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Some Fungi Attacking Corn

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Master Thesis

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Botany

Some fungi attacking corn.



SOME FUNGI ATTACKING CORN.

The fungi upon Zea mays, or Indian corn, are worthy of special note, not only because they annually cause many million dollars damage to the corn crop of the United States, but also because extensive losses of live stock are attributed to feeding horses and cattle upon the mouldy corn, and because a serious and widespread disease seems to follow the use of such corn as human food. The species of fungi responsible for these disorders, however, have never been definitely determined. The feeding experiments carried out by several State Experiment Stations have given negative results, and the stockmen and veterinarians reporting injury to stock from eating mouldy feed have almost without exception been unable to identify the moulds upon the alleged injurious feeds, simply reporting the losses as "having followed the use of mouldy feed."

The most important fungi encountered produce lesions upon the ears of corn sufficiently characteristic to render their recognition in many cases possible by both farmers and veterinarians.

It shall be the purpose of this paper, after briefly reviewing the literature concerning the toxic and pathogenic properties of the moulds, to describe the

morphological and cultural characteristics of the principal moulds found upon the ears of corn in central and western Kansas; especially those characteristics which permit the recognition of the different moulds without the aid of a microscope.

PELLAGRA.

The thorough study of the fungi attacking corn at any stage of its culture or storage is rendered many times more imperative by the existence of a very serious disease known as pellegra, attacking large numbers of the rural population of Italy, and to a less extent those of France and Greece. Pellagra is believed, by the majority of investigators, to be due to the consumption of large quantities of "spoiled corn."

Italy has at the present time about one hundred thousand cases of this disease (1). The United States was formerly supposed to be free from pellagra but it is now certain that unrecognized cases have existed for many years. The most conservative estimate of the number of cases in this country seems to be that of Dr. Lavinder, detailed by Surgeon General Wyman to the investigation of the disease. He places the number at five thousand. Others estimate as high as fifty thousand, and the number seems to be increasing.

At a recent outbreak in the Mount Vernon hospital

there were eighty-eight cases of acute pellagra resulting in fifty-seven deaths.

At the first Pellagra conference (2), held in Columbia, S. C., in October, 1908, Dr. Powers pointed out that pellagra in man and blind staggers in the horse were in all probability one and the same disease.

TOXIC POISONING BY MOULDY FEEDS.

American Work.

Although poisoning apparently resulting from the consumption of mouldy feed is of frequent occurrence, several experiment stations have been unable to produce such poisoning in experimental animals.

Delaware Experiment Station (3) procured feed from stables in which horses had died, apparently from mould poisoning, and fed it to colts, but the animals remained well. They also placed a colt in a stable in which seven horses had died of mould poisoning, fed it on the same feed, and kept it under the same conditions as the others had been, but the animal remained well. The Louisiana station (4) after investigating a severe outbreak of staggers in that state was unable to trace any definite relation between the kind or character of the feeds fed and the occurrence of the cases.

Purdue Experiment Station (5) fed horses and cattle upon naturally-occurring mouldy feed and also upon pure cultures of moulds and bacteria, but was unable to produce the disorder prevalent among the livestock of that state.

The Arkansas Station (6) fed a horse for some time upon corn badly infected with *Penicillium glaucum*. The results were negative.

The Texas Station (7) drenched an animal with large quantities of an emulsion of mould spores. The moulds employed being several species of *Aspergillus* occurring on the corn in the Pecos Valley, where many horses were dying from staggers. Results were negative.

In 1891 the Kansas Station published a bulletin claiming staggers to be a true mycotic infection brought about by eating corn badly infected by *Aspergillus glaucus*. This conclusion was drawn from the death of one colt fed on corn badly infected by this mould. Before feeding, the corn was moistened and placed in a warm place until spores began to form. The feeding of the ordinary mouldy corn ^{had} ~~having~~ produced no results. Other investigators have failed to confirm these results.

Dr. Klimmer's Observations.

The work of the foreign investigators, while carried out with other fungi than those occurring upon the

Kansas corn, show the special difficulties of this line of investigation and the necessity of carrying out observations upon large numbers of animals. Dr. Klimmer (9) a well known writer upon veterinary hygiene, has thoroughly reviewed the subject. He says in part that poisoning from moulds has often been observed in horses, cattle, sheep and hogs. Young pregnant animals appear to be the most susceptible. Cattle appear more resistant than sheep.

Mould poisoning produces symptoms of gastro enteritis, loss of appetite, colic, tympanit^sys, constipation and diarrhea, which is sometimes of a bloody nature. Often in horses an icteric appearance is produced or a peculiar action on the nervous system resulting in vertigo, unconsciousness, a staggering gait, lack of feeling, apathy, sometimes excitement, weakness, reeling and staggering, paralysis of the hind legs and bladder, paralysis of the tongue and pharynx, difficult swallowing, salivation, paralysis of the ears, also of the general body. There is excessive perspiration, with a very weak and frequent puls^e. Death frequently results in from twelve to twenty-four hours. If recovery takes place, blindness and weakness in affected regions usually remains. Post-mortem examination shows gastro-enteriti^s with dotted

lemon-colored spots, hydro-cephalus internal or external, oedemic saturations and hyper~~emia~~^{emia} of the brain and spinal cord, sometimes peritonitis, nephritis, cystitisⁱ, and acute liver atrophy. Sometimes all of the pathologic conditions mentioned are wanting.

The conditions under which the formation of poison goes on are by no means understood. It is certain that the introduction of even purely saprophytic moulds which are unable to germinate at body temperature, and can therefore, under no circumstances act other than in a purely chemical manner, can give rise to grave disorders. The problem needs further study. One thing however is known for certain, and that is, in most cases the poison is not formed. Very frequently mouldy feed has been fed without any bad results, but in other cases grave disturbances have been created.

Dr. ~~Skinner~~^{Klimmer} cites the following feeding researches carried on by Albrecht, Franck, Busch and Tubey^uf.

Ustilago Maydis.

Albrecht (10) fed Ustilago maydis to two pregnant goats, one pregnant sheep, and one pregnant dog and two hens. No injury resulted to the animals though the experience of many feeders had led them to the conclu^ssion that this fungus produced gastro-enteritis and was also one cause of abortion.

Tilletia tritici (stinking smut of wheat).

^sPusch fed great quantities of wheat badly infested with *Tilletia tritici* to two old horses, two head of cattle, six sheep, two goats, two dogs, four rabbits, six pregnant guinea pigs, three mice, four hens, and two sparrows. One of the sheep ate the feed very reluctantly and developed a foul-smelling diarr^{ea}~~rhoea~~, lost flesh and refused almost entirely to eat the smutted feed. Another one of the sheep developed a discharge from the nose and experienced considerable difficulty in breathing. One goat after some days refused to eat, drank enormous quantities of water, passed soft, foul-smelling faeces, and lost flesh very rapidly. The mice died of a bloody gastro-enteritis. One hen showed considerable nervous excitement followed by somnolence. Another died of bloody gastro-enteritis. The two sparrows likewise died of bloody gastro-enteritis. Out of the pregnant animals, (one sheep, one goat, two rabbits and six guinea pigs), five of the latter aborted. The sixth guinea pig which bore fully developed young had received feed which has^d been previously boiled for one hour^s. No symptoms of disease appeared in the other animals eating the smutted wheat nor was their general health apparently injured. Pusch also found the spores of wheat able to pass thru the alimentary canal of horses, cattle, sheep, goats and hogs without losing their power of germination; and that germination does not

take place at body temperature but only at 18 to 20 degrees centigrade. Germination within the living animal body he believes, therefore, to be precluded.

Frank (12) obtained negative results from an experiment with a yearling calf.

Albrecht fed wheat badly attacked by the same fungus to one pregnant sheep and five goats, three of which were pregnant. Occasionally the animals refused to eat their ration, but in other respects remained well and showed no symptoms of abortion. Feeding experiments carried out by Tubeuf with the same fungus failed to injure the animals experimented upon.

Dr. Klimmer in commenting upon the apparent discrepancies between the results of the foregoing experiments and the experience of many feeders that extensive losses of stock often follow the consumption of feed attacked by this fungus says; "Though the results of the feeding experiments seem to have yielded for the most part, negative results upon the larger domestic animals, they do not admit of the ^cconclusion drawn by Tubeuf that the fungus does not exert a harmful action when existing, in large quantities upon feed, for on the farms such harmful effects have been frequently observed. The apparent discrepancy between theory and practice will require further investigation..

It may be due to the different degrees of resistance of the animals concerned as it has been very frequently observed that a single animal out of an entire herd becomes affected while the majority remain perfectly well.

The feeding experiments were carried out upon too small a number of animals while in practical experience often large herds are concerned. Nor has it been observed that all of the animals eating the mouldy feed die or even become sick. Often smutted wheat has been fed to large numbers of cattle and yet no detrimental results have been observed, except perhaps abortions, which may or may not have been caused by the smutted wheat. Negative results of feeding experiments should therefore be very cautiously interpreted.

Uredineae (Rust moulds)

The hygienic significance of the rust moulds is still imperfectly understood. While Tubeuf (13) claims that they do not possess deleterious properties, Franck (14) observes that when feeding rabbits with leaves of sand oats, *Elymus europaeus*, strongly infested with *Puccinia graminis* at the stage of forming uredo spores, the animals died on the fourth day after a brief sickness, the symptoms being disturbed equilibrium resulting in the turning of summersaults, cramps of the spinal

muscles of the back, enlargement of the pupils, depressed action of the heart and lungs, and subnormal temperature. Paralysis of the bladder was found upon post mortem examination. Franck also demonstrated that the uredo spores of the rust moulds are able to pass thru the digestive canal without losing their power of germination.

