THE FUNGUS *EXOBASIDIUM VEXANS.*

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Since its arrival in Ceylon much has been written and said about the Blister Blight leaf disease of tea, but relatively little about the fungus *Exobasidium vexans* that causes it. As the rational treatment of any disease depends upon the knowledge of the causative organism an account of the fungus *E. vexans* may be of interest. At the same time we shall try to indicate how that information bears on rational treatment.

The fungus was described and named *Exobasidium vexans* by Massee in 1898 from material sent to him from Assam by Dr. Watt where it was causing serious damage to tea. The disease (blister blight) was described in North India by Watt and Mann (1903), McRae (1910) and Tunstall and Bose (1921); in Indo-China by Du Pasquier (1933); and in Formosa and Japan by Sawada (1922). It appeared for the first time in South India and Ceylon in 1946.

The disease first becomes evident on young expanding leaves as translucent spots which later become conspicuous circular 'blisters.' Normally the upper surface of the leaf becomes indented to form a circular pit and the lower surface protrudes to form the so-called blister (Plate 1, Fig. 1). Some days later the convex surface of the blister on the under surface of the leaf, becomes white and velvety as the fungus is revealed by the splitting and removal of the leaf's outer skin (the cuticle). Sometimes the convex surface of the blister is on the upper surface of the leaf ; but the fungus still appears on the under surface in the concave area Occasionally the fungus is seen on both sides of the leaf but the bulk of it is usually on the lower side. Young growing stems are also affected but they are never 'blistered' and the fungus appears on their surfaces as white velvety patches which makes the stems appear thicker (Plate 1. Fig. 2). Young stems are frequently bent and distorted. Later, the white areas turn black and dry as both fungus and plant tissues die and the dead tissues may fall out leaving a hole. Buds are also destroyed. The green outer case of the fruit is sometimes infected but the fungus has not been found on seeds.

Most fungus parasites can be grown on media other than living tissues and so can be studied apart from their hosts ; but *E. vexans* is one which will not develop fully on any known medium other than the living tea plant. The main body of the fungus is within the infected leaf or stem and lives there so long as the invaded tissues remain alive.

The living fungus is visible to the unaided eye when it is producing spores. The outer surface of the leaf has then disintegrated and the fungus can be seen as a white sheet. Spores are formed in very large numbers over the whole of this surface which is termed the *hymenium.* The velvety appearance of the hymenium is due largely to the fungus threads being rounded at the ends and standing erect in compact bundles, which are parallel and close together like the pile of a carpet (Plate 2, Fig. 14). Amongst this 'pile' are slightly longer cells (basidia) which project above the general level. They also differ from the surrounding cells in that at their apices are two, three or four minute spikes *(sterigmata)* on each of which stands a spore

