SOME EXPERIMENTAL STUDIES ON *PLEOSPORA HERBARUM* (PERS.) RABENH.

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(With Plate IV)

The strain of *Pleospora herbarum* which forms the subject of the observations recorded here was isolated from plants of *Euphorbia Paralias* growing on sand-dunes near the mouth of the Dovey Estuary, Cardiganshire.

The fungus can be found upon living plants at any period of the year, and appears to be confined, in this area at least, to *E. Paralias*, since specimens of *E. portlandica* growing in close proximity are quite healthy.

The first indication of infection is the appearance of crimson spots, often reaching a diameter of 1 mm., upon the upper surface of the younger leaves. These spots are due to the development of anthocyanin in the epidermal and immediately subjacent cells. Lower down the stem, upon older, fading leaves, the spots are brown, whilst the dead leaves at the base of the plant bear minute black perithecia. The perithecial stage is preceded and accompanied by the conidial stage, and the stem as well as the leaves may be infected. Although a complete study of the life-history of the fungus was made, no previously unrecorded features were noted, and attention was chiefly focused on its behaviour in culture.

Cultivation on Artificial Media

The fungus is easily obtained in culture upon malt extract agar (2 per cent. malt extract rendered solid by the addition of 2 per cent. agar).

Isolations were made and single-spore cultures obtained, and throughout the work subcultures from such single-spore isolations were used.

Cultures from single ascospores, kept at 22° C., show a whitish floccose mycelium within two or three days. After four or five days the mycelium begins to develop a dark pigment, and appears greenish brown when viewed from the underside of the dish.

The mycelium consists of much-branched septate hyphae, with fairly thick walls, and contains refractive oil globules and some glycogen. The older hyphae are dark in colour, owing to the development of a brown pigment in the cell walls.

The mycelium grows both on and below the surface of the agar. The aerial hyphae are profusely branched, giving the floccose appearance which is characteristic of the fungus in culture. The conidia are borne on the aerial hyphae, while perithecial primordia may be found either on or below the surface of the medium.

The conidia are of the *Macrosporium* type described by Miyabe(6) and seen on the surface of the leaves of *Euphorbia.*

Perithecial primordia, which begin to appear in cultures five to seven days old, are soon distinguished by the increased quantity of dark pigment in the cell walls; in cultures about ten days old they are visible to the naked eye as dark dots on the mycelium.

The earlier stages can easily be seen in preparations made by picking off small portions of the mycelium, staining with erythrosin and mounting in glycerine jelly. The primordia stain a deeper pink than the vegetative hyphae. They are very numerous, but of the total number of primordia laid down, not more than 30 per cent. ever reach such a size that they are visible to the naked eye as black dots on the mycelium. Even of these, only 15 to 20 per cent. ever reach full size and maturity, *i.e.* about 5 per cent. of the total number of primordia formed.

The time taken for the perithecia to mature varies considerably, but cultures about a month old usually show a number of perithecia with fully-developed asci.

The malt-agar medium has been utilised as a means of maintaining the stock cultures, since it is easily prepared, and is one upon which the fungus remains vigorous for long periods. A similar malt extract solution, without the agar, has also been used, to ascertain the effect of a liquid nutrient upon the fungal characters. In this medium the fungus forms mycelium both on and below the surface. The submerged mycelium consists of delicate, sparsely-branched hyphae. Conidia and perithecial primordia are formed only on the aerial mycelium, which forms a mat on the surface of the liquid. An additional solid medium containing a mush made from uninfected leaves of *Euphorbia Paralias* with 2 per cent. agar was also used. On this only a sparse, colourless mycelium was formed. Within a week conidia were produced in abundance, but no signs of perithecial primordia were seen for about fourteen days. Eventually a few scattered perithecia were produced, which after five or six weeks' growth formed asci and ascospores.

