PESTS, PATHOGENS, COMPETITORS AND WEED FUNGI OF CULTIVATED OYSTER MUSHROOMS (*PLEUROTUS* SPP) IN SOUTH AFRICA

by

GERTINA WILHELMINA DU PLOOY
B Sc (Hons) Pretoria

Submitted in partial fulfilment of the requirements for the degree

MAGISTER SCIENTIAE

in the Faculty of Biological and Agricultural Science
(Department of Botany)

University of Pretoria

March 2000

Supervisor: Prof Albert Eicker

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ABSTRACT

Commercial production of the oyster mushroom, *Pleurotus* spp., is barely ten years old in South Africa. Although the local industry is expanding, progress is not taking place at a satisfactory rate. Regular difficulties that are encountered put the growers into discouraging financial situations. Furthermore they rely mainly on information from abroad for the control of the pests and diseases of their crop.

This study investigated some of the problems confronting the South African cultivators. Samples were collected from farms in Gauteng and the Western Cape during various stages of production. A distinction was made between weed fungi and competitive fungi based on the incidence and severity of the infection. The presence of invertebrate pests was recorded as well. A correlation between meteorological factors and infective episodes was established.

An infective episode in the substrate often reflects the general farming hygiene. The pests and diseases of mushroom cultivation can, however, be controlled with good planning and careful management and certain recommendations are made in this regard.

OPSOMMING

Die kommersiële verbouing in Suid Afrika van die oestersampioen, *Pleurotus* spp., is skaars tien jaar oud. Alhoewel die plaaslike industrie uitbrei, is die groeitempo daarvan nie na wense nie. Kwekers ondervind gereeld probleme wat hulle in ontmoedigende finansiële posisies plaas. Verder maak hulle hoofsaaklik op inligting vanaf die buiteland staat vir oplossings rondom die beheer van plaagdiere en siektes van hul gewas.

Hierdie studie het ondersoek ingestel na sommige van die probleme waarmee die kwekers in Suid Afrika gekonfronteer word. Monsters is tydens verskillende stadiums van die verbouingsproses versamel op plase in Gauteng en die Wes-Kaap. Daar word onderskei tussen onkruid fungi en kompeterende fungi op grond van die voorkoms en erns van die infeksies, terwyl die teenwoordigheid van invertebrate plaagdiere ook aangeteken is. Daar is bevind dat 'n korrelasie bestaan tussen die meteorologiese faktore en die infektiewe episodes.

'n Infektiewe episode in die substraat is dikwels 'n aanduiding van die algemene boerdery higiëne. Die plaagdiere en siektes van oestersampioenverbouing kan egter beheer word met goeie beplanning en versigtige bestuur en sekere aanbevelings in hierdie verband word gemaak.

ACKNOWLEDGEMENTS

A sincerest "thank you" is extended to the following people without whom this project could never have succeeded:

- Prof Albert Eicker, for his tolerance and encouragement, and for leading me to an appreciation of fungi.
- The intrepid oyster mushroom growers, for allowing me to glimpse into their mushroom worlds. Without their co-operation and assistance none of this was possible.
- My family, because their love, endurance and most of all, unwavering support kept me going.
- Mr Chris van der Merwe, for patiently coaching me in the use of the electron microscopes and paying attention to the small things in my life.
- Ms Magda Nel, for safekeeping my isolates and for always being a friend.
- Ms Elfrieda Meyburgh, for her invaluable input with the literature acquisition.

CHAPTER 1

INTRODUCTION

Mushrooms have always been an important part of the human existence. When the approximately 5300-year-old frozen body of a Late Neolithic shepherd (named "Ötzi") was discovered high in the Tyrolean Alps in 1991, investigation of his knapsack revealed, amongst his equipment and weapons, two types of mushrooms (Jaroff, 1993). They were birch polypores (*Piptoporus betulinus* (Bull. Ex Fr) Karst.) and another as yet unidentified mushroom. The polypores have proven antibiotic qualities and the somewhat arthritic, whipworm infested man (http://www.gla.ac.uk/Acad/IBLS/DEEB/jd.htm) probably carried it along for medicinal purposes. Mushrooms have since become desirable for culinary as well as medicinal purposes (Stamets, 1993).

Different cultures have vastly different tastes for food. The white button mushroom (*Agaricus bisporus* (Lange) Imbach) is the most popularised edible fungus in the West. However, other cultures have many other mushrooms on their menu. As the world has become smaller in terms of travel, various cultures have learnt to appreciate and even indulge in each other's culinary delights. In recent years, what can be described as a mycophilic awakening, has gained momentum. This is confirmed by the fact that the cultivation of what is known as "speciality mushrooms" (Royse 1995) is drawing so much attention from Western cultivators. So it is that South Africa has not been completely left behind in this mycophilic awakening. Asian, Eastern and African preferences have greatly influenced our own experiences. This has lead to the local commercial cultivation of several species of the oyster mushroom, *Pleurotus* spp. (McVeigh 1993), and appears as if it could become a lucrative venture for South African mushroom farmers (Van Tonder 1995). The market has great potential and some cultivators from abroad regard *Pleurotus* as one of the easiest, cheapest and "quickest-to-the-dinner-table" mushrooms to cultivate (Northwest Mycological Consultants, unknown; Chang and Miles, 1989, (http://www.keil.ukans.edu/~fungi/).

Pleurotus spp. occurs naturally on wood where it degrades lignin as well as cellulose. This characteristic has enabled utilisation of several (often very cheap) substrates in the cultivation of these mushrooms (Poppe & Hofte 1995, Azizi *et al*, 1990). The substrate of choice for the South African *Pleurotus*-cultivator is wheat straw. This is obtainable throughout the year, since South African wheat is cultivated in both winter and summer rainfall regions. Studies have been undertaken to establish alternative, possibly indigenous substrates (Pakela, 1997). Commercial cultivating techniques are refined to an art by each individual cultivator. The basic procedures used in South African cultivation are summarised in Figure 1.

What appears to be a relatively uncomplicated produce when compared with *Agaricus*-production, however, is proving to have problems that are quite capable of devastating even seasoned cultivators. Some of the most often encountered difficulties include severe infection of the growth columns or tubes by other fungi, attack by insects and poor yields. Infections also rarely start as independent occurrences, but are often suspected to be the visible symptoms of previous errors in the farming practice itself. Exceptions will be possible whenever a coincidental vector can be indicated as the cause of the infection. In practice the solutions to these problems are not always as apparent as indicated by popular literature. The most obvious aspect to consider is the fact that the South African cultivator has to work in a climate that is not ideally suited to grow speciality mushrooms. The creation of artificial conditions does not always give the desired results and infection of the substrate sometimes leads to complete loss of the crop.

The objective of this study was to determine the most prevalent infective organisms found on the prepared substrate used for *Pleurotus* production in South Africa. A distinction is made between the occasional fungal invaders (so-called weed fungi) and fungi that actively compete for the same resources as the mushrooms (true competitors). This paper reports on the species of fungi, insects and nematodes found on the substrate. Furthermore, meteorological conditions and seasonal changes in meteorological conditions are investigated to establish whether they play any part in the occurrence of infective episodes. An attempt will be made to correlate climatic conditions, infective episodes, farming practice and farming hygiene.

CHAPTER 2

LITERATURE REVIEW

2.1 General information

Pleurotus (Fries) Kummer are popularly known as oyster mushrooms. They are Basidiomycetes and regarded as a so-called speciality mushrooms by mushroom growers. This distinction has been made of a varied group of mushrooms on grounds of the general availability (Sharma 1997a) and level of commercialisation (Royse 1995, Stamets 1993). Other members include *Lentinus edodes* (Berk.) Sing. (shiitake), *Volvariella volvacea* (Bull. ex Fr.) Sing. (straw mushroom), *Flammulina velutipes* Sing. (winter mushroom), *Pholiota nameko* (T. Ito) S. Ito ("nameko" or viscid mushroom) and *Tremella fuciformis* Berk. (jelly fungus or silver ear) (Royse 1995, Chang & Miles 1989).

In the natural environment *Pleurotus* spp are lignocellulosic fungi occurring on tree trunks in subtropical and temperate forests and causing white rot of wood. This name stems from the appearance of the wood after the degradation of the lignin and cellulose from the cell walls in wood (Worrel *et al* 1997). Fruit bodies of *Pleurotus* species have more or less one-sided fleshy caps with decurrent gills. They are slightly funnel-shaped and usually excentrically stalked, growing in clusters (Watling & Gregory 1989). Their growth habit is exploited and optimised by presenting it with an artificial environment (substrate- filled columns or tubes) resembling the log, but with increased nutrient levels (Stamets 1993).

Wood is a good source of carbon compounds for those organisms able to access these compounds, but it is poor in other nutrients such as nitrogen and phosphorous. Digestion of lignin provides nitrogen and the use of alternative substrates influence the amount of nutrients available to the fungi that decompose wood (Carlile & Watkinson 1994). It was also pointed out by Rayner and Boddy (1987) that the activity and growth rate of fungi in wood is determined by the amount of gaseous O_2 available to them. The

presence of O_2 is a reciprocal function of the amount of water present in the wood. Fibrous artificial mushroom substrates like straw is more loosely packed, allowing faster rates of gas exchange compared to wood fibre. An artificial culture environment has higher moisture content (Sinden 1946) as well as larger air spaces. This allows a larger gaseous phase within which larger volumes of O_2 and later CO_2 can accumulate, all of which is conducive to accelerated hyphal growth. The advantages of the increased gas concentrations are decreased fruition time and increased fruition levels (Rainey & Cole 1987, Royse *et al.* 1982). Vertically propped growth containers allow for the natural growth habit, but dries out more readily. *Pleurotus* growers limit this desiccation by shredding the straw and adding lime and gypsum to improve the water holding capacity of the substrate, stabilise the pH and obtain denser substrate (Van Griensven 1988). It has been found that there is a 3-way correlation between the crop yield, the size of the fruiting bodies and the pore size of the substrate (Rainey *et al.* 1987).

New species in this genus are still being discovered (Peterson & Hughes 1997; Reid *et al* 1997) but not all *Pleurotus* species are useful as commercial crops. Their brittle sporophores, excessive sporulation and rather unusual taste render some of them unpopular as consumer mushrooms (Chang & Miles, 1989). In recent years various aspects of *Pleurotus* cultivation have received a lot attention, mostly with the commercial development of the fungus in mind. However, some of the investigations are not aimed at the food basket of the consumer. The suitability of *Pleurotus* in medicine, bioconversion, bioremediation and biopulping is being investigated as well (Carlile & Watkinson 1994).

2.2 Motivation for the terminology used in this study.

As any cultivated crop, mushrooms suffer attack by other organisms. When this attack is launched by other (non-pathogenic) fungi they have been called antagonists (Baker & Cook in Fletcher 1987), indicator fungi (Harvey *et al.* 1982), weeds and competitors. Hayes & Nair (1975) referred to the group of non-pathogenic fungal fungi able to establish themselves at the expense of the crop mycelium as "competitors". According to them, a competitive fungus can either outgrow the crop mycelium, or gets an advantage by inhibition through the formation of secondary metabolites (antagonism).

Fletcher (1987) suggested the use of the word "weed" to describe fungi that are occasional or opportunistic visitors to the mushroom substrate, but do not antagonise the crop mycelium or play any role as pathogens. They are distinct from fungi that participate in the maturing process of the substrate and are also not related to specific undesirable conditions in the substrate. Both weed fungi and competitors can be indicator fungi (Rinker 1993), though not all of them can be used for this purpose (Van Griensven 1988).

Pests are regarded as substrate fauna - organisms belonging to the animal kingdom and that occur in mushroom substrate (Fletcher *et al.* 1989, Hayes & Nair 1975).

2.3 A brief look at investigations into *Agaricus* production

Much of what is known about commercial mushroom cultivation is based on knowledge gained through the production of *Agaricus* spp. The industry, the economics and the progress surrounding the cultivation of *Agaricus bisporus* are extensively documented (Vijay *et al.* 1997, Oei 1996). Detailed information on the substrate and its preparation, the relationships and interactions between the cultivated mushroom and its disorders, the physiology of *Agaricus* species, sanitation and farm management is available to all *Agaricus* growers (Rinker 1993, Wang & Wang 1993, Fletcher *et al.* 1989, Van Griensven 1988, Wuest & Bengtson 1982, Sinden 1971).

The principles underlying *Agaricus* information, however, can be applied to the commercial production of many other mushrooms as well. According to this prior knowledge we understand that the expression of disorders in mushroom crop depends upon 2 major factors (Stamets, 1993):

1. The stage of development of the crop.
2. The cause of the disorder.

Oei (1996), Eicker & Van Greuning (1991), Fletcher *et al.* (1989), Geels *et al.* (1988), Eicker (1980) and Vedder (1978) have provided comprehensive summaries of crop

disorders in *Agaricus* production. These include mycological, entomological and nematological studies as well as studies on the prevention and control of crop disorders. Chang and Miles (1989), and Stamets and Chilton (1983) have discussed various disorders in the production of *Agaricus* spp. as well as other speciality mushrooms.

2.4 Investigations into the cultivation and biology of *Pleurotus*-species

2.4.1 Suitability of a strain and substrate

The cultivation of *Pleurotus* spp. is not such an old and well established commercial enterprise as *Agaricus* production. Consequently, the competitors and other pests, the associations and interactions that exist, the possibility of indicator species and the ecology of the wheat straw substrate are not yet as well documented as for *Agaricus* cultivation (Lanzi 1986). However, Balakrishnan & Nair (1997) pointed out that the last decade has been one of intense worldwide development in *Pleurotus* commercialisation. Researchers have experimented with many strains and aspects of *Pleurotus* cultivation.

There is considerable variation in the biological and physiological characteristics of *Pleurotus* species (Vilgalys 1997). The selection of a suitable strain for every environment where *Pleurotus* mushrooms are cultivated is based on temperature, light and nutrient requirements (Hauser 1986). The suitability of each one of these strains is determined by the meteorological factors of the area, the availability of the substrate used in the specific technique and the actual technique itself (Oei 1996). Furthermore their appearance and taste is also considered when the grower finally makes a choice on the fungal strain (Stamets 1993, Chang & Miles 1989, Van Gils *et al.* 1985).

Each agricultural environment produces waste with varying possibilities of bioconversion (and therefore substrate) potential (Bisaria *et al.* 1997). This means that the substrates on which *Pleurotus* species can be cultivated are as varied as the species and strains. Poppe & Hofte (1995) combined the efforts of many researchers over as many years and compiled a comprehensive report on the results of 30 edible fungi grown on 48 different agro-wastes. They tested seven *Pleurotus* species and one hybrid with all

eight giving promising results. Several agro-residues suitable for use as a substrate are created in the South African agricultural environment (Pakela 1997), but the substrate most widely used at the moment is wheat straw (Eicker 1995). This is readily available with both winter and summer wheat strains being cultivated. The influence of cultivar variability on the mushroom crop is receiving attention (Labuschagne, 2000).

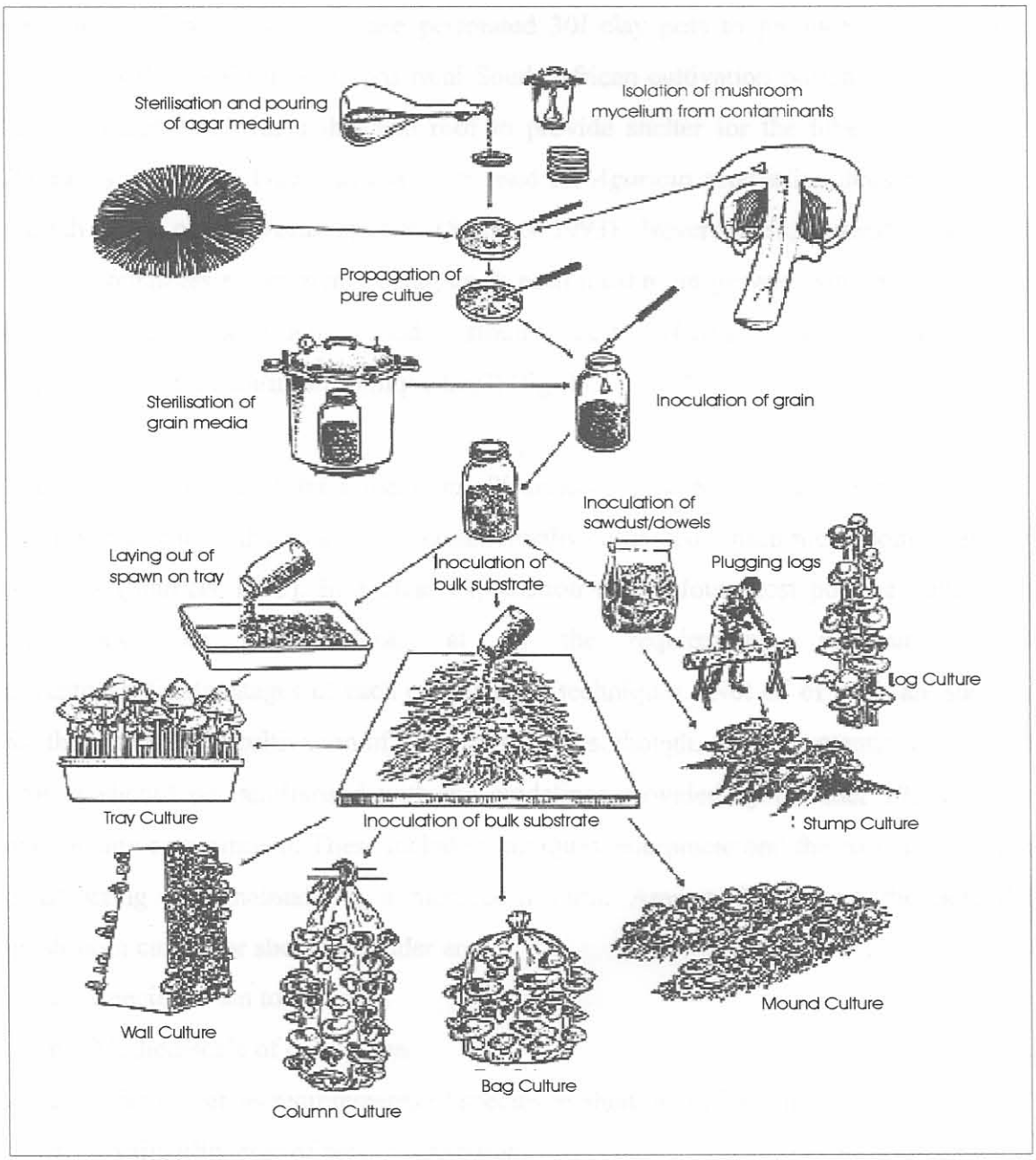

Figure 1 An overview mushroom tissue culture and cultivation.
(Courtesy of Paul Stamets, Fungi Perfecti Online 1995)

2.4.2 Cultivation Techniques

Several techniques are being used in *Pleurotus* cultivation. Suzuki and Mizuno (1997) and Date (1997) reported on the Japanese method of log cultivation of *P. sajor-caju* (Fr.) Sing. and *P. cornucopiae* Roll. respectively. This is the traditional and slowest cultivating method. It requires high humidity, moderate temperatures and good air circulation. Nigerian growers use perforated 30*l* clay pots to produce a crop (Abate 1995). Pakela (1997) investigated rural South African cultivation possibilities and used 2m deep trenches with a thatched roof to provide shelter for the tube cultivation of *Pleurotus ostreatus*. Trays such as those used for *Agaricus* production does not suit the growth habit of *Pleurotus* species (Stamets 1993). Nevertheless, several formats of growth containers are currently being used; each mushroom grower using what suits the environmental conditions and strain best (Chang & Miles 1989, http://www.fungi.com/info/technique.html) (fig. 1).

