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CHEMICAL AND BIOLOGICAL CONTROL OF MUSHROOM PESTS AND DISEASES

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CHEMICAL AND BIOLOGICAL CONTROL OF MUSHROOM PESTS AND DISEASES

Project 4095

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SUMMARY

This study set out to determine the occurrence of diseases and pests in Irish mushroom units and their method of control using chemical, biological and other means of control. It also examined the role of a combination of these methods to enable control with minimal pesticide input.
It was found that pesticides alone will never give effective disease and pest control and that they should only be considered an adjunct to the implementation of other methods. They include:

- Exclusion
- Containment of spread
- Elimination

A major factor in good disease and pest control was found to be the implementation of a good programme of hygiene which must be followed from the time of filling a tunnel to the time of emptying after cropping.
Biological systems offer good potential for control but at present are not as effective as the best chemical control methods.

INTRODUCTION

Mushrooms are subject to a range of diseases and pests which have the capacity to cause serious crop losses. For example, many growers have difficulty in producing a fourth flush of mushrooms because of the incidence of dry bubble disease caused by the fungus *Verticillium fungicola*. In addition to consumer pressure to reduce pesticide usage in food crops, there are a decreasing number of pesticides available or approved for use in mushrooms. The industry is over reliant on a small number of pesticides. Pesticide development companies are slow to seek approval for new pesticides on smaller crops because of the costs involved. Biological control systems, while successful on some crops, are not being much used in mushrooms.

The main thrust of this work was to develop systems for pest and disease control in mushrooms which minimise the use of pesticides and are environmentally friendly. This involved examining components of an integrated control system including hygiene practices, exclusion, pesticides and the use of biological controls. The development and use of such integrated disease and pest control strategies for mushrooms, with minimal pesticide usage, will allow the efficient production of mushrooms as a foodstuff into the future. It will be of great commercial benefit for Irish mushrooms to have an image of wholesomeness. This work was done partly at Teagasc, Kinsealy Research Centre and partly at commercial production units which were experiencing problems with disease and pest control. We are particularly grateful to Connaught Mushrooms Ltd. Claremorris, Co. Mayo for their help and co-operation in monitoring disease and pest levels and in applying some of the integrated control strategies under commercial conditions. Also to the Teagasc advisory service for submitting samples.

METHODS

The experimental work for this project was done in the mushroom research unit at Kinsealy Research centre. The growing tunnels used were the same as those used commercially but shorter in length.

The compost and casing used in the trials was sourced from commercial suppliers.

The mushroom strains were the same as those used commercially by growers.

Growing procedures were the same as those used commercially as outlined in the Teagasc booklet “An introduction to Production of Mushrooms in Plastic Bags and Tunnels”.

RESULTS AND DISCUSSION

DISEASE CONTROL

The four main diseases causing losses in mushrooms in Ireland since 1994 are:

Disease	Cause
Dry Bubble	*Verticillium fungicola*
Wet Bubble	*Mycogone perniciosa*
Green mould	*Trichoderma harzianum*
Cobweb	*Cladobotryum dendroides*

These diseases have varied in intensity from time to time and between mushroom units. The factors involved in this variation have been studied both experimentally and at grower level by epidemiological studies with a view to being able to put together a practical comprehensive integrated control strategy. The following are some factors identified:

Fungicides

Because the mushroom is a fungus it is difficult to produce a fungicide which is not phytotoxic to the mushroom. For this reason there are only a very limited number of fungicide types available and approved for use on mushrooms.

Efficacy

The fungicide Sporgon containing prochloraz-manganese is the one almost universally used by Irish growers. Tests were done in conjunction with disease inoculation trials to determine the efficacy of this fungicide for control of Bubble disease (*Verticillium fungicola)* and Cobweb (*Cladobotryum dendroides*). Sporgon application by incorporation in the casing or by drench after casing gave little protection when high inoculum levels were incorporated in the casing. Incorporation of the fungicide in the casing gave good protection from low levels of inoculum applied after casing. Sporgon applied as a drench (1.2 g/m^2 in 1.8 litres of water) after casing gave protection for a time but disease began to appear in the third flush. This shows that the simple use of fungicides alone will not be sufficient to obtain good disease control. It is extremely important for mushroom growers to ensure that they use clean (pathogen free) casing and that they have a very good programme of hygiene after casing.

