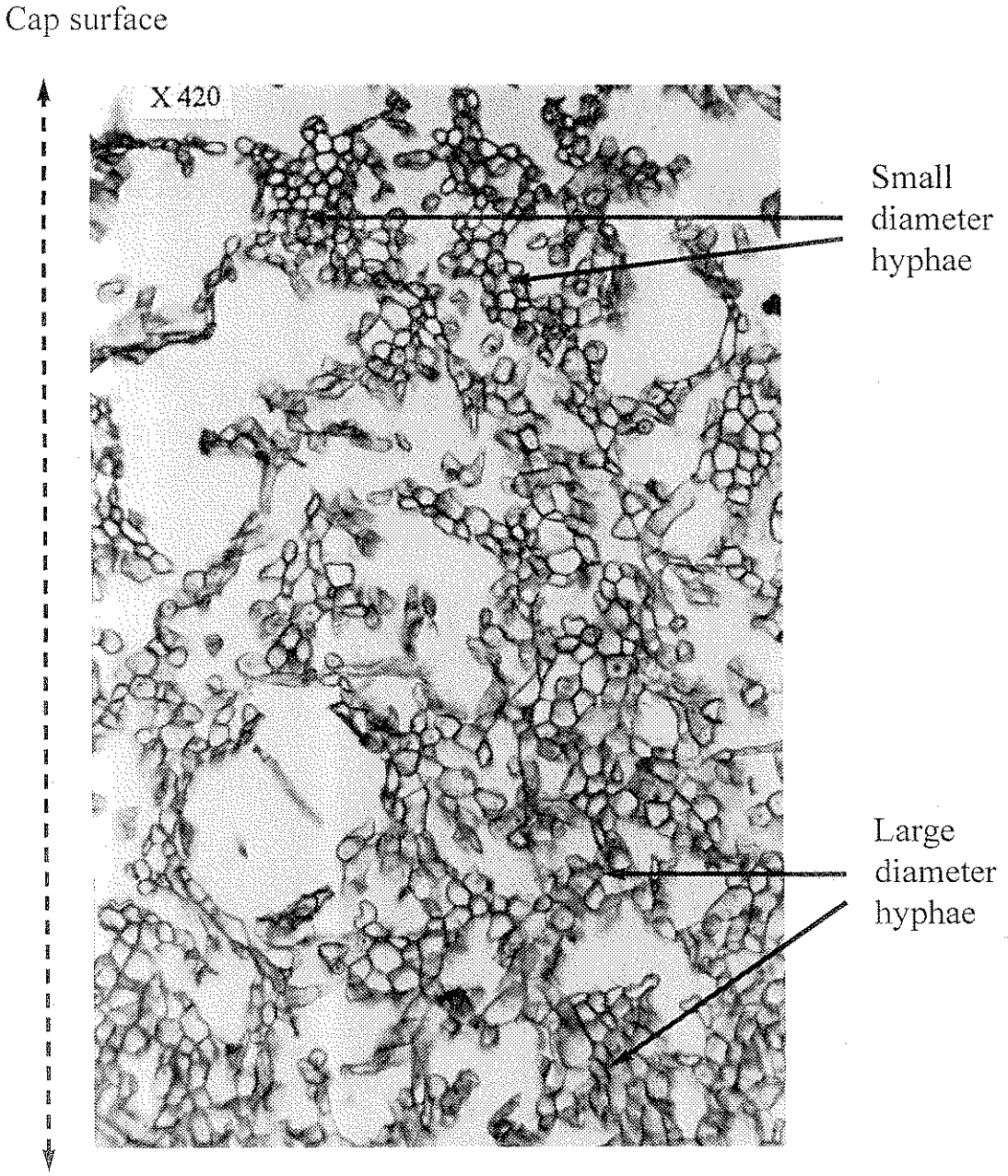


Figure 8 - Light microscopy of transverse section into mushroom cap (showing small diameter hyphae at side cap surface and larger diameter hyphae in centre of cap).



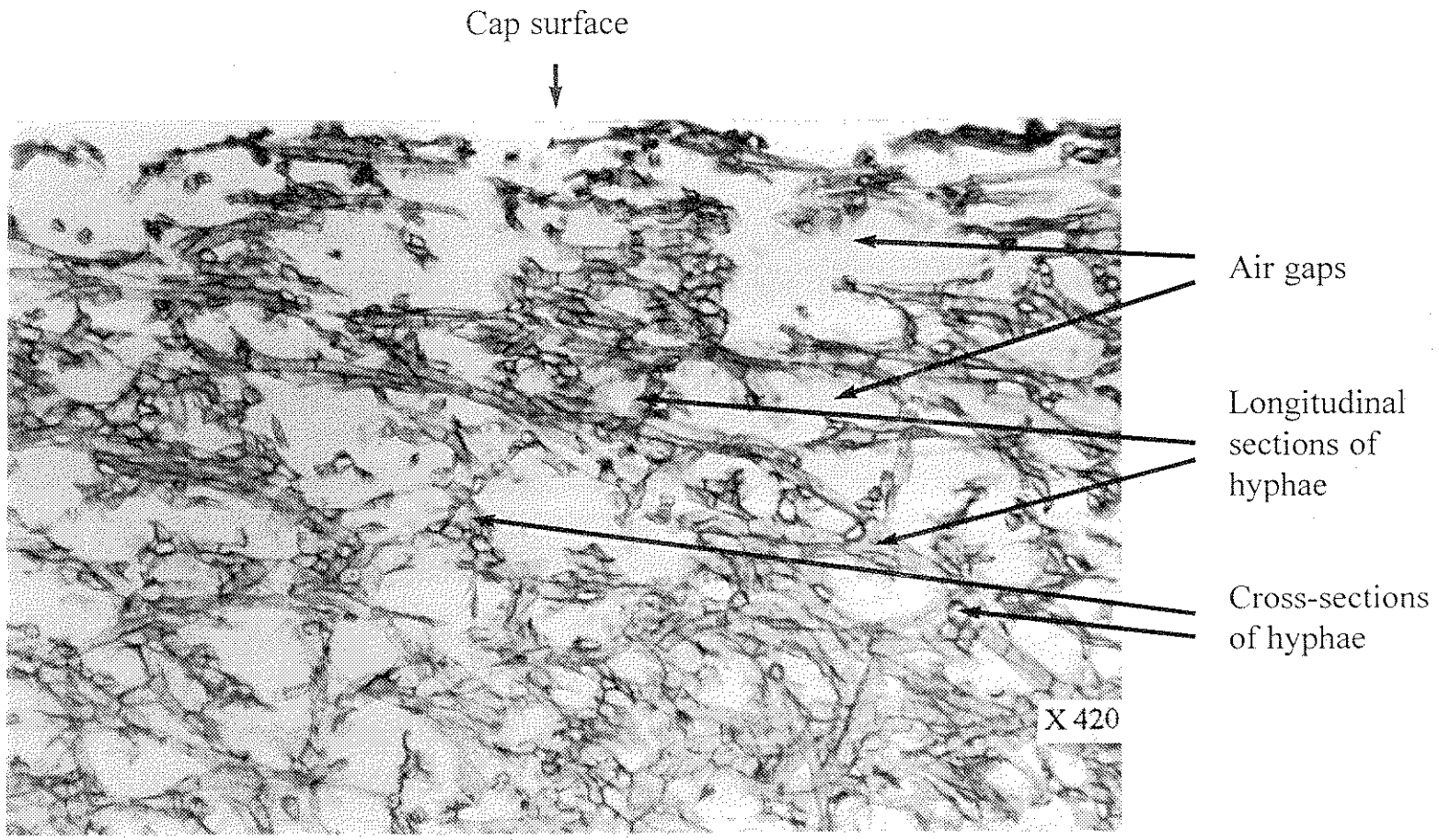


Figure 9 - Light microscopy section from the top showing a mixture of longitudinal and cross-sections of hyphae.

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.

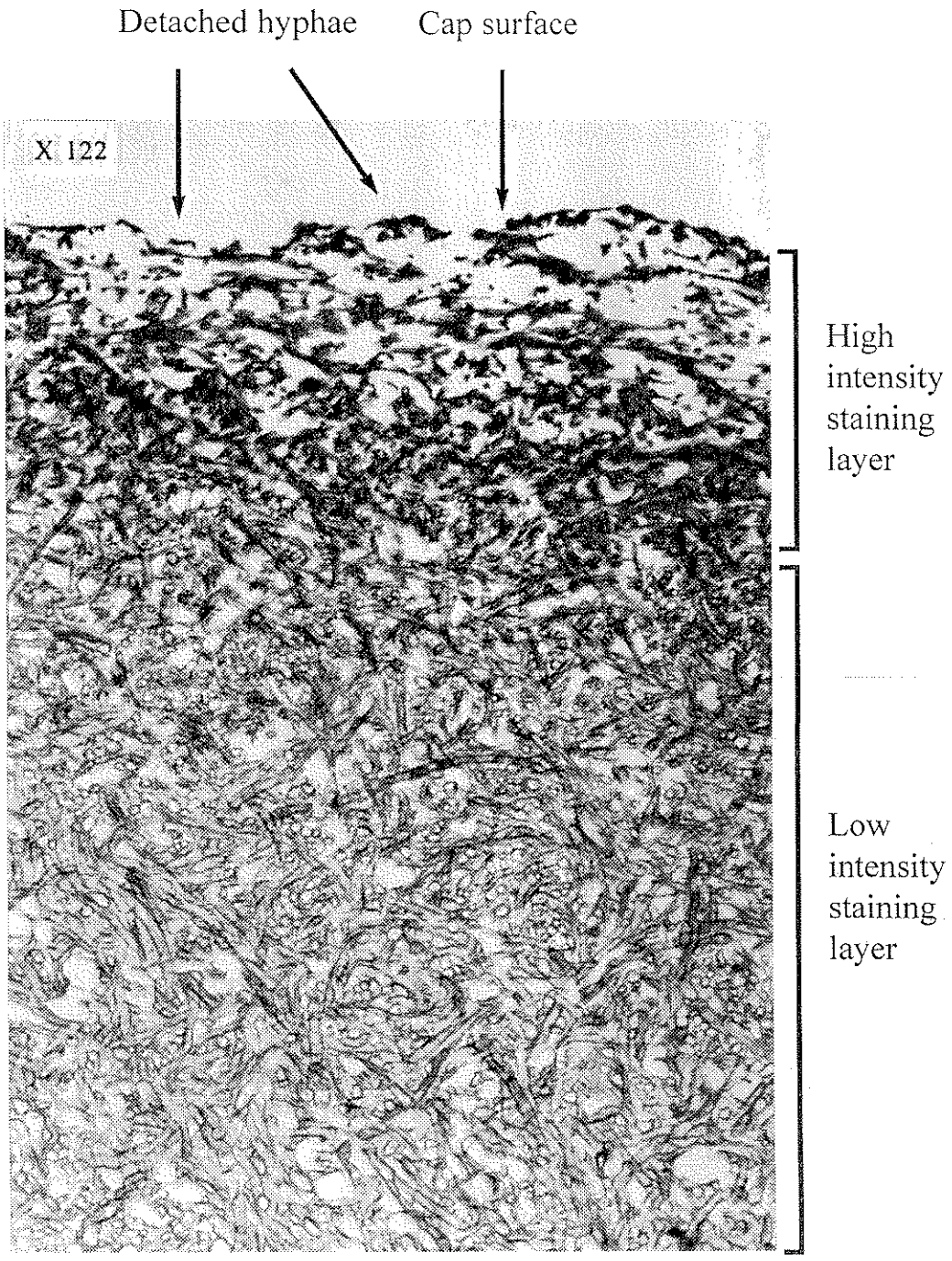
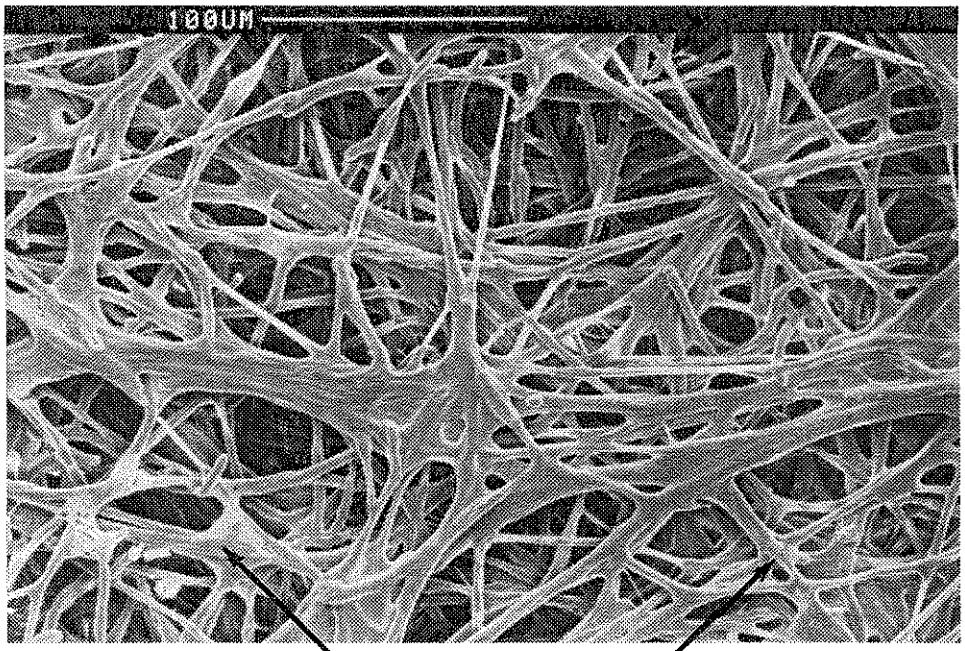


Figure 11 - Electron micrograph of the mushroom cap surface on outermost layer showing a network of hyphae.