The substrate of the oyster mushroom, *Pleurotus* spp, needs far less complicated and lengthy preparation than that of the commercially cultivated button mushroom, *Agaricus bisporus* (Stamets 1993). In a clear explanation of the four most popular cultivation techniques, Oei (1996) looks at all the requirements, preparations and advantages/disadvantages of each one of these techniques. Not all of them are suitable for the commercial cultivation of *Pleurotus* species, though, and the potential mushroom grower should be familiarised with the guidelines provided by (Vedder 1978) before making any commitment. These include techniques, equipment and the costs involved in constructing and maintaining a mushroom farm. Among the factors the potential mushroom cultivator should consider are:

a. Species/strain to cultivate
b. Method/scale of cultivation
c. Climate versus requirements of species/method of cultivation
d. Availability/cost of proper substrates
e. Land prices and availability
f. Access to unchlorinated water/feasibility of water treatment
g. Demand for mushrooms in your area
h. Practicality of distribution outside your area

i. Cost of labour
j. Cost of equipment installation (electrical wiring, laboratory and growth room construction /assembly, air system installation, etc.)
k. Method of financing the start-up of your operation

Current *Pleurotus* cultivators in South Africa encounter problems in any combination of the aspects mentioned in the list above. Each farm operation runs according to the individual business plan of the owner, but the basic principles for eventually harvesting a crop stay the same. It can be summarised as follows (Eicker 1995) (in no order of importance):

- Preparation and maintenance of suitable housing for the growth system
- Using a manageable and suitable growth system
- Regular acquisition and monitoring of suitable substrate
- Carefully monitored supplementation
- Stringent hygiene control

2.4.3 Pests and diseases and their control in the cultivation of *Pleurotus* species

South African cultivators of the oyster mushroom, *Pleurotus* spp., have to rely on European, North American and Indo-Asian information on the pests and diseases of their crop (Oei 1996, Rinker 1993, Hauser 1986, Kurtzman & Zadrazil 1982, Wuest 1982, Jandaik 1977, Kalberer 1974). Many of the pests and diseases are capable of devastating the mycelium or destroying the mushroom crop in a single outbreak or episode (http://pestdata.ncsu.edu/CropProfiles/Detail.CFM?FactSheets__RecordID=43). With huge investments in terms of time, energy and money being made, the grower has to prevent this from happening using all means available. Prevention is certainly cheaper than cure and proper sanitation, good farming practice and hygiene must be exercised (Fletcher *et al.* 1986, Stamets & Chilton 1983).

Several authors has pointed out that hygiene is the single most important factor in controlling pests on the growth container (Oei 1996, Rinker 1993, Van Griensven 1988, Clift 1987, Wetzel 1982). Six modes of transferring pathogens to the spawn have been identified (Stamets, 1993), namely:

1. The cultivator.
2. The air.
3. The media or substrate to be inoculated.
4. The tools and other equipment.
5. The inoculum, i.e. spawn material contaminated already before seeding of substrate.
6. Mobile contamination units (MCU's), i.e. vectors such as arthropods, small mammals or humans other than the cultivator.

Oei (1996) identifies three stages in the production cycle, namely sterilization, inoculation and incubation. He goes on to discuss the role that vectors play in contamination during each stage of the process and explains the role of the six transfer modes, as identified by Stamets, in each one of these stages.

By meticulous securing of the cultivation environment many vector problems can be eliminated (Vedder 1978). On large-scale farms enough capital investment can even bring about computerised monitoring (Lamber 1991). It is the small enterprise that has less financial buffering capacity that suffers most when prevention fails. Control measures can be physical, chemical or biological (http://www.agris.be/nl/).

2.4.3.1 Chemical control

Commercial growers are driven by market related forces and prefer to look at chemical control measures to provide quick and cost-efficient solutions to their pest and disease problems (Oei 1996, Hoffman *et al.* 1987, Poppe *et al.* 1985, Eicker 1984, Gandy 1981, Declaire 1978). Some of the most widely used pesticides applied to crops in the fruit and vegetable industry are based on organophosphate and carbofuran chemical structures. However, pests are becoming increasingly resistant against these chemicals. There is also growing concern about human health implications and the changing attitudes towards the environment (http://babelfish.altavista.com/cgi-bin/translate). Due to this fact, less persistent and toxic control measures such as biological control, are gaining popularity (White 1982, Stamets 1993). According to Haugen (e-mail: 1999) no pesticides have been registered specifically for use in oyster mushrooms cultivation in USA.

The problem of resistance to pesticides was quantified and comprehensively addressed by Georghiou (1986). He pointed out that up to 1984 there was 447 insect and acaride species, with 97% of agricultural or veterinary importance, which have become resistant to one or more pesticide, also called zenobiotics. Furthermore at least 100 pathogenic fungi have become resistant against fungicides, benomyl giving the worst results. The new generation of pesticides based on pyrethroids are not looking any better. Although their much shorter persistence in the environment makes them more acceptable, overexploitation of their predecessor DDT has ensured pest resistance against it. The metabolic pathways in for both types of pesticides are very similar and the gene coding for DDT resistance is apparently providing protection against pyrethroids already. Furthermore, organochlorine pesticides (like DDT) continue to impair avian reproduction, years after most organochlorine pesticide use was discontinued. Because synthetic pyrethroids have a mode of action similar to the organochlorines, they are likely to have similar effects (http://www.epa.gov/spdpublc/mbr/1997airc/108dowell.pdf).

Whilst the pests are gaining resistance, human health is damaged by many of the pesticides (Colborn *et al.* 1993). Many chemical pesticides may no longer be applied on crops in Northern America primarily due to their detrimental effects on human health (table 1), but environmental damage is also drawing more and more attention. There is a fungicide being used effectively under South African conditions, thiabendazole (Eicker, 1984) which is apparently safe for use near humans as well as other organisms (http://www.pesticide.org/factsheets.html#pesticides).

Cyromazine (White 1989, Hoffman *et al.* 1987) is an insect growth regulator (IGR) employed by mushroom growers. It inhibits the maturation processes of the larval stage in sciarid and phorid flies with no danger to humans (http://www.agris.be/nl/). The range of pesticides registered for use by South African growers of mushrooms is limited (Eicker 1984). Using substances not registered for the specific crop is not always beneficial. Van der Hoven *et al.* (1988) found that 5 acaricides registered for use on other vegetables actually reduced the mushroom crop yield.

NAME OF PESTICIDE	Regis'd for mushrooms	LEVEL	USE
1. Aldicarb: TEMIK		2, 4	Acaricide, car
2. Carbaryl: SEVIN		1, 2	Insecticide, car
3. Chlorpyrifos: DURSBAN	yes	2, 3	Insecticide, op
4. Chlorothalonil: DACONIL, BRAVO	yes	1, 3	Fungicide, op
5. Cypermethrin : CYNOFF		1, 2, 3	Insecticide, pyr
6. DDT		1, 2, 4	Insecticide, op
7. Diazinon	yes	1, 2, 3, 4	Insecticide, op
8. Dichlorvos: VAPONA	yes	1, 2, 3, 4	Insecticide, op
9. Dimethoate: ROGOR		1, 2	Gen Pesticide, op
10. Endosulfan		2, 4	Insects, acarids: o-Cl
11. Lindane	yes	1, 2	Acarids, insects: o-Cl
12. Malathion:MALATHION, MERCAPTHTHION	yes	1, 2, 3	Insecticide, op
13. Methyl bromide		3, 4	Gen fumigant
14. Prochloraz	yes	1, 2, 4	Fungicide, ia
15. Permethrin: AMBUSH	yes	1, 3	Insecticide, pyr
16. Propoxur: BAYGON		1, 4	Insecticide, op

Table 1 Some of the pesticides proven to have harmful effect on the human body.

Levels: 1 = possibly carcinogenic, 2 = mutagenic or teratogenic, 3 = neurotoxic, 4 = toxicity LD_{50}*<50mg.kg*$^{-1}$

op:organophosphate, o-Cl: organochloride, car: carbandazole, pyr: pyrethroid ia: imidazole

(Sources: http://www.ewg.org/pub/home/reports/Fruit/Figure11.html & http://ace.orst.edu/info/extoxnet/factsheets/ghindex.html)

2.4.3.2 Biological control

DDT and its damaging after effects is not the only problem in pesticide application. In her book, *Silent Spring,* Rachel Carson (Cunningham & Siago 1992) raised the issue of non-target organisms affected by xenobiotics (pesticides) as long ago as 1962. Her arguments were not initially widely accepted, but time has proved them to be accurate. This awareness has led to the development of control measures, such as biological control, that are compatible with life forms other that of the target organism (Hussey 1976). He gave a summary of current and, more importantly, a preview of future biological control developments.

Although "biological control" specifically refers to the ability of organisms to inhibit the growth of others (Carlile & Watkinson 1994), attention is increasingly focused on the utilisation of natural enemies as well as pesticides of natural origin (Georghiou 1986). This includes pest controls derived from hormones, pheromones, repellents, host specific parasites and toxins from plant extracts (Sharma 1997b). Insects and nematodes on the mushroom substrate have many predator species. Several of these species are being investigated for their potential to control, if not eradicate, the pest species by means of ecologically non-disruptive measures (Grewal & Smith 1995, White 1995).

There are a number of notable applications of biological control in mushroom cultivation. The use of *Bacillus thuringiensis* var. *israelensis* and predatory nematodes in the control of mushroom flies and predatory mites in the control of cecid larvae (Dmoch 1995, White 1995) is showing promising results. The effectiveness of predacious fungi (*Arthrobotrys spp.* and *Dactylaria candidula* (Hohn.) Bhatt & Kendrick) is being researched extensively (Galper *et al.* 1995, Glockling & Dick 1994, Van Greuning & Eicker 1992, Gray 1985, Rosenzweig & Ackroyd 1983). *Pleurotus* spp. themselves have been indicated as being able to attack nematodes in the substrate (Thorn & Barron 1884) The nematocidal principle from *P. sajor-caju* was isolated by Nath *et al.* (1999). They found it to be muscarine in nature and with decidedly nematocidal properties.

2.4.3.3 Physical Control

Physical control measures entail all actions and methods used to clean, prevent infection and to sanitise the environment for the cultivation of mushrooms. Such measures should form the first part of a strict pest and weed mould management protocol. Chemical or biological control measures should serve to augment the physical measures. Sticky pads, wire gauze enclosures in front of all doors and windows, insect traps and electrified ultraviolet light lures are effective forms of physical control (Oei 1996, Stamets 1993). Cookouts, application of heat, farm layout and vent-system filtration are more demanding physical measures but their incorporation, as part of the control programme, is not negotiable (Fletcher *et al.* 1989, Wetzel *et al.* 1982).

Another important aspect is education of the staff. The manager must ensure that workers are trained and encouraged to co-operate in terms of the use of restricted areas, daily clean overalls, headgear, breathing masks, pesticidal footbaths (Sharma 1997b).

2.5 Animal pests commonly associated with mushroom cultivation

Animal pests abound on the substrate of all cultivated mushrooms. Their prevention and control supersedes any remedial actions that may be offered (Vedder 1978, Zadrazil 1978). Reports on animal pests and their effect are mostly from the *Agaricus* cultivators (Khanna & Sharma 1997, Al-Amidi 1995, White 1995, 1991, Vedie 1991, Vedder 1978) and can be grouped as follows:

1. Nematodes / eelworms
2. Insects
3. Mites
4. Other animals such as slugs and snails.

The substrate preparation for *Agaricus* cultivation and *Pleurotus* cultivation differs markedly, but reports indicate that the same types of pests attack both substrates and crop mycelia (Al-Amidi 1995, Rinker 1993, Clift 1987). The incidence of these pests are more often than not the result of farming practice and pest control or lack thereof (Snetsinger & Wuest 1987).

2.5.1 Nematodes

Nematodes found on the substrate can be classified as saprophagous or mycophagous (Rinker 1993, Fletcher *et al.* 1989, Vedder 1978). Vedder (1978) and Wuest (1977) reported that saprophagous nematodes (mainly *Rhabditis* species) were the cause of extensive crop damage. This observation was valid but possibly misdirected, since Eddy & Jacobs (1976) and later Ross & Burden (1981) found that these nematodes feed on the readily available fine layer of biomass covering the substrate straw. This biomass sustains a population of bacteria. The nematodes remove nutrients as well as the bacteria from this fraction of the substrate, depriving the mycelium of nourishment. Later investigations by Bloom *et al.* (1984) confirmed a mutually enhancing effect of bacteria and nematodes populations. Inhibition of the mycelium and subsequently the crop results from the toxic waste products secreted by these saprophagous organisms.

Mycophagous nematodes, on the other hand, damage or devour the crop mycelium itself. Several *Aphelenchiodes* and *Ditylenchus* species have been identified as particularly problematic nematodes (Hesling 1972). Mycophagous nematodes feed by piercing the fungal hyphae with their stylet and ingesting the cell contents. Though the details of the feeding mechanisms in the two genera differ, both of them cause severe mycelium destruction (Bloom *et al.* 1984, Hesling 1972).

An unlikely occurrence is finding an ally in enemy ranks. This has been the case with a third group, the insect-predaceous nematodes (Grewal & Smith 1995, Hesling 1972). Among the species in this group of nematodes are useful parasites of mushroom phorids. A *Howardula* spp. has been investigated by Riding & Hague (1974). Two species of rhabditid nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) and *Howardula husseyi* were tested against *Megaselia halterata* (Richardson 1987). The rhabditid nematodes eventually turned out the better biological control agents and are currently being used in commercial biocontrol preparations against sciarid flies (White 1995).

2.5.2 Insects

Although *Pleurotus* substrate does not require such intensive, phased composting like *Agaricus* substrate does, sciarid, phorid and cecid flies are still very prevalent mushroom insect pests whose presence result in significant losses (Rinker 1993, Stamets & Chilton 1983, Fletcher *et al.* 1989). These authors gave comprehensive descriptions of the various mushroom flies as well as control strategies for each one. Their appearance and life histories are summarised as follows:

1. Sciarids (*Lycoriella* spp.) are the biggest mushroom flies with adults measuring approximately 5mm. These dark-winged gnats have characteristically long, segmented antennae and large compound eyes. Eggs are visible with the naked eye and the larvae have a distinctive dark head. It eats its way into the spawn grain and all stages of the developing mushroom. Bacteria and fungal pathogens quickly infect the resulting tunnels. Rinker (1993) noted their attraction to *Pleurotus* species.
2. Phorids (*Megaselia* spp.) have an arched body shape and move around jerkily on the mycelium where they feed on the growth tips. The 3mm long adults have inconspicuous antennae, except for the enlarged third segment. They are attracted by volatiles from the mushroom mycelium and are a major vector of *Verticillium* disease. These flies are frequently parasitised by *Howardula husseyi.*
3. Cecids (*Mycophila* spp and *Heteropeza* spp.) are also known as gall midges and the adults measure 1-1.5 mm, depending on the species. Their paedogenetic reproduction means that whilst few adults are flying about, a population explosion could be taking place in the growth container. The larvae can be up to 2mm long and feed on the fungal hyphae. They are vectors for browning bacteria and have been found to attack *Pleurotus* mycelium (Rinker 1993).
4. Other flies occurring on the substrate are usually Diptera - mainly Drosophilids. They are attracted by fermentation taking place in over-wetted substrate. The damage caused by them is due to the larvae devouring the crop mycelium, their bodily waste and their ability to spread bacteria and spores

from other fungi. Nematodes cling to their legs and abdomens, hitching a ride from one area to the next (Fletcher *et al.* 1989, Stamets & Chilton 1983).

Beetles sometimes find their way into the mushroom house, and graze on the weed as well as crop mycelium. There are predatory beetles that attack and consume the various life cycle stages of mushroom flies, but that is not yet an acceptable alternative (White 1995).

Springtails can be a serious predator of the mycelium (Fletcher *et al.* 1989). However, the presence of this minute arthropod is seldom reported because, according to Snetsinger & Wuest (1987) it is directly related to the efficiency of the pest control measures employed on the mushroom farm.

2.5.3 Mites

A number of mites (Acarids) are found on mushroom substrate. Red pepper mites or pigmy mites (*Pygmephorus* spp., *Siteroptes mesembrinae* Canestrini) do not feed on the mushroom mycelium, but rather on fungi such as *Trichoderma* spp. (Terras & Hales 1995, Snetsinger & Wuest 1987, Wetzel *et al.* 1982). Their presence is indicative of poor compost conditions and they can ruin a harvest completely by disseminating the spores of the weed fungi that they feed on (Oei 1996, Fletcher *et al.* 1989, Van Griensven 1988).

Van Griensven (1988) and Stamets & Chilton (1983) describe *Tyrophagus* spp. *Tarsonemus* spp. and *Caloglyphus* spp as primary mushroom pests. These mites feed on the crop mycelium as part of their saprophagous diet on the substrate. Their feeding eventually cause secondary bacterial damage to the sporocarps and there are suspicions that *Tarsonemus* spp. can transfer a viral disease (Khanna & Sharma 1997). Predatory species (*Arctoseius* spp., *Parasitus* spp.) that feeds on nematodes, mushroom flies and other mites also occur among the mites (Dmoch 1995, White 1995, Fletcher *et al.* 1989).

Lastly, it must be remembered that the consumer has the last word on the quality of the crop. Any pest or predator on the sporocarp will definitely be unacceptable in terms of marketability (Van Griensven 1988, Declaire 1978).

2.5.4 Gasteropods

Gastropods are not frequent invaders of the substrate or mycelium. Their presence always indicate poor conditions in the substrate. In too wet conditions, algae tend to grow on the substrate and mycelium. They graze upon these algae, although some of them do feed on the crop and straw in the substrate as well (Fletcher *et al.* 1989).

2.6 The wheat straw substrate: nutritious selectivity?

Most of the available carbohydrates in the substrate are present as cellulose and lignin (Chang & Miles 1989, Royse *et al.* 1982). This should create an environment selective for organisms that can utilise these exclusive energy sources. However, the addition of grain spawn and nitrogen supplements, the presence of nematodes and insects in all their metamorphic stages and the mushroom mycelium itself creates an environment with conditions conducive to the formation of short food chains (Cunningham & Siago 1992). A typical example of a food chain in this environment can be found in the wheat straw that ferments in areas of the columns / tubes where the moisture content is too high:

- *Drosophila* spp. living on mushroom mycelium and laying eggs in the fermented substrate,
- Predacious beetles and nematodes feeding on the *Drosophila* larvae,
- Predacious mites and fungi feeding on the nematodes.

Insufficient environmental control also allows other fungi (weeds and competitors) to establish themselves in the substrate (Rinker 1993, Van Griensven 1988). It is possible to trace the occurrence of the various organisms to specific conditions that occur in the substrate (Van Griensven 1988, Fletcher *et al.* 1987, Stamets & Chilton 1983). It is therefore of vital importance that the substrate is prepared by pasteurisation under strictly controlled conditions so that weed fungi, pest larvae and cysts are killed.

Wheat straw substrate is deficient in easily accessible nitrogen compounds and carbohydrates and this makes it a selective mechanism. The selectiveness of the substrate can be enhanced through proper pasteurisation, but care must be taken not to diminish

the nutrient content of the substrate (Stölzer & Grabbe 1991). Any additives must maintain the selectiveness of the substrate, but at the same time it should encourage the growth and development of the crop (Stamets 1993, Van Griensven 1988). Dhanda *et al.* (1995) reported that supplementing paddy straw actually has a negative effect on the yield of *Pleurotus* mushroom. This report is in contrast to the findings of Houdeau *et al.* (1991) and Royse & Zaki (1991). Balakrishnan & Nair (1997) also reported the successful use of several slow release supplements to increase the crop yield.

In another experiment Chitale & Singh (1995) found the chemical pesticide treatment of the straw to increase the size of *Pleurotus* mushroom yields, once again in contrast to Houdeau *et al.* (1991). This is in agreement with the findings of Van der Hoven *et al.* (1988). However, the different groups did not investigate the same pesticides, *Pleurotus* strains or mushrooms. The importance of pesticides used during the cultivation of the substrate also needs to be investigated. The origin and therefore the biochemical constitution of the straw from different wheat cultivars used possibly have an effect on the yield size (Labuschagne *et al.* 2000).

Pleurotus ostreatus (Jacq. ex Fr.) Kummer has been proven to attack nematodes in wood as well as the substrate. This is possibly a survival strategy similar to that of carnivorous plants, to supplement its nitrogen requirements in wood (Tzean & Liou 1993, Thorn & Barron 1984), but in cultivation the secondary infection problems due to nematodes render this ability less useful. The substrate is therefore supplemented with vitamins (Okwujiako 1990), slow nitrogen releasing compounds (Hazarika 1998) and pesticides (Wetzel *et al.* 1982). For *Agaricus* cultivation supplementation is best done before casing (Van Griensven 1988). *Pleurotus* cultivation techniques however do not require casing layers, so that supplementation could be done during spawning (Dhanda *et al.* 1995).

