

## 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<sub>2</sub> available to them. The

presence of O<sub>2</sub> 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<sub>2</sub> and later CO<sub>2</sub> can accumulate, all of which is conducive to accelerated hyphal growth. The advantages of the increased gas concentrations are decreased fruiting time and increased fruiting 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).

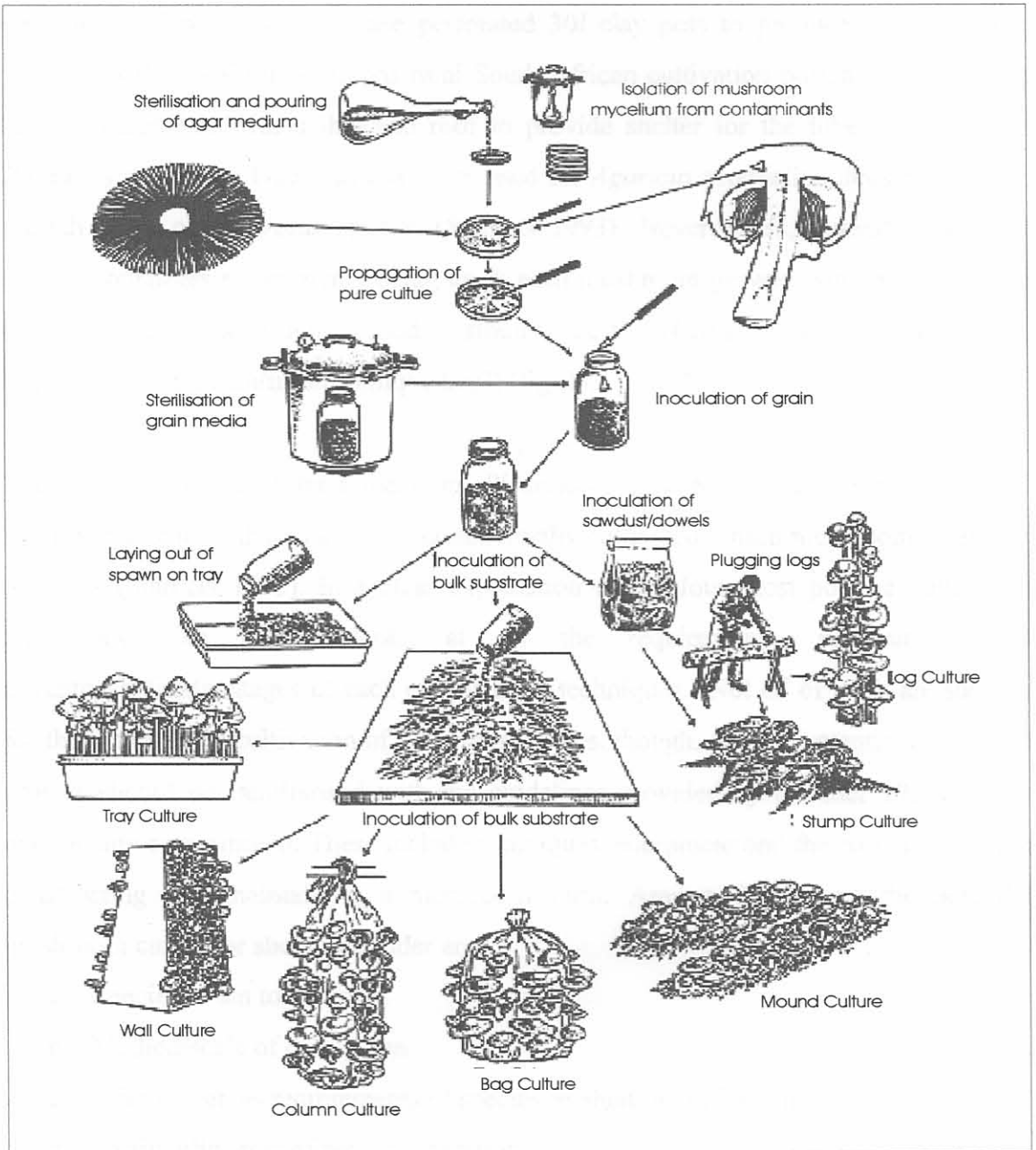


Figure 1 An overview mushroom tissue culture and cultivation. (Courtesy of Paul Stamets, Fungi Perfecti Online 1995)

## 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 30l 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](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, Wetzal 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 xenobiotics. 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/spdpublic/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, MERCAPHTHION	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  $LD_{50} < 50 \text{mg.kg}^{-1}$

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 than 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, Vedio 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 *Aphelenchoides* 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, Wetzal *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 trials 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 nephron (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 microorganisms 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 examples) 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.