The control of mushroom pests using biological control agents and environmentally acceptable products

A Review by Philip F. White

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Philip F. White

Microbiology and Crop Protection Department
Horticulture Research International Littlehampton West Sussex

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SECTION 1

DEFINITIONS OF BIOLOGICAL CONTROL

At its most basic, the term biological control is used to describe pest control by the use of predators, parasites and pathogens. In recent years, however, other means of control which can be termed 'biological' have been included within this general expression.

These include:

(i) Pheromones - volatile chemical substances which are secreted and released by animals, usually for detection and response by the same species;

(ii) Kairomones - volatile chemical substances emanating from a food source of an animal, which the animal uses for substrate location;

(iii) Repellents and anti-feedants - substances that can be either produced by the food source of an animal or manually applied;

(iv) Genetic control - for example radiation or chemical treatment to produce sterile adults.

Other methods of control, which can be described as 'environmentally acceptable' but are not necessarily biological in their mode of action, have also been encompassed within the general banner of biological control. These include physical methods of control based on exclusion or trapping principles.

In this report all of the above methods will be examined, where appropriate, and will be encompassed in the text by the phrase 'biological control'.

SECTION 2

BENEFITS OF BIOLOGICAL CONTROL

There are a number of advantages that are claimed for biological control as opposed to chemical methods of control.

These include:

(i) Pest specificity - you only affect the insect that you want to kill;

(ii) They leave no toxic residues;

(iii) The beneficial organisms can be already 'available' - they may not need to be manufactured;

(iv) Some beneficial organisms can seek out and find the host;

(v) Some beneficial organisms can increase in number and spread from the initial point of introduction;

(vi) The pest will be slow or unable to develop resistance;

(vii) Control may be self-perpetuating.

In the current political and commercial climate, where there are urgent calls for reduced pesticide inputs - especially on to rapidly-perishable food products - the aspect of most relevance to commercial mushroom growers is that which deals with toxic residues. Of the remaining aspects mentioned, pest specificity would probably be of least interest. Although it can be of great importance in an established biological control programme, in the mushroom industry, where no such programmes exist, pest specificity would not necessarily be required. Indeed it could be argued that, at this stage, the opposite would be the case.

A wide-spectrum biological control agent, therefore, might appear to be the ideal. However, it should be realised that there are disadvantages to the use of biological control agents.

These include:

(i) The speed of control - by their very nature some biological control agents can take time to have an affect;

(ii) Unless used in the manner of an 'insecticide' they may not act as an eradicant;

(iii) Due to conditions 'invisible' to the grower, they can often be unpredictable in their efficacy;

(iv) Because they <u>are</u> biological in nature, they can be expensive and difficult to develop and can require more expert supervision in their application.

Another important aspect is that as well as trying to control an insect pest population, a grower may also be attempting to control other noxious organisms, such as fungal pathogens. All these foregoing problems often mean that biological control agents cannot be used in isolation. They must mostly be used in conjunction with other control measures within an integrated pest management system.

The effect of these other control systems, invariably insecticide- or fungicidebased, on the various biological control agents will need to be assessed before embarking on such a programme.

SECTION 3

PESTS AFFECTING MUSHROOM PRODUCTION

The main mushroom pests that affect mushroom production can be described under the general headings of flies, mites and nematodes.

FLIES

All of the fly pests belong to the Order Diptera (two-winged flies). The main ones belong to the Groups Sciaridae, Phoridae and Cecidomyiidae.

Sciarids

In the UK there is only one species that is important in mushroom cultivation - *Lycoriella auripila*. At the moment, this is the most important pest of mushrooms. Uncontrolled it can completely destroy a mushroom crop (Hussey, 1973) and its affect on yield has been quantified (White, 1986). A schematic diagram, showing their development within a crop is shown in Figure 1.

Phorids

There are two phorid species that affect mushroom production in the UK and both belong to the genus *Megaselia*. These are *M.halterata* and *M.Nigra*. The latter is regarded as a minor pest (Fletcher, White & Gaze, 1989) as it only affects growing sporophores in conditions of natural daylight. *M.halterata* is the major phorid pest and can affect mushroom cropping both by direct larval browsing (Rinker & Snetsinger, 1984) and by the transmission of fungal pathogens (White, 1981). At the moment *M.halterata* is the second most important mushroom pest. A schematic diagram, showing their development within a crop is shown in Figure 1.

