INFECTION AND IMMUNITY STUDIES ON THE APPLE AND PEAR SCAB FUNGI (*VENTURIA* *INAEQUALIS* AND *V. PIRINA*).

By S. P. WILTSHIRE, B.Sc.

(With Plates XIX—XXII.)

THE diseases of apple and pear caused by *Venturia inaequalis* (Cke.) and *V. pirina* (Aderh.) exhibit many interesting phenomena with regard to the resistance and susceptibility of varieties of the hosts. The investigations described in this paper were commenced with the object of discovering the method by which these fungi attack their hosts, in order finally to investigate the features concerning the question of relative immunity. Notwithstanding the rather considerable amount of work which has been done on these diseases—especially the extensive reports of the Agricultural Stations of the United States—little definite knowledge is available, either as to the minute details of infection or the power of resistance of attacked varieties. The recent paper of Wallace¹ contains an exhaustive review of previous work on these diseases: here therefore it is unnecessary to do more than make a brief reference to those points which concern the subjects dealt with in this paper.

The previous work which has been done with regard to the infection of *Venturia* is limited to comparatively few papers. Aderhold's work² on these fungi is by far the most important that has ever been published, and to him we are indebted for most of our knowledge of them. In his description of the germ tube he states that it broadens slightly at the point of entrance, which is usually directly over the junction of two or more epidermal cells, and bores directly through it. He mentions that the appressoria are frequently surrounded by a zone of mucilaginous material, but his figures show no signs of the collar which is described below, neither do they indicate the characteristic sub-cuticular mycelium.

¹ Wallace, *Scab Disease of Apples*. Cornell Univ. Agric. Exp. Station, Bull. 335.

² Aderhold, R., *Die Fusicladien unserer Obstbäume Land. Jahrb.* xxv, 875-914.

Fischer¹ states that *Venturia* cannot attack a completely uninjured fruit without previous injury to the epidermis. The attack is dependent on the weather, cold nights followed by warm days cause injury to the epidermis. Small cracks arise which can be seen with the aid of a lens, and he explains the conditions favouring infection, as due to the injuries of the cuticle brought about by them.

Voges² notes the presence of a "gelatinous envelope" round the appressoria which he suggests are functionary in attachment of the fungus.

Wallace (*loc. cit.*), by boiling up the leaf with caustic potash and thus releasing the cuticle, gathered that the germ tube of an ascospore bores its way directly through the cuticle and continues to grow between it and the epidermis. His figure, which, as far as the writer is aware, is the only one published of the entrance of the germ tube into the host, shows the hypha growing through a crack without the formation of an appressorium and without showing any difference between superficial and sub-cuticular mycelium.

For investigation of the problems of disease resistance connected with any pathological fungus, it is important first of all to determine the actual details of infection by the fungus. Having ascertained the facts of infection, knowledge as to the fate of the fungus after penetration into the tissues of the host is required. The work described in this paper deals primarily with those two subjects. In the case of the disease under investigation, a detailed study of the germination of the spores of *V. inaequalis* and *V. pirina*, the method of penetration into the hosts and the fate of the infecting hyphae was made. The methods of investigation adopted, the results and their bearing upon the above questions are described in turn below.

Methods. The germination of the conidia was examined by the method of drop cultures, conidia being obtained both from pure cultures of the fungi on artificial media and from affected leaves and fruits of apple and pear. The same sources served for a supply of conidia for the inoculation experiments. The inoculations were carried out on cut shoots and fruits under bell jars or on shoots which were enclosed in lamp chimneys held in position by clamps on the trees. The atmosphere was kept saturated with water vapour, and the inoculations were kept moist by means of addition of sterilised water from time to time.

¹ Fischer, *Pflanzenkrankheiten*, xix, 432-434.

² Voges, *Zeitschr. Pflanzenkrankheiten* xx, 385-393.

These very artificial conditions were rendered necessary by the peculiar and special conditions under which the fungus infects the host and even under these, apparently the most favourable, infection is by no means always successful. There must remain, therefore, some slight possibility that the results obtained are simply pathological, but since the growth of the mature mycelium in nature is in many ways similar to that of the germ tube in infection, it is thought that this possibility is not very great.

