Some Experiments on the Effect of External Stimuli on the Sporidia of Puccinia malvacearum (Mont.).

BY

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With seven Figures in the Text.

In certain preliminary experiments in which the sporidia of Puccinia malvacearum were germinated near a fragment of hollyhock leaf, it was observed that all the germ tubes grew in one direction, and pointed towards the leaf fragment. This at first suggested positive chemotropism. More critical work, however, revealed the fact that it was an effect produced by the unilateral illumination of the sporidia. This led to the investigation of the tropic influences affecting the germinating sporidia of Puccinia malvacearum.

De Bary\(^1\) first raised the question as to why the germ tubes of parasitic fungi turn towards, and penetrate the tissues of their host-plants. Even before the publication of the work of Pfeffer on external stimuli de Bary suggested that 'physical irritations' or chemical stimuli might play a part in these phenomena. Later, as a result of numerous experiments on the chemotropism of Fungi, Miyoshi\(^2\) concluded that certain chemical substances attract the germ tubes of Fungi, whilst others repel them. Nordhausen\(^3\) accepted Miyoshi's results and investigated the biology of the penetration of plant tissues by Botrytis and other facultative parasites.

More recently Massee\(^4\) has concluded that the germ tubes of Fungi are attracted to their hosts by chemotropic substances secreted by the cell sap. In 1906 Fulton\(^5\) repeated many of Miyoshi's experiments, and concluded that in the light of known facts, no simple explanation such as the theory


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of chemotropism will explain the entrance of the germ tubes of parasitic Fungi into the tissues of the host-plant. He also found that various Fungi show positive hydrotropism, but that an over-abundance of moisture may cause a negative reaction in certain Fungi.

From a large number of experiments on the effect of tannin upon the germination and growth of many Fungi, Cook and Taubenhaus in 1911 found that this substance has a tendency to retard the growth of Fungi. They found also that the parasitic forms are more sensitive to the action of tannin than the saprophytic forms they used. It should be noted, however, that the parasites employed were not of the obligate class, as they could be cultivated upon nutrient media apart from the living host-plant. These investigators suggest also that tannin may, to some extent, serve as a protective agent against the attacks of parasitic Fungi.

Up to the present the Fungi used in this kind of work have, for the most part, been saprophytes or facultative parasites. We have, therefore, very little information respecting the influence of external stimuli on the germ tubes of obligate parasites. The causes which determine the penetration of the epidermis of the host-plant by the germ tube of the sporidium of Puccinia malvacearum are obviously somewhat different from those operating in the case of infection by uredospores or aecidiospores. In infection by the latter, entrance is effected through the stomata, and a very considerable amount of growth can occur at the expense of the reserve material in the relatively large spore before the fungus enters into its normal relationship with the host-plant. On the other hand, the sporidium is a small thin-walled spore with very little reserve material, and consequently, in order to succeed, its germ tube must promptly enter the tissues of the host. What determines this entry is unknown. In order to obtain some insight into the stimuli affecting the germinating sporidia, experiments were designed to test the effects of fragments of various leaves, of light, of gravity, and of water upon them.

Most of the experiments were carried out in Van Tieghem's cells, and large pure sowings of sporidia were obtained from germinating teleutospores by the device shown in Fig. 1. A small fragment of hollyhock leaf bearing a portion of a teleutospore sorus (a) was fastened to the floor of the cell by a small quantity of vaseline. Near this, but not in contact with it,

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Fig. 1. A sectional elevation of a Van Tieghem cell as used in these experiments. a, fragment of a sorus with ripe teleutospores; b, a small drop of water; c, a drop of gelatine.

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was placed a drop of water (b) to ensure a saturated atmosphere. A large rectangular cover-slip was used, and the whole slide with the cell was inverted so that the sporidia, when produced, would fall on the cover-glass (c). A good sowing of sporidia was usually obtained in twelve hours. The sporidia were found to germinate equally well when sown in a thin film of water, or on the surface of 2 per cent. gelatine. For most of the experiments the sporidia were allowed to fall on a drop of 2 per cent. gelatine spread on the cover-glass of the cell. In this way the possibility of the direction of the germ tubes being altered by the necessary movement of the cell was avoided.

