CULTIVATION OF EXOTIC AND LOCAL MUSHROOM SPECIES FOR COMMERCIAL PRODUCTION

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DECLARATION

This thesis and associated research comprises my own original work except for assistance which is acknowledged, or where due reference is made in the text. This work has not been submitted for degree purposes to any other University.

Samantha Adey

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ABSTRACT

The project was undertaken primarily to determine the potential of growing *Stropharia rugoso-annulata* and *Volvariella volvacea* at a commercial level under South African conditions. *Termitomyces umkowaani*, an indigenous mushroom, was also investigated. Mushroom culturing, spawn production and optimizing fruiting substrates were determined. The feasibility of commercial and subsistence production in KwaZulu-Natal was investigated.

Of the seven strains of *S. rugoso-annulata* purchased from CBS (Baarn, the Netherlands) that were tested, Strain 289.85 was the most vigorous. The best agar medium for culturing of *S. rugoso-annulata* was potato dextrose agar (PDA) followed by malt extract agar (MEA) and vegetable juice agar (V8). Maximum growth of the mushroom mycelium occurred from Day 7 to Day 14, as expected. The best spawn substrate tested was sorghum, and the best fruiting substrates tested were banana leaves and maize stover. As *S. rugoso-annulata* is a white-rot fungus capable of degrading lignin, this result was expected. *S. rugoso-annulata* grows best on substrates with a low C:N ratio.

Strain 1665 of *V. volvacea* was the best of the three strains obtained from CBS. The best culture medium for *V. volvariella* was PDA amended with 1% straw. V8 agar alone supported poor growth. In trials for suitable spawn and fruiting substrates for *V. volvacea*, banana leaves supported the best mycelial growth. Mycelium grown on V8 amended with 1% maize stover showed the greatest expansion on the substrates tested.

A strain of *T. umkowaani* was isolated from a fresh basidiocarp collected in Pietermaritzburg, KwaZulu-Natal. Growth of *T. umkowaani* was most rapid on a Basal Medium but mycelial vigour was inferior to that of mycelium grown on Basal Medium amended with rabbit faeces, especially at the level of 2.5%. High levels of contamination occurred when more rabbit faeces was added to the Basal Medium.

Microbial contamination was a major limiting factor to the productivity of this project. The primary sources of culture contamination were due to mites, inadequate sterile technique and a high level of *Trichoderma* spores in the Department due to an ongoing *Trichoderma* biocontrol project. Placing the bags of cultures on trays with the edges smeared with petroleum jelly effectively kept the mites away from the cultures. The contamination of a stock culture with *Penicillium* was solved with the use of Benomyl-amended agar media. Contamination problems in spawn production were the result of the inoculation process (especially when conducted by one person), the initial leaking of cotton wool caps, polypropylene bag seams and micropores and cracks in the walls of polypropylene bags. Treating the cotton wool in the caps with Busan 30A prevented the entry of contaminant bacteria and fungi through the caps. The problem of leaking seams of the bags was solved by using a longer heating period on the bag-sealer machine. SEM studies confirmed the presence of micropores and stress fractures in the walls of the bags used in this project. This problem can be solved by using high quality polypropylene with a reduced content of plasticiser.

A systems analysis of exotic mushroom production, the process of mushroom production, steps in the process, sales and constraints in the South African context, and possible solutions are discussed.

Spawn production by small growers is not economically feasible due to the capital required. Access to capital is a constraint of production in controlled environments but should not limit outdoor production. However, outdoor production is constrained by climatic requirements of the fungi. Based on optimum temperatures, GIS maps of KwaZulu-Natal were generated to display the potential areas for outdoor production. Overlap of suitable regions for production of *S. rugoso-annulata* and *V. volvacea* is limited. Outdoor production of *V. volvacea* will be limited to Northern coastal regions for only four months of the year.

A computerized decision support system was developed to answer questions of feasibility of production to the mushroom growers, based on the requirements of the mushrooms.

A current lack of marketing of speciality mushrooms is considered to be a major constraint to sales and therefore potential production, particularly for subsistence farmers.

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CHAPTER 1: INTRODUCTION

Introduction

The world population is increasing at a rate of about 80 million people per annum, which is equivalent to the entire population of a country such as Mexico. Africa has the second largest population in the world but the highest population growth (FAO Yearbook, 1990).

Conventional agricultural techniques are not able to keep up with increasing demands for food. The present situation calls for innovative thinking and strategies for alternative sources of high protein foodstuffs (Eicker, 1993).

Africa has the lowest percentage of arable land of any continent and much of the arable land is inhabited by peasant communities who produce only for subsistence. The most important agricultural products of these communities are cereals. Other crops include coffee (*Coffea arabica* L.), beans (*Phaseolus vulgaris* L.), sugar (*Saccharum officinale* L.), cotton (*Gossypium arboreum* L.), groundnuts (*Arachis hypogaea* L.), tobacco (*Nicotiana tabacum* L.), vegetables, and fibre plants. Large amounts of residues are produced from these crops, more than 75% of which remain unused, with less than 25% used domestically. With cereals, the quantity of straw and other waste equals or exceeds the amount of grain produced (Lelley, 1986). Plant wastes contain cellulose, hemicellulose and lignin. Many fungi are able to flourish on these materials. Some of these fungi are edible mushrooms (Lelley, 1986). Production of food from waste is therefore an important concept, especially in the context of the need to increase food production.

The energy crisis of the 1970's led to a great interest in the recycling of organic wastes, concurrent with concerns over environmental problems arising from the accumulation of waste materials. It was recognised that fungi could have an increased role

in the recycling of organic waste and that this was related to reducing environmental pollution (Chang and Miles, 1989).

Growing mushrooms for food protein requires less land than producing protein from animal and plant sources. Many of the mushrooms presently under cultivation contain 20-44% crude protein (Lelley, 1986). The nutritive value of foodstuffs can be expressed using a Nutritional Index (**Table 1**). This is calculated using the following formula:

$$\textbf{Nutritional Index} = \frac{\textbf{essential amino acid index X \% protein}}{\textbf{100}}$$

The essential amino acid index is calculated as a ratio of essential amino acids in food, relative to the essential amino acids required in the adult human diet (Royse and Schisler, 1980). Based on the Nutritional Index, *Agaricus bisporus* (Lange) Singer ranks above all vegetables except soybeans and spinach (**Table 1**).

Besides their protein content, mushrooms contain high levels of vitamins B, C and D, riboflavin, thiamine, nicotinic acid and niacin. Mushrooms are also a good source of iron, potassium, folic acid and phosphorus (Lelly, 1986).

In addition to their nutritional advantages, certain mushrooms have medicinal properties and have been prescribed for centuries for various ailments. Some mushrooms have been shown to reduce serum cholesterol and inhibit tumours (Stamets and Chilton, 1983), and others induce interferon formation, a defense mechanism against some viral infections (Cochran, 1978).

Table 1 Nutritive value of *Agaricus bisporus* compared with various other foods

Foodstuff	Nutritional Index
Beef	45
Pork	35
Soybeans	31
Spinach	26
Milk	25
Mushrooms	22
Kidney beans	21
Peanuts	20
Cabbage	17
Corn	11
Potatoes	9
Tomatoes	8
Carrots	6

(from Eicker, 1993)

In the process of mushroom production, environmental pollution is reduced as waste products are recycled to produce food high in protein, for human consumption. The compost generated from mushroom production is also recycled for use as animal feeds, soil conditioning and fertilizer.

Thus mushroom cultivation, which does not place significant demands on space and uses primarily wastes for the raw materials, can be of great importance for developing communities where the diets of the people are commonly low in protein.

Mushroom Production

Cultivation of almost all mushroom species follow a similar sequence of production steps:

* Acquisition of a starter culture from a mushroom laboratory or culture collection
* Establishment of a suitable growth medium for bulking-up of the culture

* Preparation of spawn using a cereal grain, or other spawn substrate
* Inoculation and incubation of spawn on a suitable bulk substrate
* Initiation of fruiting and harvesting of mushrooms.

At present, the most commonly cultivated mushroom in South Africa is *Agaricus bisporus* (button mushroom). There is also limited production of the oyster mushroom (*Pleurotus ostreatus* (Jacquin ex Fries) Kummer) by smaller growers. Mushroom production in Europe and the East extends to a number of species which are considered delicacies and fetch high prices. In the United States, small-holder production is being actively encouraged and waste materials and low-technology methods are being used in mushroom production (Stamets and Chilton, 1983). Mushrooms are been grown by subsistence farmers and by small-holders in Ghana. Similarly there is potential in South Africa to expand the number of species produced. Research is being conducted in Gauteng to promote subsistence and small-holder production (Eicker, 1993).

Ideally, new mushroom species should be amenable to being grown in a variety of environmental conditions using unsophisticated and cheap techniques. They should also be adaptable and grow on a variety of organic substrates (Eicker, 1993).

Mushroom Production and Sustainable Agriculture

Environmental protection and development are complimentary to each other. The environment is the material basis for development. Economic growth depends on the environment to supply energy and natural resource inputs, and to absorb the waste created by production and consumption (WWF, 1993).

Mushroom production is an ideal method of converting or recycling organic wastes into commercial products. This is an important aspect with regard to subsistence farmers and small-holders, because no extra costs are incurred obtaining waste materials. Plant residues from harvested crops are collected and used as organic substrates. Many of the plant residues would normally be left on the land, thereby increasing disease inoculum for

the next season. The collection of infected residues can therefore reduce disease severity in the following growing seasons.

Background to this Mushroom Project

The mushroom project was initiated several years ago and dealt with the cultivation of shii-take (*Lentinula edodes* (Berkeley) Pegler). A number of production methods were tested and it was decided that due to the capital and technological requirements of current production technology, shii-take is not an appropriate crop for developing farmers. It was then decided to investigate the cultivation of other mushrooms, more appropriate for use in developing communities.

Mushroom species selected for cultivation

The following mushroom species were selected:

* *Volvariella volvacea* (Bulliard:Fries) Singer
* *Stropharia rugoso-annulata* Farlow and Murrill
* *Termitomyces umkowanii* Heim

V. volvacea (straw mushroom) is the most important edible mushroom in the tropics and sub-tropics (Li, 1982). It is suitable for use in rural areas of KwaZulu-Natal because:

* It has a high optimum temperature for growth.
* Production requires little infrastructure.
* It is an extremely fast-growing mushroom (10-15 days from inoculation to harvest).
* It grows on uncomposted substrates such as straw, cotton waste, water hyacinth and other cellulosic organic wastes.
* It rapidly converts organic matter into compost for soil improvement in community gardens (Chang and Miles, 1989; Li, 1982).

S. rugoso-annulata is commonly cultivated in eastern European countries. The advantages of *Stropharia* cultivation are:

* Simple and inexpensive cultivation on cellulosic wastes
* Favourable palatability, consistency and appearance
* Resistance to adverse environmental conditions, disease and pests (Szudyga, 1978)
* Would work well in rotation with *Volvariella* as this is a cooler weather mushroom
* *Stropharia* has a prolonged fruiting period.

T. umkowanii (beef-steak mushroom/Ikhowe) is well-known in South Africa by local rural people and is harvested from the wild. It is a highly prized delicacy and is often sold on the road-side at high prices. There is as yet no known artificial cultivation technique and research therefore concentrated on production procedures.

Aim of the Project

The work in this project focused on attempting to grow the above-mentioned mushroom species under South African conditions. This included: laboratory facilities; available substrates; climatic constraints; water-availability; capital expenditure and marketing aspects. The project also dealt with problem-solving in mushroom production.

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CHAPTER 2: STROPHARIA

2.1 Literature Review: *Stropharia rugoso-annulata*

Introduction

Stropharia rugoso-annulata Farlow and Murrill is a mushroom commonly cultivated by Eastern European home-growers in outdoor cold frames. It has recently become popular with growers in temperate climates, including the mid-Atlantic states of North America, New Zealand and Japan. Its natural habitat is in hardwood forests, where it occurs amongst the undecomposed woody debris. *Stropharia* is extremely adaptive to outdoor cultivation and is ideal for home-growers (Stamets, 1993).

Taxonomy and Morphology

S. rugoso-annulata is also referred to as *Stropharia ferrii* Bres. and *Stropharia imaiana* Benedix. It also has a number of common names, these being: King Stropharia; Garden Giant or Gartenriese; Burgundy Mushroom; The Wine Cap; Wine Red Stropharia and Godzilla Mushrooms (Stamets, 1993).

The cap is usually 4-13 cm in diameter, but can reach up to 40cm. It is reddish brown at first but the colour fades to pale pink with age. The shape is broadly convex, becoming flattened at maturity. A thick veil covering the gills is present initially, but this breaks with age to form a thick membranous ring. The stem is thick, enlarging towards the base. Spores are purple-brown, smooth and ellipsoid (Szudyga, 1978; Stamets, 1993).

Young specimens have an excellent flavour, but this declines steadily as the mushroom matures (Stamets, 1993).

Spawn Production

In Eastern Europe, spawn is commonly produced on moist chaff of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.). Incubation takes place at 26°-27°C, for a duration of 5-6 weeks (Szudyga, 1978). For home cultivation in the mid-Atlantic states of North America grain of rye, wheat, sorghum (*Sorghum vulgare* Pers.) and maize (*Zea mays* L.) is initially used. For subsequent generations of spawn, a mixture of sawdust and wood chips, inoculated with grain spawn, is often used. Incubation for either form of spawn takes place at 21°-27°C for 3-6 weeks. It is recommended that spawn containers should be shaken every 3-4 days after inoculation to ensure full colonization, as the mycelium revitalizes after being disturbed (Stamets and Chilton, 1983; Stamets, 1993).

Substrates and Substrate Preparation

Cereal or flax (*Linum usitatissimum* L.) straw is a common substrate for fruit body production. The straw should be fresh, free from contamination and able to resist attempts at tearing. The straw should be moistened to 70-75% water content before bed preparation is undertaken. This is achieved by watering the straw 2-3 times per day for 6-10 days (Szudyga, 1978). Chopped straw can be pasteurized by submerging the straw in hot water (58°C) for 20-30 minutes. Trials showed that the use of unpasteurized straw lead to problems with insect pests and competitor moulds (Stamets and Chilton, 1983).

Sterilized sawdust or a mixture of sawdust and wood chips, with the addition of a rich soil casing layer, has also been found to be an effective substrate (Stamets, 1993).

Poppe and Sedeyn (1989) investigated the feasibility of substrate additives for earlier and double fruiting body production. Of a number of options, the use of rye grass (*Lolium perenne* L.) chaff as a fruiting substrate was found to be the most effective, leading to fruit stimulation of up to 250% of that obtained on wheat chaff. Hormone-like auxins and sterols in the *Lolium* chaff were thought to be responsible for the increase in yield.

An attempt by Reiss (1987) to grow *Stropharia* on rolls of toilet paper failed,

although extensive mycelial growth did occur.

Preparation of Beds and Production Factors:

The conventional method of growing *Stropharia,* as discussed by Szudyga (1978), is in cold frames. The frames are covered with foil or roofing paper and slope down on one side to allow rain to run off. Beds are set-up on the ground, usually on a sheet of plastic spread on the ground to prevent access by earthworms, moles, mice and insects. Moist straw is placed in layers in the frames and compressed by treading. The ideal depth is 25cm and a $1m^2$ bed uses 20-30 Kg dry straw (Szudyga, 1978). Beds are laid out in warm places, sheltered from wind. Constant shade has been found to hinder the development of fruiting bodies. Stamets and Chilton (1983) used controlled environment containers and mushroom houses. The method of bed construction in both cases was similar.

Portions of mycelium are distributed over the entire bed at a depth of 5-8 cm, or the mycelium is sown. In this case, the mycelium is crumbled and distributed uniformly over the substrate and then covered with 5-8 cm of humid straw.

A relative humidity of 90% and a temperature of 11°-14°C is required for the 2-4 week spawn run. A casing is required to initiate fruiting once the spawn run is complete. The casing requires a pH of 5.6-6.0, and typically consists of a peat/humus mixture. Calcium buffers inhibit fruiting so the pH is adjusted by varying the peat:humus ratio. The 2.5-5 cm thick casing should have a moisture content of 70-75%. The humus is pasteurized to kill nematodes, mites and other parasites. Pasteurization is used because mushrooms do not form on sterilized casing (Stamets, 1993; Stamets and Chilton, 1983).

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2.2 Evaluation of the artificial culture of seven *Stropharia rugoso-annulata* strains

Introduction

Culturing the fungus is the first step in mushroom production, and as tons of mushrooms are generated from a few petri dish cultures, it is important that this step is as efficient and effective as possible.

Starter cultures can be made from fresh healthy fruit bodies (**Figure 2.2.1**), obtained by germinating spores. Alternatively, they can be bought from a type collection. The starter cultures are grown on suitable sterile culture media.

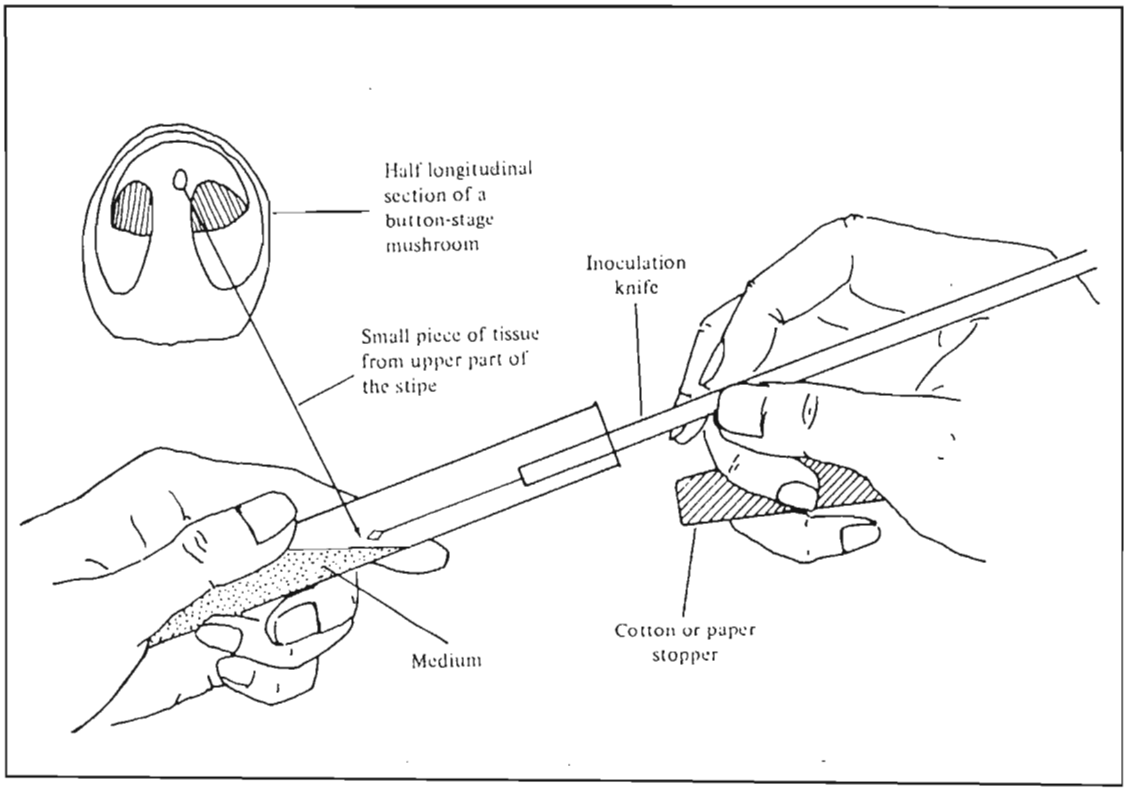

Figure 2.2.1 Obtaining a starter culture from a fresh fruiting body in the button stage (from Chang, 1982)

Media vary widely in form and composition depending on the organism to be cultivated and the purposes of cultivation. There are 3 types of culture media: natural; synthetic (defined) and semi-synthetic. Natural media are those used on the basis of experience, without an exact knowledge of their composition. These media are convenient, inexpensive and are often used for routine work. Natural and semisynthetic media are commonly used for culturing mushroom mycelia (Chang, 1982).

Once the medium for culture has been established, it is important to select a high-quality strain of culture that will prosper throughout all stages of mushroom production. A number of criteria have been established by Stamets and Chilton (1983) for assessing a strain at the culture stage for its performance potential:

* **rhizomorphism and purity of strain**

  When mycelium grows from a single inoculation site and several divergent types appear, it is considered to be sectoring. There are 2 major classes of mycelial sectors:

  1. rhizomorphic (strandy) and
  2. tomentose (cottony).

There is also an intermediate mycelium which grows linearly (longitudinally radial). Rhizomorphic mycelia run faster (spawning stage), form more primordia (fruiting stage) and yield more mushrooms than cottony mycelia. Thus, the presence of rhizomorphic mycelia with no cottony sectors is an indication of a robust, high-yielding strain.

* **lack of contaminants**

  Microbial contamination can lead to the loss of a culture, and merely the presence of a contaminating microorganism in a culture can result in low levels of performance, or diminished viability of the mushroom culture. Thus, a culture free of contaminants and with a tendency to out-compete any contaminants, should they inadvertently be introduced into the culture, would be ideal.

* **primordium formation**

  As primordium formation is more pronounced on healthy mycelium, the presence of primordia at the culture stage is an indication of a strong, viable strain. However, this is not an essential criterion for selecting a healthy strain.

The aim of this trial was to assess seven *S. rugoso-annulata* strains for viability, based on growth habit, level of contamination and colony diameter.

Materials and Methods

Seven strains were obtained from a laboratory in the Netherlands (Centraalbureau voor Schimmelcultures, Baarn). The strains obtained were: 449.87; 573.69; 288.85; 410.71; 789.73; 289.85; and 788.73. The cultures had been maintained on 2.5% Malt Extract Agar.

Three replicates with 5 plates per replicate were established for each strain. Malt Extract Agar (MEA) with 250mg of Streptomycin Sulphate per 1 dm^3 (**Appendix 2.2**) was used as the growth medium.

Two measurements of colony diameter were taken weekly, at right angles to each other. The cultures were incubated at 25°C.

The results obtained after 3 weeks of culture were analysed for statistical significance using analysis of variance and multiple range analysis.

From the growth curves, the area under each growth curve was calculated and used for the statistical analysis of the results. Area under growth curve (AUGC) was adapted from area under disease progress curve which is used for the analysis of plant disease growth rates (Shaner and Finney, 1977).

Results

Strain 788.73 showed no signs of growth and was discarded after three attempts at cultivation. The AUGC was calculated and analyzed for statistical significance using analysis of variance (**Table 2.2.1**).

Table 2.2.1 Results of analysis of variance and multiple range analysis of the growth of six *S. rugoso-annulata* strains grown on malt extract agar

Source of variation	Sum of squares	Degrees of freedom	Mean Square	F-ratio	Significance level
Main Effects					
treatment	1439469.8	5	287893.97	12.174	0.0005 **
replication	362675.5	2	181337.76	7.668	0.0096 **
Residual	236481.7	10	23648.17		
Total (corrected)	2038627.1	17			

The results of the growth trial of the strains on MEA can be found in **Table 2.2.2** and **Figure 2.2.2.**

Table 2.2.2 Results of average weekly growth and area under growth curve of strains of *Stropharia rugoso-annulata* grown on malt extract agar

Strain	Growth at Week 1 (mm)	Growth at Week 2 (mm)	Growth at Week 3 (mm)	AUGC
289.85	43.0	68.8	90.0	944.9 **a**
288.85	34.4	68.0	80.3	877.5 **a**
489.73	20.3	39.2	74.1	432.2 **b**
449.87	17.6	27.7	39.7	394.4 **b**
573.69	26.5	52.0	63.0	385.0 **b**
410.71	15.9	31.1	67.0	351.5 **b**

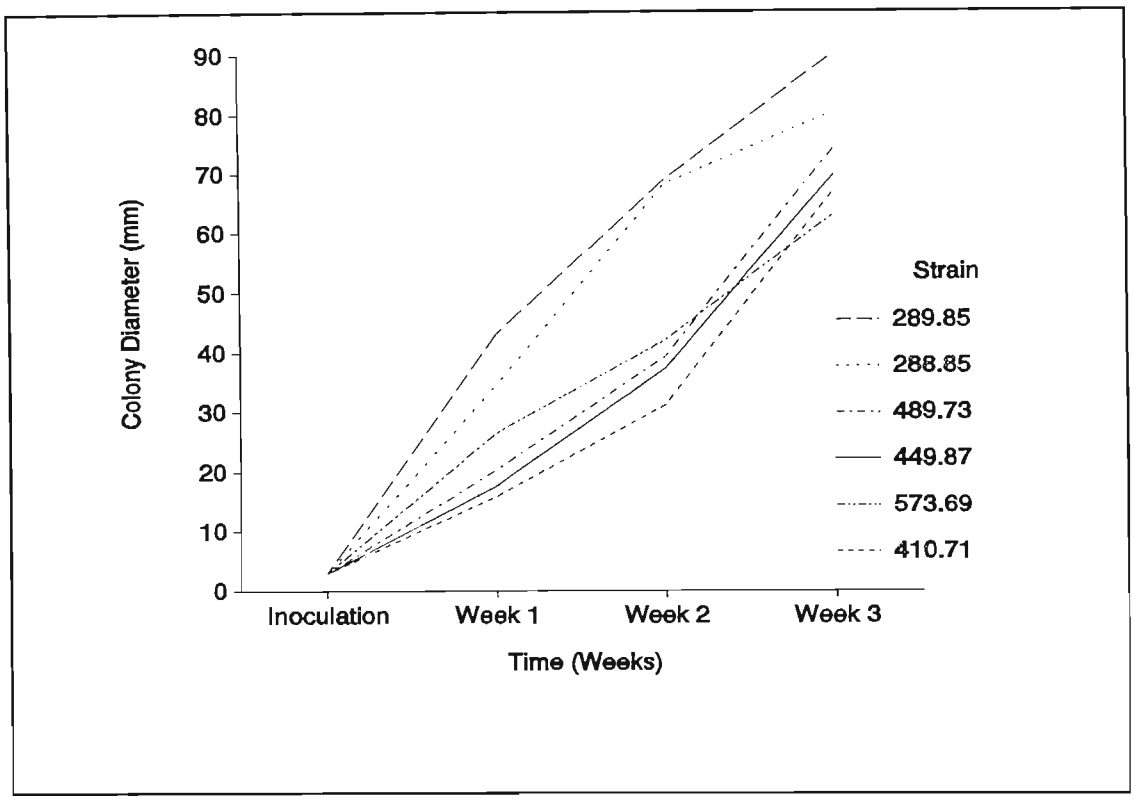

Figure 2.2.2 Growth curves of six strains of *Stropharia rugoso-annulata* grown on malt extract agar

Strain 289.85 showed the most remarkable growth of the seven strains tested, with the most rapid growth in the initial phase.

