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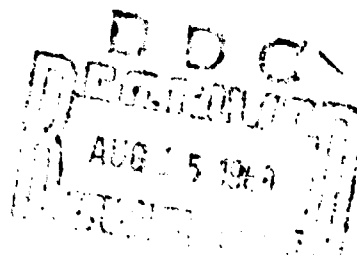
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PHOTOTROPISM OF GERMINATING MYCELIA OF
SOME PARASITIC FUNGI

[Following is a translation of an article by Gisela Gettkandt in the German-language periodical Wissenschaftliche Zeitschrift der Martin-Luther-Universität Halle-Wittenberg (Scientific Journal of the Martin Luther University Halle-Wittenberg), Vol III, No 3, Halle (Saale), March 1954, pages 691-710.]

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I. INTRODUCTION

Chapter 1: Historical Survey and Purpose of the Investigation

Very little is as yet known about the phototropic reaction effects of the germinating mycelia of parasitic fungi. As far as I know, Ward (1902), was the first to observe negative phototropic germ-tube flexures in the uredospores of Puccinia dispersa. Fromme (1915) found this to be true for P. rhamni (= P. coronata) and confirmed by Mains (1917). Robinson (1914) observed such reactions in germinated sporidia of P. malvacearum and in germinated conidia of the Hyphomycetes genus Botrytis. Stock (1931) investigated uredospores of the cereal rusts P. graminis, triticea, coronifera, and dispersa with the same results. These authors used daylight in their investigations. In addition Forbes (1939) used light filters and obtained good reactions with P. triticea using blue and violet filters.

From these reports cited it can be concluded that most research to the present time was content to establish negative phototropism, but for the problem as a whole, there is intensive as well as extensive work yet necessary. The following investigations constitute a contribution to this purpose and I hope it will stimulate further research in this area.

Up to now only a few Uredinales, and two or three fungi of other groups were examined for the presence or absence of a characteristic reaction in its germinating mycelia. Thus, we as yet do not know how widespread this capability is, nor if we can ascribe to such a reaction any actual significance for the occurrence of infection. I have checked and confirmed some of the older observations. In addition I have examined eight more Uredinales species among which some react phototropically and some do not (Chapter 4). From these results we can already derive some conclusions on the ecologic significance of these reaction capabilities.

However, the phototropism of germinating mycelia deserves our attention for completely different reasons (Chapter 5). Involved here are unicelled organisms which in their reaction mechanisms probably displace the spore bearers of phycomycetes and liverwort rhizoids. It appears desirable to subject at least one representative of this group to a precise analysis of stimulus physiology. First, the lowest illumination intensity is determined at which point there are still phototropic flexures (Chapter 6), after which an approximate determination of the wave lengths are made with the aid of compound filters (Chapter 7). The next chapters (8-10) deal with those questions which are of critical importance for the phototropism of one-celled hyaline organisms. These questions include the inversion of the phototropic flexure in liquid paraffin, the determination of the light growth reaction, and the establishment of the location the light-sensitive zone. In the final chapter (11) the results of Part IV are discussed. In this context the question of the effect of carotene

on the phototropic reaction is discussed.

The method of research is presented in Chapters 2 and 3 of Part II.

II. METHOD OF INVESTIGATION

Chapter 2. Method of Culture of Germinating Mycelia

Although, as is known, the uredospores germinate in drops of water in a short time, I chose a solid medium because it appeared most suitable for phototropic research. In a solid medium the germ tube will not alter with vibration etc. I tested, first, freshly cut young wheat blades placed on damp blotting paper under an inverted petri dish. The extracted uredospores germinated in two hours but, observation was difficult because of the thickness of the blades and the minute hairs on the surface of the blade. In addition, the scraped epidermis of Crassulaceae which was spread on a drop of malt agar proved to be a failure because of the poor germination of the spores.

In contrast good germination occurred after two hours in 2% agar and 7% gelatin without any further addition. In the agar the germ tubes have a tendency toward very tortuous growth. The 7% gelatin was found to be the best suited medium and will be used for all further research. The gelatin is spread in flat drops on an ordinary slide. The spores are inoculated directly into the hardened gel by brush tip. The slide is placed on a small stand under a dampened glass bell one half of which is covered with black paper so that the spores are only illuminated from one side by daylight.