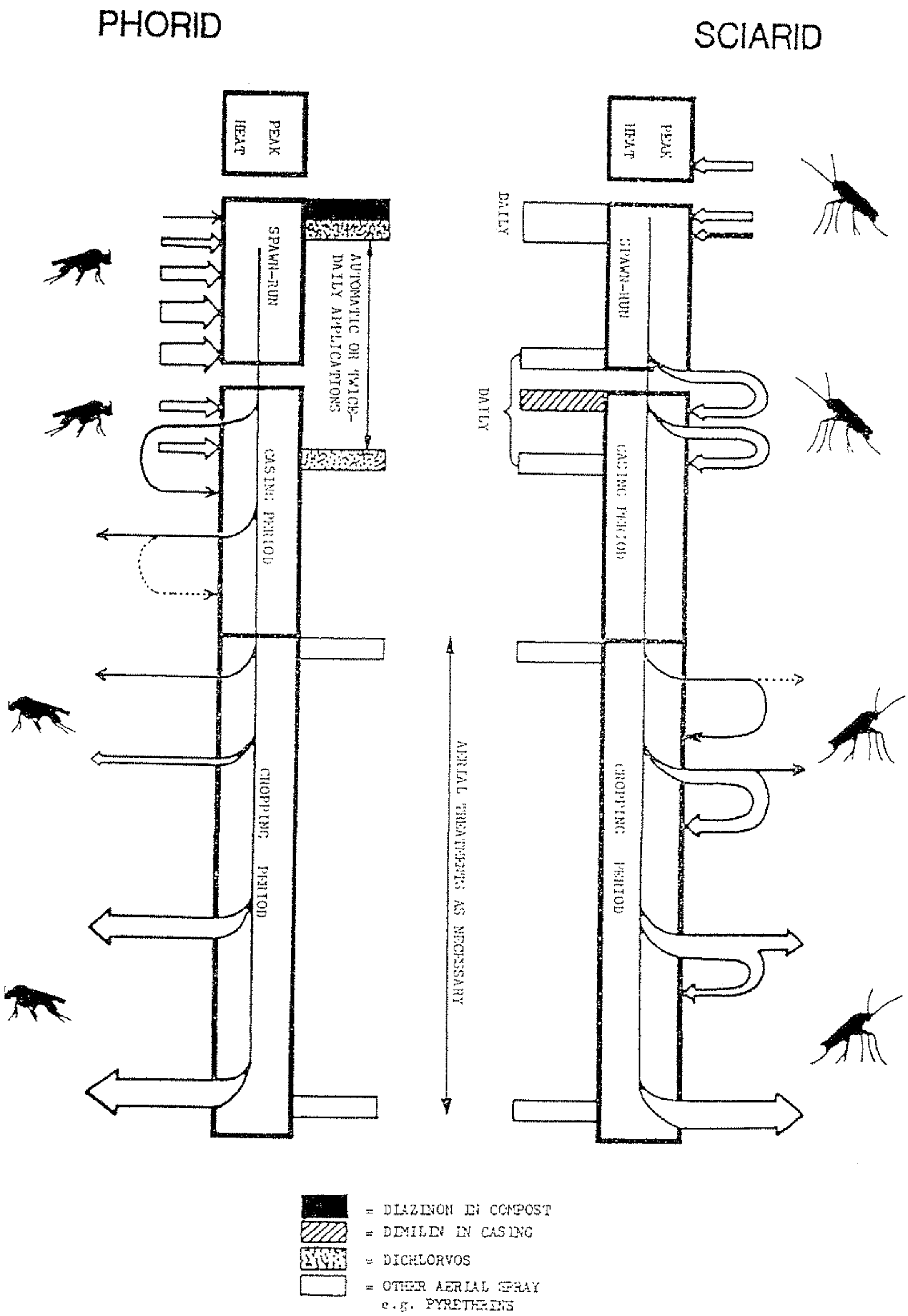

Figure 1. Schematic diagram showing population development of sciarids and phorids within a mushroom crop *(reproduced from White, 1985)*.

Cecids

There are three main species of cecid that affect mushroom production. These are *Mycophila speyeri*, *M.barnesi* (both with orange larvae) and *Heteropeza pygmaea* (with white larvae). *M.speyeri* is the most common of the three species and can cause substantial losses in yield (Wyatt, 1960). *H.pygmaea* is the next most common cecid species and can also cause substantial losses in yield (White, 1990).

Minor insect pests

There are several other organisms which are pests of mushrooms only occasionally. Most of them are saprophagous in nature and can often be associated with bad growing conditions or unconventional growing systems (Fletcher *et al*, 1989). They include Sphaerocerids, Drosophilids and Scatopsids. Because of their relative lack of importance in mushroom pest control, they will not be considered further.

MITES

There are only a few mite species associated with post phase II mushroom cultivation.

Tarsonemus myceliophagus

These tarsonemid mites are the only mite which has any evidence of primary pest status (Hussey, 1963), inasmuch as it feeds directly on mushroom mycelium. Although it has the potential to cause a lot of damage, it is only a minor pest in the UK.

Pygmephorus spp

These are the most common mites seen during mushroom cultivation and are commonly called red pepper mites. A number of species have been recorded on mushrooms in the UK and, although they can swarm over mushroom beds and trays in large numbers, they do not feed on mushroom mycelium but on various weed moulds that may be present in a sub-optimal compost.

Saprophagous mites

There are a number of mites that are saprophagous in nature and are, therefore, not primary pests of mushrooms. They include *Tyrophagus* spp., *Caloglyphus* spp. and *Histiostoma* spp. They will not be considered further.

Predatory mites?

These mites are **<u>not</u>** pests, they are beneficial organisms capable of aiding the control of several pests. However, they can be perceived as being a pest by a large percentage of a grower's workforce and are, therefore, included in this section as an indication of one of the possible problems associated with biological control. They will be considered in more detail in Section 6.

NEMATODES

There are two types of nematode that affect mushroom cultivation - mycophagous and saprophagous.

Mycophagous nematodes

As their name implies, these nematodes feed on mushroom mycelium and are, therefore, primary pests. There are two main species implicated - *Ditylenchus myceliophagus* and *Aphelenchoides composticola* (Hussey, Read & Hesling, 1969). They can destroy mushroom mycelium but with normal hygienic conditions and short cropping periods, they do not often cause serious trouble.

Saprophagous nematodes

These form the largest group of nematodes found in mushroom beds and are mostly Rhabditids. They are not normally regarded as primary pests but in certain cases they may be (Ross & Burden, 1981); and recent research has indicated that the most prevalent species *Caenorhabditis elegans* can have a deleterious effect on the crop (Grewal & Richardson, 1991).

SECTION 4

IMPORTANT FEATURES OF PEST LIFE CYCLES

Detailed descriptions of all these pests are outlined in Fletcher *et al* (1989). Consequently only the most salient features of their life cycles will be outlined below.

SCIARID - *LYCORIELLA AURIPILA*

The adult females are initially attracted by the fermentation odours **[possible kairomones]** of the compost as it is cooling down after phase II pasteurization. Egg-laying occurs over a period from up to two days before spawning until about seven days after spawning. There is evidence that after this time, a good mycelial growth through the compost will deter both further egg-laying and subsequent larval development **[possible repellent and/or anti-feedant]**(Binns, 1975).

The new generation of adults will emerge from the compost 2-3 weeks after the initial infestation. By this time, the crop has been cased and, in general, it is in the casing layer that subsequent generations develop and cause damage to the developing crop. Emergence then occurs, in steadily increasing numbers, for the duration of the crop.