Growth on Synthetic Media

A synthetic medium similar to that used by Robinson(7) in his studies on *Pyronema confluens* was made up as follows; NH_4NO_3 ($M/2000$), $MgSO_4$ ($M/500$), KH_2PO_4 ($M/500$), maltose ($M/500$) rendered solid by the addition of 2 per cent. agar. On this medium (described in the

following experiments as medium *A* 1) the mycelium is less profuse than on the malt extract agar. It is not so dark in colour, though still distinctly brown, and the perithecial primordia are less numerous than on the richer medium. A culture ten days old showed an average of fourteen primordia per sq. cm.; a culture three weeks old, about thirty-five per sq. cm. A culture two months old, examined under a low-power microscope, showed an average of forty primordia per sq. cm., of which only about half were visible to the naked eye. In the central part of the culture about five primordia per sq. cm. developed into mature perithecia (*i.e.* about 12 per cent. of the total number of primordia formed), while in the outer parts the mature perithecia were more numerous—about ten per sq. cm., or 25 per cent. of the total number of primordia laid down.

Thus it is seen that though on this medium a comparatively small number of perithecial primordia are laid down, the proportion of these which reach maturity is greater than on the richer medium. To determine whether the nitrogen or the carbon content of the medium constituted the limiting factor for the growth of the fungus, media were made up using the formula of *A* 1 as a basis, but varying the nitrogen and carbon content. Cultures on synthetic medium *A* 2, a variant of *A* 1 containing less nitrogen (NH_4NO_3, *M*/4000), indicated that decreasing the nitrogen content of the medium has little or no effect on the growth of the fungus. The growth in these cultures is a little sparser than on medium *A* 1, but a distinct brown colour is developed in the mycelium, and rings of perithecia are formed, as well as abundant conidia.

Growth on synthetic medium *A* 3, in which the nitrogen content is doubled (NH_4NO_3, *M*/1000), appears no different from that on medium *A* 1.

On synthetic medium *A* 4, in which the sugar content is decreased (maltose, *M*/1000), conidia and rings of perithecia are formed, but the vegetative mycelium is sparser and quite colourless. The dark pigment is formed only in the conidia and the perithecial primordia, and in a few adjacent cells.

That the fungus is able to grow and form reproductive structures on media extremely poor in both carbon and nitrogen is shown by the fact that cultures on *unwashed agar*, without any added nutrient, show a scanty, colourless mycelium with abundant conidia, and rings of primordia a few of which become fully developed perithecia. Cultures on *washed agar* (washed for six hours in running water, followed by twenty-four hours in distilled water), with no added nutrient, show a still sparser mycelium and fewer perithecia.

Four sets of cultures, M_1, M_2, M_3 and M_4, were made as follows, there being twelve cultures in each set; M_1, on washed agar: M_2, on washed agar with NH_4NO_3 (*M*/2000) added: M_3, on washed agar

with maltose ($M/500$) added: M_4, on washed agar with both maltose and NH_4NO_3 in the above proportions.

All plates were inoculated with single ascospores, and within two days these had germinated, an appreciable amount of mycelium being formed in M_3 and M_4. In six days a sparse mycelium was developed in M_1 and M_2, and in M_1 curious distorted hyphae were seen, probably the result of starvation.

The mycelium of M_2 was rather more profuse, and showed no signs of starvation.

After a fortnight, all cultures showed perithecial primordia, these appearing as black dots on the mycelium.

The mycelium of M_3 and M_4 was definitely greenish brown, but no pigment had been developed in the vegetative hyphae of M_1 and M_2.

After six weeks, all cultures showed fully developed perithecia with asci and ascospores, as well as abundant conidia.

The cultures of M_1 had a sparse, colourless mycelium with tufts of distorted hyphae. The perithecial primordia appeared scattered, numbering about ten per sq. cm. Those of M_2 showed no distorted hyphae, and the perithecial primordia were more numerous, about fifteen per sq. cm. The vegetative mycelium, however, was colourless.

