Trichoderma and Penicillium Diseases of *Agaricus bisporus*

The biology and epidemiology of Trichoderma diseases of *Agaricus bisporus* with some additional information on Penicillium diseases False Truffle and Pythium black compost

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INTRODUCTION

The development of bulk systems of compost production has had major effects on the UK mushroom industry. The quality of bulk produced compost has improved productivity and has also been used by compost suppliers to move efficiently into systems of production for the sale of spawned compost. With the increase in bulk compost and the sale of so called phase II compost (spawned compost) there has been a noticeable increase in mould problems. The most frequent of these has been Trichoderma spp. But False Truffle (Diehliomyces microsporus) and Penicillium Smoky mould (Penicillium chermesinum) have also been found; Pythium black compost (Pythium oligandrum) has occurred recently in traditional systems. This review looks primarily at Trichoderma diseases but briefly considers the others. The exact relationships between these various fungi and the crop (Agaricus bisporus) is either only partially understood or not understood at all. Sometimes the fungi are referred to as weed moulds but this presupposes that they have no active relationship with Agaricus. It is known that some Trichoderma species are parasitic on A. bisporus producing clearly defined symptoms (Kligman, 1950) and there are many records of species in this genus being parasitic on other fungi (Chet, 1987). Diehliomyces microsporus was reported by Bissett (1980) to be a pathogen of A. bisporus although it is generally included in the weed mould section of most mushroom books. Penicillium chermesinum is newly recorded from mushroom crops and its relationship with Agaricus is completely unknown. Finally Pythium oligandrum is a well known fungal pathogen (Veselý, 1978) but its relationship with A. bisporus has not been investigated.

TRICHODERMA DISEASES OF AGARICUS BISPORUS

The genus Trichoderma is one of the most ubiquitous of soil fungi and is found in almost all soils. It has been extensively studied as a saprophyte but also, as an antagonist and a parasite of other fungi. In the mushroom literature it has long been recorded as a source of cap symptoms (Beach, 1937) and as a green mould (Kligman, 1950). Because of the difficulties over the taxonomy of species, a number of different names have been given to the fungi involved and it is not always clear how precisely some of the named species have been associated with the symptoms described. In addition, 'physiological' forms or strains of T. harzianum species have been recognised (Seaby, 1989).

Diseases caused by Trichoderma spp

Trichoderma spot or blotch

This disease was described by Kligman (1950) who attributed its cause to T. lignorum. This species name is now considered to be synonymous with T. viride. Kligman describes this disease as being similar to Verticillium and probably widespread. The symptoms include irregular dark brown spots of various sizes - darker in colour than those of Verticillium. Sometimes the whole cap may be covered with one large blotch. The affected area becomes shrunken and depressed. The affected tissue is not wholly superficial with the underlying tissue becoming discoloured, sometimes with bands and streaks in the stem. The affected stems sometimes split or distort.

Harvey, Wuest and Schisler (1982) record Trichoderma spot, Trichoderma mildew and Green mould all under Trichoderma diseases. The pathogens associated with these are listed as Trichoderma viride and T. koningii with T. lignorum given as an outdated name.

They recognise two species associated with mushroom growing although they state that many forms can be isolated from compost and casing. T. viride is described as a pathogen which may be dark green in colour and appear on the casing at any time during crop production. Mushrooms in areas occupied by T. viride die and show signs of the dark green mould. They report the possibility of this fungus excreting a toxin into the casing which kills mushroom mycelium. Tissue death of the mushrooms starts as a mahogany brown to red brown discoloration that develops along the stipe. Dry sunken lesions develop on the cap.

Trichoderma mildew

T. koningii causes mildew which usually appears between the third break and the end of the crop. It was first reported by Sinden and Hauser (1953) who distinguished it from Trichoderma blotch. T. koningii establishes on dead tissue but spreads onto the live tissue at a fast rate. Mushrooms overgrown by the mildew develop a soft wet rot. Sage-green spores occur on the surface of the affected mushroom together with the white downy mycelium of the pathogen. It can also cause purple-brown spots on the caps of mushrooms early in the crop before mould growth occurs on the casing. Similar pale brown to purple-brown coloured spots on the caps of mushrooms have been associated with T. pseudo koningii in the UK (Fletcher, personal communcation).

Harvey et al (1982) also report the ability of the various Trichoderma species to grow on the compost particularly when carbohydrates are available or when the compost is not supplemented with sufficient nitrogen. Once the compost is colonised, Trichoderma will grow into the casing forming areas 1-2 ft in diameter. These areas turn green within several days.

Trichoderma Red Spot Trichoderma sp.

This disease was described by Kneebone & Merek (1959). The description of the pathogen suggests that it was not T. viride but could have been T. koningii or T. harzianum. They list T. koningii separately so presumably differentiated it from this species. The disease produced is described as producing reddish-brown spots on the caps which are localised and do not coalesce into blotches. There is no obvious mildew growth on the affected mushrooms or Trichoderma sporulation on the casing. The disease does not develop in the early flushes and in this respect is similar to Trichoderma mildew as described by Harvey et al (1982).

Trichoderma compost mould

This was first described by Seaby (1987) and Staunton (1987). The problem was initially associated with bag growing and in N. Ireland in the spring of 1985 bags were found which turned green 2-5 weeks after the beginning of spawn running. Similarly in Eire the disease was characterised by the rapid colonisation of spawn running and spawn run compost resulting in severe yield reduction. In the early stages of colonisation the fungus is white but rapidly turns green as it sporulates. At about the same time similar symptoms were seen in bag and block grown crops in England and subsequently on tray farms.

In all cases Trichoderma harzianum has been identified as the species involved. In N. Ireland further differentiation of types has shown there are particular physiological races of T. harzianum involved. The form most frequently found has been designated T. harzianum form 2 (Th2).

In many of the descriptions of Trichoderma diseases, red pepper mites are associated with the occurrence of the mould. These often form in large numbers on the casing surface and swarm over developing mushrooms, congregating on the cap surface. They are not a cause of damage themselves but are a source of annoyance to pickers and must be removed before the mushrooms are marketed. They probably play an important role in the epidemiology of Trichoderma diseases as they feed on the mycelium and carry Trichoderma spores.

Also, it is frequently reported that colonies of green mould occur on the remains of mushroom stalks and caps left on the casing surface. How important these are and also those colonies which grow on the wooden box sides, is not known.

Summary and Conclusions on Trichoderma diseases

1. Three species of Trichoderma are recorded as pathogens of A. bisporus, T. viride, T. koningii and T. harzianum. T. lignorum is an outdated name for T. viride. T. pseudo koningii may also be pathogenic.

2. Three distinct diseases have been described.

   (a) Blotch - caused by T. viride and characterised by dark brown sunken lesions on the cap surface and distortion of the stalks.

   (b) Spot or mildew - caused by T. koningii and possibly T. pseudo koningii and characterised by light brown coloured non-coalescing spots.

   (c) Compost - prolific white mycelial growth in the compost eventually producing the dark green spores of T. harzianum.

In addition a red spot disease caused by an unknown species has been described and many records made of Trichoderma spp on organic matter and wood in association with mushroom crops.

All three diseases have been seen in the UK. Blotch may frequently be mistaken for other diseases, eg. Bacterial blotch and spot for Verticillium. There is no mistaking the compost colonising form which is undoubtedly the most important Trichoderma disease in the UK at present.

The incidence of Trichoderma diseases of Mushrooms in the UK

Although the Ministry of Agriculture and Fisheries plant disease records for England and Wales from 1922 onwards make frequent mention of mushroom diseases and weed moulds, there are no records of any Trichoderma species affecting the crop from 1922-1942. In British Parasitic Fungi, Moore (1959) mentions T. viride occurring as a bed invader in Northants, Herts and Derby although the exact dates of these records is not given. The Mushroom Research Station Report, Yaxley for 1946-48 records T. viride in the category of "Fungi of varying or doubtful status identified in the compost and/or in the soil of mushroom beds" (La Touche, 1949). But in the 1949 report, Duncan (1950) lists T. viride in the competitors and invaders category. There are few specific references to Trichoderma problems in the Mushroom Journal and its predecessor or in Darlington's Mushroom News. Fred Atkins wrote in October 1977 "there is much T. viride about this year, on compost and then on casing" (Atkins, 1977). In 1978 Steane (1978 a) reported in the Mushroom Journal on Trichoderma diseases. He described the pathogen as probably of the T. viride type and not T. koningii. The symptoms included mycelial growth on the casing and compost showing as a dense pure white outer section in which rounded aggregates sometimes formed. With spore production the centre of the colony turns greyish green and progressively became brighter green in colour. Mushrooms near this mycelial growth showed symptoms of a possible toxin with browning, cracking and sunken lesions. The stalk was greyish-brown and powdery in appearance. Steane (1978 b) described in detail, experience with the same Trichoderma problem on R. Thompson's farm in Sussex. He related compost analysis with disease occurrence showing a relationship between the nitrogen level and the occurrence of the problem.

