OBSERVATIONS ON RESISTANCE TO POWDERY MILDEWS

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(With 2 figures in the text)

As little seemed to be known of the way in which certain varieties of a host species resist the attack of mildew, I experimented with *Erysiphe graminis*, *Sphaerotheca pannosa* and *Podosphaera leucotricha*, determining first the course of infection on susceptible varieties, then on the resistant and finally on a miscellany of inappropriate hosts. The results show that, under suitable conditions and provided that the cuticle is not too thick, mildew conidia will germinate on any plant up to the stage of penetration of the cellulose layer of the host cell and of formation of an infection papilla, but that except on susceptible varieties the penetration process is then killed before it can enter the cytoplasm or after it has formed a rudimentary haustorium. I examined with particular care the exact method of penetration of the host cell, and certain points of interest in the germination of the conidia have come to light. Zimmermann (30) in 1924 summarised all earlier work on the powdery mildews.

Methods

Leaves were inoculated by placing fresh conidia on marked areas. Rose and apple leaves were placed on damp filter-paper in Petri dishes; wheat and barley leaves were left attached to the plants, and these were covered with bell-jars. The pieces of leaf were fixed in 1 per cent. chromacetic solution and sections (3–5 μ thick) were stained, diamant fuchsin and light green being the most satisfactory combination. Alternatively, the epidermis was stripped off and stained with cotton blue and lactophenol in order to follow the germination of the conidia, or it was subjected to microchemical tests to determine the effect of the haustorial process on the wall of the host cell. The method of stripping the epidermis proving the more satisfactory from its readiness and simplicity, I generally inoculated the underside of leaves.

Erysiphe graminis

Material. Wilhelmina wheat, Spratt Archer barley and *Agropyron repens* were taken as susceptible plants, each having its physiologic form of mildew, and Norka and Persian Black as resistant varieties of wheat. The plants were grown in pots in a greenhouse. The susceptible varieties, becoming spontaneously infected, served for the supply of conidia.

Thin sections of the cuticle and epidermis of such leaves are difficult to prepare: silicification hardens the cuticle, causing it to be fractured rather than sliced by the microtome. Material for cytological purposes was therefore dehydrated in glycerine and cleared in cedar-wood oil, to obviate further hardening in alcohol and xylol, and embedded in paraffin of high melting-point. Hydrofluoric acid softens the cuticle without apparently affecting the soft tissues, but the discovery came too late to be of use.

Germination on susceptible varieties. The conidia germinate by producing from near one end a straight or somewhat flexuous, clavate, *primary* germ tube, 20–40 μ long $\times$ 4·5–6 μ wide at the apex $\times$ 3 μ wide at the base. It is cut off by a septum near the conidium and it proceeds directly to form a haustorium either from the immediate underside at 1–3 μ from the apex, or from the apex itself, if pressed into the groove between two epidermal cells, or from a small subapical appressorium, as on the vegetative hyphae. After this first haustorium has been established, the germ tube continues its apical growth as an ordinary hypha. The original "germ tube portion" becomes the first cell and haustoria subsequently arise from the third, fourth or fifth cells, rarely if ever from the second. At the same time 2–4 *secondary* germ tubes arise from near the ends of the conidium: they grow directly into hyphae and produce haustoria from their third or fourth cells, rarely from the second and apparently never from the first. Furthermore, *tertiary* germ tubes may develop. They are short, tapered processes, 4–10 $\times$ 1–3 μ, arising from any part of the spore, and generally abortive. Old conidia may develop only tertiary germ tubes, and some of them may attempt to form haustoria without success. Two to five laterals subsequently arise from the cell which was the primary germ tube.

On all three susceptible varieties the germination of fresh conidia always took this precise course, which follows, evidently, from the fact that the conidia do not contain sufficient reserves to produce directly a mycelium but, in spite of their large size, must needs draw

upon the host at the earliest opportunity. The conidia are swollen with water, having highly vacuolate cytoplasm unlike the dense contents of most spores, and this, as will be explained later, supplies the water of germination.

After 24 hours from the sowing of the conidia, at a temperature of *ca.* 20° C., and in a saturated atmosphere, penetration of the host cell is just beginning. After 48 hours, the first haustoria are more or less fully grown, and one or two secondary germ tubes may have arisen. After 72 hours, all the secondary germ tubes have developed and a fairly extensive mycelium has formed, the hyphae being 200–300 μ long (Fig. 2); the secondary haustoria are as yet immature. After 96 hours, conidial chains are formed.

Penetration of the host cell. The cell wall is penetrated and the haustorium develops in the way described by Grant Smith (26) and Foëx (9). A stylar process from the germ tube pierces the cuticle, and in its passage through the cellulose layer it is preceded by a local thickening of the layer into a papilla which it eventually pierces at the apex (Fig. 1). The process sticks like a spine into the cell wall; it becomes conical with rather a wide base, 1–1·5 μ, but the base appears to narrow again as the apex pushes into the papilla. Inside the host cell it dilates into the haustorium and in doing so it invaginates the lining layer of cytoplasm. The haustorium develops as a small pyriform body which becomes drawn out along the long axis of the host cell, and from the ends the finger-shaped appendages arise; the whole gradually enlarges into the mature organ which is 50–90 μ long, with the body 16–25 × 7–10 μ. Ultimately, according to Grant Smith, the appendages may poke through the lining layer into the cytoplasm itself. There is no obvious interaction between the nucleus of the host cell and the haustorium. Grant Smith observed no action in the case of *Erysiphe communis* on *Geranium*.

