

Studies of The Saprophytic Moulds.

The Saprophytic moulds are those that are ordinarily found growing on fruits, Jellies, Cheese, and other articles of food about the household; or in damp ^e places, on damp paper or cotton goods, where it is known as mildew. These are plants belonging to the fungi, and are closely related to the mushrooms, and their spores; many of them are also more closely allied to the parasitic fungi and have their habitat on growing plants, and produce much damage to growing crops; as the Rust, or Powdery mildew, and the Anthracis, and Furaria which produce the rot to ^{and} ^{the} disease which has destroyed so many crops. These parasites live only on living animals ^{or} plants, while the Saprophytes live on dead animal or vegetable matter only. It may be, however, that some varieties will be found to have the power of living either as saprophytes or parasites as opportunity offers. It is an observed fact however that most of the plants of the class are

so closely specialized as to be incapable of growing under other than their accustomed conditions. Many of the parasites are even confined exclusively to a single family of plants as their host. Among the saprochaitic moulds we do not know that any of them are so rigorously confined to one kind of food, but there has been sufficient observation of their habits to warrant^a the conclusion that all of the varieties will not grow equally well on a single variety of food, or soil. Yet I have thought that the mingling of animal and veget^etable matter would most nearly accomodate all; and have persued this plan in my cultivations.

The Culture Medium.

Prepare a beef broth as follows;

Thoroughly bruise a quarter pound of lean beef and digest it for one hour in one quart of water at the boiling temperature. A rice cooker, or some form of water bath should be used.

Treat a quarter pound of dried peaches, or similar fruit, in the same way. Strain out the broths thus prepared, mix them in equal parts and filter through ordinary filtering paper. Such broths are generally found to be acid in reaction and should be neutralized with bicarbonate of soda.

Now add about one ^{half} grain of pepsin to the ounce of broth and digest for six to twelve hours. Place the broth again in the rice cooker and hold at the boiling temperature for one hour.

When partially cooled add ordinary gelatine sufficient to produce a mixture that will not run at ordinary temperature. This may be made stiff enough to answer ~~the~~

for all except the warmest summer weather; say up to 85 degrees Fahr. For cultures at a higher temperature the gelatine from Irish moss may be used instead of the animal gelatine.

After the gelatine has been added and found to be about right, the whole should be strained through a close cloth, or passed through the ordinary filtering paper. This is best done at the temperature of live steam in the steam sterilizer, as but little of the gelatine will pass through the filter in the open air. However if the gelatine is nicely strained through muslin it will answer fairly well for the major part of this culture work. After the gelatine is properly cleaned it should be poured into tubes for use; and it is at this point that the greatest care should be exercised in order that it may keep perfectly. The tubes should be sealed with cotton and heated in an oven until the cotton shows a tinge of brown. Then a little of the gelatine should be poured into each of the tubes and the cotton stopper immediate-

ly replaced. After the tubes are thus filled they should be packed in crates and placed in live steam for one full hour. Immediately after they are removed from this a rubber (the ordinary rubber dam) should be securely tied over the cotton to prevent evaporation. This rubber dam should be sterilized with bichloride solution before it is put on or it will be likely to convey spores of the moulds to the damp cotton which will grow through it and reach the gelatine.

The tubes of gelatine thus prepared are to be laid away for future use. If all of the steps of their sterilization have been properly performed they will keep indefinitely. They are just as good a year or ~~two~~ two after they are put up as when fresh,

When ready for cultivations, clean one dozen, more or less, glass slides (the ordinary one by three inch are good) and sterilize them by heat. Have the tube of gelatine warmed so that it will run freely, and as soon as the

6

the slides are sufficiently cooled, pour a few drops of gelatine on each, forming a mass one half to three quarter inches in diameter. These should now be placed without delay under a dust proof vessel with sufficient water in the dish at its bottom to prevent the gelatine from drying. These are to be used for planting as occasion requires.

Collection Slides

Prepare these as for planting except that the gelatine should be spread over the slide so as to give the largest surface possible. There should be enough free space at either end for convenience in handling.

Collecting Varieties.

Varieties may be collected from places where they may be found growing; as from ~~bellies~~¹, stale bread, cheese⁵, or any thing that is found "mouldy". and the plants transferred immediately to the culture slides by means of a clean needle point, or platinum collecting wire, which is

7
better because it can always be cleaned perfectly by exposing it to a red heat.

The more successful collection of varieties, however, is done by holding a collection slide close by and stirring up a little dust from a sofa, cushioned chair, carpet, or dusting from a window sill; indeed, any where that dust collects the spores of the moulds will be found, and will be caught on the gelatine with the dust. Under favorable conditions these will grow promptly and colonies corresponding to the varieties of spores caught will develop side by side on the gelatine. These varieties are to be watched until their spores begin to ripe and then transplanted to the culture slides, and thus pure cultures obtained for observation. Often it will happen that varieties that develop slowly will be found on the same slide with those that develop rapidly, and the slow growing plants will soon be over-run by the rapid growing ones, and lost. This may often be avoided by taking up some of the mycelium of the slow growing

plant on the platinum wire and transferring that to the culture slide instead of waiting for the development of the fruit. Indeed many plants are so slow in developing their fruit that they must be transplanted in that way through several generations before their fruit will be found.

Cult^ure Dishes

A common saucer with the lid of a sm^ooth butter dish, or jelly dish (glass) makes an excellent culture dish. The glass lid should fit a little inside the rim of the saucer so that it may dip into water placed in the saucer; or if it fits fairly close it will answer well without touching the water. Place some bichloride solution in the saucer, and place in it a block of wood on which to lay the slides so as to keep them out of the water, cover this with a peice of clean white paper which has been moistened with bichloride solution, on which to lay the slides. Without this precaution of poisoning the water ~~and~~ blocks and papers the whole will soon become infes-

ted with growing moulds which will contaminate the cultures. With a sufficient number of these the cultures can be carried on indefinitely. It is of the utmost importance that the table be kept free from dust, and that the culture dishes be not opened when there is any dust ⁱⁿ the room. Also that the greatest care be had in the handling of the specimens in the open air be observed on account of the extreme liability to contamination by the introduction of spores that may be floating in the atmosphere. Another source of contamination that has annoyed me exceedingly is the microscope itself, which will become contaminated with the spores of different varieties, and convey them to the slides under examination unless great diligence in cleaning it be observed.