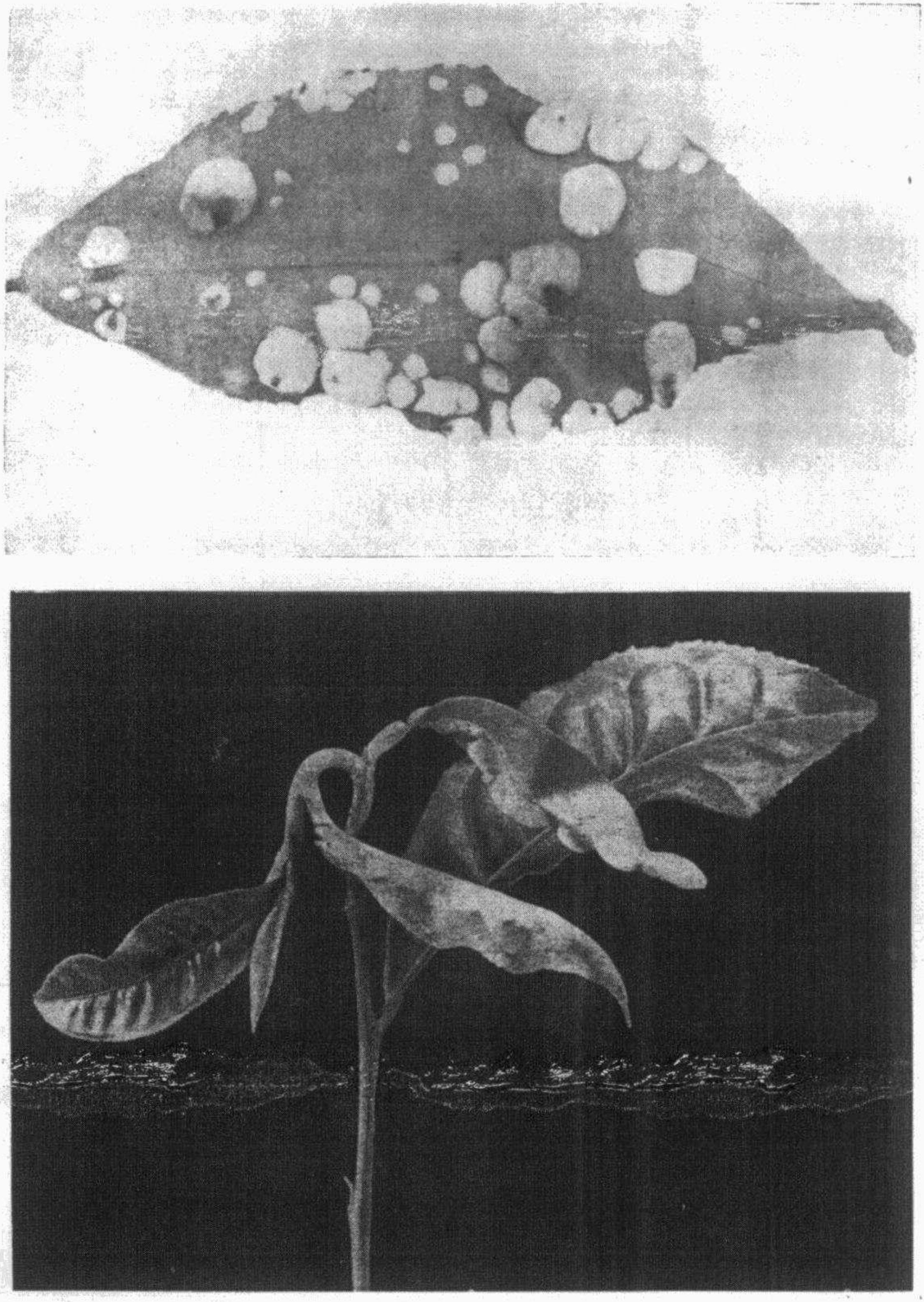

Plate 1. Blister blight on tea. (Upper) Blistered leaf. (Lower) Infected stem.