Tolerance

In 1994 a serious outbreak of cobweb disease was reported from some areas. It was particularly bad with growers in western counties. An investigation was done to determine whether (a) strains of *Cladobotryum dendroides* had developed which were tolerant to the fungicides benomyl and prochloraz, commonly used for control. (b) whether more aggressive strains of the fungus had developed. Disease samples were sent to Kinsealy, from various parts of the country, by Teagasc advisers. Isolations of the pathogen were made on to malt agar and discs from these were transferred to agar containing 10 mg/l of either benomyl or prochloraz. After four days growth at 22^{0}C measurements of the radial growth of the fungus were made. When significant growth occurred sample was deemed tolerant (Table 1).

Table 1: Survey of fungicide tolerance in *Cladobotryum dendroides* samples causing cobweb disease in mushrooms.

	% Tolerant *	
Sample source	Benomyl	Prochloraz
Cavan/Monaghan	27	11
Kildare	0	14
Mayo	73	0
Meath	100	0
Roscommon	100	0
Wexford	0	25

* Variable numbers of samples per county were received per county

The above and numerous other samples tested in succeeding years indicated that the development of tolerance to the fungicides was closely related to the frequency of use of the fungicides. The carbendazim based fungicides (benomyl) were very prone to this.

Where the use of approved fungicide with different active ingredients was rotated then tolerance was slow to develop, if at all. Thus occasional rotation of carbendazim with prochloraz or chlorthalonil would prevent the development of tolerant strains.

Phytotoxic effects

The fungicide Sporgon (prochloraz-manganese) is by far the most commonly used and most effective fungicide in mushroom growing. Growers often comment that they feel that applications of this fungicide may reduce yield. A series of trials were done to determine whether various rates of the fungicide applied either as a drench or incorporated in the casing may have phytotoxic effects on mushrooms. Only the highest rate of incorporation i.e. 9.6 g/m^2 significantly reduced yield by comparison with untreated. This is eight times the recommended rate. This shows that the fungicide has no phytotoxic effect at normal rates.

Epidemiology

Time from inoculation to disease

Experiments were done to test the effect of time and method of inoculation, of the pathogens *Verticillium fungicola* and *Cladobotryum dendroides*, on the development of bubble and cobweb diseases respectively, during cropping. Natural inoculum from diseased mushrooms and casing was used.

Inoculum put on the surface of compost, in bags, either at spawning or after spawn-run compost did not result in subsequent disease development. This indicates that spawned compost is unlikely to be a source of either of these diseases.

Application of inoculum to the casing by incorporation or surface application resulted in very rapid disease development. Bubble disease (*Verticillium fungicola)* developed when mushroom pinheads were formed on the casing in an 8 to 12 day period after casing. Development of cobweb was slower and it generally took 10 - 14 days for it to appear. The level of inoculum used in these trials was quite high and it may be that where a low level of natural inoculation occurs disease development will take longer.

Pathogen survival

Studies were done to determine the survival time of *Verticillium fungicola* inoculum. A mixture of spores and ground up mycelium were placed in distilled water and stored at room temperature. A sample was plated on malt agar every week from this. The pathogen was still alive after six months.

Disease spread

The epidemiology of the common mushroom diseases was studied on a number of mushroom units both big and small. This was done by means of spore trapping on agar strips, stationary agar plates with selective media and by swabbing structures, equipment and personnel. In addition close observations of procedures and practices in mushroom units were made to determine the major sources of risk. Factors identified are presented here in a cropping sequential format:

Pre-cropping

It is clear from our work that attention to detail at this stage will ensure that disease and pest levels start from a very low base and kept under control with minimal pesticide usage. Very strict hygiene and the adoption of good practice are the key factors at this stage:

- Personnel free of contamination. Anyone coming in contact in any way with a new crop, including filling, lining out and casing, must be free of the possibility of carrying disease spores or insects. A shower or bath plus the donning of freshly washed clothing is the ideal.