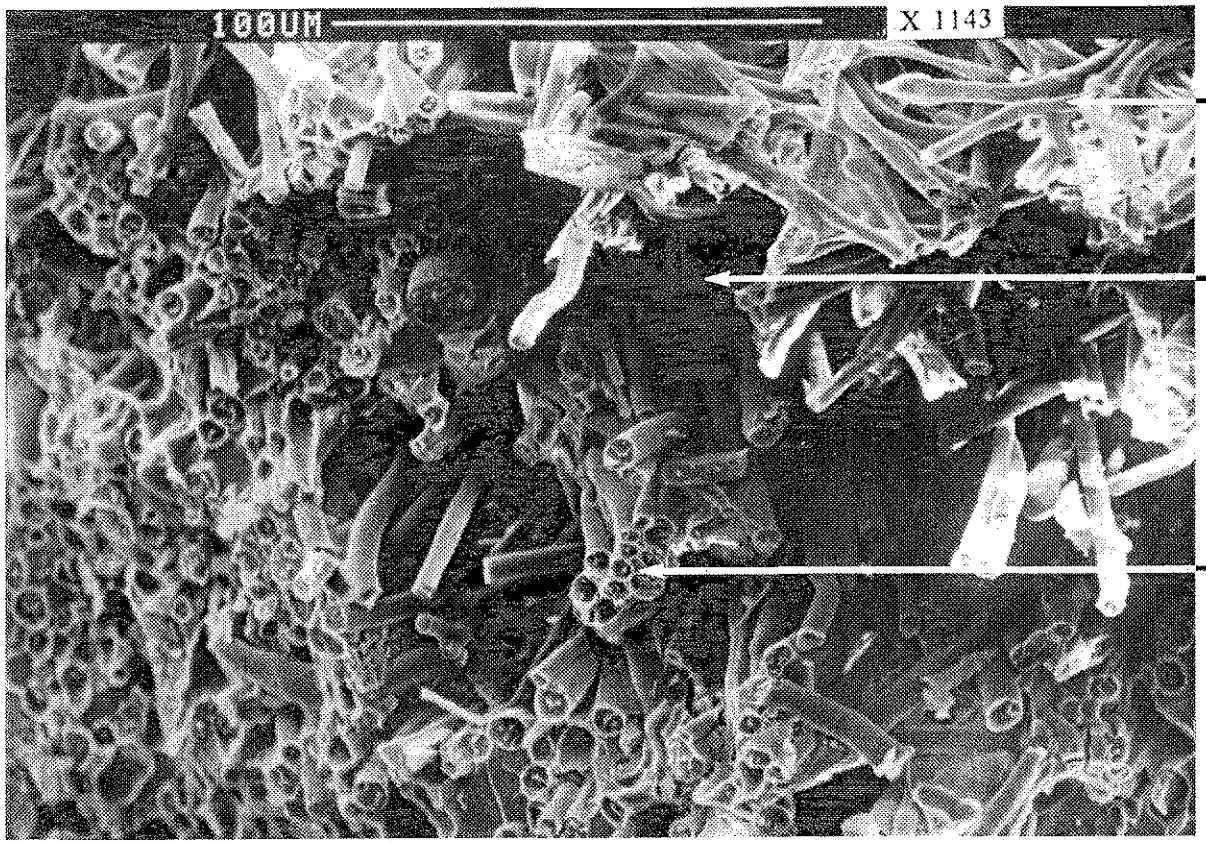


Figure 12 - Scanning Electron micrograph showing the surface and cross-fracture section of the side of mushroom cap.

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

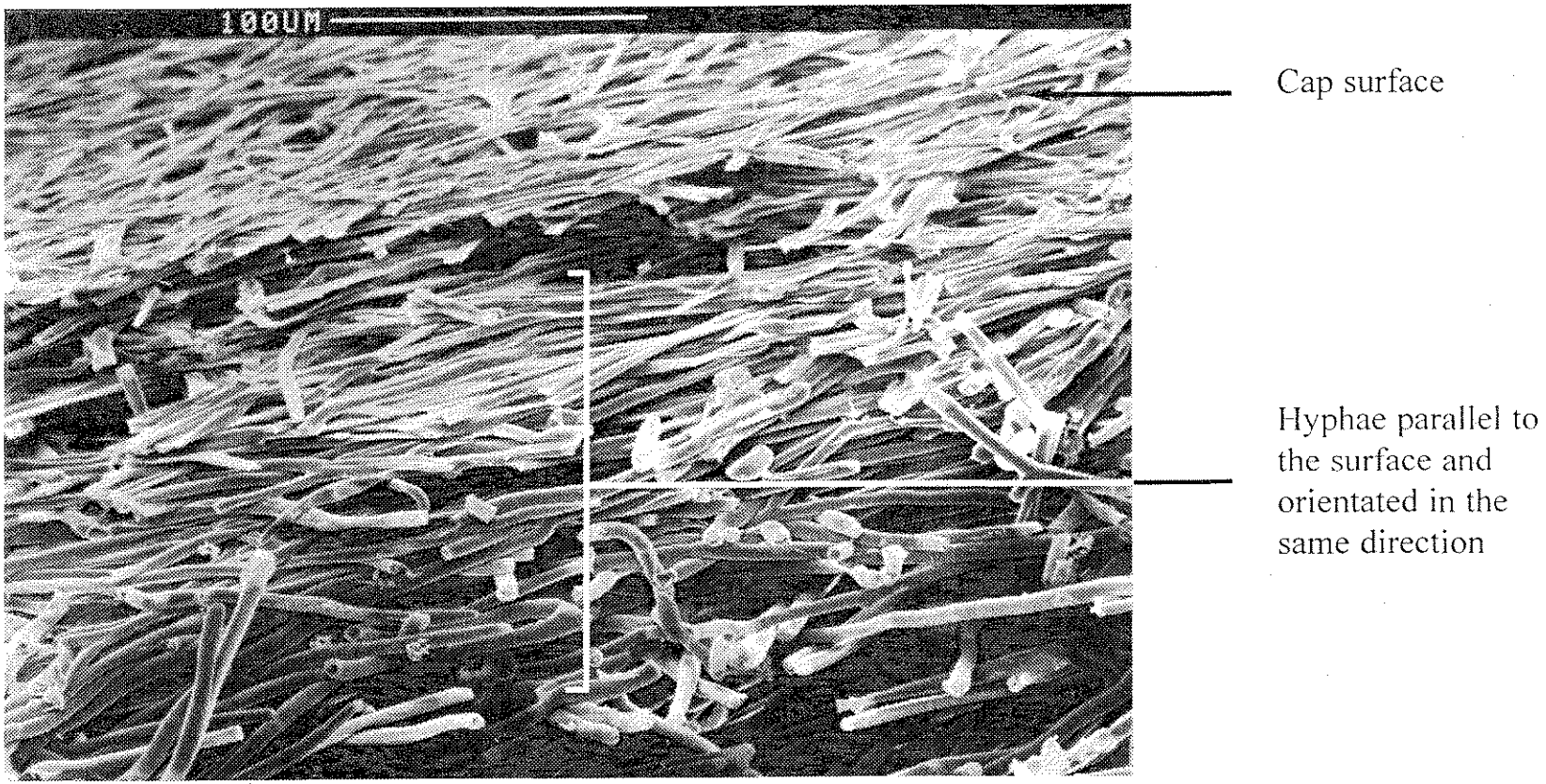


Figure 13 - Electron micrograph of a cross-fracture section from the mushroom side.

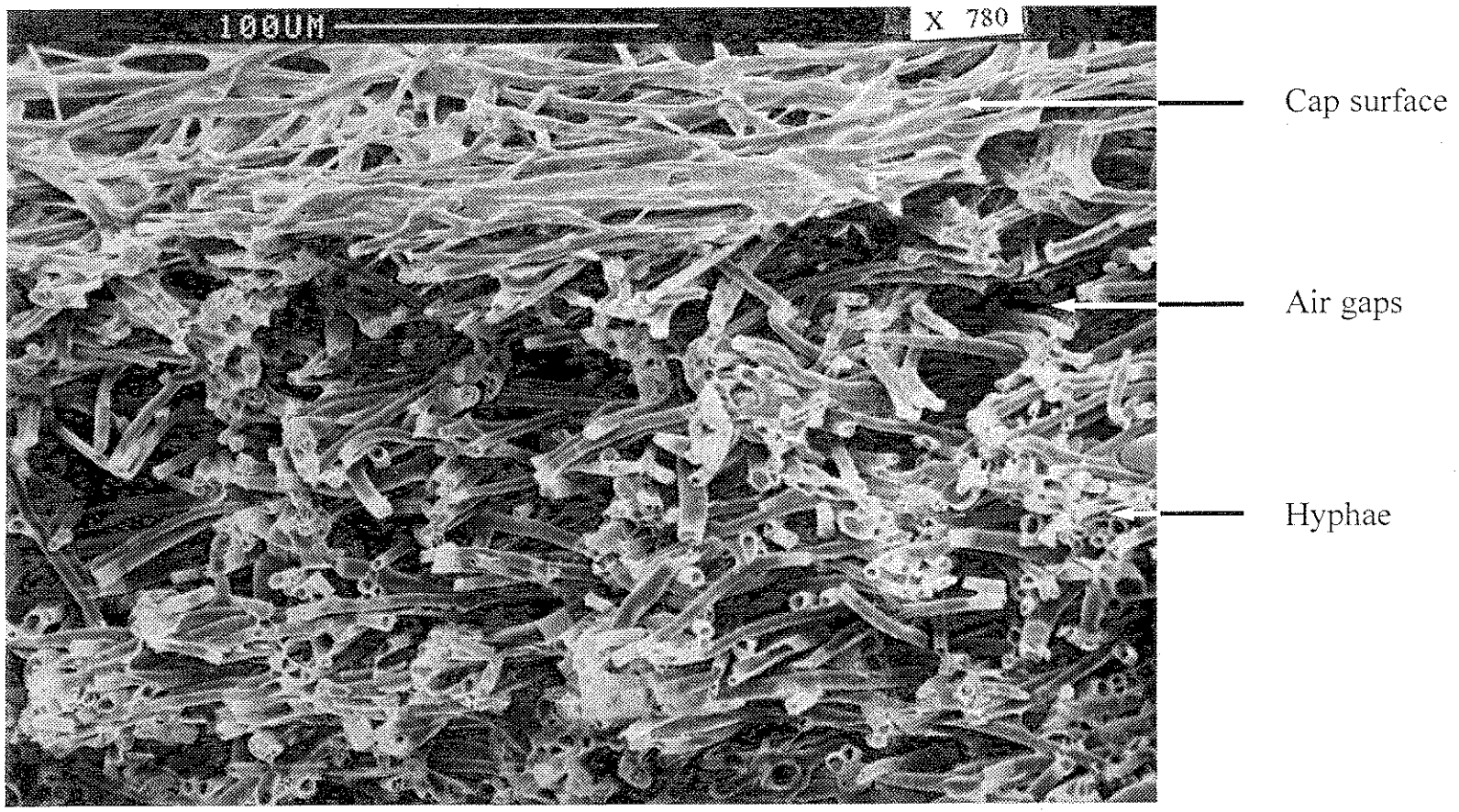


Figure 14 - Scanning Electron micrograph showing the top cross-fracture section of mushroom cap.
Note hyphae are in random directions but in plane parallel to surface.