Erysipheae (powdery mildews).

The hygienic significance of the powdery mildews also has not been conclusively determined. According to former observers they produce inflammation of the digestive organs and kidneys and in addition, abortion. Wolf (15), however, fed lupins so strongly infected that they were almost perfectly greyish, to a number of lambs without producing any disorders. Precaution however should be exercised in the use of such feed.

Epichloe typhina.

Franck (16) fed a rabbit very mouldy *Poa pratensis*. The rabbit developed a gangrene on both anterior and posterior paws, on the fourth day. Death occurred on the fifth day. Postmortem showed spotted reddening of the lungs, and hydro-cephalus. No reports have been made by practicing veterinarians.

EFFECT OF MOULD ON DIGESTION.

Mould spores (17) occur abundantly in the feed but

do not germinate even if tremendous quantities are present as in cheese and sour milk. Most observers do not attach much significance to them in human practice unless associated with other microorganisms. Von Wahl (16) observed mycosis of the stomach in which the gastric follicles were infected by the mould mycelium which had produced necrosis and inflammation^m of the parts. It was probably a species of Leptothrix.

Experiments carried out by the writer show the spores of *Aspergillus*^l *flavus* to be capable of germination after passing thru the digestive tract of rabbits and horses. Considerable quantities of pure cultures of *Aspergillus niger*, *A. flavus* and *Mucor mucedo*^M have been fed by the writer to horses and rabbits without producing deleterious results, but in view of the difficulties attending this line of research too much significance must not be placed upon the negative results of these experiments. The following table shows that the rabbits maintained approximately body weight while being forced to exist upon pure cornmeal cultures of these moulds:

No. Rabbit.	Mould Fed.	Times Fed.	Wt. at beginning.	Wt. at close.
24 $\frac{1}{2}$	Mucor ra- cemosis.	35	2030	1920
26 $\frac{1}{2}$	"	35	2005	1825
27	"	19	2405	2250
25	"	Died after three days.		
33	Aspergillus Flavus Aspergillus.	25	1980	1890
72 $\frac{1}{2}$	"	25	2130	2090
31	Aspergillus niger.	12	2210	2155
29	"	24	2950	2990.

The rabbits were weighed daily and temperatures taken frequently. In only one case did temperature or weight give evidence of any severe disturbance and in this case death may have been due to other causes. Throughout most of the experiment the cultures were prepared as follows: A large culture flask or jar containing sterile cornmeal was inoculated with spores of the mould and allowed to set in a warm place until a copious growth of mould and abundant production of spores had taken place.

The cultures usually smelled very mouldy. In a number of cases where the odor was very pronounced they were tested and found to be free from bacteria.

PATHOGENIC MOULDS.

A number of species of moulds possess the power of invading various tissues of the animal body and are therefore designated as pathogenic moulds. They have received considerable attention at the hands of the European ^{ie} scientists (19) and the symptoms and lesions produced by them both in the naturally-occurring cases and in those produced experimentally have been carefully noted. The ease with which the moulds may be demonstrated in the animal body in such cases renders very improbable the theory that the so-called mould poisoning is in reality a mycotic infection. The writer has demonstrated in four cases of staggers that no pathogenic moulds were present in the brain, liver, kidneys or spleen of the horses. The spores of some species of pathogenic moulds are well-nigh omnipresent in the air and may often be found growing upon decaying organic matter. Though the disorders produced in virtue of the pathogenic properties of these moulds lie somewhat outside the scope of this paper they will be briefly reviewed in order to show the ease with which this class of disorders may be distinguished from those in which the toxic properties of the moulds are called into question.

Perhaps the most serious of these disorders is one affecting the pigeon-feeders of France (20). These men fatten young pigeons for the market by chewing grain in their own mouths then placing the pigeons bill between their lips and forcing the chewed grain down the throat of the birds. In this way it seems the spores of *Aspergillus fumigatus*, which is prevalent on the feed in that region, find their way into the men's lungs and, germinating, produce a pseud-tuberculosis. Fragments of the mould may be found in the "sputa", and pigeons inoculated with such sputa become infected with *Aspergillus fumigatus* and succumb in a few days. Postmortem examination shows a pseud-tuberculosis from which pure cultures of the mould may be obtained. The hair-combers of Paris (21) suffer from the same disorder, due to the use of meal containing mould spores in their method of cleaning hair.

Very frequently in India (22) and to a less extent in other localities serious and painful affections of the ears are caused by pathogenic moulds. *Aspergillus niger*, *A. fumigatus*, *A. ^{albus} ~~meus~~*, *A. glaucus*, and *A. flavescens* are the species most often met with. The symptoms are a serious catarrh, occasionally a discharge of pus, accompanied by itching and hearing of noises. Deafness sometimes occurs.

In animals, ear mycoses are occasionally met with, *Aspergillus niger* usually being the causative agent. The mucous membrane of the nose (23) especially when injured, is sometimes attacked, *Aspergillus flavus* having been identified in such cases. The question has been raised as to whether the moulds are the primary cause of these troubles or only attack organs already injured. In many cases the latter seems to be the case though other cases are found in which no previous trouble can be demonstrated. Furthermore, by numerous experiments upon animals, Saxer (24) has shown conclusively that *Aspergillus*^l *fumigatus* is able to act as the primary cause of ne^cgrosis and odorless cavity formation in the lungs.

GENERAL CHARACTERISTICS OF THE MOULDS.

A number of moulds are found growing on decaying plant and animal matter wherever the proper conditions of temperature and moisture exist. Other species are found only as parasites upon trees and grains. Many serious diseases of trees and plants result from these fungi.

The moulds consist of two portions, the one being small thread-like filaments called mycelia, which are in most species highly branched and are frequently

divided into segments by cross walls or septa. These mycelial threads often rise an inch or two above the substratum and it is to this that the growth of many species of moulds owes its resemblance to cotton.

The other portion of the mould represents the part adapted for the reproduction of the organism. In its simplest form reproduction takes place by a constriction forming just behind the tip of the growing mycelium or one of the branches, as in *Fusarium*, but in the case of most of the moulds mentioned in this paper there is a special structure produced for the production of spores, or conidia, as they are termed from their manner of production. This usually consists of special branches called sporophore^s or conidiospores^h which are sometimes further branched before bearing spores as in *Penicillium glaucum*, Plate I. More often they bear upon their extremity an enlargement which may contain the spores themselves as in the *Mucor* group, Plate VI. Or they may give rise to short prominences, sterigmata, which terminate in chains of conidia. As each head bears many such chains of conidia they usually form a body, some large enough to be distinctly visible to the naked eye, thus giving the growth a peculiar granular appearance which often enables one familiar with it to recognize strange members of the genus upon microscopic examination.

As the more complex methods of reproduction are of little aid in the field methods for the recognition of these fungi they will not be mentioned at this place.

MOULD SURVEY OF KANSAS.

As the preceding review of the literature has shown, in actual feeding serious losses of stock have occurred from mouldy feed, while experiments, carried out upon the animals in question, have either entirely failed to produce sickness or else the symptoms have been those of minor disturbances of the digestive tract and have usually varied from case to case.

The writer fed corn badly attacked by moulds to several horses producing in them in most cases typical cases of staggers. Experiments are at present being carried on with pure cultures of the various fungi and analyses of feed used by various feeders are being made. The results of these experiments will be published later.

Kansas offers a peculiarly good field for the study of this problem, inasmuch as the western and especially the southwestern portion of the state frequently lose horses from "enzootic cerebritis" or "blind staggers" while other portions of the state rarely experience such losses. On this account a mould survey of the state was attempted. Field work was done by the writer

in Riley, Geary, Morris, McPherson, Reno, Pratt and Kiowa counties. As it was impossible to cover more than a small section of the state we endeavored to inspect quite thoroughly two sections of the state, one in which the disease has been prevalent, and the other in which it is of rare occurrence. The first three counties named represent the former, and the remainder the latter condition.

Of the moulds whose occurrence is frequent enough to warrant discussion *Fusarium* I, *Fusarium* III and *Penicillium glaucum* seem to be distributed quite uniformly over the territory inspected. *Diplodia zea* seems to be worse in the eastern counties, while the remaining moulds, *Aspergillus flavus*, *Aspergillus niger*, and *Rhizopus nigricans* are abundant^t in the western counties and of rare occurrence in the eastern counties. In the West often a large percentage of the ears in the fields are infected with one or more of these moulds, while in the East often ~~times~~ but a few ears in the entire field have been attacked. A few fields in Riley county growing upon very sandy soil have shown conditions similar to those found in the West. It is also significant that *Aspergillus* sp. I has been observed so far only in western counties. McPherson county has shown conditions similar to the eastern counties and for the

last few years there have been few cases of staggers in that locality. Reno county showed many fields having only the common moulds, but occasionally a very mouldy field was encountered. Thruout Pratt and Kiowa counties the mouldy fields were very numerous. A great variation, however, was noticed in different localities. Wherever the yield of corn was reduced, due to unfavorable soil or moisture conditions, there the pathogenic moulds were very abundant.