2.7 Nutritional value of *Pleurotus* sporocarps

The potential of *Pleurotus* spp. as a supplementary food in any diet has been established. Its nutritional value lies in the fact that it is an excellent source of unsaturated fatty acids, vitamins, certain minerals and all essential amino acids (Chang & Miles 1989; Crisan & Sands, 1978). It is important to note that this useful protein source can be cultivated on most lignocellulosic agricultural wastes and with low technological input. This makes it an important contender as a dietary supplement in malnourished communities where financial constraints or religious abstinence prevents ingestion of the full complement of essential amino acids (Garcha, Khanna & Soni, 1993). Consensus has not been reached on the reported nutritional values of mushrooms and will probably always differ from region to region. This is because the substrates and their feeding soils, the fungal strains and the cultivation techniques differ from one mushroom farm to the next (Crisan & Sands, 1978).

The reasons for improving cultivation techniques and enhancing the local cultivation of *Pleurotus* are not only purely commercial, but also intended to address the nutritional status of rural and underprivileged communities in South Africa (Eicker 1993). Alternative cultivation methods have been investigated with the explicit intention of making it accessible and acceptable as a supplement in communities plagued by deficient diets (Pakela 1997).

Not only protein deficiencies, but also mineral deficiencies can be alleviated by consumption of *Pleurotus* mushrooms. The mineral content of these mushrooms is due to their ability to take up both major and minor mineral constituents like potassium and zinc. Notable is the fact that *Pleurotus* species have a higher ability than other edible mushrooms to accumulate heavy metals (Chang & Miles 1989). The sporocarp may even have concentrations of metal exceeding that of the substrate it was grown on. Chiu *et al.* (1998) found that *Pleurotus* mushrooms can accumulate cadmium to such high levels as to pose a health threat to humans. Sanglimsuwan *et al.* (1993) found in trails with 13 different genera that some *Pleurotus* species had the best resistance against detrimental effects by heavy metals. Their results also indicated an exceptional ability by mushrooms to accumulate toxic heavy metals in the mycelium. Of particular importance

is the fact that *P. ostreatus* mycelium had the highest accumulation values in all the metals tested. It is not clear whether they refer to mycelium in the sporocarp specifically. Heavy metal pollution in soil and water is a common occurrence in and around industrialised areas, therefore it is important that the environmental conditions around the source of substrates are monitored.

The presence of hemagglutinins in edible mushrooms (*P. ostreatus* and *P. spodoleucus* tested positively) casts a slight shadow over the exiting nutritional discoveries being made (Ortiz *et al.* 1992). Hemagglutinins are known to cause intestinal malabsorption. Since proper cooking inactivates hemagglutinin the presence of this compound need not be a disappointing fact. Cooking (i.e. heat treatment) would also inactivate thiamintransferase that is destructive to thiamine in mushrooms. The presence of this enzyme accounts for the low thiamine values often detected in mushrooms (Kurtzman 1993).

2.8 Other aspects of *Pleurotus* under investigation

2.8.1 Allergies

Mould spores are so small that they may evade the protective mechanisms of the nose and upper respiratory tract and travel on the breathed air to reach the lungs (http://www.niaid.nih.gov/publications/allergens/mould.htm) Allergic rhinitis is a condition caused by air-borne allergens such as mould spores. It tends to occur seasonally, as do pollens from trees, grasses and weeds. In South Africa the mould season often peaks from late December to June / July, but many species are perennial since they sporulate all through the year (Eicker 1988). Mould allergies, however, are associated with occupational diseases as well (Oei 1996, Michils *et al* 1991). The allergens in this case are the basidiospores from commercially cultivated species, especially *Agaricus bisporus* and certain *Pleurotus* species and strains.

The role of the Basidiomycetes as allergens has not been investigated enough (Hebling *et al* 1999). Wild specimens of the different *Pleurotus* species produce enormous amounts

of spores (Sonneberg *et al.* 1996). In a study on extrinsic allergic alveolitis, Cox *et al.* (1988) determined that *P. ostreatus* tested positively allergenic. Hebling *et al.* (1999) showed in a well-documented case study of non-occupationally related rhinoconjunctivitis and asthma, that the airospora contained sufficient numbers of *P. pulmonarius* Fries spores to cause allergic reactions.

Although this trait is diminished through genetic manipulation of the cultivated strains (Eger 1978), workers susceptible to mould allergens still suffer from headaches, painful joints, fatigue, rhinitis, asthma and dermatitis (Rosina 1995, Chang & Miles 1989, Eicker 1988). According to Oei (1996) reports from the growers indicate that these low-spore stains give lower yields.

2.8.2 Medical properties

Mushrooms exude an array of secondary metabolites and some can be harvested and applied as medicinal preparations. Knowledge of the medicinal use of mushrooms goes back a long way in mankind's history. The use of mushroom-derived hallucinogenics in spiritual rites stem from varied and ancient cultures (Emboden 1979). In these ancient cultures medicinal, spiritual and even repressive uses were often intertwined. A new trend, however, is the zealous attempts at home-cultivation of certain mushrooms for recreational purposes (UNDCCP 1998). People conduct this pursuit with no *bona fide* interest in establishing a commercial enterprise, in the search for improvement of human medicine, or in the spiritual guidance and rituals of their faith or religion (http://www.nepenthes.com/Plants/shrooms/illustrated.html).

P. ostreatus is a traditional Chinese remedy to relax muscles and joints. It has also been proven to exhibit considerable anti-tumour activity. Other important contributions that *Pleurotus* mushrooms are making in medical terms are as hypocholesterolemics, hypotensives and immunostimulators (Rai 1997). Their high content of polyunsaturated oils is one of the reasons for their hypocholesterolemic abilities. The hypotensive effect is promising for patients suffering from renal failure, as it reduces the deterioration of the nepfron (Chang & Miles 1989).

2.8.3 Genetics

Important studies on the genetics of speciality mushrooms are being conducted. These studies seek to isolate and crossbreed desirable characteristics in lignocellulolytic fungi for several reasons:

- Identification and confirmation of species and genetic relationships. Using interspecific matings and incompatibility tests of the monokaryons, several teams succeeded in proving the taxonomic distinctness of *P. ostreatus* and *P. pulmonarius*. On the other hand, the close relationship between *P. pulmonarius* and *P. sajor-caju* was confirmed (Zervakis & Balis 1995).
- Isolation and manipulation of enzymes and lignin degrading capacity for bioremediation application (Kimura *et al.* 1990).
- Isolation and manipulation of medically valuable characteristics (Rai 1997)
- Isolation and improvement of sporeless commercial strains (Imbernon *et al.* 1991)
- Genetic manipulation to provide strains with more tolerant environmental requirements (Tschierpe 1983)
- Creating strains with higher yield capacities, more pronounced taste (Iraçabal *et al.* 1991, Magae *et al.* 1985), improved bioconversion with increased sporophore development (Jia *et al.* 1991) and other desirable market-related traits.

2.8.4 Biodegradation and Bioremediation

The age-old practice of burning agricultural waste contributes unacceptable levels of pollutants to our atmosphere (Cunningham & Siago 1992). Serious efforts at turning this waste into nutritious and more digestible animal feeds are being made. Several lignocellulosic fungi are being evaluated and the *Pleurotus* species used in these studies include *P. sajor-caju* (Karunanandaa & Varga 1996, Bisaria *et al* 1997), *P. florida* nom. prov. Eger (Wolter *et al* 1997) and *P. ostreatus* (Adamovic *et al* 1998, Colombo *et al* 1996). It has been found that *Pleurotus* species are able to digest hemicellulose and phenolic acids in rice straw (Karunanandaa & Varga 1996). According to Wolter *et al* (1997) *P. florida* degraded the added polycyclic aromatic hydrocarbons along with 40% of their wheat straw within 15 weeks.

Bioremediation as a means to decontaminate polluted soils is being investigated. Styrenes and polycyclic aromatic hydrocarbons (e.g. various phenols, pyrenes, benzenes and anthracenes) are all highly resistant pollutants in the natural environment (Braunlullemann *et al* 1997). Variable results have been obtained with the ability of several *Pleurotus* species to eliminate these compounds. The results obtained are closely correlated to the ability of each *Pleurotus* species to compete against other soil micro-organisms that are present as well (Lang *et al* 1998, Lang *et al* 1997, Inderwiesche *et al* 1996). The species used in bioremediation experiments include *P. ostreatus* (Bezalel *et al* 1997), *P. sapidus* (Schuler) Kalchbremer and *P. ostreatus* (Braunlullemann *et al* 1997, Wunch *et al* 1997). Azizi *et al* (1990) found that the ability of *P. ostreatus* to accumulate heavy metals along with its lignocellulolytic action made it suitable for application on industrial waste as well.

Biopulping, another industrial application, offers an alternative, non-chemically based, non-polluting method of digesting wood for paper production (Guillén et al. 1994). According to Camarero *et al.* (1998) biomechanical pulping of agricultural wastes (wheat straw and rice straw being only two example) offers a particularly acceptable alternative to the search for virgin fibres in paper manufacturing. This would further contribute to forest preservation. They found especially *Pleurotus eringii* Quélet to be a highly selective delignifier of straw.

CHAPTER 3

MATERIALS AND METHODS

3.1 South African cultivation of *Pleurotus* species

3.1.1 Spawn

Spawn is prepared in a very sterile and carefully monitored environment. Growers mostly prepare their own spawn, but it is possible to obtain it through a commercial spawn laboratory. Strains used in commercial cultivation are kept viable through maintaining pure subcultures at 4°C as well as in LN_2. Pre-sterilised grain (wheat, rye, millet or sorghum are all used in South Africa, rye being the preferred grain) is used in the preparation of the spawn, which is done according to standard procedures (Eicker 1995).

3.1.2 Substrate and Pasteurisation

The study has been conducted on privately owned *Pleurotus*-farms in the central Gauteng and Western Cape provinces of South Africa. The substrate of choice on all the farms is wheat straw. The wheat straw substrate is obtained in bales of between 17 kg and 350 kg sizes. The quality of the straw is variable, with the grower depending on availability and transport costs rather than using preferred suppliers. It is then stored in an area providing protection from precipitation, wind and rodents. The actual preparation of the straw is done according to a method preferred by the grower, but the principles according to which it is done is the same for all methods.

Pasteurised wheat straw is used as the substrate. The straw is chopped into 5–10 cm lengths and wetted to obtain a 65-70 % moisture content (measured w/w). Batches of straw, predetermined and sufficient for each run, is pasteurised and used immediately. With a nitrogen content of *ca* 0.5%, slow release nitrogen supplementation is needed to bring it to 4-5%.

Other additives could include vitamins, insect growth regulators (IGR's), nematocides, pesticides, fungicides and a 1:1 lime + gypsum mixture. All supplementation, if used, is added before pasteurisation. Heat labile pesticides are added later during the cooling down period. During pasteurisation heated steam (kept at 75-80°C) is forced through for 8-10 hours. Throughout pasteurisation the moisture content is kept at 70%. After cooling down polypropylene growth containers are filled with prepared substrate whilst 2–4 % spawn is added simultaneously. Before it is divided among the growth containers, a typical pasteurisation batch weighs 1.5 tonnes (Eicker 1995, Van Greuning 1995).

3.1.3 The growth system

Once again, although the same cultivation principles are applied, the cultivation methods are tailored after each individual grower's preference and facilities. Space is not a problem on most mushroom farms, so that the growing system is dictated by available labour and environmental control costs. This results in the use of mainly two different growth systems. In the one system 50 kg polypropylene columns or tubes (so-called Zadrazil sausages) are each tied onto an upright stand or support made from square tubing and standing on the ground. Smaller polypropylene bags with only an 8kg capacity are used in the other system, where tiered rows are hung, in groups of five, from the shelf above.

In all systems, new bags are filled with the prepared substrate and incubated until homogenous colonisation (spawn run) took place. Upon completion of the spawn run, the bags are pierced in several places using a 25mm punch. Clusters of sporocarps will be borne from these holes.

Incubation, growth, harvesting and packaging are done indoors. Insulated growth areas are used in order to control the climate of the crop environment. These may be permanent buildings or double-layer plastic greenhouse tunnels, where fibreglass insulation gets packed between the plastic layers. It has been found that polystyrene sheets makes good insulatory material as well.

3.2 Isolation of Animal pests

3.2.1 Materials and Methods

3.2.1.1 Nematodes and Mites

Nematodes were collected by plating the substrate directly onto CMA plates. Small pieces of substrate were placed at one end of the CMA plate only and sealed with Parafilm. The opposite part of the plate not containing the substrate was covered with foil. The plates were incubated in a room at 22°C. Nematodes moved towards the darkened area within 24–48 hours. After making sure no mites or fungal contamination was present, a small block of eelworm-infested agar was transferred to a fresh CMA plate. Plates containing live specimens were sent to the Biosystematics Division of the Agricultural Research Council of South Africa for identification.

Mites were collected in a similar way, except that water agar was used. The Petri dishes were sealed very carefully so as to prevent any unwanted mite infestation. A small block of PDA with *Cladosporium* mycelium was placed upside down opposite the substrate in the Petri dish. The plates were not covered with foil, but the mites were attracted to the mould within a few hours. They were scooped out into a clean Petri dish that was then sealed with Parafilm and sent to the Biosystematics division of the Agricultural Research Council of South Africa for identification.

3.2.1.2 Insects

The insects were captured directly from the substrate the in growth tubes as well as directly from tubes bearing fruit bodies. This was done by means of a small suction-type trap (aspirator) or "pooter", based on the method explained by Eardley & Dippenaar (1996). The trap was constructed from a McCartney bottle, short sections of glass tubing fitted through the bottle lid, fine nylon mesh and silicone tubing (Fig 2). Upon capture of a specimen, the McCartney bottle with the insect(s) was unscrewed from the pooter and fitted with a lid. A replacement bottle was put onto the trap.

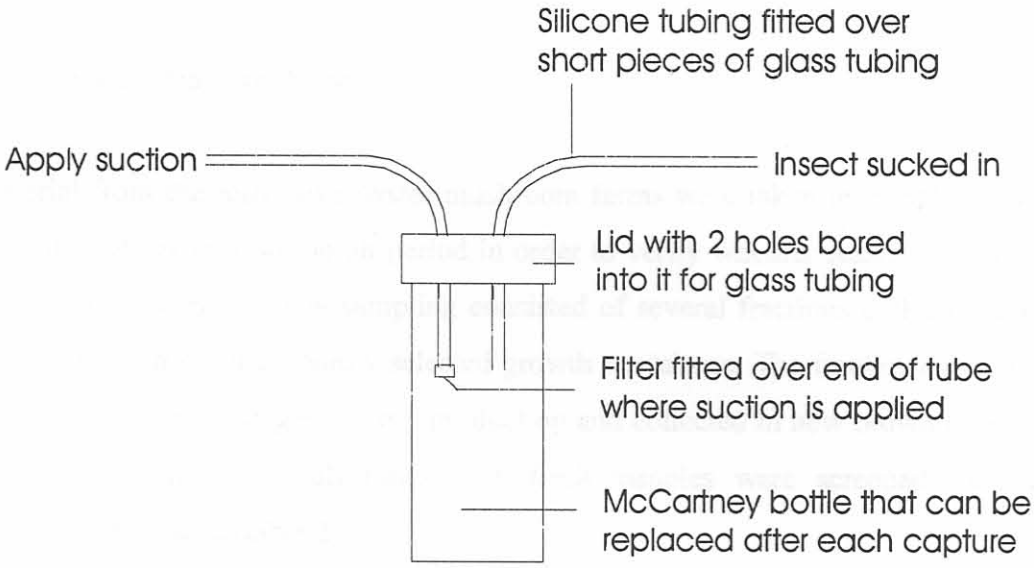

Figure 2 Outline of an aspirator-type insect trap known as a pooter

Larvae and pupae were collected in a clean Petri dish together with some moist substrate. The Petri dish was sealed with Parafilm as a safety precaution against accidental opening of the dish. The larvae and pupae were transferred to and incubated in a plastic food container with a ventilated lid in a room at 22 °C.

All insects were identified at the Department of Botany, University of Pretoria before being sent to the National Insect Collection in Pretoria for confirmation or to be identified to genus or species level, whichever was possible.

3.2.1.3 Gastropods

Slugs were collected by hand into a clean Petri dish. They were identified by the Department of Botany at the University of Pretoria. However, due to their infrequent occurrence and obvious relationship with conditions in the substrate that are too wet, the Biosystematics Division of the Agricultural Research Council of South Africa did not confirm their identification.

3.3 Competitive and Weed fungi

3.3.1 Isolation Methods

Material from the respective oyster mushroom farms were taken in samplings collected over all seasons in a 30 month period in order to verify whether seasonal fluctuation of the moulds occur. A single sampling consisted of several fractions collected at random from the substrate in randomly selected growth containers. The fractions were obtained from all the various stages of crop production and collected in new brown paper bags or sterile Petri dishes. At all times only fresh samples were screened for weed or competitive moulds (table 2).

STAGE OF PRODUCTION	PROCEDURE FOLLOWED
Sand or soil from storage facility for dry wheat	Aseptic collection of loose grain by hand, into sterile Petri dishes
Unsterile grain	
Sterile grain	
Unpasteurised wheat straw	
Pasteurised wheat straw	
Spawned substrate (in filled tubes)	Insertion of a very large pincer into randomly selected areas as well as areas with obvious contamination and chunks of substrate collected into either sterile Petri dishes or sterile brown paper bags.
Colonised substrate (in filled tubes)	
Fully productive tubes	

Table 2: Summary of the sampling procedures used to collect fractions from each monitored production stage.

During study three methods were used to isolate weed moulds from the collected material. The samples were treated as follows:

1. Dilution series of each fraction of the sample onto agar plates. The dilution series was done according to standard microbiological procedures, using a dilution range of 1:10, 1:100 and 1:1000.

2. Direct plating of each fraction of the sample onto agar plates. This was done by placing small amounts of material spaced evenly onto the agar surface.
3. Imbedding weighed quantities of fractions into cooled, but still molten agar.

The plates were incubated at 20°C, 25°C, 30°C, 35°C and 40°C. No temperature incubation lower than 20°C was done, since the environment within which the moulds are looked for, has a higher temperature range. Initial incubation was done in total darkness and once mycelial growth was observed, in black light at 25°C.

3.3.2 Media used for the isolation of moulds

For direct plating and imbedded material potato dextrose agar (PDA), Rose Bengal/PDA, oatmeal agar and water agar plates were used. O.5% Rose Bengal was added to the PDA plates only. Grain fractions were plated onto PDA with 0.25 % chloramphenicol (rather that 0.5 % Rose Bengal) added for increased bacterial inhibition.

The dilution series was done with sterile physiological Ringer's solution. 1 g material was weighed into sterile 250 ml Erlenmeyer flasks and 100ml Ringer's solution added with 1 drop of surfactant. It was shaken vigorously for 1min and the dilution series prepared from the resultant liquid. PDA plates with 0.5% Rose Bengal and 0.5 % surfactant was used for the dilution series. The surfactant in the PDA plates results in better delimitation of the fungal colonies.

3.3.3 Identification of isolates

After initial isolation and purification, the moulds were identified. Where an isolate was encountered more than once, only one isolate was kept, but the frequency with which it occurred was noted.

The slide culture method of Coetzee & Eicker (1990) was used. A 10x10mm PDA blocks were cut from a PDA plate. These blocks were stuck on the sterile inside surface of the lid of the Petri dish with the agar from which they were cut. A small quantity of spores of a mould was transferred to the four lateral sides of each block. A microscope

cover slip was placed on each agar block and the lid replaced on the dish. The agar plate + blocks was incubated in black light at 25 °C until sporulation was barely visible with a dissection microscope. At this point the cover slips was very carefully prised from the block and mounted on a drop of lactophenol blue on a microscope slide. The cover slip was sealed with a transparent nail cutex. With this method there is very little disruption of the spore-bearing structures. The agar plate ensures that the agar blocks in the lid do not become desiccated.

The semi-permanent mounts of the pure culture isolates were examined under a Nikon Optiphot light microscope, using Nomarski interference-contrast illumination. Identification was done according to several sources as applicable to each isolate.

3.4 Farming practice and hygiene

This was investigated through visitations to *Pleurotus* farms, assessment of the available facilities, interviews and monitoring of contamination by means of samples from the farms.

3.5 Meteorological information

The study has been conducted in a summer rainfall region (Gauteng), and a winter rainfall region (Western Cape). Information on the two study areas was drawn from weather stations in close proximity to each farm (courtesy of the South African Weather Bureau). In Gauteng meteorological information was therefore collected at the Jan Smuts weather station (Johannesburg International Airport) and in the Western Cape at the D.F. Malan weather station (Cape Town International Airport). The two weather stations where the data was collected are situated in the same floristic environments as the different farms.