PHORID - *MEGASELIA HALTERATA*

This pest is attracted to mushroom compost only after it has been spawned. Gravid (fertilized) females are attracted to the smell of various volatiles **[kairomones]** given off from the growing mycelium (Grove & Blight, 1983; Burrage, 1981). They prefer to lay eggs in compost which has been spawn-running for 7-12 days (Richardson & Hesling, 1978). Once the crop has been cased, fresh mycelial growth will again provide an attraction to phorid females. After this final surge of mycelial growth, the general attractiveness of the crop diminishes as cropping progresses. Fly emergence can occur at any time from casing onwards (Figure 1).

CECIDS

They can be readily found in decaying wood and rotten vegetation so the flies may give rise to an infestation on a farm by being attracted to the crop in the same way as the mushroom phorids **[possible kairomones]**. However, some recent research, to be published at this years' International Mushroom Congress in Dublin, has indicated that, with *M.speyeri*, the sexual phase of reproduction is non-functional (Dmoch, 1991). *H.pygmaea* has also a very inefficient sexual reproductive system, if one exists at all. However, cecid larvae reproduce very efficiently as larvae (paedogenesis) and it is much more likely that it will be via the larval stage that infestations will occur.

Initial, very small infestations probably arise from infested peat and it is only after going through several generations and being spread about by inadequately sterilized tray or bed timbers, or on the hands and tools etc. of workers, that the more serious and larger outbreaks of the larvae become visible. When the number of larvae reach a high enough density they start to produce flies instead of further larvae **[pheromone induced]** and only then may the minute flies be seen.

MITES

Tarsonemus myceliophagus

This mite is invisible to the naked eye but its affect on the crop is quite noticeable, causing a reddish-brown discolouration to the base of mushroom stipes. They have a relatively low reproductive rate. Their method of dispersal around a farm is unknown although it is likely that they are spread by flies and air currents.

Pygmephorus spp.

The red pepper mites are noticeable only when they are swarming from the mushroom beds. This swarming is a dispersal phase in their life cycle and, like the tarsonemid mites, they will achieve this with the aid of flies and air currents.

NEMATODES

Mycophagous nematodes

These nematodes have a normal sexual reproductive cycle. An early infestation by either of the mycophagous nematodes can result in dead, sodden patches of compost which can increase in size from the second flush onwards. They can be spread by flies.

Saprophagous nematodes

These nematodes reproduce by parthenogenesis. They are ubiquitous and, if they survive through phase II pasteurization or are introduced in a heavily contaminated casing material, will cause a reduction in mushroom cropping. They can be spread by flies.

SECTION 5

CHEMICAL CONTROL OF MUSHROOM PESTS

In the past, pest control was a simple procedure. The incorporation of cheap, toxic, organophosphorous or organochlorine insecticides into the mushroom compost and/or casing layer of the crop, with numerous and various aerial sprays being used during spawn-run and/or cropping, would control all the major pests and were all that were required. A combination of pesticide resistance, pesticide withdrawal and various 'green' issues have now made the situation more complicated.

Insecticides, such as thionazin (Nemaphos) and γHCH (γBHC, Gammacol) have been withdrawn from use or are no longer approved under current pesticide regulations. Another organophosphorous insecticide, chlorfenviphos, used to be available from two manufacturers (as Birlane or Sapecron) and was able to be used in the compost and casing layers. Now there is label approval on only one manufacturers product and its use is restricted to the compost only. There is even a question mark over the future of one of the most commonly-used insecticides in the mushroom industry, diazinon.

With these problems and restrictions in mind, only the most salient features regarding chemical control of mushroom pests are described below - the complete details being contained in Fletcher *et al* (1989).

SCIARIDS

L.auripila is now resistant to organophosphorous insecticides, such as diazinon (White & Gribben, 1989), pirimiphos-methyl and chlorfenvinphos (Binns, 1976). Larval control of this pest, at the present time, relies solely on the use of the chitin-inhibitor diflubenzuron (Dimilin) - a compound much more expensive than any of its predecessors. The same orgaonophosphorus resistance problem means that aerial control of adults is only possible with synthetic or natural pyrethrins.

PHORIDS

M.halterata, at the present, does not appear to be resistant to organophosphorous insecticides. However, as it is predominately a compost pest, larval control - even with the still-effective diazinon - is complicated by problems of inadequate mixing of the active ingredient into the compost at spawning (Wyatt & Gurney, 1974). Control of adults can be achieved with a range of products including pirimiphos-methyl (Actellifog), dichlorvos and synthetic and natural pyrethrins.

CECIDS

There is now only one formulation (a wettable powder), of one active ingredient (diazinon), which is approved for the control of these pests. Furthermore, of the three main cecid species only the *Mycophila* species are controlled to any extent by this product - *H.pygmaea* is relatively unaffected. Adult cecids are rarely seen, so aerial treatment is unnecessary.

MITES

There are no approved acaricides for the control of mushroom mites.

NEMATODES

There are no approved nematicides for the control of mushroom nematodes - either mycophagous or nematophagous.

SECTION 6

BIOLOGICAL CONTROL METHODS

In contrast to the previous sections, this one will be subdivided according to the control method rather than according to pest type.

PREDATORS

In this section are all those organisms that, for insects, operate on the macro rather than the micro scale. The predator consumes its prey, either partially or entirely. The list of predators includes: Midges; Beetles; Bugs and Mites.

Midges (Diptera)

There are numerous species of Cecidomyiidae, which in contrast to the mushroom cecids, are not pests of cultivated crops. Instead, the larvae are predatory in nature and have been shown by a number of researchers to have promise as biological control agents for a number of important horticultural crops (Nijveldt, 1988). At present, however, there is no work being done on the use of these insects to control mushroom pests.