The Saprofitic moulds are composed;

Of a delicate Mycelium which is indefinitely branched and spreads horizontally in, or on, the substratum on which it grows. Common to all of the moulds.

Of delicate filaments which spring from the mycelium and grow upward into the air. These may be branched, or simple. For all of these I shall use the term Hyphae

A few of the moulds have no hyphae

Of the fruit, or spores. Some ^{varieties} of the moulds have two or more kinds of spores or reproductive cells. Of these The recognition of three kinds will probably answer our purposes

1st. True spores. That form of the fruit which retains its vitality for an indefinite period.

2nd. Gonidia. A spore profusely budded off from the mycelium or ^{as} ~~hyphae~~ of certain varieties, and which seem destined for immediate growth, and which probably do not retain their vitality for any considerable time.

Gemmae. A spore formed by the enlargement of certain joints of the mycelial or hyphal filaments. This may be a true spore, or one of temporary functions. They seem to bear some relation to the tubers of the higher plants

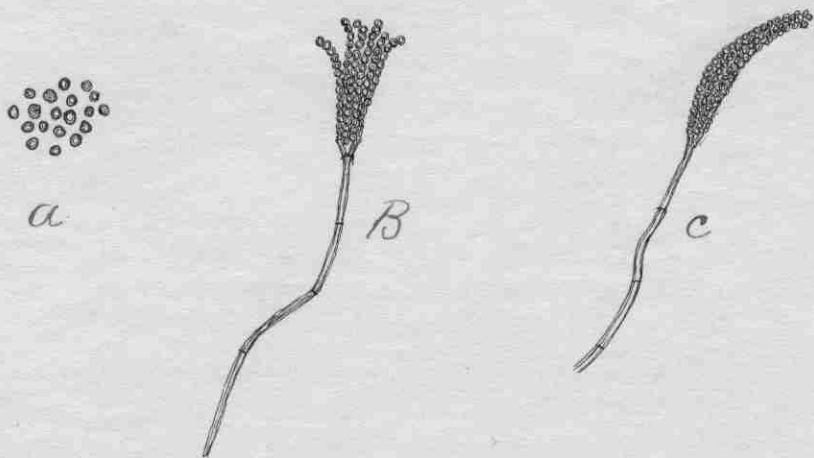
The spores may be free, or in spore cases. They may be simple, or septate; i. e. composed of several parts.

Sporophores are short stalks branched from mycelial or hyphal threads on which spores are borne. Such branches bearing gonidia will be called Gonidiophores.

These terms will probably be sufficient to describe what I have seen without the complexity of a more extended use of botanical terminology.

The principal distinguishing features of the varieties of the moulds are found in the spores, and in the manner of their formation. The greater number form their spores on the hyphae, and are termed Hypheomycetes. I know of no term having been proposed under which to group the non-hyphal moulds.

Fig 1



Penicillium Glaucum

a Spores as seen with $\frac{1}{2}$ inch lense
B spore head. The most common form
C Spore head. A frequent form

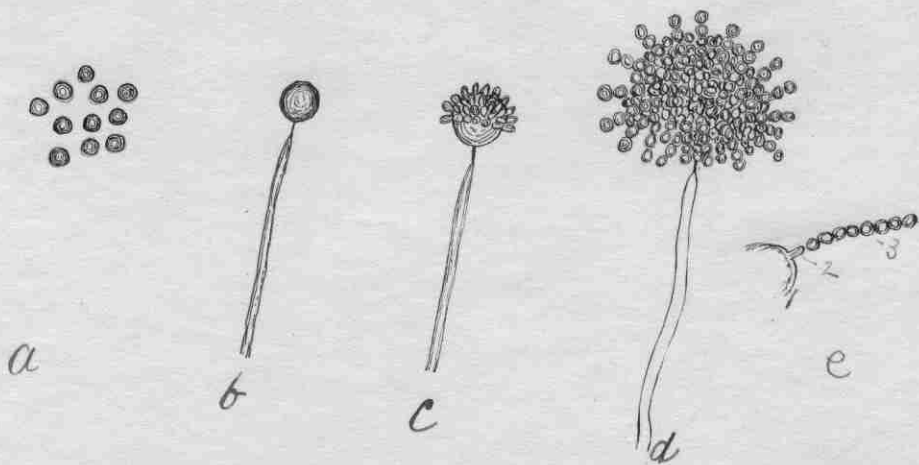
No. I. Penecyllum Glaucum.

Spores round. The mycelium infinitely branched, and divided by joints as it grows older. The hyphae rise contemporaneously with the growth of the mycelium. They branch moderately, and at the apex divide dichotomously forming from two to eight sporophores upon the apices of which chains of round spores are abjoined, six, ten, twenty, or even more in a chain. These form a kind of brush, the hyphal stem representing the handle. In some the chains of spores are more or less spread, while in others they remain in a compact bundle (Fig. ¹/_A c) looking as if they were agglutinated together at their distal ends. The spores are colorless, or white, at first, but become green as they ripen. Spores are formed promptly, and while the mycelium and hyphae are yet in active ~~use~~ growth; and as they begin to ripen in the center, or older portion of the colony, this becomes green, and shades off into the white unripe spores and finally into the margin of semi-transparent naked hyphae and mycelium. When fully

ripe, the color becomes an ashy green or grey. At this time the spores loosen readily and are apt to be carried by any breath of air.