Seasonal changes were investigated in order to find a possible correlation to infective episodes encountered in the various regions.

CHAPTER 4

RESULTS

4.1 Observation of cultivation practices

Investigation into the farming practices and the observation of procedures for hygiene application revealed a number of complicated issues. Many of them are related to financial constraints and the upgrading of the facilities.

The storage facilities used for wheat straw are open – even if tarpaulins are used to cover it or it is kept in a shed, it was found that farm and small wild animals and insects still had access to it. The wind could also still deposit its airborne charge on the bales. Dry wheat has no problem with this fact, but as soon as the wheat is exposed to moist conditions it is degraded by contaminants present in the straw. Decaying wheat attracts and harbours a high number of saprophytic organisms, including heat resistant propagules from such organisms.

Contamination of the substrate is very infrequently due to problems in the spawn. However, when these do occur, observation pointed out one of two possibilities:

1. The spawn was not handled according to strict hygiene precautions.
2. The liquid mycelium or even colonised spawn was not transferred in a sufficiently aseptic environment.

When the bags or columns are filled, the intervention of a human hand is necessary to control the process. The person(s) involved in this procedure must wear protective clothing and observe the basic rules of working aseptically, even where the filling of the bags takes place in a semi-outdoors facility. Recurrent *Coprinus* and *Trichoderma* infections could be linked directly to dusty farmyards or the presence of spent substrate together with windy conditions during filling.

The following generalised problems were observed:

1. The working as well as growing areas are not always properly sealed. Any gaps in the structures used for cultivation allow the entry of dust and insects. Wind plays a major role since fungal spores, small insects and mites can be disseminated by it.
2. The wind factor and its effect on dusty farmyards were not taken into account.
3. Spent compost stored on the premises.
4. Workers move from one farm area to the next without any precaution as far as cross-contamination are concerned. The same worker would move from straw storage to packaging and later incubation rooms without footbaths or a change of overcoat.
5. Entrances to the growing rooms are opened with direct access to the outside / inside environment. Double door trapping systems are not used on all farms.
6. Bruised crop waste is not removed immediately during harvesting. The accentuated smell of mushrooms is very evident during and shortly after harvesting.
7. Accumulation of water due to the regular, timed misting done as part of humidity control. This is especially problematic in older growth rooms preparing for 3rd or 4th (even 5th) flushes. The rooms waiting for a 5th flush are a particular cause for concern, as fermentation becomes a real issue in the growth containers here.

4.2 Animal Pests

4.2.1 Significant pest presence

Representatives from all four the animal pest groups were found on the substrate. Only two of them really occurred in large enough numbers to be of any consequence, namely nematodes and insects. A number of mites were found, but their presence did not pose a threat at the time.

Young slugs (*Limax* sp.) occasionally occurred on the substrate of the column-cultured mushrooms. It was an infrequent event and always due to wet, fermentative conditions already showing signs of algal growth as well. Their presence were therefore merely

noted as an indication of substrate condition and their identity was not confirmed by the Biosystematics division of the Agricultural Research Council (ARC) of South Africa.

4.2.2 Nematodes

Huge numbers of nematodes could be found on the substrate from time to time, but the saprophagous species were always dominant. The shiny, waving bodies on the substrate were characteristic of such episodes. The Biosystematics division of the ARC-SA identified the nematode species isolated from Pleurotus substrate (table 3).

Nematode species	Feeding habit	Western Cape	Gauteng
Rhabditis sp.	Saprophagous	Yes	Yes
Rhincorhabditis sp.	Mycophagous	Yes	Yes
Cruznema sp.	Saprophagous	Yes	Yes
Panagrolaimidae family.	Saprophagous	Yes	No

Table 3 The distribution and feeding habits of isolated nematode species.

The farms in the Gauteng region suffered more regularly under nematode infections. No nematode damage to the crop sporocarp was noted during the study. However, crop failure could be correlated to massive nematode infections, even it was saprophagous nematodes. Episodes of insect infection and nematode infection could not be correlated.

4.2.3 Mites

Contradictory to expectations, very few mites were actually observed and isolated from both regions. The only mites identified from the substrate from farms in both regions belonged to a *Tyrophagus* species. *Caloglyphus berlesei* and a *Glycophagus* sp. were isolated from substrates in Gauteng and the Western Cape respectively. The Biosystematics division of the ARC of SA identified the mite species that were isolated (table 4).

Mite species	Feeding habit	Western Cape	Gauteng
Caloglypus berlesei Mikael	Mycophagous	No	Yes
Glycophagus sp.	Oligophagous	Yes	No
Tyrophagus sp.	Mycophagous	Yes	Yes

Table 4 The distribution and feeding habits of isolated mite species.

None of the mites were found to be present in significant numbers at the time. All of them were associated with mycelium, but none were found on the sporocarps. The mites from the *Glycophagus* sp. were probably grazing on bacteria and organic debris, but both the other two species could cause damage to the crop mycelium. *Caloglyphus berlesei* is also known on *Agarics bisporus* compost in South Africa.

4.2.4 Insects

The insects were captured directly from the incubating bags or columns, as well as directly from sausages bearing fruit bodies. This was done by means of a small suction-type trap. Identification was done by the Botany Department of the University of Pretoria and the National Insect Collection in Pretoria (table 5).

4.2.4.1 Western Cape

Lycoriella mali is a well known threat to the button mushroom industry (Rinker, 1993). It was implicated in a devastating, epidemic fungus infestation on an oyster mushroom farm in the Western Cape, when the numbers of these flies where exceptionally high during the epidemic. At the time, clouds of *L. mali* were visible in the growth rooms and captured insects were carrying spores on their antennae and abdomens. The characteristic mature larvae (white, translucent body and black head capsule, 7 - 10 mm in length) were visible in the substrate as well as the sporocarp clusters. A number of weed fungi managed to successfully colonise the substrate at the time of the insect plague, and eventually overtook the crop mycelium as well. *L. auripila* are sometimes found on the

substrate, but it never observed in high numbers. It was also not observed as part of the *L. mali* plague. Another fungus gnat that regularly occurred was *M. halterata*.

Insect species	Feeding habit	Western Cape	Gauteng
Sciarid fly (Fungus gnats)			
Lycoriella mali	Mycelophilids	Yes	Yes
Lycoriella auripila	Mycelophilids	Yes	Yes
Macrocera sp.	Polyphagous	No	Yes
Phorid fly			
Megaselia halterata	Mycelophilids	Yes	Yes
Cecid fly			
None found	-	-	-
Drosophilid fly			
Drosophila sp	Oligophagous	No	Yes
Colembola: "springtails"			
Poduridae	Saprophagous	Yes	No
Entomobryidae	Saprophagous	Yes	No
Other:			
Sylvicola sp. (Wood gnats)	Saprophagous	Yes	No
Brachypephus sp. (Coleoptera)	Predaceous	Yes	No

Table 5 The distribution and feeding habits of isolated insect species.

Springtails (Colembola) are sometimes encountered and during the autumn of 1997 a serious problem was experienced when they infested the crop mycelium. During the infestation they were present in particularly vast numbers, leading to complete loss of the crop. These minute insects are difficult to eradicate as they are carried from the neighbouring alfalfa farms by wind and water. During the time of the infestation, the farming practice and control measures did not give enough protection against the insects, especially against infection during windy spells.

Fruit flies (*Drosophila* sp.) were found infrequently and are often associated with excess moisture. When the substrate is too wet, fermentation starts to take place, luring these scavengers. Also isolated from the substrate is *Sylvicola* sp. (wood gnats) and *Brachypephus* sp. (Coleoptera). The shelves are wooden structures, but their presence on the substrate is probably coincidental. Along with the fruit flies, their real danger to the crop lies in the fact that they can spread nematodes and fungal spores, leading to further decline in the crop environment (Fletcher *et al.* 1989).

Since the bags are hung, rather than stood on the next shelf, no insect problems are reported. The use of smaller growth containers also facilitates easier handling of the bags. Furthermore, any contamination has less impact, as the physical removal of contaminated bags is easier. It ensures fewer disturbances of the infective organisms and the uninfected bags.

4.2.4.2 Gauteng

Although no devastating episodes due to insects have occurred on Gauteng farms, *L. mali* as well as *L. auripila* can be found there. Another sciarid from the substrate in Gauteng is *Macrocera* sp. who, together with *M. halterata*, often are the dominant oyster mushroom insect pests in this region. Pieces of leftover material were often found on the ground, with the smell of bruised sporocarps attracting insects. Although the material is removed during the course of the morning, visiting insects find the wounded mycelium on the columns with ease. With summer temperatures reaching 30–32°C, insect pests are an abundant omni-presence.

As is the case in the Western Cape, fruit flies are always present when substrate conditions are too wet. However, they are observed more often and especially on columns presenting a second and third flush. Apart from the trapped adult flies, huge drosopholid larvae were found in pulped areas in the bottom of the columns. Their presence can be correlated with the formation of puddles around the bases of the columns. These puddles form during the regular misting of the columns to keep the humidity in the tunnels at 85 – 90 % winter and summer, since the relative humidity (Rh) in Gauteng is low (see section 4.5 for more information).

At the time of the study the spent substrate was kept within a 200 m range from the growth tunnels, providing fertile breeding place for insect pests. During September / October a definite increase in the insect numbers is observed on the bags. The outside temperatures and rainfall as well as the wind factor increase during this time, creating a cross-contamination hazard.

IGR's (Cyromazine), nematocides (Carbofuran and quinalphos e.g. Neporex), fungicides (Prochloraz, thiabendazole) and insecticides (malathion, pyrethroids, dichlorvos) are all pesticides with which varying degrees of success has been achieved on some mushroom farms. This accounts for the absence of epidemic episodes on such farms. However, these growth environments often exhibit physiological crop disorders for which no satisfactory explanation has been given yet.

4.3 Fungal disorders

4.3.1 List of the weed fungi

Weed fungi are regarded as the group of fungi that occur very infrequently. They are never been observed in any antagonistic colonisation of the substrate and only get a foothold when there is deterioration of the substrate due to the presence of more aggressive fungi, insects, nematodes or insufficient hygiene.

More than 80 possible weed species were isolated over the period of 30 months. It was decided to pay attention to only those that were found in more than 4, but less than 10 samplings done during this period. They were further differentiated according to the abundance with which they were present when they were encountered in a sampling (A, B, C in Table 6). At the time of that the fungal isolations were made the facilities for growth containers had poorly sealed entrances, allowing insect pests to take refuge inside from the harsher climate outside.

Weed fungus species	Western Cape Type	Gauteng Type
1. *Acremonium*	-	C
2. *Alternaria alternata*	C	B
3. *Arthrobotrys oligospora*	-	C
4. *Aspergillus candidus*	C	-
5. *Aspergillus fumigatus*	A	A
6. *Chaetomium cochliodes*	-	C
7. *Cladosporium cladosporiodes*	A	B
8. *Epicoccum purpurascens*	C	C
9. *Fusarium oxysporum*	B	C
10. *Penicillium fellutanum*	-	C
11. *Penicillium janczewskii*	C	-
12. *Penicillium spinulosum*	-	C
13. *Penicillium variabile*	C	C
14. *Penicillium waksmanii*	C	-
15. *Peziza vesiculosa*	-	C *
16. *Sclerotinia rolfsii*	-	C *
17. *Trichothecium roseum*	C	B
18. *Verticillium fungicola*	-	C

Table 6 Species list and distribution of weed fungus isolated from *Pleurotus* substrate. The type refers to the abundance with which the species occurred in the fractions of 4 – 10 samplings done (*: see relevant text below).

A: Present in all fractions, ≥ 3 colonies per plate

B: Present in some fractions, ≥ 3 colonies per plate

C: Present in some fractions, ≤ 2 colonies per plate

4.3.2 Brief descriptions of the weed fungi on PDA

Unless mentioned to be different, the descriptions of the colony characteristics of the

weed fungi are based their appearance on PDA, grown at 25°C for 10 days (pH = 5.8). Their spore descriptions are based on spores obtained from PDA blocks placed under black (near-UV) light until adequate sporulation was obtained (average not more than 7 – 10 days).

The use of identification sources other that Domsch *et al.* (1993) is mentioned under the remarks for each fungus. All terminology used were confirmed from Hawksworth *et al.* (1996).

n/c = no comment

4.3.2.1 *Acremonium stricum* Gams

Diameter	: 23 – 26 mm.
Colour – top	: Pinkish-white to pinkish-yellow.
Colour – bottom	: Light yellowish-pink.
Appearance	: Flat, slightly moist, cottony aerial mycelium in middle of colony.
Spores	: Conidia hyaline, smooth, 3.1-4.9 x 1.2-2.2 µm.
Remarks:	Cylindrical to oval conidia on sparse, simple phialides, forming slimy heads. No chlamydospores present.

4.3.2.2 *Alternaria alternata* (Fr.) Keissler

Diameter	: 50 – 58 mm
Colour – top	: Dark, golden brown
Colour – bottom	: Very dark brown
Appearance	: Slightly grainy, loosely matted, glistening aerial mycelium
Spores	: Dark, irregular shape, beaked, 15.2-57.8 x 7-20 µm
Remarks:	Conidia in long acropetal chains, variable number of septa, variable shape (ovoid to obpyriform) tuberculate-ornamented conidium wall.

4.3.2.3 *Arthrobotrys oligospora* Fres.

Diameter : 50 – 70 mm
Colour – top : White
Colour – bottom : n/c
Appearance : Wispy to cottony aerial mycelium in an irregular colony outline; tend to form arachnoid, trailing aerial mycelium.
Spores : Conidia obovoid to pyriform, 21.8-28 x 8-14.5 µm
Remarks: On PDA this fungus had an untidy growth habit, with tiny crystal-like clusters of conidia. It was sometimes isolated in association with other weed fungi.

4.3.2.4 *Aspergillus candidus* Link ex Link

Diameter : 25 – 32 mm
Colour – top : White
Colour – bottom : Light yellow
Appearance : Loose aerial mycelium forming small to medium granular colonies, discernable concentric rings.
Spores : Globose, smooth, diameter 2.5 - 3.5 µm
Remarks: "Balloon"-structure of individual conidial heads clearly visible under low magnification (dissection microscope).

4.3.2.5 *Aspergillus fumigatus* Fres.

Diameter : 60 -70 mm
Colour – top : Greyish blue-green
Colour – bottom : Light blue-grey
Appearance : Coarse, open colony - columnar conidial heads with dry spores visible
Spores : Echinulate, globose, 2.3 –3.0µm
Remarks: Very common on substrate in Gauteng region during the summer months.

4.3.2.6 *Chaetomium cochliodes* Pall.

Diameter : At least 70 mm on cellulose agar

Colour – top : Dark olive green ascocarps, greenish mycelium

Colour – bottom : Colourless with black spots indicating ascocarps on top

Appearance : Submerged/very flat mycelium with densely dispersed, tiny coarse brushes clearly visible. This is the characteristically hairy perithecium.

Spores : Medium brown, ovate to lemon-shaped spore, 7.8-10 x 7-8.2 µm

Remarks: This fungus was isolated from the wheat straw itself and immediately sub cultured and maintained on cellulose agar, at 25 °C. The mycelium is barely visible. Two types of hair-like hyphae growing from ascocarp: the spiralled hyphae had 3-5 relaxed coils from the middle towards the tip and were covered with globulate structures. The second type was straighter, growing from the sides of the ascocarp. No branches form in any of the hair and they are conspicuously curly with globulate structures. Evanescent, clavate asci are produced in basal tufts. The ascospores are broadly ovate to lemon-shaped.

4.3.2.7 *Cladosporium cladosporiodes* (Fres.) de Vries

Diameter : 40 – 46 mm

Colour – top : Very dark olive green

Colour – bottom : Dark, greenish black

Appearance : Soft, effuse, clearly delimited woolly colonies (lanate)

Spores : Catenate, somewhat irregularly shaped – mostly lemon-shaped to ellipsoid - with smooth walls, brownish green 4.0-9.5 x 2.5-4.5 µm

Remarks: The first isolates of this fungus was ruined by run-away mites, completely devouring the mycelium and conidia. For this reason it was later chosen as bait for isolating mites from the substrate.

4.3.2.8 *Epicoccum purpurascens* Ehrenb. ex Schlecht.

Diameter : 68 – 75 mm

Colour – top : Bright orangy-yellow with darker orange boundaries

Colour – bottom : Dark orange

Appearance : Thick matted or woolly (lanose) colonies with dark, wet irregular beads appearing in mycelium (pustules)

Spores : Dark brown, verucose, globose to pyriform with a funnel shaped attachment, numerous septa 12.5(16.0) – 23.2(26.7) µm

Remarks: The presence of this fungus is characterised by small black spots and sporodochia on the straw and sometimes even on the plastic of the container.

4.3.2.9 *Fusarium oxysporum* Schlecht. emend. Sny. & Hans

Diameter : 80 – 90 mm

Colour – top : White with feint violet tinges

Colour – bottom : Greyish-beige

Appearance : Soft, loosely tufted mycelium eventually forming a gentle mat (felted).

Spores : Macrospores 3 (5)-celled with a footcell present, fusiform, slightly curved, measurements quite variable:
(22.2) 27.0 – 45.3 (61.2) x 3.0 (3.2) – 4.8 (5.1) µm.
Microspore non-septate, ellipsoid to cylindrical, sometimes curved:
5.2 –9.8 x 2.4 – 3.7 µm

Remarks: No chlamydospores were ever observed and the colour of the colony as well as the micro- and macro-conidia was used as most important identifying characteristics.

The Plant Protection and Pest Control Division of the Agricultural Research Council identified the following five of *Penicillium* species: These species were always associated with secondary infections on the bags and were isolated from insect-damaged substrate,

sporocarps rotting due to water logging or injured mechanically as well as substrate in a generally poor condition.

4.3.2.10 *Penicillium fellutatum* Biourge

4.2.2.11 *Penicillium janczexski* Zaleski

4.2.2.11 *Penicillium spinulosum* Thom

4.2.2.11 *Penicillium variabile* Sopp.

4.2.2.11 *Penicillium waksmanii* Zalaskii

4.3.2.12 *Peziza vesiculosa* Bull. ex St. Amans

Figure 3 *Peziza vesiculosa* on wheat straw substrate.

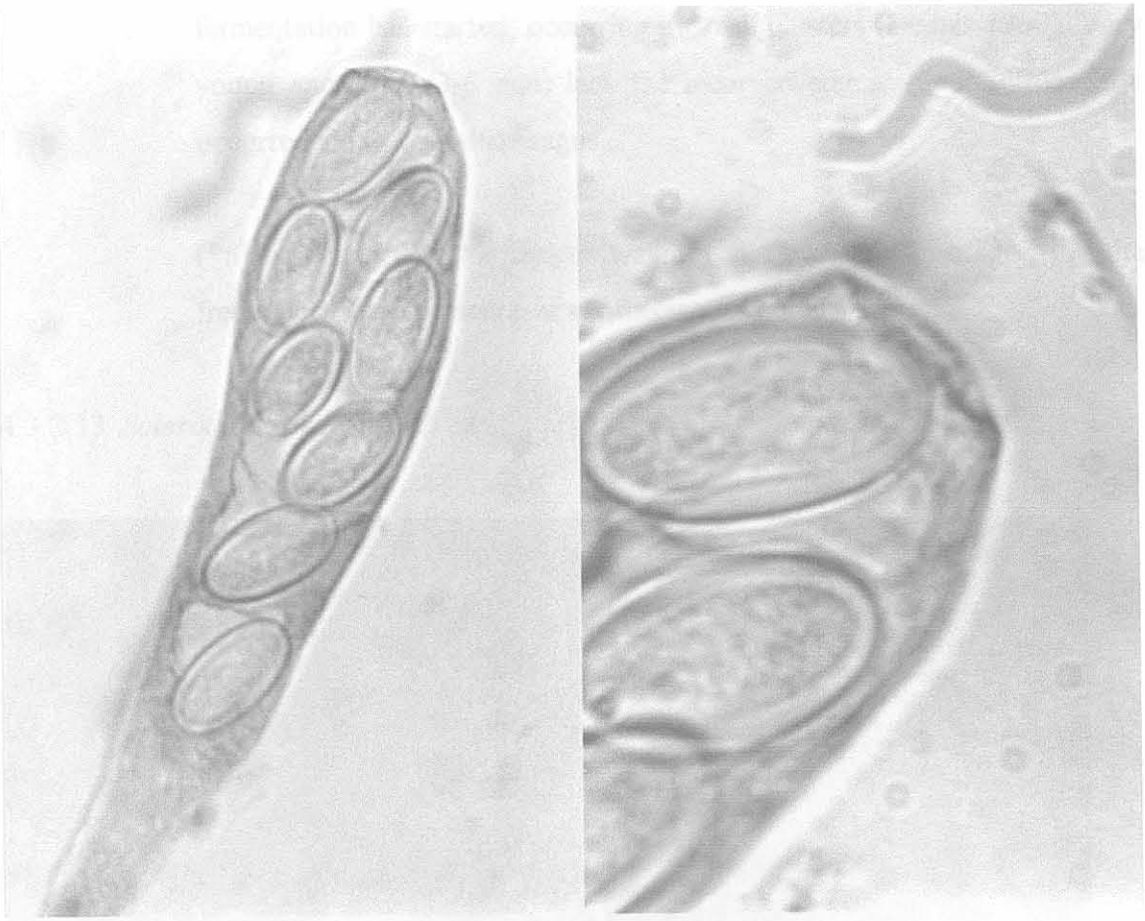

Figure 4 a. *P. vesiculosa* ascus with 8 ascospores. b. Apical ring stained blue in iodine.