In the following pages will be given such notes on the occurrence and appearance of the different moulds on the corn ears as will enable those most concerned to form some idea of the species of moulds affecting any field. Short microscopic descriptions and camera lucida drawings will also be given.

Penicillium Glaucum.

Penicillium glaucum is one of the common moulds upon corn ears, being found in every field and in every locality. Its attacks are usually limited to the area damaged by the corn ear worm, often involving but a few grains, and in many cases its growth is limited to the region upon the tip of the ear containing no well-developed grains. It forms a close velvet-like growth varying in color from drab to blue-green.

Microscopic characteristics.- *Penicillium glaucum* consists of a colorless mycelium 3 to 6 micromillimeters in diameter. It is septate from the beginning. The sporophore is from 25 to 50 micromillimeters long and 5 to 6 micromillimeters in diameter, and bears upon its end a system of branching which gives rise to conidia which are round to oval and 3 x 4.5 micromillimeters to 4 x 5 micromillimeters in diameter. The primary branches are 18 to 24 micromillimeters in length; the secondary, bottle shaped, 3 micromillimeters in diameter at largest place, with a length of 15-21 micromillimeters.

PLATE I.

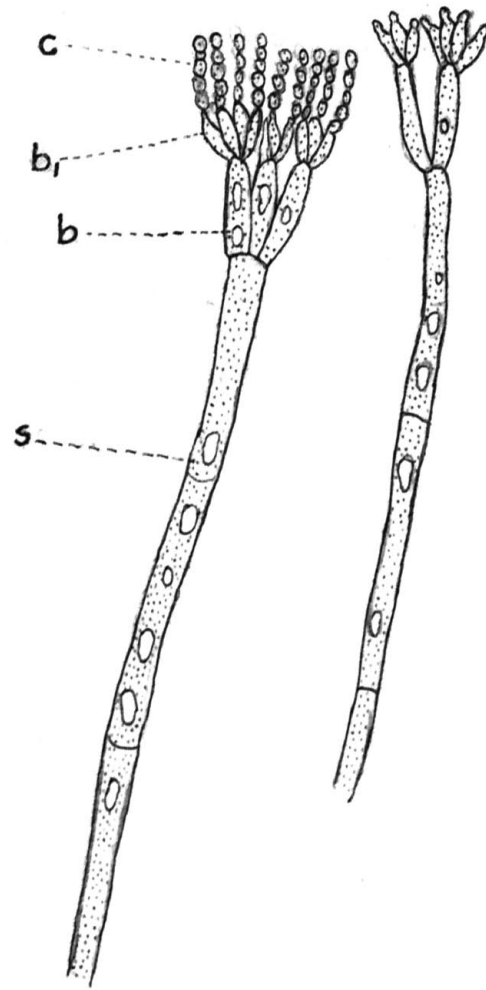
Penicillium glaucum 60 hours old.

C - chains of conidia.

B - and B' - primary and secondary branches of
sporophores.

S - sporophore.

PLATE 1



Aspergillus Flavus.

Aspergillus flavus is abundant upon the corn in the western portion of the state and rare in the eastern. The attacks of this fungus are usually confined to the grains at the tip of the ear usually following the injuries of the corn ear worm. It occasionally spreads over larger areas and involves the middle or lower portion of the ear. A few instances have been noticed in which it grew through the entire cob, similar to *Aspergillus* sp. I, and it has many times been found associated with this fungus in the interior of affected cobs.

Aspergillus flavus varies from a greenish-yellow to a golden color. It has a fine granular appearance due to the presence of the fruiting heads which are just visible to the naked eye. The spores are very light and a cloud of green dust usually appears when an ear attacked by *Aspergillus flavus* is husked. Similar clouds are produced by *Aspergillus niger*, *Aspergillus* sp. I, and to a less extent *Penicillium glaucum*. Often when young grains are attacked they shrivel up and the entire grain assumes a greenish-yellow color. This lesion seems to be characteristic of *Penicillium glaucum*, and this is the only mould which one might confuse with *Aspergillus flavus*. The *Penicillium* may be readily

recognized by its blue-green color in opposition to the yellow-green color of *Aspergillus flavus* and further by the fact that the former has a marked tendency to grow upon the tip of the cob often not attacking any grains, but confining its growth to the area on which the grains have not yet developed.

Microscopical characteristics.- *Aspergillus flavus* consists of a colorless mycelium 5 to 6 micromillimeters in diameter. It is septate from the beginning, septa are 50 to 90 micromillimeters apart. The sporophores are simple, 360 to 450 micromillimeters long, 12// to 15 micromillimeters in diameter, terminating in a swelling 15 to 27 micromillimeters in diameter. This bears simple sterigmata 7 to 9.5 micromillimeters in length and 1.5 to 2 micromillimeters in diameter. These terminate in chains of round golden-green conidia, 4 to 5 micromillimeters in diameter. Small hyphae in old cultures spring~~ing~~ directly from large hyphae 1.5 to 2 micromillimeters in diameter. Sclerotia irregularly globular, black, 350 to 620 micromillimeters in diameter.

PLATE II.

Aspergillus Flavus:

T - terminal swelling.

S - sporophore.

C - chains of conidia.

PLATE II

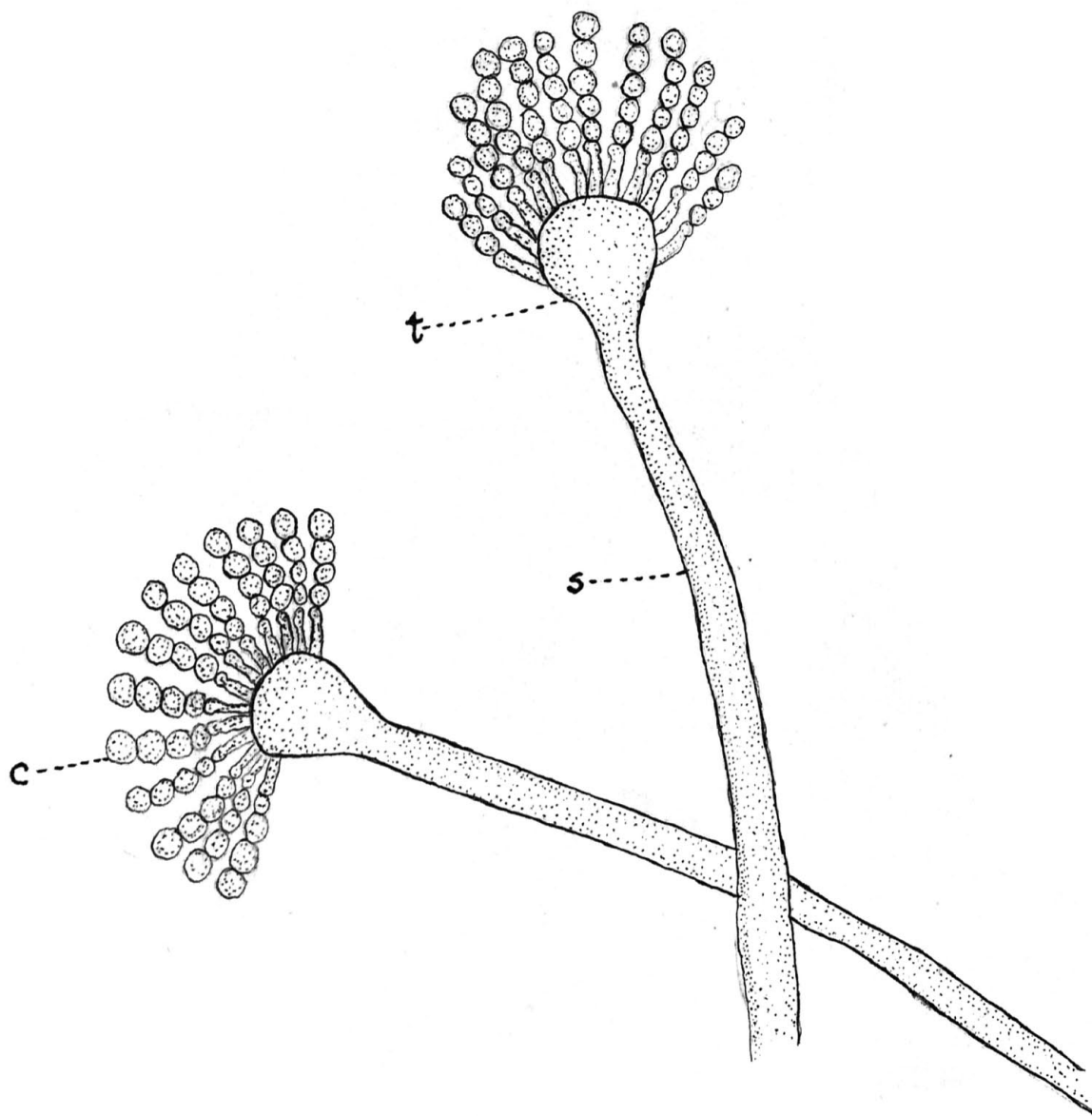
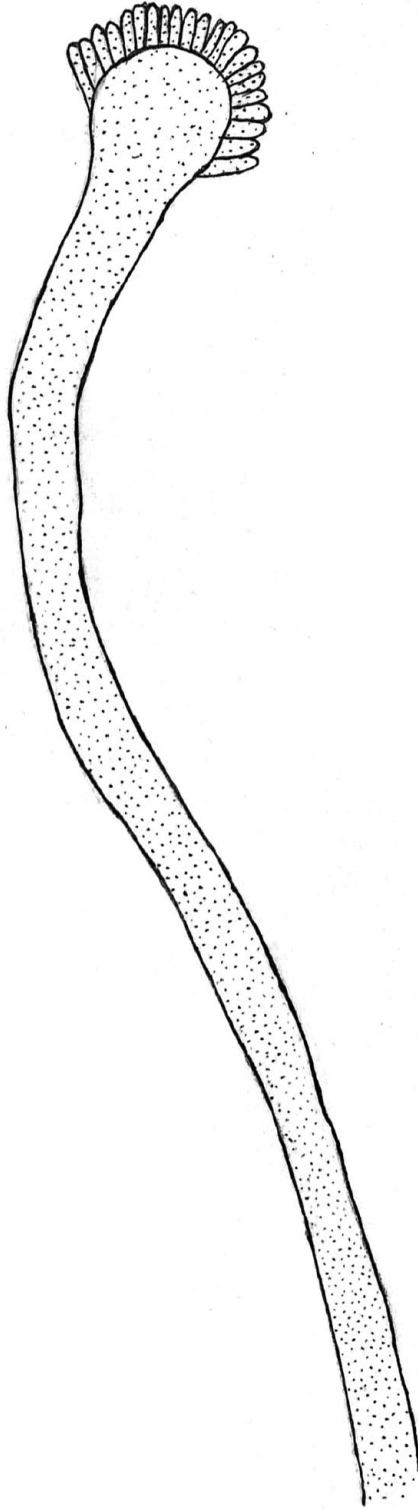