The *Penecyllum* is the most abundant of the moulds, and its spores are almost *omni-present*. A slide of gelatine exposed to the air for an hour will rarely fail to develop one or more colonies of *penecyllum*. It is there-^{fore} a great pest in the culture of other varieties, as it is continually developing where it is not wanted.

Fig 2



Aspergillus glaucum

- a Spores as seen with a $\frac{1}{2}$ inch lens
- b Spore head beginning to form
- c Spore head putting out sporophores
- d Spore head with spores
- e Portion of head with chain of spores attached to sporophore

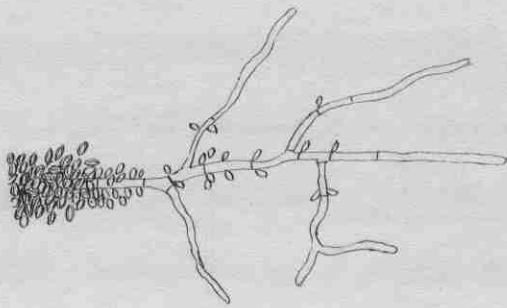
No2. Aspergillus Glaucum.

Spores round. Nearly twice as large as those of *Penecyllum*. The mycelium is profusely branched and rather stronger than that of *penecyllum*. The hyphae do not rise quite contemporaneously with the growth of the mycelium but come a little later, and are more sparsely⁵ distributed. The greater number of the hyphae are without branches, and rise straight to about an even² height and put out a rounded knob at the apex supported on a delicate terminal point (Fig. 2. b.) This enlarges, and puts out from the upper, or distal, hemisphere a number of short sporophores from the apices of which round spores are adjoined to the number of eight, ten, or even twenty, in the form of chains; the distal spores being the older. (Fig. 2. c, & d.) These spores are white at first but become green as they ripen giving the colony a greenish appearance. This ripening begins in the center of the colony and spreads to the periphery, so that the formation of the spore-heads and the ripe spores may be observed at the same time

As the spores become dead-ripe and dry they lose ~~the~~ their brightness and assume a greyish appearance. When planted on gelatine they sprout in from twenty four to forty eight hours at a temperature of 25 degrees.

This plant has also another spore occurring on another form of hyphae that arises from the same mycelium as those bearing the spore heads. In most of my cultures these hyphae have been absent or very sparsely distributed, and in only two out of twenty colonies observed have I seen this spore. The hyphae bearing them are very slender, ~~and~~ freely branched, and usually rise above the spore heads. The spores are not free but are in spore cases of an orange color, ^{which are} ~~and~~ almost large enough to be seen with the naked eye. The formation of these are well described by Sachs (p. 257.) under the name, Uroteum. I have singled out these spores and planted them carefully and have found no difference in the resulting colonies from those derived from the spores from the spore heads. Sachs regards the plant bearing these spore cases as

Fig 3



Pullularia
Mycelium with gonidia

No. 3. Pullulans. (Brown) DeBarry.

This plant has a heavy mycelium that becomes jointed early in its growth. At each joint of the mycelium oblong gonidia are abjointed and become free. (Fig. 3.) Afterward these seem to be formed from all parts of the mycelium and are given off in such numbers that the mycelium is completely obscured and seems to disappear, so that the central part of the colony is a heap of these gonidia with a fringe of mycelium around it. In some of the plants the mycelium is but spar⁵ely branched, and in that case the course of the isolated branches becomes a broad line of gonidia. No hyphea has^{ve} been observed, and no ~~other~~ other form of spores. At first the color is slightly yellowish, but as it ripens it becomes brown. These gonidia grow promptly when transferred to fresh gelatine.

In the process of ripening the gelatine melts down, or becomes fluid, and the whole becomes a slimy mass.

No. 4. This plant seems to be like no. 3 in every particular except that it is rose red instead of brown.

different from the aspergillus that does not bear them., while DeBarry and Cooke think that the plant is the same and that the orange spore cases are a result of sexual conjugation, and are therefore the true spore while those borne on the spore heads are to be regarded as gonidia. None of the colonies which I derived directly from the spore cases reproduced the *m.* (spore cases)

Aspergillus is fairly common in this locality, though it does not occur on collection slides as frequently as *penecyllum*, and some other varieties.

No. 5. Pullulans. Hypheal.

(There are several plants that I shall designate as pullulens because they resemble it more in their form of growth than any others with which I have become acquainted; though ^{of} really they are very distinct from it. I have found no description of these in the books.

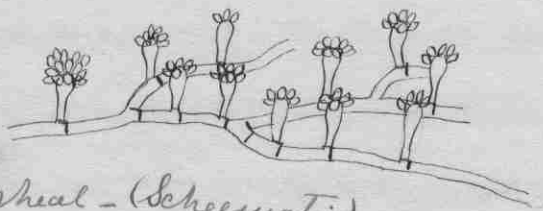
This plant has a heavy ~~intimately~~ branched mycelium that covers the gelatine very intimately. It often forms heavy expansions of the mycelium, and the mycelial threads fuse together at points of meeting in such a way as to form a very intimate plexus. It buds off no gonidia from the mycelium as the pullulans, No. 3, but instead puts up short thick gonidiaphores two to five times as long as broad, and a little inclined to be club shaped, the upper end being the larger. On the ends of these gonidia ~~are~~ are budded off in rich profusion. (Fig. 4 is a schematic representation of it.) At first two or three gonidia will be seen on the end of a gonidiaphore, the number increases until the whole upper end is covered. Then the

begin to fall away, and new gonidia are budded off in the places of those that have fallen until these cells completely cover the field and hide every thing ^{else} from view.

The gonidia thus formed cannot well be told from those formed in no. 3, and to the naked ^{eye} the plants look much the same except that No. 5 is much the denser, the cells piling up to a considerable thickness on the gelatine. As the plant ripens the color becomes a dirty yellow color, and the gelatine melts down and the whole becomes a slimy mass that will sometimes run off the slide.

