

II. THE CAUSATIVE FUNGUS

by

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My task today is to explain, in as non-technical a language as possible, the complete life-history and functions of the organism which causes the blister blight disease of tea, as we fully realise that, without this basic knowledge, very little can be achieved in the form of disease control.

As most of you are aware, the causative organism of the blister blight disease is a fungus. Now what is a fungus and what is its relationship to plant life? Even among scientists, the answers to these questions are diverse, as there is a difference of opinion as to whether some of the lower forms of plant life should be included amongst the fungi. In this connection, it is well to quote from one of the latest publications, *Plant Pathology* by Sir Edwin Butler and Dr. S. G. Jones. "The chief characteristic of the fungi is the absence of chlorophyll, even in those members which happen to be coloured green (the colour associated with chlorophyll). This carries with it a profound difference in mode of life from other plants, for they (the fungi) take their food in the form of organic matter, whereas the chlorophyllous plants (as for example the tea bush) obtain their carbonaceous food from the carbon dioxide of the air, through the energy of sun light."

In other words, the fungi in the absence of chlorophyll, which is necessary for food manufacture, must have their food already prepared for them in a form suitable for immediate digestion. Photosynthesis, which Mr. Portsmouth will deal with in his address, therefore, does not take place in the fungi.

The fungi can be classed in two distinct groups — saprophytes and parasites. Saprophytes feed only on dead matter while parasites get some or all their food from living bodies. The parasites may be still further divided into two groups — facultative parasites an example of which is *Poria hypolateritia* — a common root disease fungus of tea — can exist on either living or dead tissues. It is possible to grow the fungus under sterile conditions in a test tube of culture medium. The fungus (*Exobasidium vexans*), which causes blister blight of tea, is on the other hand an obligate parasite which is unable to live on dead material. It cannot be grown on culture media but requires a living tea leaf for growth. The fungus does not kill the part it enters, death follows in due course but not until the fungus has completed its life cycle and reproduced its kind. Now with this basic knowledge of what a fungus is and how different forms operate, we can pass on to the disease, blister blight itself.

All of you are familiar with the appearance of the fruiting body of blister blight. In fact, your presence here today is because you have seen too much of it and would rather have the effects of the disease under control.

The characteristic white blister which forms on the undersurface of the leaf, and on the young tea stems is composed of numerous white fungal strands or hyphae, at the apex of which spores or fruits of the fungus are produced. To the unaided eye the velvet-like white mass appears uniform but under fairly high magnifications you will see that in reality it consists of numerous individual compact bundles situated close together like the pile of a carpet. The white blister is the hymenium or fruiting layer of the fungus. It is the only part of a blister colony visible to the unaided eye.

For further study, at high magnifications, a transverse section of a blister is necessary. Even then it requires a trained eye to distinguish and differentiate between the structures that made up the blister colony. For your convenience, special diagrams have been prepared and I am using these as an aid to demonstration.

The first diagram (Fig. 2) shows a transverse section of part of a tea leaf. The section passes through a mature, spring blister. For the moment, we will confine ourselves to the fruiting stage of the fungus itself.

As stated previously what you now see is a highly magnified version, and of course diagrammatic.

The fertile mycelial strands of fungus or hyphae (termed basidia) on which the spores are borne are at first clavate or club-shaped. The basidia possess two nuclei which pass into the young spore when the basidium or the fertile hypha is mature. The basidium gradually widens out near the apex and forms two, or rarely three, spiked projections or sterigmata at the apex of which the spores or seeds of the fungus are borne. In a section of a white blister, spores in different stages of maturity may be seen, the very small unicellular spores being the immature ones which are very firmly attached to the basidium and fairly difficult to dislodge. When ripe the spores become two-celled following the formation of a septum or wall which divides the spore into two almost equal parts. At this stage the blister is ready to discharge its ripened spores. In the case of blister blight, as with most other *Basidiomycetes* with the hymenium or fruiting bodies borne externally, the spores are discharged from the basidia with some considerable violence. The distance to which the spores are discharged or shot away is sufficient for them to clear the hymenial surface and pass into air currents. Spores dropping from members of a related family, the mushrooms and certain bracket fungi which are common objects on decaying tree stumps, may be seen, without the aid of a microscope if a beam of light is concentrated on them. The spores appear as shining, dancing objects similar to the shimmering effects produced by dust particles in a sun beam. These fully grown spores are of microscopic size, measuring 0.006 — 0.014 mm, long by 0.0028 x 0.004 mm. wide.