Methods. Examination of inoculations was carried out by the ordinary method of serial sections, the fixatives mostly employed being chromacetic and acetic alcohol, with fuchsin and Licht Grün, and iron haematoxylin as stains. The course of infection was also watched from surface view from permanent preparations made as follows: inoculated portions were fixed in acetic alcohol for about half an hour, then washed and decolorised by absolute alcohol and finally mounted in Gilson's mounting medium, which is not so refractive as Canada balsam.

Germination. The conidia of *V. inaequalis* and *V. pirina* germinate readily in water, most of them showing a germ tube of considerable size after 24 hours, although some take a much longer time. The conidia are easily wetted, and their specific gravity is so heavy (or immediately becomes so on moistening) that on immersion in a drop of water they almost immediately sink to the bottom of it, where some change takes place, so that the conidium becomes attached to the surface on which it is resting. The comparative difficulty with which conidia about to germinate are washed from the slide on which they have rested suggests a definite attacking envelope, but although indications of an outer gelatinous layer have been seen under the microscope they are in all probability optical illusions, for similar indications are found with conidia of other fungi. In nature the germinating spore must after a short time experience desiccation and other changes due to the weather, but it is an extremely important point with regard to the beginnings of infection, that notwithstanding subsequent drying up germination can only be initiated after complete immersion in water for some time—a fact originally discovered by Aderhold, but perhaps hardly fully appreciated by the few more recent workers. It seems indeed rather curious that in an atmosphere saturated with aqueous vapour a spore will not germinate; and, as will be seen later, the correct appreciation of this fact, which has been repeatedly confirmed by the author, is of importance in understanding some of the characteristics exhibited by these fungi. Immersion in

water for a sufficiently long time having once initiated the first stages of germination, subsequent periods of desiccation seem to help rather than hinder the growth of the fungus. The appearance of the germ tube in the two species is characteristic, for in *V. inaequalis* it is rather a continuation of the spore, being formed by the more pointed end growing out as the germ tube; whilst in the case of the *V. pirina* it is sharply defined, arising as a lateral growth near to one end of the spore.

In a hanging drop, the germ tube continues to grow out as a long thin colourless hypha, but on the leaf desiccation soon brings the germ tube in contact with the cuticle and this immediately brings about the formation of an appressorium, the stimulus of contact, shown by Aderhold, causing the germ tube to swell up and at the same time to be closely applied to the cuticle. Some substance is excreted round it so that a flange-like structure is formed round the end of the germ tube, fixing it firmly to the cuticle (see Fig. 1). The changes in shape of the fungal hypha and the exact method by which the flange was formed could not be definitely followed, but the latter seemed to arise as an excretion from the fungal hyphae, no part of the cuticle being involved. At first the flat portion of the flange, which is usually very thin, especially at the edges is colourless; but it soon becomes brown and can readily be seen under the microscope. These "collars" or flanges are much more prominent in some cases than in others, apparently depending upon the state of health of the spore and the conditions of growth of the fungus. The nucleus can usually be seen to be very evident in the appressoria denoting probably great metabolic activity in this region. The formation of the appressorium is not limited to the germ tube, as the tip of any growing hypha may respond to the stimulus, although there is no evidence that the mature mycelium is capable of doing so. If after the formation of the appressorium, the spore is still kept immersed in water, the appressorium begins to put out a hypha from its upper surface, and later its appearance may suggest that it was a lateral outgrowth from the mature hypha. The germ tube generally forms only one appressorium, normally at a short distance from the spore, but sometimes it branches and even develops a short superficial mycelium producing five or six appressoria.

Aderhold and many other workers have noted that the germ tubes frequently swell up into the appressoria over the junctions of the epidermal cells, especially where three or more cells meet. Very careful examination of inoculations in superficial view has been made

and there is apparently a tendency for appressoria to be formed at the place recorded. Further details on this point are referred to later.