- Minimise dust. Dust arising from wind outside or by sweeping dry concrete inside or outside is a possible source of disease spores and must be avoided.

- Minimal bag levelling, by transferring compost from bag to bag, and the use of cacing (spawn-run material) will reduce the spread of compost green mould in particular.

- Protect casing from any possible source of disease pathogens. This includes peat extraction from bogs and subsequent handling by casing companies. Growers receiving a load of casing should cover it unless it is being used immediately.

- Fly control from an early stage. The use of fly monitoring equipment (Figure 1) has been found very beneficial as it rationalises the use of pesticide fumigants. It was clear from our studies that flies (*Sciarid and Phorid*) were gaining access to commercial production tunnels earlier than hitherto supposed. Also it was commonly found that a large fly population coincided with a disease epidemic. *Verticillium*, *Trichoderma* and *Cladobotryum* were isolated from tunnels in which these occurred.

- Many growers were found to be in the habit of leaving doors open unnecessarily. This negates the benefit of otherwise good practices.

During cropping

In this study it was found that in most cases disease starts at a very low level and then becomes an epidemic by the end of the third or fourth flush. The following factors were identified:

- Detect diseases and pests early. This requires a grower to carefully inspect the tunnel each day for the first signs of disease and pest and to take prompt appropriate action. If removing a patch or piece of disease drape a damp cloth over the affected area and then lift it into a container with liquid. Otherwise spores are released resulting in rapid disease spread. The area can then be covered with salt.

- Mushroom stumps must be bagged and removed shortly after harvest. This also includes mushrooms which fall between bags.

- People moving between tunnels are a serious source of disease spread. We have found by swabbing that disease pathogens are easily carried on hands and clothing. This is usually done unconsciously as the pathogens are microscopic. Pathogens can even be carried to canteen facilities such as tables and chairs which then act as a source of inoculum.

- A major source of inoculum, for disease spread, was identified as crops being kept too long before disposal. Some growers with a fair amount of disease in the third flush tend to keep the crop for a fourth. Usually by the time of disposal there is a huge level of disease, which creates a large inoculum source, that poses an enormous threat to other crops. Diseased crops should be killed off and disposed of immediately after taking the third flush.

<u>After cropping</u>
Our studies have demonstrated that the method of emptying and cleaning out tunnels and of spent compost disposal plays a major role in determining the level of disease in mushroom units. This is particularly so for bubble and cobweb diseases. The following is a logical sequence, with comments, based on findings from our studies.

Procedures

1) **KILLING OFF THE BAG SURFACE**
If there is some disease present, the normal procedure is to apply a strong disinfectant to the surface of the bag to kill off surface spores and

mycelium. A good drenching spray is required. It must be remembered that this will only kill to a small depth and that there will be an enormous amount of disease inoculum underneath in the casing and compost. Emptying the bag in the unit will therefore release a large amount of disease spores into the air thus undoing the benefit of the disinfectant.

2) **VENTILATE**

The tunnel must be cleared of disinfectant fumes before anyone enters to load bags. Failure to do this presents a serious health hazard.

3) **LOAD BAGS WITHOUT DISTURBANCE**

Ideally bags should be loaded on to a trailer without disturbance of the compost or casing. Emptying bags at this stage or outside in the yard creates a very serious disease hazard, which is difficult to overcome.

4) **CLOSE AIR INTAKES**

When any tunnel is being emptied the air intakes and the doors on the rest of the tunnels in the unit should be firmly closed. This will reduce the risk of spores entering clean tunnels.

5) **TAKE LOADS DOWNWIND OR <u>COVER DURING TRANSPORT</u>**

Trailer loads of used bags of compost should be taken downwind if at all possible, but this may not be practical. If so the load should be covered with a tarpaulin or sheet of plastic.