Under these conditions of side illumination the germ tubes do not grow into the medium but only along its surface. After several hours the growth slows down. For this research we are concerned only with the growth of the germ tube during the first hours, at a time when it is proceeding normally. [Note: Penetration into the gelatin takes place, in contrast if the preparation is perpendicularly illuminated from above.]

At first I was much concerned with the capriciousness of the germination of the Uredinales spores as reported by De Bary (1865), Eriksson (1896). Klebhan (1904), and Lehmann (1937). Nevertheless good uniform germination was obtained by employing ripe but not overly mature spores. For this reason only those spores were used which fell off still green blades when lightly touched.

Chapter 3. Culture of some Uredinales on Young Wheat Plants

Many problems, such as whether a phototropic condition is present or not, can now be easily solved with freshly collected material. Other problems, however, result in a series of investigations which extend over a

long period. These problems can only be solved if, at all times, good and uniformly germinating spores are available. It is considerably simpler if the fungi involved are easily cultivated in an artificial culture medium, as is the case with Botrytis cinerea. As is known, the Uredinales cannot be cultivated in this simple manner, so that it is necessary to continually reinfect the applicable host plants.

For raw material I used young grain plants injected with uredospores obtained from the local phytopathological institute and from the fields in the vicinity of Halle. Since the uredospores characteristically germinate immediately after ripening, young wheat plants could be reinfected at once. As a result it was possible to have fresh spore material available for many months. The uredospores were cultivated by methods described by Gassner and Appel (1927, p 418) and Gassner and Straib (1928, p 610). The grain blades, at times only one blade, were infected according to the usual inoculation technique of Stakmann (1935).

Infected are:

<u>Puccinia triticina</u>	on Carsten V
<u>Puccinia dispersa</u>	on Petkuse rye
<u>Puccinia simplex</u>	on Fongtien wheat
<u>Puccinia glumarum</u>	on Heines blooms

It was intentional to chose grain types which were especially susceptible to the parasites. The seed was obtained from the Phytopathological Institute of Halle.

III. DISTRIBUTION AND SIGNIFICANCE OF PHOTOTROPISM OF GERMINATING MYCELIA

Chapter 4. Confirmation of Older Data Examination of Eight Additional Uredinales

The following germinating mycelia were investigated:

a) the uredospores of:

Puccinia triticina Eriksson, on wheat
Puccinia dispersa Eriksson and Henning, on rye
Puccinia coronata avenae Eriksson, on oats
Puccinia simplex (Korn.) Eriksson and Henning, on barley
Puccinia glumarum (Schmidt) E. and H., on wheat
Puccinia antirrhini Diet. and Holw., on snapdragon
Puccinia suaveolens (Pers.) Rostr., on thistle
Puccinia menthae on peppermint
Phragmidium subcorticium (Schrank) Wint., on a wild rose

b) the aeciospores of:

Puccinia pearum Nielsen, on colt's foot
Puccinia macgilliana Kornicke, on buttercup (*Ranunculus*)

Uromyces nigri (Pers.) de Bary, on cypress spurge

c) conidia of Botrytis cinerea Pers.

1. P. triticea

Stock (1931, p 217) observed and briefly discussed a negative phototropism in a detailed study on germination and the growth of germ tubes of uredospores of some grain rusts.

The uredospores are spherical, light brown, translucent, with a diameter of $17.6 - 26 \mu$ (microns). The exospore reveals small nodelets, the endospore is smooth and is supplied with germinating pores. The spores have a reserve supply of oil droplets. They appear orange in color because they possess a coloring substance which was identified as early as 1892 by Zopf (1892) as carotene. Under most favorable conditions the spores germinate after approximately one hour. The spore content flows slowly down the germ tube which after two hours reaches an observable length (about 100μ). At first the carotene-containing droplets are thick and uniform (Figure 1). After 5-6 hours the picture has substantially changed. The carotene droplets are divided into individual zones, which appear in the germ tubes as diagonal bands (Figure 2). In all germ tubes, young as well as old, the tip zone is constantly of 4-6 μ length, is completely free of carotene. The older the germ tube becomes the less it grows and then in part ramifies. I have not been able to observe here any septation as appears in the mature mycelia of the host plants. Growth in the germ tube nearly always ceases after 10-12 hours, only very rarely does it continue to 15-18 hours.