When the conidia are sown thickly, several haustoria may develop in one cell. According to Foëx (9) the haustoria are then much smaller than when a single haustorium is present in a cell.

Microchemical tests. In epidermal strips stained with cotton blue, there is generally a blue halo, 10–25 μ wide, round the point of penetration. In strips stained with Schultz's solution, colourless circles with a colourless papilla in the centre are to be seen on a field of purple (the unaltered cellulose of the cell walls). But neither of these methods is reliable, and Schultz's solution, if allowed to act for long, causes the cellulose to break up into jagged purple strips transverse to the long axis of the cell and separated by wide colourless areas.

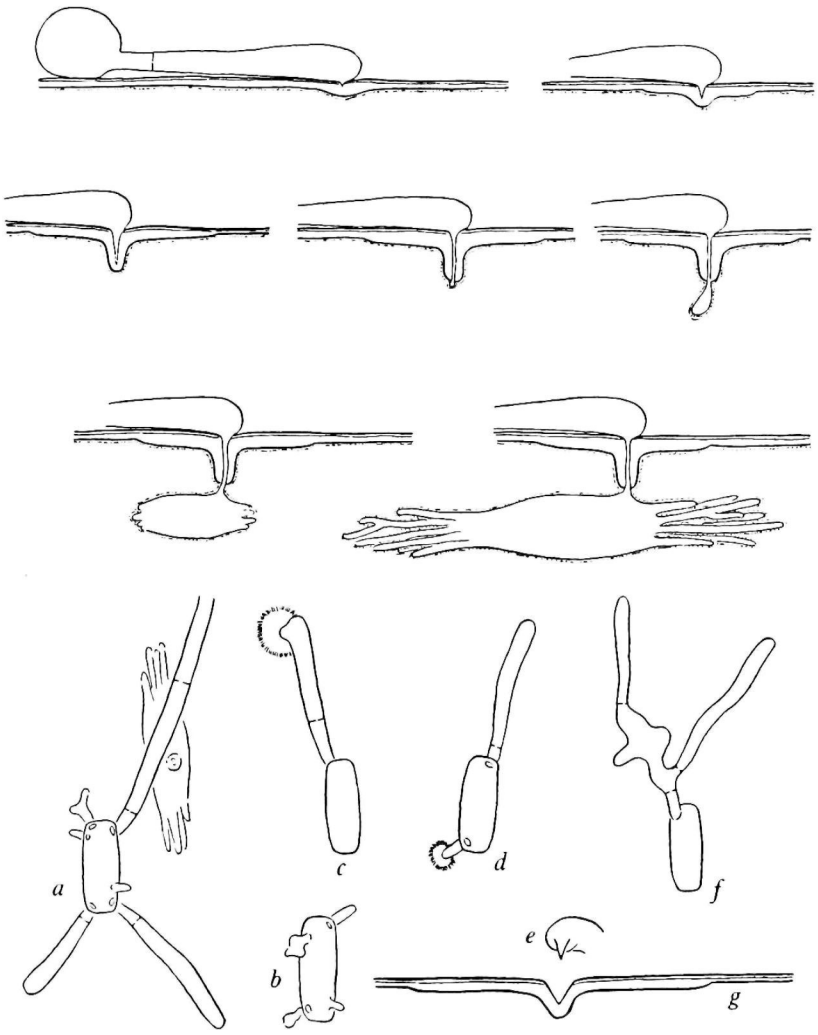

Fig. 1. *Erysiphe graminis*. Above: seven stages in the development of the haustorium, × 1000. Below: *a*, a fully germinated conidium with a primary, two secondary, and three tertiary germ tubes, × 500; *b*, an old conidium with four tertiary germ tubes, × 500; *c*, a conidium with a primary germ tube and a "cotton-blue halo", × 500; *d*, a conidium germinated on *Polypodium aureum* with a "cotton-blue halo" beneath a tertiary germ tube, × 500; *e*, the tip of a germ tube with its penetration process, × 1000; *f*, a conidium germinated on a begonia leaf, × 500; *g*, an infection papilla in section with the germ tube detached, × 2000.

A better method is to soak the strips in iodine solution for 4–5 min., transfer to 66 per cent. sulphuric acid for 5–10 min., then wash and mount in dilute glycerine. Colourless, or yellowish, circular patches, 10–25 μ wide, each with a colourless papilla in the centre, are then seen on a background of deep blue. The edges of the circles are blurred.

The colourless circles arise as minute areas, 1–2 μ wide, beneath the tips of the germ tubes before there is any sign of a papilla, although even in the smallest circles a dark spot can always be detected, which is where the penetration process pierced the cuticle. As the circle widens the papilla develops. An enzyme evidently diffuses from the process and alters the cellulose layer of the host cell wall. The altered part is distinctly swollen—it is about twice as thick as the unaltered layer—and it is probably the intense local action around the penetration process and the thrust of the process which make the papilla. Cotton blue stains only the part of the wall which is being altered, that is the circumference and papilla, so that small blue circles with dark centres in the initial stages of penetration develop into the blue haloes. The altered part stains also with stains such as haematoxylin, gentian violet, safranin and diamant fuchsin.