Diameter	: Fruiting body on substrate only: 10 mm – 67 mm
Colour – top	: Golden light brown
Colour – bottom	: Golden light brown
Appearance	: Exposed sessile apothecia, cupulate, sinuate incurved edges, blister- like pockets between hymenium and excipulum form on older specimens,
Spores	: Ascospores: smooth uninucleate, eguttulate, elliptical, 22.0–24.2 x 13.0–14.8 µm
Asci	: Unitunicate asci, simple operculate, apical ring stains blue with iodine

Remarks: This fungus was always associated with very wet substrate where no fermentation has started, occurring in small clusters (Dennis 1960). Very young apothecia (3-5 mm) lack the incurved edges. Algae infrequently occurred along with this fungus.

(*:from Table 6 – this fungus was never sub cultured on agar, but the frequency of the presence of apothecia was noted.)

4.3.2.13 *Sclerotium rolfsii* Sacc.

Figure 5 A collection of sclerotia of *Sclerotium rolfsii* on wheat straw substrate.

Although this fungus was not successfully cultured in the laboratory (indicated by * in Table 6), it was identified from the characteristic sclerotia visible in Fig. 3. Collections of light brown sclerotia (2mm diameter), trapped in arachnoid, effuse patches of white mycelium, were found on drier substrate near openings in growth containers where insects have visibly gained access.

4.3.2.14 *Trichothecium roseum* (Pers.) Link ex Gray

Diameter	: 70 – 78 mm
Colour – top	: Light apricot pink
Colour – bottom	: Pale pinkish-yellow
Appearance	: Flocculose, granular aerial mycelium, glistening spore clusters visible.
Spores	: Hyaline, 2-celled, ellipsoidal to pyriform, smooth and thick-walled with truncate bases, 11.0-27.2 X 8.0-11.5 µm

Remarks: Spores are borne in basipetal, imbricate zig-zag chains.

4.3.2.15 *Verticillium fungicola* (Preus) Hassebrauk

Diameter	: 3.1 –3.9 mm
Colour – top	: Off-white
Colour – bottom	: Beige
Appearance	: Smooth, cottony aerial mycelium
Spores	: In globose, slimy heads, conidia slightly curved, ellipsoidal to cylindrical, smooth walled, 1 or 2 guttules present, 3.5-7.0 x 1.2-2.2 µm.

Remarks: Although this fungus is a pathogen on *Agaricus bisporus*, it was found infrequently on the *Pleurotus* substrate. It was never found in association with any diseased sporocarps.

4.3.4 List of the competitive fungi

This group of fungi were often observed colonising the substrate before the crop mycelium. In cases where *Pleurotus* mycelium was already present, the competitors would capitalise on any deviation in environmental conditions to overtake the crop mycelium. Any uncontrolled fluctuations in the temperature or humidity were especially conducive to such antagonism.

During harvesting the wounded crop mycelium were often the target of insects carrying spores of other fungi. The ensuing insect damage aggravated the insecure condition of the crop mycelium and these wounded areas often became serious infection points for *Trichoderma* and *Penicillium* spp. An abundance rating for the occurrence of the true competitive fungi is given Table 7.

Competitive fungus species	**Western Cape Type**	**Gauteng Type**
1. *Cladobotryum dendriodes*	-	B
2. *Coprinus*	C	A
3. *Geotrichum candidum*	B	-
4. *Penicillium chrysogenum*	C	C
5. *Penicillium brevicompactum*	-	C
6. *Rhizopus stolonifer.*	C	-
7. *Trichoderma harzianum*	B	B
8. *Trichoderma viride*	B	A
9. *Trichurus spiralis*	A	A

Table 7 Species list and distribution of true competitive fungi isolated from *Pleurotus* substrate. The type refers to the abundance with which the species occurred in the fractions of ***all*** 10 the samplings done

A: Present in all fractions, ≥ 3 colonies per plate

B: Present in some fractions, ≥ 3 colonies per plate

C: Present in some fractions, ≤ 2 colonies per plate

4.3.5 Brief descriptions of the competitive fungi on PDA

Unless mentioned to be different, the descriptions of the colony characteristics of competitive fungi are based their appearance on PDA, grown at 25°C for 10 days (pH = 5.8). Their spore descriptions are based on spores obtained from PDA blocks placed under black (near-UV) light until adequate sporulation was obtained (average not more than 7 – 10 days).

The use of identification sources other that Domsch *et al.* (1993) is mentioned under the remarks for each fungus. All terminology used were confirmed from Hawksworth *et al.* (1996).

4.3.5.1 *Cladobotryum dendroides* (Bull. Ex Merát) Gams & Hoozemans

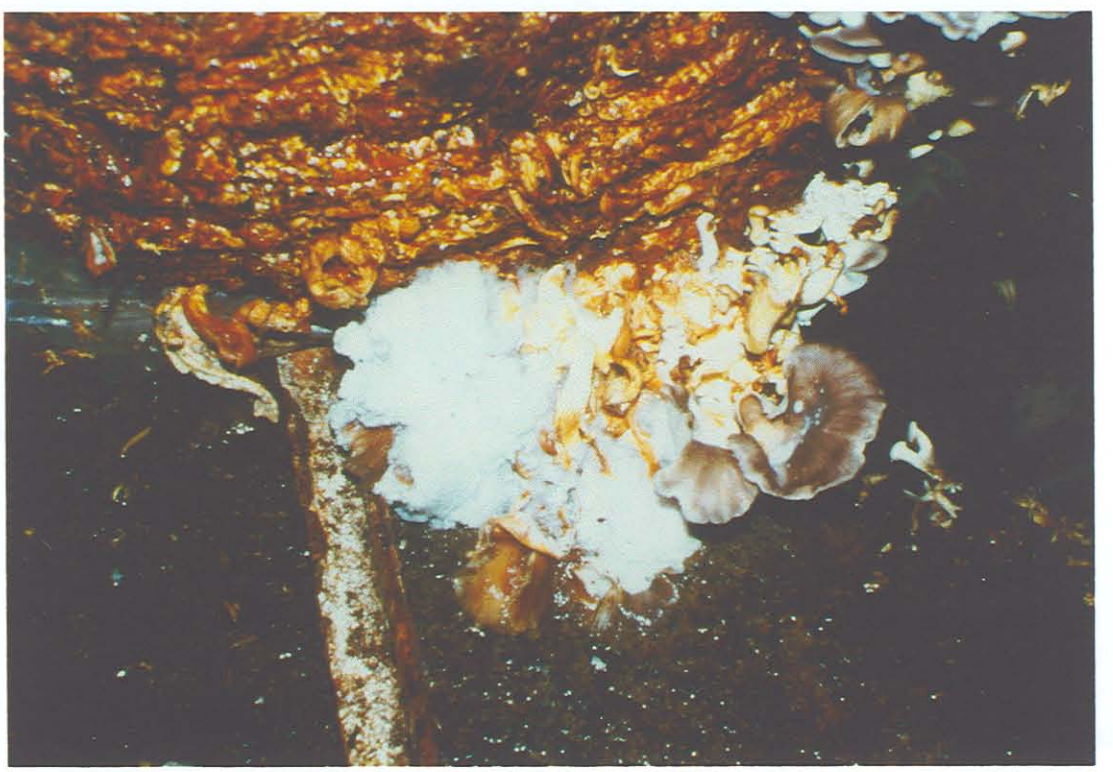

Figure 6 A cluster of oyster mushrooms, at the bottom of a cultivation column, severely infected by *Cladobotryum dendroides.*

Diameter : > 90mm

Colour – top : Dull greyish-white

Colour – bottom : Deep red

Appearance : Older parts (center) becomes lanose, towards edges more loose, spindly.

Spores : Conidia cylindrical to clavate, smooth, 2-4 celled, basal scar, thin cell walls, 22.5-26.8 x 6.9–8.8 µm.

Remarks: Conidia are borne on conspicuous conidiophores. This species was not only a competitor, but was also found to be a serious pathogen.

4.3.5.2 *Coprinus congregatus* (Bull. Ex St Amans) Fr.

Figure 7 *Coprinus* sporocarps on *Pleurotus* substrate. The inhibition zone is visible as the dark/uncolonised strip above the *Coprinus* caps.

Diameter : Cylindric-ellipsiod to ovoid unexpanded cap = 5 – 17mm, Convex expanded cap = 20 –25mm (fig.7).

Cultured mycelium (on oatmeal agar) 70mm.

Colour – cap (substr.) : Greyish buff with light brown centre

Colour – top (culture) : White

Appearance : On substrate – no veil, sulcate striate cap, gills light brown-grey turning dark later, black when deliquescent.
Cultured mycelium wispy, flattened.

Spores : Ellipsoid, pore slightly excentric, dark brown, 11.2-13.9 x 6.3-7.5 µm

Remarks: On the substrate, the fruiting bodies deliquesce rapidly inside the plastic bags. Small clumps of fruiting bodies are sometimes seen forming exceptionally long stems, looking for gaps in the bags. The bases of the stems appear to be connected. This fungus is an aggressive and hardy competitor that seems to be able to withstand the temperatures during pasteurisation. Insects can spread the spores (Orton & Watling, 1979).

4.3.5.3 Geotrichum candidum Link ex Lehman

Diameter : 70 mm

Colour – top : Dull white

Colour – bottom : White

Appearance : Flat, little aerial mycelium, fine granular texture, becoming slimy

Spores : Borne in chains, ellipsoidal to barrel shaped, arthroconidia, 7.2-15.0 x 4.1-8.3 µm

Remarks: This fungus was never isolated from Gauteng farms. It caused severe losses in production on a Western Cape farm in May 1996. The problem was remedied by severe sanitation (cook-out, destruction of substrate material, scrubbing shelves with fungicide). Although isolated afterwards, it was never again problematic.

The Plant Protection and Pest Control Division of the Agricultural Research Council identified the following two *Penicillium* species. They were isolated from harvest lesions and inhibitory zones in the substrate.

4.3.5.4 *P. crysogenum* Thom

4.3.5.5 *P. brevicompactum* Dierckx

4.3.5.6 *Rhizopus stolonifer* (Ehrenb. Ex Link) Lind.

Diameter : 90mm Petri dish overgrown in 4 days
Colour – top : White with small black spots peppered throughout
Colour – bottom : Beige
Appearance : Dense, hairy mass with black sporangia clearly visible.
Spores : Ellipsoid, textured, borne on long sporophores, 5.6-8.3 X 7.4-8.2 µm

Remarks: This fungus was very infrequently isolated from Gauteng farms. It occurred more often in the Western Cape and caused a serious problem in September 1998. Once again, the problem was remedied by careful and complete sanitation of the facility.

4.3.5.7 *Trichoderma harzianum* Rifai

Diameter : 90mm in 5 days
Colour – top : Dull green with white and lighter green patches
Colour – bottom : White / colourless
Appearance : White, hairy (tufted) mycelium covered with patches of yellow to dull green dense green dust. Conidia on aerial mycelium. White patches not bearing conidia.
Spores : Globose to sub-globose / sub-obovoid, smooth-walled conidia 2.5-3.0 x 2.3-2.7 µm (L: B < 1.25)

Remarks: Conidiophores branched, phialides short. Very regularly isolated, never on sporocarps – not pathogenic. Spores transmitted by wind, insects, water. Tend to take over the substrate, making colonisation by crop mycelium difficult.

4.3.5.8 *Trichoderma viride* Pers. Ex S.F. Gray

Diameter : 90mm in 5 days

Colour – top : Dark green.

Colour – bottom : White / colourless

Appearance : White, tufted mycelium covered with dense dark green dust.

Spores : Obovate to ellipsoidal, definite texture on spores (roughened) 3.3–5.1 x 3.1-4.9 µm.

Remarks: Phialides longer and more sparsely branced than that of *T. harzianum*, with typical coconut smell detected. Very frequently isolated, never on sporocarps – not pathogenic. Colonise substrate before crop mycelium does. Spores transmitted by wind, insects, water.

4.3.5.9 *Trichurus spiralis* Hasselbr.

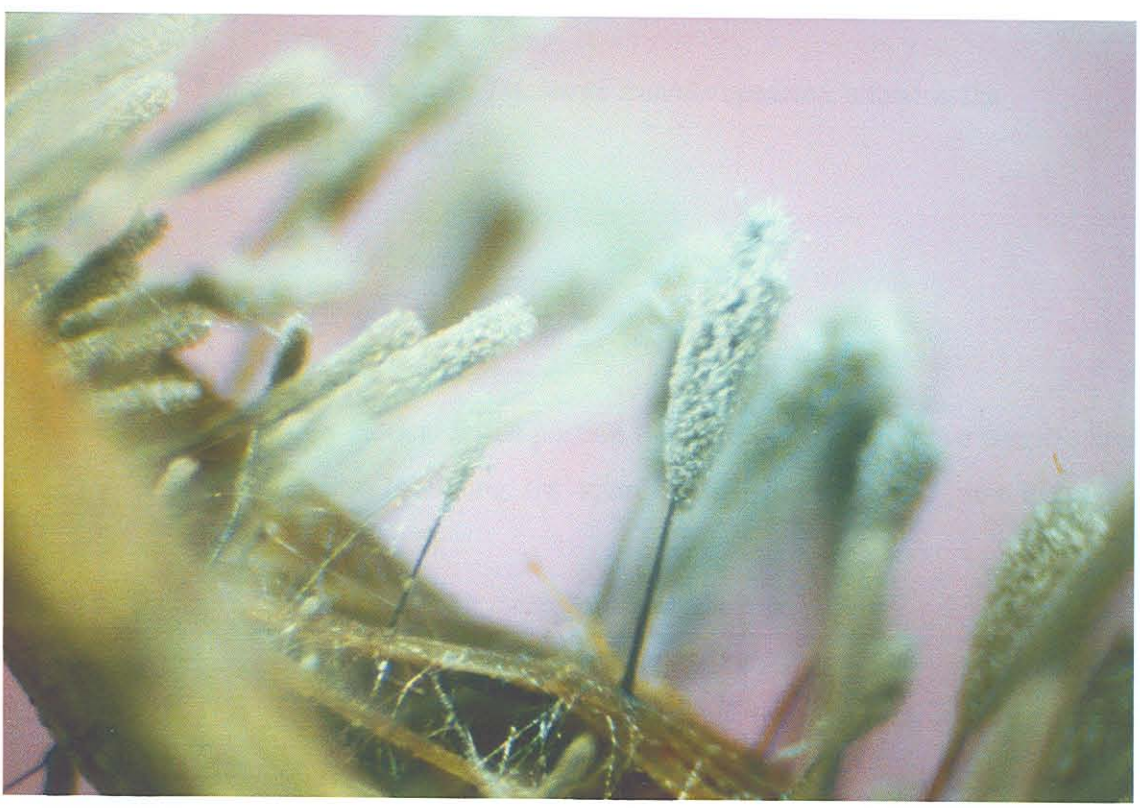

Figure 8 Dissection micrograph of the synnemata of *Trichurus spiralis* on straw fragments taken from the substrate.

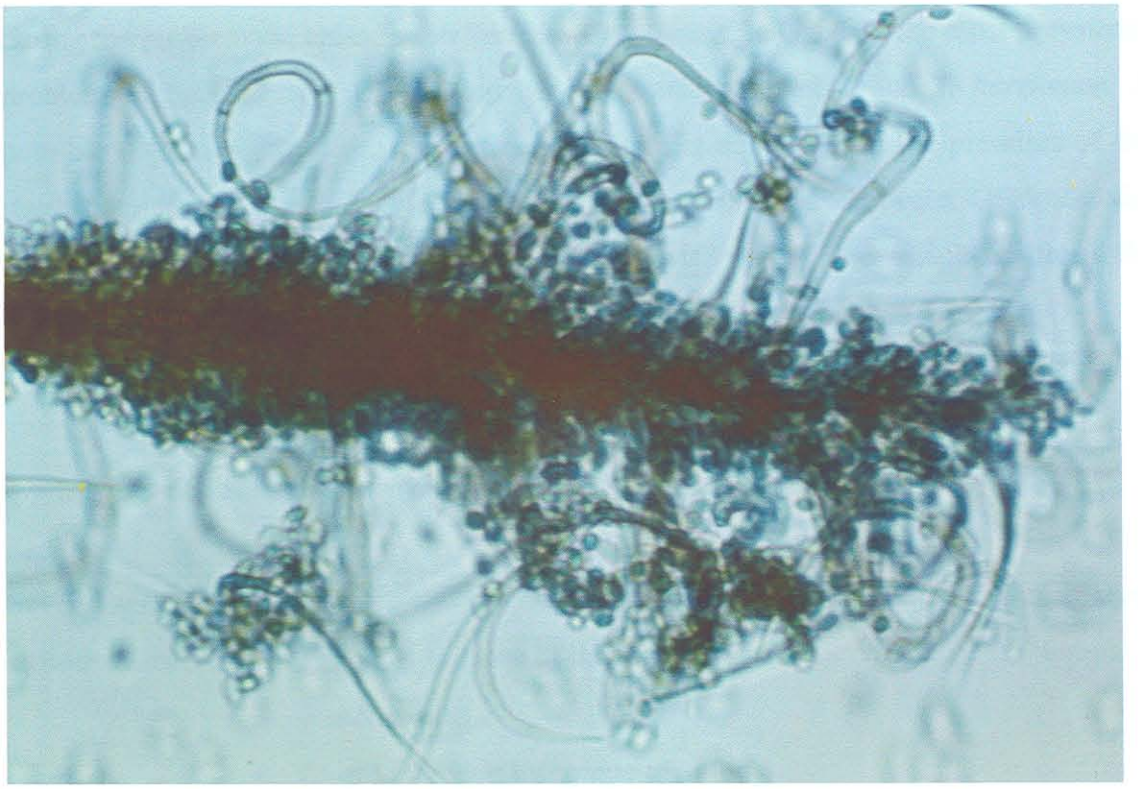

Figure 9 Light micrograph of a *Trichurus spiralis* synnema, showing the protruding spiral setae.

Diameter	: 30 - 36 mm
Colour – top	: Grey to brownish grey
Colour – bottom	: White / colourless
Appearance	: Cylindrical fertile portion with a tousled appearance due to spiral setae loosening the spore-mass, synnemata 4-7 mm including sterile stalk.
Spores	: Dry, ovoid to mitriform, truncate base, 4.5-6.7 x 3.7-4.3 µm. Conidiogenesis is annellidic and proliferous.

Remarks: This was by far the most prevalent of all fungi in both regions studies. Although it never appeared to wipe out the crop mycelium completely, it was present in every sample taken. The number of fractions from which it was isolated, varied between 25 –100 %.

T. spiralis, T. harzianum and *G. candida* were the most prevalent competitive fungi during the problematic episodes on Western Cape farms. In Gauteng the most prevalent / problematic species were *C. congregatus, T. spiralis, T. viride,* and *T. harzianum*. A possible source of infection is spent compost that is deposited on the premises of the mushroom farms. The only truly pathogenic relationship was between *C. dendroides* (Gauteng) and *Pleurotus* mycelium. Although *V. fungicola* was isolated from the substrate it was never present as a pathogen of the *Pleurotus* mycelium.

4.4 Infective episodes

Several serious episodes of infection were observed and recorded (table 9). During these episodes farms experienced one or more of the following:

- insect attacks
- fungal infection by competitive fungi
- weed fungi invading damaged or compromised bags and substrate
- nematode infections

These episodes sometimes led to the loss of a complete crop, although some part of it could often be salvaged. They were all financially damaging, nevertheless.