Penetration. A point of great importance in infection is that only young leaves or fruits are capable of being infected. This fact has been firmly established both by observations in the field and by direct experiments. In July the ordinary summer pruning was carried out in the nursery at Long Ashton, and at the end of September a good number of young shoots had developed from the dormant buds which had thus been roused into activity. On the susceptible varieties this young foliage was heavily infected while the mature foliage showed no recent infection. Aderhold has noted the liability of the young organs to be attacked, but recently Wallace cited instances where *V. inaequalis* spread rapidly amongst apples in storage. Whilst under certain conditions therefore mature organs may be successfully infected, it appeared certain in the case of the trees under inspection at Long Ashton that little late infection took place.

To avoid possible misunderstanding it should be explained that the use of the term "infection" in the previous paragraph includes not merely the penetration of the fungus into the tissues of the host, but also its development afterwards to such a degree that the infected area carrying a crop of conidia can be seen with the naked eye. It is possible that penetration may occur on older foliage and fruit: but if so, the fungus appears unable to grow to any appreciable extent in the tissues of the host.

In view of the above it is clear that normal penetration of the cuticle and further growth of the fungus must be followed on very young leaves. When the appressorium is firmly established a growth of the hypha into the cuticle takes place, beginning as a small bulge from the centre of the attached disc (Fig. 5 and compare Figs. 11, 12). The hypha usually grows directly into the cuticle at right angles to the surface of the leaf, but occasionally it is inclined obliquely (Figs. 6 and 7). As it penetrates inwards it very often becomes enlarged, possibly in order to secure the further attachment of infecting hypha. The cuticle being extremely thin in the case of young leaves, the wall of the epidermal cells is reached almost at once by the hypha which then begins to grow between the epidermal wall and this cuticle, forming a plate-like mycelium of very characteristic appearance (Figs. 2 and 3). The cells at first are very thin and the plate is on y one cell in thickness, but soon the fungus gets food from its host and the hyphae begin to

increase in size and divide so as to form a pad of cells, sometimes pushing the cuticle outwards, sometimes crushing the epidermal wall inwards. This subcuticular mycelium continues to grow out in all directions, sometimes long arms of the mycelium projecting from the original point of infection. It may at first consist of one long hypha which will divide to form the mycelium afterwards, but usually the mycelium spreads in a plate-like manner. The cells always remain hyaline and do not become brown as in the ordinary superficial mycelium. The growth of the mycelium seems to be hindered to some extent at the junctures of the epidermal cells, for it is not uncommon to find one epidermal cell covered with mycelium and most of the neighbouring ones quite free (Fig. 4).

The exterior walls of the epidermal cells are usually convex, and projecting portions of the cuticle fit in between adjacent cells. In passing from above one epidermal cell to the next the fungus almost always keeps between the cuticle and the epidermal walls without dissolving away the former to any great extent. Therefore it seems probable that for mechanical reasons it might be difficult for the fungus to pass over these junctures, and especially would this difficulty be felt by the older mycelium growing out laterally from the original invading strand rather than by the young hyphae meeting the juncture at right angles. The attack of one of these scab fungi at first has no apparent influence on the epidermal cells, the nuclei remaining in their normal position which is near the lower walls; but later various changes may take place, the cell contents being frequently conglomerated into a granular gumming substance, especially with artificial infections made in bell jars. A substance which gives the red colour characteristic of wound gum, with phloroglucin and hydrochloric acid, has also been found excreted into the intercellular spaces of the leaf of the pear when its lower surface is attacked. The final effect usually produced on the affected leaf is the extension of the palisade layers into a kind of phellogen round the scabbed area, this development frequently causing the leaf to be somewhat curled.

Sometimes in the thickened region of the cuticle above the vertical walls of the epidermal cells there appears a structure resembling a hypha cut in transverse section and a similar structure can occasionally be seen strictly following the junctions of the cells, if an inoculation be examined in superficial view (Fig. 25). It does not seem that the infecting hypha always behaves in this manner before it enters upon its true subcuticular position: but when it is remembered that the

appressoria appear to be formed over the same points, it seems to afford some significance, the importance of which is discussed below.