Although the cellulose is so clearly altered round the point of penetration, the cuticle is unaffected. In microtome sections it appears as a hyaline strip, neither swollen nor stained in any peculiar way, above the altered cellulose. In epidermal strips treated with Schultz's solution or iodine and sulphuric acid, it stains yellow whether over the altered or unaltered parts of the wall: and it does not adsorb cotton blue or nuclear stains, which it surely would do if it suffered decomposition. The cuticle must be penetrated mechanically. The tip of the germ tube is pressed very closely to the epidermis, often causing a slight yet distinct depression of the cell wall, but it has no obvious means of fixation: no mucilage sheath was disclosed by indian ink. But, as Brown and Harvey (5) have shown, the germ tube requires merely to be adhesive in a narrow ring round the point of penetration.

Germination on Norka wheat. Germination proceeded normally for 24–36 hours up to the formation of the infection papilla. There, in most cases, the penetration process was stopped and the conidium and germ tube died without further development. There were always a few cases, however, in which the penetration process gave rise to a small haustorium, with or without rudimentary appendages, and able to absorb sufficient nutriment to allow one or two secondary germ tubes to develop and produce similar haustoria. Such mycelia

grew slowly, being composed of only 4–8 cells after 72 hours (Fig. 2), and in five or six days they died. Occasionally a conidium gave rise to two germ tubes, each of 2–3 cells, which produced one or two papillae without haustoria. In three cases a normal mycelium with full-sized haustoria developed exactly as on the susceptible varieties, and conidia were produced after 120 hours. I did not experiment with these conidia.

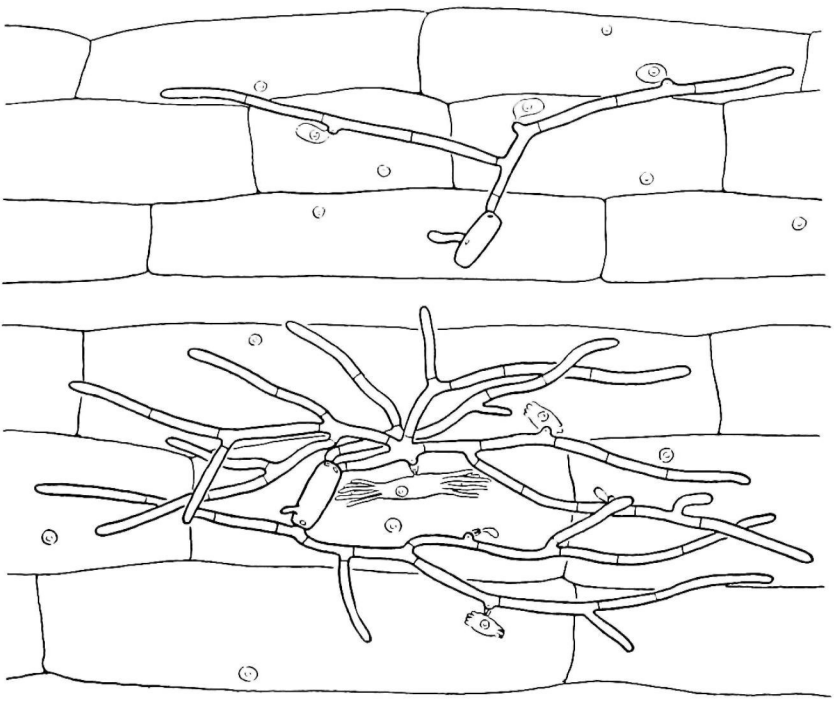

Fig. 2. *Erysiphe graminis*. Mycelia 72 hours old. Above, on Norka wheat; below, on Wilhelmina wheat: × 300.

Evidently Norka is not entirely resistant. Attempts were made to infect the glumes, as being possibly more susceptible organs, but without success: penetration stopped at the papilla stage or, as frequently, there was no penetration at all, which might have been due to the thickness of the cuticle. It should be mentioned that to avoid such a contingency in these experiments the basal part of half-grown leaves was inoculated.

Germination on Persian Black wheat. As on Norka, germination proceeded to the papilla stage, but neither haustoria nor secondary germ tubes were produced; the conidia gradually died. Evidently

the penetration process is killed before it can enter the host cell, and Persian Black is completely resistant.

Scars. Some leaves of both resistant varieties were examined four weeks after inoculation. Colourless circles, each with a papilla in the centre, were as plain as if new, and these marks of attack persist probably throughout the life of the infected part. The circles were too numerous to have been caused by casual infection.

Cross inoculations. Conidia from Spratt Archer barley and *Agropyron repens* placed on Wilhelmina, Norka and Persian Black wheat germinated up to the papilla stage without producing haustoria. Similar results were obtained with conidia from Wilhelmina wheat placed on Spratt Archer barley and *A. repens*, and from *A. repens* on Spratt Archer barley. Salmon (22) obtained similar results, observing that, after 24–36 hours, rudimentary haustoria were generally developed and sometimes a scanty mycelium which invariably degenerated in 5–6 days.

Podosphaera leucotricha

Material. Cox's Orange Pippin, Stirling Castle and Bramley Seedling apple trees were taken as susceptible varieties, Worcester Pearmain as resistant.

Methods. The plants were grown in a greenhouse; the susceptible varieties became spontaneously infected and provided supplies of conidia. As it was necessary to inoculate the upperside of the leaves on account of the tomentum underneath, the strip method was impracticable; the mesophyll cells could not be cleared away sufficiently to render the epidermis transparent. The germination of the conidia was followed either by clearing the pieces of leaf in acetic alcohol (1 : 2) or after staining with cotton blue and mounting whole.