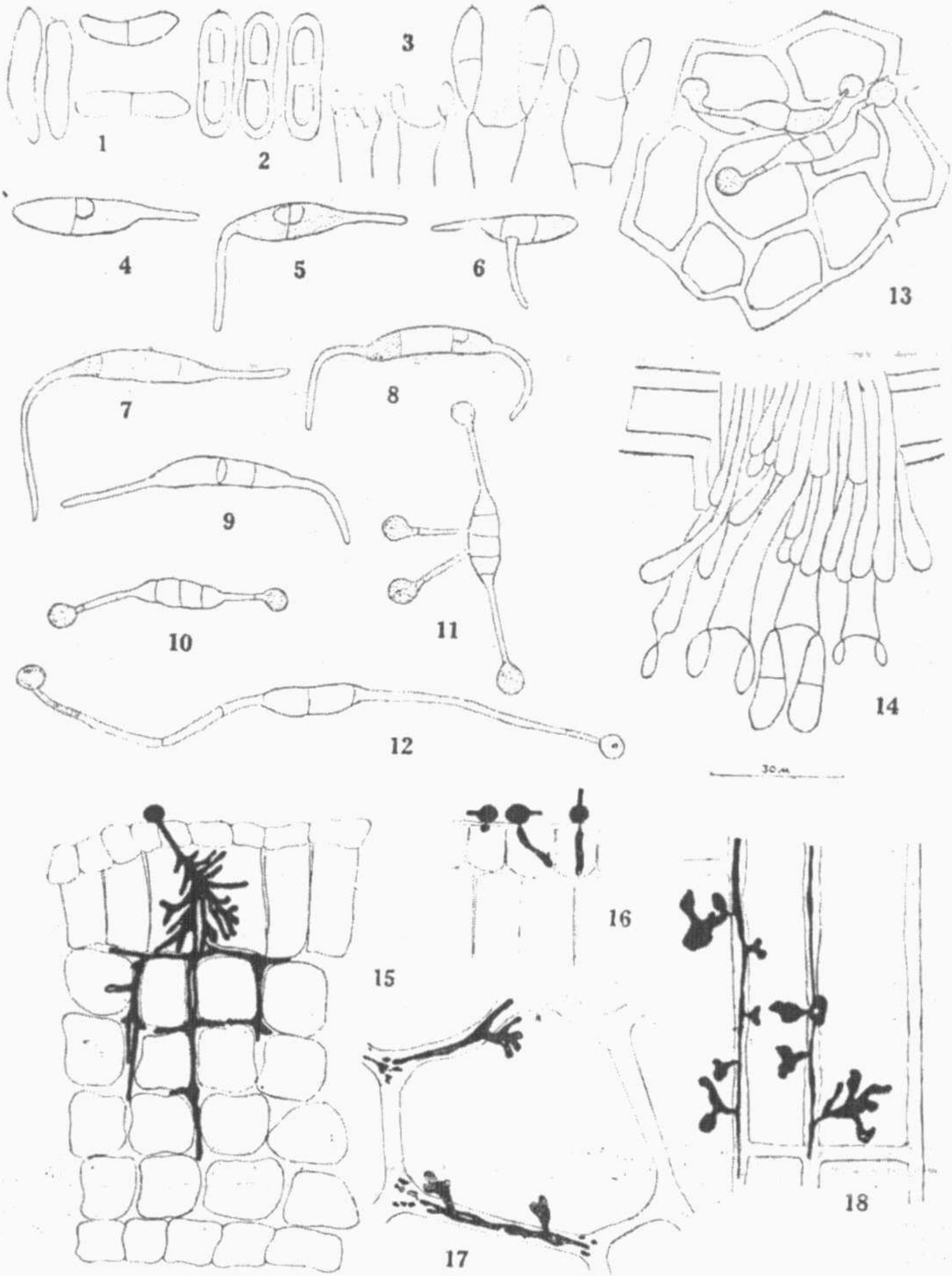

Plate 2. *Exobasidium vexans.* 1, Basidiospores. 2, Thick walled spores (abnormal). 3, Spore development on basidia. 4-9, germinating spores before the formation of appressoria. 10-12, Germinating spores with appressoria. 13, Germinating spores on leaf surface. 14, Hymenium, showing basidia and spores. 15, Section through leaf showing the fungus shortly after entry. 16, The fungus immediately after entry. 17-18, Showing the fungus mycelium between cells and haustoria within the cells.

unless it has already been discharged (Plate 2, Fig. 3). After discharging all its spores the basidium collapses. These details can be determined only with a microscope and specially prepared specimens, and even then, the ripe or nearly ripe spores are usually dislodged during the preparation. In such preparations there appear to be spores of two kinds, smaller ones attached to basidia and larger ones usually consisting of two cells, lying on the surface of the pile. It is now known (Gadd and Loos 1948) that these are but different stages in the development of the one spore. The larger ones are fully mature spores; the smaller ones are not fully grown and are more firmly attached to the sterigmata.