The subcuticular mycelium having been formed in the leaf, the uppermost cells of the stroma begin to enlarge, and push out the cuticle until they burst it open and form the conidiophores from which conidia begin at once to be cut off.

The method by which the conidiophore passes through the thick cuticle on the fruit may be of interest as affording a comparison with the method of entrance. There are two ways in which it does this: (a) by eating away the cuticle in exactly similar manner to which it infects, (b) by growth of the subcuticular stroma at right angles to the surface so that the cuticle is burst off. The fungus lives typically between the cuticle and the outer wall of the epidermal cells, but on the fruit it does not simply force its way between the layers. It also partially eats away the cuticle as it is invariably seen that this layer is thinner where it covers the mycelium. Sometimes at the edge of the scabbed patch the actual hypha can be seen completely embedded in the cuticle. Hence there is absolutely no doubt that there is some cuticle dissolving excretion exuded by the fungus in its passage through this layer.

It is not purposed to continue a description of the life-history further, since in a resistant variety the fungus never reaches a stage beyond that already described. In the following account of the resistance and susceptibility of the hosts, reference will be made again to various stages in inoculation of other portions of the plant; but the above account is typical of what happens in the normal infection of a young leaf.

The Question of Immunity.

Perhaps no other important disease exhibits such irregular behaviour as these fungi as regards their immunity. For example, it is well known that one variety may be heavily attacked one year and remain almost immune the next, although the organism may be quite near. Wallace says "it is found that certain varieties may be resistant in one year and susceptible in another year under conditions which for average varieties are as favourable to the disease in the one case as in the other." Salmon reports that at the Wye College Cox's orange pippin had almost every shoot attacked by *V. inaequalis* but at this station extremely little of this fungus was found on the variety at all during 1914. It is considered that the suggestion which at once occurs, viz. that this

characteristic behaviour of the disease may be due to variations in the climatic conditions at the time when the hosts are in most susceptible condition, is not a sufficient explanation, and Wallace thinks that the fungus itself is adapting itself to life on the "resistant" hosts. Again, too, there is great variation in the parts of the host attacked. Sometimes it is especially the stem, sometimes the leaves, sometimes the fruit. Again, the part of the host especially attacked, whether leaf, stem, or fruit, varies considerably under conditions of which we know little. The absence of adequately definite facts makes it at present unprofitable to formulate hypotheses to account for these phenomena, but on fuller knowledge it seems probable that much of the accessory factors connected with the immunity of these fungi might be explained. In the following instance some such explanation is attempted. It is well known that the pear fungus appears on the fruit first and on the foliage afterwards, whilst in the case of the apple the conditions are reversed. Now it seems likely that the very thick woolly coating of hairs on the young fruit of the apple very efficiently protect it from becoming wetted and consequently infected. Later when this covering disappears infection very readily takes place. There are many fungi in common with *Venturia* which attack their hosts in a more or less localized portion, and it is usually the young organs which are most susceptible. There seems to be a definite correlation between the age of the leaf and its powers of resistance. We might point to a parallel amongst animals in the everyday fact that the young are more susceptible to certain diseases than adults. In the case of plants it is not known whether this increased resistance accompanying age is due to the better protection of the host against attacks of fungi, such as might be afforded by the thickening of the cuticle, or whether the limiting factor is intimately associated with the life of the living cell, and the fungus is killed after penetrating the cuticle. Definite experiments were therefore made to find out what happens when spores attack the mature leaves of various varieties of some of which the young organs are susceptible. For this purpose the mycelium from a pure culture was used for inoculations, in order that the fungus might have a larger reserve of food material to sustain itself in the attack than that contained in the spore. In the case of the pear William's Bon Chretien, a susceptible variety, the fungus developed normally up to the stage of the formation of the appressorium and the issuing of the hypha from its underside into the cuticle. Instead, however, of the infection hypha penetrating completely through the cuticle, and developing