Disease records have been kept by ADAS for some years and although they cannot be quantified, they give some indication of the relative amounts of disease as well as the industry's concern at the time the reports were made. There are few specific references to Trichoderma as a problem before 1983. It is perhaps important to note that in this year bulk phase II compost began to appear on the market. In 1984 it is recorded that there were two phase II compost producers with a total output of about 300 tons of compost per week. By the end of 1984 this had risen to nearer 400 tons and in January 1990 the estimated figure was 1180 tons/wk with five phase II producers involved. Yields from such compost is reported to be in the region of 500 lbs/ton.

A few Trichoderma outbreaks were reported in 1984 but 1985 saw a large increase particularly in crops grown in blocks [the majority of the phase II producers were at this time growing in blocks]. At about the same time a major problem was recognised in N. Ireland and in Eire. In Eire it was presumed to be caused by T. viride but in N. Ireland T. harzianum was identified as the cause. The main outbreaks were largely associated with one particular phase II producer in each country. At this time it was thought that the spawn could be the source. In 1986 more problems occurred in England and Ireland causing great concern among compost producers and growers alike. There were between 15-20 reported outbreaks in Eastern England alone. False Truffle increased in incidence at about the same time and was common in phase II crops. By the end of 1986 compost suppliers were becoming very reluctant to compensate producers by replacing compost. Crops affected varied from a few to many units (bags or blocks) but those less affected often yielded very satisfactorily (500 lbs/ton). The severely affected crops showed very large reductions in yield, often producing less than a half (200 lbs/ton) of that expected.

From 1987 to the present time, outbreaks of Trichoderma as a compost problem have continued. Throughout the 1980s there were also reports of cap spotting and blotching associated with Trichoderma usually T. koningii or T. pseudo koningii, but these have not been as serious or as commonly reported as the compost invading form. Although the compost invading Trichoderma, now generally accepted as T. harzianum, was associated with phase II bag growing in Ireland and predominantly phase II block growing the the UK, it has also occurred in all systems of culture. A number of tray farms have been severely affected although these have generally, but not always, had some link with block culture or bulk phase II compost. Whenever and wherever the compost problem occurs it is closely accompanied by myriads of red pepper mites which are often the first indication of its presence.

Summary of Trichoderma diseases in the UK

1. Trichoderma viride has been associated with mushroom culture in the UK since at least the late 1940s when it was recognised as a weed mould.

2. It is a commonly occurring fungus and has been mainly linked with cap spotting, mushroom deformation and on one farm at least, in the 1970s, with compost colonisation.

3. T. koningii or T. pseudo koningii have quite often been associated with cap spotting symptoms.

4. A serious compost invading form of Trichoderma occurred in Ireland and England in 1985 and 1986 and has been present ever since. The compost form is morphologically different from T. viride and is ascribed to the species T. harzianum. Its occurrence was initially correlated with phase II compost systems but conventional systems have also been affected.

The taxonomy of the Trichoderma spp associated with mushroom culture

The genus Trichoderma is classified in the Plectomycetes division of the Ascomycotina. The familiar green sporing stage is the asexual or conidial state of the fungus, the perfect stage being in the genus Hypocrea. The perfect state of T. viride is H. rufa, but not all species of Hypocrea have the Trichoderma conidial state, eg. H. gelatinosa has a Gliocladium conidial state. Webster (1964) noted that it was unwise to press for precise nomenclature of the conidial states of Hypocrea.

The description of the genus Trichoderma according to Barron (1968) is a as follows:-

"Type Species: Trichoderma viride Pers.

Genetic Descriptions: Conidiophores erect or straggling, solitary or frequently aggregated into floccose tufts, septate, branching irregularly, or weakly or strongly verticillate, frequently branching more or less at right angles to the main axis; sporogenous cells phialides, borne singly or in clusters, hyaline, ovate to flask-shaped; when aggregated into tufts, conidiophores frequently intermixed with sinuous sterile hyphae; sterile hyphae sinuous, smooth or encrusted with wart-like protuberances, anastomosing below; phialospores hyaline or green, nonseptate, gathering in balls at the mouths of the phialides.

Diagnostic Features: Trichoderma is very distinctive in culture, producing rapidly growing, floccose colonies which are white, yellow-green or bright green in the common species".

Cooke & Baker (1983) point to the fact that the genus has been a source of confusion since it was first erected by Persoon. Of the four original species only one, T. viride, is still within the genus. Bisby (1939) found no reliable distinguishing characters among Trichoderma isolates and therefore considered it to be a monotypic genus. Most workers between 1939 to 1970 followed Bisby, naming any green spored form of Trichoderma, T. viride. Rifai (1969) revised the genus to include nine species aggregates and his work is now generally accepted.

He showed that there were some minor but reliable characters which can be used to classify these fungi but adopted the 'species aggregate' concept rather than species for his nine divisions. Such aggregates include groups of species that are "normally inseparable". Rifai justifies the use of species aggregates on the grounds that future workers may be able to sub-divide these aggregates into component species. His nine aggregate species are;-
T. hamatum, T. koningii and T. viride, which are classical species, ie. names used before. In addition he creates T. polysporum which is a previously used name but redefined and also five new species, T. piluliferum, T. aureoviride, T. harzianum, T. longibrachiatum and T. pseudo koningii. Some of the characteristics of the nine species including those that are associated with mushroom culture are listed in Table 1.

Seaby (1989) compared isolates of Trichoderma from mushroom compost studying the growth rates on antibiotic malt agar at 18° and 27°C. Many were identified as T. harzianum. He examined cultures for their smell, spore shape, morphological features of plialides (spore producing cells) and chitinase activity. His results are summarised in Table 2. In addition to these characteristics, T. harzianum (form 2) was the only one that had not formed spores in culture by the third day of incubation.

Fletcher (unpublished) using the Seaby criteria between September 1988 and August 1989 to identify strains of T. harzianum found that of the ten isolates examined that were associated with compost problems, five were in the Th3 category, four in Th1 and one in Th2. No attempt was made to relate strains to disease severity although all those sent for examination were from problem crops.

Table 1. Morphological and cultural characteristic of nine Trichoderma species

Trichoderma species	*HAMATUM	HARZIANUM	KONINGII	VIRIDE	POLYSPORUM	PILIFERUM	AUREOVIRIDE	LONGI.	PSEUDOK.
Teleomorph HYPOCREA	H.semiorbis	H.vinosa	H.ceramica	H.rufa	H.pachybasioides	H.pilifera	H.aureoviridis		
Growth agar, (oat) 20°C in 5 days	7 cm	9 cm	3-5 cm	-	-	-	-		
Colour on malt agar	Greying green	Whitish Yellowish Dull green	White greenish white dark green	Dark green bluish green Yellowish	White	White	Yellow to olivaceous green	Translus-cent white	White bright green
Conidia Shape	Short Cylindrical Smooth	Subglobose Short oval Smooth	Short Cylindrical Smooth	Globose Rough	Short Ellipsoidal Smooth	Globose Smooth	Obovoid Ellipsoidal Smooth	Elliptic Obovoid Smooth	Short Cylindrical Smooth
Size	3.8-6 x2.2-2.8	2.8-3.2 x2.5-2.8	3 -4.8 x1.9-2.8	3.6-4.5	2.4-3.8x 1.8-2.2	2.5-3.5	3.5-5x 2.5-3.2	3.6-6.5x 2.2-3.0	3.4-4.6x 2.0-2.5
Growth Opt pt	4.0-4.5	3.7-4.7	3.7-6.0	4.5-5.6	-	-	-	-	-
Opt. Temp	24°C	30°C	26°C	20-28°C	20-25°C	-	-	-	-
Max. growth Temp.	34°C	36°C	32-40°C	30°C	28-31°C	-	-	-	-

* nine species of Trichoderma, viz T. hamatum, T. harzianum, T. koningii, T. viride, T. polysporum, T. piliferum, T. aureoviride, T. longibrachiatum & T. pseudo koningii.