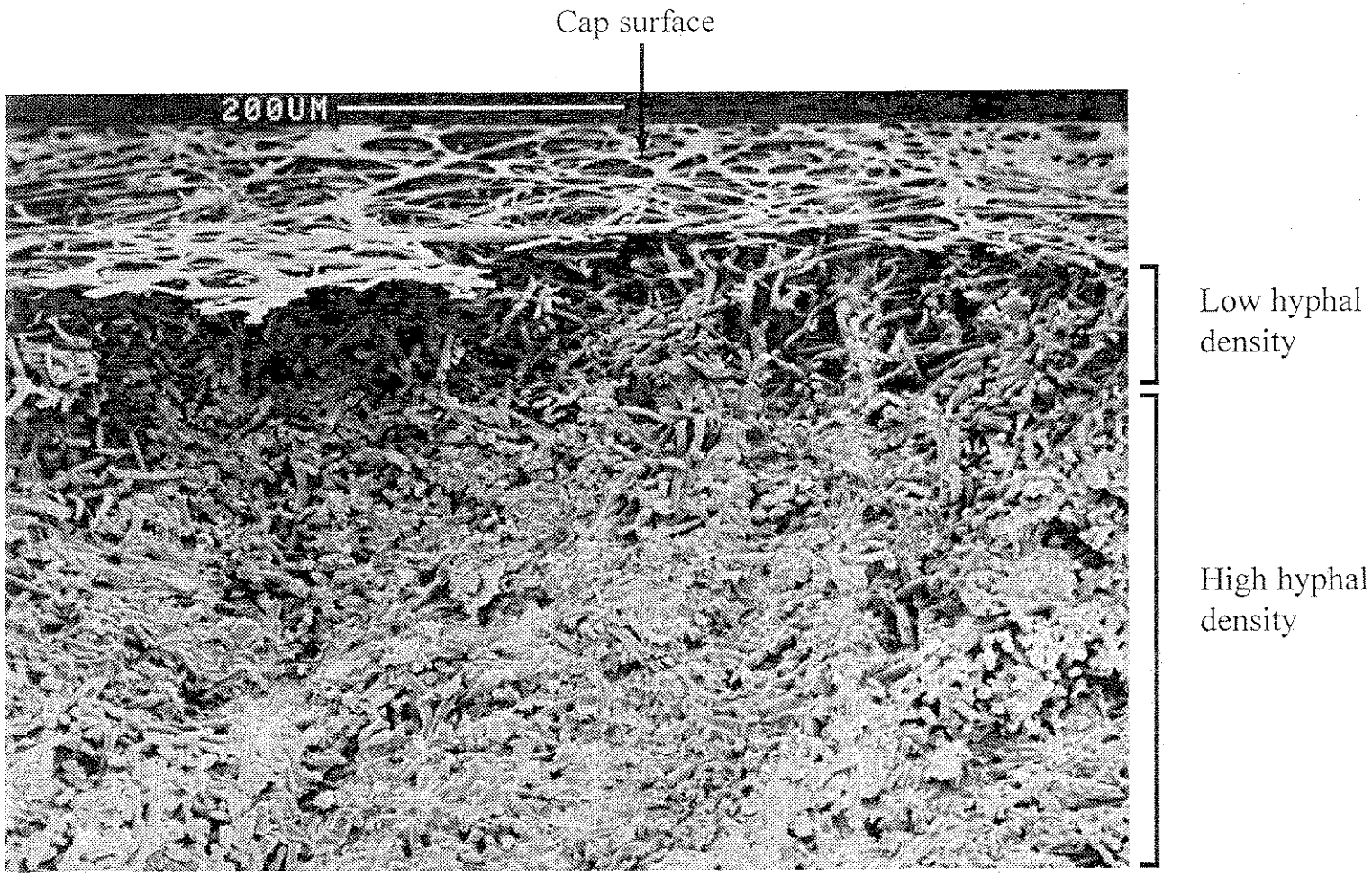


Figure 15 - Scanning electron micrograph showing fracture at the top of the mushroom cap.
Note the change in density, a low hyphal density layer near the surface and a higher hyphal density layer in the flesh

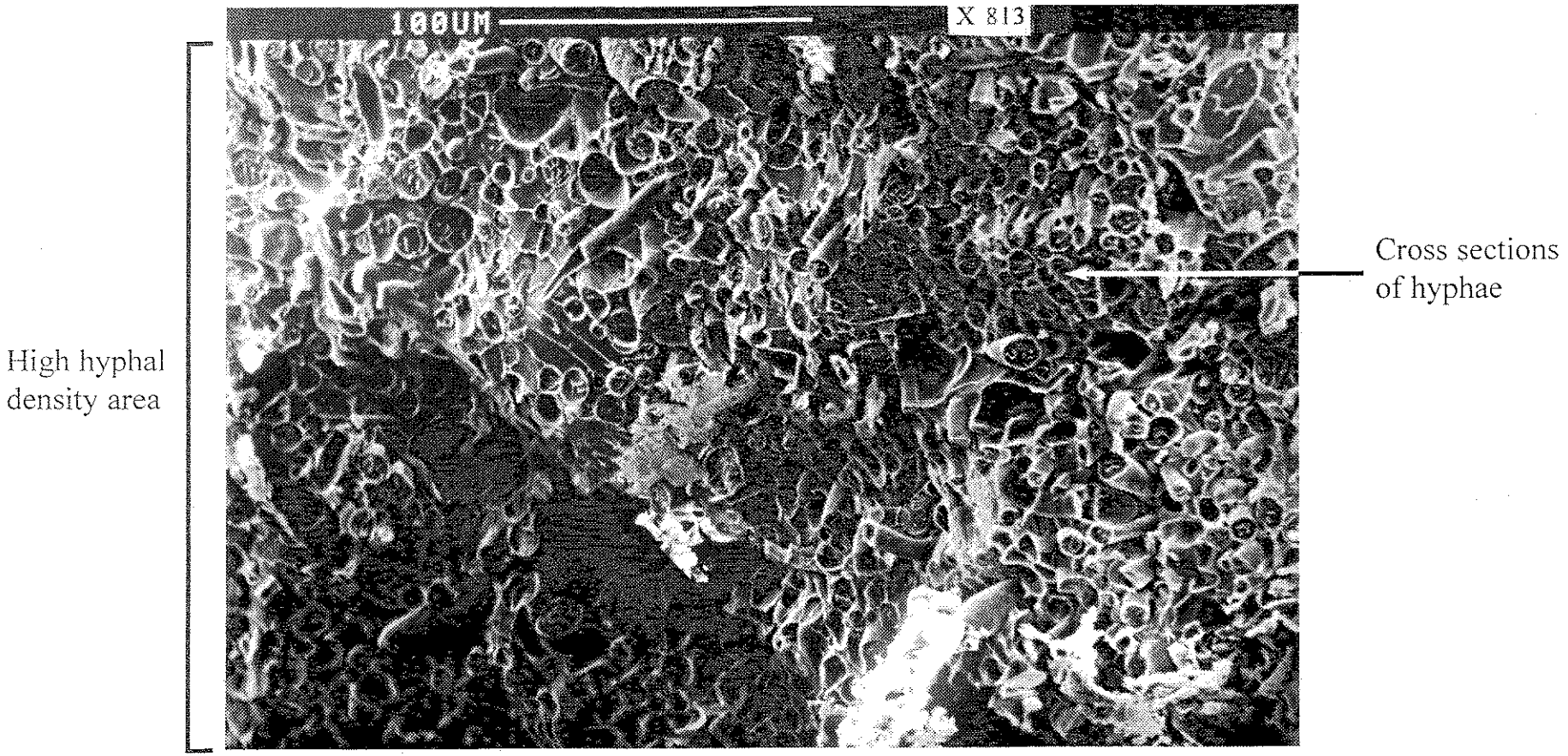


Figure 16 - Scanning electron micrograph of a cross fractured section in the deep flesh of mushroom cap showing densely packed hyphae.

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

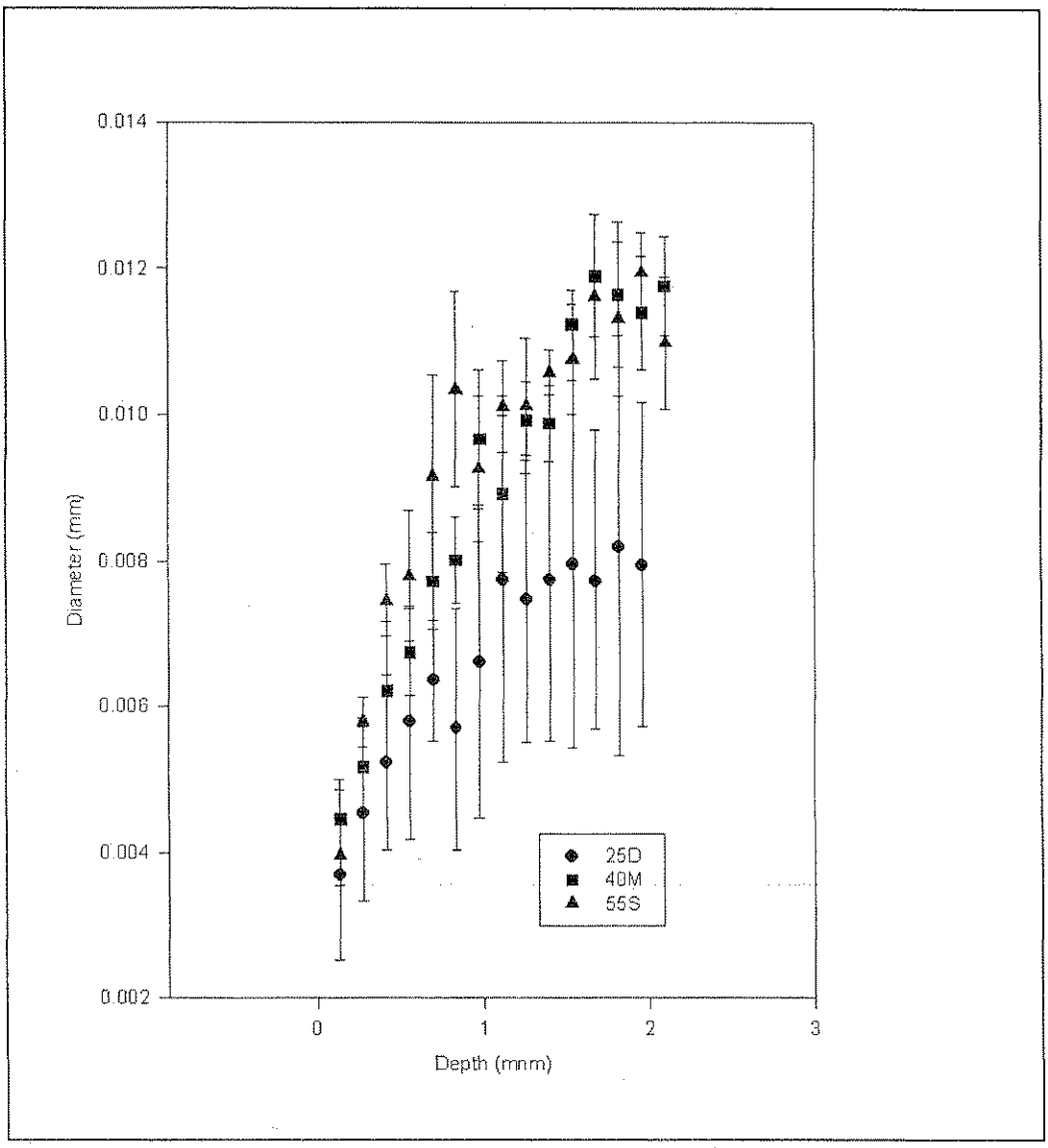
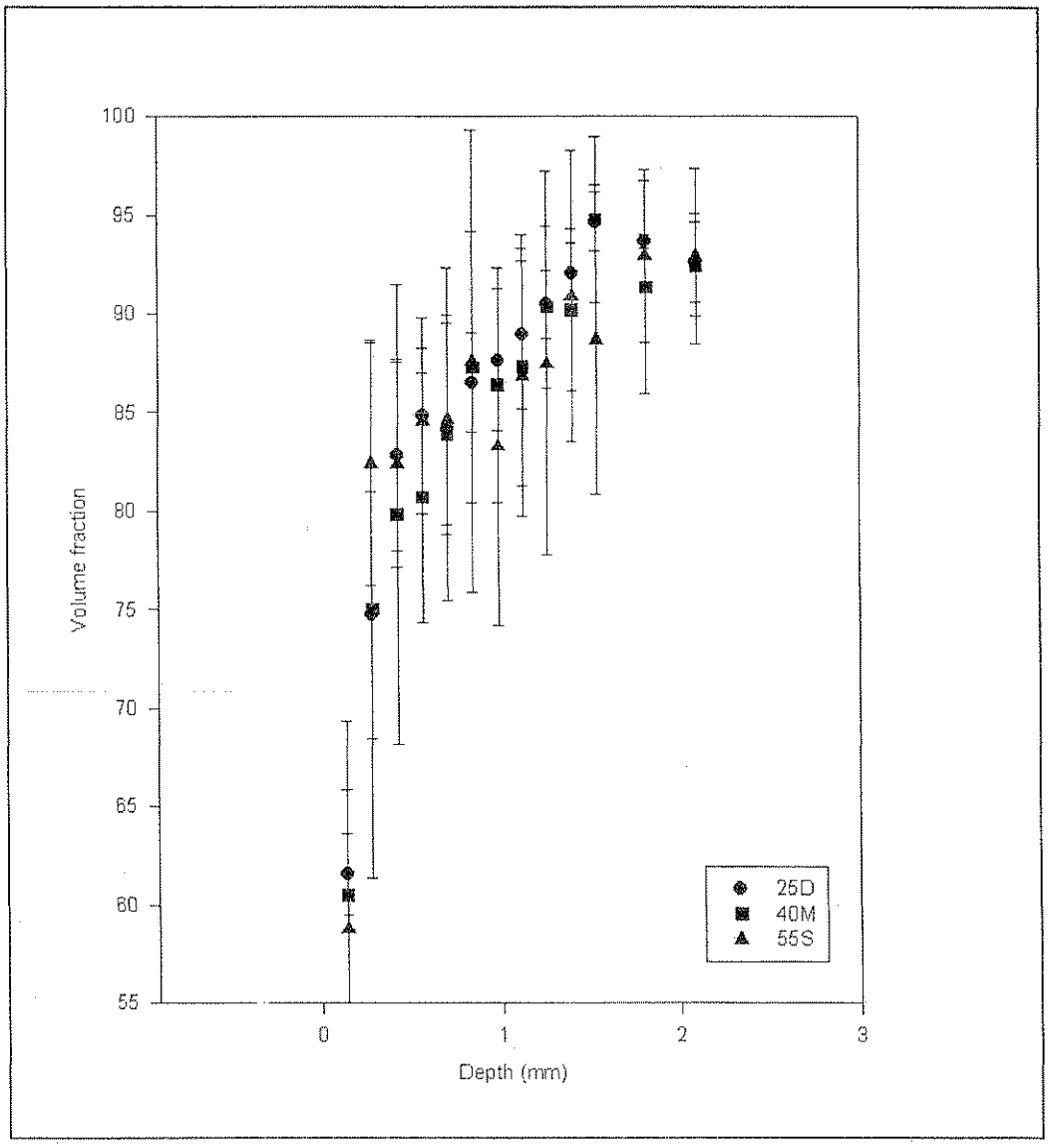