immediately a subcuticular mycelium, it showed a great tendency to grow out horizontally into the cuticle (Fig. 21), which on the older leaves is of sufficient thickness to allow this. There seems some preference of the fungus for the outer portion of the cuticle, rather than that adjacent to the epidermis, but finger-like processes, especially from the underside, are given off as branches from the main hyphae. Sometimes these processes reach the epidermal cell-wall and the formation of a subcuticular mycelium takes place: but it was never observed to reach any advanced stage in development, always remaining very simple (Fig. 22). After a varying interval of time the cells of the fungus die off, their contents becoming converted into dark brownish spherical granules. From very many inoculations on mature leaves, both in bell jars and on the trees in plantations, no conidia have ever been produced. Even when the mature leaves of a resistant variety are inoculated, the same course of events is followed. Fig. 20 shows the dead hyphae of *V. pirina* in the cuticle of the Catillac pear, a resistant variety which was very heavily inoculated with pure mycelium. It will be noticed also that the epidermal cells show the beginnings of the formation of a phellogen, which in the normal progress of the disease is developed after the attack has developed for some time, and which has been reported by Voges.

In the inoculation of the mature fruit much the same thing happens, although the cuticle here being much thicker it is much easier to follow what takes place. The infection hypha from the appressorium grows directly into the cuticle (Figs. 11, 12 and 13) and then at its innermost end swells slightly until it becomes almost spherical (Fig. 14). Growth can now proceed in all directions, but much more easily in a plane parallel with the surface of the fruit (Fig. 15). Occasionally, however, it penetrates slowly, following its original course (Figs. 16 and 17). The cuticle is apparently laid down in layers, for with all kinds of stains the innermost layers almost invariably take on a deeper colour than the outer ones, and distinct striations can nearly always be seen, especially where it covers the juncture of neighbouring cells of the epidermis. The direction of growth of the hyphae now seems to follow these striations sometimes to an astonishing length, suggesting perhaps that the physical character of the cuticle is an important factor (Figs. 18 and 19). It has been found extremely difficult to infect successfully the mature fruit (using the term "infect" as explained above); and although this is reported to occur under certain conditions the ordinary course of events seems to be for the fungus to die off in

exactly similar manner as when attacking mature leaves as above described.

Although great differences occur between the attacks on certain varieties in successive seasons, yet there are some varieties which always remain highly resistant, if not completely immune, to this disease. It was therefore interesting to determine the fate of the fungus on the young leaves of resistant varieties. For this, Bramley's Seedling and Newton Wonder apples were selected as resistant varieties for *V. inaequalis*, with Cap of Liberty, a well-known cider apple, as the susceptible variety for comparison; also the pear William's Bon Chretien, a variety susceptible to *V. pirina*, was infected, being regarded as resistant to *V. inaequalis*. All were inoculated with conidia of *V. inaequalis* obtained from the leaves of the "Cap of Liberty," and the results were very similar. Appressoria were formed as usual, infection hyphae sent out into the cuticle, but in resistant varieties the adverse effect of the host was quickly felt (Figs. 23, 24, 25) although sometimes the fungus succeeded in penetrating completely through the cuticle and forming a subcuticular pad. This usually appeared quite unhealthy, the cells being smaller and narrower. The cuticle being so thin no horizontal development of the mycelium in it could be detected except in a few cases. Inoculations of *V. pirina* also were made on the fruit of the apple, King of the Pippins, which is susceptible to *V. inaequalis*, but in no case was the infecting hyphae seen not to have penetrated into the cuticle. The apple fungus easily penetrated into the cuticle of the pear and the pear fungus that of the apple. On Bramley's Seedling the fungus exhibited a preference for that portion of the cuticle above the junctures of the epidermal cells already referred to (Fig. 25). Eventually in the resistant types the mycelium ceased to grow and practically died off. With the susceptible types on the other hand a healthy subcuticular mycelium was quickly formed and developed in the normal way described above until the production of conidia resulted.

General Considerations.