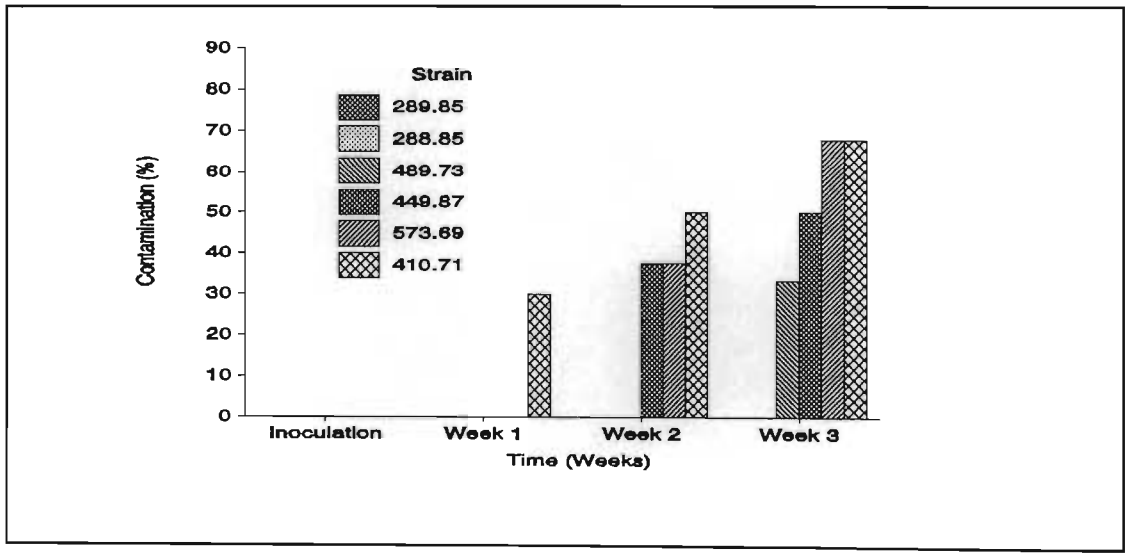

Figure 2.2.3 Weekly microbial contamination rates of various strains of *Stropharia*

***rugoso-annulata* on MEA**

Strains 289.85 and 288.85 remained uncontaminated after three weeks of culture while the other strains became contaminated from as early as Week 1 (**Figure 2.2.3**).

Discussion

The sum of squares analysis showed statistical significance between treatments and between replicates.

Multiple range analysis indicated no statistical significance between the growth of strains 573.62; 449.87; 410.71 and 489.73. And no significance between the growth of strains 288.85 and 289.85. However, the test did indicate significance between the first group of strains (573.62; 449.87; 410.71 and 489.73) and the second group of strains (288.85 and 289.85). This shows that strains 288.85 and 289.85 grew significantly better than strains 573.62; 449.87; 410.71 and 489.73.

Contamination of the cultures by *Penicillium spp.* may explain the significant difference between replicates found in the sum of squares analysis, as some replicates were more heavily contaminated than others (**Figure 2.2.3**). *Penicillium* is a common contaminant in the mushroom industry (Malay, 1974).

Strain 289.85 and Strain 288.85 showed a dichotomous growth habit, indicating healthy and viable strains (Stamets and Chilton, 1983). Strains 410.71; 573.69; 449.87 and 489.73 all showed tomentose (cottony) growth forms, indicating poor and/or declining strains.

AUGC was a useful parameter, integrating growth responses over the entire observation period.

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Appendix 2.2

Malt Extract Agar with Streptomycin Sulphate

25 g Malt Extract (Unipath Ltd)

17 g Agar (Merck Biolab)

1 dm^3 distilled water

Autoclave for 15 minutes at 121°C.

When agar has cooled to 50°C, add 250 mg Streptomycin Sulphate (disolved in 25ml distilled water) using a microbial filter.

2.3 Evaluation of various media and modified media for the artificial culture of *Stropharia rugoso-annulata*

Introduction

Mushrooms produce a wide range of extracellular enzymes that enable them to degrade complex lignocellulosic substrates into soluble substances which can then be absorbed by the mushroom for nutrition (Wood and Fermar, 1982). Consequently, growth and fruiting of *S. rugoso-annulata* depends on the ability of the fungus to utilize the cellulose and hemicellulose components of lignocellulosic materials as a nutritional source. This in turn, is determined by the mushroom's capacity to synthesize the hydrolytic enzymes necessary to degrade the polysaccharides into low molecular weight sugars which can be readily assimilated.

Cellulose is the skeletal material of plant cell walls. It is a linear polysaccharide of glucose residues joined by ß-1,4 linkages. The biological degradation of cellulose occurs most frequently via enzymatic hydrolysis catalysed by cellulase (Pigman and Horton, 1970). However, cellulose is usually intimately associated with hemicellulose and lignin. These tightly bound components limit the access of cellulolytic enzymes to the cellulose and hinder the breakdown of cell walls.

Two steps have been proposed for the enzymatic breakdown of cellulose (Reese *et al*, 1950). The first step is known as the prehydrolytic step, in which anhydroglucose chains are swollen or hydrated. The enzyme associated with this step is ß-1,4-glucan cellobiohydrolase (C_1), which attacks the highly ordered crystalline native cellulose. The second step in the breakdown of cellulose is the hydrolytic cleavage of susceptible polymers by exo-ß- and endo-ß-glucanases (C_x) (Wood and Phillips, 1969).

Wang (1982), in a study of the production of cellulolytic enzymes, indicated that these enzymes are inducible in the presence of cellulose. Mandels and Reese (1965) and Noviello (1966) supported this by showing that the presence of other carbon sources, although easily assimilated and shown to promote fungal growth, did not induce the

production of cellulolytic enzymes. From this, we can conclude that the production of cellulase is highly specific and only induced in the presence of cellulose.

The aim of this experiment was to expose the mycelium of *S. rugoso-annulata* to lignocellulosic organic wastes at the culture stage, to induce the production of cellulases and ß-1,4 glucanases for rapid mycelial growth, and to allow for a robust starter-culture for the production of spawn.

Materials and Methods

Three media were used: malt extract agar (MEA); potato dextrose agar (PDA) and vegetable juice agar (V8) (**Appendix 2.3**). Each agar medium was modified by the addition of 1% and 2% (weight/volume) each of: maize stover, banana leaves and grassland straw (*Eragrostis sp.; Hyparrhenia sp.; Themeda sp.; Setaria sp.*). This trial therefore used a factorial of three agar media, and three cellulosic additives, each at two levels.

The lignocellulosic material was chopped into 2 mm long pieces before being added to the various media. The labelling of the treatments can be found in **Table 2.3.1**.

Table 2.3.1 Labelling of treatments for the artificial culture of *S. rugoso-annulata* on various media and modified media

Agar	MEA PDA V8
Levels	Standard
Additives	Grassland Straw (S) Maize Stover (M) Banana Leaves (B)
Levels	1% (w/v) (1) 2% (w/v) (2)

There were three replications per treatment and 5 agar plates per replication. Each plate was inoculated with a 3mm by 3mm block of colonised MEA. Plates were incubated at 25°C. Measurements were taken of growth every week for 3 weeks. Two measurements were taken at right angles to each other, and the mean determined.

The pH for each treatment was determined before autoclaving, using a **Crison micro*pH*2000** pH probe (see **Table 2.3.2**).

The area under the growth curves was calculated (AUGC). Measurement of AUGC was adapted from the area under disease progress curve technique, which is used to evaluated plant disease progress (Shaner and Finney, 1977). The results were analysed using analysis of variance and non-parametric Kruskal-Wallis analysis.

Results

The analysis of results showed that there was no significance between replications nor between treatments (**Table 2.3.2**).

The pH values of the treatments can be found in **Table 2.3.4**. Mean weekly growth, mean AUGC and rank analysis are represented in **Table 2.3.3**. The results are in order of the highest value for mean AUGC to the lowest value for mean AUGC. The non-parametric Kruskal-Wallis analysis showed that there were significant differences between treatments.

The analysis of results showed that there was no significance between replications nor between treatments (**Table 2.3.2**).

The growth curves for the various treatments can be found in **Figure 2.3.1** to **Figure 2.3.4**

Table 2.3.2 Statistical analysis of results of the artificial culture of *Stropharia rugoso-annulata* on various media and modified media

Source of variation	Sum of squares	Degrees of freedom	Mean square	F-ratio	Sig. level
Main Effects					
treatments	667301.4	20	33365.1	1.428	0.165 NS
replications	28392.7	2	14196.3	0.608	0.549 NS
Residual	934457.9	40			
Total (Corrected)	1630152.0	62			

Table 2.3.3 pH values for each treatment for the artificial culture of *S. rugoso-annulata* on various media and modified media

Treatment	pH	Treatment	pH	Treatment	pH
MEA	6.10	PDA	5.88	V8	7.01
MEA + S1	5.96	PDA + S1	5.71	V8 + S1	6.91
MEA + S2	5.91	PDA + S2	5.71	V8 + S2	6.76
MEA + M1	6.64	PDA + M1	5.68	V8 + M1	6.95
MEA + M2	7.14	PDA + M2	5.61	V8 + M2	7.13
MEA + B1	6.31	PDA + B1	5.59	V8 + B1	7.00
MEA + B2	6.52	PDA + B2	5.68	V8 + B2	6.92

Table 2.3.4 Mean weekly growth, AUGC and rank analysis of growth of *S. rugoso-annulata* on various media and modified media

Treatment	Growth at week 1 (mm)	Growth at week 2 (mm)	Growth at week 3 (mm)	AUGC	Rank * Analysis
PDA + B1	47.1	85.9	90.0	1081.2	60.7
PDA + S1	46.2	84.4	90.0	1067.8	59.3
PDA + M1	45.2	82.3	90.0	1048.8	56.7
PDA + S2	43.2	75.8	90.0	996.7	49.7
PDA + M2	41.2	75.8	90.0	989.3	49.0
PDA	36.1	76.0	90.0	973.3	44.3
MEA + B2	41.0	71.4	90.0	958.4	38.3
PDA + B2	38.6	71.6	90.0	951.2	42.3
V8 + B1	32.8	70.3	90.0	922.0	29.8
MEA + S2	39.7	65.4	90.0	911.3	25.7
V8 + M2	36.4	66.7	90.0	909.6	24.5
MEA	38.1	65.0	90.0	908.7	22.3
V8 + M1	36.8	66.4	90.0	908.4	26.5
MEA + M2	36.8	65.7	90.0	903.7	22.5
MEA + M1	35.6	65.1	90.0	894.1	18.3
V8 + S2	30.0	66.9	90.0	888.2	16.8
V8 + S1	29.6	66.7	90.0	885.22	13.7
MEA + S1	36.0	63.2	90.0	883.1	14.5
V8	29.3	63.5	90.0	861.9	7.3
V8 + B2	28.0	72.8	90.0	664.2	29.0
MEA + B1	40.1	64.1	90.0	658.7	20.7

*** Rank was determined from Kruskal-Wallis analysis with the highest value indicating the best growth**

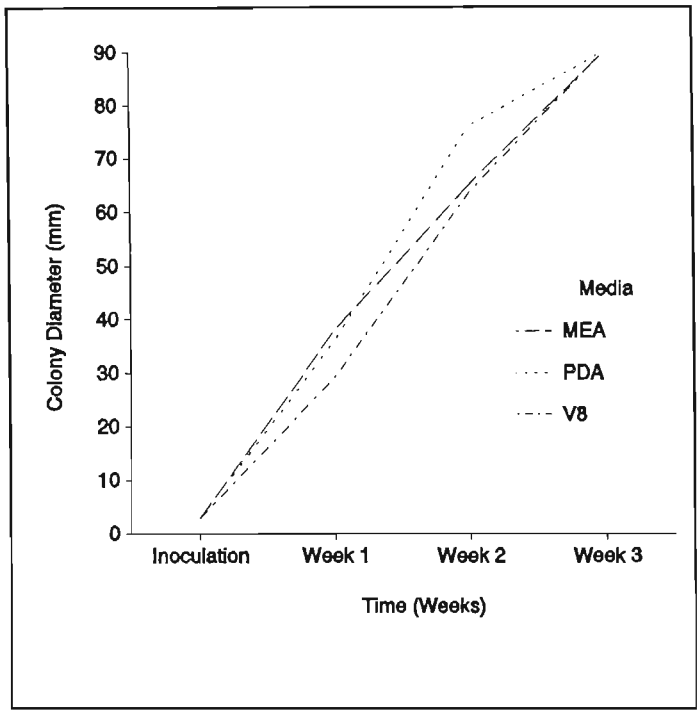

Figure 2.3.1 Growth curves of *Stropharia rugoso-annulata* on potato dextrose agar, malt extract agar and vegetable juice agar

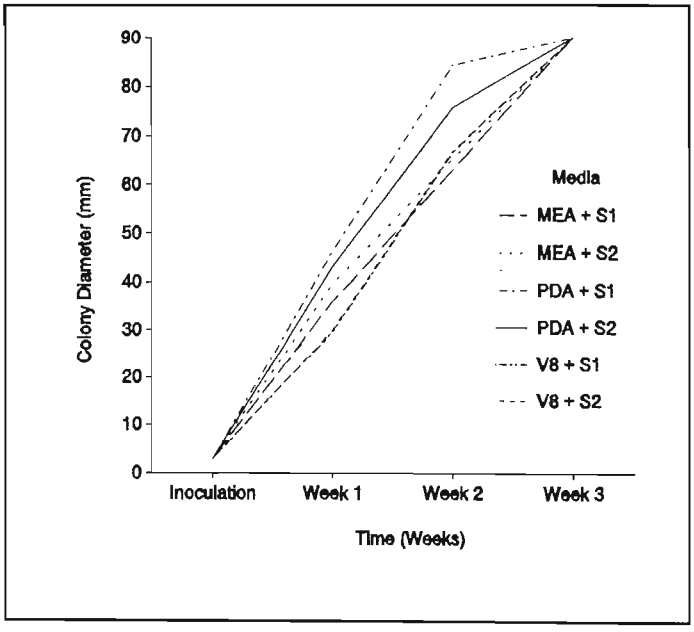

Figure 2.3.2 Growth curves of *Stropharia rugoso-annulata* on three media modified with 1% and 2% grassland straw

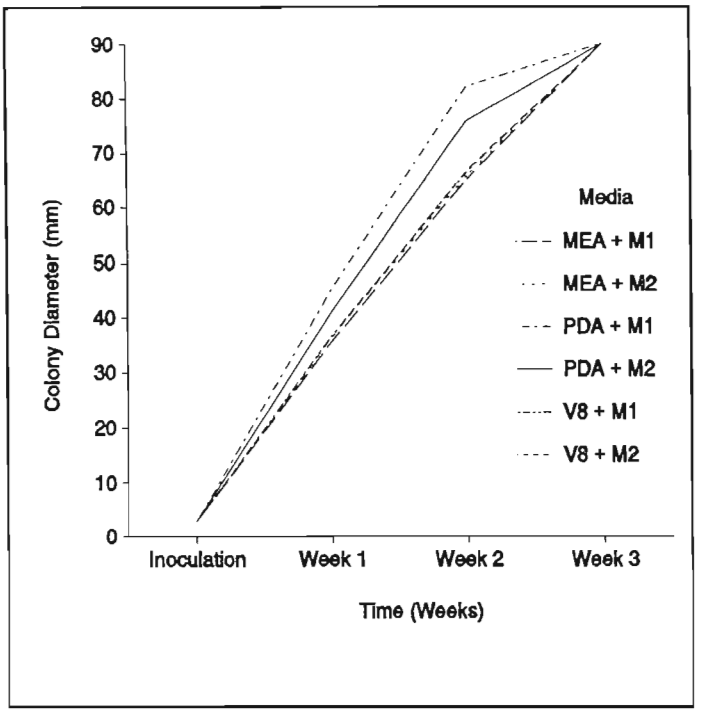

Figure 2.3.3 Growth curves of *Stropharia rugoso-annulata* on three media modified with 1% and 2% maize stover

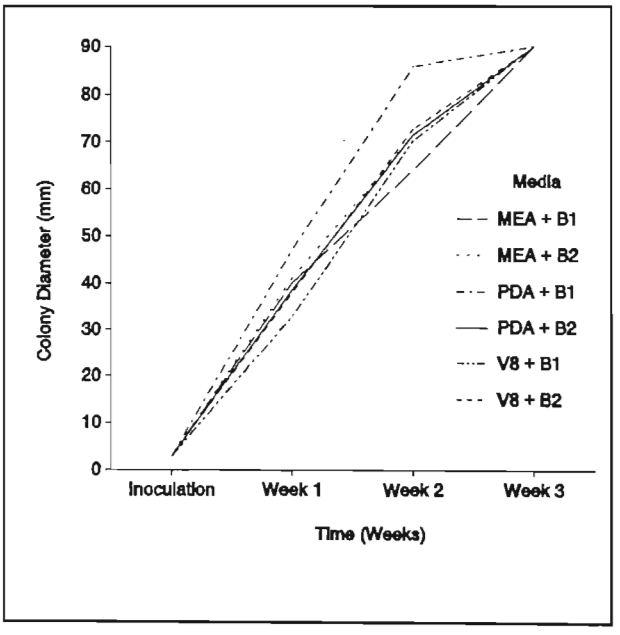

Figure 2.3.4 Growth curves of *Stropharia rugoso-annulata* on three media modified with 1% and 2% banana leaves

Discussion

Results indicated that PDA modified with 1% banana leaves supported the best growth and V8 agar alone supported the worst growth. Banana leaves contain considerably more lignin than straw-based substrates (Cherney *et al*, 1989) and as *S. rugoso-annulata* is a white rot fungus capable of degrading lignin, it is to be expected that it would show more growth on substrates with a considerable lignin content.

From the table of results (**Table 2.3.4**), the rank analysis did not always reflect the results of the mean AUGC. This is due to contamination by *Penicillium spp.* within some of the replicates. Contaminants tend to out-complete the cultured fungus for substrate availability, causing a decline in the growth of the cultured fungus (Stamets, 1993).

The pH of the growth medium has a profound effect on the production and effectiveness of the various enzymes. Cellulase performs optimally at pH 6.0; carboxymethyl-cellulase at pH 7.0 and ß-glucosidase at pH 5.8 (Wang, 1982). From **Table 2.3.3** it is clear that there was variation between treatments with regard to initial pH. This would have affected the production and effectiveness of the cellulolytic enzymes. The pH in a medium will change during the process of cellulose degradation, as phenolic monomers are produced. Thus, no conclusion can be drawn from this experiment with regard to the effect of initial pH on the production and effectiveness of the cellulolytic enzymes.

It is evident that the most pronounced growth, for all treatments, occurred from Day 7 to Day 14, where the slope of the curve is steepest. In a study of white-rot fungi, Wang (1982) showed that there is a relatively large amount of cellulase and carboxymethyl-cellulase produced by fungui, during this period. The cellulolytic enzymes reach their peak at Day 14 and ß-glucosidase reaches maximum production by Day 16.

Phenolic monomers are produced during the degradation of cellulose, and have been shown to inhibit structural carbohydrate digestion in some cellulose degrading fungi (Cherney *et al*, 1989). White-rot fungi, including *S. rugoso-annulata*, are not adversely

affected by the presence of phenolic monomers. Thus the drop-off of growth from Day 14 to Day 21 could possibly be the result of declining activity of cellulolytic enzymes, depletion of substrate and change of substrate pH to non-optimum pH.

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Appendix 2.3

Malt Extract Agar

17 g	Agar (Merck Biolab)
25 g	Malt Extract (Unipath Ltd.)
1 dm^3	distilled water

Autoclave at 121°C for 15 minutes.

Potato Dextrose Agar

200 g	potatoes
20 g	Agar (Merck Biolab)
20 g	Dextrose (D-Glucose) (Unipath Ltd.)
1 dm^3	distilled water

Peel and dice potatoes, add 0.5 dm^3 of the distilled water and autoclave at 121°C for 15 minutes. Strain through muslin cloth, top-up to 1 dm^3, add Agar and Dextrose. Autoclave at 121°C for 15 minutes.

V8 Agar

177 ml	V8 vegetable juice (Campbell's Soup Company)
2 g	calcium carbonate (Merck Biolab)
20 g	Agar (Merck Biolab)
1 dm3	distilled water

Autoclave at 121°C for 15 minutes.

2.4 Medium trials for spawn production and an initial investigation of fruiting substrates of *Stropharia rugoso-annulata*

Introduction

The cultivation of mushrooms follows a number of sequential steps. After the initial culturing of the fungus, the production of spawn is the next vital step. Mushroom spawn is used to inoculate prepared substrates. The inoculum consists of a carrier material fully colonized by mushroom mycelium. The type of carrier used varies according to the species of mushroom cultivated.

In 1932, Dr James Sinden patented the spawn making process using cereal grain as the mycelial carrier (Stamets and Chilton, 1983). A number of cereal grains are used for spawn production:

* Millet (*Pennisetum typhoideum* Rich.). It has a high number of possible mycelium expansion points but difficult to formulate as spawn.
* Sorghum (*Sorghum vulgare* Pers.). It has spherical kernels which work well It is also cheap and readily available in South Africa
* Rye (*Secale cereale* L.). This is a reliable substrate but is not available year-round. It is also more costly than sorghum.

Colonized grain is a mechanism that allows for even distribution and serves as a nutritional supplement to the expanding mycelium. The colonized grain kernel becomes a mycelial capsule, with smaller grains providing more points of inoculation per kg of substrate.

The preparation of the grains and the type of container used for spawn production are important to ensure high quality spawn. There are two common methods of grain preparation:

* soaking overnight
* boiling.

By soaking the grain overnight, heat-resistant endospores of bacteria germinate and become sensitive to heat sterilization, thus reducing the chances of bacterial contamination of the spawn.

The advantage of boiling the grain is that even moisture distribution and consistency of the spawn substrate are obtained. Care should be taken with boiling to ensure that the grain kernels do not burst. Exploded kernels expose nutrients, making the grain more susceptible to contamination, and lead to clumping of the grain and thus, sites of depressed gas exchange. The shape of the intact grain kernels with a protective outer surface selectively favours the filamentous mushroom mycelium and produces a spawn that separates readily upon shaking (Stamets, 1993).

Moisture content plays a critical role in the successful colonization of grain by mycelium. If the substrate is too dry, growth is retarded and the mycelium forms fine threads. If the substrate is too moist, the grain clumps and dense, slow growth occurs. A too moist substrate also encourages bacterial blooms. Standing water at any stage of the cultivation process encourages competitors and contaminants. The optimum moisture content is 45-55%, with the ideal being 50% (Stamets, 1993).

There are two common types of container used for spawn production:

* glass bottles

* heat-resistant plastic bags.

There are a number of advantages and disadvantages associated with each container. More grain can be treated in bags than jars in an autoclave. Broken bags are not dangerous (ie. they do not cut like broken glass). Bags are pliable and spawn can more easily be broken into individual kernels.

Stamets and Chilton (1983) recommend rye grain or wheat straw for the production of spawn. As wheat is not a major crop in this province of South Africa and wheat production is becoming more limited nationally, locally available grains were tested for their viability in spawn production of *S. rugoso-annulata*. Also, the trial tested the effect of establishing the fungus on agar modified with lignocellulosic wates, with respect to its growth

rate on spawn and fruiting substrates.

Materials and Methods

The suitability of the following locally available grains and agricultural wastes was assessed for the production of *S. rugoso-annulata* spawn: barley (*Hordeum vulgare* L.); sorghum; millet; banana (*Musa sapientum* L.) leaves; maize (*Zea mays*) stover and grassland straw (mixed species).

The relative moisture content of each substrate was assessed, and the relative moisture content with the addition of various quantities of water was determined.

The grains were prepared as for spawn production. They were soaked overnight, drained and then boiled. The barley and sorghum were boiled for 10 minutes and the millet was boiled for 3 minutes to ensure that the grains did not burst. The grains were cooled to room temperature before moisture content was assessed.

The agricultural wastes were soaked overnight and drained, which is the usual method of preparation for non-composted fruiting substrates.

Once the substrates were prepared, 0; 10; 25; 35; 50 and 60 ml of water was added to 500g of each substrate, and the moisture content was calculated using the following equation:

Moisture Content (%) = mass - dry mass / mass x 100

There were three replications per treatment, and the mean was determined to represent the result (**Table 2.4.1**).

The C:N ratio of each substrate was also determined (**Table 2.4.2**). The % ash and % N of the substrates were determined by laboratory analysis at Cedara Feed Laboratories. % Carbon was calculated using the following equation:

% Carbon = (100 - % ash)/ 1.8 (Goodall, 1994)

Table 2.4.1 % Moisture content of the substrates for the spawn trial of *Stropharia rugoso-annulata* after the addition of various quantities of water to 500g of prepared substrate

ml water added to 500g substrate	% moisture content of sorghum	% moisture content of barley	% moisture content of millet	% moisture content of banana leaves	% moisture content of maize stover	% moisture content of straw
0	51.6	55.9	42.7	79.4	74.1	83.8
10	55.0	56.5	44.2	81.0	76.2	86.6
25	55.4	59.1	45.3	82.2	81.0	86.8
35	59.1	59.8	46.2	83.8	82.6	88.8
50	60.4	61.4	47.8	87.2	84.6	89.1
60	60.8	61.5	48.3	88.1	85.1	90.0

Table 2.4.2 Carbon:Nitrogen ratios of the substrates used in the spawn trial for *Stropharia rugoso-annulata*

Barley	Sorghum	Millet	Banana	Straw	Maize
87:1*	38:1	84:1**	37:1	52:1	61:1

* Leppan and Bosman, 1923

** Oslon and Frey, 1987

Trial A:

The grains for the various treatments were soaked overnight, drained and boiled. They were cooled before weighing, and water was added to adjust the moisture content to optimum. Barley was left at 55.9% moisture content, millet was adjusted to 48% moisture content by the addition of 60ml of water to 500g of grain and sorghum was adjusted to 55% moisture content by the addition of 10ml of water to 500g of grain.