Figure 1

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Pucc. triticea, uredospores with germ tubes. $2\frac{1}{2}$ hours after insemination. Carotene content indicated by stippling. Magn. x 375

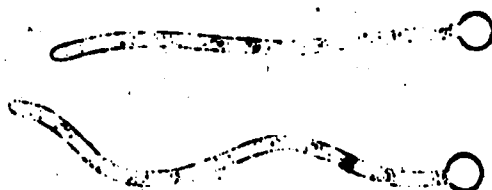


Figure 2

Pucc. triticeae, uredospores with germ tubes. 6½ hours after inoculation. Carotene zone stippled. Magn. x 250

It should be mentioned that the germ tubes grow along the surface of the gelatin in darkness or in horizontal lighting, neither penetrating or lifting off the surface. This is true for spores inoculated in gelatin in horizontal drops or in the vertical position. It may be concluded from this that geotropism has no effect upon the growth. To confirm this I placed the slides vertically in darkness. Two hours after inoculation the slides were examined closely. There was no growth direction preference recognizable. The distribution was the same as in the horizontally placed preparation (see Figure 3).

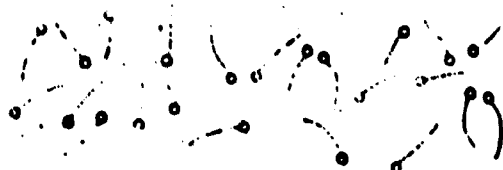


Figure 3

Pucc. triticeae. Germ tubes in darkness. Two hours after inoculation; not oriented! Magn. x 45

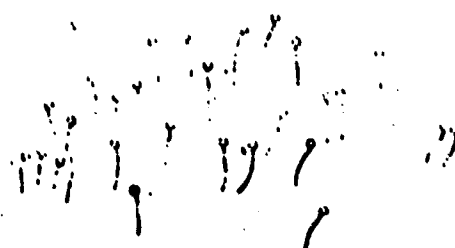


Figure 4

Pucc. triticeae, 2 hours after inoculation. Negative germ-tube phototropism under daylight from one side. Temp. 20°, Magn. x 45

After exposure to one-sided lighting the inseminated spores have quite a difference appearance. The geru tubes are now almost without exception fairly accurately directed into the path of the light source, showing by this that they possess strong negative phototropism (Figure 4). If the preparation is turned 90° and is again illuminated from one side, there soon occurs a renewed flexure. Frequent repetitions invariably produced the same results (Figures 5 and 6).

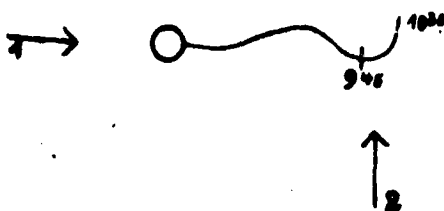


Figure 5

Pen. triticea, first illuminated in Direction 1; 2 hours after insemination turned 90° and illuminated from Direction 2. Repeated negative phototropic reaction, Temp. 19° , Magn. $\times 150$.

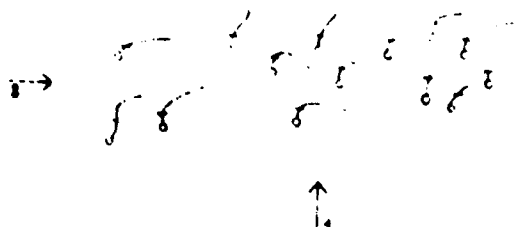


Figure 6

Pen. triticea, $1\frac{1}{2}$ hours after insemination turned 90° . Repeated negative phototropic reaction, Temp. 19° , Magn. $\times 45$.

All tests were repeated under lamplight in a darkroom using an incandescent bulb (clear glass 60 Watt, 57 HK Hefner-Kersten; Hefner candles at 213 volts) at a distance of 35 and 50 centimeters of the slides. The light on the slides arrived at a flat angle obliquely from above. A part of the light was lost in the reflection from the side of the glass ball. The germ tubes received an intensity of illumination equal to about 200 and 400 lux (HK Meter-Kersten; meter candles).

The individual germ tubes in light and in darkness showed no real difference. The carotene distribution, for example, was the

same under both conditions. The light and dark preparations differed from each other only at two points.

a) The length of the germ tubes in controlled cultures were in every case somewhat less under light conditions than in darkness.

b) The germ tube, under one-sided illumination in all cases emerged from the germ pore present on the dark side. Thus, the spores must be polarized by light even before germination. The same behavior was found for all the spores I investigated if they were capable of reacting phototropically.

