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The Mummy Disease of the Cultivated Mushroom

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INTRODUCTION

During the winter of 1935 an apparently undescribed disease of the cultivated mushroom, *Agaricus campestris* Fr., appeared in a limestone cave at Auxvasse, Missouri. The exceedingly serious nature of the disease and the lack of information concerning it prompted the undertaking of investigations on its cause, transmission, and control. These investigations have been continued, at intervals, during the past 7 years. They have not been successful in demonstrating the causal agent, but considerable information on its transmissibility and behavior has been obtained. A practicable method of controlling the disease and limiting losses to insignificant proportions has been developed. Successful control is dependent on early recognition of the disease and prompt action to stop its further spread.

The disease has been observed at Hermann, Missouri in 1936 and each subsequent year; at Quincy and Valmeyer, Illinois, in 1937, and at Kansas City, Missouri, in 1942. Dr. A. M. Kligman has reported, in correspondence, that the disease appeared at Kennett Square, Pennsylvania, in 1941. His diagnosis was based on a comparison of descriptions and photographs of diseased sporophores in Missouri with the Pennsylvania disease. Examination of photographs of Pennsylvania specimens supplies convincing evidence that the disease in Pennsylvania is identical with that in Missouri.

The only published records of the disease are by the senior author in 1937 (3) and 1940 (4). The origin of the disease is unknown. It seems unlikely that it could have been present and have escaped the attention of pathologists and mycologists for any considerable period, since the losses caused by it are very conspicuous. The symptoms are so different from those accompanying the more common diseases of the mushroom that it is also unlikely that it may have been confused with or mistaken for other diseases.

The common name "mummy disease" was suggested by Dr. Kligman. It is quite appropriate for the symptoms that develop in white strains or varieties, and, with Dr. Kligman's permission, will be used here.

[1]The authors are indebted to Mr. William Harrison, manager of the Stone Hill Farms at Hermann, Missouri, for his assistance and careful observations at Hermann and Auxvasse. Dr. W. E. Maneval and Dr. Weston Bohn gave much assistance in sectioning and staining material and in culturing fungi and bacteria.

The procedures involved in the production of mushrooms include the following: preparation of the compost, filling the beds, subjection of the compost to temperatures of preferably 130-140° F., fumigation to kill insects, spawning, growth of the fungus for about three weeks, and casing the beds with soil. Three to four weeks after casing, the first "break" of mushrooms appears. At intervals the mushrooms cease to develop, the beds are thoroughly cleaned of mushroom remnants and may be moistened. New breaks of mushrooms occur regularly about every 7-10 days until the end of the crop.

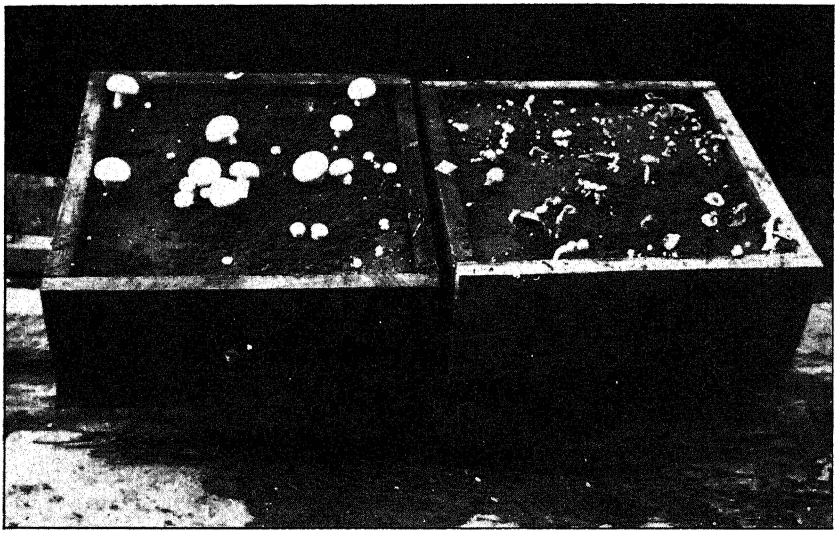

Fig. 1.—Left, normal mushrooms of a white variety. Right, diseased mushrooms of the same variety resulting from infestation with casing soil from an affected bed.

The procedure followed at Auxvasse and at Hermann, Missouri, where the investigations were made, was essentially as outlined above. It should be pointed out that the manure for composting and the soil used for casing were secured from different places for the two mushroom plants and that the soil was not sterilized. At both places the soil was screened in the field and stored for at least several weeks in the cave or in an open shed before use. At Hermann, the beds in use when the disease first appeared were about 60-80 feet long and 4-5 feet wide, and arranged in tiers about 3 feet apart. At Auxvasse, the first beds were long, narrow, and arranged on the floor of the cave or in tiers; later, wooden boxes 46 x 32 x 5 inches were used. These were arranged in tiers with a space between adjoining boxes and with the boxes in the next higher row standing above the space in the lower row. The boxes of each row rested upon short wooden blocks.

SYMPTOMS OF THE MUMMY DISEASE

The first evidence of the development of the mummy disease in a mushroom bed is the appearance, in a circumscribed area, of a few sporophores with stipes longer and smaller in diameter than normal, and often slightly curved; the pilei fail to attain normal diameter and are often in a tilted, rakish position, instead of at a right angle to the stipe. This position is apparently the result of one-sided, irregular growth of the stipe which causes uneven breaking of the

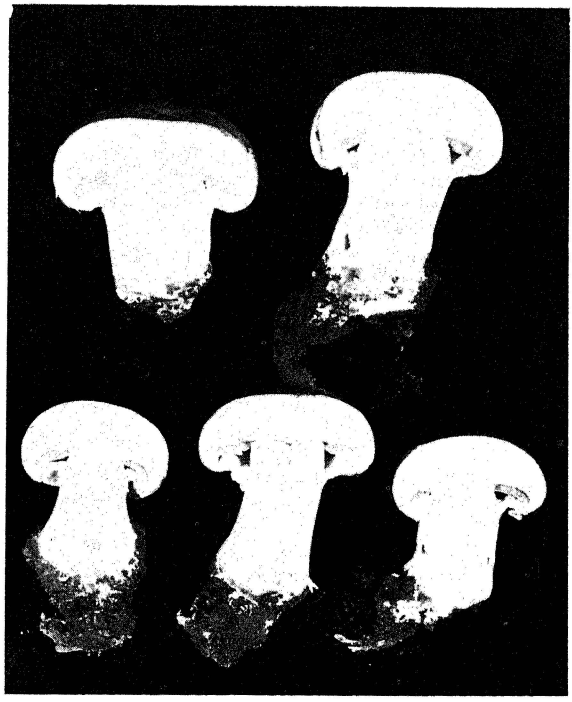

Fig. 2.—Above, normal mushrooms of a cream variety in longitudinal section. Below, longitudinal sections of young diseased mushrooms of the cream variety. Note the glassy, translucent pits and streaks in the stipes; these are packed with bacteria.

veil. The stipe elongates abnormally resulting in early rupture of the veil and premature flattening of the pileus (Figs. 3 and 5). The entire sporophore of the white varieties is slightly gray and dry in appearance. The first affected sporophores are usually few in number and confined to a small area in the bed. The disease has not been observed appearing simultaneously over large areas.