Two methods were used in this trial. In the first, the treatments were placed in polypropylene bags (Panbro plastics, Durban) to which a gas-exchange cap filled with cotton-wool was fitted (**Appendix 2.4.1**). There were 5 replications with 3 bags per replication. Once filled, the bags were autoclaved for 3 hours at 121°C and 1.5 Kg/cm^2. The bags were left to cool in the autoclave, and then were inoculated with 2 cm^2 pieces of *Stropharia rugoso annulata* culture grown on MEA. Three replications were inoculated with *S. rugoso-annulata*, one was inoculated with plain MEA, and one replication was left uninoculated. For the second method, Erlenmeyer flasks were used, with a plug of cotton wool covered with aluminium foil to seal them. The flasks were prepared as for the polypropylene bags. There were 5 replications with 3 flasks per replication. Three replications were inoculated with *S. rugoso-annulata*, one was inoculated with plain MEA, and one replication was left uninoculated.

The bags and flasks were assessed weekly for growth of *S. rugoso-annulata* and presence of contaminants. A rating scale established by Ramsden and Laing (1993) was used for this purpose (**Appendix 2.4.2**).

Trial B:

A second spawn trial was established using barley, millet sorghum, banana leaves, maize stover and grassland straw. The banana leaves, maize stover and grassland straw are considered to be agricultural waste materials. The grains were prepared and the moisture content of the grains was adjusted as for the first spawn trial. The banana leaves, maize stover and grassland straw were soaked overnight and drained before being chopped into 2cm long pieces. The moisture content of the agricultural wastes was not adjusted as it was within the optimum range (70-85%). Only polypropylene bags were used for this trial. 250ml of each type of grain was put into polypropylene bags. There were 3 replications per grain type, and 3 bags per replication. 300ml of each agricultural waste were put into polypropylene bags, with 3 replications per agricultural waste and 3 bags per replication.

The bags of substrate were inoculated with a 9 mm^2 piece of colonized agar. The two best media discovered by the modified agar trial (**Chapter 2.3**), PDA + B1; PDA + S1 together with MEA (as a control) were used to inoculate the bags.

The bags were assessed weekly for growth of contaminants and appearance of mycelium expansion on the substrate. A rating scale established by Ramsden and Laing (1993) was used for this purpose (**Appendix 2.4.2**).

The results were analyzed using analysis of variance and Friedman rank analysis.

Results

Trial A: The mean rating, rank analysis and final contamination rate of the trial can be found in **Table 2.4.A.**.

Table 2.4.A.1 Mean rating, rank analysis and final contamination rates of *Stropharia rugoso-annulata* grown on various grains in flasks and polypropylene bags

Container	Substrate	Mean Rating	Rank Analysis	Final Contamination Rate (%)
Bag	millet	1.2	4.0	77.8
Bag	barley	1.0	3.8	88.9
Bag	sorghum	1.1	3.8	66.7
Flask	millet	0.67	3.2	88.9
Flask	sorghum	0.56	3.2	88.9
Flask	barley	0.44	3.1	77.8

The mean rating, rank analysis and final contamiantion level of the trial can be found in **Table 2.4.A.2**.

Table 2.4.A.2 Mean rating, rank analysis and final contamination levels of *Stropharia rugoso-annulata* grown on various grains in flasks and polypropylene bags

Container	Substrate	Mean Rating	Rank Analysis	Final Contamination Levels (%)
Bag	millet	1.2	4.0	77.8
Bag	barley	1.0	3.8	88.9
Bag	sorghum	1.1	3.8	66.7
Flask	millet	0.67	3.2	88.9
Flask	sorghum	0.56	3.2	88.9
Flask	barley	0.44	3.1	77.8

The uninoculated flasks did not get contaminated for the duration of the trial. Only 5% on the uninoculated bags became contaminated. The flasks and the bags inoculated with agar blanks became contaminated during the trial. 45% of the flasks and 40% of the bags were contaminated.

Discussion

Trial A:

From the analysis of the results, it can be concluded that there was no difference in growth of *S. rugoso-annulata* grown in flasks or polypropylene bags.

The fact that 5% of the uninoculated bags became contaminated indicated that some contaminants were able to enter either through the cotton wool plug, or through the seams of the bags.

The fact that the inoculated flasks and bags became heavily contaminated, suggests that contaminants entered during the inoculation process. The contamination rates were considered unusually high, and the issue of contamination is dealt with further in **Chapter 5**.

Results

Trial B:

The ANOVA result indicated that differences between treatments and between replicates were statistically significant (**Table 2.4.B.1**). Friedman rank analysis showed that the differences between treatments were highly significant.

Table 2.4.B.1 ANOVA of the spawn substrate trial of *S. rugoso-annulata*

Source of variation	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance Level
Main Effects					
Treatment	162.327	17	9.5487	6.794	0.0000**
Replication	12.197	2	6.0988	4.339	0.0148**
Residual	199.580	142	1.4055		
Total (corrected)	374.1	161			

The mean rating, rank analysis and final contamination levels of the spawn trial can be found in **Table 2.4.B.2**.

Table 2.4.B.2 Mean rating; rank analysis and final contamination levels for spawn production trial of *Stropharia rugoso-annulata*

Inoculate	Substrate	Mean Rating	Rank Analysis	Final Contamination levels (%)
PDA + S1	sorghum	4.7	15.8	11.1
PDA + S1	banana	4.1	13.9	00.0
MEA	sorghum	3.7	13.1	44.4
PDA + B1	banana	3.6	12.1	33.3
PDA + B1	maize	3.2	11.4	22.2
MEA	millet	3.0	11.4	55.6
MEA	barley	2.8	11.4	44.4
PDA + S1	millet	2.8	10.5	55.6
PDA + S1	barley	2.6	10.0	22.2
PDA + B1	millet	2.4	10.0	77.8
MEA	banana	2.3	9.6	55.6
PDA + B1	sorghum	1.8	7.7	100
MEA	maize	1.8	7.4	66.7
MEA	straw	1.8	7.0	55.6
PDA + S1	maize	1.8	6.7	44.4
PDA + B1	straw	1.3	4.8	55.6
PDA + B1	barley	1.0	4.3	88.9
PDA + S1	straw	1.1	4.0	66.7

Discussion

Trial B:

The best growth was obtained from the inoculation of sorghum with PDA + S1. Straw as a substrate provided poor results with all inoculants. Sorghum, millet, banana

leaves and maize stover all supported good growth, although results were dependent on which inoculant was used. MEA showed the best results, PDA + S1 showed similar results, but PDA + B1 showed unremarkable results.

MEA performed well on the grains, but showed poor growth results on the agricultural wastes. PDA + S1 performed well on the grains and on banana leaves. PDA + B1 showed poor results on the grains, but performed well on banana leaves and maize stover.

Contamination rates were relatively high in most treatments, which may explain the significant difference obtained between replications. The problem of contamination is dealt with in **Chapter 5**.

With respect to C:N ratios, there was a trend in the four top ratings. Comparison of C:N ratios of the substrates used, shows that there was a preference for those substrates with a low C:N ratio, namely banana leaves and sorghum (**Table 4.2.2**). *S. rugoso-annulata* is considered to grow better on substrates with a low C:N ratio (Stamets, 1995. Pers. Comm.).

There was no indication from this trial that growing the cultures on agar modified with lignocellulosic material had any effect on the performance of *S. rugoso-annulata* on the substrates tested in this trial. This may have been due to the high contamination rates experienced in this trial, as no conclusive result could be obtained, due the significant difference between replications.

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Appendix 2.4.1

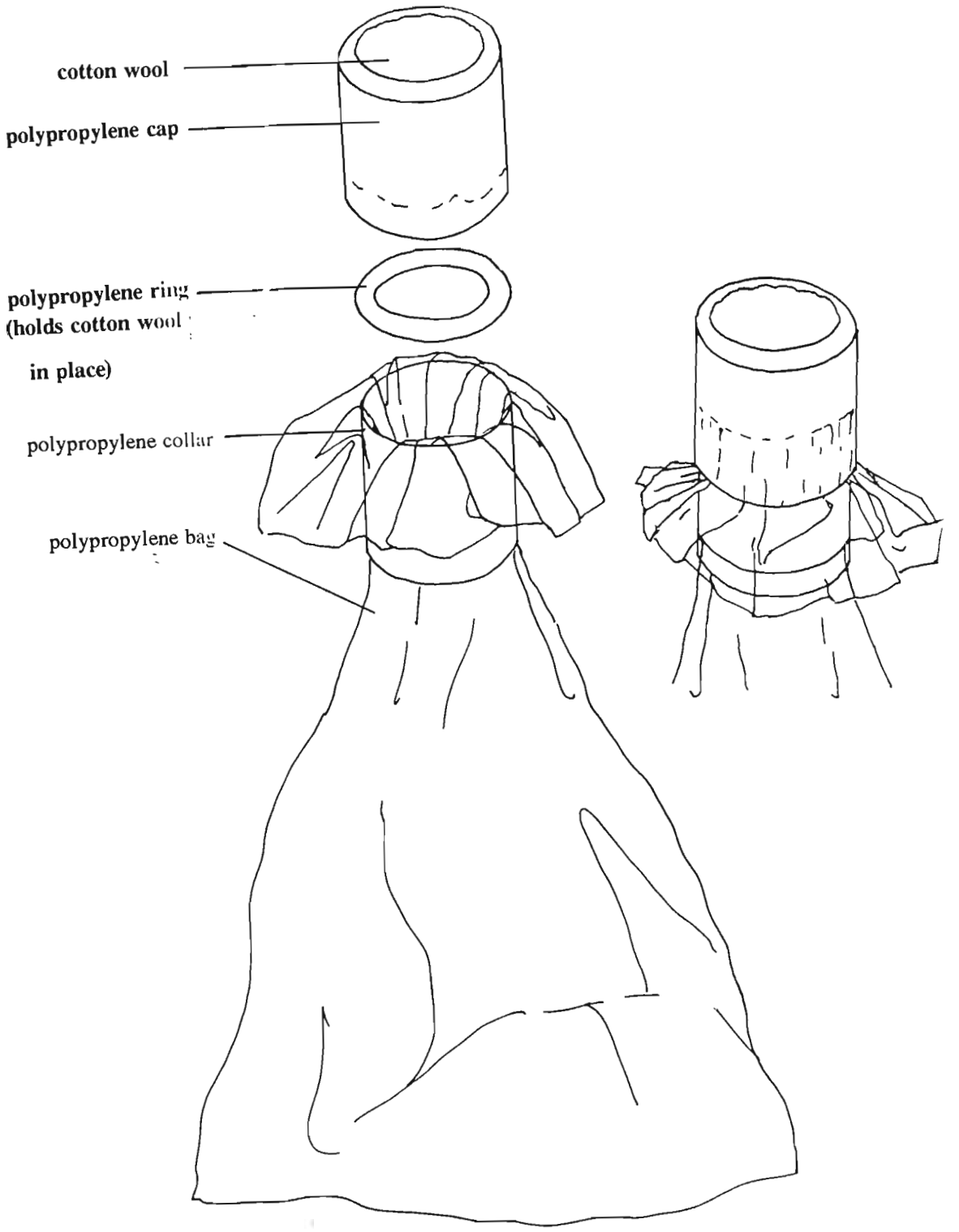

Diagram showing polypropylene bag with fitted cap containing cotton-wool.

Appendix 2.4.2

Rating scale for expansion of mycelium on substrate:

Mycelial Coverage:

1. None
2. 0-20%
3. 20-50%
4. 50-80%
5. 80-100%

CHAPTER 3: VOLVARIELLA

3.1 Literature Review: *Volvariella volvacea*

Introduction

Volvariella volvacea (Bull. ex Fr.) Sing. occurs in tropical and subtropical regions. The mushroom grows gregariously or solitary in soil, on straw, on compost and in hothouses and cellars in colder regions. It is also known as the straw mushroom, paddy straw mushroom or chinese mushroom.

There are more than a hundred species, sub-species and varieties of *Volvariella*. Several of the species are cultivated: *V. bombycina* (Schaeff. ex. Fr.) Sing.; *V. displasia* (Berk. and Br.) Sing.; *V. volvavea* (Bull. ex. Fr.) Sing. and *V. esculenta* (Mass.) Sing. (Chang and Miles, 1989).

Spawn Production

Starter Cultures

All tissues in the mushroom life cycle can be grown vegetatively in culture. There are three ways of obtaining starter cultures:

* germination of spores to make a single-spore culture
* multi-spore culture
* tissue culture from a fresh basidiocarp

Spawn substrates

A number of materials can be used as spawn substrates. These materials can be used alone or in different combinations. Chang and Miles (1989) established a number of substrates and combinations for the successful cultivation of *Volvariella volvacea*:

* Grain spawn: 100g of rye, sorghum or wheat grain with 150ml distilled water and 2g calcium carbonate. Adjustment of proportions up to 10% may be necessary

to account for differences in original moisture content of the grains.

* Straw spawn: rice straw is soaked in water for 2-4hr, then cleaned and cut into 2.5-5cm long pieces. The pieces are mixed with 1% calcium carbonate and 1-2% rice bran.

* Used tea leaves: leaves are washed and drained, and the pH is adjusted to 6.8-7.8 using 2% calcium carbonate.

* Cotton waste spawn: cotton waste is washed and drained, 2% calcium carbonate is added to neutralize organic acids.

* Manure-husk spawn: a mixture of fresh horse manure and lotus (*Nelumbo lutea* L.) seed husks are used. The lotus seed husks are soaked in water until they are saturated. Once saturated, they are mixed with the horse manure which is composted for 4-5 days in 1m high piles.

Once the spawn substrate has been prepared and sterilized, inoculation and incubation take place. The substrate is inoculated with a mother culture and incubated at 32°-34°C for 10-18 days, depending on the substrate used. The mycelium should fill the container, by which time patches of brown chlamydospores usually would have appeared on the inner surface of the container.

Fruiting Body Production:

There are three common methods for growing the straw mushroom (Chang and Miles, 1989):

1. Without pasteurization (indoor cultivation)

Two kinds of boxes are used. The first type has a wire screen at the bottom (80cm long X 80cm wide X 10cm deep). The second type is open at both ends (60cm long X 40cm wide X 30cm deep). The bedding material is cut to lengths of 20cm and is arranged parallel with the length of the box and packed tightly. The boxes are soaked for

2hr in 2% calcium carbonate solution. Excess water is drained immediately prior to planting. Thumb-sized pieces of spawn are distributed over the surface of the box and are buried 5 cm deep in the bedding. The inoculated boxes can be placed in incubation rooms at 35°-38°C at 75% rH, or they can be covered with plastic sheets.

2. Without pasteurization (outdoor cultivation)

Straw is tied in bundles of 40cm in length and soaked overnight in fresh water or in a 2% calcium carbonate solution. The beds are usually built in an east-west direction and the base is raised to ensure that flooding does not occur in the rainy season. The base of the bed is usually made from soil, but a platform of bamboo poles, bricks or wooden planks can be used to ensure that the base withstands erosion. Inoculation is the same as for non-pasteurized indoor cultivation.

3. With pasteurization (indoor cultivation)

In the case of pasteurized substrate, the mushroom beds are prepared and the growing room is set-up as for non-pasteurized cultivation. Steam is introduced into the sealed growing room using a steam generator. Pasteurization takes place at 62°C for 2 hours and then 50°-52°C for 8-16 hours. Time taken for pasteurization depends on the substrate and level of composting of the substrate. After steaming, the growing-room is left closed until the air temperature drops to 38°C. The room is then opened for inoculation of the mushroom beds.

The above methods are the three most common methods of cultivation. Other methods and substrates have also been utilized (**Table 2.1**).

Table 2.1: Methods of cultivation and substrates used for the cultivation of *Volvariella volvacea*

Substrate	Method of cultivation	Notes	Reference
rice straw cotton hulls	10 kg rice straw/m^2 soaked in water and covered with 2.5 kg cotton hulls/m^2	yield was 1-1.5 kg/m^2	Tu (1988)
sugarcane bagasse	bagasse was composted and pasteurized before inoculation	composting, temperature and bed moisture determined yield	Rivera-Vargas and Hepperly (1987)
tobacco waste	tobacco waste was not composted or pasteurized	midribs gave the best yield	Tolentino (1987)
Sorghum solid residues	residues were sterilized (moisture content 80% and pH 6.3)	fructification occurred at 28°C and a 12 hr light period	Clignez and Nsimba (1989)
sugarcane industrial by-products	sugarcane bagasse was blended with cotton waste and pasteurized	highest yield on bagasse to cotton ratio of 1:1	Khan and Dogar (1991)
wheat straw	on wheat straw after harvest in the field	20% increase in yield of subsequent wheat crop	Li (1989)
rice straw	grown in apple orchards using conventional methods	increased yield and size compared to those not grown in orchard	Hua (1991)
rice straw sheep manure rice husks	substrate was composted and different layers were used for fruiting body production	yields: inner layer 2.31 kg/m^2 middle layer 1.62 kg/m^2 outer layer 1.02 kg/m^2	Yang *et al* (1990)
rice straw	effect of Fu-Gu-Tai (substance from *Streptococcus*) on yield, using conventional techniques was determined	10 ug/ml of Fu-Gu-Tai increased yield from 68.1% - 127.8%	Lui *et al* (1990)
maize cobs soybean rattans	conventional bed techniques were used	maize cobs effective but soybean rattans not feasible	Wu and Song (1986)

Pests and Diseases

Most of the problems in mushroom cultivation occur due to the incomplete pasteurization of the substrate. This occurs when the composting substrate is too moist. The composting process does not reach a high enough temperature to kill pathogens (for example, nematodes) and these can then survive in the centre of the pasteurized substrate (Chang and Miles, 1989).

Coprinus sp. is a strong competitor fungus with the same growth requirements as *Volvariella volvacea*, but with a quicker life cycle. Thus, it is capable of out-competing *V. volvacea* for substrate availability (Rivera-Vargas and Hepperly, 1987).

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3.2 Evaluation of the artificial culture of three *Volvariella volvacea* strains

Introduction

Culturing is an important step in mushroom cultivation, as it forms the basis of the production of tons of fruiting bodies. It is important that the strain chosen is high-yielding and robust, to ensure continuous production of high quality fruiting bodies. The criteria established for strain assessment, and discussed in **Chapter 2.2**, do not all apply to the culturing of *Volvariella volvacea*. The growth form is by nature cottony and aerial and no strains, regardless of strength, produce rhizomorphic growth forms (Stamets and Chilton, 1983). However, a lack of contaminants and profuse chlamydospore formation are important criteria for strain selection. *V. volvacea* produces abundant chlamydospores in culture when the strain is healthy and the growth aerial. Chlamydospores are considered to promote rapid expansion of the fungal mycelium during spawn production and during the spawn run on the fruiting substrate.

The aim of this experiment was to assess three strains of *V. volvacea* based on colony diameter, contamination rate and chlamydospore formation.

Materials and Methods

Three strains of *V. volvacea* were obtained from Centraalbureau voor Schimmelcultures (CBS) in the Netherlands. The three strains were: 1661; 1663 and 1655. The cultures had been stored on 3.5% malt extract agar (MEA), and were cultured onto 2.5% malt extract agar on arrival. Three replicates of five plates each for malt extract agar and V8 agar were inoculated with each of the three strains.

The plates were incubated at 32°C and measurements were taken at 4; 7 and 10 days after inoculation. Two measurements of the colony were taken, at right angles to each other and the mean of the measurements determined.

The results obtained after 10 days of culture were analyzed for statistical significance using ANOVA. A multiple range analysis test was used to separate treatment

means, based on Fisher's LSD. From the growth curves, each area under growth curve (AUGC) was calculated and statistically analyzed. AUGC was adapted from area under disease progress curve measurement which is used to evaluate plant disease progress (Shaner and Finney, 1977). A Friedman's rank analysis was also run on the AUGC data.

Results

The results of the ANOVA can be found in **Table 3.2.1**.

Table 3.2.1 Results of ANOVA AUGC of three strains of *Volvariella volvacea* grown on two agar media

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance Level
Main effects					
Treatment	98912.1	5	19782.4	236.6	.0000 **
Replications	151.3	3	74.6	.9	.4354 NS
Residual	835.9	10	83.6		
Total (corrected)	99899.3	17			

Table 3.2.2 Mean growth measurements; mean AUGC and rank analysis of various strains of *Volvariella volvacea*

Strain/Media	Day 4 (mm)	Day 7 (mm)	Day 10 (mm)	Presence of chlamydospores (Yes/No)	Mean AUGC	Rank Analysis
1665 on MEA	25.1	62.9	72.8	Yes	335.6 **a***	6
1663 on MEA	23.9	45.9	55.5	Yes	256.8 **b**	5
1663 on V8	20.9	31.0	47.0	No	194.9 **c**	4
1665 on V8	22.4	30.2	33.9	No	175.1 **d**	3
1661 on MEA	15.7	22.7	36.8	No	146.9 **e**	2
1661 on V8	10.7	19.7	25.1	No	112.8 **f**	1

*** AUGC values with different letters differ at the 5% level**

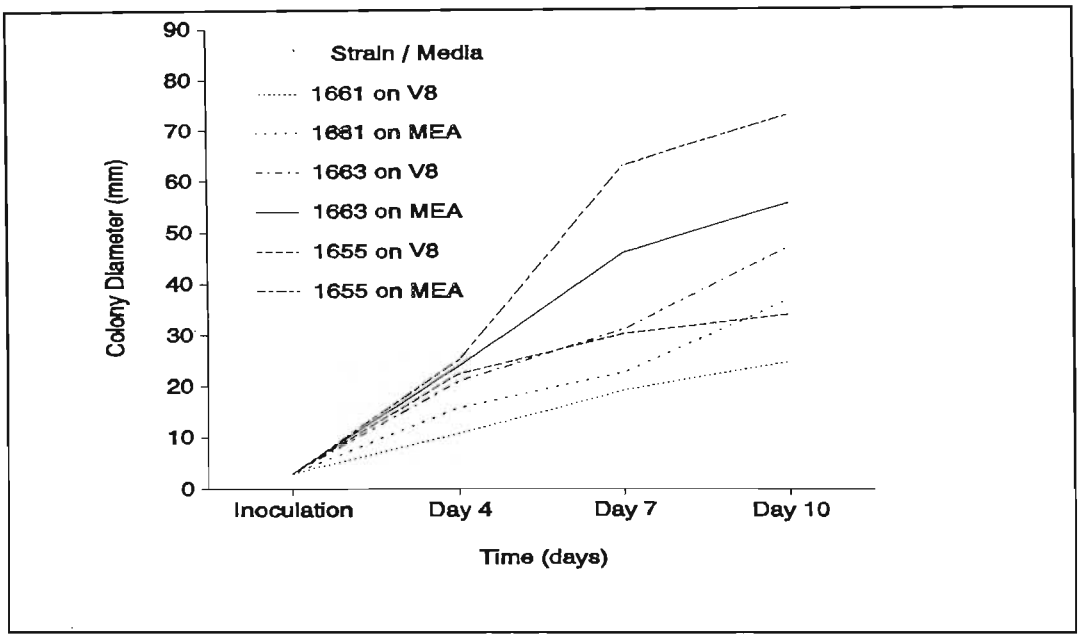

Figure 3.2.1 Growth curves of various strains of *Volvariella volvacea* on malt extract agar and vegetable juice agar

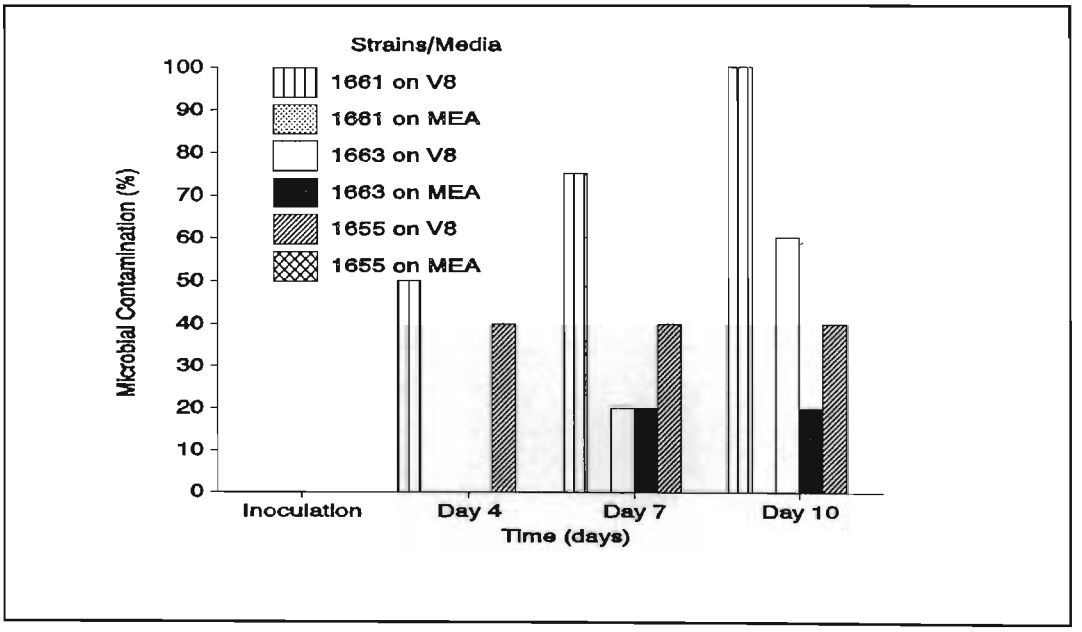

Figure 3.2.2 Microbial Contamination rates of cultures of various strains of *Volvariella volvacea* on malt extract agar and vegetable juice agar

Discussion

ANOVA (**Table 3.2.1**) showed that treatments were significantly different. Multiple range analysis indicated that results for all strains were significantly different from one another. The results of Friedman analysis were consistent with the results of mean area under growth curve, with Strain 1655 on MEA ranked best and Strain 1661 on V8 ranked worst.