As long as an infected leaf remains attached to the bush and the fungus is alive there is a continuous discharge of spores from the basidial layer but, if the leaf is plucked, discharge soon ceases unless the cut shoot is supplied with water. Spore discharge is regulated by the pressure of the cell sap upon the wall of the basidium. The spores may be collected by placing a clean glass slide a short distance below the hymenial surface, and covering the whole shoot or young plant with a large jar. The jar is used to cover the shoot in order to eliminate air currents, which would carry the very light spores away from the glass slide. In a few hours a white deposit settles on the surface of the glass slide, which, when placed under the microscope, shows the deposit to be made up of large numbers of blister blight spores. A very rough estimate of the number of spores can be obtained from measurements of the area covered. That this number probably exceeds a million can only be imagined if it is realised that 10,000 spores can lie side by side without overlapping on an area one millimetre square. From this may be gauged the potential output of spores from a single blister which may fruit, under suitable conditions, for a week. The number freed from blisters formed over an acre of unprotected tea must then be truly gigantic.

Round the spore, completely enveloping it, is a thin sheath of mucilage. The adhesive property of this sheath is of considerable importance as the spores remain fixed and cannot be dislodged even if the slide is placed in running water, as from a tap. The spores stick equally well on other surfaces such as leaves, cellophane or plastics. Even in heavy rain they remain fixed

firmly to leaves and hold their position until conditions are favourable for germination. Large numbers of course find lodgment on unsuitable bodies and only a very minute percentage of spores can ever reach tissues in which they can develop. For survival of the species gigantic numbers are therefore necessary.

The spores are colourless or hyaline. They are so light that in absolutely still air they fall very slowly and even the very small movement caused by convection currents, set up around a leaf, may carry them away. Sometime ago, in an effort to find out the distance spores are forcibly discharged, it became clearly evident how easily they are carried even in imperceptible air currents, to long distances. An infected seedling was placed at one end of a box, which could be tightly closed, and slides were placed on the floor of the box at varying intervals. The next day spores were found on the slides 30 inches away from the diseased plant. This forcible discharge prevents the spores from massing together or sticking to the hymenial layer, and is nature's method of dispersal.

The rate of fall of spores was watched by a scientist about half a century ago. He used a horizontal microscope and a revolving drum to record accurately the rate of fall. Relatively small spores fell at an average rate of 637 mm. per second. A spore takes about one hour to fall 4½ feet in still air.

Blister blight spores begin to grow or germinate under certain conditions only. A thin film of water is more favourable for germination than a drop, whilst ideal conditions are obtained when water vapour condenses as dew around the spore. This is one of the chief reasons why the disease is most prevalent in areas liable to mist and in hollows where dew forms early and persists for a fairly long period in the mornings.