In the cultures of M_3, the mycelium was much more dense, and distinctly dark in colour. The perithecial primordia averaged twenty per sq. cm. near the periphery of the cultures but were less numerous in the central parts. They were developed in more or less definite zones.

In M_4 the mycelium was more profusely branched than in M_3, darker in colour, and showed definite rings of perithecial primordia, numbering fifteen to twenty per sq. cm.

From these observations it may be concluded that the increase or decrease by small amounts of the nitrogen supply makes very little difference to the growth of the fungus, which is able to grow, though poorly, in a medium almost devoid of nitrogen. Unwashed agar contains in itself sufficient nitrogen to prevent any signs of starvation in the mycelium.

The supply of carbonaceous food material, on the other hand, affects the fungus more profoundly. Unless a certain proportion (between $M/1000$ and $M/500$) be present, the dark pigment is not formed in the vegetative mycelium, but only in the conidia and perithecia themselves.

These results indicate that in *Pleospora* a high Carbon-Nitrogen ratio in the medium is a necessary factor for the formation of pigment, the fungus in this respect resembling the *Fusarium* studied by Brown (3).

Having established the fact that the available carbon content of the medium is of very great importance to the growth of the fungus,

a further series of experiments was attempted, to find the source of carbon most suitable for growth. A series of media was made up, having the general formula of A_1, but substituting different sources of carbon, as follows: *A*, maltose; *B*, glucose; *C*, sucrose; *D*, lactose; *E*, manitol. These media were all liquid, no agar being added.

Ascospores and conidia germinated immediately in these media, and formed considerable mycelium within four or five days. The mycelium in cultures *A*, *B* and *C* was more profuse and darker than in *D* and *E*. Within a week, perithecial primordia were produced in *A*, *B* and *C*. The relative amounts of mycelium and the number of primordia formed may be represented by the following table:

Medium	After four days	Seven days	Fourteen days
A	×××	××× *pp*	××× *ppp*
B	×××	××× *pp*	××× *ppp*
C	×××	××× *pp*	××× *ppp*
D	××	×× *p*	×× *pp*
E	×	×× o	×× *p*

(×× = relative amount of mycelium formed. *pp* = relative numbers of primordia developed.)

A similar set of cultures was made up on filter paper soaked in the liquids, and showed very similar results. These results, however, were rendered suspect by the fact that in control cultures on filter paper moistened with distilled water, a certain amount of growth took place. A sparse, colourless mycelium was formed, and later a few scattered perithecia, indicating that the fungus is able to a slight extent to utilise cellulose as a source of carbon.

A few cultures were made on oatmeal agar (0·5 per cent. oatmeal flour in water, with 2 per cent. agar). In these cultures a scanty colourless mycelium was formed, producing abundant conidia and eventually perithecia. Distorted hyphae similar to those found on washed-agar cultures were seen, indicating a starved condition. The perithecia were few, but were produced in more or less definite zones. Some cultures were stained with iodine after two weeks' growth, and showed that the fungus completely consumes the starch from the medium as it covers it.

These experiments show that the fungus is able to utilise various sources of carbon, the most suitable, however, being such sugars as maltose and glucose.

Some trouble was occasioned by bacterial contamination at one point in the investigations, and it was thought that by suitably adjusting the reaction of the medium this might be avoided. The malt medium was first tested, and gave a *p*H value of approximately 6·0, while the liquid media *A*–*E* all gave a *p*H value of 7·8.

Although the trouble caused by bacteria was eliminated without the necessity for alteration of the media, this suggested the idea of

finding the possible range of acidity and alkalinity within which growth of the fungus could take place.

Accordingly a series of media was made up from the formula of medium A_1, but with *p*H value ranging from *p*H 2·5 to *p*H 9·2. The method adopted was to prepare the agar, sterilise it in test-tubes, investigate the *p*H by the potentiometer at 38° C. and apply the temperature correction. The media of *p*H 2·5, *p*H 3·0 and *p*H 3·5 were liquid, the others solid.