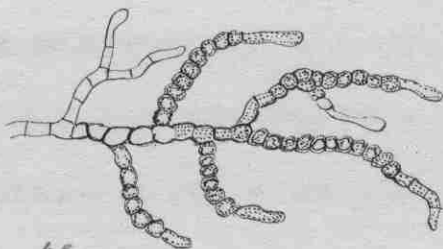
I have not certainly discovered any other form of spore for this plant, though in one case I saw some dark round bodies that were probably spores. The colony was unfortunately destroyed by an accident before I had the opportunity of proving them.

Fig 4



Pullulans - Hyphal - (Schematic)

Fig 5



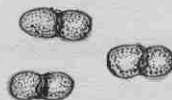
Black Pullulans. Terminal branch forming gemmae

Fig 6



Spores of black Pullulans

Fig 7



Spores in conjugation

No. 6. Black Pullulans.

In the colony two or three days old this plant is generally almost exactly like No. 8 both in its mycelium and in the gonidia formed. But at about this time it puts up an abundant hyphae, and this is of two varieties. In some of the colonies the hyphae are white, fine and fuzzy, and inclose the colony in a snow white down which remains standing after the other parts of the colony have melted down into a slimy mass. These white hyphae bear no spores.

In other colonies a heavy, jointed, stiff hyphae are put up which are black, or vary in color from a light brown to a coal black that is perfectly ^{opaque} opaque. These hyphae bear a considerable number of round black spores which seem to have a rough coating that is perfectly ^{opaque} opaque (Fig. 6.) The colonies in which these spores ^{are} ~~are~~ formed are so ^{opaque} opaque that it has not been possible for me to ascertain just how these spores are formed, or their

exact position on the hyph^{ae}~~ae~~, but from what I have been able to see by reflected light they seem to be formed on the terminal ends of the hyphae, and afterward the hyphae push them to one side and continue their growth. They are generally not very abundant, though when the colony, or rather the substratum, melts down a considerable number of them may be found in the mass. In this position many of them are found united by twos as shown in figure 7 which is probably a conjugation of the spores. In the matter of growth, however, these double spores show no differences from the single spores. Both grow readily with like results. Colonies grown from the round spores, the double spores, the gonidia, or the gemmae, which I will describe presently, present no characteristic differences

In all of the earlier stages of the growth these black hyph^{ae}~~ae~~ have a greasy, or watery appearance which causes ~~it~~ ^{the surface} to reflect light in such a way as to be very exasperating to one trying to determine the particular

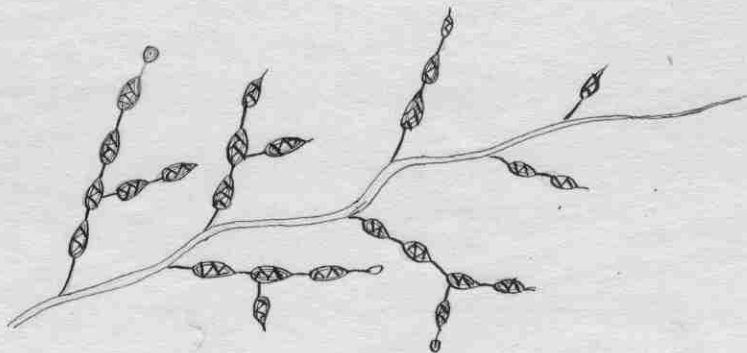
forms of the parts. But after the growth begins to over-reach the gelatine, or when the hypha^{ae} over-reach the mycelium they become dryer, and seem to assume a different form. The mass is less dense and often branches may be well seen about the margins giving the appearance shown in fig. 5. That is, they become broken up into chain of round cells. these are less densely black than the round spores. They do not very readily fall apart, but when transferred to fresh gelatine they grow promptly, manifesting all of the qualities of the other forms of spores. These are therefore germinae.

This plant seems, then, to have four kinds of spores, all of which grow with equal facility, and produce similar results. These results are, however, very perplexing, and I am not yet absolutely certain that I have not been dealing with a mixture instead of a pure growth, even though I have repeatedly singled out, and planted separately the different forms of spores. For instance, in

one case I singled out and planted round cells from a coal black colony, saw them sprout promptly, and was away four days. On my return I found a snow white colony. I examined it carefully and replaced it under cover. the next day I found the white colony surrounded by a ring of coal black growth. This was maintained during to the remainder of the growth of that colony. On several occasions I found a ring of white succeed a black colony Or there may be black splotches in a white colony, or the reverse. Or again some of the colonies are all black or all white. Or the colonies may not be very black but only look as if they had been scorched. In this case none of the round spores are formed, but there is an abundance of the gemmae.

On seeing these different colonies growing they seem to be entirely different species, yet the same spores seem to produce all of the different kinds in spite of my best endeavor to keep them free from mixtures, and I can hardly conceive that I have failed.

Fig 8



alternaria.

No. 7 Alternaria. DeBarry.

Spores septate. A rapid growing branched mycelium ~~that~~ that spreads over the entire gelatine. The hyphae rise at once with the growth of the mycelium and branch into a moderately thick web. Later the spores begin to form. These are large and prominent and occur on rather short sporophores that arise from the hyphae as lateral branches. (Fig. 8.) Up to this time the colony is white, but as the spores begin to ripen it becomes darker and finally assumes a dark brown color. This color is due entirely to the spores which are very abundant and seem to form the principle bulk of the plant. Each spore is divided ~~into~~ irregularly by a considerable number of cross markings, and when planted each of these divisions seem capable of giving rise to a sprout independently of the others; at least I have frequently noted sprouts appearing at different times from the different divisions. The

general form of the spore is fairly shown in the illustration. This is therefore a compound spore rather than a spore case filled with independent spores; and what Botanists term a septate spore.