The first series of experiments was arranged to test whether fragments of various leaves, placed near the germinating sporidia, exercised any influence on their germination or on the subsequent direction taken by the germ tubes. The leaf fragment in each case was cut with a clean scalpel, washed with distilled water, roughly dried on filter paper, and placed on the surface of a drop of gelatine on the cover of a cell. The sporidia were sown around the leaf fragment by the device already described, and all the cultures were kept in the dark. These were examined after twenty-four hours and the results were as follow. For fragments of the leaves of Althaea rosea, Ranunculus Ficaria, Stellaria media, Oxalis (sp.), Fuchsia (sp.), Solanum tuberosum, Sinapis nigra, Crataegus oxyacantha, Tropaeolum (sp.), Geranium Robertianum, Mercurialis perennis, Tradescantia (sp.), Quercus Robur, and Citrus Aurantium, vigorous normal germination was obtained. For fragments of the leaves of Primula vulgaris and garden geranium (Pelargonium) germination was, to a large extent, inhibited. Although in these cases no normal germ tubes were produced, some cases of abnormal germination were observed in all the tests. Fragments of the leaf of Eucalyptus globulus showed similar inhibitory effects, although some normal germ tubes also appeared in this case.

From these results it is clear that the germination of the sporidia in close proximity to most of the leaf fragments was quite normal and vigorous. No difference could be detected between the behaviour of the sporidia germinated near a fragment of the leaf of the normal host-plant, Althaea rosea, and that of sporidia near leaves of many of the other species used in these experiments. Whilst no positive stimulatory results were obtained, it has been mentioned that certain negative effects were seen with
leaf fragments of *Pelargonium*, *Primula vulgaris*, and *Eucalyptus globulus*. None of the sporidia germinated normally in the vicinity of fragments of the leaves of *Pelargonium* and *Primula vulgaris*. The sporidia in these cultures, however, made many abortive attempts at germination. Instead of putting out a normal germ tube the sporidium became distended, giving rise to one or more bud-like structures (Fig. 2). The contents of these were granular and contrasted strongly with the hyaline appearance of the normal germ tubes. These abortive germ tubes did not grow any further.

Similar abnormal germinations were observed where fragments of the leaves of *Eucalyptus globulus* were used, but here the inhibitory effect on germination was not so strongly marked; for some of the sporidia in each culture germinated normally. In one of these experiments with *Eucalyptus* a series of counts was made of the actual numbers of sporidia which, respectively, germinated normally, abnormally, or not at all. These counts were taken in successive areas, moving from the leaf fragments outwards to the margin of the drop. Table I shows the results of these counts.

**Table I. (Exp. 35, Series II.)**

<table>
<thead>
<tr>
<th>Area next leaf fragment</th>
<th>Total No. of spores in area</th>
<th>No. of spores showing normal germination</th>
<th>No. of spores showing abortive germination</th>
<th>No. of spores not germinated</th>
<th>% of normal germination</th>
<th>% of abnormal germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>13</td>
<td>44</td>
<td>9</td>
<td>19</td>
<td>66</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>17</td>
<td>35</td>
<td>28</td>
<td>21</td>
<td>43</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>16</td>
<td>9</td>
<td>32</td>
<td>28</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>11</td>
<td>5</td>
<td>39</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>13</td>
<td>1</td>
<td>32</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>62</td>
<td></td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area most remote from leaf fragment</td>
<td>7</td>
<td>55</td>
<td>55</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The two columns on the extreme right give percentages obtained from the figures in the corresponding counts. The percentage of sporidia which germinated at all, near the fragment, was small, but increased on moving away, until on the margin of the drop 100 per cent. of the sporidia germinated normally. More striking, however, is the fact that the percentage of abnormal germinations was greatest near to the leaf fragment, and gradually diminished to nil on the margin of the drop.

It seemed possible that in the experiments with the fragments of garden geranium leaf, the substance which affected the germination of the sporidia might be localized in the glandular hairs of the leaf. An experiment was accordingly set up, in which the sporidia were allowed to germinate near to a fragment from which the epidermis had been carefully removed. In this case excellent germination took place. It has been mentioned above that where a fragment of the leaf of *Geranium Robertianum*
was used, quite normal and vigorous germination resulted. The material of this species used had very few glandular hairs.

A number of experiments was carried out in which the sporidia were sown upon the epidermis of short pieces of the petiole of leaves of Althaea, Pelargonium, and potato respectively. After three days the epidermis on which the sporidia rested was stripped off, fixed in alcohol, and either stained in erythrosin and mounted in glycerine or embedded and cut in serial sections. No normal germination was noted in the case of Pelargonium, the sporidia having behaved as in the cover-glass cultures. On the leaves of Althaea and potato, however, the germ tubes were normal and their tips showed a slight swelling which was pressed against the epidermis (Fig. 3).