GERMINATING SPORES

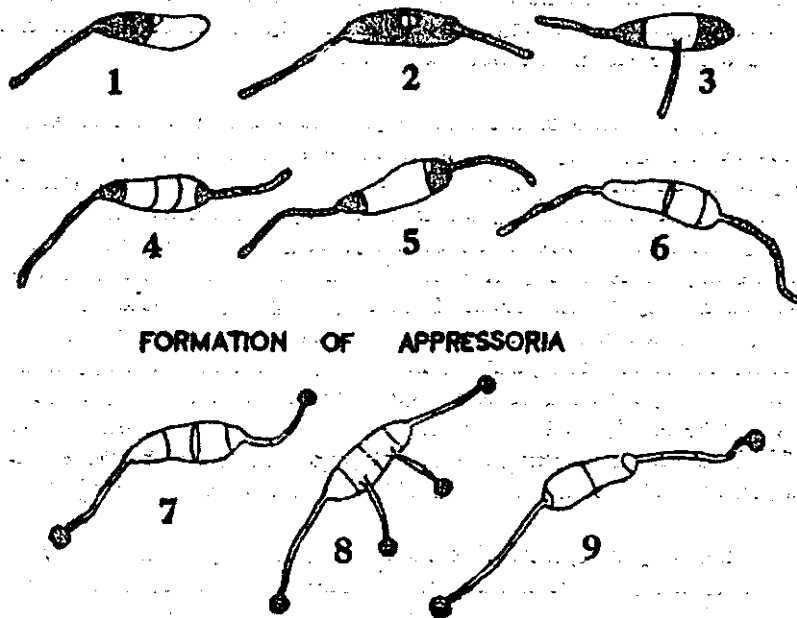


Fig. 1. 1-6. *Germinating spores before the formation of appressoria. Note movement of protoplasm into germ tubes.*
7-9. *Formation of appressoria. Spores empty of protoplasm.*

If the slide on which the spores have been deposited is wet with a fine mist, germination will commence in a few hours (Fig. 1). The first indication of germination is the emergence of a tube from one or both ends of the spore; occasionally 4 or 5 germ tubes may emerge from one spore. Germination may commence two hours after wetting. Two to three hours later a spherical body, the appressorium, is formed at the extremity of each germ tube.

At this point it is necessary to digress.

Infection with different fungi on tea leaves can be by one or two means, via the stomata, the breathing cells on the under surface of the leaf, or by mechanical penetration through the leaf cuticle. Germ tubes which enter through the stomata do not normally form appressoria, which are common in fungi where penetration is through the leaf cuticle. The formation of appressoria at the ends of germ tubes of a germinated blister blight spore therefore suggested that entry into the leaf was by mechanical means. It may interest you to know that even though blister blight was studied for over half a century, we were the first to observe this method of penetration. The observation, which was the result of careful study, completely altered the method of spray application.

In the diagrams I have prepared for you entry is shown through the upper surface of the leaf. We have found that, contrary to the old assertions, entry is mainly from the upper surface; although it is possible for entry of the infection tube to be through the lower surface as well, this method of entry is fairly rare.

Before explaining the growth of the fungus in the leaf tissue it is essential that you should now become acquainted with the internal structure of a leaf, as the eventual emergence of the fruiting bodies of the blister fungus depends to a large extent on this structure.

On the upper surface of the leaf (Fig. 3) is a continuous unbroken cuticle which covers the upper epidermal layer of cells. A similar layer of epidermal cells is present on the lower surface as well, but in this case the cuticle is not continuous but broken at irregular intervals by the formation of stomata or breathing pores through which the exchange of gases takes place. The cuticle, as its name implies is a toughened skin with the chief function of restricting transpiration mainly to the stomata. Most young organs, leaves and stems, before they have attained their full size, possess a very thin cuticle and exhibit considerable cuticular transpiration, hence the necessity of large numbers of leaf hairs on the young leaves and their absence on older foliage. The cuticle thickens as the leaf grows older and this may account for the immunity of older leaves to blister blight infection. The cuticle by reason of its thickness and toughness becomes impermeable and impenetrable.

Below the upper epidermal cells is a layer of palisade cell, which owe their name to the palisade-like structure. The manufacture of food is mostly confined to this layer and these cells are very fully stocked with carbohydrates. On older leaves there may be two or even three layers of palisade tissues. Below the palisade layer and between it and the lower epidermis are irregular-shaped cells which constitute the spongy-tissue or spongy parenchyma. There

afe wide intercellular spaces between these cells which are ideal for the formation of the fruiting bodies of the blister fungus. That is why the fungus appears on the lower surface of tea leaves — the area of least resistance.

To revert back to germination. As the germ tubes and appressoria grow a thin film of mucilage is formed around them, causing their adherence to the leaf or other surface (Fig. 4). The firm attachment of the appressorium to the leaf is of the greatest importance because the next stage in development is the formation of a very fine thread or infection tube at the point of contact with the leaf (Fig. 5). This infection thread forces its way into the leaf through the cuticle but, if the appressorium was not firmly attached, the pressure exerted would cause it to lift and entry could not then be achieved. At this stage of development the spore itself is completely depleted of protoplasm, or food material, which passes into the appressorium and is cut off from the now empty spore by a dividing cell wall in the germ tube. The time required for germination, formation of the appressorium and penetration into the leaf is approximately 16 hours.