It was found that the fungus can grow to a very slight extent upon the *p*H 2·5 medium. No sign of growth was apparent in these cultures for two or three weeks, but eventually a small colony, about 4–5 mm. in diameter, was developed. This colony consisted of tangled colourless hyphae, composed of short broad cells. No reproductive structures were developed.

On *p*H 3·0 the colonies were larger (2–3 cm. in diameter) but were still colourless, and showed no sign of reproductive structures until the cultures were over six weeks old. A few perithecial primordia then appeared, but these never matured. No conidia were observed. The vegetative mycelium was normal in appearance.

The cultures on *p*H 3·5 showed more or less normal development, but the colonies did not reach a great size. In three weeks' time a few scattered perithecial primordia appeared, and cultures a month old showed scattered perithecia (five to ten per sq. cm.) with fully developed asci and ascospores.

On *p*H 4·5 there was some development of the dark pigment, but this was more noticeable on the cultures of *p*H 5·9–7·6. On media of higher *p*H value than 7·6 the dark colour of the medium made observation of the pigmentation difficult.

On *p*H 9·0 growth was not so vigorous, and fewer perithecia were formed. Fully developed perithecia were found, however, in cultures a month old.

On *p*H 9·2 no growth occurred.

The relative amounts of growth and numbers of perithecial primordia formed, are shown in the following table:

*p*H value of medium	One week	Two weeks	Three weeks
2·5	o o	× o	× o
3·0	× o	×× o	×× o
3·5	× o	×× o	×× *p*
4·5	×× o	×× *p*	××× *pp*
5·4	×× *p*	××× *pp*	××× *pp*
5·9–7·2	×× *pp*	××× *ppp*	××× *pppp*
7·6–8·5	×× *p*	××× *pp*	××× *pp*
8·8	×× *p*	××× *pp*	××× *pp*
9·0	× o	×× *p*	×× *pp*
9·2	o o	o o	o o

(× × = relative amount of mycelium formed. *pp* = relative numbers of primordia developed.)

Thus growth of the fungus and development of perithecia attain their maximum between *p*H 5·9 and *p*H 7·2, indicating that the fungus prefers a neutral or slightly acid medium, though it is capable of growth upon a definitely alkaline medium.

After three weeks' growth, the *p*H values of the media were determined by the capillator method. In testing the solid media a piece of the agar was cut out, melted, and mixed with an equal amount of the indicator.

The results are expressed in the following table:

Original *p*H value of medium (*A*)	After three weeks' growth (*B*)	After four weeks' growth (*C*)	Change in *p*H ($C \times A$)
2·5	4·5	5·0	+ 2·5
3·0	5·0	6·0	+ 3·0
3·5	6·0	6·5	+ 3·0
4·5	6·5	7·0	+ 2·5
5·4	7·2	7·5	+ 2·1
6·2	7·5	7·8	+ 1·6
7·2	7·5	7·8	+ 0·6
7·6	7·8	8·0	+ 0·4
8·5	8·8	8·8	+ 0·3
8·8	8·8	9·0	+ 0·2
9·0	9·0	9·0	+ 0

It will be noted from the figures given in column 4, that where the initial *p*H of the medium is distinctly acid, the fungal metabolism is such that there is a shifting of the *p*H towards the point giving the optimum reaction for growth, this presumably being due to the formation of ammonia.

If, however, the initial reaction of the medium is alkaline, the formation of the alkaline by-product seems to be lessened, though the fungus does not appear to have the power of altering the reaction towards the optimum.

From the fact that the cultures on the *p*H 3·5 medium produced perithecial primordia only after three weeks' growth, by which time the reaction of the medium had been altered to *p*H 6·0, while those cultures on *p*H 4·5 required only two weeks before primordia could be formed, it appears probable that perithecia cannot be produced on a medium of a *p*H value below about 6·0.

The reaction of the sap from uninfected leaves of *Euphorbia Paralias* and from old leaves infected by the fungus was approximately *p*H 6·0.