Germination on susceptible varieties. As with *Erysiphe graminis*, the conidia produce a primary germ tube from the end of which the first haustorium arises, but only one secondary germ tube; the germ tubes grow from any part of the conidium. Penetration and formation of an infection papilla occur as in *E. graminis*. The papilla and the surrounding part of the cellulose layer, which is distinctly swollen, stain intensely with nuclear stains. The cuticle remains unaltered. The primary germ tube measures 25–40 × 4·5–5 μ at the apex, 3–4·5 μ at the base: it develops within 24 hours and the primary haustorium within 48 hours.

Apparently a mycelium cannot be established on the upperside

of mature leaves on account of the thickness of the cuticle. The conidia formed germ tubes, but I saw no sign of penetration.

These observations agree essentially with those of Woodward (28).

Germination on Worcester Pearmain apple leaves. This variety is not entirely resistant. A few opening buds were fully infected, the mycelium developing conidia. Half-grown or mature leaves were never infected, but in one instance a cluster of flowers was mildewed. I did not examine plants in the open.

Conidia germinated on young leaves up to the papilla stage and there infection generally stopped. Rarely, a normal mycelium developed and produced conidia in a week's time.

Sphaerotheca pannosa

Material. Dorothy Perkins and Crimson Rambler roses were taken as susceptible varieties, Gloire de Dijon and American Pillar as resistant. The plants were grown in pots in a greenhouse.

Methods. The same methods were used as with apple mildew. A satisfactory fixative not having been found, the details of penetration could not be made out. Chromacetic solution caused strong contraction of the radial walls and buckling of the outer wall of epidermal cells, while acetic alcohol (1 : 3) and Bouin's fluid, though causing less contraction, rendered staining difficult. The epidermis did not strip easily.

Germination on susceptible varieties. At a temperature of *ca.* 20° C. the conidia germinate rapidly. The primary germ tube and haustorium are formed in 24 hours, and in 48 hours 2–3 secondary germ tubes have arisen and produced a fairly extensive mycelium with young conidiophores of 4–5 cells; in 60 hours some conidia have matured. The germ tubes arise from each corner of the conidium, giving it a cruciform appearance. The mycelium grows rapidly on young leaves but dies on mature leaves, especially those of Dorothy Perkins, which is probably due to the thickening of the cuticle. Germination occurs readily on either side of the leaf, all the stomata being on the underside in these varieties of rose.

Microchemical tests showed a distinct alteration of the cellulose layer round the point of penetration. Staining with Schultz's solution and iodine and sulphuric acid showed colourless patches as with *Erysiphe graminis*.

Observations on resistant varieties. Germination proceeded to the papilla stage and there, in general, it stopped. With Gloire de Dijon germination was occasionally normal and conidial chains were pro-

duced after 60 hours. Furthermore, in the greenhouse, young leaves of this variety were commonly mildewed, so that it is but partly resistant, like the Worcester Pearmain apple variety. With American Pillar a few conidia managed to form a scanty mycelium, derived from 1–3 germ tubes, but it invariably died in a week without sporing. American Pillar was never seen to be mildewed, so that at most it is slightly susceptible, like Norka wheat. Under normal conditions both of these rose varieties are as resistant as Persian Black wheat.

I tried several times to infect the petals of Gloire de Dijon and the wild *Rosa canina*, but there was never a sign of penetration although germ tubes developed. The cuticle on the petals was remarkably thick, separating as a yellow pellicle on treatment with iodine and sulphuric acid.

CONCLUSIONS ON PENETRATION

The following steps can be distinguished in the penetration of the host cell by the haustorial process.

The stimulus to penetration. The tip of the germ tube and the appressoria must respond thigmotropically to contact with the epidermis. As yet, there is no evidence for positive chemotropism. As will be shown, the germ tubes will attempt to penetrate a great variety of unrelated plants, and Neger(16) found that they would form appressoria on contact with a hard surface. Foëx(9) has illustrated a conidium which has formed a characteristic appressorium on another. The germ tubes are undoubtedly thigmotropic.

Penetration of the cuticle. This is evidently mechanical, neither physical nor chemical alteration being noticeable about the point of penetration. A thick cuticle prevents penetration. Woodward(28), on the other hand, considered that it was in part chemical in the case of *Podosphaera leucotricha*, chiefly because the penetration process was slightly swollen in the cuticular layer; his figures suggest rather that the swelling occurred at the junction of the cellulose and cuticle.

Penetration of the cellulose layer. This step is clearly both mechanical and chemical. Yet, if the cuticle is pierced by pressure, why not the cellulose? Is it so much thicker and denser that it should necessitate swelling and softening, or is the production of pectinase but a relic of a former state of endoparasitism? It may be that by chemical alteration of this layer mildews avoid the necessity of deforming it, which step Brown and Harvey(5) state to be the most difficult in mechanical penetration.

Grant Smith (26) and Woodward (28) considered that the infection papilla might be formed by deposition from the host cell as a means of resistance against the parasite. If that were so, one would expect that the nucleus would be concerned, that the cytoplasm would stain deeply about the papilla and be firmly adherent to it, that the thickened part of the cell wall and the papilla would be stratified, and that not merely the base but the whole haustorium would be encased in cellulose. There are no such indications. On the contrary, the swelling and alteration of the cellulose layer in a circular patch of bleary aspect and blurred outline, having the point of penetration as the centre and the most intense action round the penetration process, conform exactly with effects to be expected from the diffusion of a cytase from the process. The secretion of cytase evidently continues after the haustorium is developed, for the papilla becomes partially dissolved and dwindles to a low collar about the stalk. Experiments with spores on strips of dead epidermis would prove the point.