Driving upwind with an uncovered load of bags with disease will result in a large shower of spores being blown back over the entire unit.

6) **CLEANING OUT A TUNNEL**

When the bags are removed the remaining dirt on the floor must be brushed out. Dampen the floor before brushing to ensure that a cloud of dust and spores are not sent into the air. Power hosing - while good for cleaning concrete does not kill spores and may spread them.

7) **DISINFECTING TUNNELS**

The floors and walls of the cleaned out empty tunnel should then be sprayed with one of the approved disinfectants such as Formalin or one of the phenolics (Sudol, Environ, Prophyl, Panacide). This will kill any spores or mycelium remaining.

If there has been any significant amount of disease in the tunnel then fumigation with formaldehyde is advisable. This will kill any spores in the ventilation duct or radiator or on the fan blades. The air temperature should be raised to over 15^{o}C and the relative humidity above 50 % with the fan on full recirculation to ensure efficient fumigation.

8) **VENTILATE BEFORE ENTRY**
All fumes must be cleared from the tunnel before anyone enters, to avoid a serious health hazard. The controls should be located outside so that ventilation can take place without having to enter a tunnel full of fumes.

9) **DISINFECT EQUIPMENT**
Any piece of equipment, which has been used in a tunnel with disease, must be thoroughly disinfected before the new crop is started. This includes hoses, roses, trays, brushes, knives etc.

10) **CLEAN PERSONNEL**
Anyone involved in cleaning out a tunnel with disease will be covered with disease spores, including clothes, hair, hands, shoes etc. Such persons must shower and have a complete change of clothes before coming in contact with other crops.

PEST CONTROL

INTRODUCTION

Mushroom pests can be loosely defined as members of the animal kingdom that damage cultivated mushrooms. In Ireland, the pest spectrum is comprised of three families of minute flies and three families of mites. A predatory mite belonging to another mite family can sometimes be a nuisance to mushroom pickers but does not damage mushrooms. Two categories of eelworms are also associated with cultivated mushrooms.

Mushroom flies and their life cycles

The main fly pests occurring in mushroom crops in Ireland are sciarids, phorids and cecids.

Sciarids (family Sciaridae), also called "fungus gnats".

Several species have been found associated with cultivated mushrooms but *Lycoriella solani* Winn. appears to be the main species infesting the

crop in Ireland. Sciarids are the most important pest of mushrooms. They are small, delicate, gnat-like flies, much longer than broad and with long thread-like antennae. Larvae are white, less than 1 cm long with a distinct black head capsule. These larvae feed on mycelium, "pinheads" and small mushrooms and also tunnel in the stalks and caps of larger mushrooms. Such feeding can result in a yield loss of 40% but a more usual figure is less than 10%. Their major significance is that adults are mainly responsible for disease spread, although the occurrence of adults in pre-packed mushrooms is also important. At mushroom growing temperatures, the life cycle takes about 5 weeks. This means that flies that enter the crop early in the spawn run period give rise to a new generation at about the first flush. Larvae and adults arising from these cause most damage.
Sciarids are predominantly casing inhabitants and the usual chemical control is by treatment of the casing with Dimilin (diflubenzuron). Adults are controlled by space treatment with insecticidal smokes or fogs.

Phorids (family Phoridae), known as "hump-backed" flies.

Adults resemble diminutive houseflies, blackish in colour, shorter in proportion to their width than sciarids and have inconspicuous antennae. Adults are very strongly attracted to compost or casing in which spawn is running. Larvae are white, about 5mm long and do not have a distinct black head. Several species have been found associated with cultivated mushrooms, the most important one being *Megaselia halterata* (Wood). Larvae feed on mycelium, mainly in the compost but their feeding does not generally reduce yield. They are not as responsible for disease spread as are sciarids.
At mushroom growing temperatures, adults entering the crop soon after spawn run give rise to a new generation of adults at or soon after casing. These in turn produce another generation of adults 4-5 weeks into cropping and these flies cause the greatest problem. Highest populations of phorids tend to occur in the autumn. Phorids are essentially compost dwellers and chemical control involves the addition of an insecticide to the compost.