Table 2. Seaby classification of isolates of Trichoderma from mushrooms

Species	Ratio growth rates 27-18°C	Coconut smell	Spore shape	Phialides	Growth rate at 27% mmh^{-1}	Chitinase activity
T. viride (4 isolates)	0.63-0.77	Strong	Round to oval Large	Long sparse asymmetrical ± single	0.28-0.34	9.8-13.5
T. harzianum(3) (7 isolates)	1.48-1.80	Slight to strong	Very short Ovoid to round medium	Large medium spare asymmetrical ± single	0.67-0.95	0.08-11.06
T. harzianum(2) (4 isolates)	1.96-2.16	Slight	Short ovoid small	Medium short asymmetrical symmetrical plump ± whorls	1.1-1.1	1.86- 4.48
T. harzianum(1) (7 isolates)	2.24-2.70	None	Short Ovoid to round small	Short medium symmetrical asymmetrical plump ± whorls	0.89-1.16	3.6-17.67
T. pseudo koningii	2.2-2.33	Sour	Narrow acute tip small	Medium thin asymmetrical ± single	1.0-1.12	1.83- 2.33

Summary and conclusions on the taxonomy of Trichoderma species associated with mushrooms

1. Before 1969 and Rifai revision of the genus, it is likely that species identification was not critical. The generally held view throughout the period from 1935-1961 was a one species genus and therefore T. viride was used by most workers for all isolates.

2. Rifai created a number of new species and reconstituted others recognising species aggregates. These he distinguished using a number of minor characters which included morphological and physiological features.

3. Seaby recognised T. harzianum as the species associated with the compost problem. Subsequently the same species has been identified in Ireland and England. Forms of this species were identified by Seaby who found his form 2 to be most frequently associated with the compost problem.

4. Precise strain differentiation of forms of T. harzianum has given somewhat variable results although consistency is reported by Seaby.

Biology of Trichoderma species

Trichoderma sp. are commonly isolated from soil (Barron 1968) and from straw (La Touche, 1949). Because of the taxonomic confusions it is not always possible to be certain of the species identification. However, in mushroom culture Trichoderma spp. are associated with the following:-

(a) Green mould growth on the wood of trays
(b) Green mould on the remains of mushrooms
(c) Spotting and blotching of mushrooms
(d) Mycelial growth in the compost and casing with prolific spore production and associated high populations of red pepper mites.

As well as being a common soil fungus it is also well documented that Trichoderma spp. are efficient mycoparasites on a wide range of fungi and this property has recently been exploited as a means of biological control of fungal plant pathogens (Chet, 1987). T. viride is reported to be an active antagonist in moist soil but is inhibited under very wet conditions when the soil has a pH of 5.4 or above (Anderson, 1962-64). T. harzianum is known to be an active antagonist at pH 6.5 and lower (Chet and Baker, 1980). There are many references to the effect of pH and it is clear from these that Trichoderma spp. are most active in acid rather than alkaline conditions.

Liu and Baker (1980) concluded that Trichoderma spp. commonly inhabit soils having a high moisture content although some strains are capable of growth in drier conditions.

Trichoderma spp. are capable of very rapid growth and prolific spore production so are able to colonise substrates chemically or heat treated. Steamed and fumigated soil quickly becomes colonised. Davet et al (1981) found that the growth of T. harzianum in soil was depressed by benomyl and stimulated by thiram. The fungus is tolerant of ammonia unlike, T. hamatum which is reported to be sensitive (Schippers et al 1982). Soil treatment with formaldehyde is reported to increase the incidence of Trichoderma spp. but is said to be very sensitive to methylisothiocyanate (Sewell, personal communication).

There are conflicting reports of the ability of species to produce antibiotics. Webster and Lomas (1964) tested a number of isolates and failed to show that any could produce antibiotics although earlier workers had reported gliotoxin production. Brian (1944) demonstrated the production of viridin but it was later shown that he was working with Gliocladium virens and not T. viride as he had supposed.

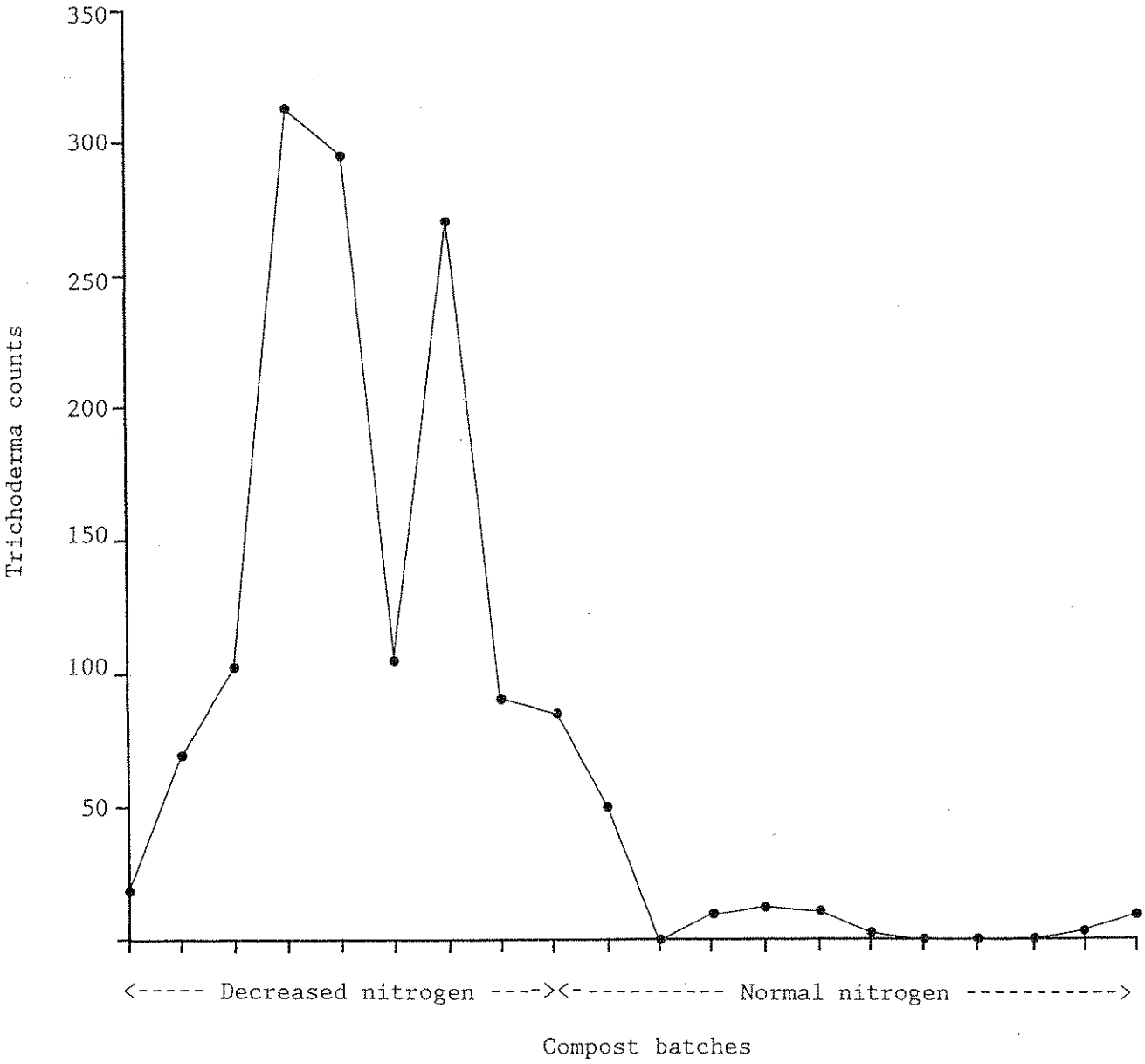
Vedder (1978) and Gandy (1977) reported excess soluble carbohydrates favoured Trichoderma growth in compost.