The fully grown spores (Plate 2, Fig. 1) are of microscopic size and about 10,000 can lie side by side without overlapping on an area one millimetre square. So long as an infected leaf remains attached to the bush and the fungus is alive there is a continuous discharge of spores from the hymenial surface; but if the leaf is plucked, discharge soon ceases unless the leaf is supplied with water. The spores can be collected by cutting a fresh blistered leaf in the evening and arranging it with its cut end in water and the blistered surface just above, but not touching, a piece of glass, so that the spores will fall on to it. The whole is then covered with a large jar or other vessel so that the air currents do not carry the spores away. The next morning a white film of spores will usually be found on the surface of the glass. A very rough estimate of their number can be calculated from measurements of the area covered; the number will probably exceed a million. Other similar collections can be obtained from the same blister. No estimate of the total number of spores formed by one blister has been made, but an experiment of this sort shows it to be gigantic.

On a few occasions thick walled spores have been observed (Plate 2, Fig. 2). These are undoubtedly a later development of the thin walled spore. As yet they have not been seen to germinate. They have been found on old dried blisters and it seems doubtful that they serve any special function.

A piece of glass bearing a deposit of spores may be placed in running water, as from a tap, without dislodging the spores. They stick equally well to other surfaces such as cellophane, plastics and leaves. This adhesive property of the spores is probably of considerable importance to the fungus as it prevents their removal from leaves by heavy rain; it holds them in position till conditions are favourable for germination. Many find lodgment on unsuitable bodies and only a minute percentage of the spores formed can ever reach tissues in which they can develop. Gigantic numbers are necessary to ensure the survival of the species.

The spores are so light that they fall slowly in absolutely still air and any small air current will carry them away. The following experiment demonstrates how small, imperceptible air currents can carry the spores appreciable distances. An infected seedling was placed with the diseased leaf held vertical at one end of a large glass sided box which could be tightly closed. Pieces of glass were arranged on the floor and in different parts of the box before it was closed. The next day spores were found on all the glasses even those on the floor 30 inches away from the leaf. Similar results were obtained when the diseased leaf was held horizontally with the hymenium downwards. As the small air currents in the box can carry away the spores it is easy to imagine what occurs in the open air. Upward currents will carry the spores to great heights and horizontal currents will convey them great distances.

How the spores reached Ceylon is largely a matter of speculation. From the evidence available it seems highly probable that they arrived by wind from South India, where the disease occurred a few months earlier than in Ceylon. They later travelled from the Western to the Eastern side in the same way. An alternative hypothesis is that they came attached to merchandise, clothing or other vehicle, but it has a serious objection. The spores would adhere firmly to such vehicles; but how would they become detached and then attached to a tea bush? Wind would carry a large number of spores, but only a very small percentage of those dropping on tea areas would lodge on tissues favourable for further growth. Infections would be likely to occur at widely separated places almost simultaneously. That is in fact what was observed. Within three weeks of the disease being first reported it was found on five widely separated estates in different planting districts. The importance of the three week period will become evident later.

Mention has been made of the sticky nature of the spores and the suggestion made that it prevented their removal from leaves by rain. It would, however, also prevent the spores from falling free into the air from the surface on which they are formed unless that surface were on the underside of horizontal leaves. Although the spores are formed on the lower surfaces of leaves, many diseased leaves and most diseased stems do not stand horizontally and so the chances of spores falling on and adhering to the leaf or stem on which they are formed are considerable. This risk is diminished very considerably by the spores being forcibly discharged into the air. The distance they are shot into the air is very small, only a small part of a millimetre, yet it is sufficient to free the spores from the surface and give them a clear start. As we have already seen even small convection currents are sufficient to carry them away. What happens later is purely a matter of chance. Some may be carried long distances and others will find lodgment nearby. Only a very small percentage will fall on young tea leaves and stems. How large a number is represented by that very small percentage is evident in the fields.

The spores do not begin to grow until they are wetted, and they germinate best in a minimum of water. A thin film of water is more favourable for germination than is a spot, and ideal conditions are obtained when water vapour condenses as dew around the spore. This is probably one of the reasons why the disease is often most prevalent in areas liable to mist, and in hollows where dew forms early.