Beetles (Coleoptera)

The most well-known example of a predatory beetle is the common ladybird, although, at present, it is not successfully reared commercially. There are numerous other beetles which have been shown to be effective predators but most research has be aimed at looking at factors that increase or encourage naturally-occurring populations within field rather than protected crops. At present there is no work being done on the use of beetle predators to control mushroom pests.

Green Lacewings (Neuroptera)

The larvae from these delicate, long-winged insects, are polyphagous predators which attack a range of soft-bodied, slow moving insects (Canard *et al*, 1984). They have been used both in field and protected crops and are in commercial production. At present there is no work being done on the use of lacewing larvae to control mushroom pests.

Bugs (Hemiptera)

There are a range of naturally-occurring bugs which are predatory in nature. Several species of anthocorids occur in the UK and research is in progress, at Horticulture Research International, to manipulate these potentially useful insects (Chambers & Long, 1991). They are voracious polyphagous predators and, as such, can consume beneficial insects as well as pest species. They are also capable of biting humans. At present there is no work being done on the use of anthocorid bugs to control mushroom pests, although there is one species (*Xylocoris galactinus*) which is called the Hot-bed bug because of its liking for compost heaps!

Mites (Acarina)

There are a number of predatory mites which are used in horticultural crops and, of the predator groups mentioned so far, this is the one which is most likely to produce a successful biological control agent. They are mostly used to control other mites which are pests although, increasingly, other pest targets are being investigated.

Mites which are known to be predacious on Diptera (flies), Acarina (mites) and Nematoda (nematodes) are obviously of most interest in the present study and, as predatory mites are often polyphagous - sometimes taking prey from all of the above Orders - this section will not be split into prey order.

A number of mites of the genus *Macrocheles* are known to attack dipteran targets (e.g. Roth, MacQueen & Bay, 1988) and Binns (1973*a*) reported finding *Macrocheles merdarius* on mushroom fly traps and in recently applied casing material. *M.merdarius* was able to feed on a number of hosts including tyroglyphid mites and nematodes.

Another genus which holds out some promise is *Hypoaspis*. Several species have been shown to be predacious on mites and are being studied for use as biological control agents (e.g. Shereef, Soliman & Afifi, 1980; Kevan & Sharma, 1964). *H.miles* has even been shown to be present in mushroom compost (Das, Somchoudhury & Mukherjee, 1987), although the authors did not realise its significance as a predator. Some current work at Horticulture Research International, Littlehampton, is aimed at studying the potential of these mites for the control of glasshouse sciarids of the genus *Bradysia* (Chambers, personal communication). *Geolaps aculeifer*, another mite predator, has also been shown to control sciarids of the genus *Bradysia* (Gillespie & Quiring, 1990).

Within the mushroom crop, several species of predatory mites have been reported and summarized by Binns (1973*a*). Three main species were described. *Digamasellus fallax*, a fairly small mite (about 0.5 x 0.25 mm), appears to accept only nematodes as food, and is phoretic on (carried by) mushroom sciarids (Binns, 1973*b*).

Arctoseius cetratus, which is about the same size as *D.fallax*, is probably the most promising of the three mites as a biological control agent. It is polyphagous and can be reared on sciarid larvae and eggs; fed on cecid larvae, tarsonemid mites (Gurney, personal communication) and rhabditid nematodes; and is phoretic on female mushroom sciarid flies (Binns, 1972).

Parasitus fimetorum is also polyphagous and can be fed on sciarid larvae, cecid larvae, mites and rhabditid nematodes and was the largest of the three (about 1.1 x 0.7 mm). They can often be seen running over the casing surface within mushroom houses but have not been shown to be phoretic on mushroom flies.

The long-legged mite, *Linopodes antennaepes*, which used to be incriminated for the damage caused by *Tarsonemus myceliophagus* (see Section 3), is actually the predator of the latter (Hussey *et al*, 1969) and is now rarely seen.

Another *Parasitus* species, *P.bituberosis*, has been shown by Al-Amidi & Downes (1990) to be predacious on sciarid larvae and eggs, cecid larvae, nematodes and springtails; and has shown promise - in both laboratory and field experiments - in the control of the white cecid *H.pygmaea*.

PARASITES

The word parasite is often used to encompass both parasitoids and true parasites. These are animals which, instead of consuming their host like a carnivore, normally develop within their host over a period of time. Depending on whether they are a parasite or parasitoid, they may eventually kill their host. An obligate parasite will live within its host without killing it, since its own survival is inextricably linked with the survival of its host. A parasitoid will live and develop within its host for a period of time before eventually killing it.

The two main groups of interest are: insects which lay eggs inside their host; and nematodes which invade their host. Both of these types are parasitoids.

Insect parasitoids

Only one parasitoidal insect is known to affect any of the main mushroom pests. This is *Asilota concolor* which is known to parasitize the larvae of the least important mushroom phorid, *M.nigra* (Hussey *et al*, 1969). However, as a parasitized *M.nigra* larva still continues to feed and develop normally until pupation, damage to the developing mushrooms continues. It is unlikely that any research will be done on this parasitoid.

Nematode parasitoids

There are several nematode parasites which affect a number of mushroom pests.

Howardula husseyi
This nematode is an obligate parasite of the mushroom phorid, *Megaselia halterata*, and extensive research has been carried out to determine its potential as a biological control agent (e.g. Richardson & Chanter, 1979). However, due to a number of factors peculiar to the life cycle and development of the parasite (Richardson & Chanter, 1981), its initial promise as a biological control agent was not realised.

***Steinernema* spp. and *Heterorhabditis* spp.**
These nematodes can be regarded as parasitoids and are likely to be important biological control agents because they can affect a wide range of insects. Their mode of action involves penetration of the hosts body cavity, release of symbiotic insect-pathogenic bacteria therein, with consequent, often rapid (within 48 hours) death of the host.

The nematodes can easily be mass-produced and stored and they can persist in the environment. They therefore possess many characteristics of chemical insecticides.

In contrast to insecticides, however, there is no evidence that these nematodes will affect plants or mammals (Poinar, 1979). The taxonomy of these nematodes is currently in a state of flux but for the purposes of this report the classifications according to Poinar (1990) will be used.