Cecids (family Cecidomyiidae), known as "midges" when adults are seen.
The adults are small, delicate, straw coloured flies. They are seldom seen as the larvae reproduce by paedogenesis - each larva has the potential to splits open and release 12-20 daughter larva every week. The usual

species attacking mushrooms is *Heteropeza pygmaea* Winn. The small featureless larvae suck the contents from mycelial hyphae, thus reducing yield. Due to their remarkable facility for population increase huge numbers of larvae migrate from the compost and spoil the crop by carrying bacteria that cause breakdown of the stem surface and gills. Larvae of the commonest species are white but occasionally species with orange larvae occur. These are much more noticeable as mushroom contaminants.

With improved hygiene and better pasteurisation of compost, cecids are less frequently seen now than in the past. Occasionally however, poor hygiene is reflected in a cecid outbreak.

There are no chemical controls for an outbreak during cropping. "Sudol" kills larvae in empty houses and on utensils.

Other flies:

Several other groups of flies can occasionally breed in mushroom compost but are not classified as pests.

Mushroom mites

Tarsonemid mites (Tarsonemidae) are minute white mites, invisible to the naked eye. These mites feed on mycelium causing the infested mushroom to develop an "off white" colour. Sometimes the base of the stem can become reddish-brown, development of mushrooms is restricted and mushrooms are poorly anchored in the casing. Usually, symptoms are not seen until near the end of the cropping cycle. Their presence indicates poor hygiene. Control is by good hygiene.

Pygmephorus spp mites or "pepper mites" do not attack mushroom mycelium but feed on other fungi particularly the green mould fungus, *Trichoderma* sp. They swarm in enormous numbers on mushrooms and on high points of the casing but they are present only for a few days. Their presence is avoided by the elimination of weed moulds.

Parasitus sp - a commonly occurring fast moving mite that is a predator on a number of mushroom pests. While sometimes a nuisance to pickers, it is a beneficial mite. It's potential as a disease carrier has not been established but, as it does not fly, is unlikely to be significant.

Eelworm pests of mushrooms

Two species of eelworms are known to attach mushroom mycelium but neither has been found in Irish mushroom units. Another category of eelworms (Rhabditidae) are common. These are exclusively bacterial feeders and do not have mouthparts capable of piercing mushroom hyphae. When they occur in high numbers they are primarily indicators of excessive bacterial decomposition of the compost. Consequently they tend to be indicators of, but not the cause of poor compost. Their presence however does not always preclude a good crop.

METHODS

The experimental work for this project was done at the Kinsealy mushroom research unit. Materials (compost, casing, synthetic and biological pesticides) were sourced from commercial suppliers. Fly numbers in plots were calculated by taking 150 ml samples from compost or casing, covering with a lid which had adhesive on its inner surface. Adult flies were subsequently counted when they emerged and stuck to the lid.

EXPERIMENTAL RESULTS

The main results obtained from the project are as follows:

Chemical controls

- Dimilin (diflubenzuron) gives 90-95% control of sciarids when added to the casing either by incorporation or as a post-casing drench. When added to the casing it does not control phorids.

- Dimilin at normal rates can reduce yields by up to 7-8%. Splitting the application avoided this reduction but is not an option on the label.

- There is no evidence of resistance to Dimilin in sciarid populations. Poor performance is usually attributable to slipshod application.

- Apex (methoprene) is not as effective as Dimilin for sciarid control.

- Wet foggers such as B+G and Tri-Jet should be accurately calibrated and every effort made to apply the insecticide evenly throughout the mushroom house.

- Fipronil (a new pyrazole insecticide) gave a level of sciarid control comparable with Dimilin. It is not registered for use on mushrooms.

Biological controls

- *Steinernema feltiae*, an insect parasitic eelworm is one of the most promising non-chemical controls for mushroom flies. It is sold as "Nemasys", "Stealth" or "Entonem". Several experiments with S. *feltiae* indicated that it gave 60-70% sciarid control when added to the casing. It is less effective against phorids.