The first indication that a spore has begun to germinate is the emergence of a thin tube from one or both ends of the spore (Plate 2, Figs. 4-9). This many occur very soon after the spore is wetted. On occasions, these germ tubes have been observed in the laboratory in one and half hours after wetting. Sometimes, a third and even fourth germ tube may emerge from one spore; these come from the side walls. The contents of the spore flow into the germ tubes leaving the spore case empty. The normal number of germ tubes is two, one from each end, and the empty spore then has one wall dividing it into two compartments. When three germ tubes arise the empty cell has two dividing walls making three compartments. The number of germ tubes arising from a spore depends upon the number of dividing walls within it, as each compartment may give rise to a germ tube.

The germ tubes normally continue to elongate until they are equal to three or four spore lengths when each tube begins to enlarge at the tip to form a ball-like termination. These spherical bodies at the end of the germ tubes are termed *appres-*

soria. When fully developed they are dark-coloured and filled with protoplasm from the germ tubes which then appear almost empty (Plate 2, Figs. 10-12). As the germ tubes and appressoria grow, a thin film of mucilage is formed around them causing them to adhere to the leaf or other surface.

The firm attachment of the appressorium to the leaf surface is of the greatest importance because the next stage in development is the formation of a very fine thread at the point of contact with the leaf. This thread forces its way through the outer covering into the leaf. This mode of entry is purely mechanical and if the appressorium were not firmly attached, the pressure exerted would cause it to lift and entry could not be achieved (Plate 2, Fig. 16).

It should be noted that entry into the leaf is gained, not through small pores (stomata) in the leaf surface as was previously supposed (Tunstall and Bose 1921) but forcibly through the cell walls. If entry was always through stomata only, infections would occur almost invariably through the lower surface of the leaf as there are very few, if any, stomata on the upper surface. For this reason, when spraying with a fungicide, growers were advised to pay special attention to the lower surfaces of the leaves as that was regarded as the most vulnerable area. In laboratory experiments we find that infection can be obtained through the upper surface as readily as through the lower. When entry is through the upper surface the white blister is still formed on the lower surface. The position of the blister and its sporing surface, therefore, afford no clue as to which surface the fungus entered.

Before describing the further growth of the fungus after entry into the leaves some further information regarding the spores themselves may be of interest.

The spores are relatively short-lived. In the laboratory at St. Coombs they normally remain viable for about a week, but freshly collected spores fail to germinate after exposure to direct sunlight or to a temperature of 35°C (85°F) for one hour. Although fresh spores normally germinate readily under favourable conditions we have on numerous occasions failed to germinate freshly collected spores though spores collected later from the same blisters have germinated normally. The method of germination, particularly the surface on which the spores were placed for germination, was suspect but such abnormal results have been obtained when tea leaf surfaces were used and particular attention paid to water requirements. No satisfactory explanation of these results can be offered though they afford evidence of the existence of an unknown factor deleterious to the spores.

The fact that the spores cannot survive high temperatures suggest that the fungus did not reach South India in the spore form by an overland route from North India. Any spores adhering to merchandise, clothing or such like vehicles would not survive the high temperature experienced during the journey. That probably explains why South India and Ceylon remained free of the disease for so many years. It has already been suggested that the spores reached Ceylon in air currents from South India but a similar explanation of its arrival in South India is not satisfactory because of the failure of the fungus to arrive that way in earlier years.

Because of the high temperatures in the Low-country the disease is unlikely to become a serious pest there. The disease will appear there from time to time when conditions are favourable but it is unlikely to assume the importance it has acquired in the higher districts.

The disease is most prevalent during periods of little sunshine, but its prevalence then is due more to other meteorological conditions, e.g. rain and mist which normally prevail during such periods, than to the absence of sunshine and its disinfecting property. Shade trees, by limiting the amount of sunshine reaching the bushes, may thereby favour the occurrence of the disease but their importance in this respect may easily be overrated.