The problems of immunity are particularly difficult because in dealing with them we have to deal with fundamental life processes which do not lend themselves to experimental conditions. All the work which has been done towards the elucidation of the limiting factors of immunity has been done with obligate parasites such as the Mildews and the Rusts, but unfortunately none of these fungi will

grow apart from the host. The facultative parasite affords a line of attack of this difficult problem, for after a determination has been made of what can be seen to happen to the fungus within the host of both susceptible and immune varieties it might be possible to submit the fungus to culture apart from the host and in media which may have been directly derived from the host. If we are to attempt to enquire whether the non-infection of an immune form is due to a toxin, or anything of a similar nature, we must follow some such line as this. *Venturia* is quite a unique fungus in its normal habitat between the cuticle and the host: and although the process of infection and subsequent manner of growth in the host may be peculiar to itself, yet it may be well to discuss it in relation to what is known of other fungi. It is not proposed to review the literature on this question in any detail, since these results would not warrant such an exposition, but a brief outline of the principal work has been done, especially that associated with *Venturia* may be given.

Büsgen¹ found with *Venturia* that the appressoria are formed frequently over the epidermal walls perpendicular to the surface, and endeavoured to show that diffusion of some substance takes place at this place and which acts chemotropically on the germ tube.

Aderhold (*loc. cit.*) holds that no particular chemotactic stimulus is necessary, but that there is simply a strong diffusion of the ingredients of the cell contents; this, however, he was not able to ascertain in demonstrable quantities in living tissues. He held that at the junctures of the epidermal cells the external cell-wall must have other properties than those of the flat surfaces of the remainder of the external walls. He records experiments which are not very conclusive but from which he gathers that the pectates (especially potassium pectate) act chemotropically on the fungus and suggests that the middle lamella may so act in nature.

In addition there is the work of Myoshi, Nordhausen, Fulton and others on general questions of chemotropism, but, so far as we know, no other paper discusses the question in relation to *Venturia*.

The question of how the infecting hyphae pass through the cuticle must take into consideration the nature of the cuticle itself. The work which has already been done does not give much information, being chiefly concerned with the distinction of cuticle from cork, whether cuticle contains any cellulose and similar chemical problems.

¹ Büsgen, M., *Ueber einige Eigenschaften der keimlinge parasitische Pilze*. *Bot. Zeit.* 1893, p. 53.

The cuticle is a protective covering layer to all young parts of plants and hence must be capable of very rapid extension. It is not known how such growth takes place, whether the outer walls of the epidermal cells become gradually converted into cutin or whether the cutin itself is a secretion of the cell. The cuticle, however, must not be regarded as being a simple homogeneous body. Von Höhnelt¹ in 1878 stated that the external layers of the cuticle are free from cellulose, whereas those layers bordering on the external walls of the epidermal cells represent cellulose saturated with cutin. More recently Gêneau de Lamartiere in 1906 has seen fit to distinguish the former as the epicuticle. With all the common stains it is found that the portion of the cuticle next the epidermal cells is coloured more deeply than the rest of the cuticle, particularly in the projections which fit between the adjacent epidermal cells; and moreover, the general staining of the cuticle suggests that it has been formed in layers. The outer layers, being continually exposed to complete desiccation, must be under greatly different conditions from those next to the epidermis. Hence it appears probable that the cuticle has a structure of its own: and this may be an important factor in the penetration of the infection hyphae through it.

The permeability of the cuticle is of interest, as regards the chemotropic attraction which certain substances have on fungal hyphae. That substances can diffuse through the cuticle in some cases cannot be doubted, since the copper of Bordeaux mixture slowly passes into leaves of certain plants, such as potatoes, which have been sprayed with it (Barker and Gimingham²). Whether any substance which may render the copper in such cases soluble diffuses out through the cuticle is not known, and the position is similar with regard to the diffusion of substances possibly chemotropic to fungal hyphae. Nevertheless it must always be remembered that such a thing may happen in nature.

The observation that the appressoria are formed frequently over the juncture of epidermal cells, seems to be justified from a careful examination of inoculations in surface view. But it is by no means the case that they are formed only in these positions. On examining 300 appressoria, 26 % were found to be over the centre of the cells and between 14–19 % did not touch the junctures although they were perhaps near them. If we take into account the relative sizes of the appressoria and the cells, this result may not appear to be particularly unexpected. The area within which an appressorium if it fell would

¹ See Czapek, *Biochemie der Pflanzen*, p. 702, 1912 Ed.