Zonation of Cultures

The development of perithecial primordia in concentric zones in cultures left on the laboratory bench has already been noted. The occurrence of zonation in fungal cultures has been described by many different investigators, and may be due to any one of many characters of the fungus, such as colour variations, branching of mycelium, and

density of sporulation, etc. These zonation effects have been attributed to various causes, such as the light relations, temperature variations, staling effects and mycelial crowding.

In *Monilia fructigena*, alternate zones of flat and conidia-bearing mycelium occur in culture. Hall (5) attributes this zonation to variations in the light relation, since an exposure of three hours daily to light gives definite rings, while cultures kept in the dark at constant temperature showed no sign of zonation. Zones could also be produced by temperature variation, but were not comparable with those produced by day and night light variations.

Bisby (2), working on *Fusarium discolor* also found that light is the chief conditioning factor causing zonation. Exposure for six minutes at 1 mm. from a 25 C.P. lamp suffices to cause zonation, but temperature is also a conditioning factor, since zonation may be produced by varying the temperature in absolute darkness.

In his work on strains of *Fusarium*, Brown (4) noted some kind of zoning in colour effects, but the most striking zonation effect is due to the arrangement of the spore masses. The tendency to form zones appears to be a function of particular strains, since one strain forms a definite number of rings corresponding to the alternation of day and night, while another strain forms one zone of spores with no relation to the periodicity of light.

Stevens and Hall (8) investigated the phenomenon of zonation for several fungi. In *Alternaria Brassicae* the end of each day's growth marks the edge of a zone which is intensified later by changes of colour. Here light is the chief conditioning factor. In *Ascochyta Chrysanthemi*, however, the zones are not due to light or temperature relations, but to the crowding of the mycelium, which shows alternate zones of profuse and sparse branching. The zonal formation of sclerotia in *Sclerotinia Libertiana* can also be attributed to crowding of the mycelium, rather than to temperature or light effects. The same conclusion probably applies to other fungi of a similar nature.

In *Pleospora herbarum* the zones are due to the formation of large numbers of perithecial primordia in definite concentric rings, separated by zones of vegetative mycelium relatively free from primordia. This gives the appearance of alternate zones of light and dark mycelium, owing to the production of a dark pigment in the primordia and associated hyphae. This pigment is also present to a lesser extent in the older vegetative hyphae. In the centre of each culture there is frequently a uniformly coloured area, with scattered perithecia, corresponding to three or four days' growth, and surrounding this area the alternate light and dark zones described. (See Pl. IV, fig. 1, of a culture on malt agar, fourteen days old, which has been left on the laboratory bench.)

The zonation effect is most pronounced in cultures growing on a

medium rich in carbohydrate, such as malt agar. On the various synthetic media the mycelium is less darkly coloured, though still distinctly brown in mature cultures, and the perithecia are produced in definite rings. Decrease of the sugar content in these media causes a decrease in the pigmentation of the mycelium, and so makes the zones less distinct. Cultures on unwashed agar without added nutrient show no trace of the pigment except in the conidia and perithecial primordia. Examination with a lens, however, shows that the primordia occur in definite rings. On washed agar with no added nutrient, the primordia are so few that it is impossible to trace any distinct zonation.

The fact that the zonation effect is seen only in cultures that are left exposed on the laboratory bench, led to the supposition that it is probably due to variations either of light or of temperature, rather than to staling effects in the medium, and it was decided to determine by experiment whether the light or the temperature relation was the conditioning factor. Accordingly a number of cultures were made on 2 per cent. malt agar, and treated as follows:

Set *A* was kept in the dark, incubated at 22° C. Here the temperature was constant, and the only variations of illumination were due to the temporary opening of the incubator for examination of the cultures.

Set *B* was incubated at 22° C., the dishes being wrapped in black paper so that both light and temperature factors were constant.