If the spore falls on a suitable leaf, and penetration through the leaf cuticle is achieved, the fungal tube widens out within the epidermal cell. At this stage the fungus is independent of external conditions, as the future growth is entirely within the leaf and all necessary food and water are obtained there. Fungicides at this stage are not effective as the growth is sealed from outside influences by the leaf cuticle.

The infection tube from the appressorium passes through the epidermal cell without branching but branches extensively within the next layer of cells near the point of entry. (Fig. 6). Later growth is, however, almost entirely between the cells or intercellular, and extends for some considerable distance to invade a fairly large area. If growth is intercellular the question may well be asked, how does the fungus extract its food from the living cell? Short irregular-shaped or lobed branches enter the cells and extract nutriment but do not immediately kill them. These irregular-shaped organs are termed haustoria or absorbing organs. The cell contents are eventually disorganised and the invaded area becomes visible as a translucent spot, with which all of you are familiar. The time taken for this disorganisation of the cell contents is 8 to 10 days and this accounts for the period taken from infection to the first indication of blister formation — the translucent spot stage. (Fig. 7). Up to this time there is no evidence of the concave depression on the upper surface of the leaf or the swelling on the lower surface.

The invaded cells are now stimulated to further growth — enlargement but not division. This causes the lower surface to bulge and forms the characteristic blister appearance. Obviously, the most convenient spot for mycelial development is amongst the spongy tissue, as the large intercellular spaces and the soft parenchyma cells do not offer the same resistance as the palisade layer.

When infection is over 10 days old the formation of the fruiting bodies begins. A dense growth of hyphae, which are in reality the immature basidia, forms under the lower epidermis. The hyphae are directed towards the lower surface and are arranged in dense tufts between the parenchyma cells. With growth and pressure of these developing basidia, the lower epidermis is pushed

cut slightly and forms small elevations which house the growing fertile hyphae. In this process some of the cells of the spongy tissue are crushed and others dislodged, while the pressure exerted in the leaf becomes greater and greater. This stage may be seen 3-4 days after the formation of the translucent spot (Fig. 8). Externally, as a result of the pressure exerted, the lower epidermis and cuticle rupture, leaving exposed the tufts of hyphae which form the white fruiting body or hymenium. (Fig. 9). These fertile hyphae or basidia produce spores at their extremities and the process of spore discharge commences.

The duration of spore discharge is governed by a number of factors. Infection with brown blight, which invades and kills the infected tissues, may shorten this period very considerably but, under normal conditions, spore discharge may continue for seven or eight days until the invaded cells become exhausted of food and the blister fungus, finding conditions unfavourable for growth and nourishment, dies.

To summarise, the life cycle of the blister fungus is —

- (1) Germination and the formation of the appressorium in a period of 6 to 16 hours. Up to this stage the fungus is very vulnerable to fungicides, desiccation and sun injury.
- (2) In 16 hours penetration into the leaf is achieved and the fungus sealed from desiccation or fungicidal action. The spore and appressorium are now devoid of food and the fungus in the leaf has become truly obligate on the leaf tissues.
- (3) In 8 to 10 days the first sign of infection is visible as a translucent spot caused by the disorganisation of the leaf cells.
- (4) 15 to 17 days after entry into the leaf, that is 7 — 9 days following the translucent spot stage, the fungus breaks through the lower epidermis and sporing commences.
- (5) The white blister is very evident 18—21 days after infection.

The effects of spraying are only visible after three weeks, as a crop of blisters is the visual result of infection three weeks earlier. If spraying commences a few days after infection blisters will still mature and spore normally. Copper spraying is protective and not curative. Your chief aim should be to commence spraying operations before the advent of a heavy crop of blisters — commencement of your spraying at a late stage serves only a limited purpose as it is essential to keep down blister formation and spore populations to a very low level for efficient protection.

BLISTER BLIGHT—LIFE CYCLE OF CAUSATIVE FUNGUS.