AUGC measurements (**Table 3.2.2**) also indicated that the best performance was by Strain 1665 on MEA and the worst by Strain 1661 on V8.

Strains 1663 and 1665 produced chlamydospores on MEA, indicating robust strains, and a preference for MEA. Thus, from the results of the trial, it can be concluded that the most robust strain was Strain 1655, whereas Strain 1661 showed signs of decline.

V8, irrespective of strain, was found to support more *Penicillium sp.* contamination than MEA (**Figure 3.2.2**). *Penicillium* contamination is considered common in the mushroom industry (Malay, 1974).

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Appendix 3.2

Malt Extract Agar

17 g	Agar (Merck Biolab)
25 g	Malt Extract (Unipath Ltd.)
1 dm^3	distilled water

Autoclave at 121°C for 15 minutes.

V8 Agar

2 g	calcium carbonate (Merck Biolab)
20 g	Agar (Merck Biolab)
177 ml	V8 vegetable juice (Campbell's Soup Company)
0.8 dm^3	distilled water

Autoclave at 121°C for 15 minutes.

3.3 Evaluation of various media and modified media for the artificial culture of *Volvariella volvacea*

Introduction

The aim of this experiment was to expose the mycelium of *V. volvacea* to lignocellulosic organic wastes at the culture stage, to induce the production of cellulases and ß-1,4 glucanases for more rapid mycelial growth, and to allow for a more robust starter-culture for the production of spawn.

An explanation of cellulase and its associated enzymes can be found in the introduction to **Chapter 2.3**.

V. volvacea differs from *S. rugoso-annulata* in that it does not degrade lignin, and therefore does not thrive on substrates with a large lignin content.

Materials and Methods

Three agar types commonly used in culture production of mushroom mycelium were used. These were also modified by the addition of ligno-cellulosic agricultural wastes, that could be used for a fruiting substrate. The agar types used were: Malt Extract Agar (MEA); Potato Dextrose Agar (PDA) and Vegetable Juice Agar (V8) (see **Appendix 3.3.1**).

The modifications were as follows: 1% and 2% (ground to 2mm long pieces) of each of the following materials were to the agar types on a weight to volume basis:

* grassland straw
* maize stover and
* banana leaves.

This trial used a factorial of three agar media, and three additives, each at two levels, as represented in **Table 3.3.1**.

The pH for each treatment was determined before autoclaving, using a Crison micro*pH*2000 pH probe (**Table 3.3.3**). Three replicates were established for each

treatment, with 5 petri dishes per replicate.

Table 3.3.1 Treatments for the artificial culture of *Volvariella volvacea* on various media and modified media

Agar	MEA PDA V8
Levels	Standard
Additives	Grassland Straw Maize Stover Banana Leaves
Levels	1% (w/v) 2% (w/v)

The cultures were incubated at 32°C. The growth of the mycelium was monitored at 3, 7 and 10 days. The diameter of the colonies was measured, with two measurements being taken at right angles to each other. The mean of these measurements was determined.

From the growth curves, area under growth curve (AUGC) were determined. Area under growth curve was adapted from area under disease progress curve, which is used to evaluate plant disease progress (Shaner and Finney, 1977).

Analysis of Variance and a Kruskall-Wallis analysis were used to determine statistical significance.

Results

The results of the statistical analysis are represented in **Table 3.3.2**.
The mean weekly growth measurements, mean area under growth curve and rank of results can be found in **Table 3.3.4**. The mean growth curve for each treatment can be found in **Figure 3.3.1** to **Figure 3.3.4**

Table 3.3.2 ANOVA table of results of the artificial culture of *V. volvacea* on various media.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Sig. level
Main effects					
treatment	442055.4	20	22102.8	1.282	0.246 NS
replications	158933.2	2	79466.6	4.608	0.015 *
Residual	689786.0	40	17244.7		
Total (corrected)	1290774.7	62			

Table 3.3.3 pH values for each treatment for the artificial culture of *V. volvacea* on various modified media

Treatment	pH	Treatment	pH	Treatment	pH
MEA	6.10	PDA	5.88	V8	7.01
MEA + S1	5.96	PDA + S1	5.71	V8 + S1	6.91
MEA + S2	5.91	PDA + S2	5.71	V8 + S2	6.76
MEA + M1	6.64	PDA + M1	5.68	V8 + M1	6.95
MEA + M2	7.14	PDA + M2	5.61	V8 + M2	7.13
MEA + B1	6.31	PDA + B1	5.69	V8 + B1	7.00
MEA + B2	6.52	PDA + B2	5.68	V8 + B2	6.92

Table 3.3.4 Weekly growth measurements, mean AUGC and rank of results of the artificial culture of *V. Volvacea* on various media and modified media

Treatment	Day 3 (mm)	Day 7 (mm)	Day 10 (mm)	AUGC	Rank * Analysis
PDA + S2	36.0	88.0	90.0	692.98	58.3
V8 + M1	40.7	84.2	90.0	685.2	56.7
V8 + B1	39.4	81.5	90.0	670.6	50.7
MEA + B2	28.5	85.2	90.0	665.1	50.0
V8 + B2	32.0	80.1	90.0	653.5	44.3
V8 + S1	33.6	79.4	90.0	649.6	32.3
PDA	28.5	80.0	90.0	641.6	41.3
MEA + B1	24.1	81.2	90.0	638.25	39.7
V8 + M2	33.4	76.6	90.0	636.42	39.3
MEA + S1	25.5	73.3	90.0	623.7	24.7
MEA + M2	21.2	78.4	90.0	620.3	32.0
MEA	16.0	79.3	90.0	613.7	28.3
PDA + M1	30.3	73.0	90.0	612.1	27.3
MEA + M1	19.8	76.5	90.0	608.8	28.0
V8 + S2	29.9	70.2	90.0	600.9	18.7
MEA + S2	17.5	74.4	90.0	594.8	20.7
PDA + M2	28.3	67.1	90.0	583.56	21.2
PDA + B1	28.4	65.2	90.0	575.1	13.0
V8	23.2	65.5	90.0	566.2	9.0
PDA + S1	25.1	86.5	90.0	423.2	19.8
PDA + B2	31.5	69.8	90.0	422.3	16.7

*** Rank obtained from Kruskal-Wallis analysis with the highest value indicating the best growth result**

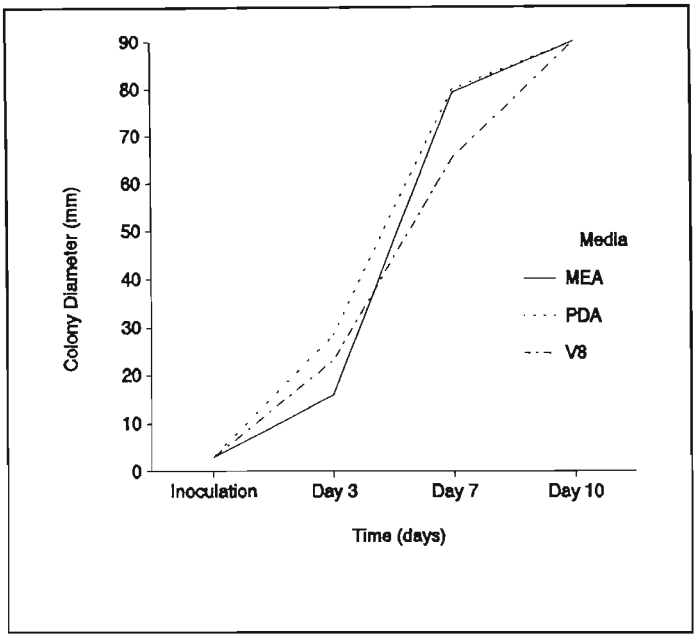

Figure 3.3.1 Growth curves of *Volvariella volvacea* on malt extract agar, potato dextrose agar and vegetable juice agar

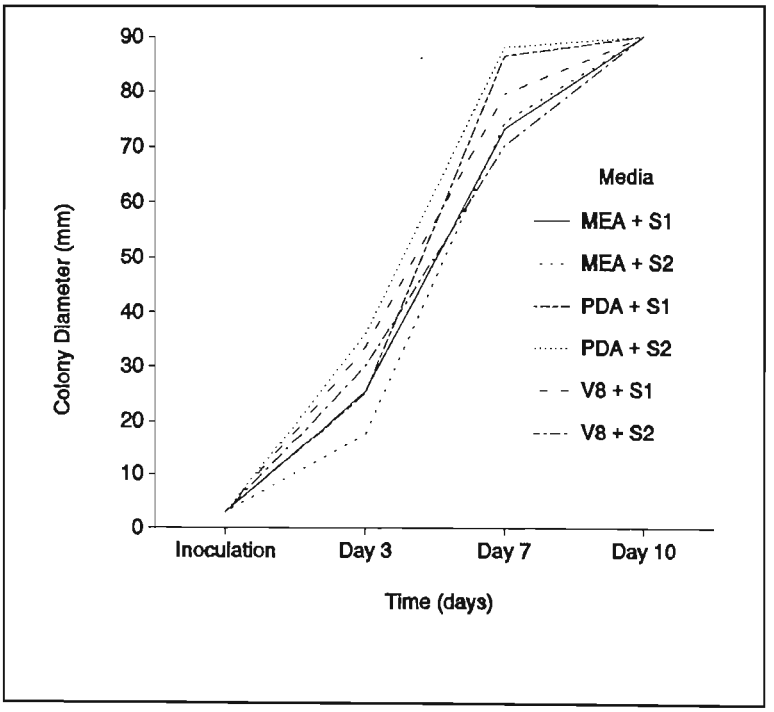

Figure 3.3.2 Growth curves of *Volvariella volvacea* on three agar media modified with 1% and 2% grassland straw

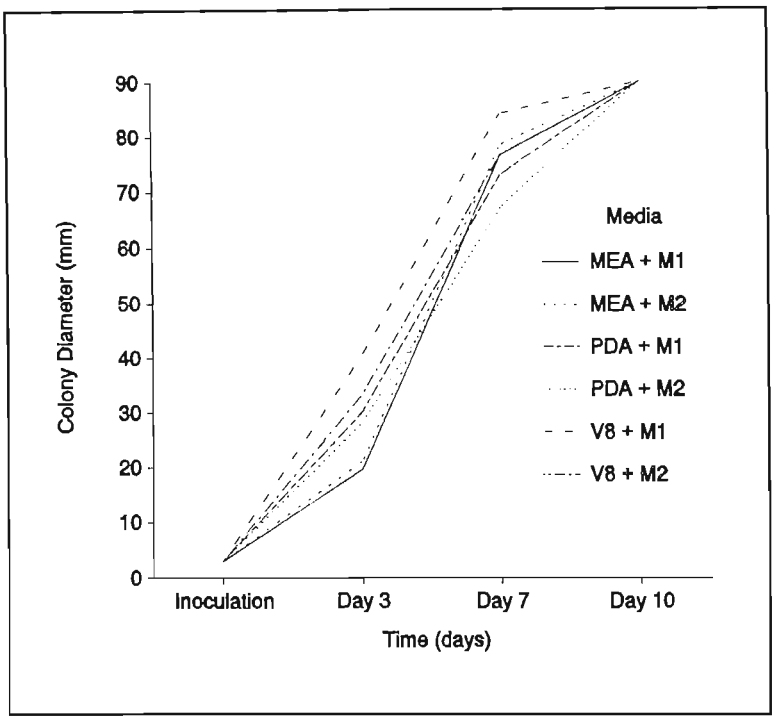

Figure 3.3.3 Growth curves of *Volvariella volvacea* on three agar media modified with 1% and 2% maize stover

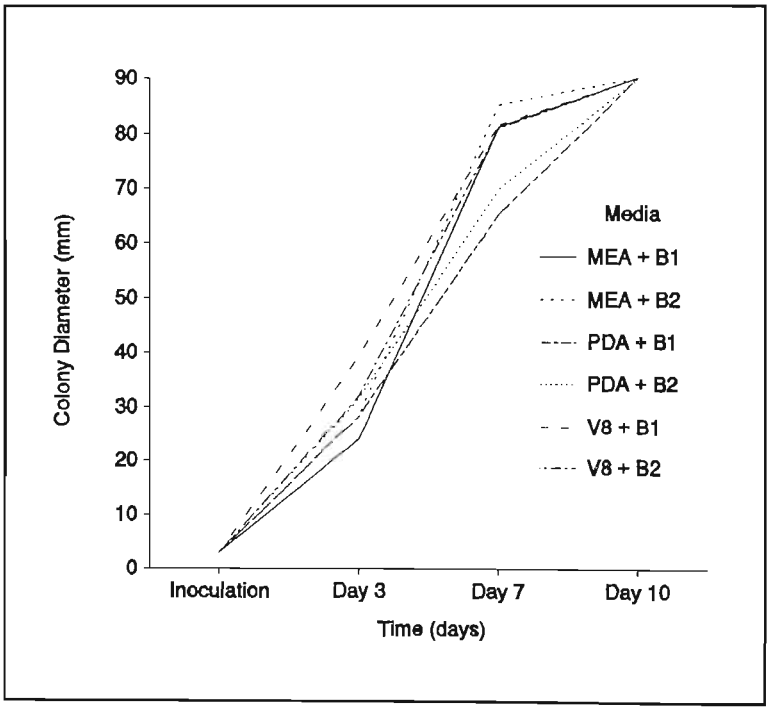

Figure 3.3.4 Growth curves of *Volvariella volvacea* on three agar media modified with 1% and 2% banana leaves

Discussion

From the ANOVA of the AUGC, there was no significance between treatments, and there was significance between replications of treatments. However, non-parametric Kruskal-Wallis analysis indicated significance between treatments, with PDA modified with 2% grassland straw supporting the most growth, and V8 agar alone the worst growth. Straw has been shown to contain moderate amounts of lignin in comparison to other lignocellulosic substances (Cherney *et al*, 1989) and as lignin-derived phenolic monomers inhibit carbohydrate digestion, it is reasonable to expect more pronounced growth of *V. volvacea* on straw-based substrates.

The pH of the growth medium has a profound effect on the production and effectiveness of the various enzymes. Cellulase performs optimally at pH 6.0; carboxymethyl-cellulase at pH 7.0; and ß-glucosidase at pH 5.8 (Wang, 1982).
From **Table 3.3.3** it is clear that there is some variation between treatments with regard to initial pH. This would have affected the production and effectiveness of the cellulolytic enzymes.

Although initial pH in the medium can be optimal, during the process of degradation, the pH will tend to change due to the presence of phenolic monomers of lignified substrates. These are the result of cellulose degradation (Cherney *et al*, 1989). Thus, the amount of lignin in the substrate will affect the functioning of the various enzymes and as a result will affect the pH of the medium. Thus, no conclusion can be drawn in this experiment with regard to the effect of initial pH on the production and effectiveness of the cellulolytic enzymes.

From the growth curves, it is evident that the most pronounced growth occurred between Day 3 and 7, where the slope of the curve was steepest. Wang (1982) showed that during this period a relatively large quantity of cellulase and carboxymethyl-cellulase was produced by fungi.

By Day 7 the cellulolytic enzymes are considered to have reached their maximum production, whereas ß-glucosidase is considered to reach maximum production by Day 8.

All treatments containing MEA, except MEA + B2, showed the most remarkable increase in growth of all the treatments, from Day 3 to Day 7. This can be explained by the fact that *V. volvacea* has been shown to have a high sensitivity to lignin and related phenolic monomers of lignified substrates (Cai *et al*, 1993). Phenolic monomers have also been implicated in the inhibition of structural carbohydrate digestion (Cherney *et al*, 1989). The growth of *V. volvacea* is also inhibited by the presence of tannin and tannin derivatives in the substrate (Cai *et al*, 1993). Thus, those substrates with a high content of lignin and substrates that contain tannin and tannin derivatives would show a reduction in the growth of *V. volvacea* compared to those substrates that do not.

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Appendix 3.3

Malt Extract Agar

25 g	Malt Extract (Unipath Ltd.)
17 g	Agar (Merck Biolab)
1 dm^3	distilled water

Autoclave for 15 minutes at 121°C.

Potato Dextrose Agar

200 g	potatoes
20 g	Agar (Merck Biolab)
20 g	Dextrose (D-glucose) (Unipath Ltd.)
1 dm^3	distilled water

Peel and dice potatoes, add 0.5 dm^3 of the water, autoclave for 15 minutes at 121°C. Strain through muslin, top-up to 1 dm^3, add agar and dextrose, autoclave for 15 minutes at 121°C.

Vegetable Juice Agar

177 ml	V8 vegetable juice (Campbell's soup company)
20 g	Agar (Merck Biolab)
2 g	Calcium Carbonate (Merck Biolab)
0.8 dm^3	distilled water

Autoclave for 15 minutes at 121°C.

3.4 Medium trials for spawn production and an initial investigation of fruiting substrates of *Volvariella volvacea*

Introduction

Spawn production is a vital step in mushroom production, and it is important that spawn is viable and free of contaminants. The requirements and standards of spawn production are discussed at length in **Chapter 2.4**.

The aim of this trial was to assess various grains for their potential as spawn substrates, and to undertake a preliminary investigation of various agricultural wastes for their potential as spawn or fruiting substrates. Further, to test the effect of establishing the fungus on agar modified with lignocellulosic wastes, with respect to its growth rate on spawn and fruiting substrates.

Materials and Methods

Barley, millet and sorghum were soaked overnight, drained and boiled. The moisture content was adjusted to optimum (see **Chapter 2.4**) by the addition of water before autoclaving. Barley was left unadjusted at 55.9%, millet was adjusted to 48% by the addition of 60ml of water to 500g of grain and sorghum was adjusted to 55% by the addition of 10ml of water to 500g of grain (see **Table 2.4.1** in **Chapter 2**).

Banana leaves, maize stover and straw were soaked overnight and drained. The moisture content was left unadjusted as it was optimal in all cases.

250ml of each treatment was placed into polypropylene bags, which were fitted with a collar and a cap filled with cotton wool (**Appendix 3.4.1**). There were three replications per treatment and three bags per replication. The bags of substrate were inoculated with a 3mm by 3mm piece of colonized agar. The two best results from the modified agar trial (**Chapter 3.3**), PDA + S2 and V8 + M1 were used, and MEA was used as the control.

The bags were assessed every four days for presence of contaminants, and for mycelial expansion of *V. volvacea*. A rating scale established by Ramsden and Laing (1993) was used to estimate mycelial expansion (**Appendix 3.4.2**).

The results were analyzed using analysis of variance and Friedman rank analysis.

Results

The results of the analysis of variance can be found in **Table 3.4.1**.

Mean rating, analysis of rank and final contamination rate (%) are represented in **Table 3.4.2**.

Table 3.4.1 ANOVA of media trial of *V. volvacea*

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance Level
Main Effects					
Treatment	37.7346	17	2.2197	3.137	0.0001 **
Replication	0.1975	2	0.0988	0.140	0.8698 **NS**
Residual	100.4691	142	0.7075		
Total (corrected)	138.4012	161			

Table 3.4.2 Mean rating, rank analysis and final contamination level of the media trials of *Volvariella volvacea*

Inoculate	Substrate	Mean Rating	Rank Analysis	Final Contamination Level (%)
V8 + M1	banana	2.8	16.1	00.0
PDA + S2	maize	2.1	12.9	66.7
PDA + S2	millet	1.8	11.9	100
V8 + M1	maize	1.8	10.5	00.0
PDA + S2	banana	1.6	10.4	44.4
PDA + S2	barley	1.6	10.2	88.9
PDA + S2	sorghum	1.8	10.1	100
V8 + M1	maize	1.4	9.8	00.0
V8 + M1	sorghum	1.4	9.8	100
MEA	sorghum	1.4	9.7	100
MEA	barley	1.4	9.1	100
MEA	banana	1.4	8.8	88.9
MEA	millet	1.2	8.6	100
MEA	straw	1.2	7.8	100
PDA + S2	straw	1.1	7.1	100
V8 + M1	straw	1.2	6.3	33.3
MEA	maize	0.8	6.2	77.8
V8 + M1	barley	0.7	5.7	100

Discussion

ANOVA (**Table 3.4.1**) showed that treatments were significantly different. *V volvacea* established on V8 + M1 and inoculated onto banana leaves showed the best growth result. *V. volvacea* established on PDA + S2 showed consistently better growth results on the substrates than *V. volvacea* established on MEA and V8 + M1. Growth of the fungus established on V8 + M1 was consistently better than growth of the fungus established on MEA.

Thus, from the analysis of the results, it is clear that establishing the fungus on agar modified with lignocellulosic materials at the culture stage, does have a positive effect on its growth at the spawn and fruiting substrate stages.

With respect to the C:N ratio of the substrates tested, there was no clear trend in the results. *V. volvacea* is considered to grow better on substrates with a high C:N ratio, with approximately 60:1 being the optimum (Torrez-Lopez and Hepperly, 1988 and Stamets, 1995, Pers. Comm.). Substrates with high a C:N ratio did feature prominently in the top 6 ratings.

The contamination rates for the trial were considered unusually high. The problem of contamination is dealt with in **Chapter 5**.

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Appendix 3.4.1

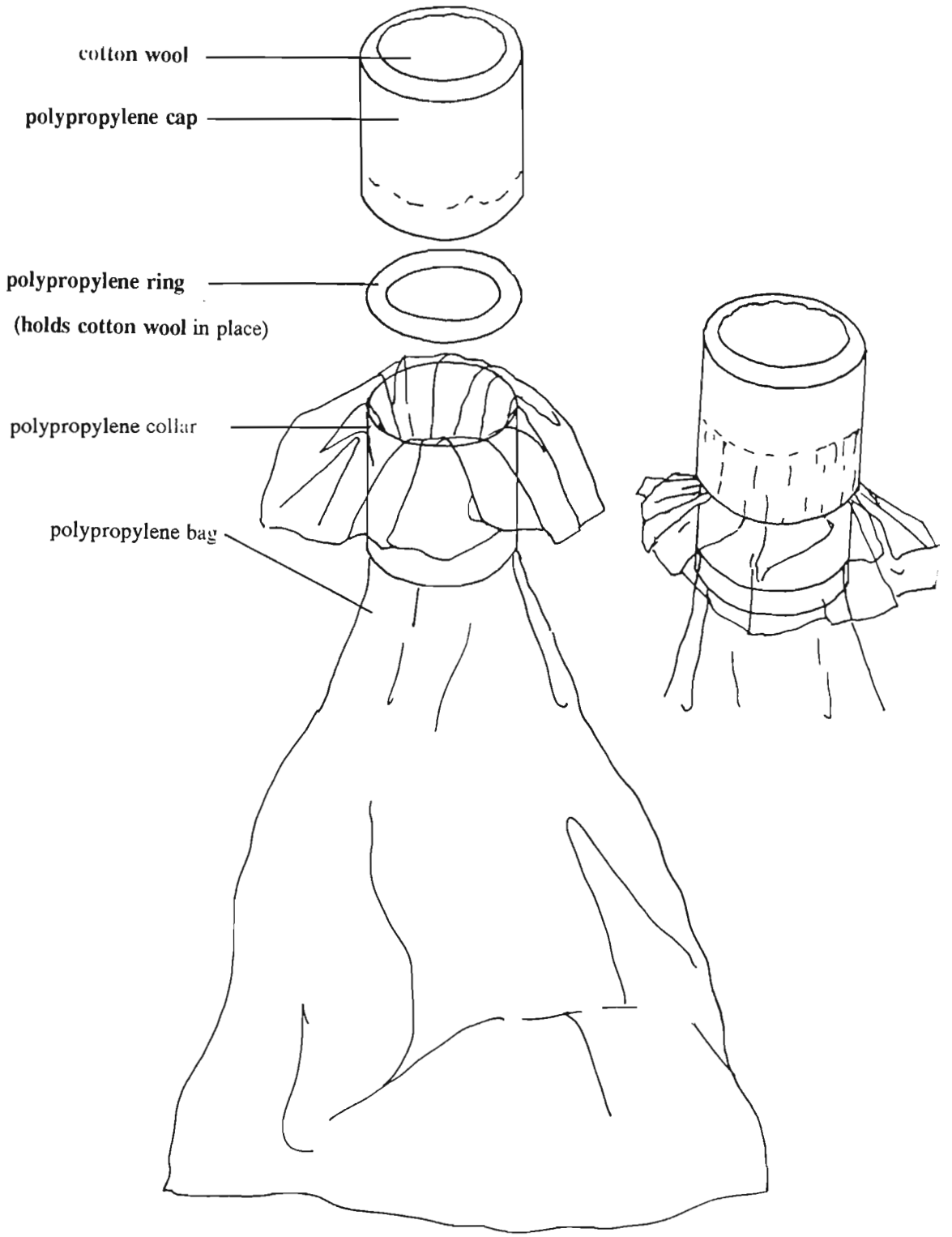

Diagram showing polypropylene bag with collar and cotton wool filled cap.

Appendix 3.4.2

Rating scale for expansion of mycelium on substrate:

Mycelial Coverage:

1. None
2. 0-20%
3. 20-50%
4. 50-80%
5. 80-100%

CHAPTER 4: TERMITOMYCES

4.1 Literature Review: *Termitomyces* species

Introduction

Termites of the subfamily Macrotermitinae are well-known in Africa and tropical Asia, as several of the species build large mounds which are often a conspicuous feature of the landscape (Sands, 1956). They construct within their nests sponge-like masses of comminuted wood fragments. On these grow fungi of the genus *Termitomyces*, which produce white spherical nodules about the size of a pin-head, consisting of conidiophores and conidia. The sponge-like masses are known as "fungus comb" or "fungus gardens". Although the *Termitomyces* species normally fructify in the form of conidial spheres, on occasion during the rainy season, they develop higher forms of fructification on the soil surface. Two types of agarics occur. The first arise from beds of fragmented comb deposited on the surface above the nest by the termites. The second arise from the comb in the nest and thrust their way upward through the soil to fruit on the surface.

Taxonomy and Morphology

Alasoadura (1966) examined species of macrofungi associated with termite nests. Three species of *Xylaria*, twelve of *Termitomyces* and one of *Podaxis* were found in this environment.