Fig. 3. Sporidia germinating on the epidermis of a fragment of the petiole of a potato leaf. Normal germination with a slight swelling at the tip of each germ tube pressed against the epidermis, but no penetration of the cuticle. × 300.

No case of penetration of the epidermis by the germ tube was seen in the case of the potato, though on Althaea the tip of the germ tube had penetrated the epidermis and formed an infection vesicle within the epidermal cell. This normal infection has already been described and figured by Kellerman, Rathay, Eriksson, and myself.

My attention was called to the negative heliotropism of the germ tubes by a preliminary experiment in which they appeared to be directed towards a fragment of leaf on the drop of gelatine. More critical examination of

4 On Some Relations between P. malvacearum and the Tissues of its Host-plant. Memoirs of the Manchester Literary and Philosophical Society, 1913.
this experiment suggested that the direction was influenced by the shadow cast from the leaf fragment and not by the substance of the leaf. The effect of light on the direction taken by the germ tubes was therefore investigated.

Several pairs of cultures were set up in which sowings of sporidia were made on the surface of a drop of gelatine as already described. One from each pair was placed on the laboratory bench in such a position that it was illuminated from one side by the light from the window. The control cultures were placed in the dark. After 16 hours all the sporidia in every cell had germinated equally well. Whilst the germ tubes of those in the dark were indifferent as to direction, the germ tubes of those which were illuminated from one side had all grown away from the light. Fig. 4,

![Fig. 4. A. Sowing of sporidia showing germ tubes growing away from the light of the window. The arrow indicates the direction of the light. B. Control to the experiment figured in A carried out in the dark: the germ tubes are growing in all directions. $\times 45$.](image)

A and B, represents one pair of these tests. In this figure and in Figs. 5 and 6 the position of the spores and the direction of the germ tubes are accurately indicated, the figures having been drawn with the aid of the camera lucida.

In order further to test this effect of light on the direction of the germ tubes another double series of cultures was set up. One from each pair of these was placed on the bench as before in front of the window. The controls, instead of being placed in darkness, were rotated horizontally on a klinostat which was placed in front of the same window. As before, all the germ tubes in the cultures at rest were directed away from the light, while in the control cultures they had grown in all directions. The resulting appearance was so similar to that shown in Fig. 4 that illustration is unnecessary.

A still further confirmation of this effect of light was obtained by the following experiment. One of the cells was arranged as before, except that a minute partition of tin-foil, about 1 mm. high and 1 cm. long, was fixed on the surface of the gelatine. The culture was placed before the window with
the partition parallel to the window. Fig. 5 shows the result; the germ tubes on the light side (A) of the partition (p) are all growing towards the latter, i.e. away from the light. On the other side (B), however, the spores lying in the zone of shadow have their germ tubes directed towards the partition, i.e. further into the shade. A little further away they are growing in all directions, whilst entirely beyond the influence of the shadow they are pointing away from the light of the window. A control culture with a similar partition in the dark showed, as before, the germ tubes growing indifferently in all directions.

Miyoshi\(^1\) found in his work on the chemotropism of Fungi that light exercised no influence upon the germinating spores of the Fungi he used in his experiments. It should be noted, however, that he only experimented with one obligate parasite, viz. the uredospores of *Uredo linearis*.

For purposes of comparison similar series of experiments to those described were set up in which aecidiospores of *Puccinia poarum*, the conidia of *Botrytis* (sp.), *Alternaria*, *Penicillium glaucum*, and *Peronospora parasitica* were employed. The results of these experiments testing the effect of light, as well as those already described for the sporidia of *P. malvacearum*, are summarized in Table II.

**Table II.**

<table>
<thead>
<tr>
<th>Spore</th>
<th>No. of tests separately made</th>
<th>Medium of hanging drop</th>
<th>Effect of unilateral light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporidia of <em>P. malvacearum</em></td>
<td>25</td>
<td>2 % gelatine</td>
<td>All germ tubes directed away from light.</td>
</tr>
<tr>
<td>Aecidiospores of <em>P. poarum</em></td>
<td>6</td>
<td>2 % gelatine</td>
<td>Indifferent to light.</td>
</tr>
<tr>
<td><em>Botrytis</em> (sp.)</td>
<td>12</td>
<td>2 % gelatine</td>
<td>All germ tubes directed away from light.</td>
</tr>
<tr>
<td><em>Alternaria</em> (sp.)</td>
<td>4</td>
<td>2 % gelatine</td>
<td>Indifferent to light.</td>
</tr>
<tr>
<td><em>Penicillium glaucum</em></td>
<td>6</td>
<td>2 % gelatine</td>
<td></td>
</tr>
<tr>
<td><em>Peronospora parasitica</em></td>
<td>6</td>
<td>Water</td>
<td></td>
</tr>
</tbody>
</table>