According to Harvey et al Trichoderma spp. thrive in the mushroom compost when carbohydrates are available or when the compost is not supplemented with sufficient nitrogen. It can also grow on the various supplements that are added such as soya bean meal, cotton seed meal, Spawn Mate, etc. Once it has colonised the compost it will grow into the casing. Harvey et al (1982) also report that mushroom spawn may recolonise such Trichoderma affected areas, although this is not the case with recent experience (Seaby 1987).

Steane (1978 b) monitored disease levels on a mushroom farm in Sussex, England where Verticillium and Trichoderma were particularly troublesome. The Trichoderma problem (identified as T. viride) originated from the compost and had been a constant problem on this tray farm. Although it rarely caused measurable crop loss, colonies of the fungus up to 6 cm diameter were frequently seen on the casing before the first flush. When Agaricus bitorquis was grown this mould became more troublesome as it attacked mushrooms on beds causing symptoms not unlike those of Verticillium.

During the summer 1977 Steane reports a farm policy of decreasing the rate of nitrogenous supplements in the compost (deep litter chicken manure) because analysis had shown a high level of nitrogen in the horse manure. He speculated that some of this nitrogen in the horse manure had not been available and there was, therefore, an increase in the C:N ration. This change had little effect on the incidence of Trichoderma in the A. bitorquis crops but in crops of A. bisporus, the return to higher nitrogen levels resulted in a dramatic reduction in Trichoderma incidence (Fig. 1).

Fig. 1 TRICHODERMA COUNTS IN RELATION TO WEEKLY COMPOST BATCHES (STEANE 1978b)



The effect of the C:N ratio on Trichoderma incidence is also noted by Perrin & Gaze (1989). Two composts with high C:N ratios (21.9 and 23.3 to 1 respectively) supported weed mould growth including Trichoderma spp. Horsfall (personal communication) showed the optimum C:N ratio of Trichoderma mycelial growth to be 25:1. Brian & Hemming (1950) studied various nutritional factors which affected spore production in T. viride. They concluded that strains differed in their detailed response but in general a pH 4.0-6.0 was preferable, 0.1-10.0% glucose in the medium increased sporulation, variations in the nitrogen level had most significance compared with the other major nutrients - the lower the N level the greater the sporulation. Conditions favouring vigorous mycelial growth tended to reduce sporulation.

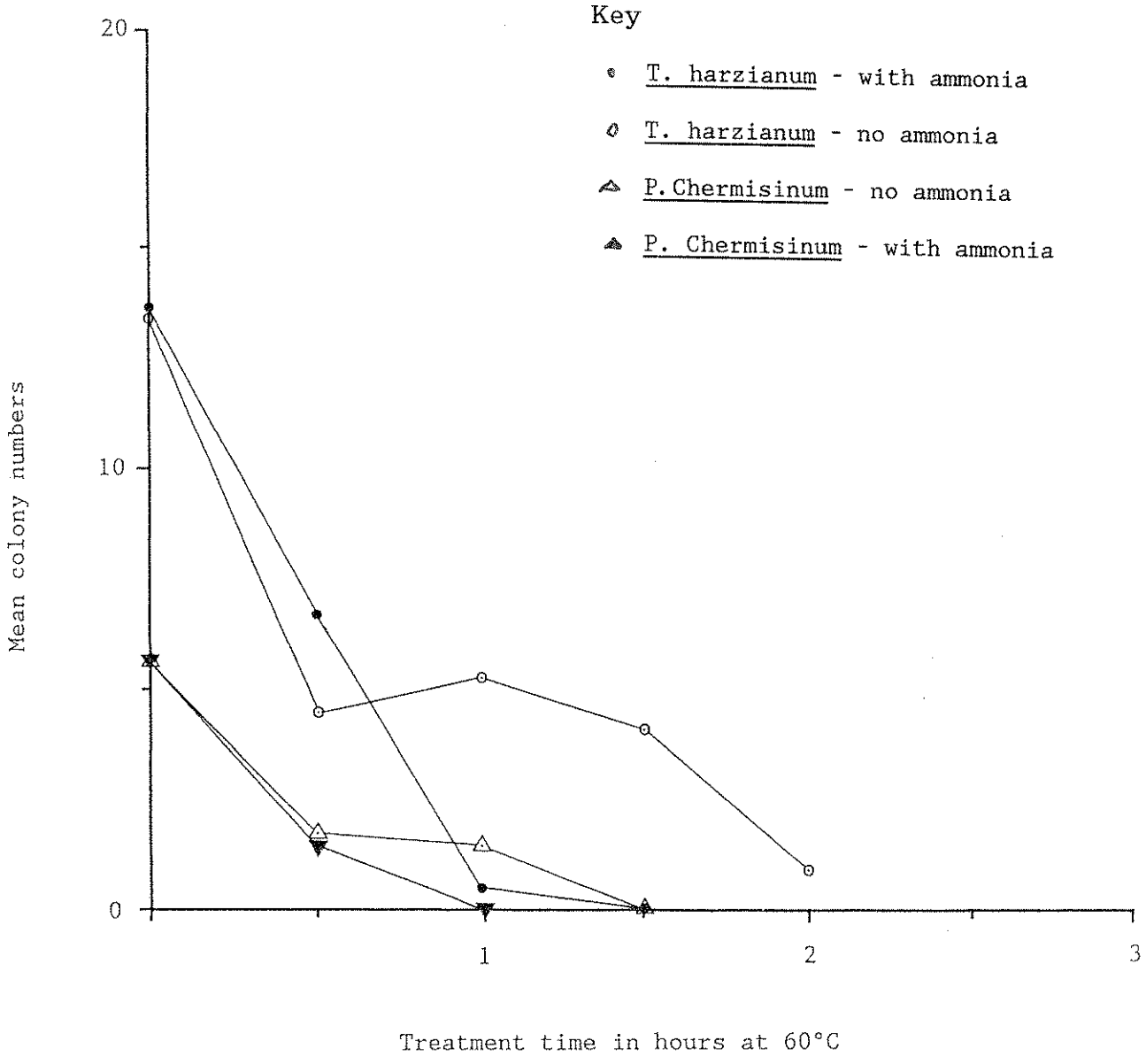
Seaby (1987) working in N. Ireland has made detailed studies of the compost colonising form of T. harzianum. He found three strains of T. harzianum to be associated with the compost problem and in particular one form which he designated Th2. This particular strain was characterised by its slowness to sporulate in culture and it also required more light to induce spore production. Its growth rate was optimum at 27^{o}C and was in excess of 1 mm per hour. This form was commonly found in compost but was the least common in the compost ingredients or from lagoon water and poultry litter. Seaby concluded that Th2 is unusual in that it is closely adapted to spawned mushroom compost.

In experiments at ADAS, Wye on compost colonisation by Trichoderma isolates, phase II compost was modified by the addition of acid (acetic), sucrose and water. Davenport (personal communication) reported experiments in which 10 or 20% sucrose (100 ml) was added to a 1 kg of spawn run compost. Water was also added at 100 and 200 ml rates. Three isolates of Trichoderma were used to inoculate the amended compost, one from N. Ireland believed to be Th2 and two others from compost problems in the UK. Trichoderma grew very poorly in the unamended compost but the addition of sucrose not only increased the growth of Agaricus, it also resulted in growth of Trichoderma. The 20% sucrose treatment gave Trichoderma growth in all five replicates. The addition of water at the 200 ml rate also resulted in some Trichoderma development but not in all replicates.

Smewin (personal communication) examined the effect of pH and sucrose on unspawned phase II compost which was simultaneously inoculated with Trichoderma and Agaricus. She found that good Trichoderma growth occurred only when the pH of the compost at the time of inoculation was between 4.0-5.0. The addition of sucrose did not result in Trichoderma growth neither did a nitrogen source, acetamide. Davenport worked with 8 different isolates of T. harzianum, all associated with compost problems.