PLATE III.

Aspergillus Flavus:

Showing very young sterigmata in the process of formation. X 450.

PLATE III



PLAT IV.

Aspergillus flavus:

Young mycelium X 450. Magnified about 450 diameters.

PLATE IV

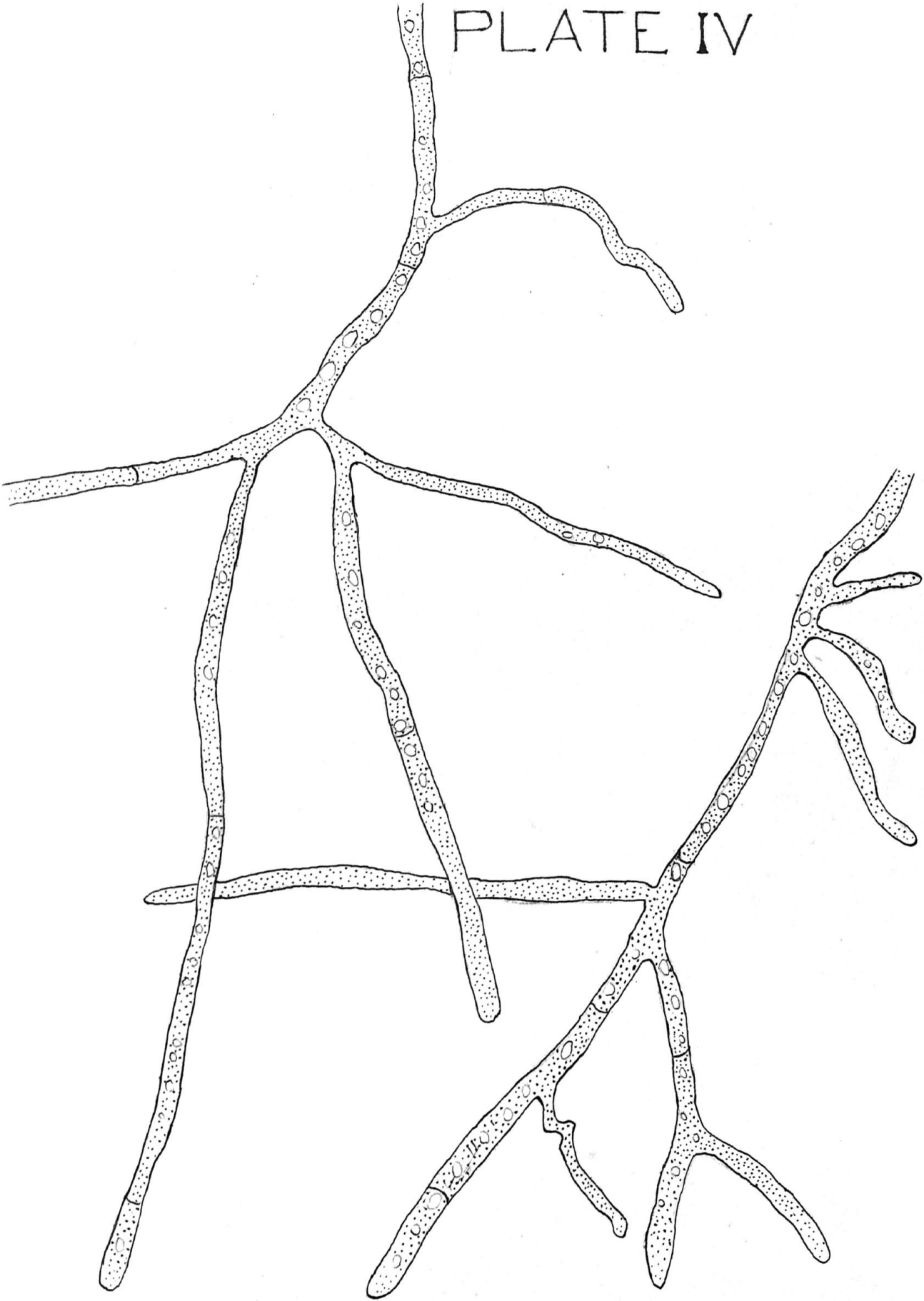


PLATE V.

Old mycelium of *Aspergillus flavus*. X 450.

Rhizopus nigricans.

Rhizopus nigricans occurs abundantly in the western portions of the state. The growth usually covers a large portion of the ear as a matted filamentous growth of a dirty greyish-white color. It often involves the husk causing it to adhere to the ear. Occasionally only the tip or small areas are attacked. The growth in some cases is less compact and the sporangia may then be readily distinguished as small black specks, about the diameter of a common pin, scattered abundantly thruout the growth. Rhizopus nigricans is distinguished from Diplodia zea, which also frequently attaches the husk to the ear, in that ^{adherence of the husk to the ear} ~~this property~~ is much more pronounced in the latter. Also the sporangia may readily be observed on the Rhizopus mycelium. Furthermore, the ears attacked by Diplodia are usually light weight. These facts are usually sufficient to allow of the accurate classification of the fungus in question. This may be confirmed by an examination of the cob, ^{on} breaking the ear, ~~upon~~ the occurrence of a row of black specks about the size of pin heads, close to the outer edge of the cob, ~~which~~ is characteristic of Diplodia zea.

Microscopical characteristics.- Rhizopus nigricans consist of a colorless non-septate mycelium, 9-21 micromillimeters in diameter. Sporophores arising ^e in tufts

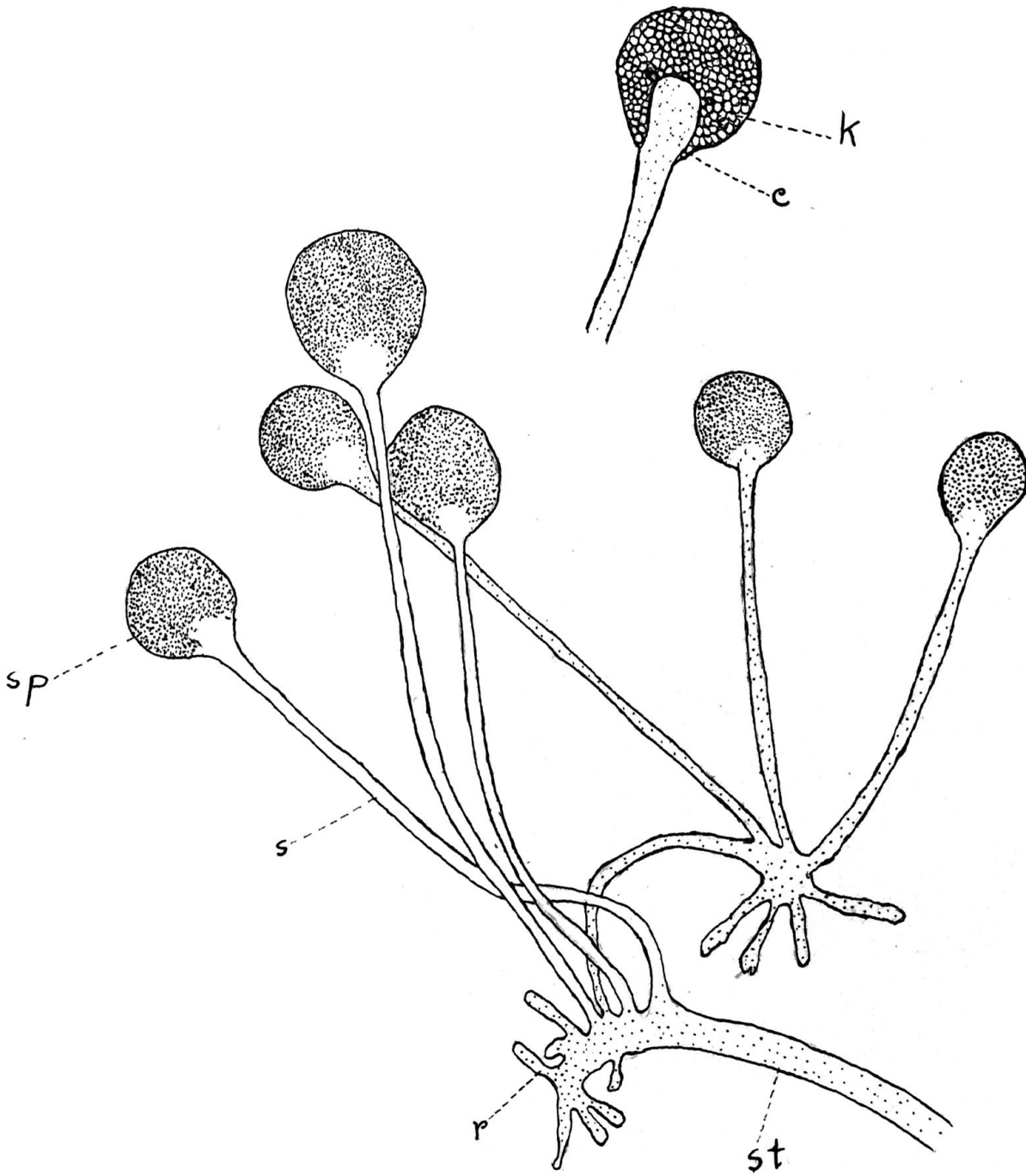
from the ends of stolons which originate in a preceding center of growth. and ^{which} after growing aerial for a short distance touch ^{the} the substratum and give rise to rhizoids penetrating the substratum. 4 Sporophores reach the length of 1 to 7 mm, and attain a diameter of 12 to 18 micromillimeters. Upon the tip of this sporophore is borne a black globular sporangium 120-210 micromillimeters in diameter. Beneath and enclosed by this sporangium is the columella 45-90 micromillimeters in diameter. Spores for the most part are globular, occasionally irregularly ellipsoidal, 4-5 x 6-9 micromillimeters.