When the next break appears, after 7-10 days, the area producing diseased mushrooms has increased greatly in size and extends 5-10 feet in all directions from the original site. At this time the

symptoms are more pronounced. Nearly every sporophore in the area of disease is affected; a normal mushroom is rare. The stipes are long, slender, and often markedly curved. The pilei are small, sometimes barely one half inch in diameter and usually in a tilted position on the stipe (Figs. 5 and 7). The gills and annulus are often poorly developed. In the white mushroom varieties the entire sporophore is gray or light tan. The tissues are firm at first, but become somewhat spongy and slightly more moist externally than normal; finally they become spongy, dry and tough. When the stipe of an infected sporophore is cut across with a sharp knife there is a gritty sound and feel as if the stipe contains some granular material. Normal mushrooms cut smoothly and easily. A con-

Fig. 3.—Young, diseased mushrooms of the cream variety. Note the rakish position of the pileus, the curved and somewhat narrowed stipe.

spicuous symptom at this time is the abnormal development of rhizomorphs attached to affected sporophores. When a diseased mushroom is removed from the bed it is accompanied by a large mass of numerous rhizomorphs and adhering soil. This is especially marked in contrast with the smaller number of rhizomorphs subtending normal sporophores (Fig. 7). The general appearance of the mushrooms in an affected area and the contrast with those in a normal area is well illustrated by Fig. 1.

When the diseased sporophores and buttons are removed from a bed and the bed is watered, if needed, a new break of pinheads develops after the normal interval. The break appears normal in every respect, and at this time an affected bed cannot be distinguished from a normal bed. However, the development of the sporophores ceases very soon. Most of them fail to grow beyond the button stage and many remain in the pinhead stage. These become mummified, dry, spongy and shrunken. A few develop further into slender, punky sporophores with the characteristic tilted pilei scattered sparsely over the bed (Fig. 7). No marketable mushrooms are produced and the bed is a complete loss.

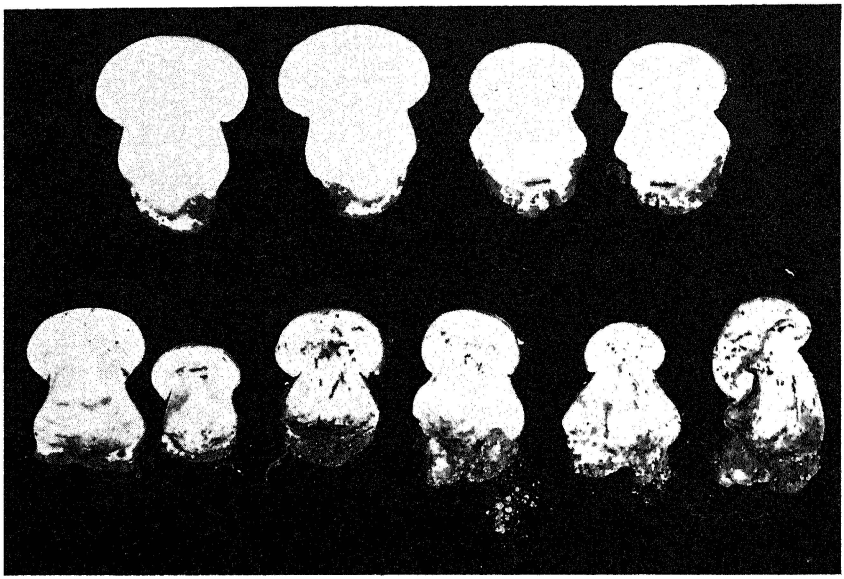

Fig. 4.—Above, longitudinal sections through normal mushrooms of a white variety. Below, longitudinal sections through young, diseased mushrooms of the white variety. Note again the rakish pilcus, the narrowed stipe and the bacteria-filled pockets.

Fig. 5.—Above, external appearance of diseased sporophores; these may be considered typical specimens from beds in later stages of the disease. Below, external appearance of normal sporophores.

Longitudinal sections through diseased sporophores in the earliest identifiable stages of the mummy disease show colorless, translucent, watersoaked dots or streaks in the white tissues of the stipe and, frequently, of the pileus. They are most pronounced near the base of the stipe, and at the point of attachment to the largest rhizomorph there is usually some browning and development of a wet rot (Fig. 2). The discolored areas swarm with bacteria which are apparently secondary, invading and rotting the abnormal tissues. Sections through sporophores with elongated stipes (Fig. 6) reveal a light tan color which is more marked in the stipe than in the pileus.

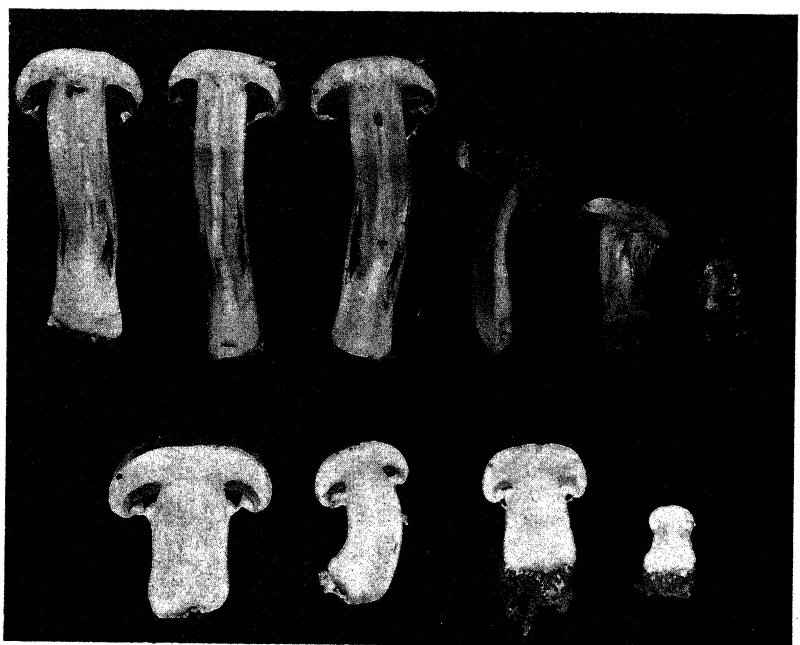

Fig. 6.—Above, longitudinal sections through diseased sporophores showing occurrence of bacteria-filled pits and decomposition developing in advanced stages. Below, longitudinal sections through normal sporophores.

The tissues are slightly spongy and dry and have undergone some shrinkage, resulting in the pulling of the central tissues of the stipe away from the more compact cortical region. The central core of less compact hyphae becomes more conspicuous than in a normal stipe. At this stage bacteria-filled cavities may be numerous in stipe and pileus or there may be none. Sections through buttons from beds in advanced stages of the disease are often honeycombed with dark cavities filled with bacteria (Fig. 4). However, the cavities are not always present and sections through the buttons may show only tan, spongy fungous tissue. The brown discoloration at the point of attachment to the rhizomorph is characteristic, regardless of the occurrence of bacteria-packed cavities in the stipe and pileus.