Western Cape	Gauteng
	May 1995
August, September 1995	September 1995
March, April, May 1996	April, May 1996
August, September 1996	August 1996
March, April 1997	May 1997
September 1997	August, September 1997

Table 8 Periods during which infective episode were experienced on farms in the two regions.

4.5 Meteorological information

Two regions were studied, each one with very distinct weather patterns. The Western Cape is a winter rainfall region and Gauteng gets summer rainfall.

In the Western Cape summer is characterised by the high incidence of extreme winds (often more than 28.8 km/h) (Fig.10). These winds then blow hot, dry air into the region (Fig's. 11 & 12) and have a pronounced effect on the other meteorological factors for this region. During this period the relative humidity (Rh) and cloud cover is also low (Fig's. 13 & 14), despite the coastal proximity of the region.

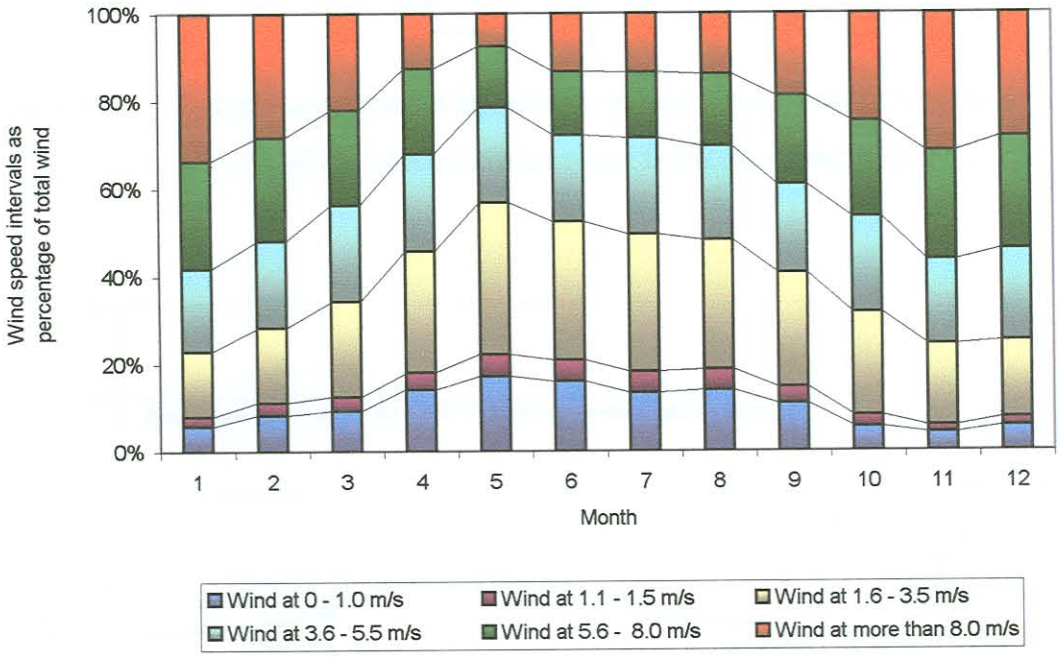

Figure 10 Hourly wind analysis to determine the percentage frequency of the occurrence of wind speeds in the specified categories (m/s) in Cape Town (n=10).

The reciprocal pattern develops when the rainy season begins. As the temperatures drop, the frequency of extreme winds also decreases. Cool, moist air moves in and the region experiences more cloud cover, fewer hours of sunshine and higher levels of Rh.

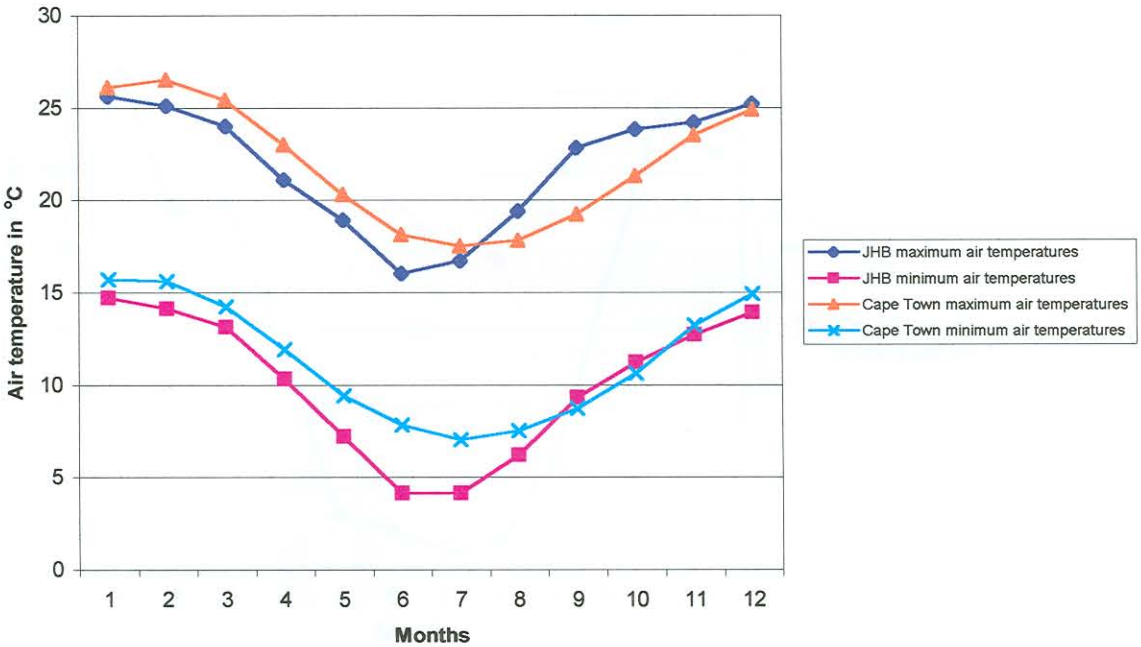

Figure 11 Comparison between the average air temperatures (in °C) of the two regions within which the mushroom farms are situated (n=29).

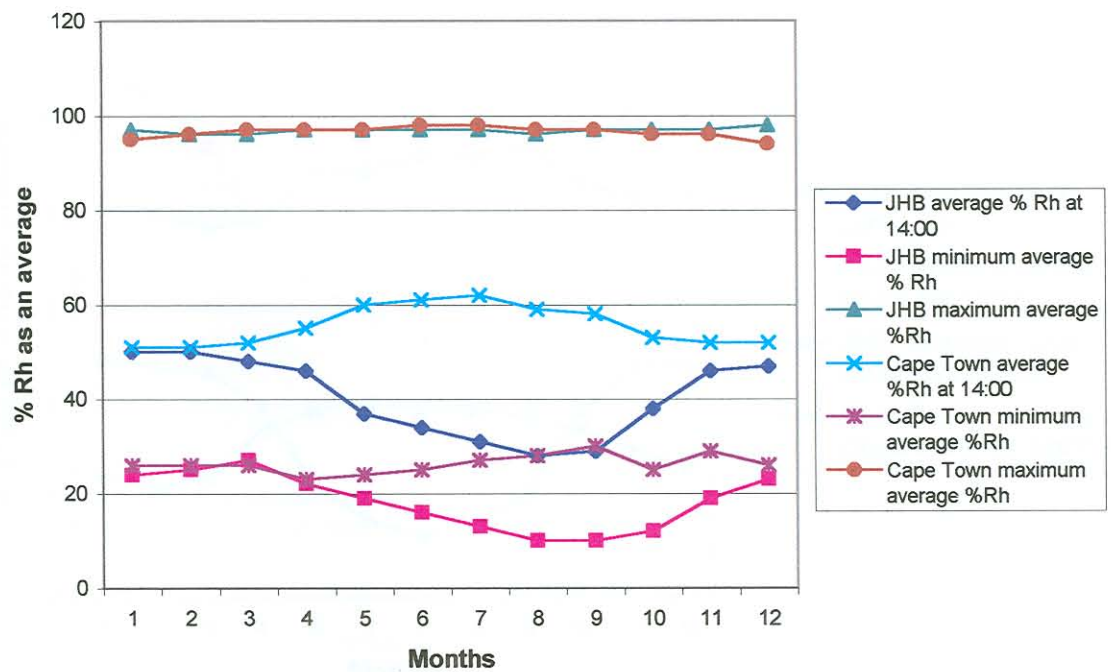

Figure 12 Comparison between the average relative humidity (% Rh) of the two regions within which the mushroom farms are situated. Averages of the measurements taken at 14:00 as well as the minimum and maximum values are compared (n =28).

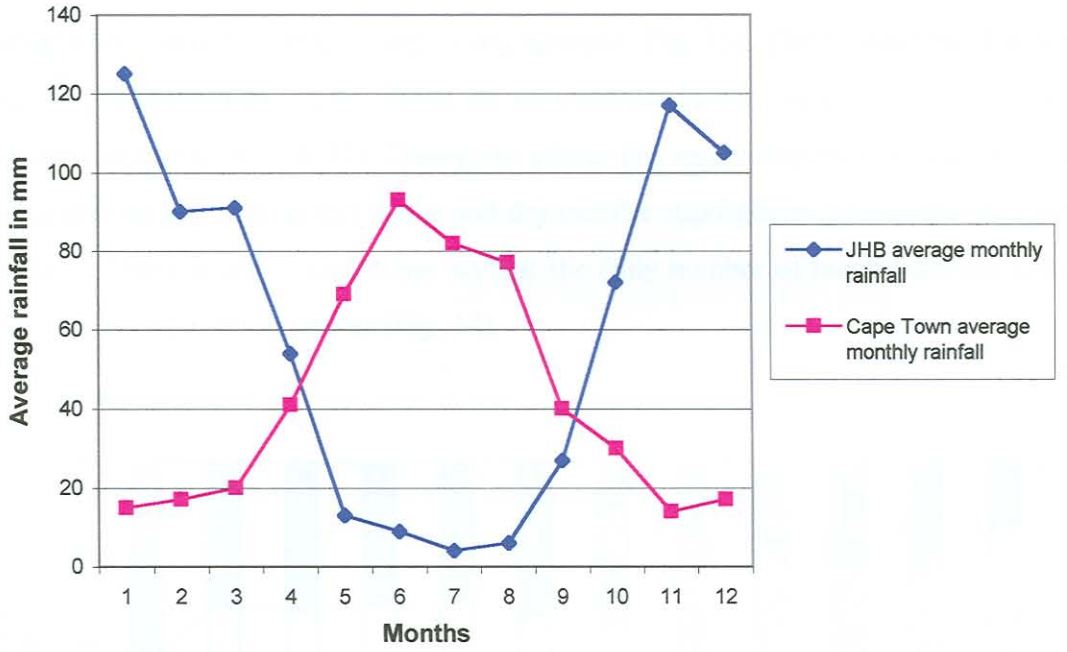

Figure 13 Comparison between the average monthly rainfall of the two regions within which the mushroom farms are situated (n=26)

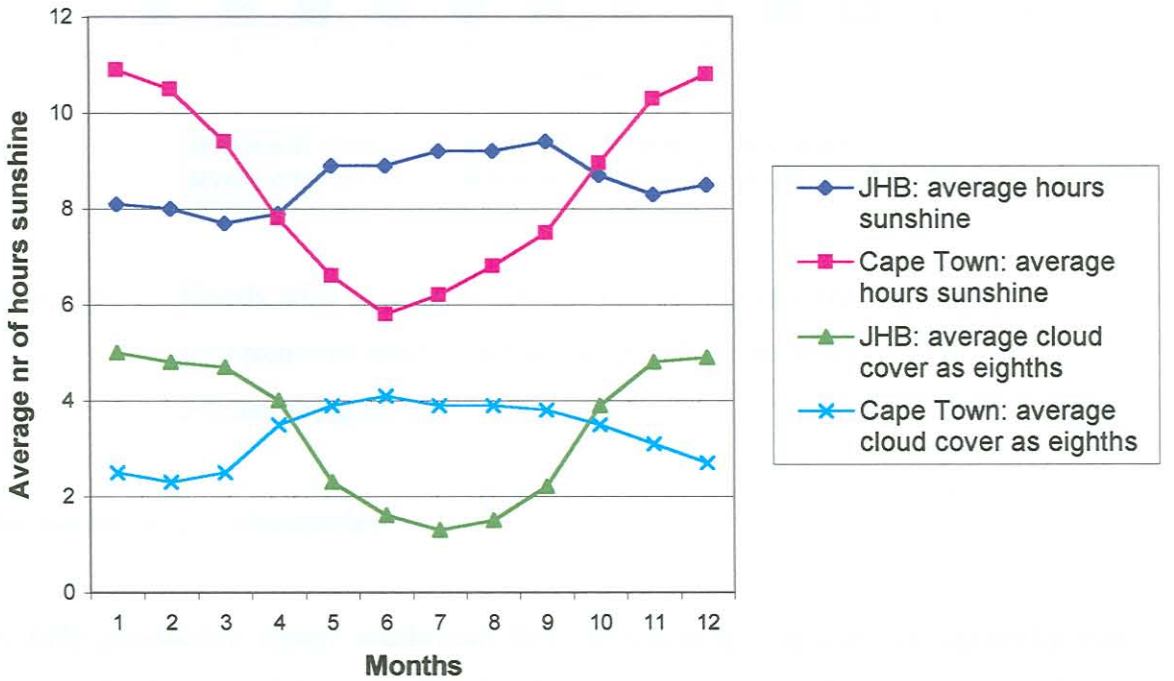

Figure 14 Comparison between the average number of hours sunshine and the average cloud cover of the two regions within which the mushroom farms are situated. Cloud cover is not measured in hours, but as eighths of a whole day.

In Gauteng the limited occurrence of extreme winds coincides with the onset of thunderstorm activity during spring / early summer (Fig. 15). The summer rainfall period (Fig. 13) is marked by warm, moist air as opposed to the summer conditions in the Western Cape (Fig.'s 11 & 12). During the winter this region experience moderate winds that aggravate the cold temperatures and dry out the atmosphere causing the Rh to drop very low. There is little cloud cover so that the daily number of hours sunshine actually increase, despite it being winter (Fig. 14).

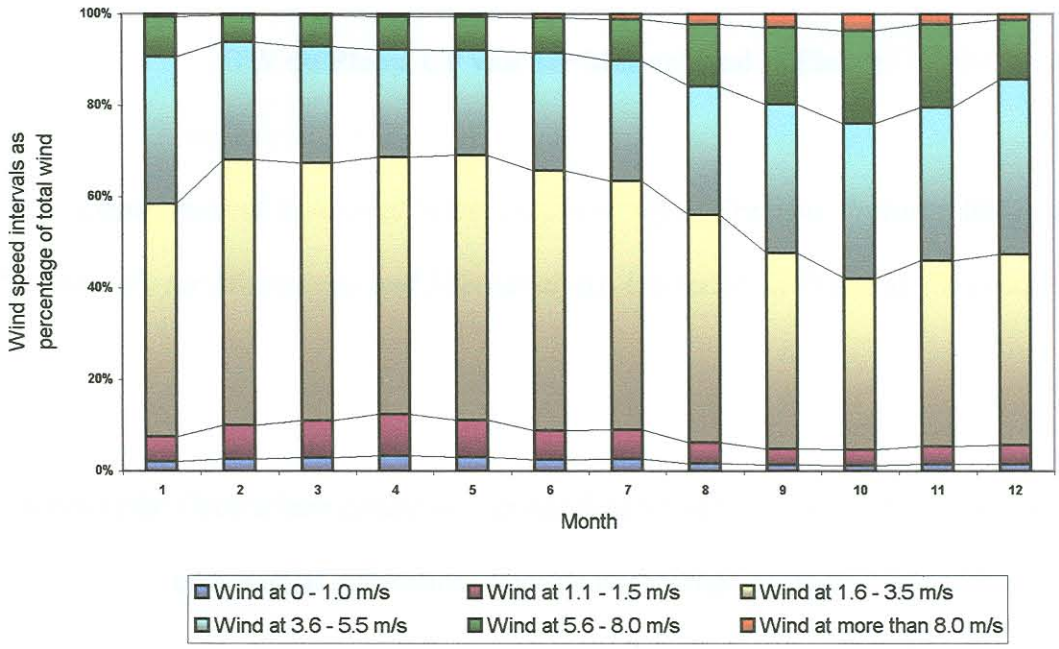

Figure 15 Hourly wind analysis to determine the percentage frequency of the occurrence of wind speeds in the specified categories (m/s) in Johannesburg (n=10).

4.6 Latest fungal adversaries

A fully productive oyster mushroom farm in Gauteng (capacity 200kg/week) was completely devastated by an outbreak of *Monilia sitophila* (Montagne) Sacc. during September 1999. It was established that the fungus came onto the farm through straw that had become wet before it was delivered to the farm. Even double pasteurisation of the straw could not eradicate the fungus.

CHAPTER 5

First report of *Geotrichum candidum* associated with *Pleurotus* cultivation in South Africa and microscopical comparison with *Sporendonema purpurascens*.

GW du Plooy, CF van der Merwe* and A Eicker

Department of Biological Sciences, University of Pretoria, Pretoria, 0001

**Laboratory for Microscopy and Microanalysis, University of Pretoria, Pretoria, 0001*

Keywords: *Geotrichum candidum*, *Sporendonema purpurascens*, *Pleurotus* species, commercial cultivation, electron microscopical study

First report of *Geotrichum candidum* associated with *Pleurotus* cultivation in South Africa and microscopical comparison with *Sporendonema purpurascens*.

ABSTRACT

***Geotrichum candidum* has been isolated from the substrate of a commercially produced *Pleurotus* species. Mushroom growers still confuse this fungus with *Sporendonema purpurascens* due to its popular name (red geotrichum). A morphological comparison between the two fungi was done in order to verify the substrate isolate.**

INTRODUCTION

Several competitive fungi occur on the substrate of commercially produced mushrooms. Two types of mushrooms are cultivated commercially in South Africa, namely *Agaricus bisporus* (Lange) Imbach and several *Pleurotus* (Fries) Kummer species [2, 3]. Each one of them has a unique environment within which it will bear a crop. The different substrates invite different competitors and often the presence of these in their respective environments is an indication of a sub optimal condition in that substrate environment [13]. Among the many competitive mould already described on Agaricus[3], are two often-confused species.

Geotrichum candidum Link ex Lehman has been described on the substrate of *Agaricus brunescens* [12]. Mushroom growers, however, know *Sporendonema purpurascens* as the lipstick mould or red geotrichum. Both of these fungi are usually associated with problems in the casing soil. Although Wood pointed out in 1957 that the two species are not related[17], Vedder still noted them as synonyms [16] and Stamets & Chilton, in a very popular and widely read book [14], also gave the common name of *Geotrichum* as lipstick mould. Hoog *et al.* reviewed *Geotrichum* teleomorphs distinctly apart from *S. purpurascens* [7] but in an important reference book Fletcher *et al.*[5] again used the popular name "red geotrichum".

In the summer of 1996/1997 an infection of *G. candidum* was identified as the cause of a total loss of production of *Pleurotus ostreatus* in the Western Cape. It has not previously been associated with infection of the *Pleurotus* spawn or substrate. Based on so many confusing references, it was decided to confirm the identity of the isolated fungus with electron microscopy.

MATERIALS AND METHODS

Isolation and culturing of G. candidum

G. candidum was obtained from samples from an infected commercial operation for the cultivation of *Pleurotus*. Production was halted and a range of samples was collected in order to determine the origin of the infection upon failure of the spawn run. The samples consisted of material taken from various stages of production and included raw straw, substrate in production, spent substrate, new grain, spawn, waste from the preparation chamber and soil from the storage facility. This range was selected in order to determine the origin of the infection. The samples were processed using both direct plating and dilution series onto potato dextrose agar (PDA) plates to which either a solution of 0,5% Rose Bengal or 0.25% chloramphenicol was added. All the plates were incubated at 25°C for a maximum of 7 days. Sporulation was encouraged using near ultraviolet light (black light). Individual colonies were identified and aerial mycelium was removed from each and sub-cultured on PDA, water agar with 10% cellulose and oatmeal agar.

S. purpurascens was obtained from the mycology collection at the University of Pretoria (UP-127) and sub-cultured on normal PDA. Both transmission and scanning electron microscopical studies were undertaken on actively growing colonies of *G. candidum* and *S. purpurascens*. Very thin blocks of agar (3 x 3 mm) containing actively growing mycelium from the cultured plates of each species were prepared for electron microscopy.