PLATE VI.

Rhizopus nigricans:

- Sp. - sporangia.
- C - columella.
- S - sporophore.
- R - rhizoids.
- St. - ^astolon.

PLATE VI



Aspergillus sp. I.

Of the moulds playing the rôle of true parasites upon corn *Diplodia zea* and the three species of *Fusarium* have been recognized by several observers. The remaining moulds attacking corn seem to be largely saprophytic in their nature, attacking the corn only when it has been injured and in most cases confining themselves to the injured ~~territory~~^{portions} or to the grains immediately adjacent. In vigorous large ears the mould is not able to extend its growth to any extent upon the uninjured portion of the ear, while in the small ears resulting from unfavorable conditions of soil or climate a large portion, or in fact, the entire ear may be affected. This is also true of the small ears formed at the close of the season by vigorous stalks. As these ears are often left in the field they form an important element to be considered in the study of forage poisoning. As has already been mentioned the different species of moulds under the same conditions vary considerably in their ability to extend their growth beyond the area damaged by the worm. The three moulds possessing this power to the greatest extent are *Aspergillus sp. I*, *Rhizopus nigricans* and *Aspergillus flavus*. In *Aspergillus sp. I* this property is so prominent that the slightest injury is sufficient to cause moulding of

^{the}entire ear. Indeed, ears are often found attacked by this mould in which no previous injury can be demonstrated. *Aspergillus* sp. I, usually appears upon the tip of the ear as a black velvety growth extending for considerable distances between the rows of grains. In most cases the butt of the ear is also attacked. Such ears are usually light in weight and are very readily broken in two, when it becomes apparent that the mould has grown through the entire length of the cob, ^cconnecting the moulded areas upon the ends. Often *Aspergillus flavus* is associated with it both in the lesions upon the external ear and those in the interior of the cob. Its habits of growth must be largely relied upon to differentiate it from *Aspergillus niger*, although its sporophores are somewhat shorter and the fruiting heads a little smaller than those of *A. niger*.

Microscopical characteristics.- *Aspergillus* Sp. I. does not differ morphologically from *A. flavus*. Its mycelium is 3-5 micromillimeter in diameter; septa 30-50 micromillimeters apart; conidia, globular, black, 4-5 micromillimeter^s in diameter; conidiophore 440- in length, and 12-15 micromillimeter^o in diameter. Terminal swelling 24-45 micromillimeter^s in diameter. Sterigmata unbranched, 12-18 micromillimeter^s long, 2.5-3 micromillimeter^s in diameter. Whether or not this mould proves to be a new species or a variety of *Aspergillus niger*

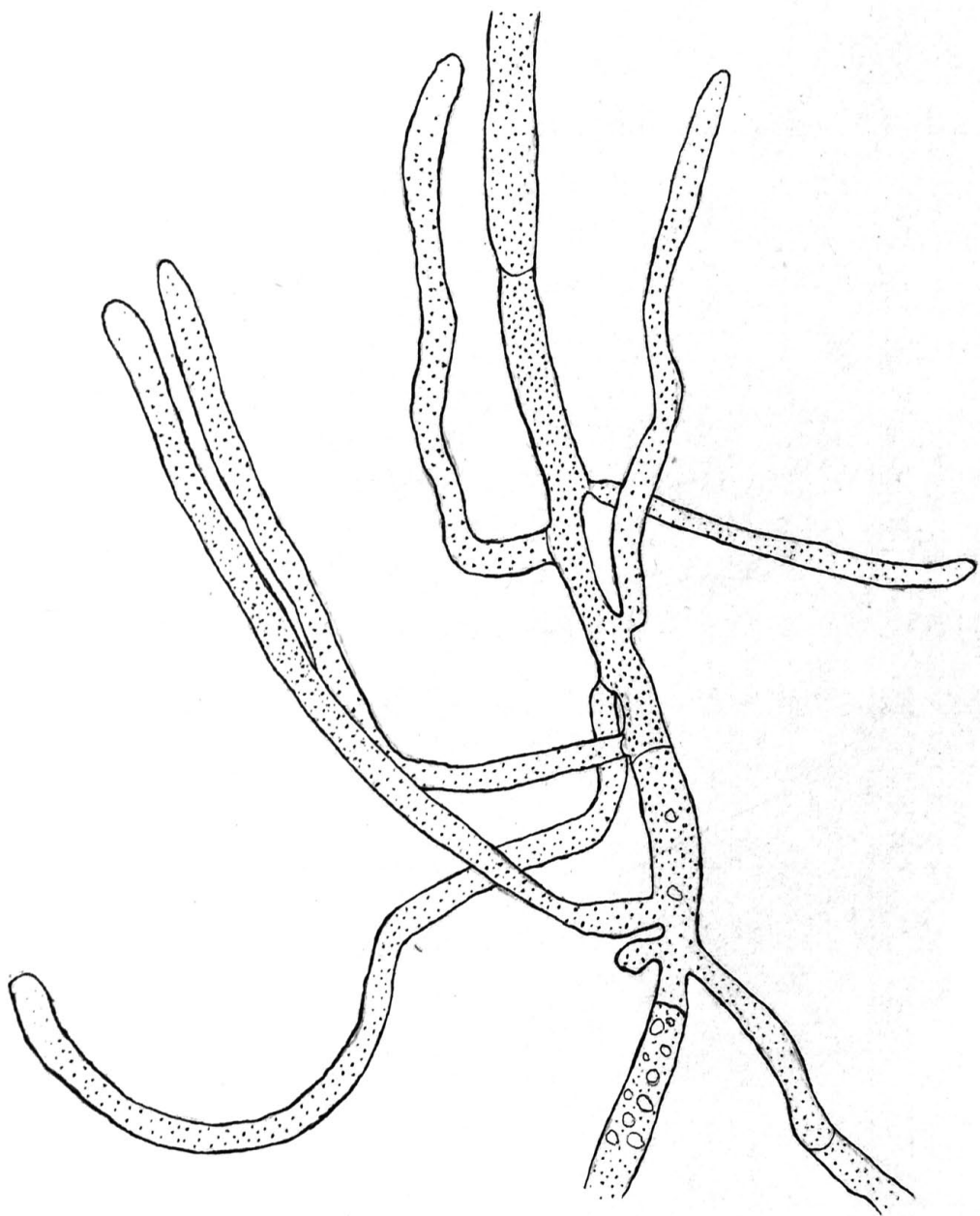
must be determined by additional study. Until this point is settled it will be referred to as *Aspergillus* sp.I.

PLATE VII.

Aspergillus sp. I:

Young mycelium magnified about 450 diameters.

PLATE VII



Aspergillus Niger.

Aspergillus niger occurs abundantly upon the corn in the western portion of the State and occasionally in other sections. It is of a deep jet-black color, affecting the ear mainly in the injuries produced by the corn-ear worm but sometimes spreading out upon sound grains and covering a large portion of the ear. It has a granular appearance due to fruiting heads which may readily be distinguished without the use of a lens upon specimens which have not been too roughly handled. Superficially it is differentiated from *Ustilago maydis*, or corn smut, by the following characteristics: smut has rather a brownish color; is composed of successive layers of a thin paper-like substance and brownish black matter. The absence of fruiting heads, the brownish color and the fact that smut causes the grains attacked, and often the whole ear, to grow to many times their natural size, renders its differentiation very easy.