The oldest spore in any given chain is that next to the hypheal branch, while the most distal is always the youngest. Also the growth of the one is usually completed before the next is budded out from its distal extremity, and it is not uncommon to see new spores growing at the extremity of the chain, or budding out from the side of a mature spore when most of the spores in the chain seem to be fully ripe. The form of the colony is neat and compact and usually free from projecting hyphea. It is only occasionally that a few stray hyphea will produce a grey streak above the mass of spores.

I have found this plant quite commonly on my collection slides. It presents three varieties that have

distinctly maintained their differences through twelve generations, and it is quite probable that there are other varieties that I have not seen.

No. 8. Alternaria. Buff.

Spores of the same form as those of number 6. The hyphae grow considerably longer but the colony is much less dense. The spore clusters are less dense and when ripe are lighter in color inclining to a yellow or buff.

No. 9. Alternaria. Grey.

The form of the spore is the same as in the brown variety and are of an ash grey color when ripe. The colony is very light and flockulent, and the hyphae are very long, often extending out over the margins of the slide in every direction. The hyphae^{ae} of this variety continue to grow after the completion of the formation of the spores and covers them in with a light

flocculent mass that is nearly snow white.

No. 10. Bead mould. Brown. No Author.*

Spores round, and about one third as large as those of *Penecyleum*. They grow readily and form a short, ~~but very~~ dense mycelium which rarely spreads over an area of more than an eighth inch in diameter. The hyphae rise contemporaneously with the growth of the mycelium, are short and straight, without branches, are abundant, forming a dense mass, and each adjoins single a chain of round spores from its distal extremity. These chains contain from ten to twenty spores each. The color of the colony is white at first but becomes brown, or almost black as the spores ripen. It obtains its full growth in from three to five days.

No. 11. Bead Mould. Grey. No Author.*

This mould is similar to number ten only that the color of the ripe spores is grey instead of brown.

* By the term, or phrase "No Author" I mean simply that I have found no description of the plant in the Books. My search, however, has been

No. 12. Bead Mould. Red. No Author.

This mould is similar to number ten only that the color of the ripe spores is rose red instead of brown.

No. 13. Snap Mould. Grey. No Author.

This little plant, though its colony is, to the naked eye, so very similar to that of many of the moulds as to deceive any but an expert, probably belongs to a very different class of ~~plants~~. Properly it has neither mycelium ~~or~~ hyphae.

The spores vary from round to oblong often being twice as long as broad, and the round ones are generally about two thirds as large as the spores of penicillium. They grow promptly when placed on gelatine, and form a short thread no thicker than the spore, and only from four to six or eight times its length. These extend horizontally on the gel^latine or rise into the air. They generally extend horizontally from the mar-

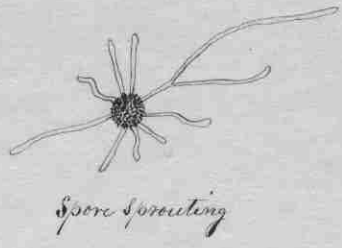
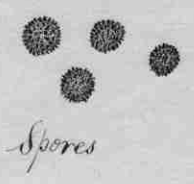
gins of the colony and rise into the air in the central parts. On the end of each one of these threads from three to five spores are formed, both on the horizontal and standing threads. There is no movement of the spores to be seen, and yet free spores are continually seen scattered around the margins of the colony and these at once begin to grow, and thus rapidly extend the colony. In this way the colony will sometimes extend to as much as an inch in diameter in a week or ten days. How are these spores scattered? This I have been unable to determine. The colony ~~becomes~~ becomes so compact that nothing can be done with ~~trans~~ transmitted light, and I only know that in watching the margins and fixing the position of the outlying spores there soon comes a little flash across the field, and a spore is lying where there was none the moment before. They are evidently snapped out from the standing threads in some way; but what may be the mec.

anism by which it is accomplished I have thus far been unable to determine. The color of the colony is a light grey, attaining a tinge of yellow as it gets a little old.

No. 14. Snap Mould. Red. No Author.

This plant is similar to number thirteen except that it is red instead of grey.

Fig 9



Red Cromogen.

No. 15. Red Cromogen. No Author.

The spores are round and coal black. They seem to be covered closely with very short protuberances, and are large enough to be seen with a good hand magnifier. They are probably spore cases inclosing a number of small spores. The mycelium is intimately branched and spreads quickly over all of the gelatine on the slide whether it be much or little. The hyphae rise contemporaneously with the growth of the mycelium are long and branched, form a very thick web that grow out over the margins of the slide in every direction and if the papers on which the slides are laid are not poisoned it will attach itself to the paper and spread on that also. The growth is white at first, but as the growth proceeds it becomes a beautiful pink pink. If now the slide is turned over it will be ~~found~~^u found that the whole of the gelatine has become an intense carmine color, and a closer examination shows

that the pink tint is caused by the color showing through the hyphal web, and that the hyphae have not changed their color. As the colony becomes older and more dense this pink color of the colony fades and is replaced by a light yellow hue which gradually deepens as the plant grows older. Wherever it grows out onto the paper this is also stained an intense red. The hyphae^{ae} of this mould are often a full half inch long, and in old colonies little tufts of fresh hyphae of a lighter color than the old spring up here and there and varigate its surface.