Figure 18: Volume fraction from the surface to 2 mm in the flesh of mushrooms from treatment 25D, 40M and 55S.



II.4 Discussion

II.4.1 Arrangement of hyphae on the surface

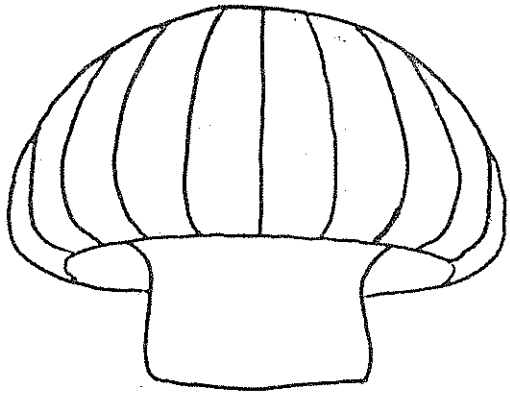
The outermost surface layer was observed to be a network of hyphae (Fig. 11). With the naked eye it was very difficult to differentiate this outer layer from the rest of the cap as it is a thin layer of about 15 □m, with no clear delineation with the underlying tissues. There is speculation about the 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 a mushroom cap grows. The splitting does not reach 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.

II.4.2 Orientation of hyphae under the surface layer

The light micrographs (Fig. 6 & 9) and electron micrographs (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



II.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 features e.g. staining intensity, hyphal density and orientation (Fig. 20).

II.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 easy 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 to your fingers. The electron micrographs 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 to 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

II.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 □m thick. Hyphae are radially orientated in the cap, the hyphal diameter is small.

II.4.3.3 Layer C

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

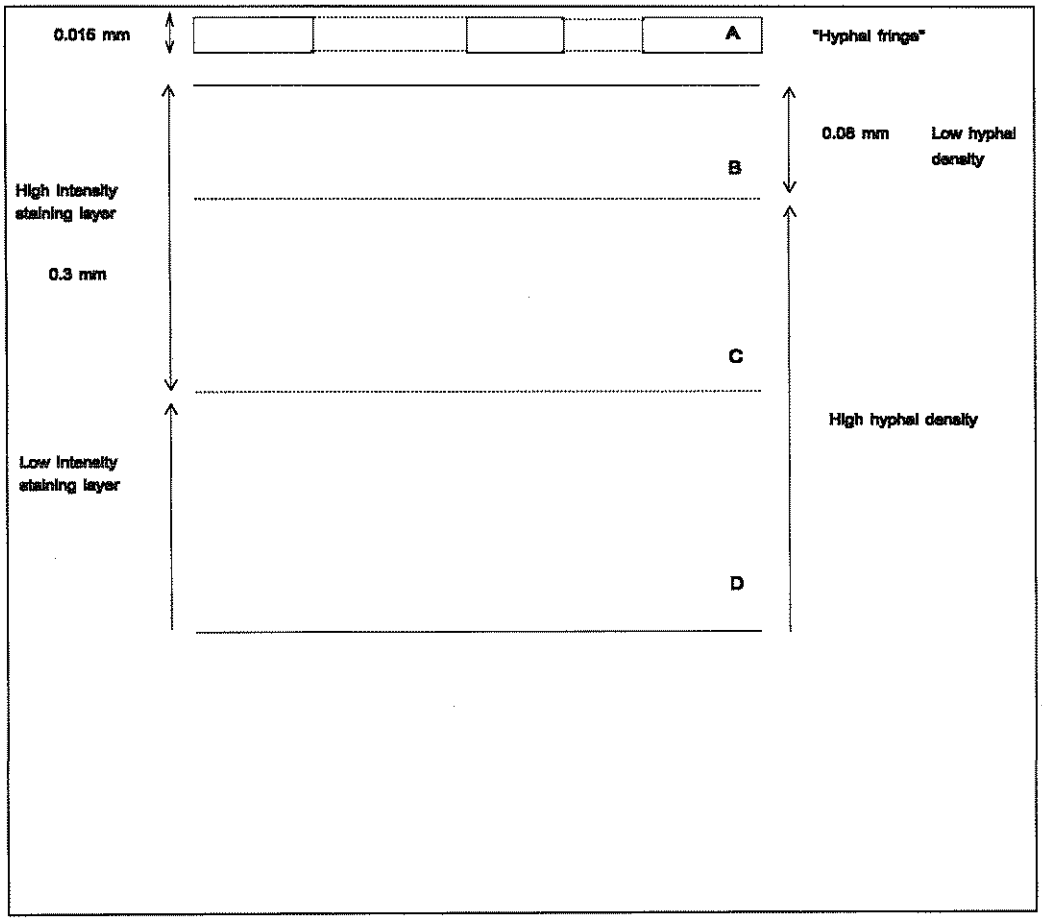
II.4.3.4 Layer D

This layer has low intensity staining and high hyphal density. At the moment it is not known how deep this layer is. Hyphae here have a larger diameter than hyphae of the

previous layers. Most of the hyphae are parallel to the surface but there are others which go 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.



B. PART III

Mechanical properties of the sporophore

III.1 Introduction

There is every reason to believe that biological structures are as carefully designed in Nature through the process of evolution, as structures 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 Part II, mushrooms also have a complex structure which gives a mushroom the ability to support its own weight, develop and grow, disperse spores above ground level and force the growing structure through compacted soil. The mechanical properties of mushroom tissue assist in the understanding of mushroom texture and quality.

Mushrooms are very susceptible to bruising caused by mechanical damage during handling and transport. Other fruit and vegetables like potatoes, apples, peaches, pears etc, are also very susceptible to bruising 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 commonly 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.

III.2 Materials and methods

III.2.1 Relationship of stiffness to hyphal orientation

Cylindrical cores of mushroom tissue were taken at different positions and orientations (Figure III.1) 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.

III.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.

III.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 mushroom 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 and 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)

III.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

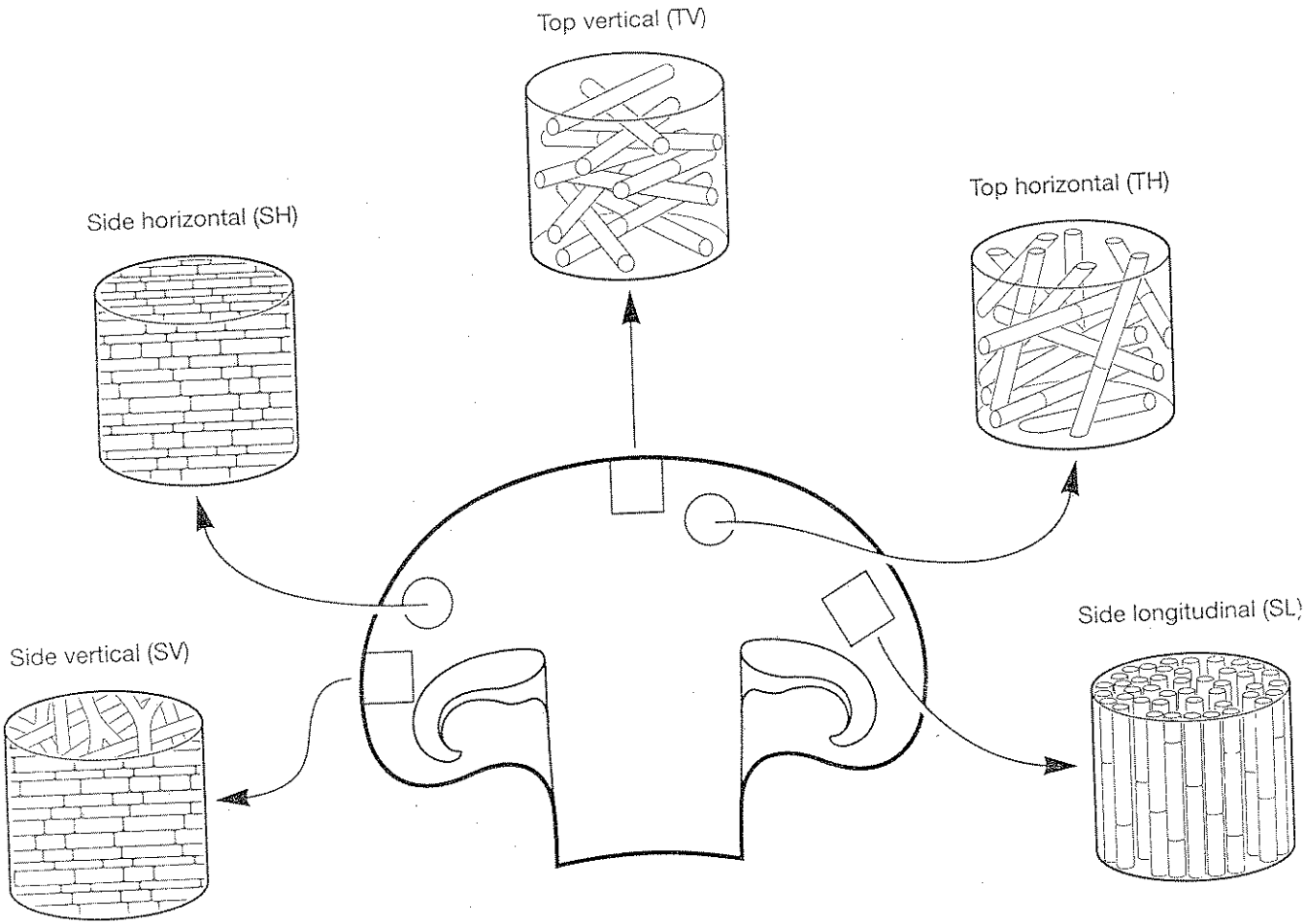


Figure III.1: Cylinders position and orientation in the cap

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

III.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 (Figure III.2).