Aspergillus niger may be distinguished from *Aspergillus* sp. I by the shorter length of its sporophores and the smaller size of its fruiting heads. The most characteristic difference is, however; the ability of *Aspergillus* sp. I to attack

the cob. However, in many cases a microscopic examination will be necessary in order to ascertain which species of *Aspergillus* we are dealing with.

Microscopical characteristics.- Morphologically *Aspergillus niger* differs from the members of the genus already described only in possessing branched sterigmata. Mycelium 5-6 micromillimeters in diameter; ~~spora~~ ^{septa} 21-36 micromillimeters apart; sporophore 8-12 micromillimeters in diameter, 520-1050 micromillimeters long; gross diameter of fruit heads, 60 hours old, 36-90 micromillimeters. Sterigmata branched; primary sterigmata 8-10 micromillimeters long, 2.5 to 3 micromillimeters in diameter; secondary sterigmata 4-5 micromillimeters long, 1.5 micromillimeters in diameter. Spores globular, 4-5 micromillimeters in diameter.

PLATE VIII

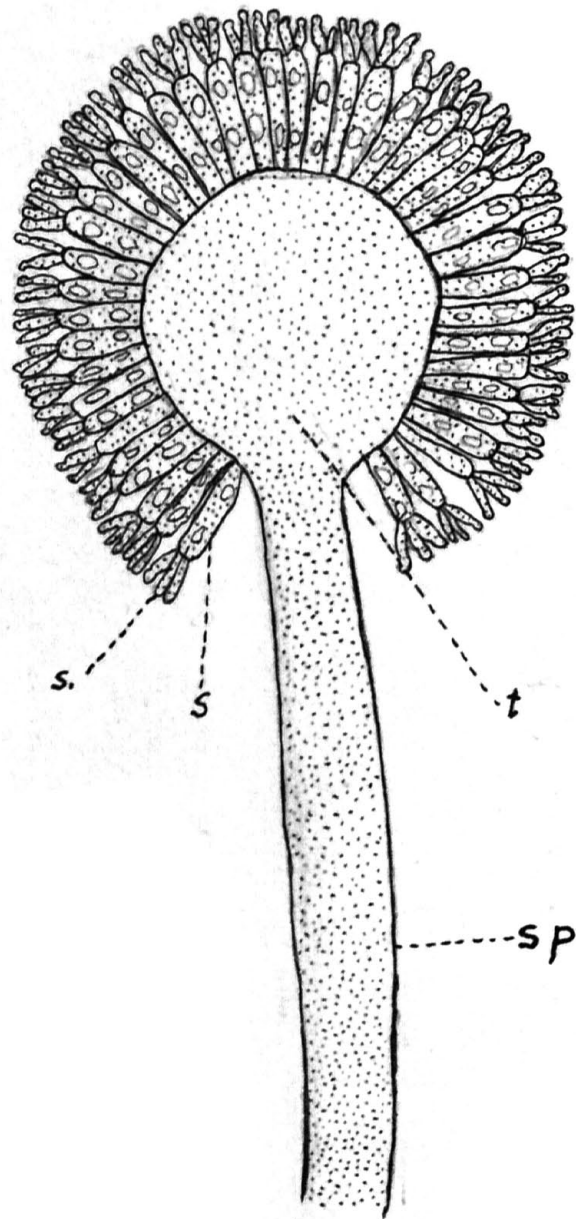


PLATE VIII.

Aspergillus niger - with conidia removed showing
branched sterigmata.

Sp.- portion of sporophore.

T - terminal swelling.

S - primary sterigmata.

S' - secondary sterigmata.

PLATE IX

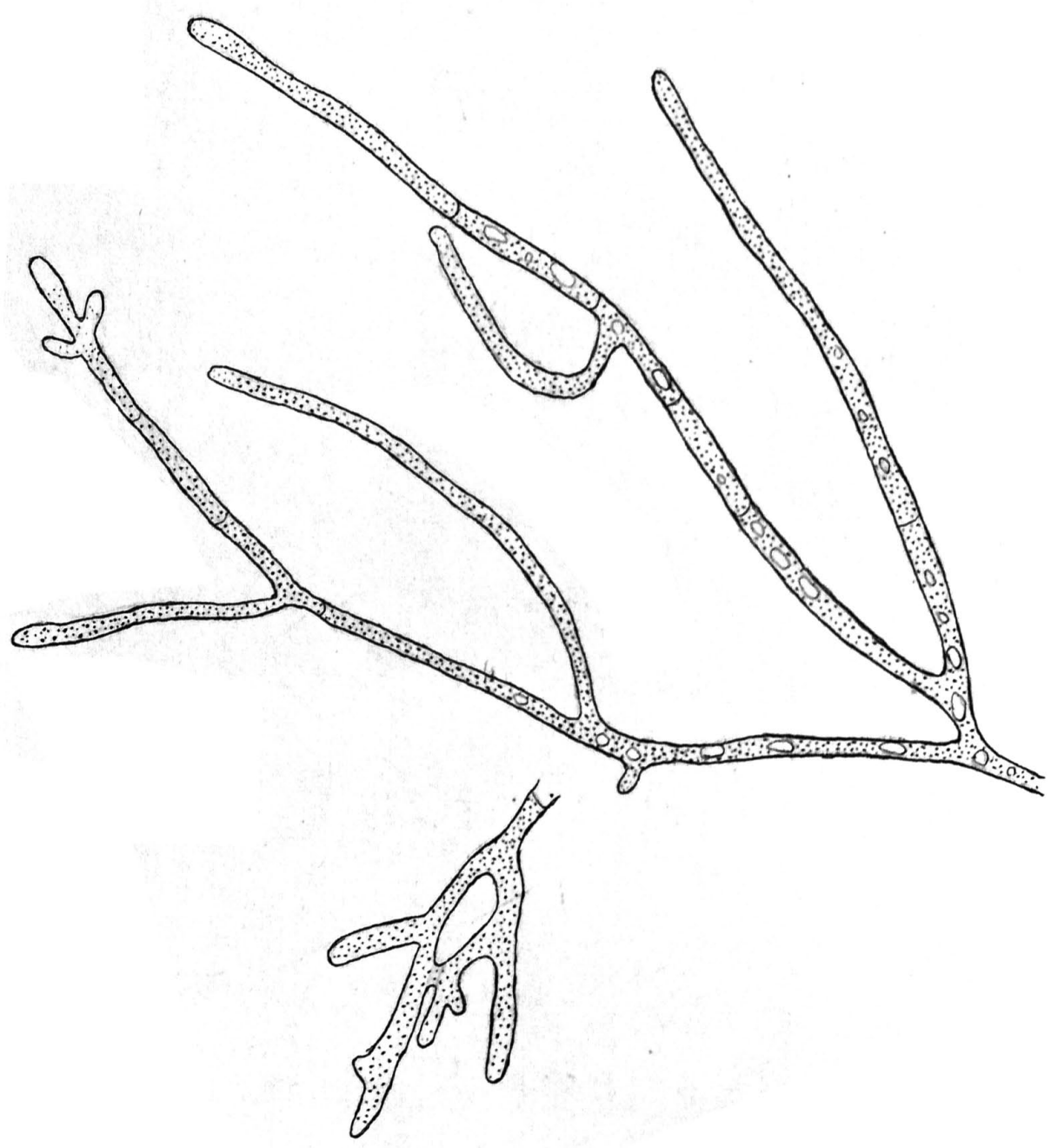


PLATE IX.

Aspergillus niger:

Mycelium 40 hours old X 450.

Other moulds than the species so far described are occasionally met with. Their occurrence has, however, been rare and for practical purposes may be neglected. They will however be described although their recognition would be difficult if not impossible for one not equipped with a microscope.

Aspergillus sp. II.

This mould has been rarely found on corn. It usually confines its attack to the tip of the cob, similar to *Penicillium* ^g*glaucum*. It is at first sky-blue, later changing to a dark, dirty-yellow color. The fruiting heads are small, the sporophores short, the growth compact, resembling *Penicillium* *glaucum*, from which it is often difficult to distinguish in old growths.

This fungus has been found more frequently on corn stalks which have moulded in the shock, and may be of more importance in this connection.

PLATE X.

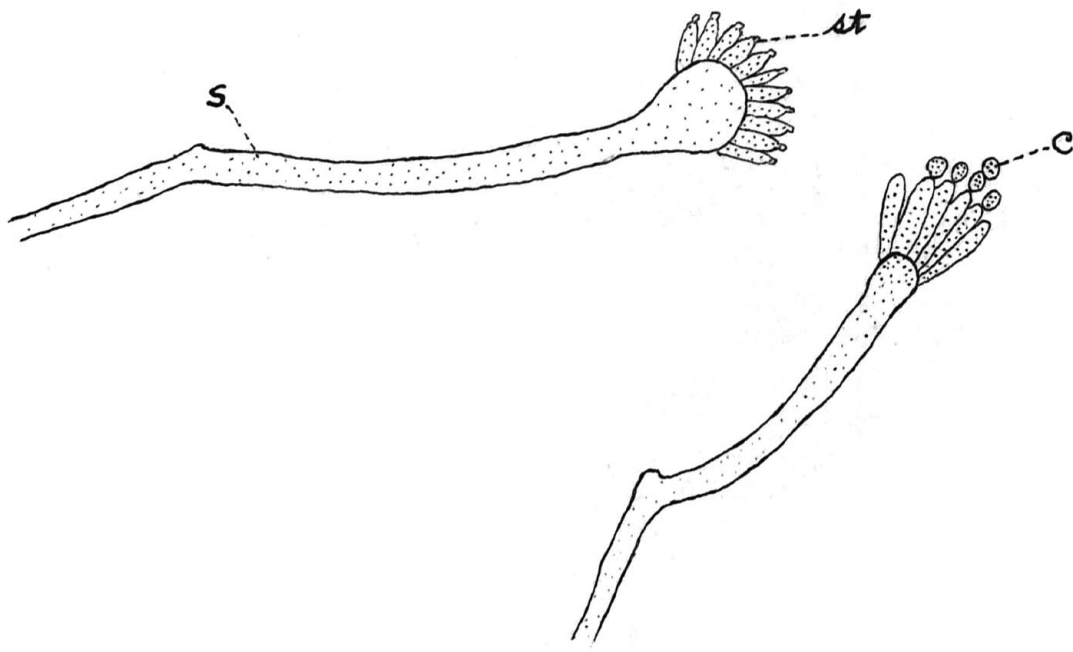
Aspergillus sp. II.