Gemmae are formed rather sparingly appearing as occasional short rounded joints on the hyphae^{ae}. These sprout promptly when placed on fresh gelatine, but they are often difficult to find, and as the spores are rare I have been compelled to use mycelium frequently for transplanting it and this is so uncertain of growth that it has occasioned much difficulty. I found the first spores after keeping it under ob-

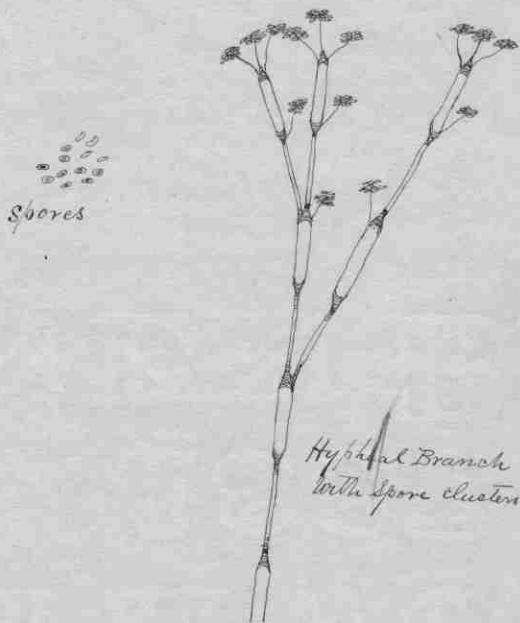
servation for two months, and in the twentyfirst generation, these having been planted partly from mycelium and partly from gemmae. In this colony the spores ~~occ~~ occurred thickly enough to give a distinct black color over about one fourth of the ^usurface, and in isolated groups that could be seen with the naked eye over the rest of it. These spores have all been formed in the thick hypneal web where it has been impos^sible to determine the manner of their formation, or their relation to the hyphea. They occur in bunches of from three or four to a dozen in close contact, though seemingly not joined together,

Some of these spores were singled out and freed from every thing else and several plants made. They sprouted promptly, each one giving rise to from four to eight or ten sprouts, ^(Fig 9) and the resulting colonies were in no wise different from those grown from the hyphea or the gemmae. None of them reproduced the

black spores. I found this plant on three of five collection slides, indicating that its spores are not rare, and although it grows luxuriantly on my gelatine I must conclude that this material is not well suited for the development of its spores.

I have allowed five colonies grown from the spores to stand until they have withered away and have not discovered a single spore among them

Fig 10



Buff Flox.

No. 16. Flox. Buff. No Author.

The spores are discoid, oval, and a little smaller than those of penecyleum. They show what would seem at first sight to be a nucleus, but I am of the opinion that this is the point of attachment to the adjoining spore during their formation, and that it is therefore a marking on the exterior. This is always on the flattened side. When planted they sprout within fifteen hours at 85 degrees. They give rise to an abundantly branched mycelium that spreads quickly over the whole of the gelatine, even though it covers the whole slide. However, at first the mycelium grows long rather than thickly branched, and ^{afterward} fills in a close web by the growth of lateral branches. The mycelium is unusually delicate. The hyph~~s~~^{ae} are put up slowly, occurring mostly after the mycelium has spread over the entire gelatin. They are long and delicate with but few lateral ~~branches~~ branches, and often rise to a quarter inch in height.

The hyphae of this family are of peculiar form. The stems are flat and jointed; and the long diameter of each succeeding joint is placed at right angles with the long diameter of its predecessor. The conjunctions of the parts are of darker color than the parts themselves, and a little extended instead of being the usual simple cross marking. In this extension what would otherwise be abrupt corners on the flattened parts are rounded away making a smooth transition from the one flattened part to the other. The colony is white at first but as the hyphae rise they become yellow or buff.

The fruit begins to form at the extreme ends of the hyphae. Then several lateral branches are put out just below ^{each of} which also bears a bunch of fruit. Often a single hyphae will have a dozen clusters of fruit.

The fruit is white at first and being seated on the mass of buff colored hyphae gives a strong likeness to
~~ness to~~

bunches of flowers. The color becomes of a dull greyish yellow as they ripen, and ^{the spores} are inclined to fall away and are easily scattered about by a breath of air.

The exact form of the spores cannot be determined as they are united in the bunches. They are evidently joined by their flattened sides but seem to be very irregularly placed. Their relation to the sporophores is also difficult to determine. Indeed they look like an irregular accretion of the end of the sporophore. The hyphae as seen en masse are more yellowish than the ripened spores so the the colony becomes lighter in color as the spores ripen.

No. 17. Flox. Grey. No Author.

This plant is similar to number 16 except that the the growth is in every way heavier, thicker, and lighter in color under the same conditions. Before the

Fig. 11



Green Floy

ripening of the spores the color is a light grey, but the spores take on a yellow tinge in ripening, and then the color of the two plants is nearly the same. The spores and their manner of formation seem to be the same.

No. 18 ~~BBB~~. Flox. Green. No Author.

This plant has no similarity to the two preceding except in the form of its fruitage and its spores. In form the spores could scarcely be told from the other two varieties, but they are of a pea green color. The mycelium spreads rapidly over the entire gelatine and out over the sides of the slide often growing one half or three quarter inches on the naked glass. It is extremely delicate, ⁿ finely branched, and with all so ~~delicate~~ transparent and inconspicuous that with the naked eye it is often difficult to determine its presence on the gelatine. The mycelium

generally covers the gelatine completely before any hyphae are seen. In a strong growth with plenty of gelatine it finally puts ~~film~~ up filmy hyphae, in clusters here and there first, and later often webs over the whole of the gelatine. But there is apt to be naked spots. Under less favorable conditions, as with only a thin film of gelatine, there will only be clusters of hyphae here and there, and especially about ~~to~~ the margins of the gelatine; and it is usually on these that the first fruit forms in clusters similar to those of the last two varieties only that they are confined more closely to the apices of the hyphae. The fruit is white at first but soon becomes of an ~~in~~ intense pea green color that contrasts strongly with the general absence of color in the colony. This fruit seen by the naked eye in fringes about the margins of the gelatine looks very much like concretions of sulphate of iron. In stronger growths the fruitage is

more general over the surface giving the colony a splotchy appearance, and in some of the best growths a solid green was obtained. I have noted that the colonies that have grown rapidly with a large supply of gelatine produce a less intensely green spore than those grown with a scant supply.

This mould has not the peculiarities of the jointing of the hyphae that is seen in numbers I6 and I7, which of course marks it as belonging to a distinct family notwithstanding the close similarity of the spores.