Botha and Eicker (1991a) studied the macro- and microscopic characters of basidium context cultures of: *Termitomyces umkowaani* (Cook + Massee) Reid; *T. reticulatus* v.d. Westh. and Eicker; *T. sagittaeformis* (Kalchbr. and Cooke); *T. clypeatus* Heim and *T. microcarpus* (Berk et Br.) Heim. Macroscopic characters were very similar but microscopic characters differed markedly. It was possible to distinguish between different species by relying strictly on microscopic characters. Growth characters did not change when the nutrient medium and incubation conditions were standardised, and proved

to be a reliable taxonomic criterion.

Botha and Eicker (1991b) did further studies on the genus *Termitomyces* and observed macro- and micromorphology of comb sporodochia. Sporodochia were isolated from combs taken from termitaria of *Macrotermes natalensis* Hav. and *Odontotermes badius* Hav. The stroma of sporodochial cultures from *M. natalensis* had a farinaceous, granular texture, while the stroma of the cultures from *O. badius* combs was excessively raised, cerebriform and tuberculous. Sporodochial mycelium of both isolates produced sympodially branched, holoarthric conidial chains and huge inflated, thin-walled conidia (sphaerocysts).

Purkayastha and Chandra (1975) documented the discovery of *Termitomyces eurhizus* (Berl.) Heim in India. The pileus is usually 3-9.5 cm wide , convex, later becoming expanded with a prominent umbo. The stipe is central, up to 20 cm long and 1.5-2.5 cm thick. The annulus is persistent, and the volva is absent.

The 'Omajowa' or 'Termitenpilz', *Termitomyces* species of Namibia were studied by van der Westhuizen and Eicker (1991).
Sporocarps of 15-28cm and up to 40 cm appear in groups of 5-10 around the lower part of the termite mound during the rainy season. *Macrotermes michaelseni* (Sjöstedt) is believed to be the associated termite.

A number of *Termitomyces* species occur in East Africa. These are: *Termitomyces magoyensis* Otieno; *Termitomyces microcarpus*; *Termitomyces badius* sp.nov.sp.nov.; *Termitomyces clypeatus*; *Termitomyces rabourii* Otieno and *Termitomyces robustus* (Beeli) Heim. Bull. Jard. Bot. Etat Brux. (Otieno, 1968).

Ecology and Symbiosis

Heim (1941, cited by Sands, 1956) established the identity of the fungi associated with Macrotermitinae, as belonging to the genus *Termitomyces*. He stated that the "fungus comb" is part of the normal architecture of the nest, and is attacked by the cavernicolous fungi simply due to its suitability as a medium. He considered that in some cases the other

fungi are tolerated by the termites, whereas in other cases, only so long as the growth of the fungi remains within convenient limits. He stated that *Termitomyces* has become adapted to this particular habitat to a greater or lesser extent, and is not cultivated in any way by the termites.

Grasse (1945, cited by Sands, 1956) pointed out that quantities of spores and hyphae are found in the gut of workers and nymphs of most of the Macrotermitinae. The small quantity of fungus available prevents its use as a bulk nourishment, and he suggested that it may be a source of vitamins or other important substances.

Ghidini (1938, cited by Sands, 1956) considered the function of the fungus comb to maintain a constant state of high humidity. Luscher (1951) observed that the fungus nodules are only occasionally eaten, and suggested that the fungus combs play a part in controlling the temperature, by producing heat, possibly by bacterial fermentation, since in the case of *Sphaerotermes*, the combs bear no fungal mycelium.

Sands (1956), in a study on the factors affecting the survival of the termite *Odontotermes badius*, states that although *Termitomyces microcarpus* may not in itself be sufficient to support life in *O. badius* for prolonged periods, it is extremely important to the termites, since without it they are unable to survive any longer than they do when completely starved. He concluded that if the fungus were only a cavernicolous form making use of a suitable medium, and tolerated by the termites, it would be difficult to explain the marked effect it shows on termite survival. This may have been the state of affairs at some period during the evolution of *Termitomyces* and the Macrotermitinae, but the relationship between them now appears to be one of symbiosis.

In the case of *Odontotermes obesus* (Rambur.), nymphs and workers of the termite were observed eating spherules of its mutualistic fungus *Termitomyces albuminosa* (Berk.) Heim. These viable spherules were also transferred among individual termites and were transported from an exposed portion of the fungus-comb substrate to a more sheltered area. This behaviour pattern resembled some aspects of the method of culturing mutualistic fungi used by the fungus-growing attine ants (Batra, 1975).

In the case of some species of *Odontotermes* and a species of *Microtermes* from tropical India, the defensive oral secretions of soldiers, and the soil that has recently been manipulated by workers, exerts a fungistatic effect on contaminant fungi within the fungus comb (Batra and Batra, 1966). This factor suggests that the fungus is specifically cultured.

Laboratory colonies were established by Batra and Batra (1966) to investigate termite-fungus mutualism. Data were obtained on the division of labour; royal couple; feeding habits; proctodeal exchange; soldier secretion and behaviour; nutrition; activities regarding the mutualistic *Termitomyces* and *Xylaria* fungi; regulation of growth of these fungi in the comb; microhabitat of termitarium and chemical composition of the comb and its role in recycling nutrients. The result of these investigations showed that true mutualism existed between the fungus and the termite.

Cheo (1948) discussed the interaction of the termites with *Collybia albuminosa* (Berk.) Petch and *Xylaria sp.*. *C. albuninosa* is considered to "depend" on the termites, although the chance of sporophore formation is much reduced by the termite's feeding on the conidial spheres. Doflien (1905, cited by Cheo 1948) stated that *C. albuminosa* remains only in pure culture through the activity of the termites. The growth of *C. albuminosa* and the simultaneous suppression of growth of *Xylaria* on the same termite-inhabited comb are believed to be interdependent, not two separate constant features. On artificial culture media, and in the absence of termites, *Xylaria* grows much faster than *C. albuminosa*. This indicates that under natural conditions, termites promote the growth of *C. albuminosa* and suppress *Xylaria*. The simultaneous presence of two fungi in association with termites must be attributed to more than a physical mechanism.

Rohrman and Rossman (1980) also noted that when the comb is removed from the nest, it appears to be covered with a pure culture of *Termitomyces*. Once separated from the termites however, the comb becomes overgrown by *Xylaria* within two days. Subsequently, a number of other fungi also appear. This again indicates that the termites select for *Termitomyces*.

El Bakri *et al* (1989) investigated colony foundation and development in *Microtermes* sp. nr. *albopartitus*. The fungus combs are formed by female reproductives depositing a pillar of soil consisting of faecal pellets. The faecal pellets contain the asexual spores of *Termitomyces* and are ingested from the fungus combs of the parent colony before swarming, and carried as a bolus of spores in the gut throughout swarming and the early stages of colony development.

Cultivation

The cultivation of mushrooms requires a number of steps. The first of these is the acquisition of a viable culture.

There are two methods for the development of a selective medium for the isolation of viable mycelium/spores:

* include substances which inhibit the growth of unwanted organisms
* include energy sources/nutrients available only to a restricted range of organisms, including the preferred one (Tsao, 1970).

Thomas (1985) established a selective medium for the isolation of *Termitomyces* from termite nests. Substances for inclusion were screened by measuring the radial growth of *Termitomyces* in their presence and absence. Substances which did not inhibit the growth of *Termitomyces* were further tested for their effects on common contaminant fungi. Those substances which inhibited the growth of the common contaminant fungi were tested for their effects on the germination of spores of *Termitomyces* and contaminant fungi. After a number of trials the medium was refined and the final selective medium was determined.

Batra and Batra (1979) tested the effect of several media on the growth of *T. albuminosus*. These media included oat meal, Sabouraud glucose, potato-dextrose, yeast and malt extract, fungus comb filtrate and comb residue suspensions. The growth response on basal medium with various vitamins was also determined. They concluded that *Termitomyces* mycelium grows slowly on the basal medium, is heterotrophic for

thiamine and utilizes carboxymethyl cellulose and Walseth cellulose, though not cotton fibre, as carbon sources.

Once a viable culture has been obtained, the next step is to determine which substrate is suitable for the production of fruiting bodies.

Sengupta *et al* (1984, cited by Botha, 1989) investigated the growth of *T. clypeatus* mycelium on various natural substrates. These included: sawdust; wheat bran; green coconut coir and bagasse. All agrowastes except sawdust supported good mycelial growth. However, no attempt was made to induce basidiocarp formation.

Quimio (1977, cited by Botha, 1989) attempted to induce basidiocarp formation. Mycelium was grown on a substrate of rice bran, corn meal, mud press and grated coconut meat pulp. To induce basidiocarp formation, he used ordinary soil, termite house soil and mud press as a casing. The casing was watered with soil extract, termite extract, termite house extract and plain water. However, basidiocarp formation did not occur with any of the treatments.

Botha (1989) investigated the influence of three environmental factors on the mycelial growth of eight species of *Termitomyces*. Optimal mycelial growth was observed at pH 5-7, a temperature range of 20°-30°C and under completely dark conditions. Natural cellulose fibres supported mycelial growth of all species. A wide range of carbon sources supported mycelial growth with the exception of certain amino sugars, sugar alcohols, dicarboxylic acids and uronic acids. Nitrogen sources such as complex amino acid mixtures and inorganic ammonium sources supported good mycelial growth. Although *T. umkowaani* grew well on a mixture of wheat, sorghum, sawdust and digestive bran, growth remained vegetative and no fruit body formation occurred.

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4.2 Adaptation of an established basal medium for the artificial culture of *Termitomyces umkowaani* (Cooke and Massee) Reid. mycelium.

Introduction

Termitomyces (Heim) exists in a symbiotic relationship with certain species of fungus-growing termites. The termites produce C_x-cellulases and β-glucosidases in the midgut epithelium and salivary glands. These enzymes lead to the partial digestion of plant material, which is then excreted and used to construct the fungus combs. Fungus combs are highly convoluted and resemble coral. The fungus combs are inoculated with spores of *Termitomyces* which the termites carry in their gut. The fungus then produces C_1-cellulases which further degrade the partially digested plant material of the fungus combs (Martin and Martin, 1978).

Termitomyces is difficult to isolate from the natural habitat due to the presence of many other fungal propagules (Thomas, 1985). However, a basal medium (BM) for the artificial culture of *Termitomyces* was established by Botha (1989). Unfortunately growth of *Termitomyces* on this agar is very slow, allowing for growth of contaminants.

Rabbits are considered to be coprophagous, in that they consume one form of their faeces to redigest partially digested cellulosic material. Coprophagous pellets contain three-and-a-half times the crude protein but only one third the fibre of the redigested secondary pellets (Sanford and Woodgate, 1979). Rabbit faeces were therefore added to the basal medium as they contain a certain percentage of undigested plant material. We speculated that the presence of partially digested cellulosic material in the agar would stimulate the production and utilization of C_1-cellulases by the fungus, and thereby lead to more rapid growth than currently occurs on an unmodified basal medium.

Materials and Methods

The basal medium, as established by Botha (1989), was modified by the addition of rabbit faeces in varying concentrations.

Botha's basal medium has the following ingredients: 0.9g K_2HPO_4; 0.7g KH_2PO_4; 0.75g $MgSO_4$; 0.3g KCl; 2.00g NH_4NO_3; 20.0g Agar; and 1 dm^3 double distilled water. Rabbit faeces was added at 2.5%, 3.5%, 5% and 7% weight/volume. The rabbit faeces used in the agar was a mixture of the initial coprophagous pellets and secondary faeces. A mixture was used as the secondary pellets contain more fibre. It is difficult to get more coprophagous pellets because the majority of them are taken straight from the anus, with only a small percentage available for collection from the bedding material (**Table 4.2.1**). The pH of each agar was determined before autoclaving (**Table 4.2.3**). The rabbit faeces agar was autoclaved for 15 minutes at 121°C.

Table 4.2.1 Total nutrient content of rabbit faeces used in Rabbit Faeces Agar

Nutrient	% on dry matter basis	Nutrient	% on dry matter basis
Carbon	50.63	Sodium	0.22
Protein	11.75	Potassium	1.54
Nitrogen	1.88	Ash	8.87
Calcium	2.04	Zinc	345 ppm
Magnesium	0.64	Copper	47 ppm
Phosphorus	0.85	Manganese	413 ppm

Three replicates of 5 plates per replicate were established for each formulation of Rabbit Faeces Agar (RFA), and were inoculated with *Termitomyces umkowaani* mycelium. Unmodified basal medium was used as a control. The *T. umkowaani* mycelium was originally isolated from a fruiting body onto unmodified basal medium. The inoculated plates were incubated at 30°C.

Growth of the colonies was monitored every two days and the diameter of each colony was measured weekly. Two measurements were taken perpendicularly to each other and the average size of the colony was calculated from these measurements. Area under the growth curve (AUGC) of each treatment was analyzed. The area under

growth curve measurement was adapted from the area under disease progress curve technique, which is used to evaluate plant disease progress (Shaner and Finney, 1977). ANOVA was conducted on the AUGC data. Data was further analyzed using Friedman's ranked analysis.

Results

Growth curves were plotted for each treatment (**Figure 4.2.1**), using the average values for each week of growth. Mean weekly growth; AUGC and rank analysis are presented in **Table 4.2.2**.

Table 4.2.2 Mean weekly growth, mean area under growth curve and rank analysis of growth of *T. umkowaani*

Treatment	Growth at Week 1 (mm)	Growth at Week 2 (mm)	Growth at Week 3 (mm)	Mean AUGC	Rank Analysis
BM + 2.5%	14.9	52.3	74.3	678.0 **a** *	4.5
BM + 5%	13.9	29.7	71.3	505.8 **b**	3.5
BM + 3.5%	15.7	33.3	55.7	482.5 **c**	3.5
Basal Medium	90	contaminated	contaminated	378.0 **d**	2.5
BM + 7.5%	6.9	7.5	13.8	122.3 **e**	1.0

* AUGC values with different letters differ at the 5% level

Table 4.2.3 Relative pH values for each agar type before autoclaving

Agar	pH
2.5% RFA	7.19
3.5% RFA	7.17
5% RFA	7.29
7% RFA	7.45
Basal Medium	6.65

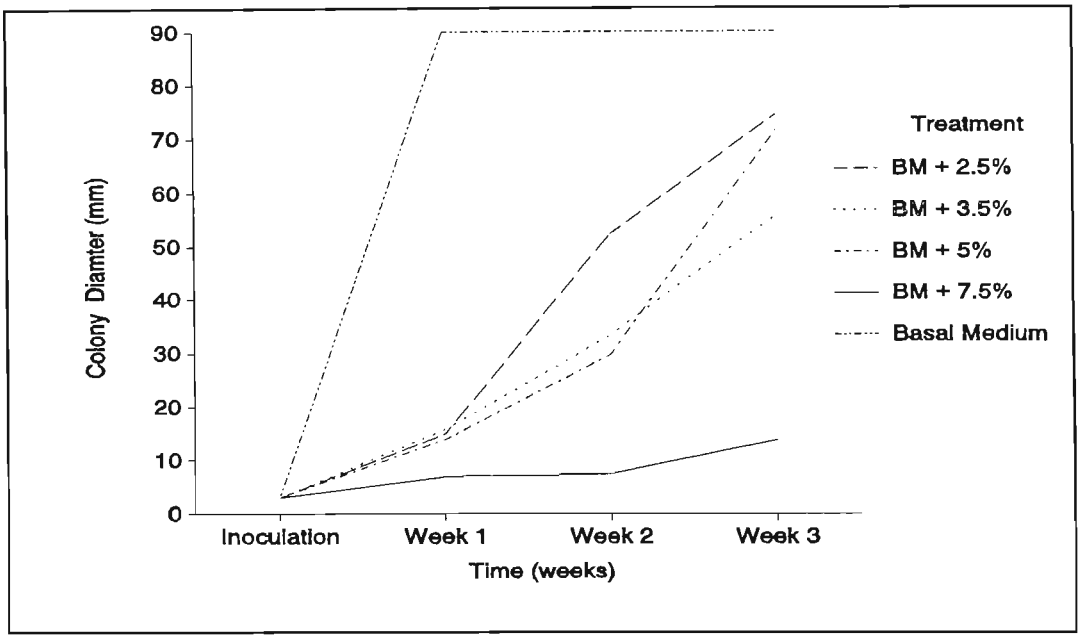

Figure 4.2.1 Growth curves of *T. umkowaani* on Botha's basal medium modified with rabbit agar in various concentrations

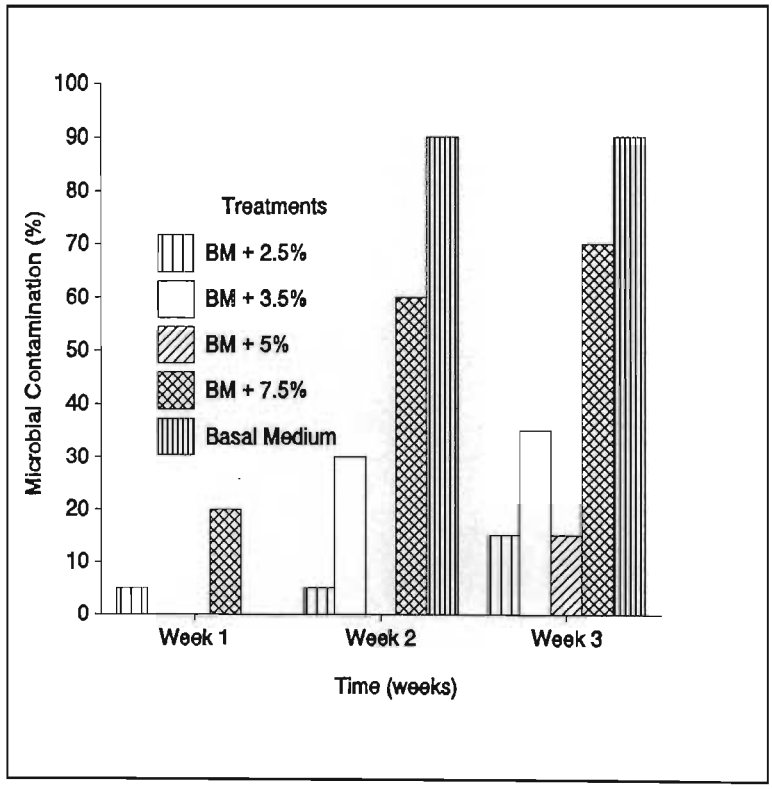

Figure 4.2.2 Weekly microbial contamination of cultures of *Termitomyces* on Basal Medium and Rabbit Faeces Agar

Discussion

Differences between treatments were found to be statistically significant. Growth of *T. umkowaani* mycelium was the most rapid on Basal Medium (BM). However, the growth was inferior to that on the RFA with respect to density and appearance. The growth on BM was sparse and hyaline, with the growth on the modified agar being dense, fluffy and off-white.

Differences in growth on the various agar types do not appear to be due to pH values. *Termitomyces* is said to prefer a slightly acidic medium as the fungus comb found in termite nests is said to be acidic, pH 6.5 (Mohindra and Mukerji, 1984). The most acidic medium used was Basal Medium (**Table 4.2.3**) which did not support the best growth.

All the BM plates were overgrown with contaminants within one week after inoculation with *T. umkowaani*. Some of the plates on the modified agar were overgrown with contaminants. The worst case of contamination of modified agar occurred with 7% RFA where 70% of the plates were contaminated by Week 3 (**Figure 4.2.2**). The contamination of the 2.5% RFA appears to have been due to handling of the plates as the level of contamination did not increase from Week 1 to Week 2, and only rose slightly at Week 3.

From the analysis of the results, the addition of rabbit faeces to Basal Medium did improve the growth of *T. umkowaani* on the medium, and the optimum level of addition was 2.5%. This will be valuable for future research on the commercialization of *Termitomyces*.

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CHAPTER 5

CONTAMINANTS, CONSTRAINTS AND STORAGE

5.1 Contaminants

In the process of mushroom production contaminants can be problematic right from the initial culture stage through to fruiting body production. The presence of contaminants is due mainly to non-sterile technique. This is particularly common in the culture and spawn stages, where sterile technique is essential.

Stamets (1993) identified six vectors of contamination, which can play a significant role in all steps of the production process. These vectors are:

1. The cultivator
2. The air
3. The media
4. The tools
5. The inoculum
6. Mobile contamination units.

In the life of this project, contaminants impacted significantly on the results of most trials. In many cases results were only obtained after the third attempt at a trial, due to the excessive levels of contamination in earlier trials. This chapter identifies possible points of entry of contaminants in all steps of the production process (**Figure 5.1**), and also discusses the role of vectors.

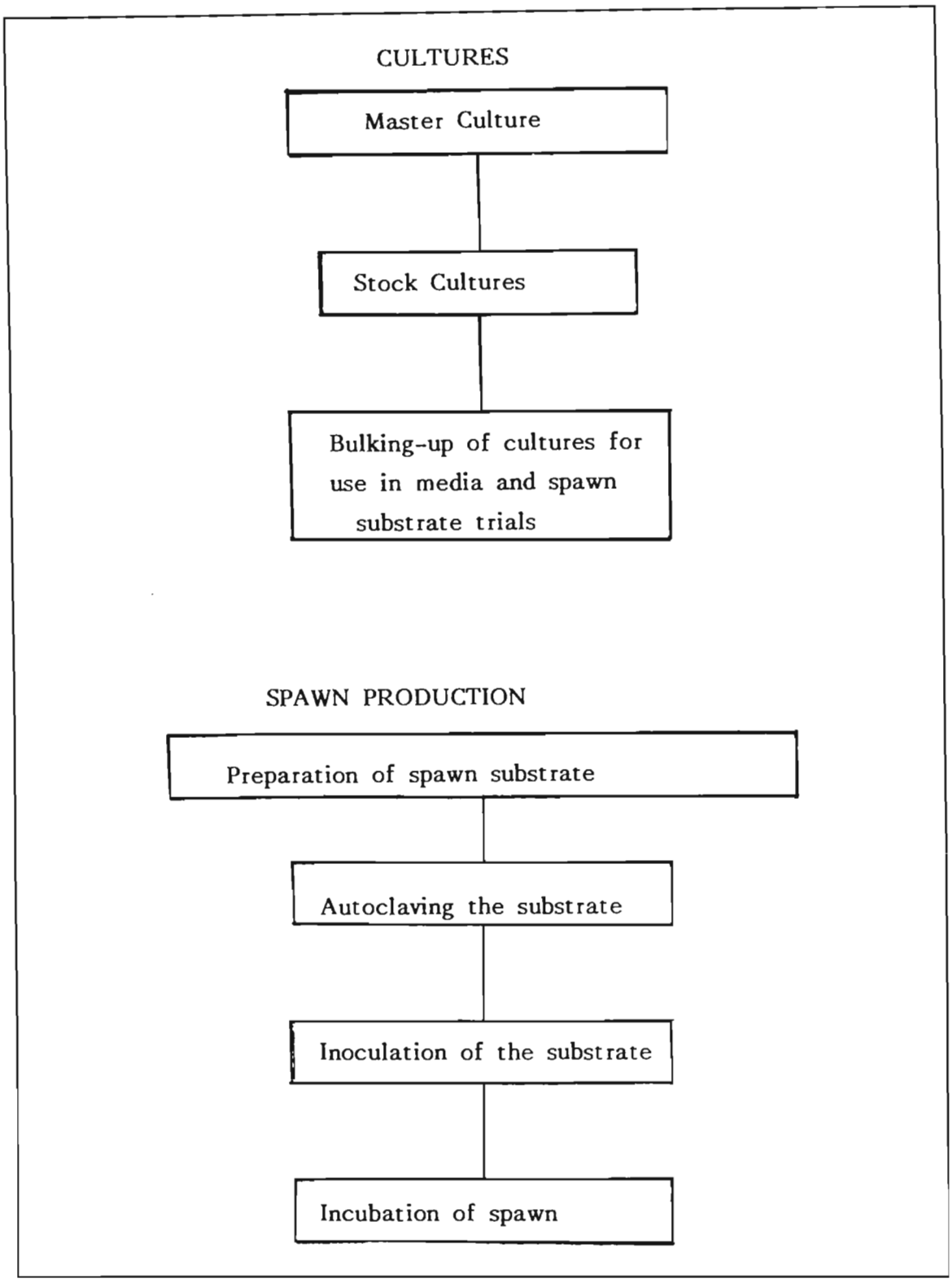


Figure 5.1 Steps in the process of mushroom production covered in this project that could lead to potential contamination problems

5.1.1 Contaminants in Culture

From **Figure 5.1** it is apparent that the entry of contaminants is possible right from the stage of transferring the master culture to stock cultures (slants).

Contamination problems can arise due to:

* The hands of the person performing the transfer being inadequately sterile, or pieces of skin flaking-off onto the cultures being transfered.
* Faulty filters on the laminar flow bench, which allow dust and spores to enter onto the work surface.
* Insufficiently sterile culture medium.
* Non-sterile tools.
* Contaminated starter-culture.
* Mobile contamination units (example, mites) carrying contaminants onto the cultures during incubation of the cultures.

The contaminants encountered in the culture stage of this project were:

* *Penicillium sp*;
* *Aspergillus sp*;
* *Trichoderma sp*; and
* various bacterial species.

Pests, such as mites, were also encountered in the culture stage.

Mites

Mites were more active in the *S. rugoso-annulata* incubator and usually only when the temperature rose to 27°C (due to a faulty thermostat). Mites were even present on cultures sealed in plastic bags. The contaminants were carried on the legs and feet of the mites, and were deposited as they walked and fed on the cultures. Mite contamination was characterized by trails of mixed species of contaminants, mainly bacteria, over the surface of the culture and the culture medium.

The incubator was washed-down with 10% bleach solution, followed by 70% ethanol. The cultures were then stored on glass trays, with petroleum jelly smeared around the edges to prevent mites reaching the cultures. These measures were effective in controlling the mites.

Bacterial contaminants

The level of bacterial contamination was relatively low. When bacterial contamination was prevalent, the flaming of tools for longer periods and pouring of agar at higher temperatures soon solved the problem. Bacterial contamination was more prevalent in *V. volvacea* cultures than in *S. rugoso-annulata* cultures. If bacterial contamination reaches undesireable proportions, antibiotics (example, Streptomycin Sulphate) can be added to the culture medium, through a microbial filter, prior to pouring the plates.