The symptoms described above pertain particularly to the white varieties of the cultivated mushroom, which make up the bulk of the commercial crop. The mummy disease has also appeared in cream varieties at Hermann, Missouri. The general course of the disease is identical with that in the white varieties but there are some minor variations in the development of symptoms. As in the white varieties the disease in the cream types can be recognized by the crooked stipes, tilted pilei, and arrested growth. However, the cream varieties are apparently much more susceptible to the secondary bacterial rot. The white types usually become dry and shrunken, but the cream sporophores become soft and watery and have an offensive, putrefactive odor. In most instances they are watery and partially liquefied in the interior tissues as early as the disease can be identified from external symptoms. Brown watery cavities occur in many otherwise apparently normal sporophores growing at the periphery of an infected area. The tissues swarm with bacteria.

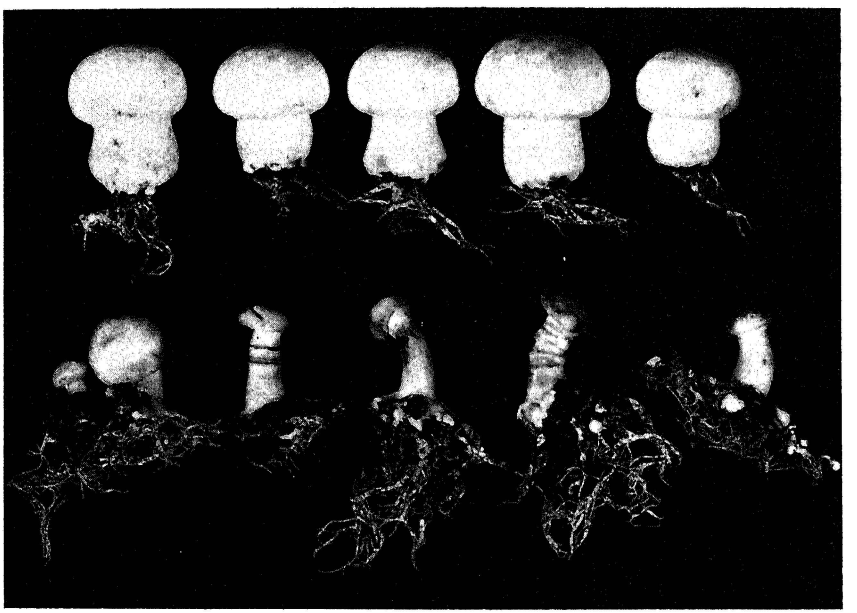

Fig. 7.—Above, normal sporophores. Below, diseased sporophores. Note the small, tilted pilei, curved, slender stipes, and profuse development of rhizomorphs and fungous tissue at the base of the stipes.

A conspicuous symptom occurring in the cream types, but not in the white types, is the exudation of drops of liquid from the pileus and the veil region. The exudate from small buttons is sometimes almost colorless, but from most buttons and older sporophores it is yellow to dark amber. A row of droplets, filled with bacteria, often appears around the stipe at the veil. Two days after appear-

ance of the first symptoms most of the sporophores collapse and are involved throughout in a watery rot. When diseased sporophores are broken from the rhizomorphs a drop of exudate quickly appears on the newly exposed brown-discolored tissue. The affected sporophores break away from the rhizomorphs in a brittle fashion instead of coming out of the bed with some rhizomorphs attached as is usual in normal cream and white types; the masses of rhizomorphs and soil which come out of the bed with diseased white sporophores are not characteristic of the cream varieties.

Microscopic examinations of fresh and fixed material from diseased sporophores reveal that the small discolored cavities are filled with a gray slime composed of motile bacteria. Long, non-septate hyphae are present in the stipes and pilei; they extend through the tissue in various directions, often forming tangled masses; they are especially common at the base of the stipe and near the bacteria-filled cavities. The hyphae stain deeply in lactophenol preparations and resemble the vascular hyphae that occur in rhizomorphs (Buller) (1). Large, non-septate hyphae occur in smaller numbers in normal sporophores. However, these appear to be formed by the end to end fusion of cells and partial dissolution of septa (Hein) (2), and have ring-like thickenings not found on the more abundant non-septate hyphae in the diseased sporophores. Furthermore, the large hyphae in normal sporophores appear as single strands rather than in groups or clumps.

In occasional diseased sporophores ovoid to spheroid, hyaline, thin-walled, one-celled bodies with dense, granular contents are associated with the large, non-septate hyphae. The structures resemble the chlamydospores or unfertilized oogonia of Pythium and Phytophthora. The size of the bodies is 30.4 - 35.9 x 27.6 - 31.0 micra, with the average dimensions 32.4 x 28.6 micra. Neither oospores nor antheridia were seen. The hyphae and spore-like bodies occur in both white and cream types.

Numerous irregular crystals of an unidentified material occur in the tissues of diseased sporophores. They are probably responsible for the characteristic "gritty" sensation experienced in cutting the stipes.

The rhizomorphs bearing diseased sporophores appear normal externally. In cross-section near the base of the stipe the interior of the rhizomorph is brown, soft, and teeming with bacteria; the appearance contrasts markedly with the white, firm, turgid appearance of the tissue of a normal rhizomorph. The development of rhizomorphs is not inhibited. As has been mentioned, repeated breaks may be secured on beds many weeks after infestation has destroyed their capacity for production of normal mushrooms.

There is often a profuse development of fungous tissue about the base of the stipe of diseased sporophores; a dense mat of tissue to which numerous rhizomorphs are attached usually develops about

the base of the stipe of sporophores exhibiting arrested growth (Fig. 7). This abnormal development suggests that the critical phase of the disease occurs in the large rhizomorphs subtending the sporophores or at the base of the stipe. The transfer of water and nutrients into the young sporophore is apparently prevented by the disorganization of the conducting tissues; the excessive development of tissue about the base follows the interruption of the flow into the sporophore. Observations on many beds have failed to reveal other abnormalities in the development of the host which might account for the cessation of growth characteristic of the mummy disease. The mycelium in the compost remains vigorous, as attested by the new breaks that occur in affected beds long after the disease has appeared.

Repeated examinations of casing soil and compost from infested beds, and comparisons with soil and compost from nearby normal beds loaded, spawned and cased at the same time, have revealed no differences in color, moisture content, texture, odor, reaction, abundance and character of mycelial growth, occurrence of other fungi, nematodes or insects which would permit distinguishing the infested soil and compost from the normal samples. It seems clear that the causal agent does not inhibit the development of mycelium in the compost or the development of rhizomorphs in the casing soil.

On April 21, 1937, hydrogen-ion determinations were made electrometrically on samples of soil and compost from 6 infested beds at Hermann. Control samples were taken from adjacent normal beds loaded from the same lot of compost as the infested beds and maintained under the same environmental conditions. The infested and control beds selected for sampling were located in 5 cellars.

TABLE 1.—HYDROGEN-ION DETERMINATIONS ON SOIL AND COMPOST FROM MUSHROOM BEDS INFESTED WITH THE MUMMY DISEASE, AND FROM NORMAL BEDS. HERMANN, MISSOURI. APRIL 21, 1937.