Preparation for scanning electron microscopy

Two techniques were used to prepare the material for Scanning Electron Microscopy (SEM). Since the procedure for critical point drying disrupts many of the delicate conidial structures [6, 8], it was decided to use simplified versions of the standard procedures. For the first technique the agar blocks were placed in a new Petri dish and 1 ml of 1% aqueous OsO_4 placed in the lid of the Petri dish as scattered small drops. The lid was inverted over the excised blocks and the material fixed by exposing the mycelium to OsO_4 vapour for 24 hours. Thereafter it was mounted on a SEM stub and dried in a desiccator for another 24 hours. Finally it was sputter coated with gold using a Polaron E5200 sputter-coater and viewed with a JEOL 840 SEM.

It has been shown that specimens can be prepared for the SEM without chemical treatment[11]. Therefore, as the alternative method for the SEM study, the 3 x 3mm agar blocks containing active growth were freeze-dried. This was done by mounting the agar blocks on Perspex strips (8 x 5mm) and rapidly plunging them in the cryo chamber of a Reichert KF80 plunge freezer containing liquid propane at –180°C. The frozen Perspex strips containing the material were removed after 10 minutes and transferred to a pre-cooled slotted copper block (63 x 63 x 15mm) immersed in LN_2. The copper block was fitted with a thermocouple and heater and the whole assembly transferred to a Fisons high vacuum unit. The temperature of the copper block was still under –100°C when a vacuum of 1×10^{-4} Torr was reached [9]. After ± 16 hours the temperature of the copper block was at 0°C and it was accepted that freeze-drying was completed. At this stage the block was

heated to 25 °C before the material was removed from vacuum. The Perspex strips were mounted on SEM stubs and coated with gold using a Polaron E5200 sputter-coater.

Preparation for transmission electron microscopy

Primary fixation was done in 2 ½ % glutaraldehyde plus 2% formaldehyde (prepared from paraformaldehyde) in 0.075M sodium phosphate buffer at pH=7.4 for 2 hours. After fixation the material was rinsed 3 times in 0.075M sodium phosphate buffer and then fixed in 1% aqueous OsO_4 for 2 hours. The material was rinsed 3 times in distilled water and dehydrated in a series of Ethanol concentrations (30%, 50%, 70%, 90%, 100%). It was finally embedded in modified Quetol 651 epoxy resin[15].

Thin sections were made with a Reichert Ultracut E Microtome using a diamond knife. Sections were contrasted with 4% aqueous uranyl acetate (10 minutes) and lead citrate [10] for 2 minutes. It was viewed with a Philips EM301 transmission electron microscope.

RESULTS

Isolation and culturing of G. candidum

It was found that *G. candidum* was present in the sample range from newly prepared spawn onwards. No contamination by *G. candidum* was detected in the soil or unseeded straw samples. In view of this, it was argued that contamination took place during the inoculation process itself. This conclusion was substantiated upon investigation of the method used during spawn transfer. The rye grains tended to stick together after sterilization in plastic bags. It was the practice to break the lumps apart by hand before adding the *Pleurotus* mycelium. Even with proper sterilization of the hands, fungal spores can be transferred from air, clothing, hair, or any other surface and quickly colonize the spawn bags. Upon visual inspection, the rye was colonized by a white growth, but the grains were not properly matted down. The grain became slimy when handled. Small amounts *Pleurotus* mycelium was still present, but *G. candidum* forms prolific numbers of spores, out-competing *Pleurotus.*

Scanning electron microscopy

The first method using 1% aqueous OsO_4 vapour as a fixative rendered the best results for *G. candidum*. Pitting was observed on the material prepared according to the second (freeze drying) method. This was probably due to an effect of the liquid propane on the thick mucilaginous layer that covers the hyphae of this fungus. The results on *S. purpurascens* were similar in both cases. The hyphal damage seemed excessive and the reason for this was determined with the TEM study.

Arthrocatenate conidia (*G. candidum*) are shown in figure 1. These are formed through holoblastic conidiogenesis. Due to random schizolytic secession hyphal fragments are not of constant size and the separation of the septal walls of adjacent conidia can be seen. The mucilaginous layer is also clearly visible. The enteroblastic conidia of *S. purpurascens* are trapped in the collapsed conidiogenous cell within which they were formed (Fig. 2). The desiccated nature of the sample clearly indicates the dry nature of the mycelium. Individual conidia, rather than chains, are formed by the disintegration of some conidia during formation thereof (arrows, Fig. 2).

Transmission electron microscopy

The initialization of the *G. candidum* conidia from the inner cell wall can clearly be seen in the lateral blastoconidium being formed in figure 3. The outer cell wall of the hyphae starts to split apart once the distal septa are completely sealed off. Remnants of both cell walls can be seen at the slightly convex septum end of each barrel-shaped conidium (Fig 4). Although the conidia are not always of equal size, there is no disintegration of adjoining cells. Each conidium has a thick, multilayered wall whereas *S. purpurascens* has thinner walls and the conideogenic nature of the outer layer is clear (arrow, Fig 5)

The damage to the hyphal structures of *S. purpurascens* can be ascribed to the empty hyphal sections that are apparent with the TEM studies (Fig. 6). These empty sections originate when the cytoplasm of one conidium in a pair of adjacent conidia dies and disintegrates. In this way the hyphae content is fragmented. Figure 6 shows the differentiation of the conidium from the inner hyphal wall. The neighboring cell

disintegrates and the mature conidium is left in a collapsed tube consisting of the outer hyphal wall.

DISCUSSION AND CONCLUSION

The incorrect and confusing use of the popular name for *S. purpurascens* led to misidentification of *G. candidum* in the past [5, 12, 14, 16, 17]. Two very important features are useful in differentiating between the two species concerned. Firstly, mature colonies of *G. candidum* stay always white, but become mucilaginous on PDA. Mature colonies of *S. purpurascens* turn red on the substrate and a brownish-red on PDA and bright red on the substrate, therefore the popular name lipstick mould. It does not become mucilaginous like *G. candidum* either, but stays dense and wooly. Secondly, holoarthric conidiogenesis is a diagnostic feature of *G. candidum*, distinguishing it from *S. purpurascens* with enteroarthric conidiogenesis. Conidiogenesis of *G. candidum* and *S. purpurascens* is discussed in detail in Cole & Samson[1].

These problem fungi were usually associated with *Agaricus* cultivation and have been kept under control by improved cultivation techniques [3]. In this case *G. candidum* was proven to be the organism infecting the spawned grain, killing the crop mycelium. It is the first record of *G. candidum* on *Pleurotus* spawn and as well as substrate. Non-lignocolous and non-cellulolytic fungi find very little easily accessible carbohydrates in straw. *G. candidum* cannot utilize cellulose (Eveleigh & Brewer 1964), but wheat grains are filled with simple carbohydrates constituting the endosperm. The severity of the infection and

complete loss of production was due to the fact that damage was incurred during the spawning phase already.

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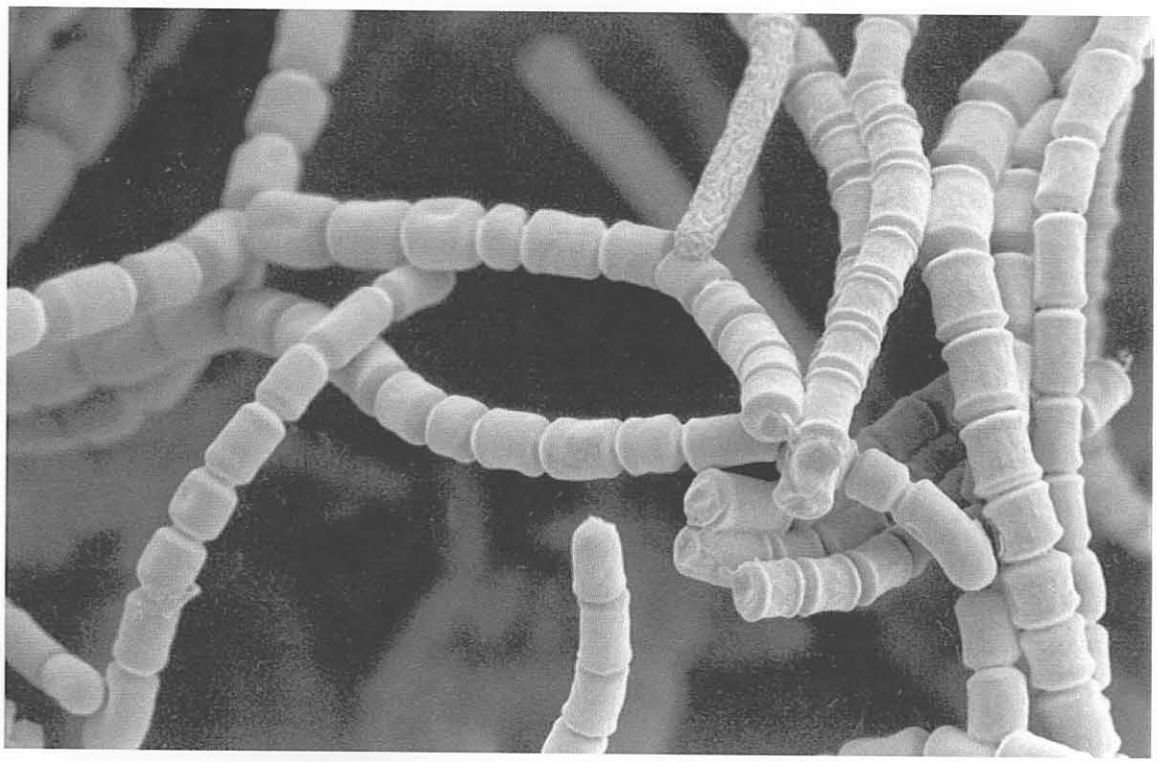

Figure 1

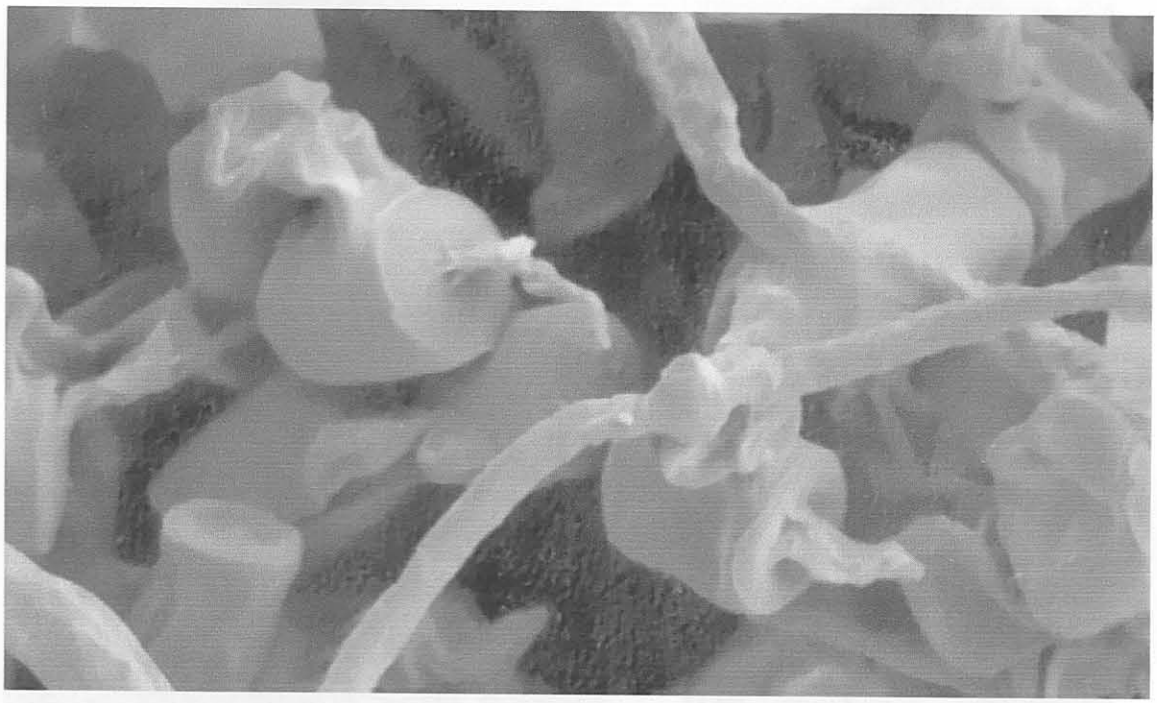

Figure 2

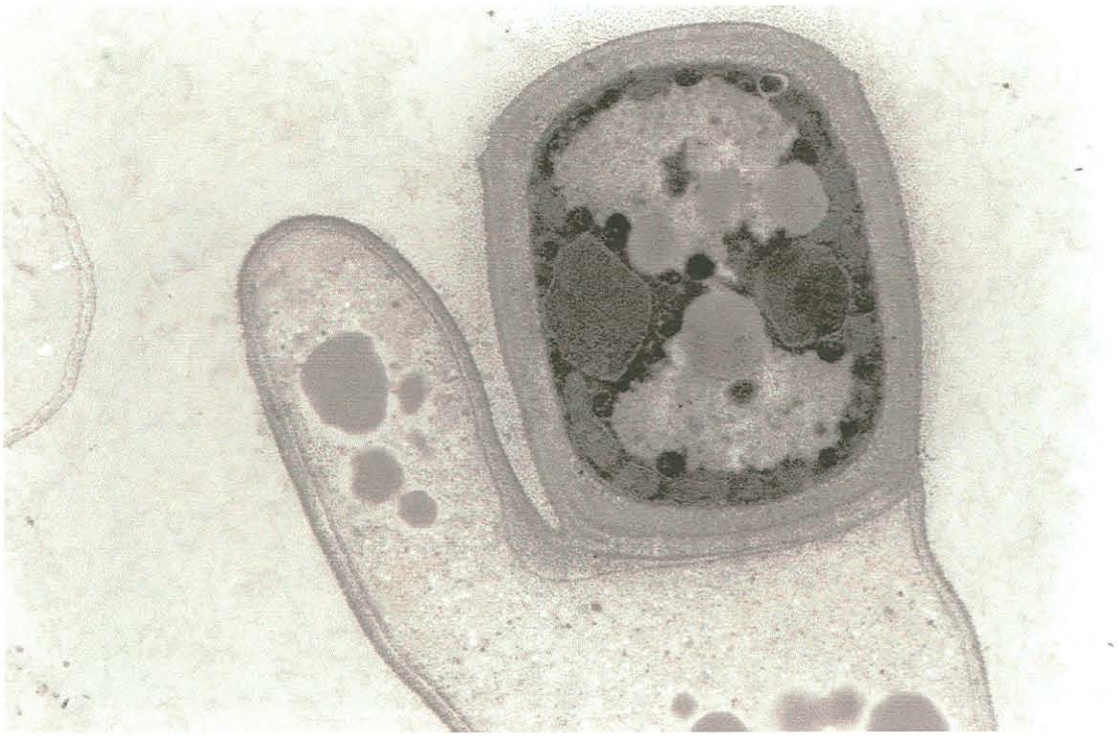

Figure 3

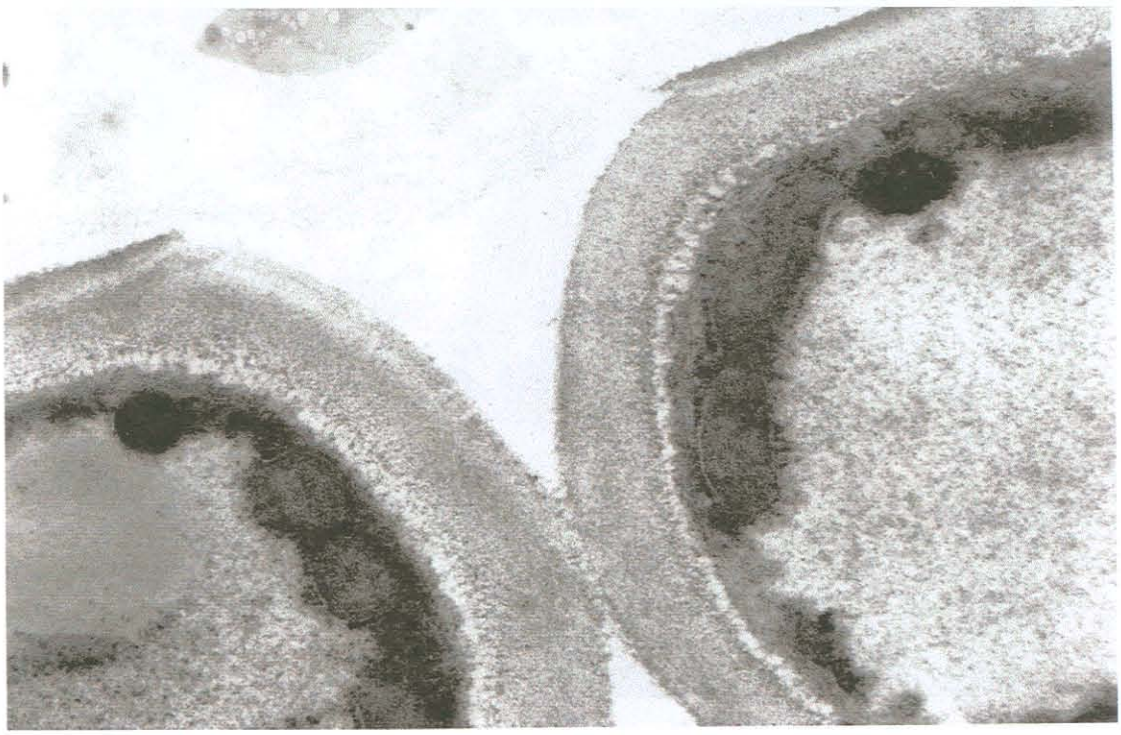

Figure 4

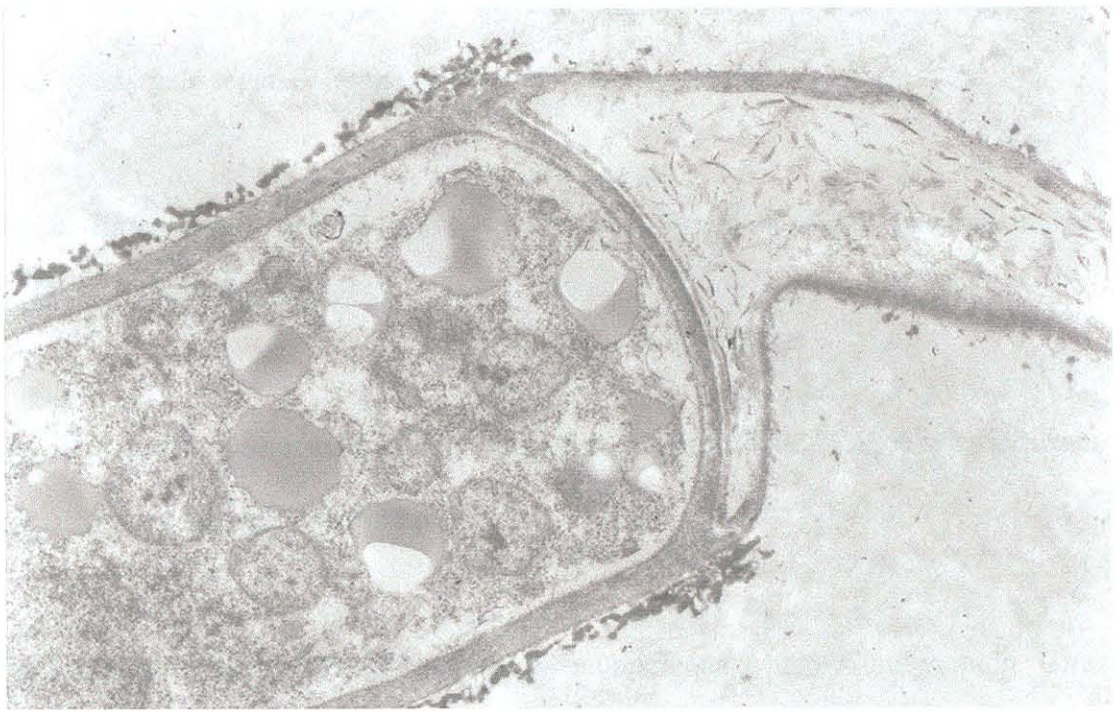

Figure 5

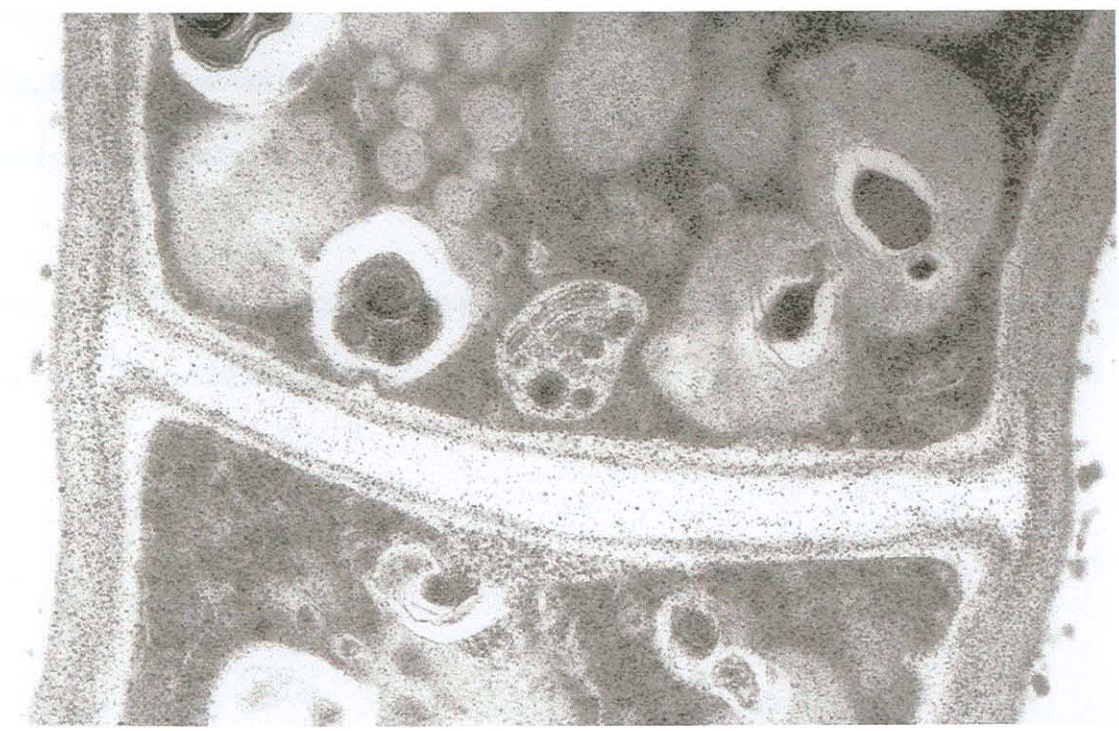

Figure 6

Fig. 1: Arthocatenate conidia of *G candidum*. Barrel-shaped conidia separate from each other, barely held together by the gelatinous layer covering them.

