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.
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 *Trichoderma* 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 chemoreceptive 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 recurrent 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 (*C₂H₂*) and carbon dioxide (*CO₂*). 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, disfluubenuron (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.