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A TRANSMISSIBLE DISEASE OF CULTIVATED MUSHROOMS ('WATERY STIPE')

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'Watery Stipe' is one of three names given to a type of mushroom disorder which is of considerable economic importance to the industry. The other two are 'La France disease' and 'Brown disease'. There has been some controversy over the question of whether three separate disorders exist, or whether they are simply different manifestations of the same one.

'La France disease' was first described by Sinden & Hauser in 1950, but very briefly, which made the description of little use for the identification of the disease by others. Sinden identified 'La France disease' in the United Kingdom 2 or 3 years later and sporadic outbreaks of suspected 'La France' were reported up to 1957. In that year, however, an unidentified disorder spread to most parts of the country where mushrooms were grown

and reached epidemic proportions. As the symptoms differed slightly from those described by Sinden the disorder became known firstly as 'Brown disease' and later 'Watery Stipe'. As might be expected the symptoms observed in outbreaks on commercial farms varied, but can be classified into four groups:

(1) Much reduced cropping. At the height of the 1957 outbreaks crops were down to a quarter or less of normal.

(2) A large proportion of the sporophores which do develop die whilst immature, thus decreasing the crop still further. Environmental conditions seem to influence considerably the general appearance of such sporophores.

(3) The production of abnormally shaped sporophores. The most usual deformity is an elongation of the stipe, which is also often bent at right angles. These elongated sporophores usually have small pilei and are aptly described as 'drum-sticks'.

(4) The watery stipe symptom is caused by waterlogging of the sporophore tissues, which appears usually as translucent longitudinal streaks on the stipes. These streaks frequently enclose a cavity in the tissue.

It is obvious from a survey of several outbreaks that no single symptom can be regarded as diagnostic. Thus, identification of 'Watery Stipe' has been based upon an assessment of these different symptoms.

Two theories were developed as to the cause of 'Watery Stipe', both based largely upon observation of affected crops in 1957. One was that it was caused by environmental conditions, in particular high temperature and humidity, and insufficient ventilation; it was shown that outbreaks were most frequent during periods of warm humid weather when ventilation in mushroom houses was most likely to be unsatisfactory. The other view was that 'Watery Stipe' was a pathological condition as it often appeared first in a restricted area, then spread to the rest of the house and finally to the whole of the farm. No improvement could be obtained by increased ventilation, but if the farm were cleared and disinfected the trouble disappeared. These two views are not altogether incompatible because if a pathogen is present, its behaviour and the type of symptom could be greatly influenced by the environment.

The investigation of 'Watery Stipe' at the Glasshouse Crops Research Institute began with the object of determining whether the condition was transmissible. Casing and compost were obtained from nine crops suspected of having the disorder. Owing to the difficulty of obtaining suitable material when required, only one importation of material has been made so far, the material for later experiments being obtained by saving compost and casing from the preceding one.

In the first series of experiments a comparison was made between the effect of inoculating freshly spawned trays of compost with (1) the material mentioned above and (2) water extracts of this material, the extracts being filtered to remove micro-organisms larger than yeasts and fragments of mycelium. The incorporation of BHC at the rate of 50 p.p.m. into the compost prior to filling, controlled the multiplication of pests introduced with the material. Periodic sampling of the plots showed that the numbers of pests present were well below the danger level.

The results of the first series of experiments were striking, the control and water extract plots giving similar yields, in contrast to the plots inoculated with material which yielded one-third of the number and one-fifth of the weight of the controls. Mycelium in the plots inoculated with material began to degenerate in some instances within 2 weeks of the first picking and in all had completely disappeared by the end of the experiment. There was no corresponding deterioration in the water extract or control plots.

The inoculation of plots with material has been repeated several times, with similar results. There have been no differences in the behaviour of the material from different sources. Although the mushrooms produced in the treated plots were often of poor quality, they were so few that it was impossible to estimate the frequency with which the macroscopic symptoms described earlier occurred. Abnormal mushrooms were found also on control plots, indicating either that the disorder had spread or that they were caused by environmental conditions.

The plots (6 sq.ft.) were too small to study rates of spread, if any. More recently, when shelf beds 12 × 4 ft. were used, no mushrooms were produced within a radius of about 3 ft. from the site of the inoculation. Beyond this area one true flush of mushrooms was produced and thereafter only scattered sporophores of very poor quality, but there was no marked increase in the size of the bare area. It is possible therefore, that the disorder spreads most rapidly when the mushroom mycelium is growing most actively, but that when the substrate has been fully colonized and fruiting begins, there is little further spread. If this is so, the severity of an outbreak would be related to the time of infection, which may explain some of the contradictory statements which have been made about the behaviour of the disorder, particularly rates of spread. Further experiments are planned to elucidate this point.

During all the experiments mycelial isolations were made at intervals and plated out on 2½% malt agar. In many instances the mycelium was in a poor condition and failed to regenerate, but the cultures which were obtained were of two types; (1) normal; producing white, fluffy mycelium and thick rhizomorphs radiating from the point of inoculation; and (2) an abnormal type: in which the mycelium was adpressed, without rhizomorphs, frequently buff coloured and very slow growing.