In mushroom pest control, most research has been done on *Steinernema feltiae* (=*S.bibionis*) and *Heterorhabditis bacteriophora* (=*H.heliothidis*). Mushroom sciarids, phorids and cecids were shown to be susceptible to parasitism by both nematode species (Richardson, 1987). Richardson & Grewal (1991) have also shown that *S.feltiae* can give a similar level of control of the mushroom sciarid *Lycoriella auripila* compared to that achieved by the standard insecticide, diflubenzuron.

MAFF-funded research with these nematodes for use against mushroom cecids is continuing at Horticulture Research International, Littlehampton. The Agricultural Genetics Company Ltd. (AGC) are planning commercial grower trials in the second two quarters of this year; and the launch of a commercial product should occur in the next 6 - 12 months (R.Jenkins, AGC, personal communication).

PATHOGENS

In this group of potential biological control agents are the bacteria, fungi and viruses - micro-organisms that can infect insects and other animals and cause their subsequent death.

Bacteria

The most common bacteria used for pest control around the world is *Bacillus thuringiensis* and it is the species upon which most research is being carried out. *B.thuringiensis* bacteria produce a protein crystal and a vegetative spore - both held within the cell. Both are capable of having insecticidal action, although it is the crystal that generally produces most activity. The crystal only becomes 'insecticidal' after it has been broken down by the alkaline conditions within an insects gut. It is not toxic to mammals.

Bacteria for dipterous insect control

Bacillus thuringiensis israelensis (*B.t.i.*) is a dipteran-active strain of *B.thuringiensis* which is highly active against mosquito larvae (Goldberg & Margalit, 1977) and has been used in various parts of the world in an attempt to control other dipteran pests. Osborne *et al* (1985) showed that *B.t.i.* could exert control over a glasshouse sciarid, *Bradysia coprophila*; while in the mushroom area of research, Cantwell & Cantelo (1984) showed that *B.t.i.* had promise as a control agent for the American mushroom sciarid *Lycoriella mali*, although the dose rate used was not economic. More recently Keil (1991), using an experimental formulation of *B.t.i.* in commercial trials, demonstrated that control of *L.mali* and the mushroom phorid *M.halterata* could be achieved at the same level as grower-applied control measures.

White & Jarrett (1990) and Pethybridge, White & Jarrett (1991) have demonstrated that a number of *B.thuringiensis* isolates gave control of *L.auripila* larvae, superior to that achieved by *B.t.i.* and comparable to that of diflubenzuron; and work continues with these isolates, and the search for superior ones, at Horticulture Research International, Littlehampton. Commercial exploitation of an isolate for mushroom pest control should occur within the next 2 - 3 years.

Bacteria for nematode control

There is also the possibility of using *B.thuringiensis* to control nematodes. Osman *et al* (1988) have shown that two species of plant-parasitic nematodes were controlled, to varying degrees, by two different formulations of *B.thuringiensis* and Bone *et al* (1988) have shown that *B.t.i.* also had an effect on an animal-parasitic nematode.

Research in this area has developed to the extent that the Mycogen Corporation (a U.S. Bio-control company) have filed a patent on novel *B.thuringiensis* isolates for the control of nematodes (Mycogen, 1988). However, there are no reports of any similar research being carried out on the various mushroom nematodes.

Bacterial exotoxins

In addition to producing a protein crystal and a vegetative spore, *B.thuringiensis* can produce a number of exotoxins. In contrast to the crystal and spore, the exotoxins are general toxins and, in consequence, can have a wide range of activity, including mammalian toxicity.

Exotoxin for dipterous insect control. Although active against a range of insects, the beta-exotoxin was only active against the mushroom sciarid, *Lycoriella auripila*, at very high doses (Jarrett, personal communication).

Exotoxin for mite control. There are a few cases where a product (thuringiensin) containing the stable beta-exotoxin of *B.thuringiensis*, has been shown to be effective against various plant mite pests (Neil *et al*, 1987; Royalty *et al*, 1990). At present there is no work being done on the use of exotoxins to control mushroom mites.

Fungi

A number of pathogenic fungi are capable of invading the bodies of insects, mites and nematodes. The pest comes into contact with either the spore or the vegetative mycelium of the pathogenic fungus, which then penetrates the cuticle of the pest and develops within its body. After the death of the pest, the fungus produces spores on the surface of the cadaver, thus spreading the disease further.

Fungi for dipterous insect control

A number of fungi have been tested for their efficacy against Dipteran pests and have shown promise as potential biological control agents. Species from the genus *Entomophthora* commonly affect a wide range of targets including carrot flies (Eilenberg, 1988) and house flies (Mullens, 1986).

Verticillium lecanii, in addition to controlling aphids and whiteflies (Hall, 1982), has also been shown to affect the mushroom sciarid *Lycoriella auripila* (Matthews & White, unpublished observation). However, *V.lecanii* is a hyperparasite of various other fungi, including various *Agaricus* species (Samson & Rombach, 1985); and laboratory tests by Matthews (personal communication) confirmed that a commercial preparation would produce the same symptoms on an *Agaricus bisporus* sporophore as would be produced by the fungal pathogen *V.fungicola*. It seems unlikely, therefore, that *V.lecanii* would be suitable as a mushroom pest biological control agent

There are many other genera shown to parasitic on dipteran insects including *Hypomycetes* (e.g. Bordat *et al*, 1988) and *Metarhizium* (e.g. Samuels *et al*, 1989) but, within the sphere of mushroom research, it is the development of a species of *Pandora* that is showing most promise. *P.gloeospora* has been shown by Keil (reported in an article by Kukich, 1991) to be active against the mushroom sciarid *L.mali*, killing adults within 24 hours. Semi-commercial trials, in conjunction with Ecogen (a biotechnology company in the U.S.) are planned in the near future and its use against public health pests is also being investigated.