S - sporophore.

St- sterigmata.

C - conidia.

PLATE X



Mucor Erectus.

Mucor erectus is a white filamentous growth occurring chiefly on the portions of the ear injured by the ear worm. The growth is almost ^rpure white and has a very loose fluffy appearance. The fruit heads are very inconspicuous and can be seen with the naked eye only on the closest inspection. It never covers a large portion of the ear and is not frequently encountered. Mucor erectus is hard to distinguish from Fusarium. Its color is usually whiter than the Fusarium and it has a looser, somewhat more luxuriant growth.

PLATE XI

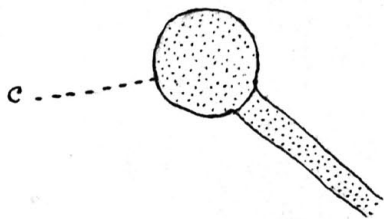
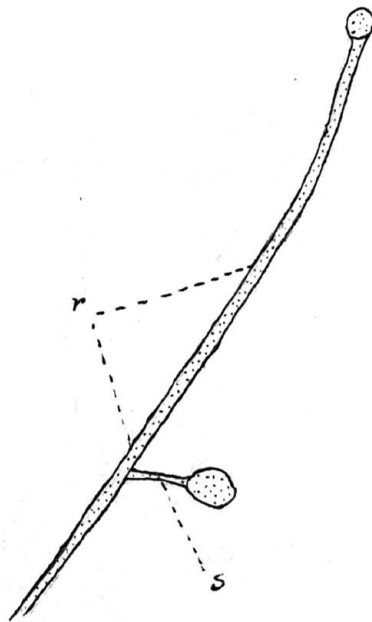
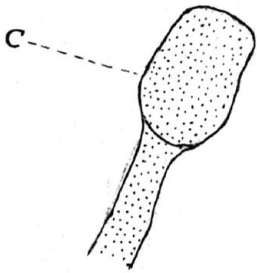


PLATE XI.

Mucor erectus:

C - common form of columella - X 450.

C' - occasional form of columella - X 450.

R - raceme - X 96.

S - sporophore - X 96.

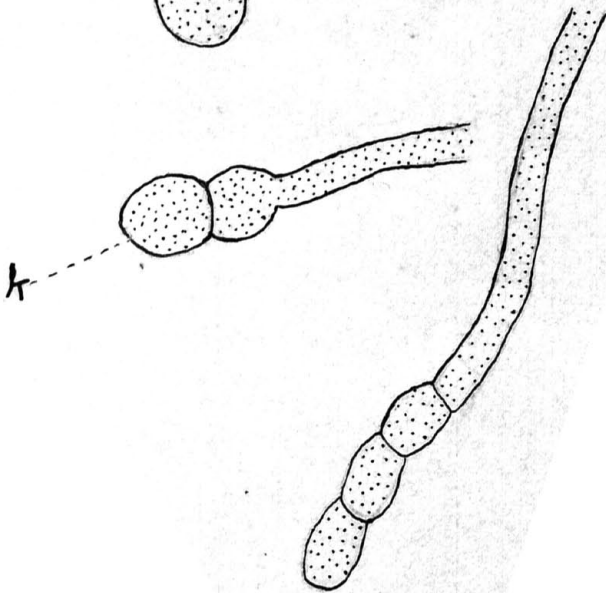
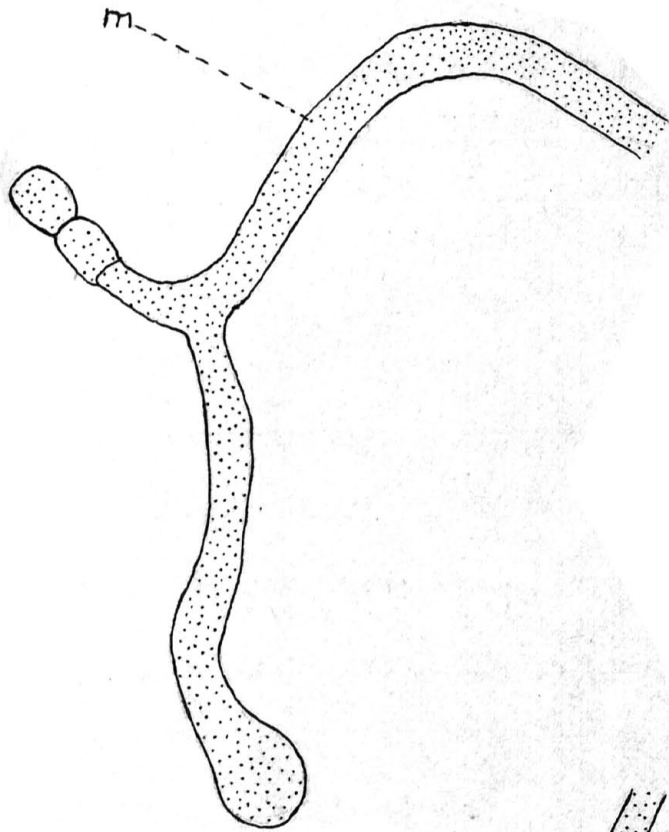
PLATE XII.

Mucor erectus:

M - mycelium.

K - conidium formation.

PLATE XII



Trichoderma lignorum.

Trichoderma lignorum has been found occurring abundantly in a few fields in Riley county. It is seen as a light-green growth somewhat similar to Penicillium glaucum but of a brighter, more definitely green color. It always affects a large part of, and often the entire, ear. The growth occurs largely between the rows of grains and between the individual grains in the same row. The species seems to be essentially a wet rot and often some of the grains on the ear have germinated.

Its parasitic habits as well as the yellow-greenish color opposed to the blue-green color of the Penicillium and the large amount of filamentous growth between the grains readily distinguish it from Penicillium glaucum.

PLATE XIII.

Trichoderma lignorum:

M - mycelium.

C - conidia.