Infested and control beds Pair Nos.	Soil		Compost	
	Normal pH	Infested pH	Normal pH	Infested pH
1	6.87	6.95	6.47	6.49
	7.02	7.22	...	6.15
2	7.95	8.01	6.82	6.30
	7.60	8.05	6.80	6.38
3	7.62	7.07	6.34	6.90
	7.40	7.22	6.40	6.33
4	7.38	7.07	6.22	6.15
	7.13	6.89	6.50	6.15
5	7.97	6.87	6.50	6.38
	8.05	6.83	6.56	6.35
6	7.63	7.23	6.70	6.30
	7.28	7.18	6.47	6.38

In 4 of 6 comparisons of soil from infested and normal beds the infested soil was somewhat more acid than the soil from normal beds. In comparisons of compost the same relationship was observed in 5 of 6 determinations. However, the differences were usually small, and samples of soil and compost from some normal beds were more acid than some samples from infested beds. Infestation apparently does not have a marked effect on the reaction of either compost or casing soil. So far as can be determined by visual examination the hyphae of the Agaricus develop normally in the compost of infested beds for several weeks after the disease appears.

Hydrogen-ion determinations were made on tissues of normal and diseased sporophores of white and cream types. The tissues were macerated in water and the filtered liquid was used. The white sporophores were in the light brown, dry, spongy stage and there were few bacterial pockets in the interior. The cream sporophores were undergoing the rapid breakdown characteristic of the variety; numerous areas of wet, brown rotting tissue occurred in the stipes and pilei.

TABLE 2.—HYDROGEN-ION DETERMINATIONS ON TISSUES OF NORMAL AND DISEASED SPOROPHORES OF WHITE AND CREAM MUSHROOM TYPES. EACH DETERMINATION REPRESENTS ONE SPOROPHORE. APRIL 21-27, 1937.

Variety	Condition	
	Normal	Diseased
White	6.50	6.55
	6.42	6.57
	6.30	6.41
	6.41	6.65
Cream	6.31	7.48
	6.38	7.40
	6.43	6.61
	6.39	6.85
	...	7.11

In both types the tissues of the diseased sporophores were more alkaline than those of normal sporophores. In the white type, showing little evidence of bacterial decomposition, the changes were not large. The cream type, which was decomposing rapidly, showed a very marked development of alkalinity. It is probable that this is caused by the occurrence of amino compounds or ammonia produced by the activity of proteolytic enzymes of the secondary bacteria, and that the change cannot be attributed to the action of the causal agent of the mummy disease.

Under natural conditions in caves and houses the disease has appeared at the third break or later. Old beds approaching the end of production are very frequently affected. The initial site of infestation is small, involving only a few square feet. The spread of the disease through the bed is rapid, and each succeeding break

shows a much larger area of infestation. Many observations on the rate of spread indicate that the progress of the disease is at the average rate of about one foot per day. When the disease appears near the middle of a 50-foot bed the entire bed is likely to be invaded in about 3 weeks.

The mummy disease is not dependent upon, or especially favored by, any particular set of environmental conditions. It has appeared in caves at an almost constant temperature of 53° F., and in houses at temperatures up to 65° F. The location of the bed in the tier does not affect the likelihood of infestation; the disease appears as frequently in middle and top beds as in ground beds. In large establishments the disease occurs at random in beds distributed throughout the houses or caves. Fortunately, the number of initial infections has been limited and a majority of the beds have remained normal.

The mummy disease does not spread readily from bed to bed. This behavior has made the control of the disease fairly simple and inexpensive. Many cases have been observed in which the disease spread rapidly through a long bed on the top or in the middle of a tier early in the season, yet the other beds in the tier remained normal until the end of the crop. Indeed, the remaining beds in the tier seem no more likely to become infested than beds in remote tiers, provided reasonable care is used to prevent transferring infectious material from the diseased bed. The disease does not show a very marked tendency to become concentrated in particular houses. No instances are known in which infestation occurred in a large percentage of the beds in one house of an establishment while the other houses remained free from the disease.

LOSSES

The losses to mushroom growers resulting from the mummy disease were extensive until control measures were used. The amount of damage is dependent on the number of beds that become infested and their age when the disease appears. When the disease has progressed through a bed it is permanently out of production. Small abnormal sporophores may continue to develop over a period of weeks or months, but no marketable mushrooms are produced.

In numerous instances 50-foot beds have been completely invaded about 6 weeks after the first break. The disease appears to be one of the most destructive known to affect mushrooms.

TRANSMISSION OF THE DISEASE

Experimental work on the transmission and control of the mummy disease was carried on with long beds at Auxvasse and Hermann, and with small beds, 18 inches square, which were prepared in

large numbers, filled, heated, spawned, cased and handled, in general, like the large beds. Most of the inoculation experiments were performed with the small beds at Hermann.

1. Isolations and Inoculations.—The constant association of bacteria with the mummy disease has been noted. Isolations from stipes and pilei with discolored spots in the tissues invariably yielded bacteria. Isolations from specimens collected at the same time from a single bed usually yielded the same species in most plates, but lots from other beds, or from the same bed collected on another date, often yielded other species. Among approximately 25 lots of sporophores at least 6 species of bacteria were predominant in one or more lots. Failure to demonstrate the constant association of a particular species with the disease suggests a saprophytic relationship, with the predominant species influenced by the environmental conditions prevailing at the time.

The media used included mushroom decoction agar, potato agar, potato dextrose agar, asparaginate agar, tap water agar and nutrient agar. When the media were acidified various fungi were secured. Tissues from diseased pilei usually failed to yield growth of any type in acid agars. Attempts to secure pure cultures of Agaricus from diseased sporophores were not successful, although similar trials with tissues from normal sporophores were successful. Isolations from the basal region of the stipe yielded many cultures of species of Trichoderma, Mucor, Fusarium, Aspergillus, Verticillium, Penicillium, Rhizopus, and Cephalosporium. At least 2 species of Pythium were obtained, one of which produced fruiting bodies in culture which resembled those seen in diseased sporophores.

Isolations from normal and discolored rhizomorphs on acid agars yielded the same fungous flora. As rhizomorphs develop the Agaricus hyphae engulf soil particles and the isolates obtained were representative of the common soil flora.

Small beds producing normal mushrooms were used to test the pathogenicity of the isolates. Three beds were infested with each species. The fungi were grown on agar slopes and the entire contents of 3 tubes were buried in each bed. The cultures were placed in the casing soil in contact with the compost and covered with soil. The bacteria were incubated in nutrient broth and the bacterial suspensions were poured on the soil and into the compost. The suspensions were also injected into normal sporophores and buttons.

Compost from an infested bed was placed in a jar of autoclaved compost. A species of Fusarium invaded the autoclaved compost and grew quickly to the bottom of the jar, which was broken and a bit of compost was transferred to a second jar of sterile compost. When the Fusarium permeated the medium, the compost and Fusarium were placed in a normal bed. No evidence of pathogenicity was obtained. A portion of the compost from the first jar was

removed carefully to avoid securing compost which was in contact with the original inoculum (compost from an infested bed). It was divided and placed in the compost of two normal beds. After 4-5 weeks some abnormal, but not typically diseased, sporophores developed in both beds; later production was normal. The mummy disease is not caused by a Fusarium and apparently has little resemblance to the Fusarium disease reported by Wood (5).