The effect of temperature on the survival of spores of Th2 have been examined by Seaby (1987) and Hilton (personal communication). Seaby reported no survival of spores placed in compost and subjected to peak heat temperatures (up to 9 hours at 58-60^{o}C followed by 3.5-4.5 days at c. 46^{o}C). But Hilton found that some spores survived laboratory treatment of two hours at 60^{o}C but did not survive 1.5 hours at this temperature in the presence of ammonia (Fig. 2). In this experiment the ammonia level peaked at 1600 ppm, was down to 900 ppm after 15 mins. but was maintained at a level above 800 ppm for the whole of the two hour treatment.

Fig. 2 NUMBER OF COLONIES OF TRICHODERMA HARZIANUM AND PENICILLIUM CHERMESINUM FOLLOWING TREATMENT AT 60°C (HILTON, PERSONAL COMMUNICATION)



Summary and conclusions on the biology of Trichoderma species

1. Trichoderma species are commonly found in soil and on organic matter.

2. They grow best at a low pH (4.0-6.0)

3. They are often associated with high carbohydrate levels

4. They frequently occur when nitrogen levels are low

5. They have been found in mushroom compost where the C:N ratio exceeds 20:1

6. T. harzianum isolates associated with compost problems have an optimum growing temperature of 27°C

7. Spores of T. harzianum have been reported to be killed at peak heat temperatures but only in the presence of ammonia levels which were slightly in excess of those that occur during peak heat

8. Isolates do not readily grow in phase II prepared compost even in the presence of spawn but can sometimes be induced to colonise compost by manipulation of the pH or the carbohydrate status.

Epidemiology and control of Trichoderma diseases

Studies have not been made of the epidemiology of the spot and blotch diseases but recently some work has been done on the compost problem. Seaby has reported his observations in the Mushroom Journal (1987, 1989). He examined various possible sources of the fungus including dust particles, clothing, animals, airborne spores, spawn, contaminated surfaces, compost, casing and machinery. He subsequently also examined compost materials and lagoon water. Circumstantial evidence implicated airborne dust as a source of spores contaminating the freshly opened phase II tunnels. Also the first loads out each day were most likely to be affected implicating contamination of the conveyor or machinery systems. Dry windy conditions were often associated with the worst outbreaks.

Spores were found to be difficult to detach by air flow and so airborne spores were not considered to be a very important source.

Workers' clothing was commonly found to be contaminated, 85% of all tests being positive. Red pepper mites were found to be very heavily laden with Trichoderma spores. Dead mites formed ideal 'light dust' particles for the dispersal of Trichoderma spores. Sciarid flies also picked up large numbers of Trichoderma spores when exposed to colonies of the fungus.

Spawn is a potential source of various mushroom pathogens and Seaby reports finding T. harzianum and T. viride on spawn but speculates that it probably was introduced after the spawn was bagged and on the farm. There is no proof that spawn is a primary source of the compost Trichoderma problem. Surface spawning which resulted in spawn handling and handling compost was considered to be an important source of contamination. In laboratory and farm experiments Seaby showed that the likelihood of compost colonisation decreased with time. After 12 days, inoculum failed to colonise compost unless a very large inoculum load was added. This was also the case when run compost was used.

Most equipment on the farm was found to be contaminated as was lagoon water. Th3 predominated in the water samples examined.

Seaby examined over 50 compost samples from affected and unaffected crops and could find no relationship between the moisture content, the N content or the pH and Trichoderma incidence. Occasionally there were indications that dry compost (below 65% moisture) and high nitrogen and high moisture were associated with Trichoderma growth. Freshly peak-heated compost was not colonised with Trichoderma unless mushroom mycelium was present and similarly fully run compost was difficult to infest. Temperature had a marked effect on the colonisation of compost - the nearer the compost temperature was to 30°C the more likely was poor mushroom growth and good Trichoderma growth occurred.

Seaby (1989) recognised various "infection" routes according to the system of culture on the farms he examined. He found Trichoderma on various surfaces and accounted for the problem by identifying weakness in the hygiene procedure. Some examples within the different systems were:-

Shelf system: the shelf structure, dust, mites, the trailer delivering the compost, concrete in adjoining yard, ineffective use of formaldehyde at crop termination.

Tray system: compost line, pickers' overalls, concrete surfaces.

Bag system: open vents allowed contaminated flies in the crop, the compost, clothing, workers, animals and mites.

Observations made by Fletcher (personal communication) suggests that brown strains of Agaricus bisporus may not be severely affected by the compost Trichoderma disease. On one farm where all white strain crops were severely affected by both Trichoderma and red pepper mites 4 brown strain crops were unaffected.

Control by the use of chemicals has not been successful although many isolates are known to be sensitive to the benzimidazole fungicides. When tried in compost at 100 ppm benomyl did not give an effective control.

To date, severe outbreaks of the disease have only been controlled by the use of very strict hygiene procedures (Seaby 1989, Fletcher, White and Gaze, 1989).

Summary and conclusions on the epidemiology of Trichoderma diseases

1. No work has been done on the epidemiology of the spot and blotch diseases.

2. Investigations of the compost disease have been made, in particular by Seaby.

3. Compost Trichoderma has been of greatest concern in bag and block systems.

4. Circumstantial evidence strongly supports the compost as a primary source.

5. Once established on a farm all surfaces become contaminated.

6. Dust, pickers, mites and flies are particularly significant vectors.

7. Trailers, equipment, shelves, trays, ladders are also important sources.

8. Ready to spawn compost does not support the growth of Trichoderma.

9. Fully colonised compost does not generally support the growth of Trichoderma.

10. The disease develops when the mushroom mycelium and the Trichoderma grow simultaneously.

11. The optimum temperature for the development of T. harzianum in compost is $27^{\circ}C$ - a temperature frequently occurring in hybrid spawn runs.

12. So far there have been no reports of severe compost Trichoderma in crops of brown strains.

13. Fungicidal control by surface treatment is not effective although the fungus is sensitive to benomyl.

14. Once established on a farm the disease can only be eliminated by strict attention to hygiene.

Discussion of Trichoderma diseases and identification of areas for further work

Although occasionally troublesome, the cap spotting or blotch diseases are not of major importance and where they do occur they are well controlled with the use of fungicides particularly the benzimidazoles. The same is not true of the compost problem which can cause very severe crop loss and is difficult to eliminate once it has become established. It seems likely that the compost problem is a new disease caused by a previously unrecognised pathogen as stated by Seaby (1985) although there is some confusion over the taxonomy of the causa organism. As Chet (1987) reports, many isolates do not fit into the nine aggregate species described by Rifai (1969) but are often on the borderline between two species. It is frequently the physiological properties of isolates that relates to their significance as pathogens or antagonists and it is these features that must be characterised if any relationship between species and disease problems is to be identified. Seaby (1989) describes how he determines three types of T. harzianum and reports consistent results in relating the type: to disease severity. Using his method at ADAS Wye a different pattern has been found. This may reflect minor differences in the technique used but if such is the case it is unlikely that the technique has widescale use in the consistent identification of strains. Although Steane reported a Trichoderma compost problem in the 1970's, it did not completely agree with the present day problem substantiating Seaby's claim that the present compost problem is a new disease. What has not been demonstrated so far is how a compost becomes predisposed to the growth of T. harzianum. Seaby (1989) states that it is not possible to gro Th2 in unspawned compost and generally not in spawn run compost, and Fletcher (personal communication) has found the same. Neither will it establish easily if inoculum is used simultaneously with spawning (Fletcher, personal communication). Seaby implies an essential relationship between spawn and Trichoderma growth, the Trichoderma being totally dependent upon the actively growing mushroom mycelium. He speculates that this may be explained by the antagonistic effects of the mushroom mycelium on the

Trichoderma inhibiting bacteria in the compost. On mushroom farms the problem often appears in the first flush or even during spawn-run and may continue to appear through the subsequent flushes. There is no clear evidence of spread within a crop but certainly sequential manifestation takes place.

Compost temperatures during spawn running particularly with the now commonly grown hybrids, are frequently in the region of 27-28°C or even above. These temperatures are common in spawn run in all systems of mushroom culture and are certainly not confined to bag or block culture. But the majority of serious Trichoderma outbreaks have occurred and continue to occur in bag and block produced crops. Trays and shelf growers also experience the problem but there are fewer with serious problems. Generally a wide variety of white strains have been affected but Trichoderma appears to be more infrequent in brown crops. Brown strains are generally very vigorous in the initial spawn run stages and this might be a factor.