Woodward (28) states that with *Podosphaera leucotricha* the cellulose layer may be acted upon by diffusion from the hypha before the cuticle is pierced. In the case of *Erysiphe graminis*, I never observed alteration of the layer in strips stained with iodine and sulphuric acid without there being a dark point in the centre of the patch indicating penetration; it was necessary to examine the strips with an immersion lens, as the penetration process is at first exceedingly fine. With rose petals the cuticle was not pierced nor was the cellulose layer below the germ tube in any way affected.

Intrusion of the haustorium. Unless the turgor pressure in the penetration process is greater than that in the host cell, the process could not swell into a haustorium without piercing or killing the cytoplasm, which it appears unable to do. That turgor pressure may be an important factor in resistance has been shown by Hawkins and Harvey (11) in experiments with *Pythium de Baryanum* on potato tubers: certain resistant varieties of tuber were found to have a higher pressure in the cell vacuole than the parasite. Moreover, it is known that plants in a flaccid state tend to be more susceptible to mildew: Rivera (19) and Volk (29) have studied this problem in connection with *Erysiphe graminis*. But the flaccid state may assist rather the entrance of the penetration process by facilitating the deformation of the cell wall and the development of a steep-angled cone of penetration (5).

These results on the penetration of the epidermis by mildews

agree essentially with those obtained by former investigators with *Botrytis cinerea* (1), *Colletotrichum lindemuthianum* (8), *Sclerotinia libertiana* (2), and with the basidiospores of *Puccinia graminis* (27). They conform also with the theory of mechanical penetration of the epidermis put forward by Brown and Harvey (5). Considering how different these fungi are, one is led to suppose that such is the general method of entry by parasites which pierce straight into the host and that it may not be as difficult as one would at first imagine. The infection papilla is characteristic, however, of mildews, with the single exception of the bryophilous discomycete *Neotiella crozalsiana* (7).

CONCLUSIONS ON RESISTANCE

Each stage in penetration may be opposed by a barrier. Too thick a cuticle or cellulose layer or a high turgor pressure may debar the haustorial process, and on entry into the host cell it may encounter toxins. That the process should be stopped at the papilla stage in all cases of true resistance, after overcoming the obstacles in the cell wall, suggests that in the main toxins are the basis of resistance. From the point of view of the host plant the means of resistance is highly satisfactory; the parasite never obtains a footing in its tissue and there is no question of a prolonged struggle or the construction of an expensive barrier of dead cells.

As regards the chemical nature of resistance, Marañon (13) has put forward an ingenious hypothesis based on his analysis of the leaves of varieties and hybrids of *Oenothera*, susceptible and resistant to *Erysiphe polygoni*. Resistance is correlated with an appreciably higher tannin content and water-soluble acid content, and it is suggested that the tannin kills the fungus, being a protoplasmic coagulant to which many fungi are susceptible, and that the acid prevents the action of the tannin on the cytoplasm of the host. It remains to show how the hyphae of mildews react to different concentrations of tannin. On such a basis of quantitative differences in the toxin, one can readily explain the partial infection and occasional full infection of resistant varieties of a host species, such as happens in the case of Norka wheat, Worcester Pearmain apple or Gloire de Dijon rose, but to be of general application the hypothesis must needs be qualified *ad extremum*. Mere quantitative differences, no matter what the toxin, could never account for the sum total of resistance to mildews when the high specific infectivity of the whole family is considered. Those very varieties of *Oenothera*, for example, which are susceptible to that physiologic form of *Erysiphe polygoni* are at the

same time resistant to every other species of mildew, and yet that physiologic form of *E. polygoni* cannot infect one of the hosts of the other mildews. There must be qualitative differences in the toxins by which different species of host plant resist different physiologic forms of mildew. And one may doubt, indeed, whether by bulk analysis such differences could be detected or even the nature of the toxin.

Salmon has shown that when experimenting with biologic species (physiologic forms) one must beware of a subinfection of resistant hosts caused by a heavy inoculum. If several germ tubes attempt to enter one epidermal cell, that cell may be unable to resist so many, and one or more may develop haustoria and a scanty mycelium, which soon dies, however, with or without sporing. Such may have been the case in some of my observations on the partial resistance of Norka wheat, Worcester Pearmain apple and Gloire de Dijon rose; in others, as shown in Text-fig. 2, one conidium was certainly able of itself to invade a host cell and even develop a normal mycelium. These varieties may fairly be judged imperfectly resistant in contrast with Persian Black wheat, which was never infected, though subjected to equally heavy inoculation. American Pillar rose occupies an intermediate position, for what scant mycelia it might support were sterile. Proof of the nature of subinfections must lie in inoculation with the spores, which should show whether a new and more virile strain had arisen, as that of the wheat mildew which Salmon (23) educated on to barley leaves. On the other hand, with *E. polygoni* on cultivated Brassicae, Searle (25) considered that the fungus over-wintered by means of autumnal subinfections, which developed fully in the following spring.