None of the organisms used proved pathogenic. In every case the inoculated beds continued production of normal mushrooms. Control beds on which a double handful of casing soil from an infested bed was placed developed typical symptoms of the mummy disease in about 3 weeks. Repeated trials have failed to secure in culture an organism which could be proved to be the causal agent of the disease.

2. Transmission by Casing Soil and Compost.—The only method by which the mummy disease has been transmitted from diseased to normal beds involved placing casing soil or compost from a diseased area in contact with a normal bed. In 1935 small sections (about 2 sq. ft.) were cut out of affected beds with a saw to avoid disturbing the compost and casing soil. The sections were placed in close contact with undisturbed normal beds, compost to compost, and soil to soil. In every trial the disease was transmitted to the normal beds. The first symptoms usually developed about 3 weeks after the infested material was placed in contact with the normal bed. There was some variation in the period involved, with 17 days the shortest and 29 days the longest periods noted. After invading the normal bed the disease progressed rapidly throughout the bed, with the development of typical symptoms identical with those of the infested beds from which the sections were cut.

When similar experiments were set up with a portion of the casing soil removed to permit contact between affected and normal compost only, the disease was transmitted as readily as when the casing soil was in place.

Infested sections were placed 1 foot from normal beds. The intervening space was filled with fresh (unused) casing soil. After 36 days the disease appeared in a normal bed. Examination of the casing soil between the beds showed growth and intermingling of rhizomorphs and hyphae from the beds.

A quantity of soil and compost from an affected bed was placed in a large box containing a layer of moist, fresh (noninfectious) soil approximately 6 inches deep. After 12 days samples from the original inoculum and from the soil at distances of 1, 2 and 3 feet from the inoculum, were transferred to normal beds. The sample of the inoculum transmitted the disease but the samples of soil did not. There was no discernible development of mycelium or rhizomorphs in the soil samples. After 21 days a portion of the original inoculum transferred to a normal bed failed to transmit

the disease. Samples of soil from areas 1, 2 and 3 feet distant from the inoculation again failed to transmit the disease. After 51 days a third attempt to transmit the disease with a portion of the original inoculum again gave negative results.

Experiments involving the placing of casing soil or compost from affected beds in normal beds resulted in the appearance of the disease after about 3 weeks, with the first diseased sporophores developing in the area in which the infective material was placed. The evidence indicates that the causal agent is in the soil and compost or in the rhizomorphs and mycelium transferred with the soil.

Diseased sporophores cut just above the base of the stipe were macerated in tap water. The suspension was mixed into the casing soil and compost of normal beds. Entire diseased sporophores were buried in normal beds. The results of repeated attempts to transmit the disease through diseased sporophores were invariably negative.

Rhizomorphs subtending diseased sporophores were used in attempts to transmit the disease. Soil from an affected bed at Hermann was taken to Columbia, the rhizomorphs were removed, washed in water, stored over night at 4-5° C., and placed in the casing soil or compost of normal beds. Other rhizomorphs were ground in a mortar, stored as above, and placed in normal beds. In each case the whole or ground rhizomorphs failed to transmit the disease to the normal beds. Rhizomorphs transferred directly from affected to normal beds also did not transmit the disease. Attempts to transmit the disease were made by removing from affected to normal beds: (1) the basal portion of affected stipes with their rhizomorphs (2) diseased sporophores cut into small pieces in water and allowed to decompose 3 days at 20° C. (3) large numbers of small, entire, diseased sporophores (4) large quantities of large, crushed, diseased sporophores. All results were negative; the beds continued in normal production.

Approximately 1 kilogram of casing soil from an affected bed was stirred with 2 liters of tap water and allowed to settle. The supernatant liquid was decanted off and divided into 2 parts; one part was filtered through Whatman No. 50 filter paper. The filtered and unfiltered leachates and the soil that settled out were placed in normal beds. The leachates were used at the rate of 250 ml. per bed. After 4 weeks the mummy disease appeared in the beds in which the soil was placed. Those in which both filtered and unfiltered leachates were placed remained normal.

The disease has not appeared prior to the third break in commercial houses. However, it may be induced at the first break if casing soil from an affected bed is placed on a normal bed at the usual time for casing. On Feb. 3, 1937, 3 beds were cased with soil from an affected bed. The first breaks appeared on March 1,

March 10 and March 13. All three beds produced typically diseased mushrooms. Samples of soil and compost were transferred from the beds to normal beds and the disease was again transmitted.

Soil removed from an area of diseased mushrooms and placed in a normal bed has been shown repeatedly to transmit the mummy disease. It has also been proved that soil removed from an apparently normal area contiguous to a disease area may also transmit the disease. For example, soil removed from among apparently normal sporophores a few feet from an area of diseased sporophores almost invariably transmits the disease. On April 2, 1938, a long bed was infested with infectious casing soil at one end. On May 1 unmistakable symptoms appeared in the infested area. Samples of soil were taken at one foot intervals from the margin of the area of visibly affected sporophores and placed in normal beds. On June 4 the disease had appeared in beds infested with soil from distances of 1, 2, 3, 4 and 5-feet. Beds that received soil from a site 6 feet or more from the disease area remained normal. This experiment substantiated numerous observations on the behavior of the disease in experiments on its control, to be discussed later, in which it was found that trenches across an affected bed 6 feet in advance of visibly diseased sporophores usually stopped the spread of the disease through the bed, while trenches 3 feet from the disease area were not effective. It seems clear that the pathogenic agent extends through the bed, either in the substratum or in the Agaricus hyphae, to a distance of 3 to 6 feet beyond the margin of the area in which affected sporophores can be identified.

Infectious soil or compost was placed in contact with wheat grain spawn in beds at spawning. There was a normal development of mycelium and rhizomorphs. A break appeared at the usual time (after 10-11 weeks), but all the sporophores were diseased.

A side board was removed from an affected bed, washed with cold water, and used to replace a side board of a normal bed. The disease was not transmitted.

The numerous experiments on the transmission of the mummy disease indicate that it is transmitted from infested to normal beds very readily with casing soil and compost, and that it cannot be transmitted through diseased sporophores. The experiments suggest that the pathogen does not spread through the bed independently, but is dependent on the presence of living Agaricus mycelium or rhizomorphs, which must be transferred in a condition permitting resumption of growth to secure transmission of the disease.

The failure of the disease to spread readily from bed to bed in mushroom houses is in accordance with this hypothesis. Insects of various types are usually more or less abundant, and a disease comparable with those caused by *Verticillium spp.* and *Bacterium tolaasi* (Paine) Elliott, with abundant development of readily disseminated spores or cells, would be expected to spread very rapidly

throughout the houses. Transmission of the mummy disease by insects or air currents is highly improbable.