PLATE XIII

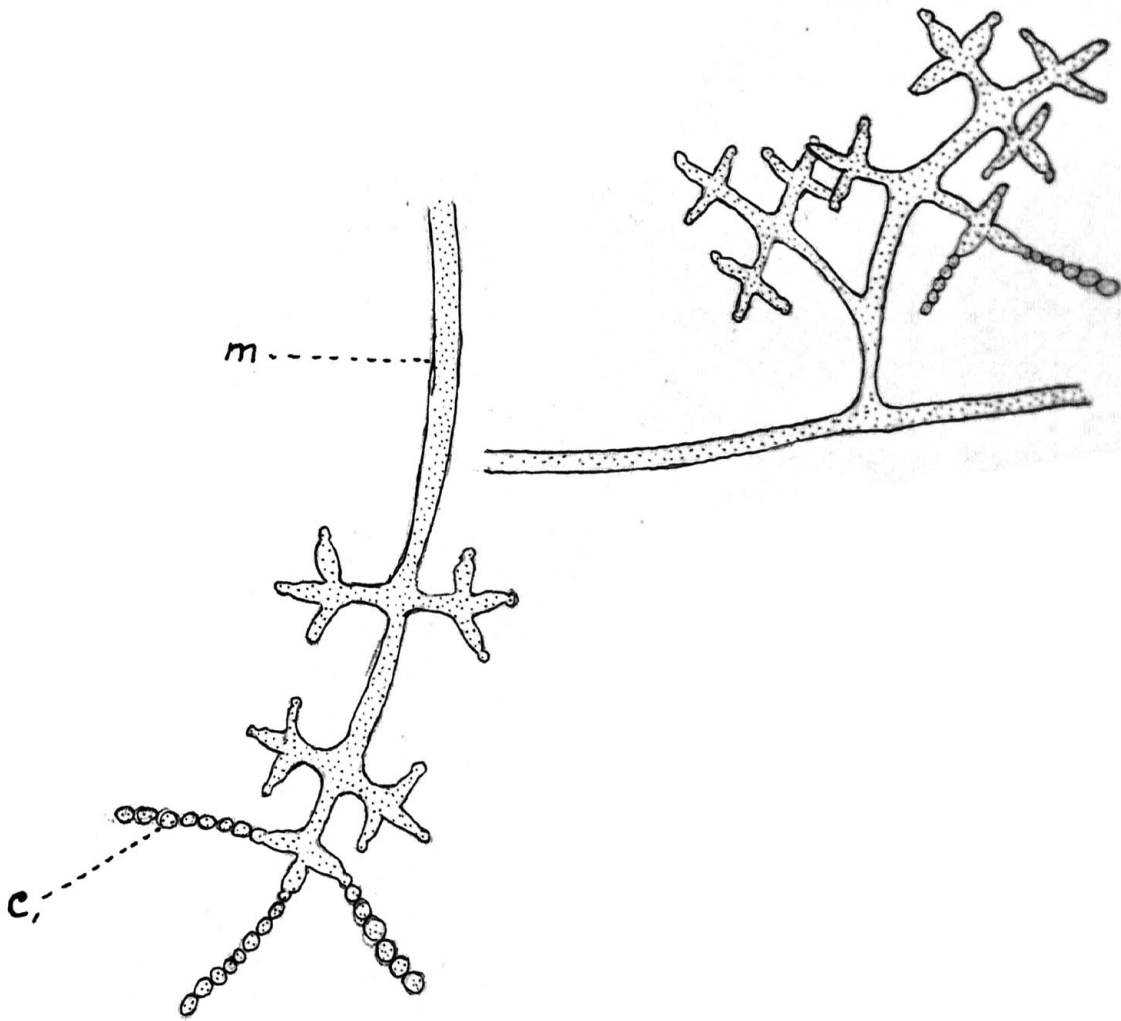
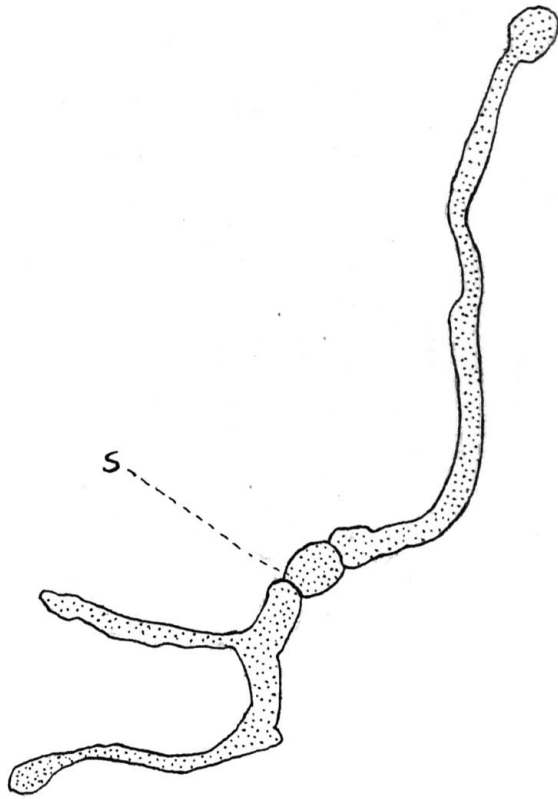


PLATE XIV.

Trichoderma lignorum:

36 hours culture - germinating spore.

PLATE XIV



Fusarium ^I/~~II~~.

Examination of many mounts of the faeces of the corn-ear worm has failed to show a single instance in which the spores of this mould were not present. In most cases they have germinated and formed a very readily visible white growth which usually confines its ^{elf}~~growth~~ to the grains in the immediate vicinity of the injuries produced by the worm, causing them to shrink up and become very brittle. The growth is usually white but often has a pinkish cast. It can not be considered a serious parasite upon the ear, as the attacks rarely involve much territory not already injured by the worm. It has been found to about the same extent in all areas investigated.

Microscopic characteristics.- Fusarium II consists of a white or slightly pinkish mycelium, which gives rise to numerous side branches each terminating in one or more fusiform conidia.

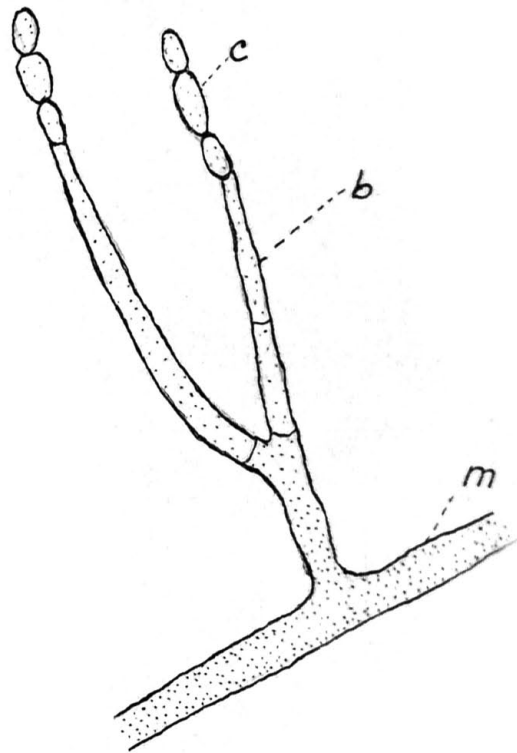
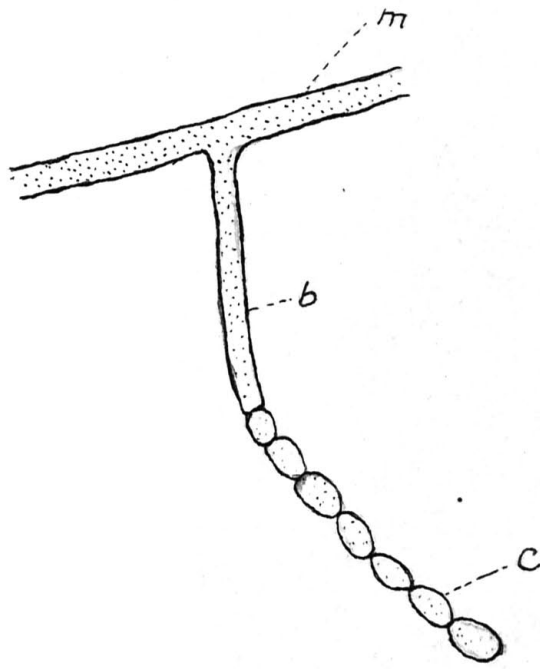
PLATE XV.

Fusarium I (Burrill).

M - mycelium.

B - later branch with chain of conidia.

PLATE XV



POPULAR KEY TO THE MOULDS UPON INDIAN CORN.

- A. - Black.
- B. - Colorless (white, pinkish or gray).
- C. - Colored, blue, blue-green, or golden-green.
- A. - Jet-black in appearance. Heads of spores visible in undisturbed growths.

(A)-Growth extending through all or part of the cob resulting in more or less disintegration of the cob.

Aspergillus sp. I.

- B. - Colorless (white, pinkish or gray). Sometimes discolored in old growths.

^a
~~(A)~~-White or pinkish powdery in appearance; usually following the worm injuries and attacking several grains in the same place.

Fusarium I (Burrill).

^b
~~(B)~~-Almost always quite pinkish in color; growth not filamentous in appearance, usually attacking single isolated grains anywhere upon the ear causing them to shrivel up.

Fusarium II (Burrill).

^c
~~(c)~~-White, often discolored, brownish-black in old growths; filamentous appearance especially between grains and between the ear and husk when the latter is adherent to the ear, as is often the case. Growth usually attacks the entire ear which is rendered light in weight and brittle.

Diplodia zea.

(D)-Gray or dirty white.

- I. - Nearly pure white, downy growth, usually tiny black specks (sporangia) thruout the growth. Injuries on the ear rarely cover much area.

Mucor erectus.

- II.- Gray, matted growth, usually affecting a large portion of the ear. Generally grows thru the husks binding them to the grains. Black specks abundant and readily visible thru the growth.

Rhizopus nigricans.

C. - Highly colored (blue, blue-green or golden-green).

(A)-Blue-green to dirty-slate color.

- I. - Growth compact, following injuries, or on tip of cob.

Penicillium glaucum.

II.- ^{loosely} ~~tangled~~ filamentous growth between and over grains, generally affecting the cob also.

Trichoderma lignorum.

(B)-Light, sky-blue, velvety in appearance. Fruit heads usually difficultly visible. Usually growth is confined to tip of cob where no grain has developed.

Aspergillus sp. II.

(C)-Golden-green or yellowish color, velvety, heads readily visible on uninjured specimens. Growth usually in injuries and region adjacent to them. Sometimes causes grains attacked to shrivel and be stained yellow; occasionally found producing lesions in cob similar to Aspergillus sp. I and frequently associated with it in these lesions.

Aspergillus flavus.

CONCLUSIONS.

First, *Aspergillus flavus*, *Aspergillus I* and *Rhizopus nigricans* are very abundant in the regions troubled with enzootic cerebritis or blind staggers.

Second. Feeding experiments carried out upon horses with corn rich in these moulds resulted in the death of most of the horses fed from enzootic cerebritis. ~~X~~The corn also contained considerable quantities of other moulds~~X~~.

Third. The negative results obtained by some investigators may be due to the use of too small a number of animals, but more probably to continuing the experiment for too short a period of time.

Fourth. Results should be confirmed by observations upon large numbers of animals under field conditions.

Fifth. The large value of the moulded feed prevents its destruction and from the fact that the poorest grade of feed produced seems to be safely fed to hogs, and in most cases to cattle, the recognition of the species of moulds injurious to horses is of great importance.

Sixth. This paper does not attempt to designate certain moulds as injurious and others as harmless, but aims only to furnish an aid to the busy veterinarian

and stockman in recognizing the species of moulds on the feed concerned, and to call attention to the above-mentioned species on account of their abundant occurrence in certain localities in which the disease is prevalent.

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