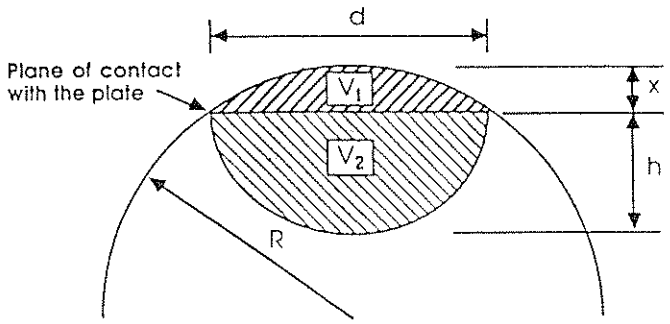
Figure III.2: Calculation of bruise volume (Holt and Schoorl, 1977)

$$V = V_1 + V_2$$

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

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

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



III.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 of J. Smith). Cylinders were taken in the cap in the top vertical (TV) and side vertical (SV) position (see Figure III.1). Ten mushrooms from flush 3 were tested.

III.3 Results

III.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 tissue stiffness depending on the orientation and where the tissues were cut from the sporophore (Figure III.3). 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.

III.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 (Figure III.4).

III.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 (Figures III.5 & III.6). There is a linear regression between the percentage of plastic deformation and the displacement, up to 4 mm compression (Figure III.5). 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 (Figure III.6). In proportion, it requires a lot more energy to create an 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 obtained is a typical load (or energy)-displacement curve (Figure III.7).

III.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 (Figure III.8). The energy absorbed increases linearly when the total energy supplied to mushrooms during rapid or slow compression 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 than bruise volume resulting from rapid compression test for the same amount of energy absorbed (Figure III.9).

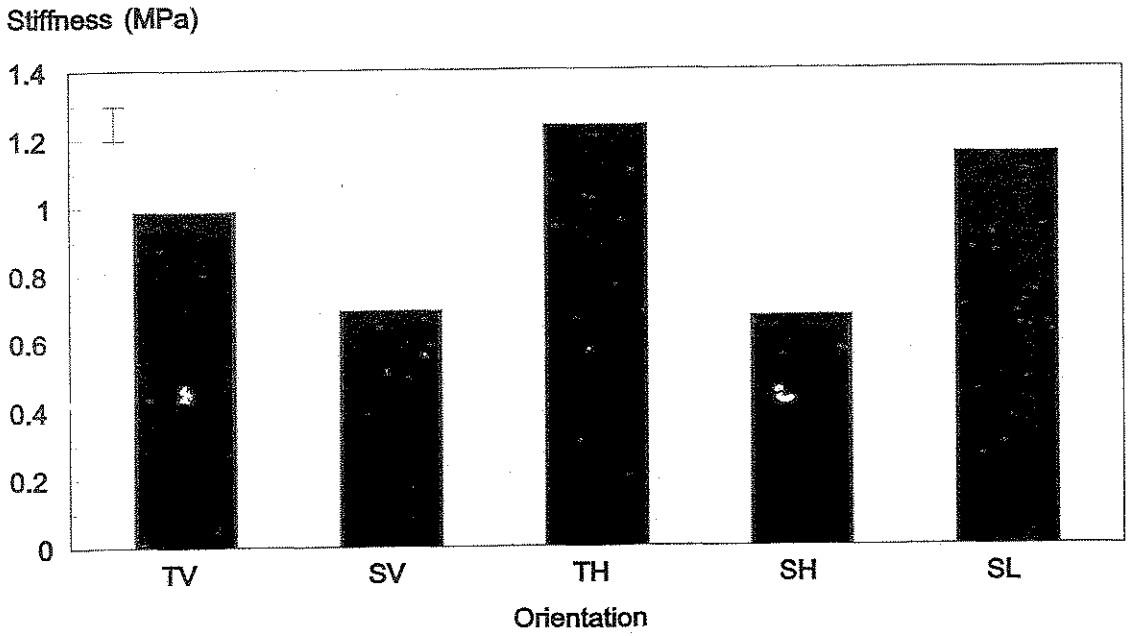


Figure III.3: Stiffness of mushroom tissue taken in different position and orientation in the cap (see Figure III.1)
TV: Top Vertical, TH: Top Horizontal, SV: Side Vertical, SH: Side Horizontal, SL: Side Longitudinal.
Bar indicates Least Significant Difference (0.05)

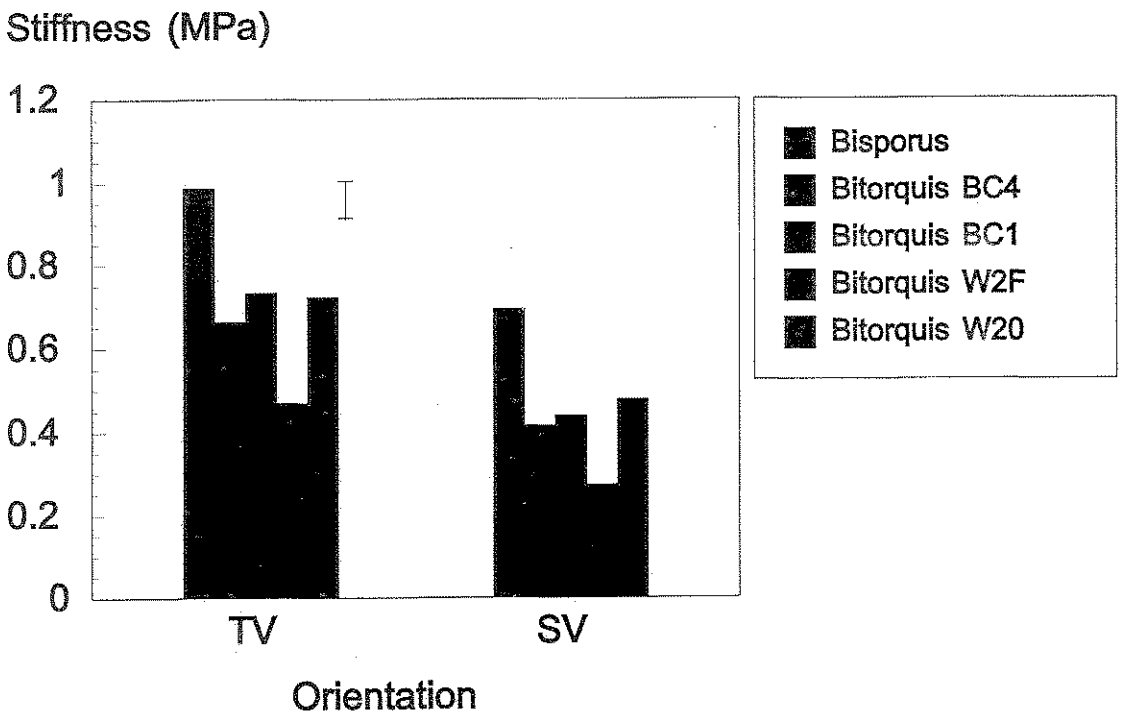


Figure III.4: Stiffness of the mushroom tissue in species *A. bitorquis and bisporus*
TV: Top Vertical, SV: Side Vertical. Bar indicates Least Significant Difference (0.05)

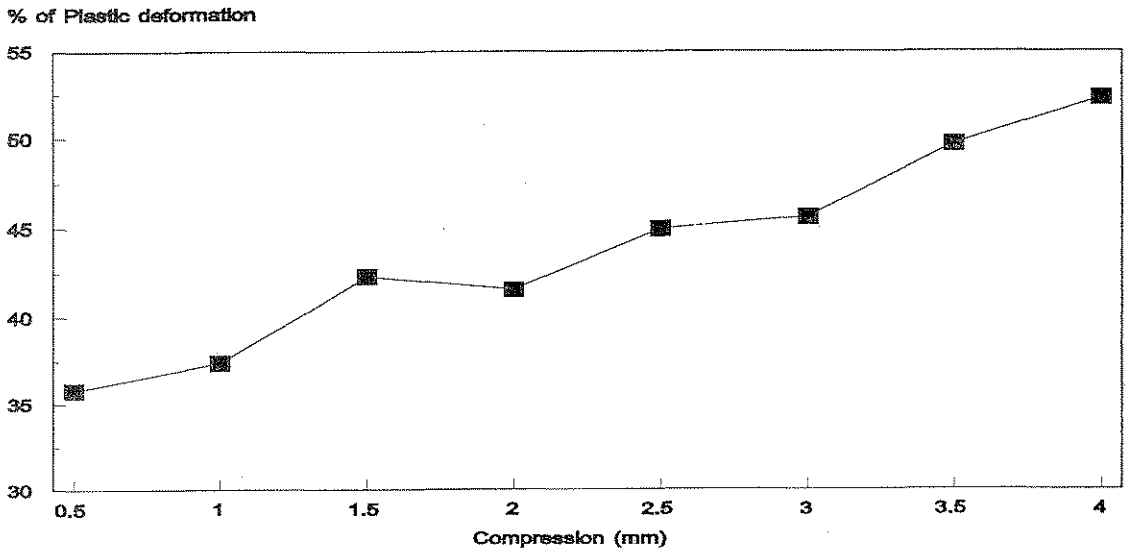


Figure III.5: Percentage of plastic deformation produced on mushrooms cap, under compression at increasing displacement.

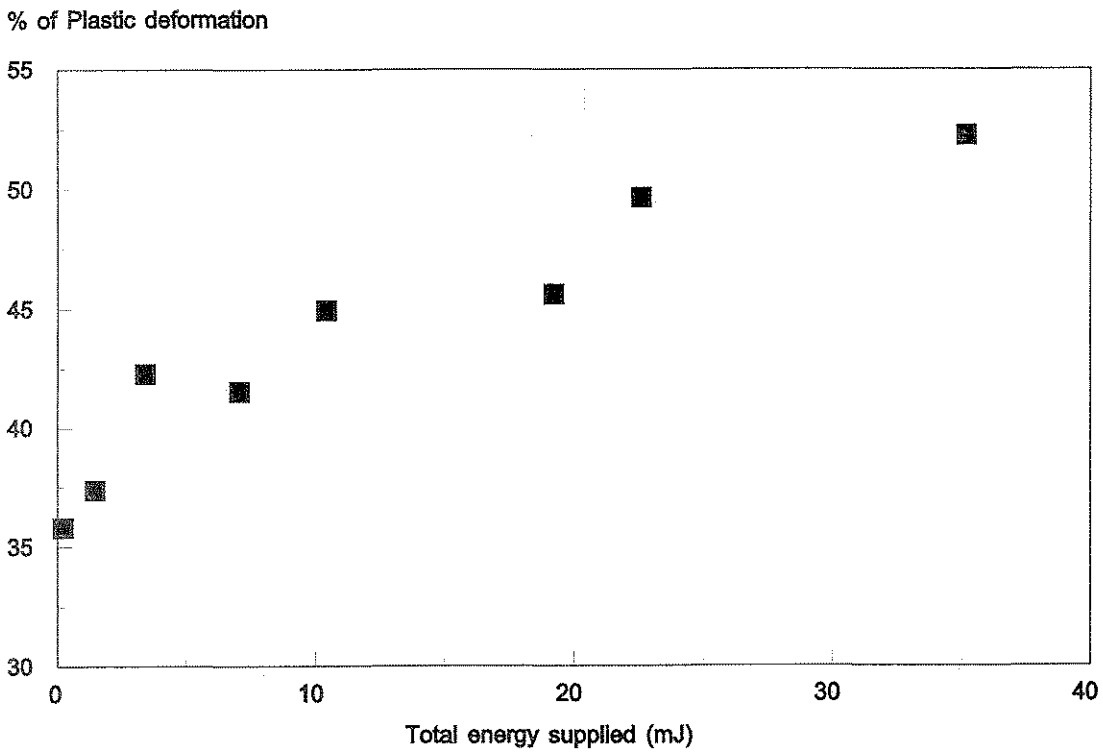


Figure III.6: Total energy supplied to mushroom cap to produce a plastic deformation.

Figure III.7: Total energy supplied to mushroom cap during compression at increasing displacement.