***Aspergillus* contamination**

Few problems occurred with *Aspergillus* contamination. The fungus was localised to the outer edges of the petri dishes when it did occur. *Aspergillus* contamination was considered to be as a result of handling of unsealed petri dishes.

To reduce the incidence of contamination due to handling, petri dishes can be sealed using masking tape or a plastic-based sealing tape.

***Trichoderma sp.* contamination**

The Department of Microbiology and Plant Pathology at the University of Natal has for a number of years conducted trials on the biological control of a variety of fungal plant diseases using *Trichoderma*. As a result, there was a large population of air-borne *Trichoderma* spores present in the Department. This proved to be a problem of considerable proportions at the initial stages of this mushroom project as much as 90% of

mushroom cultures became contaminated with *Trichoderma*.

The level of *Trichoderma* contamination decreased steadily over the duration of the project. This indicated a drop in the number of spores present in the air. This was probably due to there being no *Trichoderma* project being conducted in the Department during the second year of the project. The only means of avoiding *Trichoderma* contamination was to restrict the *Trichoderma* project to a laboratory which was physically removed from the laboratory where the mushroom project was being undertaken.

Penicillium contamination

The level of *Penicillium* contamination became progressively worse during the duration of the project. The worst case of contamination was the contamination of a stock culture of *S. rugoso-annulata*.

This created many problems at the stage of spawn production. Initially it appeared that the problem of contamination of the spawn was due to non-sterile technique. Approximately three weeks after the stock culture had been re-cultured, fruiting bodies of *Penicillium* appeared on the agar in isolated areas.

Two possible areas of entry of the contaminant were investigated:

* ineffective filters on the laminar flow bench
* mixed culture of *Penicillium* and *S. rugoso-annulata*.

The effectiveness of the filters on the laminar flow bench was tested by the following method:

5 petri dishes of MEA were left open on the laminar flow bench for each of the following time intervals: 1 minute; 2 minutes; 5 minutes and 10 minutes.
The plates were then incubated at 25°C for 3 weeks. However, no growth appeared after this time.

The conclusion then was that the contamination of the spawn was due to a mixed culture containing the *Penicillium* and *S. rugoso-annulata*. As the master culture had been used for numerous stock cultures, and was running very low, it was decided to attempt to rectify the problem of the mixed stock culture:

Benomyl is a fungicide effective against *Penicillium spp* and other hyphomycetes, but does not affect wood decay fungi, including *S. rugoso-annulata* (Malay, 1974).

MEA with 1 ppm Benomyl (Agricura) was used therefore to purify the cultures. The Benomyl was added prior to autoclaving as it is considered to be heat stable (Malay, 1974). This was an advantage as it decreased the chances of contaminants entering as Benomyl did not have to be added when the agar was cool.

The dual culture was inoculated onto the Benomyl-Malt agar. The *Penicillium* did not grow, and after a week of growth, pure mycelium of *S. rugoso-annulata* could be transferred to MEA.

5.1.2 Contaminants in Spawn Production

There are considerably more areas of possible contaminant entry in spawn production, than in the culture stage.

From **Figure 5.1**, the first possible entry point is insufficient autoclaving time of the spawn substrate. The second is the entry of contaminants during the inoculation of the spawn medium. The third is the entry of contaminants during incubation of the spawn.

Contamination at all these stages can be due to

* The cultivator
* The air
* The substrate
* The tools
* The inoculum
* Mobile contamination units.

Although the problem of contaminated spawn was caused by a contaminated stock culture, once the stock culture was purified, there were still considerable contamination problems with the spawn. Both *S. rugoso-annulata* and *V. volvacea* spawn was usually heavily contaminated.

As all possible precautions were taken with regard to sterile technique, the cultivator, the air and the tools used were ruled out as being vectors of the contamination problem.

The substrate was autoclaved for three hours and was thus also not considered a possible source of contamination.

It was then apparent that the contamination was entering the bags either at the time of inoculation, or through various entry points in the bags. A number of possible entry points were identified:

* cotton-wool plugs,
* pores in the polypropylene bags,
* seams of the polypropylene bags.

Cotton wool plugs

As the bags were always left in the autoclave until they had cooled to room temperature, entry of contaminants could not have been due to the cotton-wool being wet and therefore losing its electrostatic charge. However, a trial was established to determine if the point of entry was through the cotton-wool plugs.

Materials and Methods

Treatments:

1. Cotton wool treated with Busan 30A (Buckman Laboratories, Hammarsdale, KwaZulu-Natal) a heat stable contact biocide.
2. Cotton wool covered with aluminium foil during and after autoclaving.

3. Cotton wool left untreated.

4. Substrate treated with 1ppm Benomyl.

There were three replicates per treatment, and three bags per replicate. The substrate used was sorghum, which was prepared as for the spawn media trials (**Chapter 2.4 and Chapter 3.4**). The bags were inoculated with *V. volvacea* and incubated at 30°C. The bags were assessed at four-day intervals to determine mycelial growth and to check for the presence of contaminants.

The results were analysed using ANOVA with a Fisher's LSD test for means separation and Friedman's rank analysis.

Results

Mean rating, rank analysis and contamination levels (%) for the contamination trial are represented in **Table 5.1.2.1**.

The analysis of variance showed that the treatments and replications were not significantly different (**Table 5.1.2.2**).

Table 5.1.2.1 Mean rating; rank analysis and % contamination of the contamination trial

Treatment	Mean Rating	Rank Analysis*	% Contamination
Benomyl in substrate	4.6	3.6	00.0
Busan 30A and foil	3.67	3.2	22.2
Busan 30A	4.0	3.2	33.3
Cotton wool and foil	3.1	2.6	44.4
Cotton wool	2.67	2.4	55.5

* from Friedman rank analysis

Table 5.1.2.2 ANOVA of the contamination trial.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	Significance Level
Main Effects					
Treatment	33.2	4	8.3	1.929	0.1254 **NS**
Replications	2.3	2	1.2	0.269	0.7659 **NS**
Residual	163.5	38	4.3		
Total (corrected)	198.9	44			

Discussion

The results were not statistically significant. However, the trends apparent in the results are clear.

The cotton wool treated with Busan 30A and covered with foil had lower contamination rates than Busan 30A alone and cotton wool left untreated. This indicated that Busan 30A did prevent contaminants entering through the cotton wool. Most importantly contamination did occur with biocide-treated cotton wool, proving that there were contaminants entering through the bag walls or seams.

Pores in the bags

From the results of the Cotton Wool Trial, it was clear that the contaminants were entering through the bag walls or seams.

Scanning Electron Microscopy (SEM) was conducted on the polypropylene bags at various stages during the spawn process to identify possible points of entry.

Materials and Methods

The treatments were:

* unautoclaved bag,

* autoclaved bag,

* inside surface of a contaminated bag and

* outside surface of a contaminated bag.

The bags of the various treatments were air dried and then cut into 3mm by 3mm pieces. Four pieces per treatment were stuck onto brass viewing stubs using double-sided tape. The samples were then sputter-coated with gold palladium using a Polaron E5100 sputter-coater. The samples were viewed under a Hitachi S-570 scanning electron microscope at 8.0 KV.

Results

The results of the SEM viewing are represented in **Plate 5.1**.

Discussion

From the photographs, it is clear that contaminants were able to enter through various holes, cuts and stress fractures in the polypropylene bags (**Plate 5.1.a**). There were more stress fractures and holes in bags that had been autoclaved (**Plate 5.1.b**), than in the bags that had not been autoclaved (**Plate 5.1.a**). The outside surface of the contaminated bags had a moderately high population of contaminant spores present (**Plate 5.1.c**). The inner surface indicated stress fractures where spores had entered and germinated (**Plate 5.1.d**).

The results of this trial were confirmed by a mushroom rearcher from Strathclyde, Scotland (Smith, 1995. Pers. Comm.). Similar contamination levels were experienced in spawn trials conducted for a mushroom project. After some investigation, it was found that the contaminants were entering through pores and holes in the polypropylene bags. The spores of contaminants were literally being sucked through the pores and holes when the bags cooled to room temperature.

A further factor unearthed was that the polypropylene roll used to manufacture the bags was of a reject batch. This factor was only discovered after the trials had been

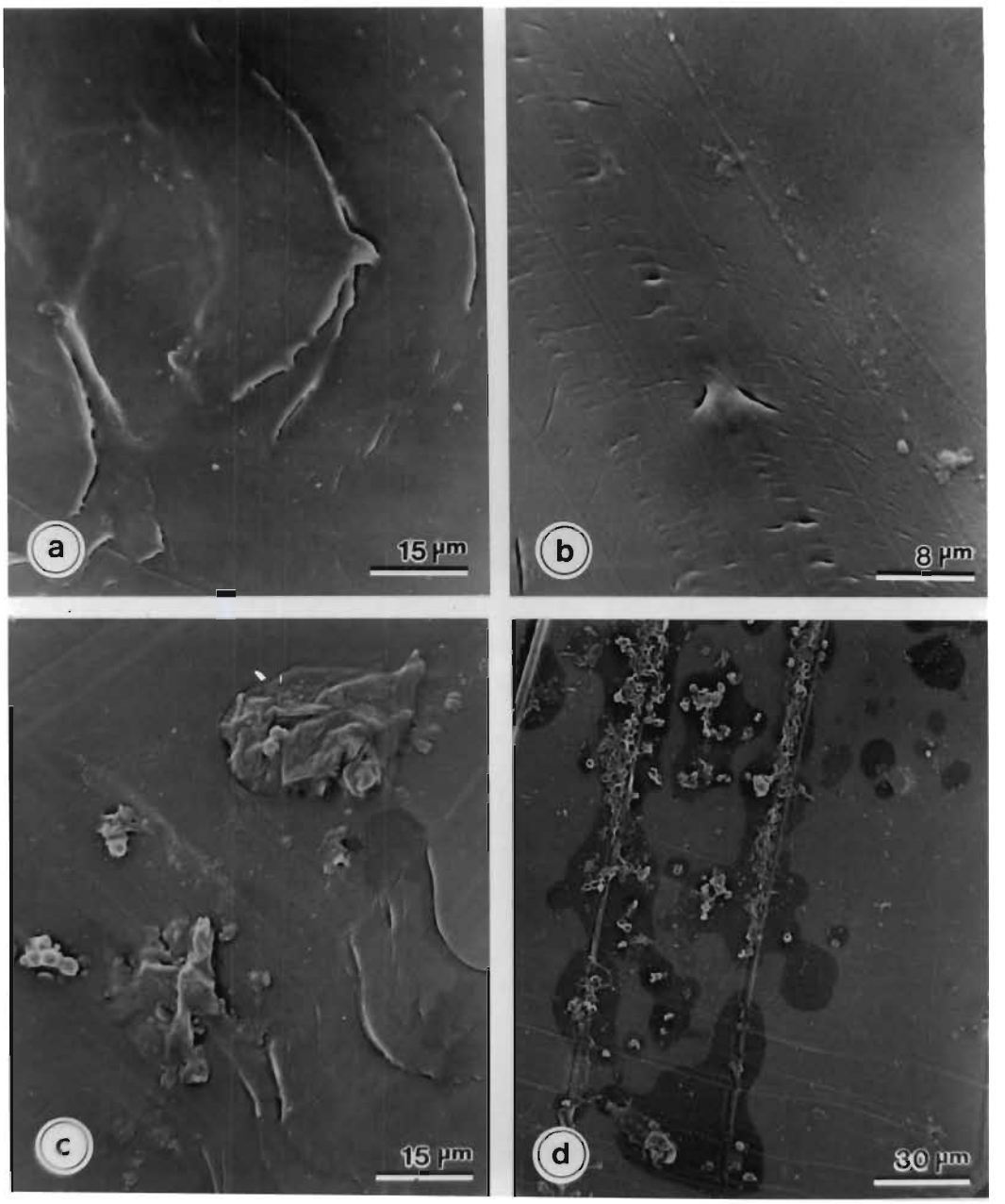
Plate 5.1 Scanning Electron Microscopy of:

(a) surface view of a polypropylene bag

(b) surface view of an autoclaved polypropylene bag

(c) outer surface of a contaminated polypropylene bag

(d) inner surface of a contaminated polypropylene bag


a
15 µm
b
8 µm
c
15 µm
d
30 µm

conducted, when the economic analysis of the trials was being undertaken. The reject polypropylene roll was purchased on the basis that it was cheaper than standard quality polypropylene. Smith (1991, Pers. Comm.) stated that top quality polypropylene with reduced plasiticiser was required for spawn bags.

Seams of the bags

The integrity of the seams of the bags was determined. Ten bags were sealed using the standard method with double seams on a Tish 400 impulse sealer (Tew Electric Heating Company). Each of the bags was filled with 800 ml of water. Eight of the ten bags leaked through the seams. A higher heat setting and longer period of sealing was employed, which proved effective in ensuring the integrity of the bag seams.

5.2 Constraints

This is a very general category which refers particularly to logistical problems. **Figure 5.2** shows the steps in mushroom production where logistical problems occur, and steps that can be made more efficient and effective by various means.

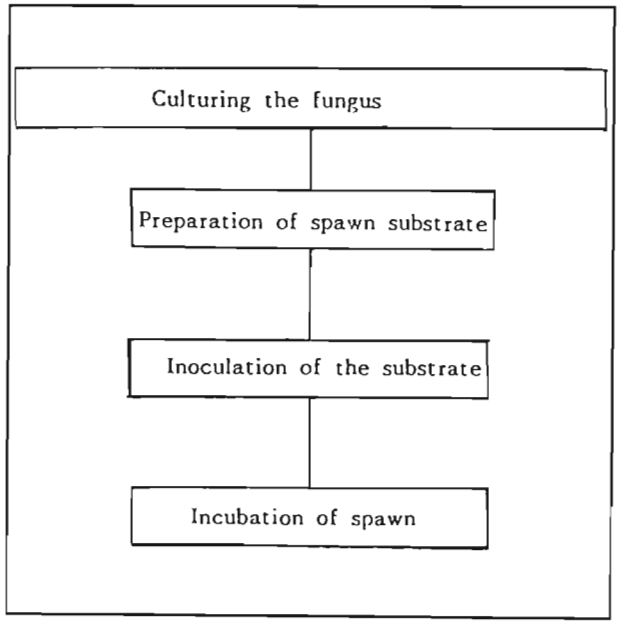


Figure 5.2 Steps in the process of mushroom production covered in this project where logistical constraints were encountered

The culturing of the fungus did not present any logistical problems and could effectively be undertaken by a single person.

Most of the constraints were encountered at the stage of spawn preparation. There are a number of processes that constitute spawn preparation:

* soaking the substrate
* draining and washing the soaked substrate
* boiling and cooling the substrate
* filling the caps with cotton wool
* filling the bags with prepared substrate
* fitting the collars and the caps to the filled bags.

Most of these processes are tedious and time consuming, especially if conducted by only one person. It would be feasible for a number of people to conduct a number of these steps concurrently. For example, the bags and caps can be prepared at the same time as the substrate is being prepared, so that the bags can be filled as soon as the substrate has cooled.

One of the most obvious difficulties experienced was inoculating spawn grain. Due to the nature of sterile technique, no bottle-lids, inoculating tools or petri-dish lids can be placed on the laminar flow bench top. This created considerable difficulty when inoculation of spawn grain was being conducted by only one person. It was found that contamination was greater when inoculation was performed by one person, than when it was performed by two people.

When two people were inoculating spawn substrate, it was possible for the lids and inoculation tools to be flamed for longer, and kept off the laminar flow bench surface. Inoculation time was also cosiderably reduced when performed by two people.

Many of the procedures involved in spawn production are time consumming, especially when performed by one person. This can become a problem if time is limited or large quantities of spawn need to be produced. Spawn-making can be very efficient if

the various stages and processes are performed by a number of people, so that a "production-line" effect is produced.

5.3 Storage:

Sexually reproducing organisms are limited in the number of cell replications they can undergo. If a further recombination of genes does not occur, cell lines decline in vigour and eventually die. Mushrooms are no exception to this, and strains will die unless precautions are taken to maintain cultures in storage.

Cultures are typically stored in test tubes (culture slants). The need for gas exchange during storage on slants is minimal, provided the growth of the culture is slowed down by timely placement into cold storage. Culture slants stored at room temperature have a maximum life of six to twelve months and under refrigeration up to five years or more (Stamets, 1993).

All strains of *S. rugoso-annulata* were stored on slants of 3.5% MEA, at 5°C. Twenty slants of each strain were prepared.

V. volvacea was also stored on 3.5% MEA slants, but at 10°C as the fungus dies at temperatures below 7°C.

The master culture was used to prepare numerous stock cultures. The stock cultures were then used to bulk-up cultures for the trials conducted (see **Figure 5.3**). One stock culture was used for each trial to prevent the cultures attenuating, or losing viability.

All the cultures used for the trials proved viable and thus the storage method used was effective.

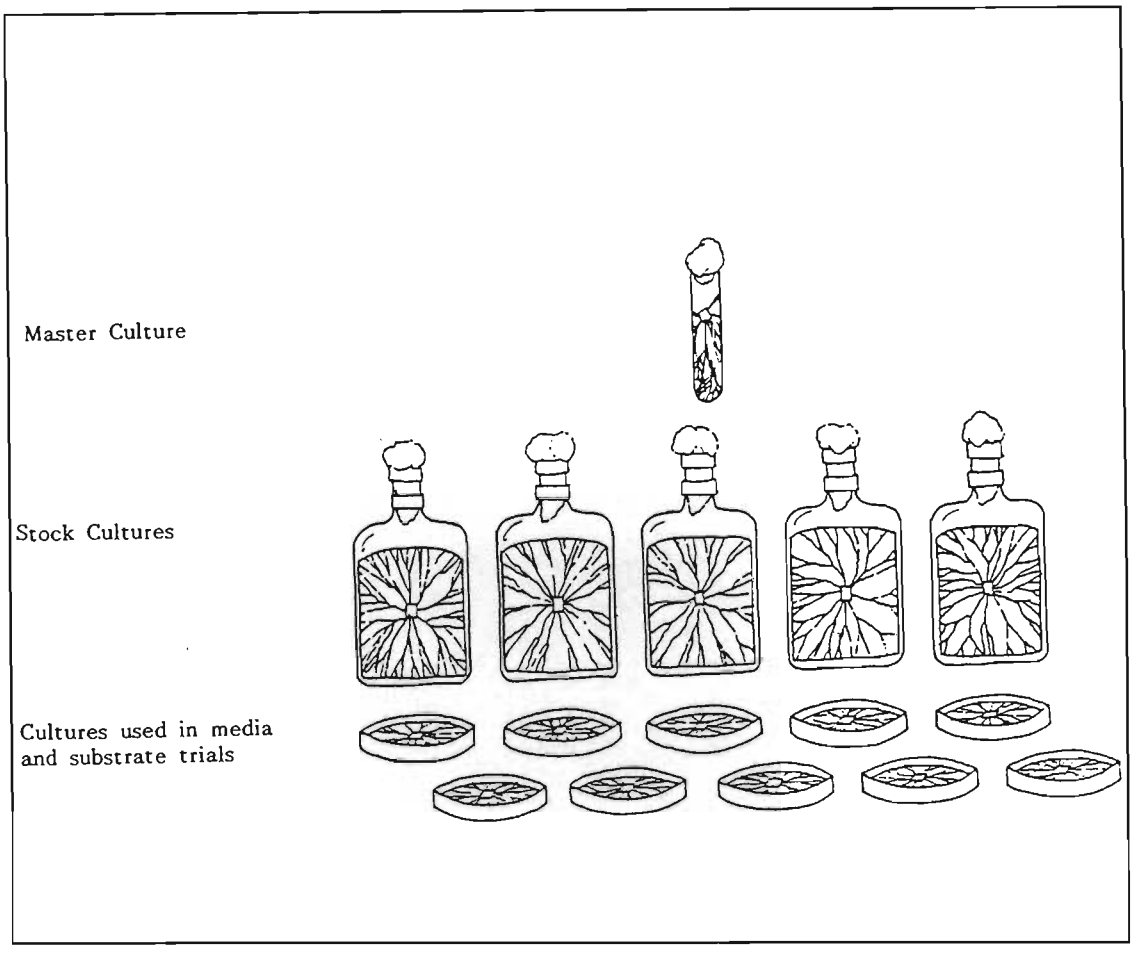


Figure 5.3 Diagramatic representation of culture maintainance for the mushroom project

References

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CHAPTER 6

ECONOMIC ANALYSIS, FEASIBILITY AND MARKETING ASPECTS OF *Stropharia rugoso-annulata* AND *Volvariella volvacea* PRODUCTION.

6.1 Introduction

Cultivation techniques have been developed for various mushroom species to increase production and yield. Cultivation technique determines amount of production, but it is not the only factor determining success in the industry. A number of factors need to be considered, and a number of obstacles need to be overcome before mushroom production can be deemed feasible and economically viable.

6.2 Economic analysis

Economic feasibility needs to be determined right from the beginning of the production process, and economic analysis must include all aspects and costs involved in production.

A record should to be kept of all variable and fixed costs of production. Determining the economics of production is relatively simple, and can be done using the following two equations.

Gross Margin = Gross Output - Variable Costs **[1]**

Net Farm Profits = Wholefarm Gross Margin - Fixed Costs **[2]**

Table 6.2 gives an example of costing for the production of $1m^2$ of mushrooms.

Using the figures supplied in **Table 6.2:**

From equation [1]:

Gross Margin = R 280.00 (Gross Output) - R 165.50 (Variable Cost)

Gross Margin = R 114.50

Table 6.2 Economic analysis of mushroom production for 1 m^2 of fruiting substrate

	Amount (R) Debit	**Amount (R) Credit**
Income: sales of crop		(8 kg @ R 35/kg) R 280.00 *
Purchase of spawn	R 7.50 **	
Fruiting body production		
Substrate (straw)	R 12.00	
Water	R 8.00	
Plastic sheeting	R 18.00	
Labour	R120.00	
Total:	R165.50	R 280.00
Total Gross Margin:		R 114.50

* based on current costs of *Pleurotus ostreatus*

** from Oei, 1991

The figures used in the economic analysis were based on actual costs calculated for the project, unless otherwise stated. As spawn production is difficult and a high risk, it would be more economically viable to buy spawn from a commercial spawn producer. Further, to establish a spawn laboratory would involve a considerable amount of capital.

The estimated cost of a small-scale spawn production plant is R 5 512.50 (Oei, 1991). This does not include the cost of a sterile laboratory nor a laminar flow bench. The cost of

a sterile laboratory would vary, depending on its design and construction. A figure of not less than R20 000 is estimated due to the need for air filters, positive pressure in the room and other equipment needed to maintain sterile conditions.

The approximate cost for buying spawn from a supplier is R7.50 per litre (Oei, 1991). Thus, unless a considerable amount of capital was available to produce spawn, if there was a very large demand for spawn, it would be more feasible to buy spawn from a commercial supplier.

6.3 Feasibility of production

The feasibility of producing mushrooms depends on a number of factors. These include:

* available capital
* climatic suitability
* availability of water
* availability of labour
* expertise in production methods.

6.3.1 Available capital

The amount of capital required would depend on the type of production being pursued. Commercial production using controlled environments would require at least R75 000 to establish the mushroom farm (information supplied by a small-scale commercial grower who wishes to remain anonymous). Loans for this purpose can be secured from various financial institutions, but interest on these loans can be considerable. With regard to the economics of production, the interest on the loan is calculated as a fixed cost and is subtracted from the gross margin (**6.2** Equation [2]).

In the case of subsistence or cash crop farmers, loans are not always readily available. Subsistence farmers may be able to receive funding through various aid projects, feeding schemes, or farmer's organizations.

6.3.2 Climatic suitability

An unsuitable climate can be overcome by the use of controlled environments. However, this is expensive and more capital would need to be available than for a climatically suitable location.

Temperature range maps were obtained from the Geographical Information Systems Unit at Cedara Agricultural College, Pietermaritzburg. The maps were produced using a GIS system which determines areas suitable for the production of various crops, based on the climatic requirements of the crops concerned. **Figure 6.3.1** to **Figure 6.3.3** show optimal areas for *S. rugoso-annulata* production, for each month of the year in KwaZulu-Natal. The areas are determined by a temperature range of 12°C to 25°C. These temperatures are considered optimal for the growth of *S. rugoso-annulata* (Szudya, 1978; Stamets and Chilton, 1983) and no environmental control would be neccessary for the production of *S. rugoso-annulata* in these areas.

Figure 6.3.4 shows optimal areas for *V. volvacea* production in KwaZulu-Natal. The areas are based on a temperature range of 20°C to 40°C. Only four months of the year are suitable for *V. volvacea* production, and the areas concerned are limited to the Northern coast.

From the information provided on the maps, it would be feasible for growers in Northern coastal areas to rotate production, growing *S. rugoso-annulat1a* during June, July and August and *V. volvacea* during December, January, February and March. Production during April, May, September, October and November would require the use of a mushroom house or contained environment.