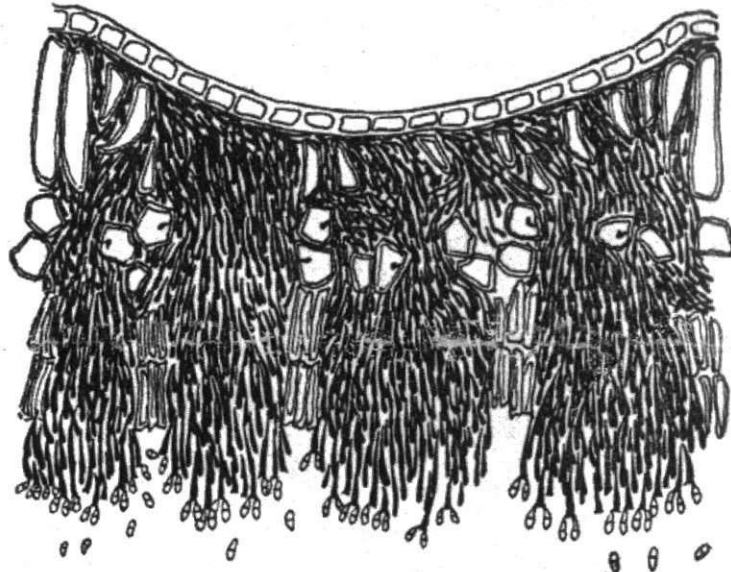


Fig. 2. Transverse section of a white spring blister showing concave depression of upper surface of leaf, fungus in the leaf and spores on basidia.

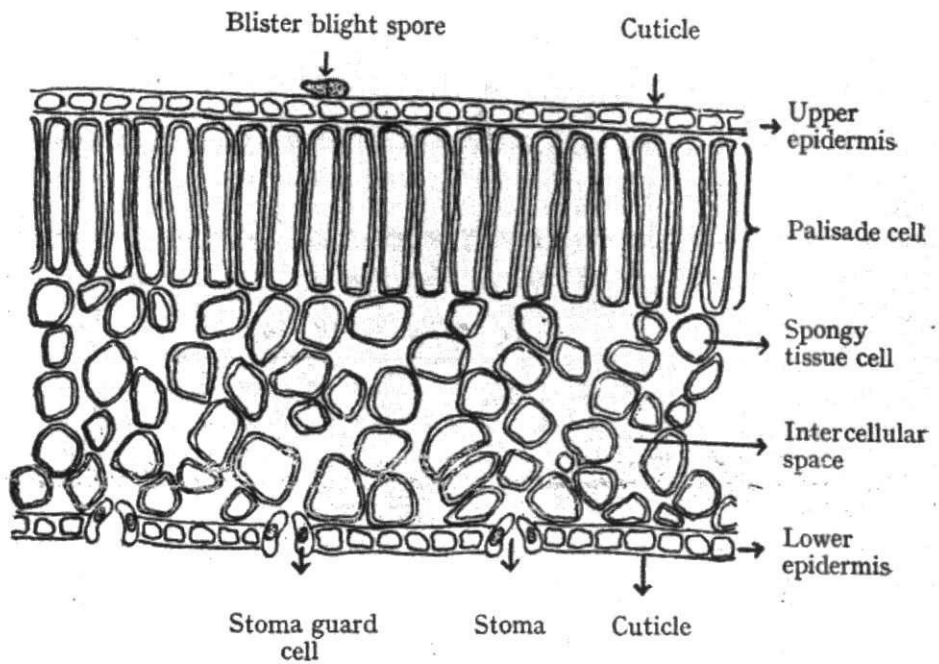


Fig. 3. Transverse section of young tea leaf showing internal structure. Ungerminated blister blight spore on upper surface of leaf.

BLISTER BLIGHT—LIFE CYCLE OF CAUSATIVE FUNGUS.