So varied may be the degrees of infection caused by a mildew, not merely on different host species or varieties, but even on exactly similar plants of the same species, wild or cultivated, that it is impossible at present to arrive at any conclusion concerning the nature of parasitic specialisation in the family. Many are the records of anomalous infection. With *Erysiphe cichoracearum* Neger (17) has described from the same host species instances of full infection, subinfection and no infection, as determined microscopically; conidia from *Sonchus asper* caused subinfection on *S. oleraceus* in summer and full infection in autumn, likewise the conidia of *Sphaerotheca humuli* from *Epilobium montanum* on *Taraxacum officinale*. In such cases the inhibitory effect of high temperature must be taken into consideration. Similarly, with *Erysiphe polygoni*, Searle (25) found that

conidia from the swede might infect plants of *Brassica sinapis* normally or not at all. Most remarkable are some of Neger's early experiments(16). A mildewed plant of *Spiraea ulmaria* was grown for three weeks under a bell-jar in contact with a healthy plant and, whereas the leaves of the mildewed plant were spontaneously infected as they unfolded, the healthy plant was not once attacked: so, too, with mildewed and healthy plants of *Ranunculus repens* and of *Plantago major*. Biologic specialisation seeming to be much less strict in the mildews than in rusts, one could wish for inoculation experiments with pure strains of the fungus derived from single spores, conducted under comparable conditions and with a microscopical examination of the extent of infection.

It appears that other hosts may have a rather different, if less effective, method of resistance from what I have described. Neger(17) found, in the cases of subinfection caused by *Erysiphe cichoracearum*, that the rudimentary haustorium from the primary germ tube was entrapped by a dark brown, gummy substance which was deposited round it on entry into the host cell; the haustorium was killed but the host cell also died. Woodward(28) found a similar occlusion of the haustoria of *Podosphaera leucotricha* in connection with the browning of apple leaves; browning of the leaf cells occurred in the immediate vicinity of the occluded haustoria, the extent of browning varying with the number of haustoria, so suggesting the escape of toxic substances from them.

If the stimulus to penetration rests with the thigmotropism of the germ tube, conidia must attempt to penetrate any solid object on which they can germinate. I experimented therefore with a number of inappropriate hosts, using the strip method of examination, to discover to what extent they might be infected.

Experiments with inappropriate hosts

Erysiphe graminis on Impatiens Sultani. A primary germ tube developed in 24 hours and gave rise to an infection papilla by penetrating the cuticle and cellulose layer in the normal way. There was no further development, and I could see that the stylar process from the germ tube just reached the tip of the papilla. Cotton blue gave a small blue halo, Schultz's solution and iodine and sulphuric acid a small colourless circle, round the point of penetration. Some processes were apparently unable to penetrate, and the best marked cases of alteration of the cellulose layer were on the elongated cells overlying the veins of the leaf: there the colourless patches were

10–20 μ wide. Grant Smith (26) found in *Phyllactinia corylcae* that the hyphae entering the stomata generally grew straight towards a vascular bundle if there was one in the immediate neighbourhood, forming large haustoria in the cells of the bundle sheath: and Salmon observed the same tendency in *Erysiphe graminis* when induced to grow endophytically from wounds (24).

Erysiphe graminis on Cobaea scandens. Infection was stopped at the same stage as on *Impatiens*, but the stylar process, which was very fine indeed, penetrated in fewer cases, and the papilla and altered area of cellulose were much smaller.

Erysiphe graminis on apple, rose, broad bean, hyacinth, tulip, and begonia. A primary germ tube developed with the tip closely pressed to the leaf, but there was no sign of a papilla or of zoning on treatment with cotton blue. These experiments were performed before I realised the uncertainty of staining with cotton blue and should be repeated with iodine and sulphuric acid. On apple and begonia leaves the germ tube became strongly lobed and distorted after 48 hours, and gave rise to one or two short hyphae of one or two cells, although no secondary germ tubes developed (Fig. 1).

Erysiphe graminis on Polypodium aureum and Polypodium sp. (allied). Infection proceeded normally to the papilla stage where it was stopped as on *Impatiens*. The cell wall round the point of penetration showed in unstained strips a bright yellow-brown halo which might be 20 μ wide. The haloes corresponded with colourless patches in strips treated with iodine and sulphuric acid and they developed from small yellowish circles like the cotton-blue haloes. A remarkable thing was noticed in this experiment, which was performed twice on each host at an interval of a week, each time with several leaves and a large number of spores. The penetration process always arose from a small tertiary germ tube: the primary germ tube lay along the leaf without apparently the least attempt at penetration (Fig. 1). The arrangement was so peculiar as to suggest that the host had had some effect on the germ tubes, and one could hardly suspect every conidium of the same irregularity.

Erysiphe graminis on Adiantum reniforme. Conidia would not germinate on old leaves, although these were placed on damp filter-paper in Petri dishes. On young leaves germination proceeded as readily as on *Polypodium aureum* up to the papilla stage, where it was stopped. The papilla was abnormally large, like a hemispherical pad; it was pierced nearly to the apex by a stout stylar process which, on falling out, left a relatively large conical hole. The surrounding

cell wall was discoloured bright rusty yellow as with *P. aureum*, but the altered area was smaller and the penetration process arose from the primary germ tube. Microtome sections of these papillae would be instructive in showing the exact shape of the penetration process.

Evidently the cellulose layer of the epidermal cells of these fern leaves differs chemically from that of the flowering plants with which I experimented, for such yellow discoloration was never observed with the latter. Yellowing, or browning, is a common feature of fern tissues, and the cytase from the penetration process may give some clue to its nature.