Set *C* was kept on the laboratory bench, the dishes wrapped in black paper, so that variations of temperature could affect the growth of the fungus, while the light factor remained constant.

Set *D* was also kept on the laboratory bench, the dishes wrapped in white, translucent paper. (This white paper was used in order to keep these cultures in the same conditions as the others with regard to humidity, air supply, etc.)

It was found that in cultures from sets *A* and *B* there was no sign of zonation (see Pl. IV, fig. 2). In both the mycelium appeared uniform in colour, with the perithecia more or less evenly scattered. The cultures of sets *C* and *D* both showed definite zonation.

Cultures kept at constant temperature, but exposed for a definite period (six hours) each day to daylight, showed no sign of zonation, nor did cultures kept at constant temperature but exposed to diurnal light variations.

From these results it may be concluded that variation of temperature is an important factor, if not the chief factor, in determining the zonation of cultures of this fungus, variations of illumination having no apparent effect. If this assumption is correct, it should be possible to induce artificial zonation by varying the temperature conditions.

Plates of 2 per cent. malt agar were inoculated and kept for alter-

nate two-day periods at 22° C. and 14° C. The diameter of each culture was measured at each change. The rate of growth of the mycelium was more rapid at 22° C. These cultures, examined after three weeks' growth, showed alternate light and dark zones of mycelium, the dark zones corresponding to the periods at the lower temperature. The dark zones consisted of mycelium with a greater development of the dark pigment and with more numerous perithecial primordia. A greater amount of aerial mycelium is also formed in these zones, giving the appearance of floccose rings on the upper surface of the culture, corresponding to the dark rings on the under surface (Pl. IV, figs. 3 and 4).

The experiment was repeated, the cultures this time being kept for alternating two-day periods at 28° C. and 22° C. The rate of growth was considerably greater at the lower temperature, from which it may be surmised that there is an optimum temperature for vegetative growth at about 22° C., any variation from this temperature causing a slowing-down of growth. (A further experiment, involving a series of cultures grown at temperatures ranging from 14° C. to 35° C., and measured at regular intervals, confirms this conclusion.)

These cultures, examined after three weeks' growth, also showed the alternating zones of light and dark mycelium, but the darker zones here corresponded to the periods at 28° C.

Other cultures were kept at a constant temperature of 22° C. for different periods, e.g. seven days, fourteen days, and then subjected to variations of temperature. No zones were formed in the central parts of these cultures (*i.e.* in the parts developed at constant temperature), but zones appeared as soon as the temperature was varied.

It was found that in cultures kept at 22° C. exposure for one hour each day to temperatures of 14° C., 25° C., 28° C. or 35° C. gave a definite zonation, the effect being most pronounced on exposure to 35° C.

It may therefore be concluded that there is an optimum temperature for vegetative growth at about 22° C. Exposure to higher or lower temperatures slows down vegetative growth, and this check is accompanied by the production of numerous perithecial primordia. The high concentration of the dark pigment in the walls of these, and of adjacent hyphae, gives the effect of the dark zones.

It is probable that any check to the vegetative growth of the mycelium will be followed by the production of increased numbers of reproductive structures, though all these may not necessarily mature.

A similar conclusion has been reached with regard to other fungi. Robinson (7), for example, finds that in *Pyronema confluens*, reproductive organs are formed as the result of any check upon vegetative growth. In *Pleospora herbarum*, such a check is not necessary to cause the

formation of reproductive bodies, but has the effect of increasing the number produced.

This conclusion is supported by the fact that in many cultures, especially on media poor in nutrient material, a greater number of perithecial primordia are laid down near the edge of the dish. This is especially noticeable in cultures on agar without added nutrient, where often only a few scattered primordia are formed in the centre of the culture, and a far greater number occur in a zone about 5 mm. wide near the periphery. Here vegetative growth is checked when the hyphal tips touch the glass, and an increased formation of reproductive structures follows.

Occurrence of Saltation in Cultures

The appearance of saltants in a single-spore culture of a fungus is of common occurrence, described by many investigators.