Spores of Trichoderma will probably survive peak heat temperatures on most farms. It seems important that ammonia levels are high enough in peak heat to add to the effects of high temperatures. The observations of Steane (1972) are interesting in this respect because he relates incidence of Trichoderma to low nitrogen compost levels resulting from reductions in the chicken manure content of the original mix. Nitrogen content influences the amount of gaseous ammonia although it need not have a major effect on the nitrogen level at spawning. The relationship between ammonia levels during kill and the survival of weed mould spores needs further investigation. It is also of interest that Steane reported a major affect of nitrogen on the incidence of Trichoderma in A. bisporus crops but not in A. bitorquis presumably growing in the same compost. Was this result related to the different speed of colonisation of the compost by the two species?

Carbon: nitrogen ratios are yet another aspect of compost analysis which have been related to Trichoderma incidence with reports of ratios of 23:1 favouring Trichoderma and the normal ratios of 14-15:1 not being favourable. Carbohydrate levels are clearly an essential component for Trichoderma growth and high carbohydrate levels would be expected to support weed moulds. But many of the bag and block composts that has been affected are said to have had ratios near to or normal rather than being high in carbohydrates.

The fact that Trichoderma is more of a problem for some growers than others, may reflect the differences in the thoroughness of hygiene once the damaging strain is established. Batches of compost split two or three ways are sometimes reported to give problems on one of the farms but not on the others. There is clearly a farm factor as well as a compost factor involved in this complex disease.

The initial association of the Trichoderma compost problem with the phase II systems using polythene bags in N. Ireland and Eire and bags and polythene wrapped blocks in England is significant. There are features of these systems that differentiate them from other forms of growing, although some of the individual components are used in other systems. For instance, the compost is always produced in a bulk phase II tunnel but this system of production is not unique to the phase II compost producers and where it is linked with shelves or trays very little Trichoderma has been seen. Is there an essential difference in the compost of the phase II producers which makes it more likely to support the growth of Trichoderma? Compost analyses show no obvious differences but phase II producers are sometimes accused of producing 'weak' compost. Yet many of the crops with a low incidence of Trichoderma yield 500 lb/ton. It is possible that the borderline between a productive compost and one that supports Trichoderma growth is not far apart?

In order to generate a Trichoderma problem in phase II systems it seems from work so far that it is essential for the Trichoderma spores to be present at the time the spawn is introduced or if not then, to be introduced within a few days of spawning.

The recirculation of lagoon water is commonly practised on many farms including those of the compost producers and as Seaby found Th3 to be a common contaminent and Fletcher found Th3 to be most commonly associated with compost problems, such lagoon water could be a very important source of spores for the compost.

The ingress of a few Trichoderma spores after phase II seems to be unlikely to cause a problem judging from the experience of experimenters so far, so contamination would have to be on a massive scale, eg. large numbers of red pepper mites carrying the spores or extremely dusty conditions allowing inoculum to blow around or large numbers of spores surviving phase II.

The first potential point of contamination is the phase II producers farm and if the phase II compost is contaminated either as a result of dust blow causing contamination at cooldown, or of low ammonia levels during kill allowing survival of spores, the compost could be contaminated at spawning. Once the compost is spawned and wrapped or bagged on the phase II farm it is reasonably well protected from contamination unless inoculum levels on the growing farm are extremely high.

Once inside the polythene the compost cools and a film of water is produced around the bag or block. Such water condensation does not occur in tray or shelf systems. Following transportation the bags/blocks are incubated on the growing farm and it is often difficult in warm weather to get adequate temperature control. Optimal temperature for Trichoderma growth (27°C) is general during spawn-run on many shelf/tray farms as well as in bags and blocks.

Once established on a farm the fungus will be virtually everywhere and the primary source of individual outbreaks is then more difficult to ascribe to any one source.

It would seem from work so far that the vulnerable phase in mushroom production is from cooldown of phase II to casing. This presupposes that the compost is not already contaminated at cooldown of phase II. In order to control Trichoderma every effort has to be made to protect the compost through this vulnerable period and so far this can only be done by the strictest attention to hygiene.

But the fundamental questions of compost vulnerability or selectivity remains and information is needed on what constitutes a Trichoderma favourable compost because when this is understood every effort can be made to produce compost that is not favourable but is capable of providing high yields of Agaricus.

Further work

1. Detailed studies of compost taking into account C:N ratios, ammonia during phase II and other nutritional factors linked with T. harzianum growth. Sequential sampling from phase II producers would be an integral part of this study.

2. The interaction between temperature and ammonia concentration in relation to the viability of T. harzianum.

3. A detailed study of the interaction of T. harzianum and A. bisporus to define the relationship both in vitro and in vivo.

4. Further characterisation of the strains of Trichoderma involved with the compost problem in order to identify isolates with greater certainty.

5. An examination of spawn strains and Trichoderma to establish difference which might exist between the interaction of white, cream and brown forms of A. bisporus.

6. The role of red pepper mites with a study of their feeding habits, the viability of spores they carry and the life expectancy of the mites and the spores.

7. The sensitivity of Trichoderma isolates to fungicides and disinfectants.

8. On farm studies of mechanisms of contamination and the epidemiology of the disease including the significance of compost ingredients, lagoon water, etc. as sources.

PENICILLIUM DISEASES OF AGARICUS BISPORUS

The genus Penicillium is perhaps the most ubiquitous of all fungi and is found from the equator to the polar regions although perhaps it favours the temperate and colder regions (Barron, 1968). It has presented mycologists with considerable taxonomic and nomenclature problems. It is a form genus based on its conidial morphology and some species have perfect states that can be assigned to different genera, eg. Eupenicillium, Talaromyces and Carpenteles. However, most of the known 100 species of Penicillium have no known ascocarp stage (Webster, 1980). Classical works on the taxonomy of the genus have been published by Raper & Thom (1949) and by Pitt, (1979).

Penicillium species grow on all kinds of decaying materials and spores are universally present in the air. It was the airborne spores of P. notatum that contaminated bacterial cultures and led to the discovery of penicillin by Fleming (1944). Other species are used in cheese making (P. camemberii and P. roqueforti) and as sources of antibiotics (P. grisio-fulvum for grisio-fulvin). Other species are known to cause fruit rots (P. italicum and P. digitatum).

Penicillium species have long been associated with the production of mushrooms. Some of the earlier observations were summarised by Kneebone and Merek (1959). They describe Green mould caused by Penicillium spp. as causing green colonies on the casing surface, on woodwork and on dead mushroom tissue and being saprophytic and of no significance other than as a source of confusion with Trichoderma spp. Atkins (1974) makes similar comments but adds there are species which are believed to produce allergic reactions of a hay-fever nature among sensitive operators.

Fletcher, White and Gaze (1989) record two types of Penicillium mould in the UK. The first is as described previously but the second, Smoky mould, caused by Penicillium chermesinum, is a previously undescribed mould of the crop. They associated the presence of this mould with large numbers of spores which are disturbed and become air-borne when the compost is handled, appearing like a smoke arising from the affected material. This problem is sometimes associated with large yield reductions and it is not clear whether it is a pathogen, antagonist or a competitor.

There are many similarities between the problem described by Fletcher *et al* and Vern Astley disease (Sinden and Hauser, 1950). This disease is reported to be caused by *Spicaria* sp which, according to Barron (1968) is a genaric name synonymous with *Paecilomyces*. This latter genus has been included in the *Penicillium* group by some (Pitt, 1979). Also *Penicillium simplicissimum* described by Thom and other *Penicillium* species have previously been ascribed to the genus *Spicaria*. There are obviously close relationships between *Penicillium* spp. and *Spicaria* spp.

Vern Astley disease is characterised among other features by the production of a spore cloud from the compost. The spores are produced in single penicillia-like heads and the conidia measuring 2-5.5 μ are borne in chains (Table 3).

There are no other records of *Penicillium* spp. or related species associated with mushroom problems.

The Penicillium compost problem in the UK

Smoky mould has been recorded four times in the past four years. On one farm in the north of England it has been a persistent problem and associated with considerable reductions in yield. In the midlands, a tray farm suffered somewhat similar yield reductions as did a small farm in Sussex. One other record has been made but in this case the effect on yield is not known.