The time taken for the spores to germinate and gain entry into the leaves or stems varied with external conditions, particularly temperature and the presence of water. In one experiment spores from the same blisters were germinated on glass at 15°, 20° and 25°C. (59°, 68° and 77°F.). The spores germinated best at 25°C; after 4½ hours fairly long germ tubes were visible whereas the spores germinating at 20°C were just starting to grow. The spores at 15°C did not begin to grow for about 7 hours. The times reported here are, however, not constant as spores have been seen to begin germination in 1½ hours after being wetted at room temperature (*circa* 20°C.).

The time taken for the fungus to gain entry into the tea plant is of interest though it is impossible at present to state with any accuracy the minimum or average times for any set of conditions. The shortest time observed is 8 hours under laboratory conditions with the temperature about 20°C. That observation shewed that a few hours of favourable conditions are sufficient to allow infection. When the fungus has entered the host plant external climatic conditions are of little importance for its survival.

The essential requirement for the germination of a spore is water. Spores may find lodgment on suitable leaves but they cannot begin to grow till they are wetted. But very little water is required. In the evening when dew forms, the spores' requirements are fulfilled and growth may start. A few hours later an appressorium is formed from which a penetration tube is sent into the leaf. During this time the germinating spore must be moist. The interval between dew formation and its dispersal next day is often sufficient for the fungus spores to grow and enter leaves as shown by the later formation of blisters. That is to be seen frequently in the fields, most frequently in shaded places. Such observations lead to the conclusion that shade favours the disease.

That conclusion cannot be disputed, though it might be well to consider it in greater detail before remedial measures are based on it. We have seen that shade may prevent the sun from exercising a disinfecting action on the dry spores which may have lodged in the bushes, and that by delaying the evaporation of dew it may prolong the period of favourable conditions thereby allowing more spores to gain entry into the bushes. The times when Blister Blight is most to be feared are when there is little or no sunshine and there is sufficient rain or mist to keep the bushes wet. Then the presence of shade trees is not of the least importance, as they do nothing to increase the incidence of the disease. Shade as a factor affecting the incidence of the disease is, therefore, of importance only at times, in the main, unfavourable to the disease. At such times diseased leaves can be found in shaded areas when less shaded areas are free from the disease.

It may be argued that if the fungus survives in shaded areas at times when it is being exterminated elsewhere by unfavourable conditions, a source for later reinfection of the freed area is maintained. That is true and it would be of greater importance if shaded areas were the only possible sources for reinfection. The complete removal of shade from all tea areas would *not* result in the eradication of the disease, and should not even be contemplated. It is doubtful that a good case could be made out for the complete removal of shade from any tea area. The benefit derived from shade trees in the form of organic matter for incorporation in the soil as a rule far outweighs the additional Blister Blight damage resulting from their presence. What is required is control and not elimination of shade trees, so that sunlight is admitted into the area at times when it is likely to be beneficial.

After this digression we return to the fungus which has just entered a leaf. A very fine tube from the appressorium has been pushed through the leaf's outer covering, the cuticle and cell wall, and has enlarged to normal thickness within an epidermal cell (Plate 2, Fig 16). At this stage the fungus is independent of external conditions as future growth will be all within the leaf, and the necessary food and water will be obtained there. The tube (hypha), which has entered the epidermal cell continues to grow and leaves the cell without branching. The fungus frequently branches extensively *within* the next layer of cells (Plate 2, Fig. 15) but later growth is almost entirely *between* the cells (Plate 2, Figs. 15-18). By further growth between the cells a more or less large area is invaded. Short irregular branches *(haustoria)* enter the cells and extract nutriment but do not immediately kill them. The cell contents are disorganised, and the invaded area becomes visible as a translucent spot. At the same time the invaded cells are stimulated to further growth — cell enlargement but not division. This causes the invaded area to bulge, usually towards the lower surface and so forms the blister. Translucent spots become visible after about nine days from infection and the blister formation can be dectected a few days later.