The factors causing saltation are in most cases unknown, though Barnes (1) has produced, experimentally, variations in *Eurotium herbariorum* by the effect of heat on the spores, and Brown (4) suggests that saltations in *Fusarium* are most likely to occur on media which combine high concentration with minimal staling capacity.

Saltation was first noted in *Pleospora herbarum* when certain cultures showed a sectored effect (Pl. IV, fig. 5). Some parts of such a culture are composed of normal mycelium, bearing conidia and perithecia, while other parts are composed of colourless, much-branched hyphae forming a white, floccose mycelium which shows no sign of forming conidia or perithecia even after some weeks' growth, and which produces only a negligible amount of the dark pigment in the vegetative hyphae.

A large number of subcultures were taken from the normal part of such a culture, and of these about one-third showed the same appearance, the remainder being normal. Subcultures taken from the non-sectored parent of the first sectored culture were nearly all normal, but a few varied in the same manner. All subcultures taken from the white sectors produced the same white, highly floccose mycelium, incapable of forming conidia or perithecia, and in repeated subcultures on different media showed no sign of reverting to the normal, nor of giving rise to further saltations.

An explanation is thus offered of the occcasional tufts of white, floccose mycelium sometimes seen in otherwise normal cultures (Pl. IV, fig. 3). These tufts occur very frequently on the rich malt extract media, and less frequently on the synthetic media. No signs of saltation were observed on the media extremely poor in nutrient material, but the saltant when grown on these media retained its characters.

Subcultures were made from these tufts, and from the normal mycelium near them, and again the two strains were obtained. Thus the white, non-fertile strain may arise in culture on any medium containing a sufficiency of nutrient material for normal growth, and may appear either in small tufts, or in definite wedges, giving the culture a sectored appearance.

Conclusions

Pleospora herbarum, a facultative parasite upon *Euphorbia Paralias*, can easily be obtained in culture upon artificial media, both conidia and ascospores germinating readily. The fungus can grow and form reproductive structures upon media extremely poor in nutrient material, but on such media the mycelium is sparse (in extreme cases showing signs of starvation), and the perithecia are few, although abundant conidia are produced. The variation of the nitrogen content of the medium does not affect the fungus as profoundly as the variation of the carbon content. It is the latter which controls the pigmentation of the fungal mycelium, and to a large extent the production of perithecial primordia. Unless a sufficiency of available carbon is present, no pigment is produced except in the reproductive structures, and these are few in number; while the richer the medium, the more numerous are the perithecial primordia, and the more pronounced the pigmentation of the mycelium. In very rich media, the primordia are so numerous that only a small proportion can develop, whereas in poorer media the actual number of primordia formed is smaller, but a greater proportion of these reach maturity.

The fungus seems able to utilise any source of carbon, if necessary, but the most favourable media for growth and reproduction are those rich in such sugars as maltose.

Vegetative growth can take place on strongly acid or alkaline media, but no reproductive structures are developed on the former until the fungus has been growing for some time. During growth the fungus produces some substance of an alkaline nature, probably ammonia, which causes a decrease in acidity, and reproductive structures are not formed until the medium has attained a suitable reaction (roughly *p*H 6·0 which corresponds to the *p*H value of the sap of the host plant).

The zonation effect seen in many fungal cultures is very pronounced in *Pleospora herbarum*, giving the appearance of alternating zones of light and dark mycelium. This effect is caused by natural or artificial temperature variations, any variation from the optimum temperature causing a check to vegetative growth, which is accompanied by the formation of increased numbers of reproductive structures. This in turn involves increased formation of the dark pigment, giving the effect of dark zones in the cultures.

The production of reproductive organs as the result of a check to vegetative growth has been described for other fungi, and may well be so in *Pleospora herbarum*. It must be noted, however, that such a check is not necessary for their formation, but only causes an increase in the numbers formed.