Fungi for nematode control

There have been a number of attempts to control nematodes by parasitic fungi. Various species of the *Arthrobotrys* genus have been used against plant-parasitic (Cayrol, 1983*a*; Leuprecht, 1988) and other nematodes (Cayrol, 1983*b*). They have also been attempts to control both mycophagous and saprophagous nematodes in mushroom crops with species from this genus. In reviewing their use, Hesling (1978) was of the opinion that although there was some evidence that *A.robusta* could kill both types of nematode, their use in mushroom culture was not justified. Notwithstanding this, a grain spawn of *Arthrobotrys irreglaris* is still commercially available as S350 from Somycel S.A..

Not all nematodes are pests, of course, and it is interesting to note that Poinar & Jansson (1986) have shown that two parasitic fungi, including a species of *Arthrobotrys*, can kill insect-parasitic nematodes of the genera *Neoplectana* (=*Steinernema*) and *Heterorhabditis*. This could limit the effectiveness of any combined use of nematode-parasitic fungi and insect-parasitic nematodes.

Viruses

Viruses are widely researched and studied within certain dipteran Groups, notably the Lepidoptera. Very few viruses are known to infect dipteran insects and those that do mostly affect various species of mosquito. Some nuclear polyhedrosis viruses (NPVs) have been shown to infect a few sciarid species of the genus *Rhynchosciara* (Morgante *et al*, 1974) but these occurrences have been used to study the action of the NPVs, rather than to determine their potential as bio-control agents.

PHEROMONES

Pheromones are volatile chemical substances which are secreted and released by animals. Their function is usually to aid detection and/or to provoke a response by the same species and are often sex-orientated. In biological control regimes they can either be used 'live' (i.e. using captured insects to attract others) or, more normally, a synthetic analogue of the natural pheromone is used for the same purpose.

They can be used either for monitoring pest populations, so that other control methods can be used more effectively; or they can be used as control methods in their own right. In the latter case, they can be used in mass-trapping exercises to remove one of the sexes of a pest or in such a way as to prevent the two sexes finding each other. This is aimed at limiting or entirely stopping an infestation by that pest.

Pheromones are safe to use and are normally extremely species specific. This can be a positive feature if the aim is to control certain pest species without harming other biological control agents.

Pheromones in dipterous insects

Numerous substances have been identified as being active as sex-pheromones in Diptera and have been synthesised. The economic importance of a pest has played a role in determining which pheromones have been most researched. Thus, there are numerous references in the literature to the use of Trimedlure, an attractant of the Mediterranean fruit fly, *Ceratitis capitata* (e.g. Leonhardt *et al*, 1989; Cunninham & Couey, 1986). Other important pests, such as mosquitoes, have also been targeted (Laurence & Pickett, 1985).

Pheromones in sciarids

In the Sciaridae, Alberts *et al* (1981) showed that the female of *Bradysia impatiens* (a glasshouse sciarid) - mated or unmated - emitted a sex-pheromone which attracted the male. In the mushroom sphere of research (but still with the Sciaridae), Girard *et al* (1974) demonstrated the existence of a sex-pheromone in the mushroom sciarid, *L.mali* and Kostelc (1977) later determined the pheromones constituents, heptadecane being the most active. However, Binns (1976) had used this compound in earlier trapping experiments, with no success, so it may be that a 'cocktail' of compounds is necessary to bring about a response. MAFF-funded work, by the author, on this area of research is in progress.

Pheromones in phorids

In the UK, work initiated by the author on the mushroom phorid, *Megaselia halterata*, showed that a sex-pheromone was produced by virgin females (Burrage, 1981). Subsequent isolation, identification and synthesis of the presumed pheromone by Baker *et al* (1982), however, produced a compound that, in commercial trials, was not as active as the natural pheromone (White, unpublished data).

Pheromones in cecids

A more unusual pheromone-induced behaviour occurs with the mushroom cecids. Panelius (reported by Wyatt & Binns, 1975) demonstrated that *Heteropeza pygmaea*, under certain conditions, produced larvae which were destined to become adults rather than paedogenetic mother-larvae. This phenomenon has the potential, if it could be manipulated, of preventing the normal larval population build-up of this difficult-to-control cecid.

Panelius was able to postulate the following sequence of events, based on pheromone-induced dispersive behaviour: each paedogenetic larva produces a pheromone (or pheromone-like) substance; if there are enough larvae in the immediate environment the concentration of the pheromone builds up to a 'critical' (but undefined) level; once this 'critical' pheromone concentration has been reached or exceeded, it induces the larvae to 'switch' from being a larva destined for paedogenetic reproduction, to a larva destined to produce an imago larva.

This imago larva produces a pupa in the 'normal' insect way and an adult fly subsequently emerges. Similar processes are thought to exist in the *Mycophila* species. As already implied, this could have an important impact on cecid control Cecid larvae are difficult to control but the adults are not. In addition, as already mentioned in Section 4, cecid adults may even be sterile (Dmoch, 1991) thus obviating the use of insecticides entirely.

Pheromones in mites

There are many fewer instances of pheromone-induced behaviour in mite pests. Some of the lesser important genera associated with mushroom growing (e.g. *Caloglyphus* and *Tyrophagus*, Fletcher *et al*, 1989) have been shown to have sex-pheromones (e.g. Leal *et al*, 1989) and alarm-pheromones (e.g. Kuwahara & Sakuma, 1982) but these are unlikely to be important in mushroom mite control.

KAIROMONES

These are volatile chemical substances which are produced by the food source of an animal and are used by that animal for the location of the food source. They are not produced intentionally by the food source to attract attention (as is the case with scents given off by flowers).

Kairomones and insects

There are a number of kairomones associated with insects. Most of them consist of hydrocarbons and their chemical ecology and biochemistry is discussed at length by Howard & Blomquist (1982).