- Differences in the level of control given by different commercial brands of S. *feltiae* were not significant.

- Applying *S. feltiae* by casing incorporation, as a post-casing drench or either of these treatments augmented by an additional drench treatment one week later did not improve the level of control they achieved.

- Occasional failure of *S. feltiae* to give any control of sciarids has been attributed to poor quality control by the manufacturer.

- Good exclusion of flies from the growing house improves the performance of *S. feltiae.*

- *Bacillus thuringiensis* var *israelensis* ("Bactimos"), a strain of an insect bacterial disease specific to flies, gave poor control of sciarids and did not augment the effectiveness of *S. feltiae*.

- Neen seed extract (contains azadirachtin) gave poor control of sciarids and did not augment the effectiveness of *S. feltiae*.

- *Hypoaspis miles*, a mite that feeds on the larval stages of all the mushroom pests was assessed for sciarid control. When introduced after spawning it reduced sciarid numbers by 48%: when introduced after both spawning and casing it reduced sciarid numbers by 60-65%.

Cultural Controls

The impact of cultural methods of control on insect populations was monitored on an ongoing basis throughout the duration of the project. The most important of these are:

- Hygiene - precrop cleaning and disinfection of growing tunnels, outside apron, utensils and containers is an important aspect of cecid and tarsenemid mite control. In fact such measures are the only steps one can take to avoid such pests. Discarding severely infested crops prematurely is an integral part of good farm hygiene.

- Exclusion of flying insects - this is a most important aspect of fly control in mushroom houses. Air inlets should be fitted with a mesh with apertures not greater than 0.5-0.6mm. Doors should be seated against an intact rubber seal, like that of a commercial refrigerator. It is important to keep doors closed insofar as possible, particularly during spawn run.

- Quick transport of spawned compost to the house avoids exposing it to egglaying by sciarids and phorids.

- Regulation of human traffic between crops (allowing only traffic from young to old crops) helps to reduce pest problems.

- Regulating house temperature to achieve a quick spawn provides competition for pests for resources in their ecological niche.

- Reducing the production cycle to reduce the potential for pest build-up can be a very useful pest suppression tool-terminate severely infested crops early.

- Pest problems increase with increasing size of the mushroom farm; the biggest problems arise when a number of growers share the same site.

- Using traps to monitor fly numbers increases growers' awareness of low fly populations. Low fly populations especially during spawn run are of major importance as they give rise to the initial infestation which culminates in the high populations that occur later in the cropping cycle and result in economic injury. The Pennsylvania black light trap is highly suitable for monitoring mushroom pests.

- It is also important to continually check crops for pests, determine the extent of the problem, treat as appropriate and evaluate the effect of the treatment.

- If pesticides must be used, use the most appropriate one, apply evenly, apply at the correct dosage, the right time and under the right conditions.

- Avoid routine use of pesticides during times of the year when the pest pressure is low e.g. winter and early spring.

CONCLUSIONS

The use of fungicides alone will not be sufficient to obtain good disease control.
Many of the common fungal pathogens of mushrooms have the capacity to become tolerant of them, if used frequently. Alternation in the use of fungicides with different active ingredients should be practised.
In general the approved fungicides were found to be non-toxic to mushrooms except when used at an extremely high dosage.
Diseases develop about 8 to 14 days from the time of inoculation but this depends on the temperature and level of inoculum.
Verticillium inoculum can survive for a very long time in water.
The study on disease epidemiology revealed that there are very many vulnerable parts in the growing procedure. The method of emptying and cleaning out tunnels was found to have a major impact on disease levels in subsequent crops.

For pest control results from the project indicate how a considerable reduction in synthetic insecticide use in mushroom growing could be achieved by adhering to a number of cultural measures which could be summarised under the heading "good agricultural practices". Chemical insecticides approved for use on mushroom crops are already severely restricted and the introduction of new ones is not expected in the short term. Alternatives to chemical insecticides e.g. biological pesticides are being developed but at present any combination of them do not give pest control comparable to that of chemicals.

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