At Auxvasse some circumstantial evidence was obtained that the disease may occasionally be transmitted from infested to normal beds with soil carried on the shoes of workmen. It was noted that a large percentage of newly infested beds appearing in the cave showed the first development of diseased sporophores along sideboards on which workmen stood most frequently in picking, watering and cleaning the beds. Although the amount of soil transferred in this manner would be small, it seems reasonable to assume that living rhizomorphs or hyphae may occasionally be transferred and resume growth in the new bed. However, a single small experiment in which small amounts (one-fourth handful) of infested soil were transferred to two normal beds gave negative results, while larger amounts of soil transmitted the disease.

Effects of Various Factors on the Infectivity of Soil and Compost

1. Aging.—The effect of the age of compost and soil from affected beds on their infectivity has not been demonstrated very clearly by experiments. Other factors, such as changes in the moisture content and temperature variations during the storage periods, may have affected the survival of the pathogen to as large an extent as aging.

A bed was infested with infectious soil on February 3, 1937. At the first break on March 1 the sporophores showed typical symptoms. The bed was moistened occasionally to June 1, when it was placed in a fairly humid cellar at Hermann and held until December 21, 1937, when samples of soil and compost were removed and placed in duplicate normal beds. On February 15, 1938, the mummy disease developed in one bed infested with compost. The other bed to which the compost was added, and also 2 beds on which the soil was placed, remained normal.

On February 25 casing soil was taken from the bed which developed symptoms of disease on February 15. The soil was placed on 3 normal beds; on March 24 typical diseased sporophores were developing on all 3 beds.

Casing soil from 2 naturally diseased areas was placed in barrels on June 1, 1937, and held in the cellar to December 21, when it was used to replace the casing soil removed from one-half the area of normal beds. The beds did not develop the disease. Normal sporophores developed on the portions recased with soil from the affected beds.

On June 20, 1938, an infested bed was placed in a cellar and moistened occasionally to maintain the water content at approximately the level of a normal producing bed. On February 15, 1939, soil and compost were removed and each was placed in 3 normal

beds. On March 22 all beds which received either soil or compost were developing sporophores with typical symptoms.

The experiments show that soil and compost in beds kept moist retain their infectivity at least 8 months. In beds allowed to dry the casing soil had lost its ability to transmit the disease after about 7 months, but one of two samples of compost from a dry bed did transmit the disease.

In the above experiments the old infested beds were held at normal cellar temperatures.

On December 7, 1936, approximately 20 liters of casing soil from an affected bed were placed in a tightly closed container and held at room temperature (about 25° C.) to January 25, 1937, when a portion of it was used to case 3 small beds. The mummy disease did not appear. Beds cased at the same time with soil transferred directly from the same infested bed developed typical symptoms.

The causal agent of the mummy disease exhibits little resistance to aging in casing soil removed from infested beds.

2. Temperature.—Containers with approximately 15 liters of soil and compost from an infested bed were stored from June 20, 1938, to October 19. Heavy paper was tied securely over the top of each container. One container each of soil and compost was stored at —12° C., 4.5° C. and at cellar temperature (15-23° C.). The last were moistened occasionally. On October 19 portions of soil or compost from the stored containers were placed in normal beds. The disease was transmitted by both soil and compost stored at 4.5° C. and at cellar temperatures, but not by soil or compost stored at —12° C. The last samples were frozen and thawed quickly.

On June 30, 1938, samples of soil and compost from the infested bed mentioned above were placed in liter bottles and stored under various conditions, as follows: Soil—(1) suspended in 3 times its volume of tap water. Sealed and stored at room temperature (25-37° C.). (2) Soil not covered. Stored at room temperature. (3) Not covered. Stored (in electric refrigerator) at 4° C. (4) Suspended in water as in (1) and stored at 4° C. (5) Moistened, sealed and stored at 4° C. (6) Moistened, sealed and stored at 35° C. (7) Moistened, sealed and stored at 30° C. The compost was stored as follows: (1) Moistened, sealed and held at room temperature. (2) Not covered. Stored at room temperature. (3) Not covered. Stored at 4° C. (4) Moistened, sealed and stored at 4° C. (5) Moistened, sealed and stored at 30° C. (6) Moistened, sealed and stored at 35° C.

On October 19 the entire contents of the bottles were placed in normal beds. No infection resulted in any case. It is of particular interest that in the entire series, including the larger quantities of soil and compost, the disease was transmitted only by the soil and compost stored in quantity at cellar temperatures and at 4.5° C. The smaller lots, in open containers, dried out in storage to a

much greater extent than the larger quantities. This factor may have influenced their loss of infectivity. The samples in sealed bottles were not subject to drying out and their loss of infectivity cannot be attributed to lack of moisture.

Heating infested casing soil renders it non-infective. Several experiments gave uniform results. There was no transmission of the mummy disease through soil heated in a drying oven at 55° C. for 2 hours, steamed at 100° C. for 1 or 2 hours, or autoclaved 20 minutes at 15 pounds pressure. The infectivity of compost from affected beds was destroyed by autoclaving or steaming at 100° C. for 1 hour.

Soil and compost were rendered non-infective by increasing the temperature slowly over a 3-day period to 46-55° C., which temperature range was maintained 24 hours. Drying compost at 60-80° C. also resulted in non-infectivity.

Suspensions of infested soil in water were maintained 10 minutes at temperatures of 47°, 57° and 67° C. The suspensions were cooled quickly and placed in normal beds. No infection occurred.

The experiments indicate that the causal agent is rendered inactive very quickly at moderately high temperatures. The heating which the compost normally undergoes during the composting process and in the beds is sufficient to destroy the disease agent. It seems very unlikely that the compost can be regarded as a possible source of infection.

3. Chemicals.—Infective casing soil was removed from beds, treated with various chemical compounds, and used to replace a portion of the casing soil of normal beds. In each series of experiments untreated soil was used to infest normal beds as controls. Only experiments in which the control beds developed typical symptoms of the mummy disease are regarded as significant.

On April 8, 1938, a series of beds was infested with equal amounts of casing soil treated and held in tightly covered containers 48 hours. Final results were observed on May 28. Formaldehyde (1 part 40% formaldehyde to 5 parts water) was mixed thoroughly with the soil at the rate of 20 ml. to 18 l. of soil; in another series the same solution was used at the rate of 35 ml. to 18 l. of soil. Neither treatment destroyed the infective principle; the disease appeared in triplicate beds simultaneously with its appearance in the control beds. Carbon bisulphide was mixed with infective soil at the rate of 1 oz. to 324 cubic inches of soil. The treatment rendered the soil non-infective; beds infested with the soil remained completely normal.

Compost samples were treated with formaldehyde as above with the same results. Control beds with untreated infective compost also became diseased, and beds in which infective compost steamed 1 hour at 100° C. was placed remained normal.

On March 29, 1939, lots of 1500 grams of infectious casing soil were treated with various chemicals. Each lot was divided into 3 parts of 500 grams each and each part was placed in a normal bed. The treatments used were as follows:

1. Check. No treatment.
2. Carbon bisulphide, 5 ml.
3. Formaldehyde, (1 part to 5 parts water), 10 ml.
4. Calcium cyanide, 5 g.
5. Trichlorethylene, 2 ml.
6. Tetrachlorethane, 2 ml.
7. Pentachlorethane, 2 ml.
8. Perchlorethylene, 2 ml.
9. Ceresan (5%), 2 g.
10. Pyroligneous acid (16 parts to 100 parts water), 40 ml.
11. Ammonium hydroxide (17.5%), 5 ml.
12. Acetic acid (1.2%), 80 ml.