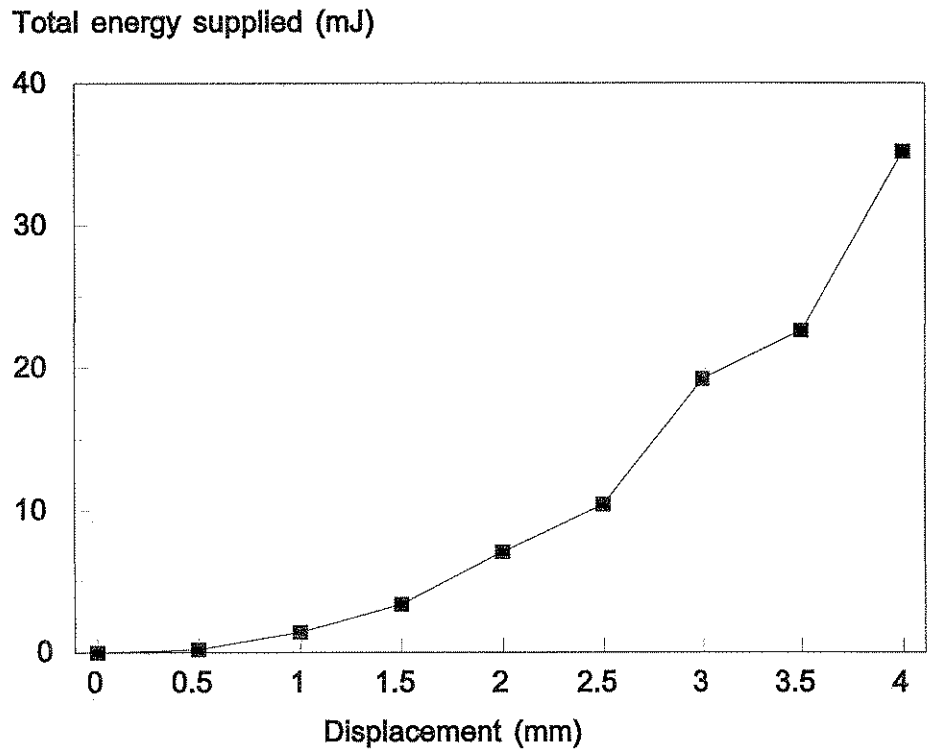


Figure III.8: Energy absorbed by mushroom caps when subjected to slow and rapid compressions.

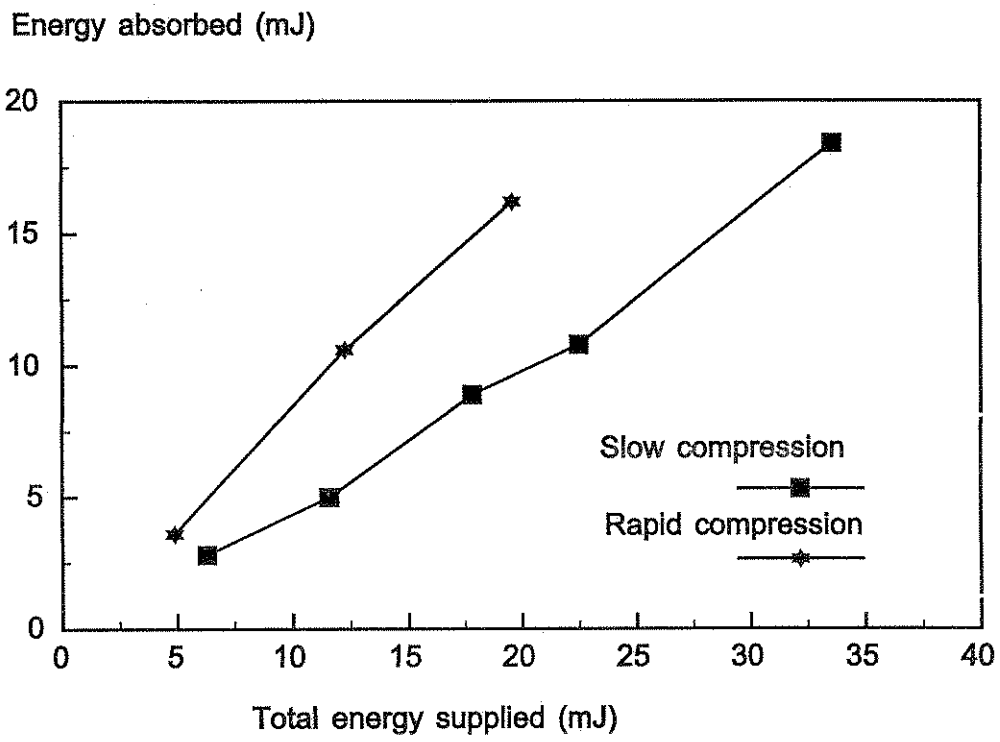
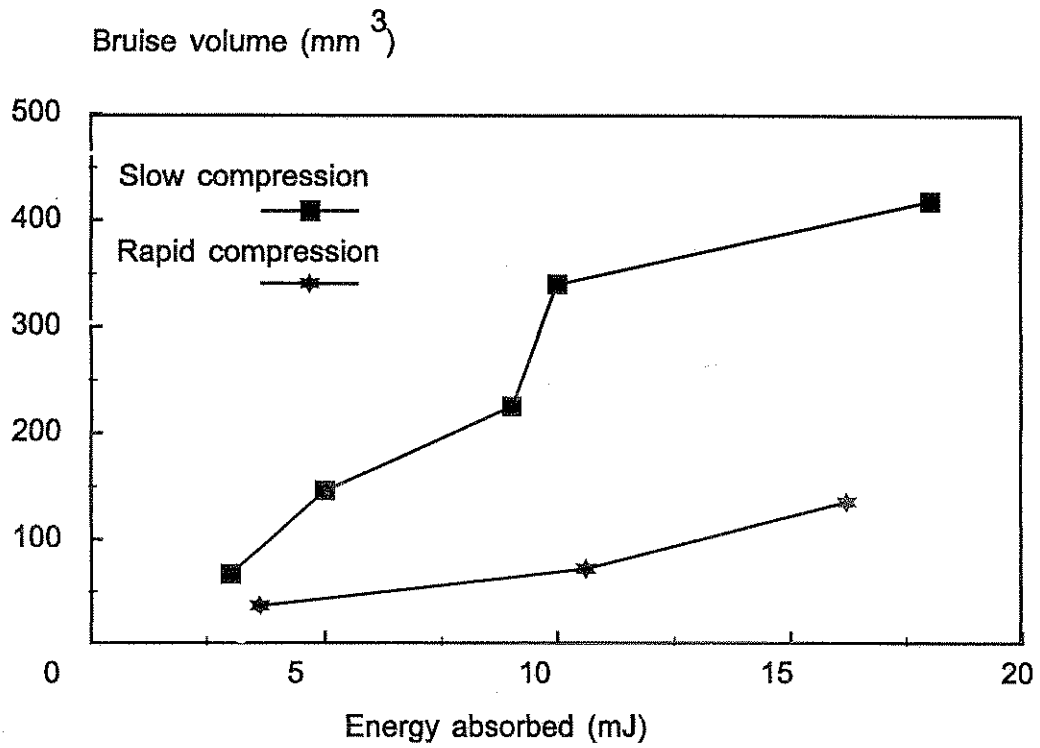


Figure III.9: Bruise volume developed in mushroom caps after either a slow or a rapid compression.



III.4 Discussion

III.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* 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 Figure III.1), 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 Chapter 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 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.

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

The percentage plastic deformation increases with increasing compression displacement (Figure III.5). 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 is larger than near the surface, these hyphae might be more brittle and would burst under the compressive forces.

III.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 (Figures III.8 & III.9). 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) hypothesised 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.

B. PART IV

On-farm textural measurement

IV.1 Introduction

After mushrooms are harvested on farms, they are held at cool temperatures until sold to retailers. A quality assessment is done shortly after harvest. Some of the criteria for their quality are maturity, colour, stalk length and appearance-damage. According to a consumer survey, the major quality attributes for mushrooms are colour, maturity, firmness and flavour. As these parameters are very important to the consumer it seems reasonable that they should be part of the quality assessment. Colour and maturity are part of the quality assessment in most farms, however firmness and flavour are not. Mushrooms have a very strong and specific flavour so there are not major differences between one lot of mushrooms to another. Firmness, however, can vary from crop to crop and farm to farm, but there is not a readily available method to quantify texture.

One of the aims of this project is to produce a technique, which would enable growers to assess the textural quality of mushrooms on the farm. For this technique to be successful, it must be reasonably accurate, reliable, cheap (in terms of materials, equipment and labour) and fairly rapid. It is hoped that a technique to assess texture would be of use for growers:- (a) in assessing quality of mushrooms sent out to retailers/wholesalers as part of the quality control procedure and (b) in measuring how quality might be affected by changes in farm practice/agronomy (different casings, composts, CO_2 levels, use of supplements, agrochemicals, watering practice, etc).

A number of ideas were considered and these were reduced down to two techniques. The first of these is a procedure and formula which estimates mushroom firmness to a reasonable degree of accuracy using easily obtained fresh weight and dry weight measurement. This technique is described in detail below.

There is of course a crude method of evaluating firmness by squeezing mushrooms between your fingers but this method is very subjective and dependent on operators, so by the means of a device, the quality assessment, regarding firmness, will be more objective. In our laboratories it is possible to measure firmness by the mean of the Instron machine. However, this machine costs about £20K so it is uneconomical

having such a machine on mushroom farms. We have been looking for an alternative method to estimate mushroom firmness which could be easily used by growers. Previous experiments done on mushroom stipe (McGarry and Burton, 1994) have shown that the stiffness of the stipe was related to the sample density. Other work (Part **V**) have shown stiffness of the cap tissue could either be related to its dry matter content ($r^2 > 0.9$) or not be related to it ($r^2 < 0.2$). As mushroom density is proportional to its dry matter content and to its water content it is reasonable to find a relationship between mushroom stiffness and both its dry matter and its water content. The purpose of this investigation was to use a simple formula with easily obtained measurements such as dry matter content and water content of mushroom tissue to estimate mushroom stiffness. As the water content is calculated from the fresh weight minus the dry weight, it was more convenient to take in account the fresh weight instead. So the challenge was to relate the mushroom stiffness to its fresh weight and dry weight.

1 A formula was determined empirically from a large number of mushrooms with varying textural properties where on each mushroom the stiffness was measured and the fresh weight and the dry weight were also determined.

2 The formula was tested on farms and to do so the growers were asked to measure the fresh weight and the dry weight of mushrooms and to give a subjective evaluation of the firmness. An estimated stiffness was then calculated and compared with the evaluated firmness. Some aspects, like the strain and the flush number, were also taken in account for the validity of the formula.

The second technique stems from the finding of the project that the mechanical process most damaging to mushrooms (in terms of bruising) is the process of 'slip-shear'. An example of when slip-shear force is applied is when one slides one's finger over the surface of the mushroom with some downward force. This occurs during picking and to a lesser extent when mushrooms rub against each other before or after harvest. However, no slip-shear device is currently available for experimentation. We at HRI have been collaborating with the Mechanical Engineering Department of Coventry University to design and build devices to inflict a controlled amount of slip-shear force on to the surface of mushroom. Two design teams, each of four people, have been working on the production of two such devices, which we are calling 'bruisometers'.

IV.2 Materials and Methods

To produce mushrooms with a wide range of texture (soft to firm), they were cultivated either in standard conditions and also in experimental conditions involving variations in the casing and compost depth. The mushroom strain U1 and abnormally firm mushrooms, provided by a farm, were also tested. Tissue stiffness, dry weight and fresh weight were measured on mushrooms grown for all the different treatments.

IV.2.1 Mushroom cultivation

IV.2.1.1 Mushroom cultivation in standard conditions

Mushrooms were grown as per method in part I but either the casing or the compost composition changed:

- Four types of casing containing varying amount of peat were used.
- Four different types of compost which contained:

- High nitrogen level
- High nitrogen level plus recycled phase 2 compost
- Low nitrogen level
- Low nitrogen level plus recycled phase 2 compost

Mushroom strain U1 grown in standard conditions and abnormally firm mushrooms provided by a commercial farm were included in the experiment.
For each treatment ten replicates of mushroom were tested.

IV.2.1.2 Mushroom cultivation in experimental conditions

Mushrooms were grown on polypropylene trays (600 mm x 400 mm) filled with deep or shallow compost (260 mm with 19 kg and 110 mm with 4.5 kg) and covered with 25 and 55 mm deep casing layer. The factorial combination was:

- Deep compost and 25 mm casing

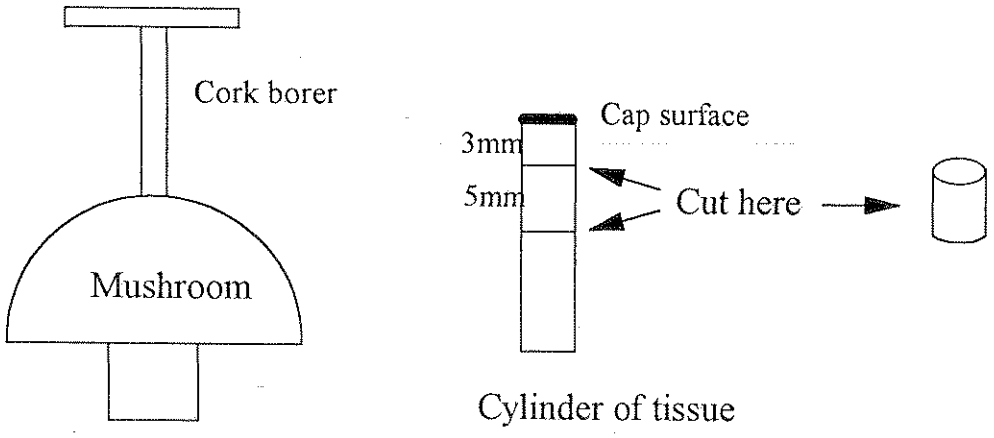
- Deep compost and 55 mm casing
- Shallow compost and 25 mm casing

Ten replicates of each treatment were tested for the first three flushes.