Kairomones and sciarids

Lycoriella auripila flies first infest mushroom compost as it cooling down after Phase II pasteurization (Fletcher *et al*, 1989) and they can sometimes be seen congregating around the doors of pasteurization rooms at this time (Hussey, 1969). It has also been noticed that, unless a source of infestation was close, all the invading flies were female (Binns, 1979). It is a reasonable assumption that this behaviour is induced by some kairomonal activity present in the exhaust gases of the pasteurization process. The females respond to this stimulus for the location of a food source for their progeny. However, there appear to be no published records on the natue or identification of these substances.

Manipulation of such a kairomone within an integrated pest control programme could result in more effective control of this damaging pest.

Kairomones and phorids

Female *Megaselia halterata* flies are attracted to the smell of growing mycelium (Binns, 1976). The oviposition attractant has been isolated from the air in spawn-running rooms (Grove & Blight, 1983) and bioassayed in wind-tunnels (Burrage, 1981). However, there has been no further work done with these potentially useful compounds.

Kairomones and mushroom mites

It is unlikely that, if they exist, kairomones will play any role in the control of these relatively unimportant pests.

REPELLENTS AND ANTI-FEEDANTS

These substances can sometimes be produced by the food source of an animal, which in some way repel the animal either before (repellent) or after (anti-feedant) it has fed and can be regarded as the food source's self-defence mechanism. A repellent acts as a dispersant of the potential feeder, while an anti-feedant results in the animal staying where it is, gradually starving to death. These substances can be isolated, synthesised and subsequently used against a potential pest in a similar way to insecticides.

Pesticides themselves can act as repellents; for example a proportion of the effectiveness of permethrin against *Lycoriella auripila* was attributed to the repellent nature of the product (White, 1977). This sort of repellency, however, cannot be described as biological control.

Repellents/anti-feedants and sciarids

In his work on the substrates of *Lycoriella auripila* larvae, Binns (1975) suggested that a degree of 'cultural' control of sciarids could be achieved due to an apparent antagonism between sciarid larvae and mushroom mycelium. The mycelial metabolic by-product, calcium oxalate, was later shown, in laboratory tests, to act as a repellent (Binns, 1983).

Recent semi-commercial experiments by the author (in collaboration with the A.D.A.S.) have shown that this substance does have a degree of activity but probably at an insufficient level to act as a stand-alone control measure. As with the use of kairomones, however, calcium oxalate may find a useful place within an integrated pest control programme.

GENETIC CONTROL OF INSECTS

This type of control is based on the inundative mass-release of sterile insects (normally males) into a natural population. The large majority of wild females, therefore, mate with infertile males thus causing a subsequent collapse in the population. Insects can be sterilised by either radiation or chemical treatment. Because of the enormous cost of undertaking such a programme, however, its use has been confined to pests of great economic importance. It is most unlikely that such a programme could be envisaged for mushroom pest control.

'PHYSICAL' CONTROL OF INSECTS

In this section can be included all those methods of control which, although not necessarily based on 'biological' principles, are otherwise environmentally acceptable. Methods based on hygiene principles will not included as they are covered quite adequately in Fletcher *et al* (1989).

Exclusion of insects

At first sight it might appear reasonable to try and exclude flying insects from the critical phases of commercial mushroom production, such as the cool-down from Phase II pasteurization, spawn-running and pre-cropping. However, to completely stop the incursion of 2-4 mm flies, who are positively attracted to the crop at these stages, is more or less impossible. That is not to say that an attempt should not be made, but the limitations should be realised. The use of mesh screening is commonplace but the mesh must be fine enough to stop the progress of flies (16 mesh/cm) but not too fine to prevent the flow of air into the room.

Monitoring techniques, to aid growers in determining when to apply insecticides, can be of the passive type (Jansson, 1986), or of the active type (e.g. the use of 'blacklight' traps; Cantelo *et al*, 1977). Other trapping systems have tried to exert a degree of control over the flying pest population by the use of suction traps (Lelley, 1984) or sticky traps (Wardlow & O'Brien, 1988). Both techniques had a measure of success, although the 'stick' in the latter technique also contained a synthetic pyrethroid.

The efficiency of these trapping systems would undoubtably be greatly improved if used in conjunction with an active pheromone attractant, preferably a 'cocktail' which would attract both phorid and sciarid adults. Even more so than pheromones and kairomones, trapping techniques are unlikely to be effective as stand-alone pest control measures. However, used in an integrated pest control programme, they will find their place.

SECTION 7

SOURCES OF BIOLOGICAL CONTROL AGENTS

The latest worldwide compendium of agro-biological products is published by CPL Press Ltd (Newbury, UK)(Lisansky, 1990). It contains a list 197 companies that are involved in, research on, manufacture or supply biologically-based methods of control, although not all of them relate to the control of pests. Unless otherwise stated, the information in this section is taken from this publication.

The number of companies that are, or become, interested in the bio-pesticide market is in a constant state of flux. It is likely that numerous companies examine the practicality of marketing and/or producing bio-pesticides every year.

PRODUCTS WITH RELEVANCE TO THE MUSHROOM INDUSTRY

Mites

The only soil-dwelling predatory mite that is being mass-reared, is produced by Applied Bio-nomics Ltd. (Canada). It is a species of *Hypoaspis*, which is marketed as a control agent for glasshouse fungus gnats (probably *Bradysia* spp.), and its potential against the same genus is to be evaluated at Horticulture Research International, Littlehampton (Chambers, personal communication).

Bacillus thuringiensis

From the commercial viewpoint, Abbott Laboratories (USA) is the leading bio-pesticide company, supplying 75% of the existing *B.thuringiensis* market (total value about $20=22 million). They market twelve formulations of *B.thuringiensis* and produced the experimental formulation used by Keil (1991) against mushroom phorids and sciarids in the USA. Sandoz (Switzerland) is the second biggest bio-pesticide company, again with most of its bio-products based on *B.thuringiensis* (sixteen formulations).

The strongest of the newcomers to the bio-pesticide market is Novo BioKontrol (Denmark, five formulations), while Ecogen (USA, four formulations) and Mycogen (USA, two formulations) also have strong interests in the *B.thuringiensis* market.