On May 11 the mummy disease had developed in the check beds which received untreated soil, and in the beds with soil treated with pyroligneous acid and ammonium hydroxide. All other beds remained in normal production.

The disease agent is sensitive to chemicals, and the infectivity of casing soil is destroyed by chemicals ordinarily considered to have but slight fungicidal or bactericidal action at the concentrations used.

THE CAUSAL AGENT

Experiments have failed to establish the identity of the causal agent. Isolations from diseased material have failed to yield an organism capable of initiating the disease in normal beds, and one may only speculate regarding the nature of the pathogen. The evidence indicates that any factor resulting in the killing of the Agaricus mycelium destroys the infectivity of soil or compost, and suggests that the pathogen is an obligate parasite dependent on living fungous tissue. Transmission of the disease apparently depends upon the transfer of living, infected Agaricus tissue which is capable of renewed growth in the new environment. The transmission of the mummy disease to normal beds may be dependent on anastomoses between normal and parasitized hyphae. It has been observed repeatedly that transmission of the disease occurs when there is an intermingling of hyphae from the inoculum with those of the normal bed.

The incubation period of the disease is usually about 3 weeks. During this period the pathogen extends through the bed to a distance of approximately 4-5 feet in all directions from the site of infestation with infectious soil or compost, although the first sporophores showing symptoms of the disease are restricted to a much smaller area. After the appearance of abnormal, diseased sporophores the disease spreads through the bed rapidly, at the

rate of about 1 foot per day. This rapid spread is difficult to reconcile with the known growth rates of fungi. The presence of hyphae resembling those of a Phycomycete in diseased sporophores has been mentioned; however, soil infestations with 2 species of Pythium gave negative results, but it is not certain that the fungus observed in the sporophores has been obtained in culture.

The rate of spread of the mummy disease and the possibility that anastomoses between infected and normal hyphae may be necessary for its transmission suggest that the pathogen may be of the virus type. There is a rapid movement of water and solutes through the hyphae and it appears probable that virus particles might be carried at a rate comparable with the observed spread of infection. Attempts to secure pure cultures of Agaricus from diseased sporophores and rhizomorphs have not been successful. Such cultures, if obtainable, may prove helpful in determining the nature of the pathogen. There is insufficient evidence to warrant definite conclusions regarding the nature of the pathogen at this time.

THE SOURCE OF INFECTION

The source of the causal agent of the mummy disease has not been determined with certainty. The casing soil is suspected as the bearer of the pathogen, but the evidence is largely circumstantial. The compost used at the various establishments is obtained from widely separated points, yet the disease has shown no inclination to appear more frequently in beds filled with a particular lot of compost than in those filled with other lots. Furthermore, the heat through which the compost passes appears, on the basis of experimental evidence, to be sufficient to inactivate the causal agent.

The disease has showed no tendency to persist from year to year in certain beds or houses. The pathogen apparently does not survive the cleaning and drying of the beds and houses. Initial infections are believed to be due to the introduction of the pathogen from outside the houses.

The transmission of the disease by insects may apparently be disregarded. The failure of the disease to spread readily from infested to normal beds in spite of the presence of numerous insects seems to preclude the possibility of the introduction of the pathogen by insects.

The casing soil remains as the most likely source of the disease. If the conclusion that the disease can be transmitted only through living rhizomorphs or hyphae is correct, it must be assumed that the mummy disease occurs in nature in a species of Agaricus or another fungus, and that transmission through the casing soil involves the transfer of infected, living material from the field to the mushroom beds. The routine practice in handling casing soil in areas where the disease occurs in Missouri includes screening the

soil in the field and storing it in bins under shelter for some time before it is used. In different seasons it has been observed that the number of initial infections developing in the beds varies considerably. This may be due to (1) variations in the amount of the infected hyphae in the soil (2) variations in the survival of infected hyphae during the storage of the casing soil.

The susceptibility of the causal agent to heat and drying demonstrated experimentally suggests the practicability of controlling the disease by sterilization or pasteurization of the casing soil. Although this method of attack seems promising the growers have preferred to divide their beds into short sections by barriers, or to use the box type of culture to limit losses. Should initial infections become numerous other methods of control must be adopted.

CONTROL

Early attempts to control the mummy disease were directed toward securing renewed production of normal mushrooms in affected beds. Large beds at Auxvasse, out of production for 2 weeks or more, were divided into plots of 10 square feet. The abnormal sporophores, buttons, pinheads and clumps of rhizomorphs were removed carefully. Various chemicals, in several dosages, were worked thoroughly and uniformly into the casing soil. The beds were then watered. The materials used were: formaldehyde dust, Dubay 738, 5% Ceresan, sulphur, lime, and dry lime-sulphur. Several replications were prepared for each type of treatment. In no instance was the use of chemicals in the casing soil of the slightest value. No normal mushrooms appeared in any of the treated plots. In some plots the development of a new break was inhibited for a month or more by the chemical, while in others a new break developed after the usual period.

In some beds the casing soil was removed together with a small amount of the upper portion of the compost, and the beds were recased with unused soil. Recasing had no effect on the development of the disease. At the first break after recasing all sporophores were diseased.

It has been mentioned that the mummy disease invariably progresses throughout beds in which infection occurs, but does not spread readily from infested to normal beds. Attempts were made to delimit the disease to a small area and prevent the destruction of entire beds. A solution of mercuric chloride in water (1:250) was used to wet a strip of casing soil and compost about 6 inches wide across infested beds. The solution was poured into shallow trenches until the soil and underlying compost were thoroughly soaked. The disinfected barrier strips were placed at various distances, 2, 4, 6 and 8 feet, in advance of the sporophores identifiable as diseased. The progress of the disease was stopped, at least

temporarily, by the chemical barriers at distances of 6 and 8 feet, while those at 2 and 4 feet exerted no influence on its spread. However, it proved necessary to renew the mercuric chloride barriers at weekly intervals to arrest the progress of the disease permanently. The brief period of effectiveness of the chemical may be attributed to dilution in the soil and compost, and to the combination of the mercuric salt with the colloidal material of the soil and compost.