A series of isolations from plots showed that before inoculation the mycelium produced normal colonies on agar, but usually after inoculation the mycelium would eventually yield the abnormal type of culture, even though it had not shown visible signs of deterioration. Even more interesting results were obtained from plots in which the mushrooms showed a gradation from healthy to dead. Mycelium from the areas with healthy mushrooms produced healthy cultures, but those from areas with dead mushrooms produced abnormal growth, and sometimes these isolations were made from areas only 6 in. apart. Isolations from control plots, which had produced much heavier crops, showed no signs of degeneration.

The abnormal cultures differed from the normal ones microscopically. The mycelium was much sparser, especially the aerial part, and the individual cells were sometimes slightly swollen, giving the mycelium a somewhat gnarled appearance. Fewer calcium oxalate crystals were produced, so the mycelium lacked the furry appearance of the healthy one. In neither stained or living mycelium could any contaminant fungi or bacteria be found. It was reported in a private communication that some mushroom strains may be contaminated by a parasitic bacterium but that the culture could be cleaned by treatment with rose bengal. Treatment of the abnormal cultures with rose bengal at several strengths failed to produce any improvement in growth. Growth was also consistently poor on media of different richness. Inoculation into beef broth with abnormal mycelium gave no indication of the presence of bacteria.

If two healthy compatible cultures were inoculated on to an agar plate, the mycelia met and the rhizomorphs anastomosed. But when a normal and an abnormal culture were inoculated on to the same plate, the mycelia were slow to touch, a clear zone remaining between them for some time. The growth of the normal colony which took place subsequent to the touching of the two colonies was often weaker than usual, although no apparent change occurred in the appearance of the older mycelium. But when this older mycelium was removed and grown in isolation, its new growth was slow and abnormal. By isolating mycelium from different parts of the colony, it was possible to trace the spread of the infection throughout the mycelium. The transmission of the slow-growing condition to the healthy cultures has been demonstrated repeatedly in these experiments. This technique is now being employed for the identification of 'Watery Stipe' outbreaks; its greatest drawback is slowness, about 4 weeks being needed to complete the whole process.

Transmission to normal cultures has been obtained also by macerating abnormal mycelium in sterile water and dipping disks of normal mycelium in this suspension for a few seconds before plating out. If the liquid is filtered to remove the fragments of mycelium, infectivity is lost. This confirms the results obtained with water extracts of material in the first series of experiments in beds. The extracts so far used have contained fairly large fragments of mycelium which were capable of regeneration. It remains to be seen whether much finer suspensions will give the same results.

The experiments described so far were done at a temperature of 25° C., At 33° C. there

was a marked difference in the response of normal and abnormal cultures. With normal cultures, growth rate was slowed, no rhizomorphs were produced, and the colony was much denser than at 25° C. However, the growth rate of most abnormal cultures at 33° C. was increased and the mycelium became white and fluffy, so that it was indistinguishable from the normal mycelium grown at the same temperature. When young mycelium from these high temperature colonies was subcultured and incubated at 25° C., the normal colonies reverted to the original type of growth, but the abnormal colonies did not, and instead produced normal fluffy white mycelium. When tested by the double-inoculation method, these cultures failed to transmit any disorder. Very occasionally, heat treatment failed to improve the growth of abnormal cultures. These continued to give a positive reaction to the transmission test. Also the original inoculum in the heat-treated abnormal cultures remained infected. When abnormal mycelium was killed by heat, infectivity was lost.

This investigation is still far from complete so it is not possible to do more than hazard a guess as to the cause of 'Watery Stipe'. Lindberg (1959) described a disease of *Helminthosporium victoriae* which caused abnormal growth of this fungus and which was transmissible to normal strains. He has not so far been able to discover the cause of the disease. The similarity between the two diseases is so striking that one is tempted to think that the cause in both cases is similar. One of the main objects of future work will be to try to answer a question which has long been in the minds of many mushroom growers, namely, is it a virus?

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CECIDOMYIIDAE AS PESTS OF CULTIVATED MUSHROOMS

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Since its discovery by Wagner in 1862, paedogenesis (i.e. reproduction by immature forms) has been regarded as little more than a biological curiosity by entomologists. It is only in recent years that this phenomenon has assumed any degree of economic importance. The first reference to Cecidomyiidae attacking cultivated mushrooms was by Barnes (1926) and soon afterwards (Theobald & Barnes, 1928) paedogenesis was first recognized in a mushroom-infesting species. With the rapid post-war expansion of indoor mushroom cultivation, the Cecidomyiidae have suddenly come to the fore as major pests of mushrooms. In many higher plants, intensive cultivation brings with it ideal conditions for the rapid multiplication of pests, and similarly some mycophilous species are favoured by modern methods of mushroom culture.

Prepared mushroom compost is placed in trays or on shelves in a mushroom house and the whole is then thoroughly pasteurized at 130° F. This treatment not only completes the composting process but effectively eliminates all insect life. Most growers imagine that this is a good safeguard against insect pests being introduced from the compost stack. However, we have been unable to find any of the regular mushroom pests either in the fresh compost or in the original horse manure. After pasteurization mushroom spawn is introduced to the beds and the mycelium begins to run through the compost. It is apparently the smell of this rapidly growing mycelium which attracts the majority of Dipterous pests to the mushroom house. A few cecid flies can be caught in light traps at this time. On most commercial holdings several houses are cropped consecutively and throughout the year. Thus there is