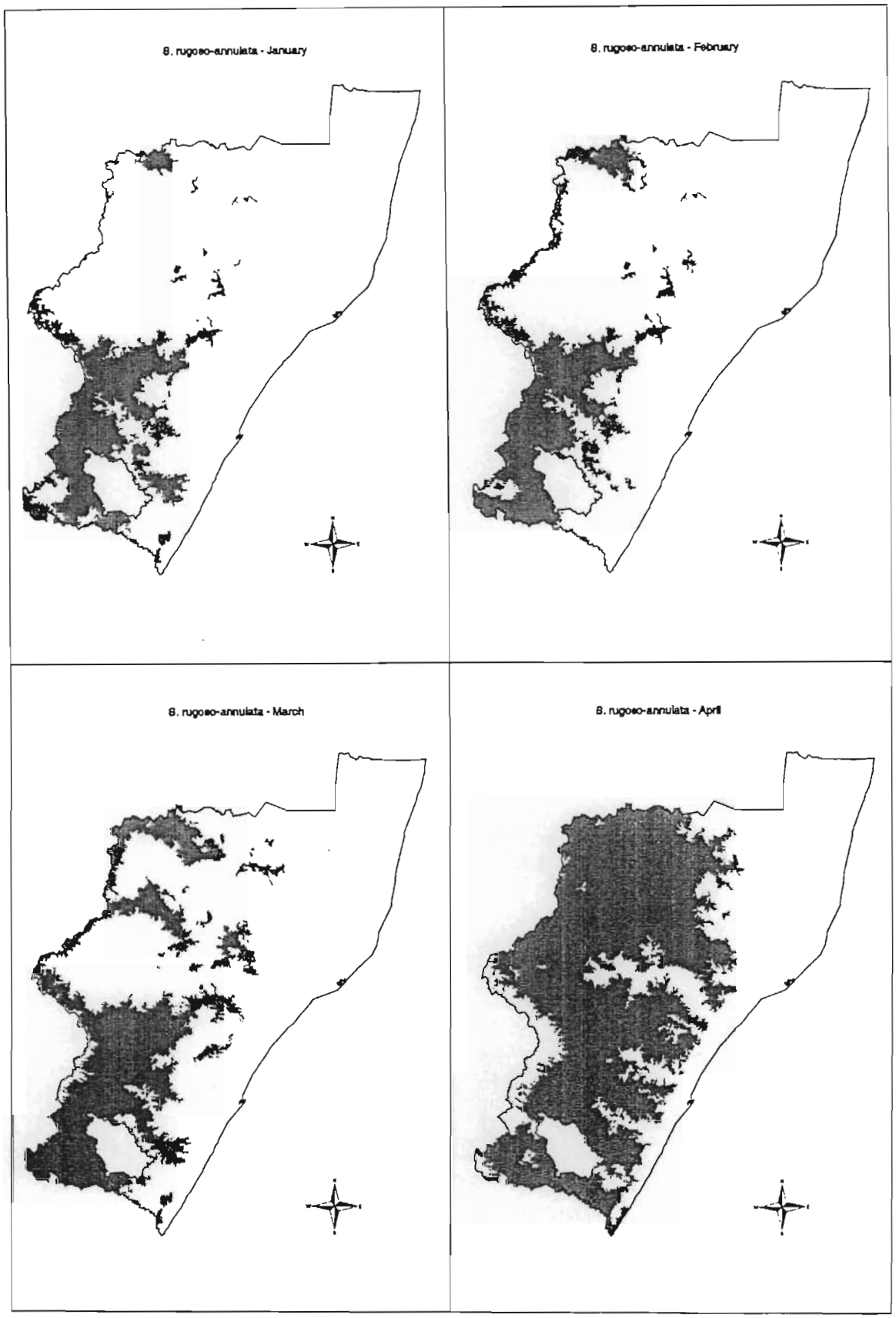

Figure 6.3.1 Maps of KwaZulu-Natal showing areas with optimal temperatures for the production of *S. rugoso-annulata* during January, February, March and April

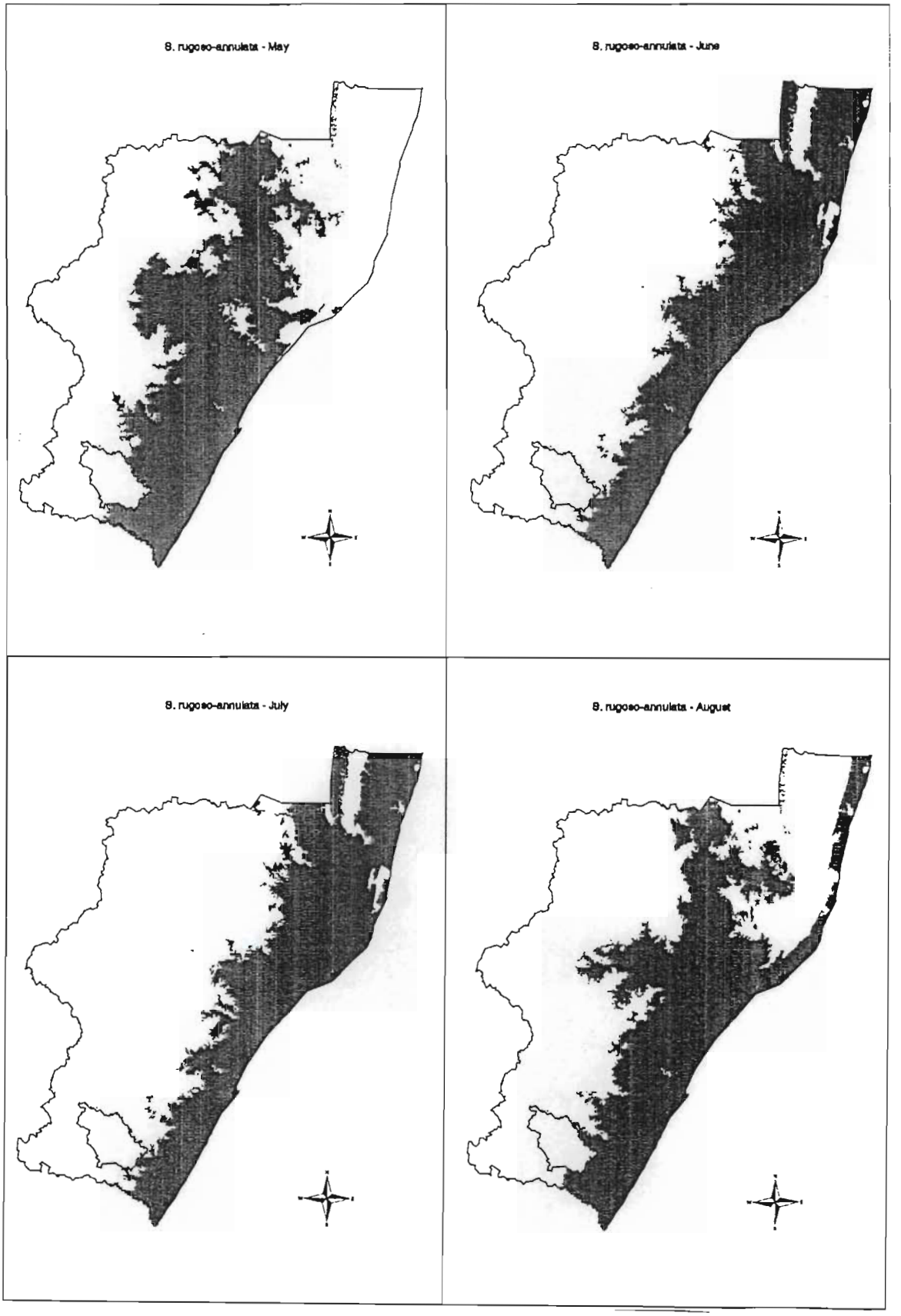

Figure 6.3.2 Maps of KwaZulu-Natal showing areas with optimal temperatures for the production of *S. rugoso-annulata* during May, June, July and August

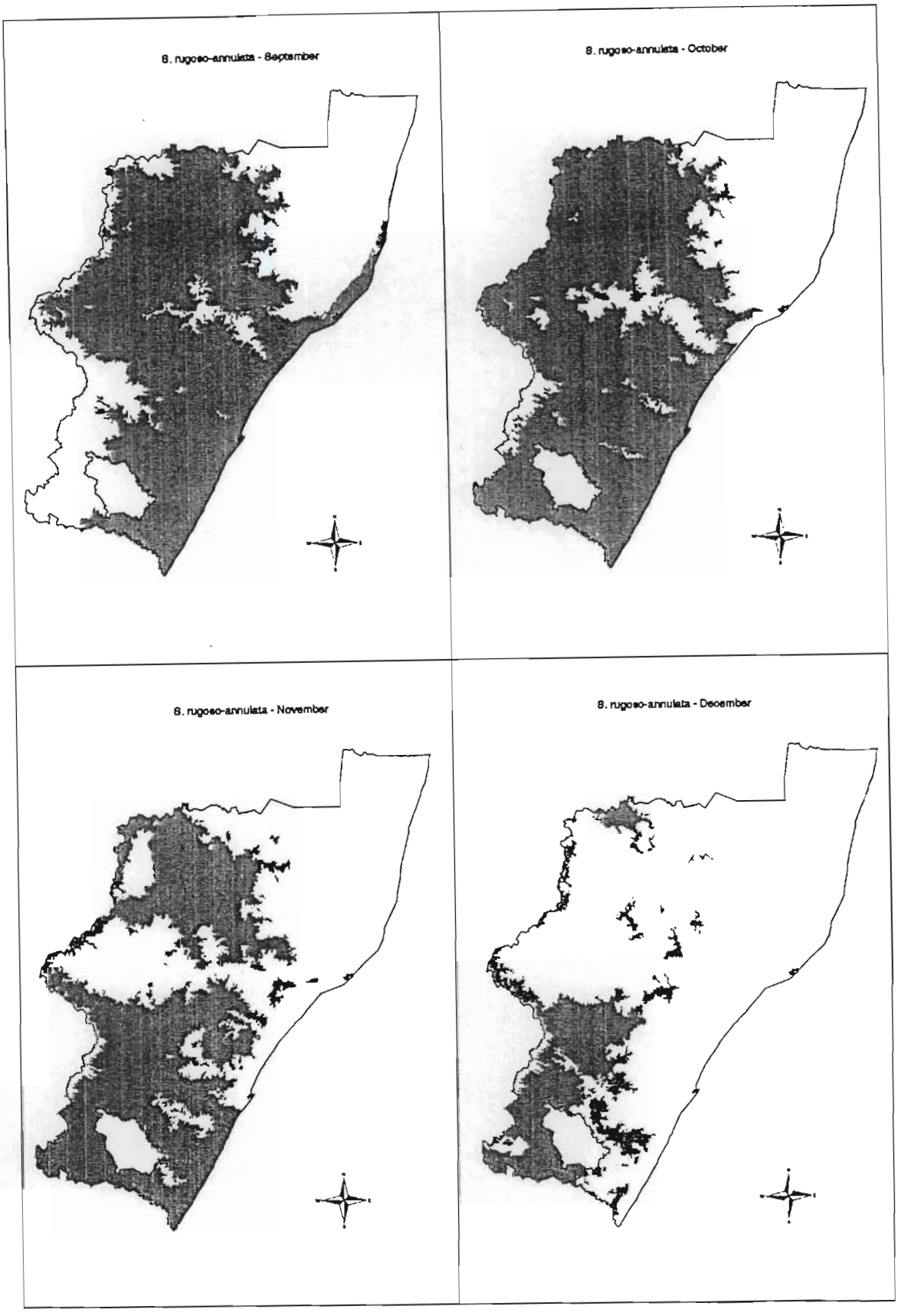

Figure 6.3.3 Maps of KwaZulu-Natal showing areas with optimal temperatures for the production of *S. rugoso-annulata* during September, October, November and December

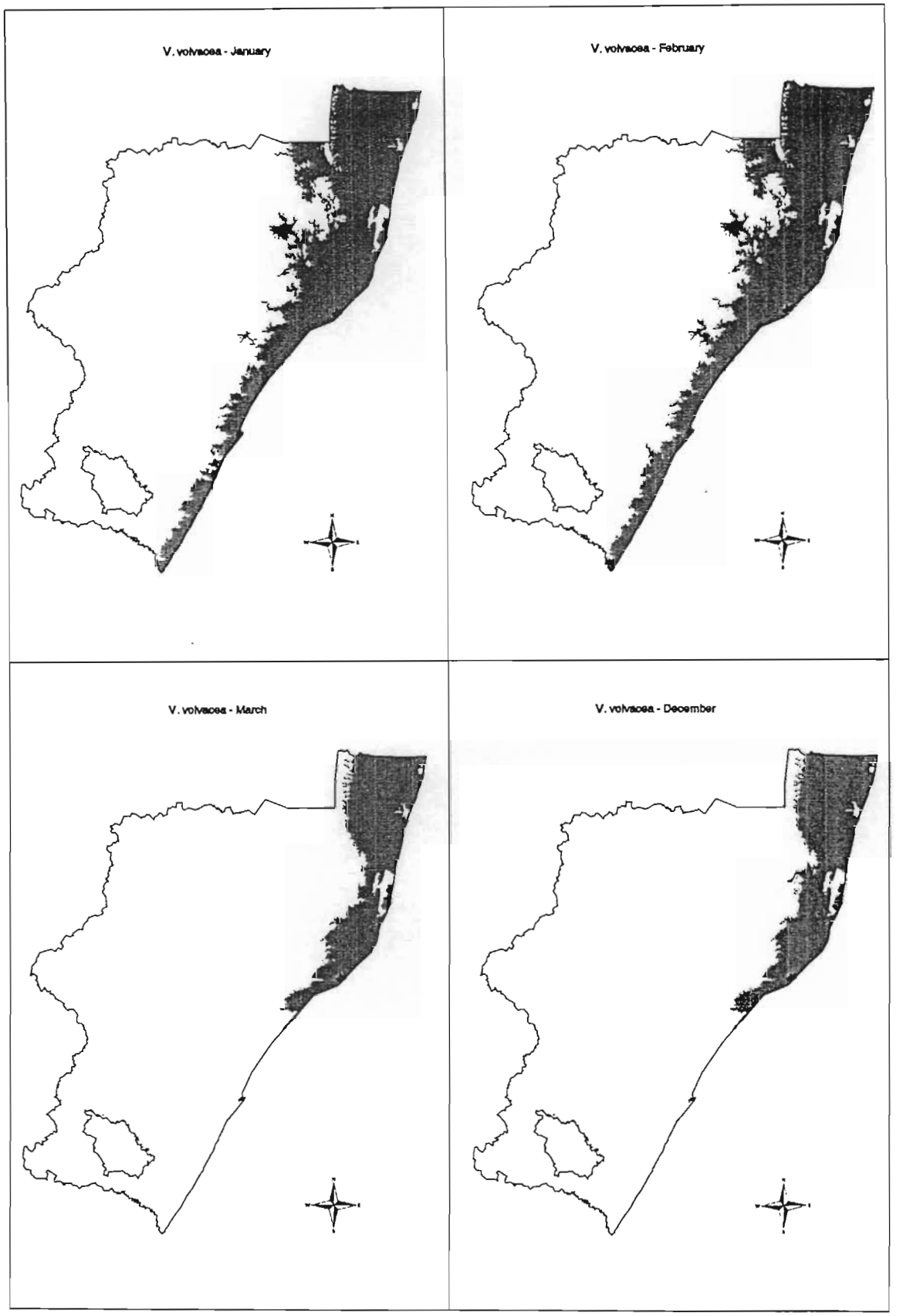

Figure 6.3.4 Maps of KwaZulu-Natal showing areas with optimal temperatures for the production of *Volvariella volvacea* during January, February, March and December

Climatic conditions other than temperature that would also need to be considered would include prevailing winds, aspect and humidity.

Prevailing winds would play an important role in the spread of contaminants on a mushroom farm. The farm lay-out should be such that any areas of likely contamination are down-wind from area where critical processes, such as spawn production occur. Prevailing winds could also serve to heat or cool buildings, depending on the nature of the wind.

Aspect would have an effect on temperature and light control, and whether mushrooms are grown in houses, shelters or in the open. Positioning beds in an East-West direction would allow for more heat on the beds during the day. For mushroom houses, if the door is East-facing, the entry of light and heat could be used to aid in fruiting.

Areas with generally higher relative humidities would require less humidity regulation and use less water.

6.3.3 Availability of water

Mushroom production can require large quantities of water. For commercial growers in developed or semi-developed areas in KwaZulu-Natal, water is usually supplied by Umgeni Water, or from bore-holes. For smaller growers in rural areas, water often has to be carried a considerable distance.

Water availability could be a limiting factor, but means do exist to increase water availability without undue effort of the household or community. These include: rainwater harvesting; fog interception (Alcock and Verster, 1987) and roof run-off (Alcock *et al*, 1988). The implementation of spring protection schemes (Alcock, 1987) and major reticulation schemes (Alcock *et al*, 1988) in these communities would serve to lessen the burden of water availability and make mushroom production more feasible.

6.3.4 Availability of Labour

Mushroom production is a labour intensive process. For subsistence and cash crop farmers, labour availability is not a problem as farming in their communities is a family

undertaking, unless family members are migrant labourers.

In South Africa, unskilled manual labour is relatively cheap compared to western countries. Due to the large population, labourers are readily available, so employing labourers for commercial farms should be easy. However, some skills are required and workers need to be hard-working and dedicated to producing a high standard of mushroom crop. Thus workers would have to be paid well or other incentives offered.

6.3.5 Expertise in production methods

Mushroom production requires skilled operators and managers to ensure that the process runs smoothly. There are not many people trained for mushroom production in this country as it is a relatively new technology and industry. There is thus a need for training and skill acquisition, to ensure high quality production.

6.3.6 Towards the development of a Decision Support System

Determining the feasibility of mushroom production is a complex process as many different factors need to be considered. Once a small-holder or subsistence farmer had decided to explore the possibility of mushroom production, there are a number of questions that need to be answered before the decision can be made. The decision, based on the answers to the questions, can usually only be made by someone with a detailed knowledge of mushroom production.

Experts on mushroom production are not readily available in South Africa and there needs to be a system that contains the knowledge and reasoning of an expert, but is also always available. Such a system is known as a Decision Support System or an Expert System. Expert systems are a branch of artificial intelligence that uses specialized knowledge to solve problems at the level of a human expert (**Figure 6.3.6.1**)

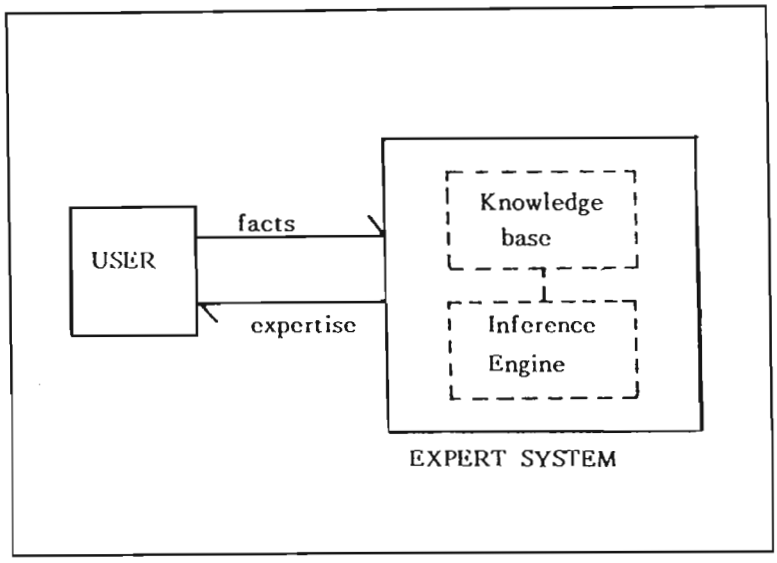

Figure 6.3.6.1 Basic concepts of an Expert System function

(from Giarratano and Riley, 1989)

The user supplies the facts or other information to the expert system and receives expert advice in response. The knowledge base of the expert system contains the knowledge from which conclusions are drawn. Conclusions are the expert system's responses to the user's queries for expertise (Giarratano and Riley, 1989).

The simplest method of representing qualitative arguments that lead to a decision, is a decision tree. One starts at the top and answers questions, as directed by the arrows, until led to a decision. Decision trees are easy to use but unless they are simple, they can be difficult to build and modify. A more pliable representation is needed if the model is to be amended in the light of experience (Starfield and Bleloch, 1986). Such a system is a decision table.

The decision table contains exactly the same information as a decision tree. The questions are written down the left-hand side of the table in any order, but are rewritten as statements that can be either true or false. Decision tables are easy to modify and implement on computer but can become cumbersome. If-then rules and expert systems would be a better method of representing the questions (Starfield and Bleloch, 1986).

The process of building an expert system is called knowledge engineering as is

undertaken by a knowledge engineer. Knowledge engineering is the acquisition of knowledge from an expert or other source (example, literature) and its coding in the expert system (**Figure 6.3.6.2**) (Giarratano and Riley, 1989).

Expert systems are generally designed very differently from conventional programs as the problems usually have no algorithmic solution and rely on inferences to achieve a reasonable solution.

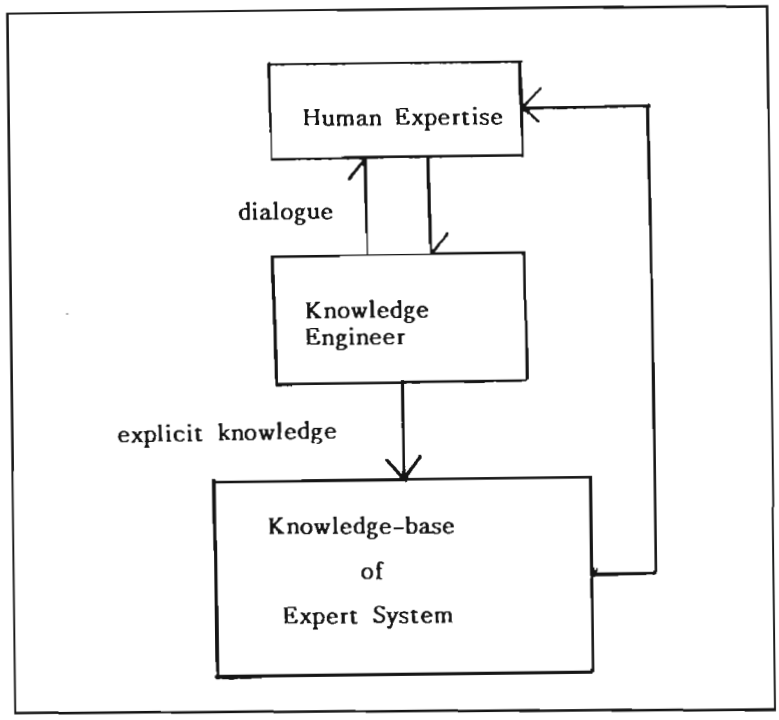

Figure 6.3.6.2 Development of an Expert System

(from Giarratano and Riley, 1989)

It is essential to decide if an expert system is an appropriate paradigm. That is, should an expert system be used instead of an alternative paradigm such as conventional programming:

* Can the problem be solved efficiently by conventional programming (expert systems are best suited to situations in which there is no efficient algorithmic solution)?
* Is the domain well balanced (it is important to have well-defined limits on what the expert system is expected to know and what its capabilities should be)?
* Is there a need and a desire for an expert system?

* Can the expert explain the knowledge so that it is understandable by the knowledge engineer?
* Is the problem-solving knowledge mainly heuristic and uncertain (if the problem can be solved simply by logic and algorithms, it is best solved by conventional programming)? (Giarratano and Riley, 1989)

There is a need for the development of a Decision Support System for potential mushroom cultivators in KwaZulu-Natal as many variables need to be considered for successful mushroom production. Climatological data, economic data and logistics can be entered as a data base from which decisions can be made.

A number of decision trees were constructed to determine:

* which technology to apply (**Figure 6.3.6.3**)
* which species of mushroom to grow (**Figure 6.3.6.4**)
* whether to buy or produce spawn. (**Figure 6.3.6.5**).

From these, a computer program was written that requires the user to answer questions that appear on the screen. In each category, a decision is made based on the user's answers to the questions. The program was written by M.D. Relihan using DBXL, and a copy of the program is available on computer disk in the back cover of this document. The program is available for review in Appendix 6.3.

The program is certainly an oversimplification of the system and an extensive data base on climatological and economic information is required. However, the acquisition of such a data base was beyond the financial and time constraints of this project.