IV.2.2 Stiffness, fresh weight and dry weight measurements

A cylinder of tissue was taken on each mushroom by driving a cork borer (n°2) into the top of the mushroom cap. A 5 mm height cylinder was cut off with parallel blades at about 3 mm from the cap surface (see Figure IV.1). The fresh weight of the cylinder was determined and the stiffness of the tissue was measured as per method **II.6**. The cylinder was let to dry 48 h in an oven at 70° C and the dry weight was measured.

Figure IV.1: Removal of a plug of tissue from the mushroom cap.



IV.2.3 Statistical analysis

The formula was determined empirically by Genstat analysis. All the other data were also analysed by Genstat.

IV.2.4 On farm evaluation of the formula to estimate stiffness

The obvious experiment to do to evaluate the formula would be to calculate the stiffness by the formula and compare it with the stiffness measured with the Instron machine. This is unfortunately not possible because:

a) The farms are more than 3 hours driving distance from HRI and by the time mushrooms would have been tested they would have lost their textural properties.

b) The Instron machine is big and fragile so it is not recommended to transport it.

Therefore an experiment was performed to:

a) examine how easily such a test could fit into existing quality assessment procedure b) see how well the estimated stiffness agrees with subjectively obtained measures of texture.

Two major mushroom farms kindly agreed to take part in this evaluation. Growers were asked to collect mushrooms and to record certain features like the room number, the mushroom strain, the maturity and the flush number. They were also asked to give a subjective idea of the mushrooms' firmness (soft, medium or firm) just by gently squeezing them between fingers. The mushroom appearance that includes the overall colour and the presence of bruise patches were recorded as well as any changes in the cultivation (e.g. casing, compost, supplements) compared to the standard cultivation.

The test provided to the growers consisted of harvesting a sample of 10-20 mushrooms at stage 2 from each plot (i.e. mushrooms grown in a particular growing room and harvested at the same time). A sample of mushroom tissue was taken from the cap as in method described in Figure IV.1. The fresh weight of the cylinder was quickly measured and it was left to dry in an oven at 70°C for about 48 h. After that the cylinder was weighed for its dry weight.

The estimated stiffness was calculated with the formula using the fresh weight and the dry weight of the mushrooms.

IV.3 Results

IV.3.1 Determination of the formula to estimate mushroom stiffness

IV.3.1.1 Correlation between stiffness and fresh weight

The measured stiffness of the mushroom cores is plotted against the fresh weight of the cores (Figure IV.2). The stiffness of the cores was found to be proportional to their fresh weight. The correlation coefficient found is $r^2 = 0.52$.

IV.3.1.2 Correlation between stiffness and dry weight

The measured stiffness of the mushroom cores is plotted against the dry weight of the cores (Figure IV.3). The stiffness of the cores was found to be proportional to their dry weight. The correlation coefficient found is $r^2 = 0.50$.

IV.3.1.3 Determination of the empirical formula

Thus the stiffness of the mushroom cores was found to be proportional to both their fresh weight and their dry weight. This can be represented by the following equation:

Stiffness (MPa) = a x Dry weight (mg) + b x Fresh weight (mg) + c
(a and b are the regression coefficients, and c is a constant)

The genstat analysis gave the following results for a, b and c.

	Value	Standard deviation
a	0.117	0.0101
b	0.0205	0.0016
c	- 1.35	0.134

The equation can be now be rewritten as:

Estimated Stiffness = 0.117 x Dry weight + 0.0205 x Fresh weight - 1.35

Figure IV.2: Relationship between the stiffness and the fresh weight of mushroom cylinders.

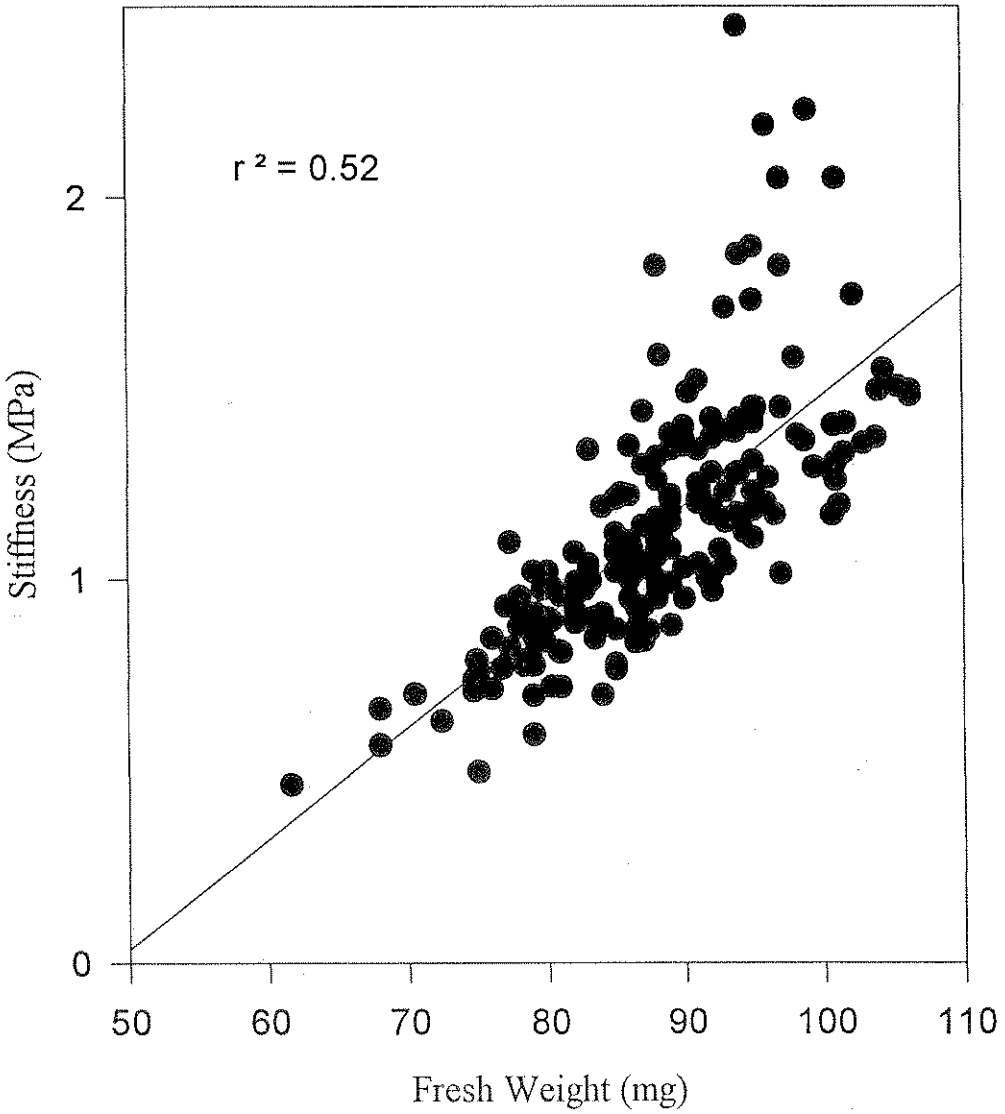
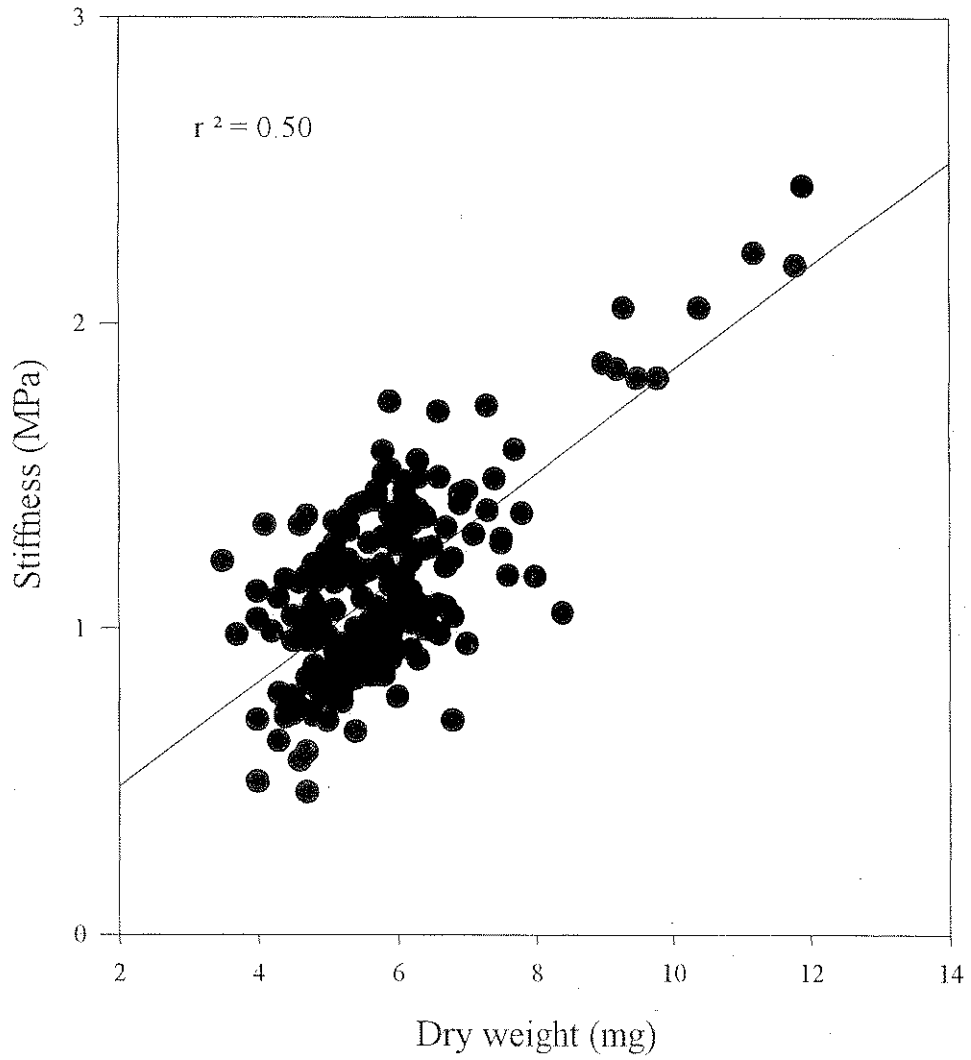


Figure IV.3: Relationship between the stiffness and the dry weight of mushroom cylinders.



IV.3.1.4 Correlation between the measured stiffness and the estimated stiffness

"Estimated stiffness" will be used to refer to the stiffness calculated from the formula using the fresh weight and the dry weight of mushroom tissue.
The data of fresh weight and dry weight used to determine the formula were used to calculate the estimated stiffness.
Figure IV.4 shows the estimated stiffness plotted versus the stiffness measured with the Instron machine. A correlation coefficient of $r^2 = 0.72$ was found between the estimated stiffness and the measured stiffness. This correlation in much higher than the correlation between dry weight and measured stiffness ($r^2 = 0.50$) or the correlation between fresh weight and measured stiffness ($r^2 = 0.52$). Therefore taking in account both the fresh weight and the dry weight gives a better evaluation of mushroom firmness than taking in account only one of these parameters.

IV.3.2 Experimental evaluation of the formula

This formula was tested using independent mushrooms grown at HRI mushroom unit under standard conditions (the data collected from these mushrooms were not used to establish the formula). The stiffness and the fresh weight and the dry weight of the mushrooms cores were measured using the method described in IV.2.2. The estimated stiffness was calculated using the formula and is plotted against the stiffness of the cores measured with the Instron machine (Figure IV.5). The correlation coefficient between the estimated stiffness and the measured stiffness was found to be $r^2 = 0.62$.

IV.3.3 Test on farms

The data of fresh weight and dry weight of mushroom cylinders measured on farms were put into the formula to determine the estimated stiffness. The results obtained in farm A and B are summarized in Figure IV.6.