Penicillium chermesinum has not previously been recorded from mushroom culture, and there are very few international records of its occurrence in any situation. It is thought that, in the current outbreaks, the mould may have originated from dirty straw. It has not been found on wood or other materials within crops. The P. chermesinum problem is characterised by a dramatic reduction in yield which, at the extreme, can be 80%. Symptoms become apparent in the first flush where there is an edge break of more or less normal mushrooms, with no cropping in the middle of the shelves or boxes. If the compost is examined in the non-cropping areas, large clouds of spores looking like smoke can be seen as the compost is disturbed. The compost has a mouldy smell. In less severe outbreaks, the first and second flushes may be reduced in yield and, by the third flush, non-cropping areas occur, together with the characteristic spore production from the compost. When the affected compost is examined very carefully, numerous penicillate sporing structures are seen. These are white in colour but, with age, turn brown. On agar, the fungus has a very characteristic slow growth rate, and on potato dextrose agar, is grey-brown in colour.

The spores of P. chermesinum are slightly oval in shape and are just under 2 µm at their narrowest point. It is, therefore, possible that some could pass through a 2 µm filter.

It is likely that there is an association between P. chermesinum and mushroom mycelium, perhaps similar to that already reported for Trichoderma harzianum. When maximum damage occurs, it seems highly likely that P. chermesinum spores must be present in large quantities within the compost at the time of spawning and the mushroom mycelium is either parasitized or very potently inhibited. Bulk phase II and/or bulk spawn-running systems are ideal for spore distribution and, as the Penicillium has an optimum growth temperature of 28°C, it is ideally adapted to compete with, and perhaps attack, the mushroom mycelium during the initial stages of mycelial colonisation.

Control of Smoky mould

Controlling this problem has proved to be extremely difficult, particularly in situations where there is a continuous flow of bulk compost within an area where spawning also occurs.

Filtration of the air used in phase II is obviously vitally important and any small cracks or gaps within the system will result in compost contamination. Filters must be carefully checked to make certain that they are efficient and replaced at the correct intervals.

Areas outside the bulk tunnels must be regularly disinfected and, if within a sealed spawning area, fumigated regularly with a disinfectamt to make certain that airborne mycelial fragments and spores are killed that have settled on ledges. It is particularly important to do this every time the tunnels are emptied, whether they are for phase II or bulk spawn-runs.

Some experimental work has shown that benomyl added to the compost at spawning gives an effective control, but this procedure should not be considered as a routine as it is likely to result in fungicide resistance.

Table 3 - Comparison of Smoky mould with Vern Astleys disease

	Smoky mould	Vern Astley
Symptoms mushrooms:	First flush may be normal although distortion may be present at the bed edges. Mushrooms open prematurely and may crop earlier at the bed sides.	Patchy distribution of mushrooms, cap scaly and later the affected mushrooms are flat, and the cap small in relation to the stipe. Immature opening. Stem tissue spongy and discoloured in the upper and peripheral parts.
Compost:	Clouds of spore produced when the compost is moved.	Clouds of spores produced when the compost is moved.
Allergic reactions:	Workers report irritation of the nose and throat in the presence of spores.	Choking congestion in the chest; a dry hacking cough and partial paralysis of the larynx- vocal chords. Not all workers susceptible
Appearance of mycelium in compost	Not detected. Mushroom mycelium diminishes in quality.	Like mushroom spawn but more granular and slightly grey. Mushroom mycelium dies.
Spore size and shape:	Ellipsoidal 2-2.5 x 1.5 -2.00 μ in chains.	Globose to elliptical; 2 x 5.5 μ in chains.
On PDA	Growth slow. Grey brown in colour, pale yellow reverse.	Growth slow. dense felt of white mycelium which soon becomes grey with slightly greenish tinge.

Future Work

Nothing is known of the biology, epidemiology, relationships with Agaricus mycelium, conditions favouring attack and predisposition of the compost to the smoky mould problem.

Its infrequent occurrence so far suggests that it may not be of major significance but once it gets established it is very difficult to eliminate. For this reason alone serious consideration should be given to essential work on this problem as it clearly has immense potential to cause very large crop losses.

FALSE TRUFFLE (DIEHLIOMYCES MICROSPORUS) IN RELATION TO THE OTHER COMPOST PROBLEMS

The incidence of False Truffle has increased since the development of the phase II compost system and the disease has been frequently found in block grown crops. Although Kligman (1950) reported that the spores of this fungus are very resistant to heat requiring 180°F (82°C) for five hours to kill them, more recent work by van Zaayen and van der Pol-Luitem (1977) and Bissett (1980) showed that they could be killed by peak heat treatments (60°C). Such a wide variation in results is unusual in biology and although there is no reason to doubt the results of the recent workers, experience indicates that Diehliomyces does survive phase II compost treatment.

In order to investigate this aspect further and also other relevant features relating to False Truffle development a series of experiments were done at ADAS, Wye by M.W. Wood, a sandwich course student from Hatfield Polytechnic.

A difficulty workers have experienced with D. microsporus is obtaining reliable conditions which test the viability of the ascospores. Most workers have test-germinated ascospores on agar and have reported low levels of germination. Wood attempted spore germination on agar with similar results. It is questionable whether the viability of the spores is being properly tested if the detectable germination levels are low although the spores are produced in such large numbers that a few survivors may be all that is necessary to initiate the disease.

Wood, heat treated the contents of disintegrated ascocarps which he first filtered to remove the mycelial fragments. Spore suspensions (15 ml) were treated in a water bath for the prescribed time and used in incubated spawned compost which was cased after spawn-run. The number of replicates producing ascocarps of Diehliomyces was a measure of spore survival. Using this technique, Wood showed that ascocarp formation occurred following ten minute heat treatment of spores at 40 to 60°C but not at 70 to 80°C. When spores were treated at 60°C for ten minutes, 1, 2, 4 and 6 hours, they survive up to the 1 hour treatment but not 2 hours or more.

Wood's results can be summarised as follows:-

1. Ascocarp formation reached a maximum level (100%) at 30°C after 13 days incubation. At 25°C and 22°C there was 37% and 0% respectively but over a longer period of time some ascocarps formed at 22°C but never reached more than a third of the number formed at 30°C.

2. A one day incubation period at 30°C returning to 22°C increased the ascocarp number to two thirds of that of the 30°C treatment. Returning to 15°C after a one day treatment at 30°C resulted in no ascocarp formation.

3. Pseudomonas putida, a common inhabitant of the casing, was found to be associated with the decay of ascocarps.

4. A biological factor was identified in casing which induced ascocarp formation.

The significance of the current False Truffle problem

It seems likely that the increase in spawn running temperatures possibly also aggravated by two hot summers has lead to an increase in the incidence of False Truffle. There is also a link between the phase II compost system and the incidence of this disease which may again reflect the lack of ability of the growers to control compost temperatures.

There is a possibility that ascospores may survive peak heat temperatures if all parts of the compost are not treated at 60^{o}C for two hours, although Wood's experiments do not take into account the effects of compost volatiles and in particular ammonia. Work needs to be done to establish the effects of these volatiles on the survival of ascospores and also to examine the effects of time of compost contamination on False Truffle development. Temperature of compost during spawn-run is clearly important and Wood's work on short durations of high temperatures needs to be extended to enable a better understanding of the biology of this troublesome disease.

PYTHIUM - BLACK COMPOST

Pythium black compost has recently been described by Fletcher et al (1989). It is not a common problem but occurs sporadically and sometimes causes up to 25% loss in yield. The same disease problem was described in the USA some years ago (Fergus, Sinden, Schisler & Sigel, 1963). Recently some work has been done in the UK (Fletcher, Smewin & O'Brien, 1990) which provides some extra information (Appendix 1). They found that Pythium oligandrum was constantly associated with black compost and this fungus had a marked inhibitory effect on the development of mushroom mycelium. Its effect was markedly by enhanced by excessively high levels of nitrogen.

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Pythium oligandrum associated with a cropping disorder of Agaricus bisporus

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Pythium oligandrum was consistently isolated from black patches of mushroom compost from two farms. When used in experiments it inhibited the growth of mycelium of Agaricus bisporus and this effect was enhanced in the presence of a compost supplement which contained high levels of nitrogen. The common name pythium-black compost is proposed for the problem.