No. 19. Arbora. Brown. No Author.

(This family I have called Arbora on account of the remarkable likeness of its fruit laden hyphae to the branches of a bush in full leaf.)

Spores round, and a little smaller than those of penecyleum.

The mycelium is intimately branched and very thick, the hyphae rise contemporaneously with the growth of the mycelium. The fruitage begins almost at once, occurring on sporophores arising as lateral branches from the hyphae. (Fig. 12) each sporophore bearing a cluster of spores. These are in branching chains of from two to six or even eight in some instances. It is not uncommon for two spores to arise from one, producing a branching of the chains. The distal spore of the chain is the younger. These are colorless at first but quickly become of a dark green and shade off into a dark brown as they ripen. They are so numerous that they dominate the color of the colony. The hyphae

and the mycelium continue their growth as the spores are forming so that the young colony has a center of dark brown shading off into a fringe of white. This plant forms a very neat compact colony which when mature has a very soft velvety appearance, and is one of the most beautiful of the ^{or}dark colored moulds.

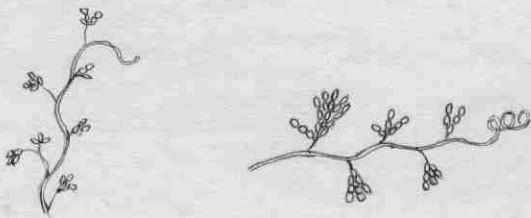
No. 20. Arbora. Buff. No Author.

This mould is the same in its general form and fruitage as the last, but the spores are larger, and oblong instead of round, and of a dark buff color instead of brown. The fruitage also begins much later in the growth. It colors the gelatine black, and liquifies it late in the growth, as do all of the Arbors.

No. 21. Arbora. Drab. No Author.

The spores of this mould are almost exactly the same in form and color as those of number 19 but they

Fig. 12.



Hyphae with spore clusters

Arbora

^{are} too few in number to give more than a slight shade of coloring to the colony. It has finely branched mycelium of rather slow growth. The hyphae rise contemporaneously with the growth of the mycelium and keep the latter well covered in. The hyphae are very fine and delicate, and greatly inclined to curl, often forming spirals of some length. The gelatine is colored intensely black early in the growth and this showing through the web of hyphae gives the colony a peculiarly soft brownish appearance. The fruitage does not occur until very late in its growth, so much so that I had planted with mycelium a number of times before I had discovered the fruit. The spores are born on sporophores that occur as lateral branches from the hyphae similar to those of number 19 but the spores are in more considerable clusters. These clusters are so sparsely^s distributed, however, that generally only one or two will come in the field of the microscope at one time. As the colony grows older the web of ~~the~~

hyphae becomes thick enough to hide the black color of the gelatine and it then becomes a light drab or grey color. The colony is unusually neat in its form being free from unevenness, or of bunches of hyphae straying about its margins.

What has seemed to be other varieties of this family have been seen during my cultivations, especially one with a much larger oblong spore, and one with a redish tint, together with some that presented less prominent differences; but none of these were cultivated sufficiently to determine their characters.

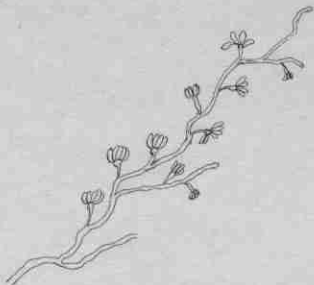
Fig. 13.



Mycelial gonidia



Hyphal gonidia



Hyphal branched gonidia

Comma mould

No. 22. Comana Mauld. No Author.

The true spores of this plant had^{ly} probably not been found. It bears gonidia of two forms very abundantly, however, and these grow promptly when placed on gelatine. The forms of these gonidia are shown in figure 13. The larger is long shaped, curved upon itself and marked with three cross bands that are distinctly darker than the other parts. Within^{each of} the two central divisions there is a nucleus, and in^{each of} the end divisions there are two tolerably distinct nuclei. These gonidia are born on the mycelium. The smaller gonidia are born on the hyphae. These are long shaped many are curved (but not all) are marked by a broad shaded cross band and show a nucleus in each of the end divisions. Each of these varieties grow readily and produce similar colonies. ~~==~~

The mycelium grows rapidly, spreads over the ~~entire~~ entire gelatine given it and onto the naked glass

about a quarter inch. The mycelial threads are peculiar in that their contents is distinctly granular in all their parts; a very unusual condition.

The hyphae rise contemporaneously with the growth of the mycelium and form a snow white flocky mass. The hyphae have not the granular appearance observed in the mycelial threads. Very soon after the hyphae begin to rise they put out lateral gonidiophores the distal ends of which become slightly club shaped and upon which two to six of the smaller gonidia are budded off, ~~but remain attached~~ These seem to fall away after a time and new ones to appear in their places. While this is going on among the hyphae a similar process is taking place on the mycelium by which the larger gonidia are formed. After some days the hyphae become less fruitful, but continue to grow and form a thick web of snow white hyphae, ~~and~~ ⁴ the gelatine melts down without changing color, and is apt to run from

under the colony carrying with it myriads of the gonidia.

I have now cultivated this plant to the tenth generation by means of the gonidia without finding the true spores. I have given it temporarily the name, ~~of~~ comma, on account of the peculiar form of the gonidia this being the term applied by Koch to curved micro-organisms.

No. 28.

This mould has been but imperfectly studied; for though I have cultivated it to the twelfth generation planting from the mycelium and certain parts of the hyphae, often having much difficulty and many failures in getting growths, I have as yet found no form of reproductive cells. The plant forms an intimately branched mycelium that is very delicate and from this hyphae rise promptly and in most cases become compacted together in stiff spike like bundles that rise to a height of a quarter inch or more and then form a thick web of intimately interwoven white fibers that incloses the whole in a snow white mass. After a week or ten days the gelatine becomes fluid with little change of color and the whole colony becomes saturated with this fluid and sinks down. I have ~~searched~~ searched ~~them~~ carefully for spores without result. I have, however, generally obtained a new growth by

laying some of the hyphae from the central parts of it the spike like bundles on the fresh gelatine.