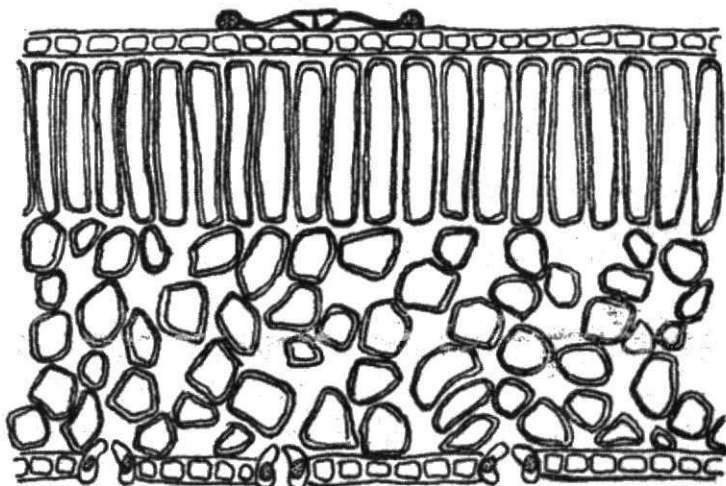


Fig. 4. *Transverse section of young tea leaf showing formation of appressoria of germinated blister blight spore.*

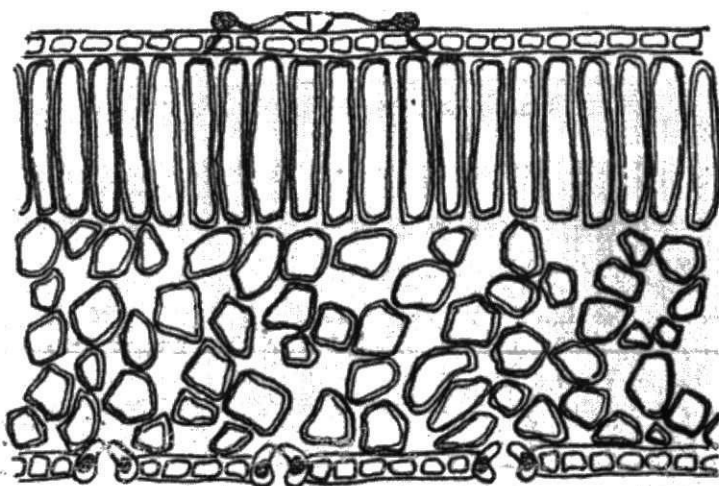


Fig. 5. *Formation of infection tubes from appressoria. Infection tubes have passed through the upper cuticle of leaf.*

BLISTER BLIGHT—LIFE CYCLE OF CAUSATIVE FUNGUS.

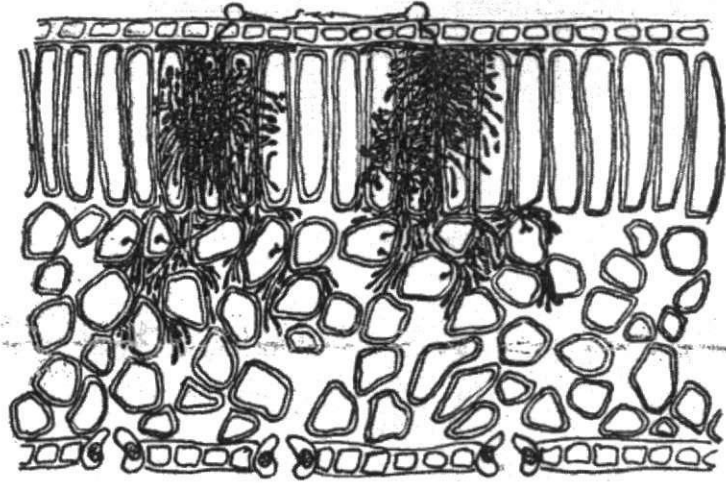
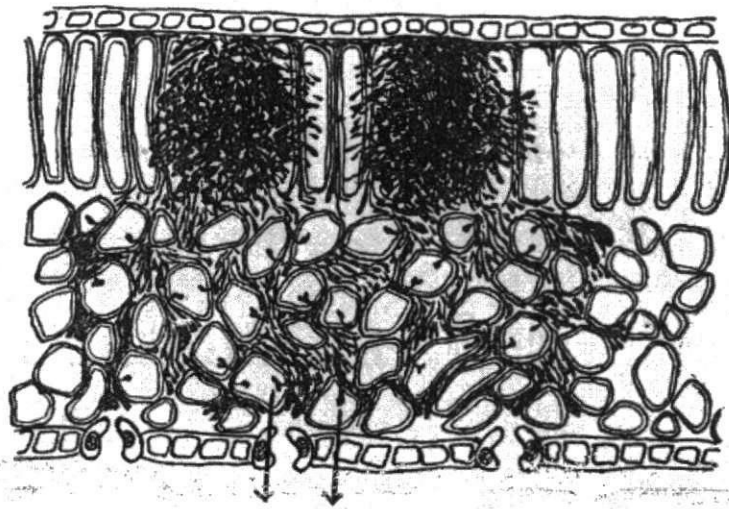