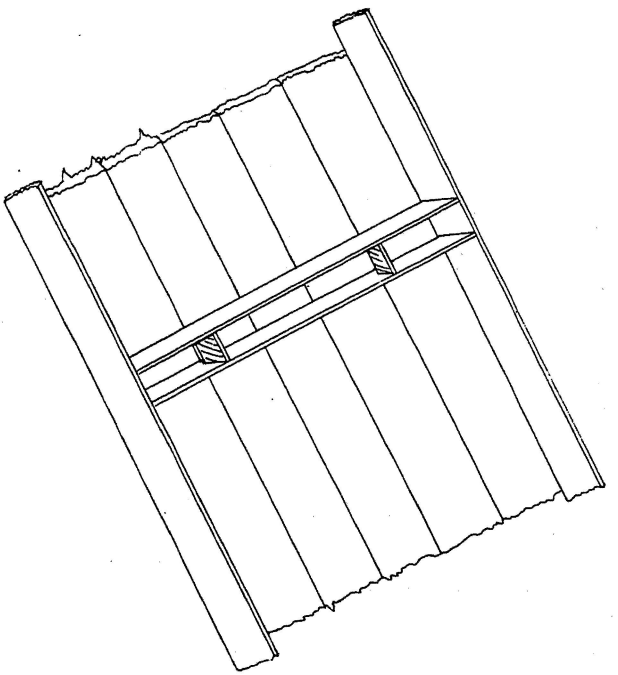

Fig. 8 — Sketch of portion of a bed with a trench designed to prevent passage of the causal agent of the mummy disease from one section of a bed to adjoining sections. Note that the small blocks holding the cross-members apart are raised several inches above the floor of the bed. Scale: approximately 1/2 inch = 1 foot.

Narrow trenches, 4 to 6 inches wide, across infested beds proved completely effective, when placed 8 feet in advance of diseased sporophores, in preventing further spread of the mummy disease. In numerous experiments the trenches placed 6 feet in advance of abnormal sporophores usually proved effective, but in a few instances the disease developed beyond the trench. Those made 8 feet in advance of the area of abnormal mushrooms almost invariably prevented further progress throughout the period of production. The control achieved by the method is most striking.

On the "diseased" side of the trench appear only the misshapen, dwarfed, and useless sporophores, while on the "healthy" side the bed continues in normal production over a period of weeks or months.

The effectiveness of the trench method of limiting the spread of the disease prompted the use, at Hermann, of cross-boards to divide the long beds into 8 ft. sections. The cross-boards were of the same width as the side boards. Two boards separated by wood blocks, were placed across the bed (Fig. 8). The blocks to hold the cross-boards apart were placed about 3 inches above the floor boards to facilitate removal of compost or soil that might fall into the barrier during loading and casing. It is essential that the barrier be kept clean. Three years experience has provided adequate proof of the value of dividing the beds into short sections. The mummy disease has continued to appear in an occasional section, but the barriers have almost invariably limited it to the one section. The very rare instances in which the disease has spread to an adjacent section were found to be the result of accumulation of soil or compost in the barrier. In each instance rhizomorphs and mycelium were found in the material in the barriers. The observations confirm other experimental evidence indicating that the disease is transmitted only through living rhizomorphs or hyphae. At Hermann the use of discarded crankcase oil on the floor boards in the barriers has proved effective in preventing the growth of hypae across the barrier.

The method of production used at Auxvasse, involving the use of small beds, has limited losses from the mummy disease to negligible proportions. In the units of 250 small beds the numbers of beds developing the disease have varied from none to 6. When a bed develops symptoms it may be removed and destroyed to eliminate the possibility of transmission of the disease to other beds.

It is apparent that the effectiveness of the barrier or small bed method in combatting the mummy disease is dependent on the infrequency of initial infections. Under conditions resulting in a high degree of infestation of the casing soil used to case the beds it is possible that a high percentage of the sections or small beds might develop the disease, necessitating the use of other methods of control. However, in the houses and caves under observation the numbers of sections and small beds becoming infested have been so small that their loss has not been important.

Tests of resistance of various strains of white mushrooms to the mummy disease showed no differences in susceptibility. In 1935 a large bed at Auxvasse was spawned by placing pieces of spawn of a cream variety at wide intervals among the white spawn. The mummy disease later developed in the bed and spread through its entire length among the white mushrooms. The scattered "islands" of cream mushrooms continued to produce break after break of

normal sporophores. In the following season several beds at Hermann were spawned with spawn of the same cream variety. The mummy disease appeared in a bed early and spread rapidly. Indeed, after the disease appeared the sporophores of the cream variety underwent more rapid and complete destruction than those of the white varieties. In one bed of the cream variety in which some pieces of spawn of a white variety were placed, normal white sporophores continued to develop during several weeks after all the cream mushrooms were affected.

SUMMARY

The mummy disease of the cultivated mushroom has caused serious losses in Missouri since 1935 when it was recognized as an undescribed disease.

The disease occurs in caves and houses. It develops under a fairly wide range of temperature and humidity conditions.

Infection causes the development of abnormal sporophores, with elongated, slender stipes and small, tilted pilei. Uneven growth of the stipe tissues often causes considerable curvature. In more advanced stages most of the sporophores are arrested in development in the button stage, becoming gray or brown, dry, spongy, and mummified. Bacteria-filled cavities often appear in the sporophores; the cream strains are subject to a soft, wet type of rot resulting from secondary bacterial invasion.

The disease spreads through beds at the rate of about 1 foot per day. Affected beds are permanently out of production. The beds are completely invaded and a normal sporophore in an affected bed is very rare.

Transmission has been secured only by transferring casing soil or compost from affected to normal beds. Cultures of several species of fungi and bacteria secured from diseased sporophores failed to initiate the disease.

Affected beds cannot be distinguished from normal beds by the appearance of the compost or casing soil. New breaks develop in affected beds, but the sporophores cease growth in the pinhead or button stage.

The disease does not spread readily from bed to bed, even in the same tier.

The causal agent is present in the casing soil and compost to a distance of 4 to 6 feet in advance of the youngest visibly diseased sporophores.

The usual incubation period of the disease is about 3 weeks.

There is no evidence that the disease is transmitted by insects.

The infectivity of soil and compost from affected beds is destroyed quickly by drying, by heating to moderate temperatures, and by

treatment with chemicals. Any treatment that kills the Agaricus mycelium probably renders the soil or compost non-infectious.

The rate of spread and difficulty of transmission suggest the possibility that the disease is caused by a virus, and that transmission is secured only by anastomoses between infected and normal hyphae.

The probable source of infection is believed to be the casing soil.

Attempts to reclaim affected beds by recasing and by treatment of the casing soil with chemicals were not successful.

The progress of the disease through the beds was stopped by the use of mercuric chloride barriers, and, more effectively, by narrow trenches across the beds 6 to 8 feet in advance of visibly affected sporophores.

Division of beds into short sections by double cross-boards with a narrow air space between them has reduced losses from the mummy disease to unimportant proportions in commercial houses in Missouri.

LITERATURE CITED

1. Buller, A. H. R. 1931. Researches on Fungi. Vol. 4. Longmans, Green and Co., 329 pp. Illus.
2. Hein, Illo. 1930. Studies on the mycelium of Psalliota campestris. Am. Jr. Bot. 17: 197-211. Illus.
3. Tucker, C. M. 1937. A destructive disease of mushrooms. Proc. Mo. Acad. Sci. 3: 71-72 (abstract).
4. Tucker, C. M. 1940. A disease of cultivated mushrooms. Mo. Agr. Exp. Sta. Bull. 413: 32-34.
5. Wood, F. C. 1937. Studies on "Damping off" of Cultivated Mushrooms and its Association with Fusarium Species. Phytopath. 27: 85-94. Illus.