A few of the colonies, instead of forming the upright spikelike bundles, have formed a complex network of anastomosing bundles in which many of the ~~ea~~ compound fibers are large enough to be seen with the naked eye. This is gradually covered in with a web of delicate white fibers. Such colonies occupy all the gelatine given them, but in none have the looked for spores been forthcoming.

General observations.

During the time of making these cultivations I have seen a considerable number of other varieties of saprophytic moulds that I have not been able to study sufficiently to determine even their most general characteristics with any degree of certainty. Some of these have seemed to grow fairly on the gelatine offered them but have gradually withered after two or three generations, indicating that the substr^um was not suitable for their maintainance. Others have failed to form spores or gonidia, and plants from the parts at hand have failed of growth. Others still have been lost by being ~~over~~, over-run by varieties ~~to~~ that have accidentally found lodgement on the gelatine with them. In these several ways quite a number of what seemed to be new varieties have been lost.

Furthermore, this effort does not represent any thing that may be considered ~~an~~ exhaustive effort

ⁱⁿ ~~to~~ obtain^{ing} the varieties that might be found, even about my own household; for in obtaining these I have exposed only five slides for the collection of varieties. Of these four were in my own rooms and one in my barn.

To render these cultivations satisfactory the plants should be offered various substances belongⁿing to our foods on which to grow, and the characters of ~~the~~ the growth on each ~~be~~ noted, such as cheese, meats bread, boiled potatoes, fruits, vegetables, &c. It would probably be found that some of the moulds that have refused to form their spores on the gelatine, or that have done so tardily, ~~might~~^{would} form them promptly if grown on some other substance. This would also give some clew to the tendencies of the different moulds as to their favored habitat.

One observation is of importance as regards the growth of the moulds on fruits in our cans, and on jellies, and such articles as are sealed or closely

covered. In an effort to grow some of the moulds in small cultivation tubes that were sealed to prevent drying of the gelatine I found that while the ~~hyphae~~ mycelium and hyphae would grow readily no spores would be formed. But if the rubbers were removed, admitting fresh air for several hours, the spore would be rapidly formed. This was the case with penicillium and other varieties that form their spores readily and quickly in my culture dishes. Now the injury done to the substratum on which these moulds grow, the decomposition, is mostly during the formation and ripening of the spores. This has been the case with about three fourths of the varieties I have studied. It is for this reason that our sealed ^ejellies and fruits on which we find growths of moulds are ^{so often} not injured. The moulds can do only a small part of their work without fresh air.

Another point that is sure to attract the attention of any one who undertakes the study of the moulds

is the formation of bladder like enlargements on the hyphae. This seems to occur on any of the moulds, and I have been unable to find it to be of any special significance. Sometimes these appear as a considerable enlargement of the apical end of a hyphal branch, but they are more frequently seen in the course of the branches. In many cases the branch may be seen coursing its way through the apparent enlargement giving the idea that it is nothing more than the condensation of a drop of moisture in the form of dew, which is probably correct. But many of these are true swellings of the branches in which the membranes are clearly seen to be expanded enormously. Furthermore I have on several occasions observed the formation of these enlargements which have occurred very rapidly. In these cases an unusual motion of the contents of a branch was observed and its course being followed it was found to lead to one of these expan-

sions which was observed to be rapidly filling with the fluid contents of the branch. I have watched these enlargements to find their function, if any, and have failed to discover that they have been of any use whatever. In a number of instances they have seemed to occur as a kind of blight affecting a portion of a colony. In these cases the branches become matted together in the multitude of these enlargements by each branch sticking to it when it happens to come in contact with it. A considerable number of these may occur, however, among the branches of the most flourishing colonies.

Movement phenomena are common among the moulds. The heads of *Aspergillus* have during the ripening stage an almost constant rotary motion. This is reciprocating, the extent of the motion often being as much as a revolution and a half, and in one instance I witnessed two and quarter revolutions. These movements are

slow but sufficiently distinct to be easily followed. Movements of the terminal branches of the hyphae are common to most of the moulds. These must not be confounded with movements caused by air currents, for they are something different. The most of them are oscillations that take place with a fair degree of regularity, and are so slow as not to be likely to attract attention if not looked for. Some are of a very curious sort. In the comma mould particularly I have noticed the habit of doubling the terminal ends of the hyphae upon themselves, and also of suddenly forming knees ^{or abrupt bends.} At a point where a knee is to be formed a little swelling will occur and suddenly the twig will bend sharply to something near a right angle. I have not been able to determine satisfactorily to myself whether these remain permanent or not. The appearances of the plant would indicate that they straighten out after a time, but I have not been able to witness the movement.

The same plant also exhibits during a certain stage of its growth phenomena similar to that so remarkable among what is known as sensitive plants. On disturbing it the upright hyphae fall down. I found this plant so sensitive that it was difficult to examine it with the microscope with its branches extended.

These movements were not suspected in the first part of my work, and have only lately forced themselves on my notice. They have therefore not been at all sufficiently studied.

In these cultivations I have had no intention of studying more than the most salient features of the moulds. Many questions of great interest from the scientific point of view I have made no endeavor to touch for the reason that they would require more time than I could devote to the work. One of these is the matter of sexual reproduction. Another the different forms of the molds ~~on~~ when growing on different substances. Also the chemical nature of the decompo-

sitions induced by the moulds. On this point I have this observation. I slipped^{ly} the colonies of a number of the moulds from my slides into half drachm bottles and corked them with the view of saving the spores. Plants made from these ^cceased to grow after about a month. It is evident that confin~~ments~~ with their own excretory products soon destroys their vitality.

G. V. B.

Jacksonville June 3rd 1892