Insect-parasitic nematodes

Most companies involved in producing or supplying insect parasitic nematodes are based in Europe, although the largest one (BioSys) is based in the USA. The others are: Agricultural Genetics Company Ltd. (MicroBio Division)(UK); Applied Horticulture (UK); Chr.Hansen's Bio Systems (Denmark) and SIAPA SpA (Italy).

Parasitic fungi

Ecogen (USA) are involved in the development of *Pandora gloeospora*, a fungus shown to be effective against *Lycoriella auripila* (Kukitch, 1991). Somycel S.A. (France) market a grain spawn containing the nematode-trapping fungus *Arthrobotrys irregularis*.

Pheromones and kairomones

The technology of commercial use of these products is fairly specialized and, at present, the markets for their use are small compared to those for chemical pesticides. There are a number of companies offering pheromone-based products around the world but the one likely to be of most relevance to UK interests is AgriSense - Biological Control Systems Ltd (UK). This company has shown interest in developing mushroom-related products and has a worldwide distribution system. Most companies involved with pheromones also supply various trap products.

ADDRESSES OF COMPANIES CITED

Abbot Laboratories
14 Street & Sheridan Road, North Chicago, IL 60064, USA

Agricultural Genetics Company Ltd. - MicroBio Division
Unit 126, Cambridge Science Park, Cambridge, CB4 4FZ, UK

AgriSense - Biological Control Systems Ltd.
Treforest Industrial Estate, Pontypridd, Mid Glamorgan, Wales, CF37 5SU, UK

Applied Bio-nomics Ltd.
P.O. Box 2637, British Colombia, Canada, V8L 4CL

Applied Horticulture (A Division of Fargro Ltd.)
Toddington Lane, Littlehampton, West Sussex, BN17 7PP, UK

BioSys
1057 East Meadow Circle, Palo Alto, CA 94303, USA

Chr. Hansen's Bio Systems
10-12 Boge Alle, DK-2970, Horsholm, Denmark

Ecogen Inc.
2005 Cabot Blvd West, Langhorne, PA 19047-1810, USA

Mycogen Corporation

5451 Oberlin Drive, San Diego, CA 92121, USA

Novo BioKontrol

Novo Nordisk, Novo Alle, DK-2880 Bagsvaerd, Denmark

Sandoz

Agro Division, 4002 Basle, Switzerland

SIAPA SpA

Via Yser 16, 00198 Rome, Italy

Somycel S.A.

B.P. 25, 37130 Langeais, France

SECTION 8

FUTURE WORK

Research into biological methods of control of the main insects pests is likely to produce the best economic return. Basic research into the biological control of the minor pests, no matter how scientifically interesting, is unlikely to give value-for-money unless an *a priori* reason exists for such a course of action. For example, the organic production of mushrooms, i.e. without the use of pesticides, may throw up new pests, or increase the importance of existing minor ones. The only answer for the control of that pest may well be biological.

However, for the purposes of this review, it will be the assumption that, at the present time, no such reasons exist and it is on the major insect pests that the focus for biological control research rests. A codicil to such a statement would be that the mushroom pest complex is a dynamic one and a 'weather eye' should always be kept out for new developments.

In making suggestions for future work, it can be difficult to judge the distinction between the desirable and the possible. This judgement the author has attempted to make, based on numerous discussions with scientific colleagues and several years experience in mushroom pest control.

PHEROMONES AND KAIROMONES

There are a number of subjects within this area which can be regarded as suitable for industry funding.

Sciarids

MAFF-funded work on sciarid pheromones, by the author, has just been initiated at Horticultural Research International. However, there are certain aspects of this work that fall outside the specific remit of this project - but are complementary to it - and would be suitable for industry support. This would be to look at the kairomones, present in the fermentation odours from cooling compost, responsible for attracting the females to the crop.

Phorids

The presence of mycelium-produced kairomones and a female-produced sex pheromone has been demonstrated in mushroom phorids. Some of these compounds have been tested in bioassays but further research and development work on these compounds has not been carried out. As there is a question-mark hanging over the future of the main control measure for phorid larvae, diazinon, it would be a subject suitable for industry support.

Cecids

The presence of a pheromone-like substance has been demonstrated in mushroom cecids and some preliminary research, by the author, has been carried out with the white mushroom cecid *Heteropeza pygmaea*. Cecid control is extremely difficult with the one chemical control measure that is available to mushroom growers. As that chemical is diazinon, the same potential problem that exists with phorid control, exists with cecid control, only more so. With phorids (and to a lesser extent, sciarids) there is at least the opportunity to control the vulnerable flying stage with a variety of products. This opportunity does not exist with cecids. One of the major problems that can often face pest research programmes, is insect rearing. This problem does not exist with mushroom cecids. Therefore, this would be a subject suitable for industry support.

Predatory mites

Work with predatory mites is increasing in several areas of the world. It has already been demonstrated that mites can play a role in the control of the mushroom cecid *Heteropeza pygmaea* and the mushroom sciarid *Lycoriella auripila*; and that work is being carried out on a number of mites that might control other sciarid species. This latter work (at HRI) is, at present, confined to looking at sciarids in glasshouse crops. However, there is the opportunity that, with some additional industry funding, the work could be extended to include mushroom pests.

FUNGI

Research into the *Pandora* species of entomogenous fungus, by Keil in the USA, is advancing successfully, with financial support from the US biotechnology company, Ecogen. There appears every likelihood that, in the near future, *Pandora* will be developed commercially. It would seem appropriate, therefore, to determine its effectiveness against mushroom pests under UK conditions.

INSECT-PARASITIC NEMATODES

The insect-parasitic nematodes that are being developed for sciarid control do not function as well in the compost (against phorids and cecids) as they do in the more moist casing. There is a need to select, using breeding or natural selection techniques, for an nematode isolate that performs well in compost conditions.

SECTION 9

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