The fact that in many cultures, especially on media poor in nutrient substances, an increased number of perithecia are formed near the periphery of the dish, supports the above conclusion.

Saltation frequently occurs in cultures, resulting in the production of a white, non-fertile strain which appears either in small tufts or in wedge-like sectors. No other saltations were observed. The new strain is easily isolated, and retains its characters on any medium. The factors which cause this saltation are not known.

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EXPLANATION OF PLATE IV

Fig. 1. Culture exposed to day and night temperature variations, showing zonation.
Fig. 2. Culture kept at constant temperature, showing uniformly scattered perithecia.
Fig. 3. Culture kept for alternate two-day periods at 22° C. and 14° C., showing artificial zonation (upper surface of culture).
Fig. 4. Culture kept for alternate two-day periods at 22° C. and 14° C., showing artificial zonation (under surface of culture).
Fig. 5. Saltation. Culture showing appearance of new strain in wedges, giving sectored appearance to mycelium.

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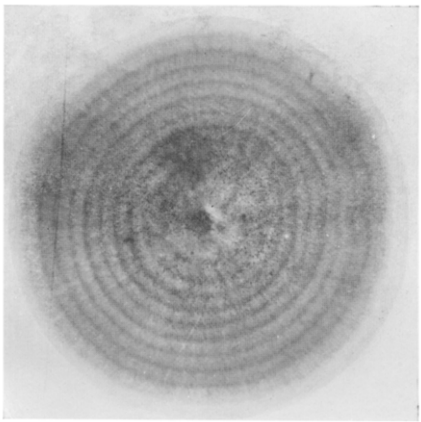
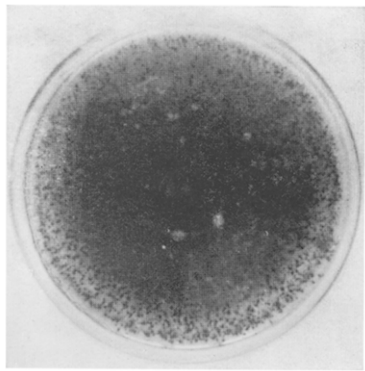


Fig. 1.



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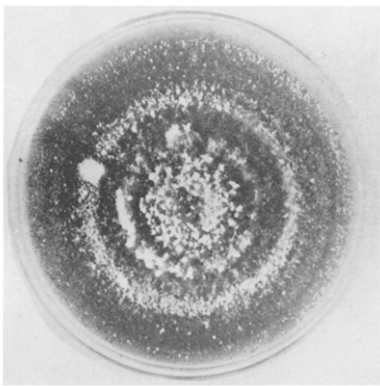

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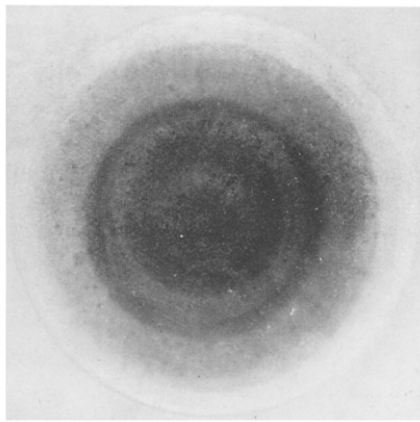

Fig. 4.

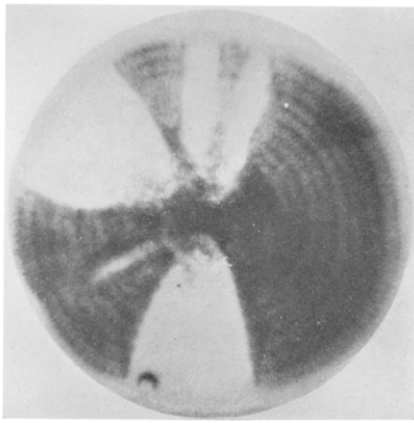

Fig. 5.

Pleospora herbarum (Pers.) Rabenh.