Fig. 2: Enteroblastic conidia of *S. purpurascens*. The collapsed hypha has trapped the solitary cylindrical conidium.

Fig. 3: Lateral blastoconidium formation in *G candidum.* The arrow indicates the abscission area between adjacent cells.

Fig. 4: Barrel-shaped to ellipsoid conidia have undergone schizolytic separation. The solid arrow indicates the gelatinous attachment at the slightly convex septum end. The line arrow indicates where the cell wall of the conidiogenous cell is visible.

Fig. 5: An enteroblastic conidium of *S. purpurascens* with the collapsed adjacent area. It is void of any cell contents, indicating that some cells degenerate within the hyphae.

Fig. 6: The hypha wall surrounding the two living conidia next to one another is clear (arrow). The conidium wall of *S. purpurascens* is thin compared to that of *G candidum*.

CHAPTER 6

DISCUSSION

6.1 Weed fungi

The majority of the fungi that were isolated in both geographical areas were opportunistic saprotrophs that will not be able to compete with *Pleurotus* mycelium in a healthy crop environment. They were never isolated as regularly as the aggressive competitors and their presence is regarded as being co-incidental. They can be regarded as true weeds of the crop and are therefore named as such. This group of fungi has ubiquitous existence and could have been isolated from the substrate for a number of reasons:

1. Improper pasteurisation.
2. Random inoculation by visiting insects.
3. Spores drifting on air currents and entering through ventilation system.
4. Spores introduced by workers moving between the bags.
5. Spent substrate kept in close proximity of the cultivating operations.
6. Water accumulation on floor and/or in substrate.
7. Too mush nutrient additives – especially nitrogen.
8. Temperature fluctuations.
9. Waste material after harvest left lying around.

The presence of these opportunistic saprotrophs can therefore be linked to the general standing of the farming practice. During times that problems in the production of the crop were encountered, large numbers of these fungi could be isolated from the substrate. This is despite the fact that none of them were observed to be able to compete aggressively on its own, indicating their opportunistic nature (refer Table 5). Many isolates were not identified because they occurred very sporadically in a few fractions.

6.2 Competitive fungi

There is a small group of serious competitors that are quite able of diminishing the size of the harvest, if not altogether making it fail. This group includes parasitic species and aggressive invaders of the substrate. *Trichurus spiralis*, *Trichoderma harzianum* and *Geotrichum candidum* were the most important species encountered in the Western Cape crop. The different conditions on the Highveld led to *Trichurus spiralis*, *Trichoderma harzianum, Trichoderma viride* and *Coprinus congregatus* being the most prevalent competitors in this area. Two parasitic species, *Cladobotyum dendroides* and *Verticillium fungicola* was isolated from Gauteng farms. Only the first species ever appeared as truly problematic. The second species was always present as a weed mould, and was never actually found on the sporocarps.

The fungi regarded as competitors are encountered frequently and tend to completely take over the substrate in localised spots on the columns. Any irregularity or disturbance in the substrate condition, even after harvesting has started, can lead to invasion by the fungi in this group. The same reasons listed in 6.1 above, applies to this group.

Pesticide-biotransformation by all the organisms involved should be investigated in a further study in order to determine more specific control measures, especially when unregistered pesticides prove to be effective. The use of registered pesticides is a market-related requirement. With increasing pressure to use chemical measures that are environmentally less costly, the effect of *Spirulina* algae as an additive in the control of infection by *Trichderma* spp. is an exiting area that needs closer attention as well (http://www.fungi.com/info/technique.html).

A number of problem species have been identified, but the ecology of the various infections has not been researched extensively. As with *Agaricus*-production it will be possible to correlate most of the problem species with specific conditions in either the substrate itself or in the preparation of the substrate (Van Greuning, 1989). This will require more careful observation by growers exposed to the daily cultivation of *Pleurotus* species.

6.3 Animal pests

The occurrence of insects, nematodes, mites and other invertebrate animals on *Pleurotus* substrate can be traced to the farming practice of each grower. Flying insects present a particular problem as far as the facilities are concerned. Their mobility and chemo receptive capacity enables them to find the smallest flaws in the mushroom grower's control measures. Unfortunately they are not the only pests to capitalise on negligence in this department. A single mycophagous nematode species was found, but it was the massive numbers of saprotrophs that caused problems on several farms. This should alert growers to the fact that absence of parasites is no guarantee for success.

Weed and competitive fungi, insects, mites and nematodes have been identified as recurring problems of local production practices. Climatic factors sometimes aggravate the problems and cause them to appear as a sudden breakout of pests and diseases. Closer inspection, however, will reveal that the substrate is in an epidemic state: the disorder only becomes apparent during the log-phase of the infection, which means that the condition had been building up for some time before the actual devastation becomes apparent (Agrios, 1988). Insufficient control can be pointed out as the single most important reason for such outbreaks.

6.4 Farming Practice and Hygiene

Due to the big differences in summer and winter temperature, it is practice to use different strains suitable to the various temperature conditions in each season (Chang and Miles 1989, Hauser 1986). However, in setting up suitable constructions for the commercial cultivation of *Pleurotus* spp., the control and maintenance of humidity and temperatures stays the most capital-intensive exercise for the South African cultivator. Keeping climatic conditions within the preferred range of the crop requires careful operational control and good insulated housing, since the repercussions of providing inadequate facilities and ill-matched climatic conditions can wreak havoc on a crop. Whilst it is true that many of the difficulties experienced by local cultivators can be ascribed to meteorological factors, there are several instances where the individual

cultivator also contributes to some difficulties. Numerous practice related reasons for infection of the substrate exist (Rinker, 1993; Fletcher *et al*, 1989; Stamets and Chilton, 1983), but all of them fall into only two categories namely, hygiene and farming practice.

Regardless of the substrate and the eventual cultivation technique the grower uses, from the preparation to the harvesting of the crop, there are certain basic principles that must be followed. All infections depend on the success of an invasive organism in the specific environment. In the case of mushroom production, the character of the available environment changes as the chronological events progress. This series of environments start with spawn production in a nutrient rich medium continues until inoculation of the substrate leads to a sealed plastic tube. This tube has very high humidity and very low rate of gaseous exchange. Aggressive competitors like *Coprinus* spp. and *Trichoderma* spp. can gain a foothold in early stages, killing or out-competing the crop mycelium. This environment is eventually changed again when the bags are pierced and pinning takes place. Then the humidity drops and the rate of gaseous exchange increases. During pinning there is also an increased demand for space and nutrients. If all went well during colonisation, the biggest threat now comes from insect vectors and predators. Once the sporocarps start to develop, the formation of ethylene and increased carbon monoxide levels changes the environment a final time, attracting larger animals such as fruit flies and slugs. The presence of these animals heightens the danger of cross-contamination.

6.4.1 Transfer of the infective organism

As mentioned in chapter 2, Stamets (1993) identified six modes of transferring pathogens to the spawn, which can be regarded as the first in a series of very different environments that the mycelium occupies during mushroom cultivation. However, these six modes are valid throughout the complete cultivation process and should be taken into consideration right to the point of shipping the harvested product. Since the mushroom mycelium is physically handled until the final environment (the growth container) is reached each one of these environments is prone to invasion by weed fungi or pathogens. Furthermore, not only pathogens, but also other contaminants can be transferred in these six modes.

The chain of possible events in the contamination of the substrate starts with the straw that is pasteurised and not sterilised. When insufficient heating and agitation takes place pasteurisation can actually trigger the germination of spores buried in deeper layers of substrate. Pasteurisation facilities must be kept scrupulously clean. Conglomerates of the leftover substrate provide very suitable habitats to contaminants. Contamination by dust and water from outside the production facility must be minimised. All floors should be of such material that they could be washed and sterilised frequently, the exception being the storage facility of new straw. Here the important point is to keep the straw dry and dust-free. Water that accumulates due to humidification must be washed out and the floors sterilised in-between runs. Strict adherence to good hygiene is very important.

The handling of the especially the spawn itself requires great care and meticulous attention to hygiene. All equipment must be sterilised in-between the preparation of batches. Hands must be disinfected and no conversations should be allowed during spawning. All used plastic bags, rubber bands, cotton wool and unsuccessfully colonised grain must be destroyed and not used again. Strict measures must be taken against mite infestation and any casual insect visitation. There must be no draughtiness or dust accumulation.

6.4.2 Adherence to hygiene

As far as the substrate itself is concerned, once again all hands touching it must be disinfected from the point of pasteurisation onwards. Workers should wear protective clothing and facemasks upon entering a growth room. These should be removed once outside, in order to reduce the risk of cross-contamination and exposure to allergens. All growth rooms should be sealed carefully and the entrances should be fitted with an effective trapping system that will prevent the entering of insect pests. Each batch of fresh substrate should be controlled all the way from a point at an outside storage facility to the final filling of the tubes. This control must be in terms of aeration, personnel access and proximity to unpasteurised or spent substrate.

Sealing each growth room perfectly is not only important in terms of pest control measures, but also in terms of the maintenance of constant temperatures and humidity.

Climate maintenance is one of the most cost-intensive areas in the production of the crop and by taking care here, the cultivator can make substantial savings. Sealing and insulating the rooms is expensive, but a commercial undertaking cannot be successful without it. The choice of material and the structural design is up to the cultivator, as long as maintenance and hygiene requirements are met adequately.

6.4.3 Farming Practice

A number of situations that led to actual problems were identified.

1. **Too high moisture content in the substrate.**

   This causes fermentative conditions to arise. Algal growth can take place inside as well as on the outside of the tubes, attracting herbivorous insects and slugs. Serious infections by *Peziza vesiculosa* could also be ascribed to wet substrate conditions.

2. **Wheat straw not chopped into small enough pieces.**

   Large pieces of wheat straw are more difficult to hydrate. The moisture in the substrate is therefore not distributed homogeneously. This causes the colonisation of the substrate to be impeded. Although the *Pleurotus* mushroom is a particularly strong competitor, the delay in the time it takes to colonise the bags gives the opportunistic saprotrophs a gap to establish themselves. Most of them have relatively short doubling time for biomass production and prolific sporulation abilities. Their sheer numbers cause an initial obstruction for *Pleurotus* colonisation of the substrate. In time, however, it has been observed that *Pleurotus* will overpower most competitive fungi with the exception of *Trichoderma* species.

3. **Growth tubes not properly packed.**

   If the tubes are packed too loosely, it can contribute to desiccation, poorly balanced stacks and leaching of nutrients. However, too densely packed tubes can cause resilient competitors to gain a foothold over the advancing crop mycelium, outcompeting it for space and nutrients.

4. **Post harvest crop waste allowed lying around.**

   Waste should be cleared away before the picking area is evacuated for the day. The mature tissue in harvested material continues to produce ethylene (C_2H_2) and carbon dioxide (CO_2). These are attractants for many saprotrophs.

5. **Insufficient access control for movement in and out of the growth chambers.**

   This is very difficult and costly to control, but it is one of the most effective ways of spreading an infection. Salary demands dictates the number of hands available and the number of hands available dictates the application of staff. The ideal situation will be to have each staff member dedicated to a task. This is not possible in most instances. An important consideration is the use of double entry doors to trap vectors and footbaths to prevent transmission of spore-laden dust. It does carry a cost penalty, but reliable yields and good turnover could be the pay-off.

6. **Sanitation difficulties and conflicts**

   Along with access control, sanitation can be quite difficult to impose on a mushroom farm. As people move around, their shoes carry potential contamination along, so a copper solution footbath will seem quite logical. However, it is useless without the simultaneous use of double doors and protective clothing. After each completed harvest the growth chamber should be disinfected and washed out. It is the practice on some *Agaricus*-farms to "cook" the growth chambers with steam blown into the room. This takes care of spores and conidia floating in the air, and pests that have invaded the cropping area. This also increases the operational cost, so that the initial lure of a cheap crop is lost even more. A more subtle and yet equally important area that requires good sanitation is the workers' themselves. Their hands, faces, hair and clothes must all be kept clean or protected. This is important not only for healthy crop production, but also for their own health since *Pleurotus* spores have a cumulative allergenic effect.

7. **Incorrect use of pesticides**.

   It is imperative that the cultivator should not deviate from a strict program of pest control. Incorrect use of chemical pesticides and fungicides can render the desired effects useless and is a waste of money. Some of the products favoured by South African cultivators are registered on other vegetable crops, but their suitability for use on mushrooms is uncertain. Physiological disorders and acquired resistance are the greatest danger to the inappropriate use of pesticides. Acquired resistance to these products will eventually be to the detriment of all the farmers in the vicinity. Oei (1996) provides some useful guidelines on the application and use of pesticides, but simply following the manufacturer's instructions will cover most of them.

The preparation of the spawn and the substrate, maintenance of the bags during production and post-harvest procedures are all sensitive periods when contamination can occur. Furthermore, the contribution of vectors should be minimised by secured housing conditions. There is no single key to success, but hygiene in the farming practice certainly ranks as highest priority.

6.4.4 Fungicides and pesticides currently employed

Prochloraz and thiabendazole have been registered as fungicides suitable for mushroom cultivation (Bot 1999), while dichlorvos, diflubenzuron (a chitin inhibitor) and mercaptothion is registered for insects (fungus gnats) (Nel *et al.* 1999). Personal preference will dictate the combination of pesticides used by each cultivator. Benlate has always been very popular, but with increasing pest resistance across many crops against the product it is of little value. Insect growth inhibitors (IGR's), insecticides, fungicides and nematocides must be administered not only in the correct dosages, but also at the correct timing during the preparation of the substrate. Cyromazine is an IGR that has given satisfying results in Europe (White 1989, Hoffman *et al.* 1987), but it is not yet approved in South Africa for use on mushrooms.

6.5 Relevance and importance of meteorological aspects

Weather is a major contributing factor to many of the difficulties in local cultivation and Agrios (1988) pointed out that the following five aspects need to be considered in any investigation into the influence of weather on crop production:

1. air temperatures
2. relative humidity (Rh)
3. rain
4. wind
5. cloud cover and sunshine

Each one of these factors has an influence on the others, creating the overall climate. South African conditions are quite harsh due to the specific combinations of the five factors listed above.

The seasonal occurrence of fungal disease episodes was revealed during the course of the study period (Table 9). It appears that spring and autumn are the periods of highest susceptibility to infection on the farms. This is when the inversion of the seasons takes place. The wind is a prominent feature of the differences in the regional climates (Fig. 10 and Fig. 15) and it carries air-borne particles into the cultivation facilities. It therefore plays a significant role in the amount and type of contamination and insect vector problems experienced on the farms.

The mushroom farms are situated in two areas that are very different as far as the surrounding flora is concerned. The study area in the Western Cape has coastal Fynbos on sand and limestone (veld type 47). The Gauteng study areas are situated in the Eastern and Central variations of Bankenveld (mixed grassveld type) with sandy, acid soils (veld type 61) (Acocks 1988). This means that the air borne particles such as pollen, seeds, plant and even animal debris in the two areas will differ accordingly. The differences in the floristic elements and soil types certainly contribute to the differences in contamination profiles. This will have to be investigated by taking air samples throughout an extended period. The variation in terms of pests, weed fungi and competitor fungi present in the regional infections that are being reported, is clear already.

ABSTRACT

Commercial production of the oyster mushroom, *Pleurotus* spp., is barely ten years old in South Africa. Although the local industry is expanding, progress is not taking place at a satisfactory rate. Regular difficulties that are encountered put the growers into discouraging financial situations. Furthermore they rely mainly on information from abroad for the control of the pests and diseases of their crop.

This study investigated some of the problems confronting the South African cultivators. Samples were collected from farms in Gauteng and the Western Cape during various stages of production. A distinction was made between weed fungi and competitive fungi based on the incidence and severity of the infection. The presence of invertebrate pests was recorded as well. A correlation between meteorological factors and infective episodes was established.

An infective episode in the substrate often reflects the general farming hygiene. The pests and diseases of mushroom cultivation can, however, be controlled with good planning and careful management and certain recommendations are made in this regard.

OPSOMMING

Die kommersiële verbouing in Suid Afrika van die oestersampioen, *Pleurotus* spp., is skaars tien jaar oud. Alhoewel die plaaslike industrie uitbrei, is die groeitempo daarvan nie na wense nie. Kwekers ondervind gereeld probleme wat hulle in ontmoedigende finansiële posisies plaas. Verder maak hulle hoofsaaklik op inligting vanaf die buiteland staat vir oplossings rondom die beheer van plaagdiere en siektes van hul gewas.

Hierdie studie het ondersoek ingestel na sommige van die probleme waarmee die kwekers in Suid Afrika gekonfronteer word. Monsters is tydens verskillende stadiums van die verbouingsproses versamel op plase in Gauteng en die Wes-Kaap. Daar word onderskei tussen onkruid fungi en kompeterende fungi op grond van die voorkoms en erns van die infeksies, terwyl die teenwoordigheid van invertebrate plaagdiere ook aangeteken is. Daar is bevind dat 'n korrelasie bestaan tussen die meteorologiese faktore en die infektiewe episodes.

'n Infektiewe episode in die substraat is dikwels 'n aanduiding van die algemene boerdery higiëne. Die plaagdiere en siektes van oestersampioenverbouing kan egter beheer word met goeie beplanning en versigtige bestuur en sekere aanbevelings in hierdie verband word gemaak.

ACKNOWLEDGEMENTS

A sincerest "thank you" is extended to the following people without whom this project could never have succeeded:

- Prof Albert Eicker, for his tolerance and encouragement, and for leading me to an appreciation of fungi.

- The intrepid oyster mushroom growers, for allowing me to glimpse into their mushroom worlds. Without their co-operation and assistance none of this was possible.

- My family, because their love, endurance and most of all, unwavering support kept me going.

- Mr Chris van der Merwe, for patiently coaching me in the use of the electron microscopes and paying attention to the small things in my life.

- Ms Magda Nel, for safekeeping my isolates and for always being a friend.

- Ms Elfrieda Meyburgh, for her invaluable input with the literature acquisition.

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