Fig. 6. Proliferation of the fungus in the leaf tissues—four days after entry into leaf. Note that spore and its appendages have collapsed. The fungus at this stage is obligate on leaf tissues.



Haustoria.

Fig. 7. Translucent spot stage showing formation of haustoria (food absorbing organs).

BLISTER BLIGHT — LIFE CYCLE OF CAUSATIVE FUNGUS.

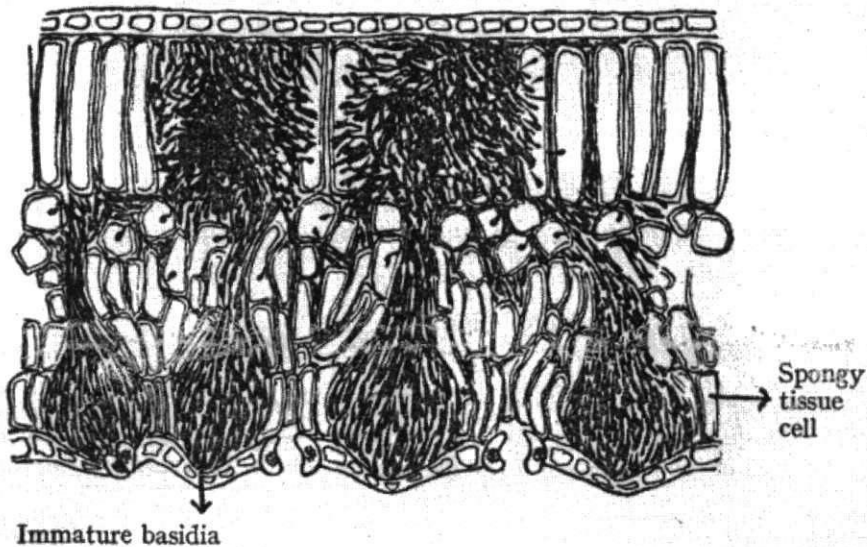


Fig. 8. Formation of basidia or fertile hyphae. Note elongation and distortion of spongy tissue cells.

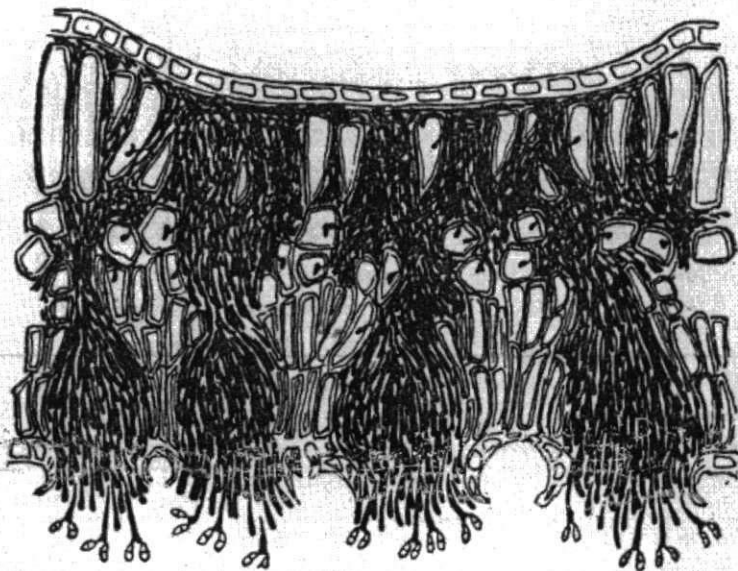


Fig. 9. Formation of fruiting layer. Basidia have broken through the lower surface of leaf and formation of spores has begun. Note concave depression on upper surface of leaf.