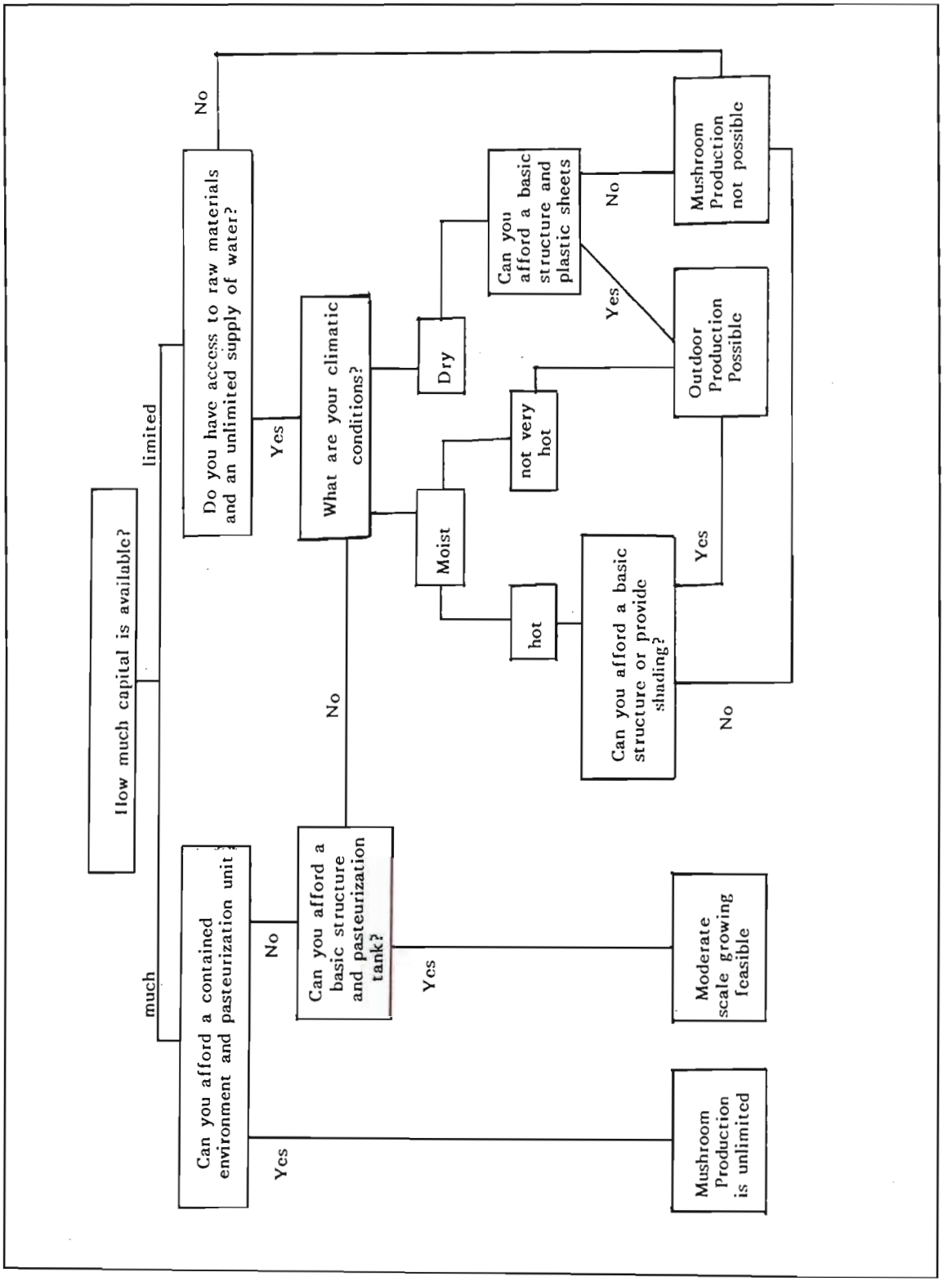

Figure 6.3.6.3 Decision tree for determining which technology is appropriate for mushroom production

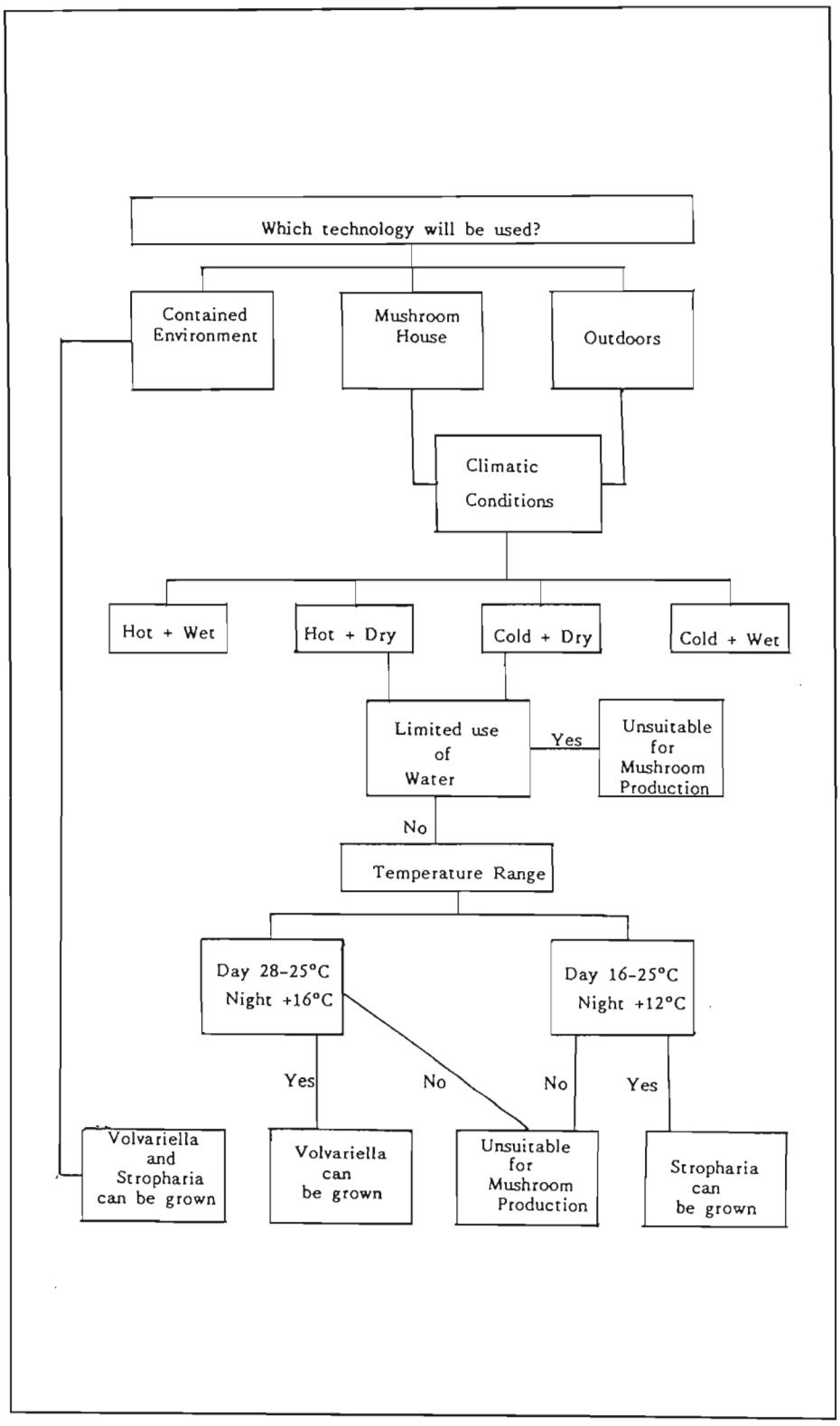

Figure 6.3.6.4 Decision tree to determine which species of mushroom should be grown

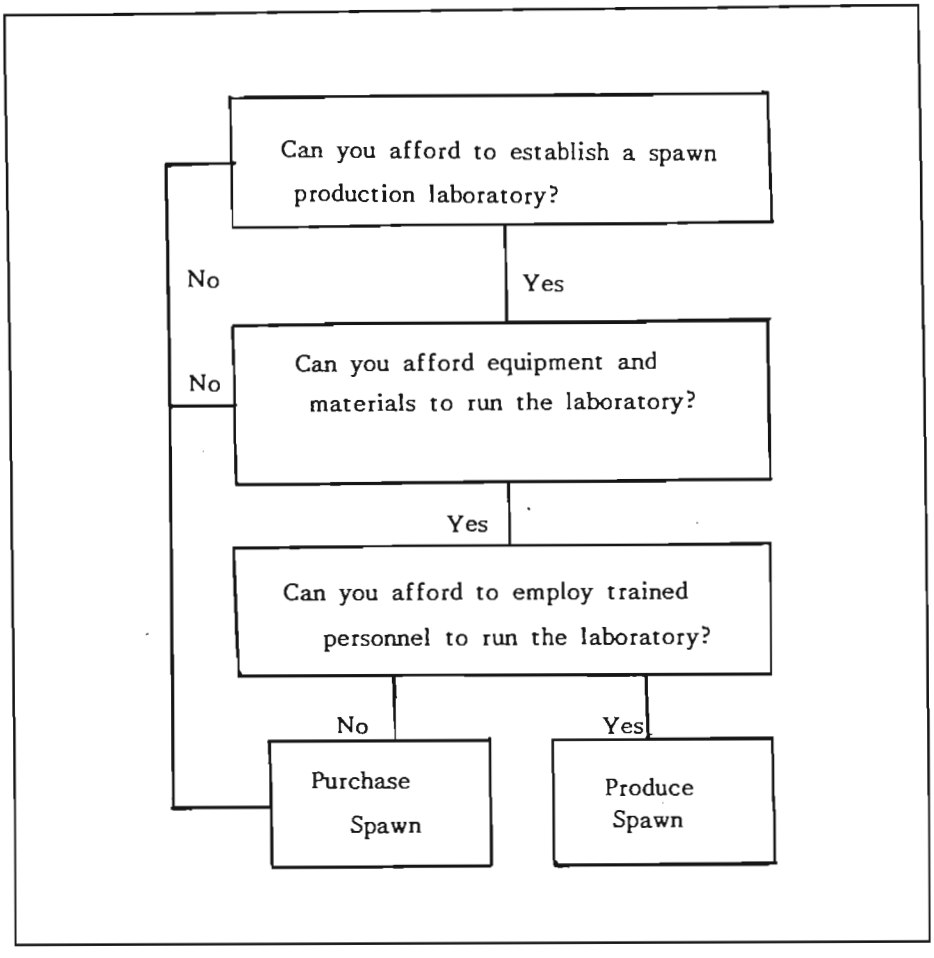

Figure 6.3.6.5 Decision tree to determine whether to buy or produce spawn for mushroom production

6.4 Marketing aspects

Mushrooms are an important crop in the United States and many European countries. The value of mushrooms increased from $68 million in 1969 to $368,547 million in 1979 in the United States of America. This increase indicates a rapidly growing interest in mushrooms as food and suggests that there may be a market for additional mushroom species (Farr, 1983).

There has been an increase in the interest of mushrooms by the South African public. New books are regularly published on mushroom identification, growing mushrooms and cooking with mushrooms. An increased awareness of their nutritive and medicinal value by

the general public would help to increase consumption and further expand the opportunities for alternative mushrooms.

A "profile of fresh consumers" (Tomes, 1991) indicated that the top 4 factors in fresh produce selection are:

1. taste/flavour;
2. freshness/ripeness;
3. appearance/condition and
4. nutritional value.

Price was rated very important by 66% of consumers. At present speciality mushrooms are costly due to low levels of production. If consumers were informed why speciality mushroom cost so much, they may be more prepared to pay the higher prices.

Most mushrooms are sold fresh, which certainly adds to consumer appeal. Distance from market thus becomes an objective as the shorter the distance, the fresher the mushrooms can be delivered and the better they will look and taste.

Most retailers say that sampling and cooking demonstrations are the best way to introduce new food types to the consumer. Obviously then this would be the first step in an education-based mushroom merchandising campaign for westernised consumers.

Packaged mushrooms not only look good, but can also supply the consumer with information about the mushroom's nutrition, cooking ideas and recipes and the shelf-life of the mushroom. Packaging may also sell more mushrooms as the prices do not seem overwhelming when the units for sale are small.

The above discussion only really states what westernised consumers want and not how the smaller growers should deal with marketing their product.

Possible outlets are restaurants; wholesalers; supermarkets and individuals at a market. It is possible for smaller growers to undertake an effective promotion campaign, but they must first localize the target. A method of promotion would be advertising in newspapers and printing information leaflets. However, the community would have to be largely literate, so many rural communities would have to rely on information supplied by

word of mouth and by cooking demonstrations at local markets (Oei, 1991). Transport may be a problem and a number of small farmers would need to collaborate.

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Appendix 6.3

Program for the initial development of a Decision Support System for mushroom cultivation

```
* MUSHQ.PRG
* MUSHROOM QUESTIONS
* TO ASK THE QUESTIONS
  CLEAR
  IF VERDICT = '?'
  @ 20,3 SAY 'O to quit'
    IF MX[QUES]=' '
    @ 18,3 SAY 'Y = YES,  N = NO'
    ELSE
    @ 18,3 SAY MX[QUES]
    ENDIF
  CHOICE = ' '

  @ 16,5 SAY MQ[QUES] GET CHOICE PICTURE '@! X' VALID CHOICE$MQ[QUES]
  READ
    IF .NOT.CHOICE='O'
    STORE STUFF(MA,QUES,1,CHOICE) TO MA
    ENDIF
  ELSE
  @ 10,10 SAY 'THE VERDICT IS:'
  ? VERDICT
  STORE VERDICT TO VERD[MAIN]
  VERDICT = '?'
  @ 20,5 SAY 'Do you want to change an answer for re-decision (Y/N) ';
    GET CHANGE PICTURE '@! Y'
  READ
    IF CHANGE='N'
    CHOICE = 'O'
    ENDIF
  CLEAR
  K = 1
    DO WHILE CHANGE='Y'
    @ 3,1 SAY ' '
      DO WHILE LEN(NUMBERS[MAIN])>(3*K)-2
      M = VAL(SUBSTR(NUMBERS[MAIN],(3*K)-2,2))
        IF .NOT.SUBSTR(MA,M,1)='U'
        ? STR(M,2) + '. ' + MQ[M]    && + '    ' + SUBSTR(MA,M,1)
        ENDIF
      K = K+1
      ENDDO
    STORE 0 TO SUB
    @ 20,0 CLEAR
@ 20,3 SAY 'Select the question to re-answer (0=quit) ' GET SUB PICTURE '99' ;
VALID (STR(SUB,2,0,'0')$NUMBERS[MAIN].AND..NOT.SUBSTR(MA,SUB,1)='U').OR.SUB=0
    READ
      IF SUB = 0
      STORE 'N' TO CHANGE
      ELSE
      @ 22,3 SAY 'O to quit'
       IF MX[SUB]=' '
       @ 21,3 SAY 'Y = YES,  N = NO'
       ELSE
       @ 21,3 SAY MX[SUB]
       ENDIF
```

Appendix 6.3

```
* MUSHINI.PRG
* MICHAEL RELIHAN
* The initial values of the following arrays are assigned below
* MQ : the question structure
* MO : the answer option structure
* MX : the structure for explanation of the options
***
MO  = 'YNO'                    && DEFAULT ANSWER OPTIONS
Mx  =                          && EXPLANATION STRUCTURE SET TO SPACE
XTRA = 'OO'                    && EXTRA QUESTION NUMBERS OUTSIDE RANGE
* WHICH TECHNOLOGY TO APPLY
START[1] = 1
END[1] = 6
MQ[1]  = 'How much raw materials & water can you access ?                '
MO[1]  = 'ABCO'
MX[1]  = 'A = large amount, B = moderate amount, C = little or none'
MQ[2]  = 'Can you get contained environments & pasteur. units ? '
MQ[3]  = 'Can you get a basic structure & a pasteur. tank ?      '
MQ[4]  = 'What are your climatic conditions ?                    '
MX[4]  = 'A = moist, B = dry'
MO[4]  = 'ABO'
MQ[5]  = 'Are you likely to have very hot weather ?               '
MQ[6]  = 'Can you afford a basic structure & plastic sheeting ? '
* BUY OR PRODUCE SPAWN
START[2] = 7
END[2] = 9
MQ[7]  = 'Can you afford to set up a laboratory ?                '
MQ[8]  = 'Can you afford to employ people to produce spawn ?     '
MQ[9]  = 'Do you have the equipment to produce spawn ?           '
* WHICH MUSHROOMS TO GROW
START[3] = 10
END[3] = 14
MQ[10] = 'Which technology do you plan to use ?
MX[10] = 'A = Contained environment, B = Mushroom house, C = Outdoors'
MO[10] = 'ABCO
MQ[11] =  What are your environmental conditions ?               '
MX[11] = 'A = hot & dry, B = hot & wet, C = cool & dry, D = cool & wet'
MO[11] = 'ABCDO'
MQ[12] = 'Do you have unlimited access to water ?                '
MQ[13] = 'Are your temperatures: days=28-35 C, nights >16 C ?    '
MQ[14] = 'Are your temperatures: days=16-25 C, nights >12 C ?    '
* ALLOCATING QUESTION NUMBERS PER CATOGARY
STORE '*' TO NUMBERS
STORE 1 TO K, MAIN
  DO WHILE (K <= N).AND.(MAIN<4)
    IF (K>=START[MAIN].AND.K<=END[MAIN]).OR.(STR(K,2)$XTRA[MAIN])
    STORE NUMBERS[MAIN] + ',' + STR(K,2,0,'0') TO NUMBERS[MAIN]
    ENDIF
    IF K = N
    STORE STUFF(NUMBERS[MAIN],1,2,'') TO NUMBERS[MAIN]
    MAIN = MAIN+1
    K = 0
    ENDIF
  K = K + 1
  ENDDO
STORE 1 TO MAIN
```

Appendix 6.3

```
* MUSH.PRG
* MICHAEL RELIHAN
*************************************************************************
SET ECHO OFF
SET TALK OFF
SET STATUS OFF
SET HEADING OFF
CLEAR
SET BELL OFF
SET CONFIRM OFF
SET EXACT OFF
SET DELETED ON
SET SAFETY OFF
SET DOHISTORY OFF
*SET ESCAPE OFF
SET DATE BRITISH
SET TYPEAHEAD TO 4
*SET DEFAULT TO C:
*RUN C:
*RUN CD\TEMP
*SET PATH TO C:\DBXL
*************************************************************************
@ 10,10 SAY 'Initialising ...'
STORE 14 TO N   &&   The number of questions
DIMENSION MQ[N], MO[N], MX[N]
DIMENSION VERD[3], START[3], END[3], NUMBERS[3], XTRA[3]
STORE REPLICATE('U',N) TO MA
STORE 1 TO MAIN, SUB, QUES
STORE ' ' TO CHOICE, VERD
STORE 'N' TO CHANGE
STORE '?' TO VERDICT
DO MUSHINI
*SET PROCEDURE TO MUSH
  DO WHILE .T.
  CLEAR
  @ 4,1 SAY ' '
  TEXT
        0. Quit the program.
        1. Choose the technology to apply.
        2. Choose between buying and producing spawn.
        3. Choose which mushrooms to grow.
        4. Clear previous answers
  ENDTEXT
  @ 4,3 TO 12,65 DOUBLE
    @ 11,6 SAY 'Enter your selection (0-4) ' GET MAIN PICTURE '9' RANGE 0,4
  READ
  CHOICE=' '
  CLEAR
    DO CASE
*-------------------------
   CASE MAIN=0
     QUIT
*-------------------------
   CASE MAIN=1
```

Appendix 6.3

```
    DO WHILE .T.
      IF CHOICE='0'
      EXIT
      ENDIF
      DO CASE
     CASE LEFT(MA,1)='U'
      QUES=1
     CASE LEFT(MA,1)='C'
      VERDICT = 'Do not attempt to grow mushrooms'
     CASE LEFT(MA,2)='AU'
      QUES=2
     CASE LEFT(MA,2)='AY'
      VERDICT = 'Production is not limited'
     CASE LEFT(MA,3)='ANU'
      QUES=3
     CASE LEFT(MA,3)='ANY'
      VERDICT = 'Large scale production is possible'
     CASE (LEFT(MA,4)='ANNU').OR.(STUFF(MA,2,2,'')='BU')
      QUES=4
     CASE (LEFT(MA,5)='ANNAU').OR.(LEFT(STUFF(MA,2,2,''),3)='BAU')
      QUES=5
     CASE LEFT(MA,5)='ANNAN'
      VERDICT = 'Outdoor production is possible'
     CASE (LEFT(MA,6)='ANNAYU').OR.(LEFT(STUFF(MA,2,2,''),4)='BAYU');
      .OR.(LEFT(STUFF(MA,5,1,''),5)='ANNBU')
      QUES=6
     CASE (LEFT(MA,6)='ANNAYY').OR.(LEFT(STUFF(MA,2,2,''),4)='BAYY');
      .OR.(LEFT(STUFF(MA,5,1,''),5)='ANNBY')
      VERDICT = 'Limited production is possible'
     CASE (LEFT(MA,6)='ANNAYN').OR.(LEFT(STUFF(MA,5,1,''),5)= ANNBN')
      VERDICT = 'Do not attempt to grow mushrooms'
     CASE (LEFT(MA,1)='B').AND.(SUBSTR(MA,4,1)='B'.OR.'N'$SUBSTR(MA,5,2))
      VERDICT = 'Do not attempt to grow mushrooms'
     OTHERWISE
     ? MA + '    QUES = ' + STR(QUES,3)
     WAIT
     ENDCASE
    DO MUSHQ
    ENDDO
*-------------------------
   CASE MAIN=2
     DO WHILE .T.
       IF CHOICE='0'
       EXIT
       ENDIF
       DO CASE
      CASE SUBSTR(MA,7,1)='U'
       QUES=7
      CASE SUBSTR(MA,7,1)='N'.OR.SUBSTR(MA,7,2)='YN'.OR.SUBSTR(MA,7,3)='YYN'
       VERDICT = 'Buying of spawn is recommended'
      CASE SUBSTR(MA,7,2)='YU'
       QUES=8
      CASE SUBSTR(MA,7,3)='YYU'
       QUES=9
```

Appendix 6.3

```
      CASE SUBSTR(MA,7,3)='YYY'
       VERDICT = 'Spawn production should be feasible'
      ENDCASE
     DO MUSHQ
     ENDDO
*-------------------------
   CASE MAIN=3
     DO WHILE .T.
       IF CHOICE='0'
       EXIT
       ENDIF
       DO CASE
      CASE SUBSTR(MA,10,1)='U'
       QUES=10
      CASE SUBSTR(MA,10,1)='A'
       VERDICT = 'Recommend Volvariella sp. in summer & ';
       + 'Stropharia sp. in winter.' + chr(13)+chr(10) + 'Both can be grown.'
      CASE SUBSTR(MA,10,2)$'BU,CU'
       QUES=11
      CASE SUBSTR(MA,10,3)$'BAU,BCU'
       QUES=12
      CASE SUBSTR(STUFF(MA,12,1,''),10,3)='BBU'
       QUES=13
      CASE SUBSTR(STUFF(MA,12,2,''),10,3)$'BDU,CDU'
       QUES=14
   CASE SUBSTR(MA,10,2)$'CA,CB,CC'.OR.SUBSTR(STUFF(MA,12,2,''),10,3)$'BDN,CDN'
       VERDICT = 'Conditions are unsuitable for any mushroom production'
      CASE SUBSTR(MA,10,3)$'BAN,BCN'.OR.SUBSTR(STUFF(MA,12,1,''),10,3)='BBN'
       VERDICT = 'Conditions are unsuitable for any mushroom production'
      CASE SUBSTR(MA,10,3)='BAY'.OR.SUBSTR(STUFF(MA,12,1,''),10,3)='BBY'
       VERDICT = 'Volvariella volvacea can be grown'
      CASE SUBSTR(STUFF(MA,12,2,''),10,3)$'BDY,CDY'
       VERDICT = 'Stropharia rugoso-annulata can be grown'
      ENDCASE
     DO MUSHQ
     ENDDO
   CASE MAIN=4
   STORE REPLICATE('U',N) TO MA
   ENDCASE
  ENDDO
RETURN
```

CHAPTER 7:

THE FUTURE DIRECTION OF RESEARCH AND DEVELOPMENT OF EXOTIC MUSHROOM PRODUCTION IN KWAZULU-NATAL

Mushroom production can assist in alleviating protein shortages in some communities, but a number of problems have to be solved before mushroom production can fulfil its potential (**Table 7**). Each situation should be critically assessed before mushroom production is initiated.

The predominant constraint on mushroom production identified in this project was the occurrence of contaminants at the spawn stage. The contamination of the spawn consistenly impacted on the results obtained in the trials. This is an area that would need to be investigated before small-holder and subsistence production could become feasible, as it is unlikely that spawn production would be possible by these farmers.

However, collaboration between commercial spawn producers and small-holders and subsistence farmers could solve the problem of contaminated spawn. Commercial farmers would be able to produce contaminant-free spawn as they could afford to establish spawn laboratories, and they could supply the small-holders and subsistence farmers with spawn.

The availability of substrates is not considered to be a constraint to mushroom production, as many agricultural wastes are potential mushroom substrates. As crop residues are considered to be waste materials, they would be readily available for mushroom production.

The geographic distribution of small-holder and subsistence mushroom farmers would be determined by climatic conditions, in particular, temperature range. The distribution of commercial farmers would not be affected in this way, if they used temperature controlled chambers.

Water-availability would be a determining factor for mushroom production. Mushroom production can require a considerable amount of water, and if this is not readily available, mushroom production would not be feasible.

Table 7 Systems Analysis of Exotic Mushroom Production in KwaZulu-Natal: potential constraints and suggested solutions

Processes and Factors in Mushroom Production	Constraints	Solution
Cultures	acquiring cultures	from a type collection
Spawn	producing or acquiring uncontaminated spawn	establish a laboratory or buy from a commercial producer
Production Systems	identifying which system is suitable for given conditions	consider capital, climate and technological ability
Correct Environment	ideal environments are limited	use contained environments, or areas of optimal climate
Water availability	mushroom production requires considerable amounts of water	use supplied water, bore-hole water or collect rain and fog
Substrate Materials	availability of materials	most agricultural wastes are readily available
Capital	availability	farmer's associations and Aid schemes
Production Technology	is the technology appropriate to the farmer?	can use education and extension to introduce mushroom technology
Transporting the crop	transportation not always readily available	smaller farmers can co-operate
Markets	is there a market for the product?	market research needs to be undertaken
Price	will the price of the product at market cover production costs?	this will be determined by market research and consumer awareness

Appropriate technology is an important aspect of introducing mushroom production to small-holders and subsistence farmers. Appropriate technologies:

* are labour intensive
* have comparatively light capital costs
* are understood, used and maintained locally
* are compatible with local social conditions
* make optimum use of locally available resources

(Committee on Science and Technology, 1978).

Appropriate technology is important for the acceptance by communities of mushroom production as a viable option and the successful implementation of the process.

Mushroom production could be introduced to small-holders and subsistence farmers by demonstration trials run at research farms and also by farmer's associations. Farmers could get hands-on experience, without the risks involved of crop failure, before attempting production themselves.

Mushroom production does not rely on many external inputs. Most of the materials and resources required can be obtained as a result of agricultural activities carried out in the community. These materials and resources include crop residues, animal manures, labour and water.

Increased mushroom production can be obtained by adjustment of the immediate environment, and does not require the use pesticides or chemical fertilizers. Thus, mushroom production does not threaten to harm the environment.

Mushroom production does not erode the fundamental basis of farming communities as it poses no threat to the family unit. It can help to solve the problem of malnutrition and in certain cases, may add to the income of households without corruption of values.

Based on the research done in this project on the cultivation of edible mushrooms, it is suggested that mushroom production be introduced into developing-agriculture as an alternative protein and vitamin source, and possibly as a cash crop.

The mushroom species considered were:

Stropharia, *Volvariella* and *Termitomyces*.

Stropharia can be grown on most agricultural wastes and does not require a high level of technological input. The production of *Volvariella* is very simple, and mushrooms can be grown in newly harvested fields (Li, 1989) and in orchards (Hua, 1991). This factor would help to alleviate the demand for arable land. Unfortunately, *Volvariella* production does require a large amount of water. *Volvariella* is a tropical mushroom and will therefore flourish in the sub-tropical zones of KwaZulu-Natal.

The cultivation of *Termitomyces* is still in its infancy, due to the symbiotic relationship between the fungus and termites. Once the link between them has been elucidated, commercial production may become a viable enterprise. Presently, *Termitomyces* are collected from the wild during the fruiting season by people in all areas where they occur, both in Africa and in India. The introduction of *Termitomyces* as a crop would be easier than for *S. rugoso-annulata* and *V. volvacea*, as the fungus is already familiar to those who would participate in its production.

Mushroom taxonomy, physiology and culture are still open for further explorations and the success of mushroom production depends on applied research. More research into production in African countries is needed as the environmental conditions differ from those in Eastern and Far-Eastern countries, where most of the research is now taking place. The breeding of strains capable of surviving African conditions is an important aspect of this research.

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