A dense growth of hyphae forms under the lower epidermis. Here the hyphae are directed towards the lower surface and are arranged in dense tufts between the cells (Plate 2, Fig. 14). Some of the plant cells are crushed and others dislodged. The leaf's outer skin, the epidermis and cuticle, is broken and flakes off leaving exposed the tufts of hyphae which form the white hymenium. The hyphae which form the hymenium cease growth and become basidia when they produce spores at their extremities. The time from infection till spores are discharged from the hymenium varies from 14 to 21 days, an average time is about 18 days.

The time during which spores are produced from the hymenium varies considerably, it is shortest in very wet or very dry weather but may continue for 7 or 8 days. By that time the invaded leaf cells are exhausted. They die, become black and later fall out leaving a hole in the leaf.

It should be noted that spores are not formed till about 18 days after infection occurred. For that reason fresh blisters may be observed at times when the weather is fine and apparently quite unfavourable for the disease. One has, therefore, to consider climatic conditions about three weeks prior to the occurrence of the sporing blisters. Towards the end of a dry season fresh blisters are usually very scarce and difficult to find. Relatively few spores are in the air and very few find lodgment on suitable tissues. Many are killed by sunlight, by high temperatures or by old age as the spores normally do not survive more than a week or two. Yet a wet day, mist or a prolonged dew which provides enough water long enough for spore germination and penetration results in a fresh though small crop of blisters three weeks later, no matter what the intervening weather has been like. In this way a very heavy attack can be built up in 8 or 9 weeks. It may build up more rapidly if conditions remain favourable after the first three weeks.

Mention was made earlier of the improbability of spores reaching South India alive by an overland route. But it will be evident that the fungus could be carried across India unwittingly in a living tea plant. We do not suggest that the disease was in fact introduced into South India in that way as we have no evidence of it; we merely indicate the possibility. It would be equally possible to introduce it into Ceylon in the same way if the plant was smuggled past Customs officials. It is not uncommon to hear grumbles regarding the difficulties of getting plants into different countries. When there are regulations against the import of certain plants there are usually very good reasons for those regulations, hard as they may appear. The regulations are made in the interest of the community as a whole and the planting community in particular, the deliberate breaking of them may prove disastrous.

All tea leaves and stems are not equally susceptible to attack by *Exobasidium vexans*. The older the tissues the less liable they are to infection. The most susceptible are the young leaves enveloping buds; and it is easier for the fungus to enter a first leaf (counting from the bud) than a second leaf. Usually infections do not occur on the third leaves. This does not mean that blisters are never observed on third or older leaves. They are seen in such positions but the leaves were younger and much nearer the bud when the infection occurred. The interval of nearly three weeks between infection and the complete formation of a white blister must not be forgotten. In that interval several leaves may unfold.

Moreover some bushes are more susceptible than others. Whenever the disease occurs, even mildly, certain bushes display numerous blisters while adjacent bushes are apparently free. On the other hand in heavy infections some bushes carry few or no blisters at the time surrounding bushes are heavily infected. Such bushes should be sought for when the disease is very prevalent and the best of them propagated vegetatively for supplies. The importance of resistant stocks for supplies and replanting needs no emphasis. The search for and propagation of resistant clones is already progressing satisfactorily on many estates.

The fact that young tissues are susceptible whereas old leaves and stems are resistant affords the reason why the time of pruning is of great importance. The reason for the advice that pruning should be timed to obtain a dry weather recovery will be self-evident from the foregoing. The loss of young foliage and stems as a consequence of attacks by this fungus not only results in delay in cropping but may also result in the death of bushes unless they have abundant starch reserves. Successive defoliations would be disastrous. The aim must be to get a cover of resistant foliage as early as possible but that can be done only at times unfavourable to the fungus.