² Barker and Gimingham, *Ann. Applied Biology*, Vol. 1. p. 19.

touch the juncture of the epidermal cells would be bounded by a line, the breadth of the appressorium within the edge of the cell; and taking the relative sizes of the diameters of the cells and appressoria roughly as 6 : 1, we should find this area would be $\frac{1}{4}$ that remaining. Hence we might expect the appressoria to touch the junctures of the epidermal cells more often than not. On the other hand, however, other fungi as well as bacteria are said to show a similar liking for that part of the cuticle above the junctions of the walls. The elaborate paper of Fulton, in which he calls in question the whole of the work done on chemotropic attraction of fungal hyphae, makes us suspicious of accepting altogether the works of earlier investigators and it becomes more and more evident that a further study of chemotropism is necessary for the progress of enquiry into the question of the attack of parasitic fungi. It is not yet established whether chemotropism does exist at all; and not until we know, not only whether it exists but also whether it plays any part in infection, can we hope to attempt to solve the question why the fungal hypha enters the host.

If some chemotropic substance does influence the direction of growth of the germ tube, it must either be the exterior layers of the cuticle or something formed interior to them and then excreted. There is no reason to suppose that the exterior of that portion of the cuticle covering the perpendicular epidermal walls has peculiar properties. Nordhausen has suggested that the mechanical checking which the germ tube undergoes on crossing a groove or the collection of water in it may account for the predilection of the appressoria for this position, yet the behaviour of appressoria is quite similar on varieties like Port and William's Bon Chretien which have no grooves (see Figs. 26, 27 and compare Fig. 28). That the cuticle which covers the junctures of the epidermal cells is, however, different from the remaining is shown by the different staining properties it possesses; this fact, taken in conjunction with the occasional occurrence of hyphae embedded there, suggests rather pointedly that this may be a substance chemotropically attractive to the germ tube. At the best, however, *this* chemotropism can be only an accessory factor in infection since hyphae can penetrate the cuticle quite well in the middle of the epidermal cell-walls.

The growth of the infection hyphae from the appressoria through the cuticle itself is interesting in connection with the part played by chemotropism. If the hypha entered solely because attracted by the substances in the epidermal cells or cell-walls of the host, we should suspect that it would bore directly through the cuticle to reach the

subcuticular position. But although the cuticle of organs susceptible to attack is very often so thin as to prevent the horizontal growth of the infection hyphae in it, yet occasionally one finds stages as represented in the Figs. 6 and 7. These figures suggest that there is no guiding influence towards the epidermis, but that the fungus simply eats its way slowly through the cuticle until it arrives between the cuticle and epidermal cell-wall. With mature organs of both susceptible and resistant varieties where the cuticle is thicker, we have still more interesting cases. How can the long horizontal growth of the infection hyphae in the cuticle (a very frequent occurrence) be explained? Does it not rather suggest in place of an attracting substance the presence of something which is repulsive to the invading hypha? The physical layering of the cuticle may have something to do with the direction of growth, but this horizontal habit is not confined to fruits but is also exhibited in mature leaves, where the layering of the cuticle is not nearly so evident on staining. Of course, the tendency is most clearly developed in resistant organs because the young susceptible parts of plants have very thin cuticles, but that it should occur at all strongly negatives the view of the germ tube being attracted by the cell sap of the host. The conclusion seems unavoidable that the fungus infects *in spite of* the host, and that chemotropism if it acts at all must play a very insignificant part in the process.