Sphaerotheca pannosa on Impatiens Sultani. Infection to the papilla stage with alteration of the cellulose layer took place exactly as with *Erysiphe graminis.*

Sphaerotheca pannosa on Polypodium spp. and Adiantum reniforme. Infection to the papilla stage with rusty yellow discoloration of the cellulose layer took place exactly as with *Erysiphe graminis.* All infections arose from the primary germ tube.

Observations with other mildews

Oidium euonymi-japonici. Conidia from *Euonymus japonicus*, germinated on the leaves of the host, produced a short, lobed, primary germ tube from which the first haustorium developed with the formation of a papilla and alteration of the cellulose layer as with *Erysiphe graminis* on a normal host. On *Impatiens Sultani* and *Adiantum reniforme* the conidia behaved exactly like those of *Erysiphe graminis* on these hosts.

Erysiphe cichoracearum. Conidia were taken from *Myosotis collina* and *Anchusa* sp. They infected the leaves of the host species exactly as in the case of *Erysiphe graminis* on a normal host. On *Impatiens Sultani, Taraxacum officinale, Convolvulus arvensis* and the three species of fern the conidia behaved like those of *Erysiphe graminis* on *Impatiens Sultani* and the ferns except that, after one penetration process had arisen from the germ tube and had been stopped at the papilla stage, a second and sometimes a third would arise from some point nearer the conidium, to be stopped at the same stage; in strips stained with iodine and sulphuric acid 2–3 colourless intersecting circles with a papilla at the centre of each, would be seen beneath the germ tubes. This is the only mildew in which I observed multiperforation: it is clearly connected with frustration because it did not occur on *Myosotis collina* or *Anchusa*. Conidia from *Myosotis collina* were also placed on leaves of *Veronica beccabunga*: after 48 hours

some primary germ tubes showed no sign of penetration, others had formed an abortive haustorium and yet others had developed normally to give rise to a full-sized haustorium and a secondary germ tube of 1–2 cells. It appears therefore that some conidia of this strain from *Myosotis collina* can parasitise *Veronica beccabunga*. As already noted, biologic specialisation in this species of mildew is particularly complicated; it would repay a detailed study.

The conical hole bored into the papilla by the stylar process was often of striking size on the leaves of *Impatiens* and *Taraxacum*. *Erysiphe graminis* also produced a large hole on *Impatiens*. The texture of the cellulose layer may differ in widely different plants.

Conclusions concerning the inoculation of inappropriate hosts

Given the right conditions for germination, mildew conidia will attempt to infect any plant on which they alight. If it can pierce the cuticle of an inappropriate host the penetration process is then stopped at the papilla stage and probably killed by toxic substances in the host cell. Whether the cellulose layer can act as a barrier is uncertain. The cytase, diffusing from the penetration process, may not be able to act on all kinds of cellulose, so that it would be interesting in this respect to germinate conidia on the leaves of horsetails, lycopods, bryophytes and gymnosperms. Brown (3, 4) observed that the extract of the germ tubes of *Botrytis cinerea*, which contained a pectinase, had no effect on the species of bryophyte, fern and *Spirogyra* which he tried, though highly active in macerating phanerogamic tissue like that of potato tuber, leaves and petals. The cytase from the penetration process appears to be the same in all six species of mildew, as it produces the same effects. Chona (6) and Menon (14) have recently shown that the macerating enzyme, pectinase, is essentially the same in a variety of parasites.

That the germ tube should begin to penetrate on such a variety of unrelated hosts tends to prove that the stimulus to penetration is not provided by a chemical substance emanating from the host, but that it is merely a thigmotropic response. That a papilla should be formed in such a variety of hosts tends to prove that it is an effect of the penetration process on the cellulose layer and not a particular reaction of the host cell against the attack of mildews: the phenomenon of intersecting circles caused by multiperforation in *Erysiphe cichoracearum* also supports this contention.

NOTES ON THE GERMINATION OF MILDEW CONIDIA

It is known that the conidia of mildews do not germinate properly in water (10, 15, 16, 28). The conidia of the six species with which I experimented produced, when immersed in water, a short germ tube or commonly none at all: the vacuolate structure of the cytoplasm gradually collapsed into a granular homogeneous state, and after 24 hours, at *ca.* 20° C., the conidia were dead. Immersion of 1–3 hours was sufficient to destroy their power of germination. Spore emulsions are therefore useless for inoculation, and this peculiarity may explain Woodward's difficulty in obtaining germination (28).

On dry cover-slips in a saturated atmosphere, the conidia of all six species germinated normally. The apex of the germ tube was often capitate or lobed, as Neger (16) found, as if forming an appressorium on the cover-slip. One or two secondary germ tubes developed, but they remained short.

On the surface of water, however, the conidia germinated readily. They were dusted on to a drop in a watch-glass which was placed on water in a Petri dish. After 24 hours at *ca.* 20° C., in all six species, a single, unbranched, 1–2-septate germ tube, 100–200 μ long, had grown from each conidium vertically into the air. How the germ tube was balanced other than through sheer dexterity in apical growth it is impossible to say: isolated conidia produced such germ tubes and a tap on the watch-glass rolled them over. Abortive tertiary germ tubes had grown into the water in many cases and these might have assisted in the balancing by pressing against the underside of the surface film. As I looked at the forests of germ tubes, I saw that bubbles formed and burst round the conidia as if the water were beginning to boil. The bubbles must have been the carbon dioxide of respiration escaping from the water.