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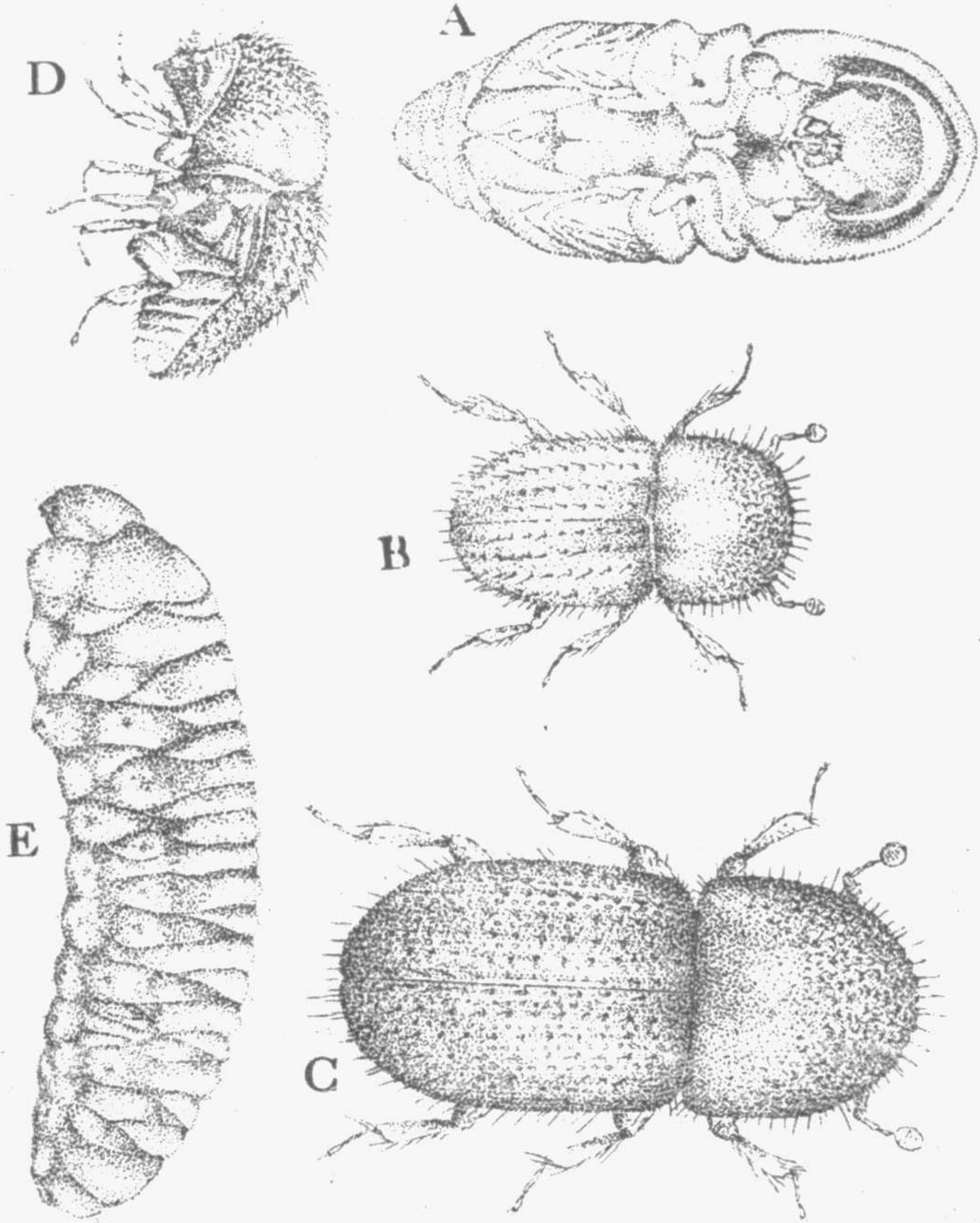

Plate 1. The shot-hole borer of tea (*Xyleborus fornicatus*)
Male B and D. Female C. Pupa A. Larva E.
All x 30. (Drawings by W. T. Fonseka)

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