Fulton\(^2\) in investigations on the chemotropism of Fungi obtained results which he regarded as negativing Miyoshi’s conclusions. He did not specially test the effect of light, but states that it probably does not enter as a tropic factor. This appears to be so for some cases. My experiments for *Botrytis*,

\(^1\) Loc. cit.  
\(^2\) Loc. cit. p. 105.
Fig. 6. A. Sowing of the conidia of *Botrytis* showing germ tubes growing away from the light of the window. The arrow shows the direction of the light. × 45. B. Control experiment carried out in the dark; the germ tubes growing in all directions. × 45

Fig. 7. Sporidia germinating in droplets of condensation water. All the germ tubes are directed out of the droplets which are indicated by the dotted lines. × 100.
which was one of the species Fulton used, show conclusively that the direction of the growth of the germ tubes was influenced by light. Fig. 6A represents one of these experiments with Botrytis and Fig. 6B is the corresponding control which was kept in the dark.

The effect of water on the germination of the sporidia is of some interest. In certain preliminary experiments where the sporidia germinated in the droplets of water which condensed on the cover of the cell, it was observed that the germ tubes were invariably directed towards the margin of the droplet and often grew out of it (Fig. 7). It was not decided how far this was an expression of negative hydrotropism or merely of the need of the growing germ tubes for oxygen. It may be mentioned that where the sporidia were germinated in an atmosphere saturated with water vapour they frequently produced germ tubes which formed a slight swelling at the tip and grew into the gelatine.

The growth and direction of the germ tubes did not appear to be influenced in any way by gravity.

**Summary and Conclusion.**

The results of the experiments made on the effect of various stimuli on the germinating sporidia of Puccinia malvacearum may be briefly summarized thus:

1. The sporidia of P. malvacearum have been shown to be negatively heliotropic, and this is also the case for the conidia of a species of Botrytis; whilst with conidia of Penicillium, Alternaria, Peronospora, and aecidiospores of Puccinia poarum no irritability to light was apparent.

2. Other influences shown to affect the germ tubes are moisture and contact. As regards moisture, the germ tubes tend to grow out of a drop of water into the moist atmosphere around. On the other hand, germ tubes, on the surface of gelatine in a moist atmosphere, tend to penetrate the gelatine. These influences are difficult to analyse satisfactorily. With respect to contact, the tip of the germ tube swells and becomes closely applied to the epidermal surface of both the host and non-susceptible plants. This may, perhaps, be a result of the contact.

3. On the normal host-plant a very slender growth from the swollen end of the germ tube penetrates the cuticle and outer wall of the epidermis, and this brings about true infection. This has not been seen in any case on plants other than the normal hosts although numerous attempts at infection were made.

4. No evidence of chemotropic influences radiating from a fragment of leaf laid on a drop of gelatine could be obtained. There were no indications of positive chemotropism of the germ tubes towards the normal host or of negative chemotropism of the germ tubes towards fragments of non-susceptible leaves.
5. In the case of certain leaves (Pelargonium, Eucalyptus, Primula) with glandular secretions, a definite toxic effect was evidently exerted on sporidia and germ tubes in the immediate neighbourhood of the fragment of the leaf. This is a special phenomenon presumably related to the presence of glandular secretions and cannot be extended generally to leaves that are not susceptible to infection.

So far as the results bear on the question of why the normal host should be infected whilst other plants are not, they simply confirm this fact without explaining it. They do not support any of the explanations so far offered of this natural immunity or susceptibility, nor do they indicate the direction in which an explanation is to be sought.

The experiments, however, result in indicating that the germ tube is irritable to light, to a certain degree of moisture, and to contact. These irritabilities are possibly advantageous in bringing the tip of the germ tube of a sporidium into the most favourable position for infection, but the problem as to what permits or determines actual infection still remains.

In conclusion, I wish to acknowledge my indebtedness to Professor W. H. Lang for suggesting this investigation and for his continual advice and assistance in the course of the work. I am also grateful to Mr. D. Thoday for his helpful interest in the experiments.

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