It has been shown that immunity does not depend on freedom from the initial attack, any more than in the case of the rusts; immunity there depends upon the non-entry of the infection tube into the host. What the limiting factor in immunity is, we can only speculate—it may be simple starvation of the fungus, as suggested by the gradual reduction of growth, or it may possibly be the formation of some toxic substance. With such possibilities existing it might be well to mention here some experiments which were made on the growth of the conidia of *V. inaequalis* in the expressed sap of the young leaves of certain varieties of apples. Young leaves of King of the Pippins (susceptible) and Bramley's Seedling (resistant) were pounded in a mortar and the sap then squeezed out of them. Conidia were then sown in the sap and hanging drop preparations made which, although by no means free from bacteria and yeasts, remained sufficiently pure for the one or two days necessary for the experiment. Control drops were set up with water instead of the sap medium. In all cases the growth of the conidia in the sap was very much slower than in water and a much larger percentage did not germinate. On diluting the sap, the conditions

of germination were gradually improved until in media of which the ratio of sap to water was 1 : 10 no difference from the water controls could be observed. A few further experiments were made with Newton Wonder (resistant) and Cap of Liberty (susceptible) which as far as they went coincided with the results given above. In these sets of preparations the conidia appeared to grow better in the sap of the susceptible varieties than in that of the resistant kinds: but the approach of winter rendered it impossible to carry this work any further for the time being. The little which has already been done seems to support the view obtained from the study of microscopical preparations that the host is antagonistic to rather than attractive towards the parasite.

The facts which are recorded in this paper are therefore considered to suggest that the appressorium, being formed as the result of mechanical stimulus as shown by Aderhold, penetrates into the cuticle, feeding on it as it goes and finally reaching its normal habitat between the cuticle and epidermis where it flourishes, if the attacked variety is susceptible.

Immunity is shown not to depend on the protection of the cuticle and indications are recorded which suggest the probability of the cell sap of the host being in all cases antagonistic to the fungus.

In conclusion I should like to express my deepest thanks to Professor B. T. P. Barker, M.A., at whose suggestion the work was begun, and who throughout has rendered invaluable aid by helpful criticism and advice. I am also indebted to him for facilities for working at the Agricultural and Horticultural Research Station of the University of Bristol.

LIST OF FIGURES.

- Fig. 1. Spore of *Venturia inaequalis* germinating on the fruit of apple Stirling Castle. $\times 1600$.
Fig. 2. Spore of *Venturia pirina* germinating on leaf of pear Louise Bonne de Jersey. $\times 1600$.
Fig. 3. Same showing later stage. $\times 1600$.
Fig. 4. Mycelium of *Venturia inaequalis* in leaf of apple Cap of Liberty, surface view showing delimitation of mycelium juncture of the epidermal walls. $\times 1600$.
Fig. 5. Appressorium of *Venturia inaequalis* on the leaf. $\times 1600$.
Fig. 6. Entrance of infection hypha into the cuticle on leaf of apple Cap of Liberty. $\times 1600$.
Fig. 7. Same. $\times 1600$.
Fig. 8. Same, later stage. $\times 1600$.
Fig. 9. Same. $\times 1600$.
Fig. 10. Penetration of mycelium of *Venturia pirina* from cuticular to subcuticular position in young leaf of pear William's Bon Chretien. $\times 1600$.

- Figs. 11, 12, 13. Entrance of infection hyphae of *Venturia inaequalis* into the cuticle of the fruit of apple Stirling Castle. $\times 1600$.
- Fig. 14. Same at a later stage. $\times 1600$.
- Fig. 15. Same showing horizontal intracuticular development of the mycelium. $\times 1600$.
- Figs. 16 and 17. Same growth of infection hypha directly into the cuticle. $\times 1600$.
- Figs. 18 and 19. Growth of mycelium of *Venturia inaequalis* in the cuticle of apple King of the Pippins, showing how it roughly follows the layering of the cuticle. $\times 1600$.
- Fig. 20. *Venturia pirina* on mature leaves of pear Catillac (resistant). $\times 1600$.
- Fig. 21. *Venturia pirina* on mature leaves of pear William's Bon Chretien (susceptible in young leaves). $\times 1600$.
- Fig. 22. Same as Fig. 21. $\times 2050$.
- Figs. 23, 24, 25. *Venturia inaequalis* on the young leaves of apple Bramley's Seedling (resistant). $\times 2050$.
- Fig. 26. Cuticle of pear William's Bon Chretien.
- Fig. 27. Cuticle of pear Port.
- Fig. 28. Cuticle of pear Catillac.