IV.3.3.1 Farm A

On average there is a great similarity between the firmness evaluated by the operator and the estimated stiffness measured by our formula. There are however some

Figure IV.4: Correlation between the measured stiffness and the estimated stiffness

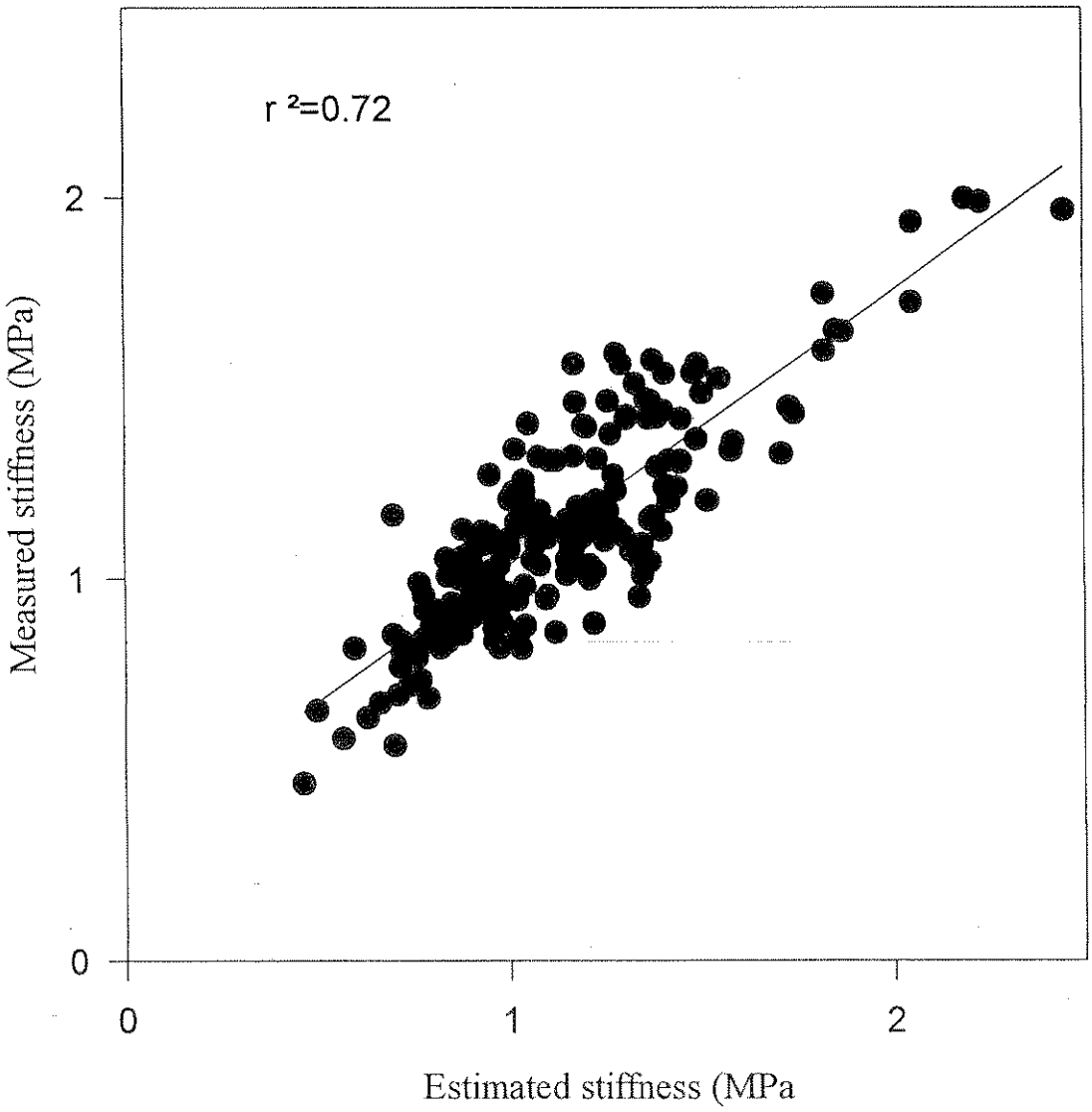
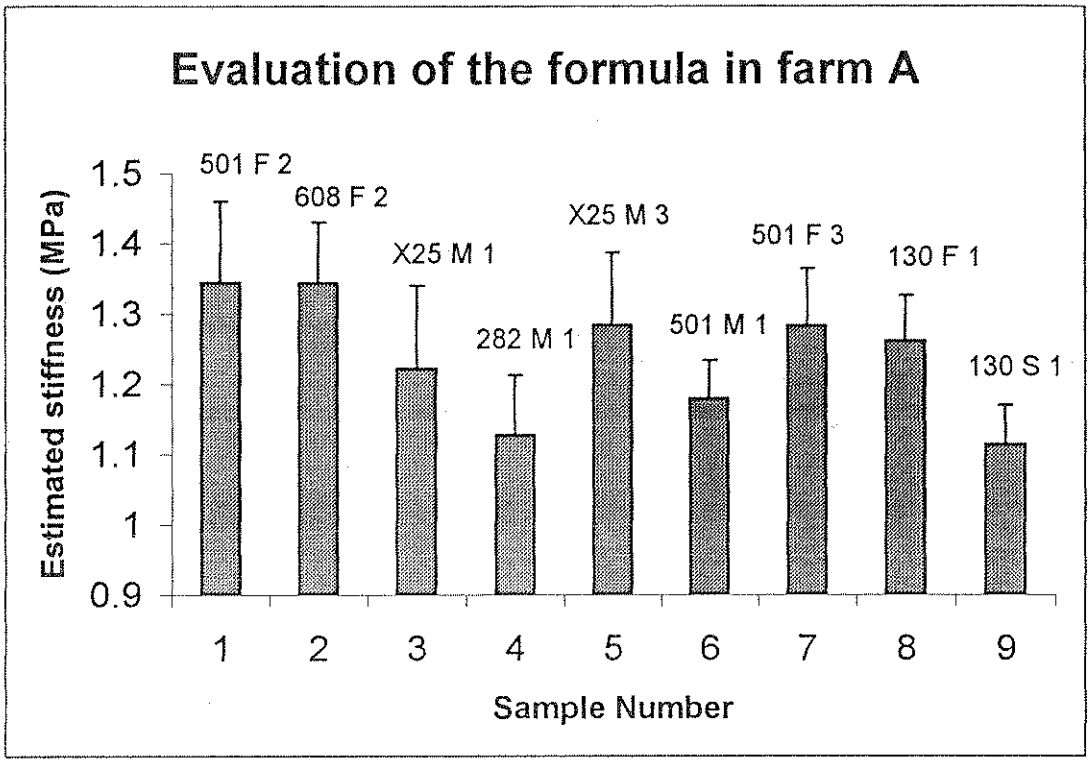
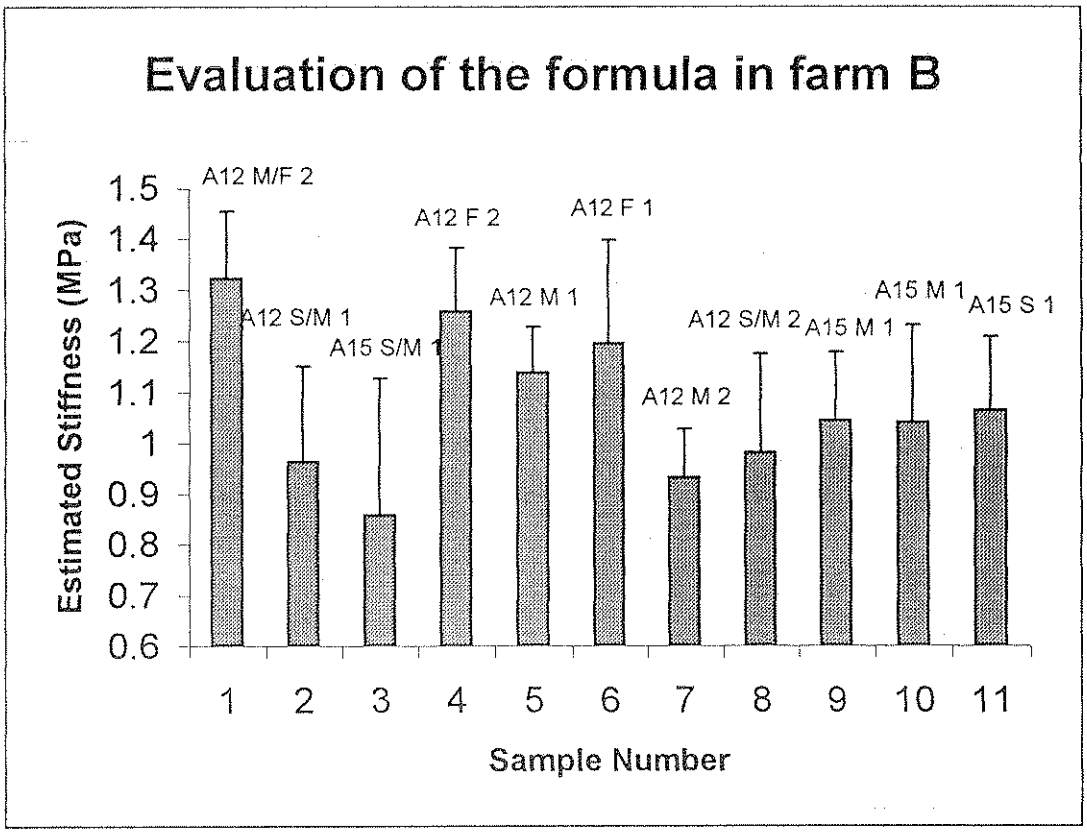


Figure IV.6: Estimated stiffness measured in mushroom farms





examples where there is a disagreement between the estimated firmness and the evaluated stiffness. Samples 7 and 8 with an estimated stiffness of 1.28 and 1.26 were evaluated to be firm whereas sample 5 with an estimated stiffness of 1.28 was evaluated medium. Equally, sample 4 with an estimated stiffness of 1.12 was evaluated medium whereas sample 9 with an estimated stiffness of 1.11 was evaluated soft.

The differences in the estimated stiffness and the evaluated firmness are probably just a question of reference on the day of assessment. The good point however is that no sample was evaluated firm and was found to be soft, and vice versa.

For a given strain there is usually a variation in the stiffness, with the flush. Flush 1 usually produces soft mushrooms, flush 2 firm mushrooms and flush 3 firm or medium mushrooms. This pattern was found for mushroom strain 501 where flush 1 was soft (sample 6), flush 2 was firm (sample 1) and flush 3 was medium (sample 7).

The same was found for mushroom strain X25 where the first flush (sample 3) was found to have a lower estimated stiffness than flush three (sample 5)

There is however a surprising result between samples 8 and 9. There are both strain 130 at first flush but sample 8 was found to have a higher estimated stiffness than sample 9.

IV.3.3.2 Farm B

All the samples which were found to be firm by the operator were also found to be firm using the formula. The samples were evaluated by the operator to be soft/medium; this again is matching the result found by measuring the firmness with the formula. One of the conclusions from these results is that the mushroom strain A15 was found, on average, to be softer than the strain A12.

IV. 4 Mushroom bruisometer

IV 4.1 The Design Process

Two design teams from Coventry University, each of four people, have been working with Kerry Burton and Tanouja Rama of HRI on the design of the bruisometer. The design process involved meetings with HRI where the design problem was identified and specified. Constraints and criteria of the design solution were identified and then ranked in importance using weighting tables.

Other than meetings with HRI, the two teams worked largely independently of each other. As a result, the two designs were different even though they both contain features which are in principle similar (a) the application of variable force, (b) a means of holding the mushroom, and (c) a way of moving the mushroom relative to applied force. Both bruisometers are portable and electrically operated using batteries.

IV 4.2 Bruisometer One

This device applies a variable force using a constant force spring attached to a pivoted bar which is in contact with the mushroom via a contact patch. The force can be varied by changing the position of the spring along the bar onto notched locations. The lowest available force is 0.396 Newtons (N). There are 15 increments of 0.196 N to a maximum force of 3.34 N.

The mushroom is held cap downwards in the device by a triangular arrangement of spikes. The mushroom can then be moved to the contact patch using a rack and pinion operated by a thumb-wheel via a gearbox.

The slip-shear treatment is delivered by rotating the mushroom while the constant force spring is applying force radial to the mushroom via the contact patch. The rotation was driven by a battery-operated electric motor geared to a maximum of a complete rotation in one second. Different speeds are achieved using three alternative sets of resistors which can be switched to apply different voltages to the