These simple experiments may be of interest in the physiology of mildews. For a fungus, growth of the germ tube into the air is clearly abnormal. The bubbling suggests that it is growth away from a high concentration of carbon dioxide to a high concentration of oxygen, and the relatively rapid death of the conidia when immersed in water suggests that they are killed from lack of oxygen rather than through any action of the water itself. One is led to postulate that mildews require a low carbon-dioxide tension and a high oxygen tension in their hyphae, wherefore they cannot normally enter stomata or become endoparasites, but for the most part are compelled to lead an ectoparasitic existence. Unlike most fungi, whose spores are packed

with dense cytoplasm and which absorb water and swell up on germination, mildews carry their water of germination in the highly vacuolate cytoplasm of the conidium in place of food reserves, and on germination they develop a haustorium at the first opportunity. That mildews will thrive in a hot, dry summer in England, when other parasitic fungi suffer through drought, is undoubtedly connected with these two facts: the ability of the conidia to germinate on a dry surface, and their inability to withstand prolonged wetting; rain must kill many spores if not the hyphae themselves. And so, too, with *Oidium heveae* in the eastern tropics, which in monsoon countries with a dry season may become a pest in rubber estates, yet rarely does so in regions of evergreen rain forest such as Malaya, Sumatra and the west of Java. The respiratory requirements of mildews in the vegetative state seem to be the antithesis of those of yeasts; in the ascigerous stage these requirements must be changed, for the hyphae combine to form an ill-aerated tissue.

Conidia which appear to be fresh may vary greatly, for no obvious reason, in their power of germination. I noticed this with rose and apple mildew. Conidia from vigorous young patches of mycelium would give perhaps 1 per cent. germination; under similar conditions I found 10–20 per cent. to be the average and 30–40 per cent. the maximum. With *Erysiphe graminis* I frequently obtained 100 per cent. germination, and this species was accordingly used in preference to the others. These irregularities have been noted by other investigators (10, 16). The life of mildew conidia seems to range from 2 to 3 days to a week, and a sample from an old patch of mycelium will always give a low percentage germination. The conidia germinate as soon as they are detached and they seem unable to withstand desiccation; long-distance infection by conidia is thus improbable.

Summary

Experiments were made with *Erysiphe graminis*, *Podosphaera leucotricha* and *Sphaerotheca pannosa* to determine at what stage of infection resistant varieties of the host plant checked the attack of mildew. Norka and Persian Black were taken as resistant varieties of wheat, Worcester Pearmain of apple, and Gloire de Dijon and American Pillar of rose. Particular attention was given to the exact method of penetration of the host cell by the haustorial process.

The conidia germinated characteristically with a short, unicellular, primary germ tube from the tip of which the first haustorium

developed. On completion of the haustorium the apex of the primary germ tube resumed its growth and 1–3 secondary germ tubes grew out from the spore directly into hyphae. Abortive, simple or lobed, tertiary germ tubes might also arise.

It was concluded that the stimulus to penetration was thigmotropic, that the cuticle was pierced mechanically, that the cellulose layer of the host cell was swollen by a cytase diffusing from the penetration process, and that the infection papilla was formed by the intense local action of the cytase and the thrust of the process. The papilla and surrounding part of the cellulose layer were so altered by the cytase that they no longer responded to microchemical tests for cellulose, but the nature of the altered matrix was not determined.

On resistant varieties infection was generally stopped at the papilla stage without penetration of the host cytoplasm. Persian Black wheat was completely resistant. Norka wheat, Worcester Pearmain apple and Gloire de Dijon rose were imperfectly resistant in that subinfections with scant mycelium and rudimentary haustoria might develop on them and occasionally a normal mycelium with conidia.

On cross-inoculation with the physiologic forms of *Erysiphe graminis* from wheat, barley and *Agropyron repens*, the conidia also germinated and initiated penetration to the papilla stage on the wrong hosts; the process of penetration was then stopped and no haustoria were formed. The same results were obtained on a variety of inappropriate hosts, e.g. *Impatiens*, *Cobaea*, *Polypodium* and *Adiantum*, provided that the cuticle was not too thick. On *Polypodium* and *Adiantum* the penetration process turned the cellulose layer yellow-brown.

A few experiments with *Oidium euonymi-japonici* and *Erysiphe cichoracearum* corroborated these results, which seem of general application to mildews.

The problem of resistance to mildews has been discussed. It is considered that resistance is primarily caused by toxins in the host cell, but that environmental and structural factors may also be operative, at least in cases of subinfection.

A short period (1–3 hours) of immersion in water was found to be sufficient to kill the conidia. When germinated on a water surface each conidium sent a long germ tube into the air. It is surmised that mildews required a high oxygen tension and a low carbon-dioxide tension in their cytoplasm.

These investigations, which were undertaken at the Cambridge Botany School during the year 1928, it has been impossible through change of circumstances to complete. But I wish to express my thanks to the Department of Scientific and Industrial Research for a grant in aid of the research, and especially to Mr F. T. Brooks, F.R.S., to whom I owe not only the suggestion of the problem but guidance and supervision, without which the results now presented could not have been obtained. It was Prof. W. Brown's invigorating address on the Mechanism of Disease Resistance in Plants to the British Mycological Society at Newcastle in September, 1933, which has prompted publication of my observations.

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