INTRODUCTION

Mushrooms (Agaricus bisporus (Lange) Imbach) are produced in the UK by a variety of methods but the tray system predominates (Gaze, 1985). Compost, which is usually a mixture of horse manure, straw and chicken manure, is inoculated with mushroom mycelium and compressed into wooden trays. Approximately two weeks after inoculation, a layer of peat and chalk is placed over the colonised compost in order to induce fruiting (Fletcher, White & Gaze, 1986).

In 1986 cropping problems occurred in two widely separated tray farms. Symptoms shown by the affected crops were very similar to those described by Fergus, Sinden & Schisler (1963) for a tray-grown crop in the USA where Pythium artotrogus (Mont.) de Bary was isolated from the affected compost.

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The English crops showed black completely uncolonised compost in affected trays with an almost identical distribution to that described by Fergus et al. These crops were slow to develop particularly in the first two flushes but later showed signs of recovery. The nitrogen analysis of eight samples of the affected and also five from the adjacent unaffected areas of compost on one farm showed a mean of 3.6% (2.6-4.3) and 3.2% (2.7-3.1) respectively. The usually recorded level at spawning on this farm is 2.5% and is followed by the application of a nitrogenous supplement.

In order to investigate possible causes of the problem, the affected compost was examined for the presence of Pythium spp. and various experiments were done to try to reproduce the symptoms.

MATERIALS AND METHODS

Isolation

Two techniques for the isolation of Pythium spp. were attempted (Johnston & Booth, 1983). Firstly, a small sample (c. 10 g) of compost was placed in a Petri dish and surface sterilised for 3 min. using 5% sodium hypochlorite followed by a repeat treatment and washing in sterile distilled water. Similar samples were examined without surface sterilisation. Small sub-samples of the compost were placed in Petri dishes, covered with distilled water and 3 cm-long pieces of boiled grass leaves (5 to 10) put into the water with the compost. The dishes were incubated at room temperature (c. 21°C). In the second method, autoclaved sand was mixed with the affected compost in equal amounts and wetted using sterile distilled water. The mixture was placed in sterile plastic boxes (135 x 75 x 55 mm) to a depth of 40 mm. Soaked and autoclaved maize seeds (15 minutes at 121°C) were placed on the sand-compost mixture and left overnight but not longer than 16 h. The seeds were then surface sterilised using sodium hypochlorite, sliced and the pieces plated onto tap water agar. The plates were incubated at 21°C.

Inoculation experiments

Interactions between Agaricus bisporus and Pythium oligandrum isolated from the compost were studied using prepared mushroom compost, commercial spawn growing on wheat grains and water agar cultures of P. oligandrum. Clear plastic boxes (135 x 75 x 55 mm) were used and 135 g of prepared compost was compressed into each box leaving a 10 mm space above the compost. Ten grains of spawn were placed in each box in two rows of five. Five 4 mm disks of water agar were cut from the edges of Pythium cultures and placed equidistantly between the two rows of grain. A. bisporus and P. oligandrum were inoculated either simultaneously or one after the other. Each treatment was replicated five times. The boxes of compost were incubated on the laboratory bench at c. 21°C and examined at intervals for the extent of the mycelial growth of A. bisporus. Colonisation of the mushroom compost by A. bisporus was assessed visually by estimating the percentage area covered on the top surface of the compost.

The effect of the addition of a nitrogen supplement on the interaction between the two organisms was studied by adding an organic nitrogen source, Betamyl 1000 (Spawn Mate UK Ltd, Yaxley, England), to the compost before inoculation. Rates used were 5-40 g Betamyl to 700 g of compost, the recommended rate being 5-7 g.

RESULTS

A Pythium sp. was consistently found in the black compost using both systems of isolation. Surface sterilisation of the compost was not necessary in order to obtain isolates. Cultures sent to the Commonwealth Mycological Institute were identified by Dr Jean Stamps as Pythium oligandrum Drechsl. No other species was isolated.

In experiments, P. oligandrum was readily re-isolated from compost 3 weeks after initial inoculation. When inoculated before, with or after A. bisporus, the effect on A. bisporus mycelial growth was greatest when the two were inoculated simultaneously or if the P. oligandrum was inoculated 3 or 5 days before (Table 1). Inoculation 5 days after had no apparent effect on the growth of A. bisporus. The differences were most marked in the first three weeks of incubation and became less so during the following 9 days.

When Betamyl was added to compost and A. bisporus and P. oligandrum were inoculated simultaneously there was an inhibitory effect on A. bisporus growth. After 17 days, the Betamyl treatments significantly reduced the extent of colonisation by A. bisporus compared with that of the unsupplemented control (Table 2). Betamyl (1 g) restricted to the surface of the compost affected growth as much as the highest rate of Betamyl mixed throughout the compost.

DISCUSSION

Discrete areas of black compost in mushroom crops where most of the adjacent compost has been colonised by A. bisporus and had become light brown in colour have been reported by Fergus et al (1964) and associated with Pythium artotrogus (Mont.) de Bary (an outdated name for P. hydnosporum (Mont. apud Berk.) Schroet). We examined compost samples from two widely separated tray farms in England and found similar black patches occurring just below the compost surface. Some crops showed such patches in almost every tray and these yielded very poorly compared with the unaffected with yield reductions of about 25%. Circumstantial evidence from one farm indicated the affected black compost had a raised nitrogen level compared with the surrounding compost. Our experiments indicate that the presence of P. oligandrum, when

inoculated with or before A. bisporus, has a marked effect on the growth of A. bisporus although this effect may be temporary. The magnitude of the effect could be increased by applying high rates of Betamyl supplement throughout the compost well in excess of those recommended by the manufacturer of the product or by making local applications of smaller amounts.

We have attempted to examine the relationship of P. oligandrum and A.bisporus The mycoparasitic behaviour has been investigated by various workers and exploited for the control of pathogenic soil-borne Pythium spp. (Veselý, 1988). An antagonistic effect seems to occur in mushroom compost although this may be transient. Our isolates of P. oligandrum grew well on agar at temperatures of 25 and 30°C. Such temperatures commonly occur during the initial phase of spawn growth. High nitrogen levels may in themselves have an inhibitory effect on the growth of A. bisporus but our experiments demonstrated that P. oligandrum alone has an inhibitory effect. Locally high levels of nitrogen can occur in mushroom compost for various reasons but one is the uneven mixing of nitrogen supplements. P. oligandrum is a commonly occurring fungus which, in suitable conditions (which could be the presence of high nitrogen), can colonise mushroom compost and its presence then adversely affects the development of A. bisporus. We were unable to determine the source of P. oligandrum but on one occasion did isolate it from pasteurised compost. Straw used to make compost is commonly contaminated with soil and P. oligandrum is known to be soil-borne (Waterhouse & Waterston, 1966).

The common name, pythium black compost is proposed for the disorder of the mushroom crop.

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Table 1. Colonisation of mushroom compost by Agaricus bisporus after 19 days

	Colonisation percent*
Pythium only	0^{a}
Pythium/Agaricus simultaneously	56.7^{c}
Pythium followed by Agaricus 3 d. later	15.5^{b}
Pythium followed by Agaricus 5 d. later	18.7^{b}
Agaricus followed by Pythium 5 d. later	90.0^{d}
SED	5.7

Treatments with the same letters are not significantly different at the 5% level using the Duncans Multiple Range Test.

* Angular transformations.

Table 2. Colonisation of mushroom compost by Agaricus bisporus after 17 days

	Colonisation per cent**
Agaricus only	80.3^{d}
Agaricus/Pythium simultaneously	31.9^{c}
Agaricus/Pythium simultaneously + 5 g* Betamyl	23.4^{b}
Agaricus/Pythium simultaneously + 10 g Betamyl	19.7^{ab}
Agaricus/Pythium simultaneously + 20 g+ Betamyl	20.9^{ab}
Agaricus/Pythium simultaneously + 40 g Betamyl	16.5^{ab}
Agaricus/Pythium simultaneously + 1 g Betamyl/surface	12.9^{a}

* Rate per 700 g compost

** Angular transformations

\+ Betamyl at 20 g resulted in a compost nitrogen content of 3.4%

Treatments with the same letters are not significantly different at the 5% level by the Duncans Multiple Range Test.