2. *P. dispersa*

Ward (1902) reported on a phototropic reaction. Although he noted on p. 268 that this reaction was not completely convincing and that a more precise investigation was required. Stock (1931) confirmed Ward's observation. My research material was collected in June 1951 from a rye field near Halle-Buschdorf. The uredospores are round to elliptical, have a length of 24-26 μ , width 22-26 μ , are brownish, finely spined. With one-sided illumination from both daylight and lamplight I was able to observe a negative phototropic reaction of the germ mycelia. The germ tubes reached a length of about 80-120 after two hours. The investigation in darkness, as a control, revealed that the germ tubes had grown on all sides. The carotene distribution was as in *P. triticea*.

3. *P. coronata*

Fromme (1915), Mains (1917), Stock (1931), and Forbes (1939) observed a negative phototropic reaction also for these mycelia. The uredospores are spherical to ovoid, 16-26 μ long and 14-24 μ wide, finely spined, and yellowish-brown.

Locality: In the vicinity of Halle-Hohenthurm on Avena in July 1951. After three hours the germ tubes reached a length of about 100-130 μ and displayed negative phototropism under one-sided illumination. The carotene distribution was determined to be as in *P. triticea*. The same reaction was observed for *P. coronata* on Lolium perenne which was abundantly infected in the botanical garden.

4. *P. simplex*

The research material comes from the phytopathological institute and from the vicinity of Halle-Buschdorf. The spores were collected and investigated in July.

[The uredospores are round, spherical, brownish-yellow, exospore]

spiny. Its germ mycelia show negative phototropism under one-side illumination (Figure 7). After 2-3 hours, the germ tubes were not quite so long as in species, Nos. 1-3, that is 50-60 μ . They also branch earlier. The carotene distribution is as in *P. tritici*.

As a result of the earlier branching the negative phototropism reaction is less conspicuous than they are in Nos. 1-3 however this reaction was clearly established in many repetitions.

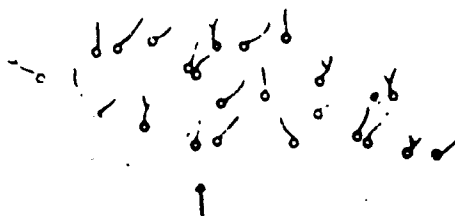


Figure 7

Pucc. simplex, 2 hours after inoculation, one-sided daylight.
Temp. 21, Magn x 45

5. *P. glumarum*

The source of the spore material was the phytopathological institute. The spores are roundish, 18-22 μ broad, light yellow. As compared to the rusts of Nos. 1-4 these spores germinate much later. Only after 12-14 hours do the germ tubes reach a length of 80-100 μ .

Under one-sided illumination no negative phototropic reaction could be observed (Figure 8). The investigation was repeated about 40 times using daylight and lamplight of various intensities. At no time was there even a hint of a negative phototropic reaction. Carotene was also found in the germ tubes and its distribution was as in *P. tritici* (the tips free of carotene; when older, the carotene droplets in conal arrangement).

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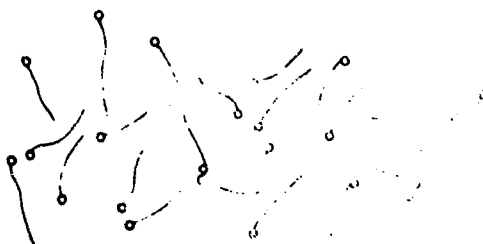


Figure 8

Pucc. glumarum, 16 hours after insemination, no phototropic arrangement (lamplight, about 200 lux, Temp. 21°, Magn. x 45)

6. P. antirrhini

Snapdragon rust is spread fairly well in the vicinity of Halle. The leaf underside, the stem, and the leaves of the calyx are especially thickly covered by chocolate brown pustules. The spores are round to oval, 20-24 μ broad, 20-28 μ long, chocolate brown, and surrounded by a compact exospore. Under favorable conditions the germ tube reaches a length of 60-100 μ after 2-3 hours. When illuminated from the side, no negative phototropism was observed as in the preceding species (Figure 9). In contrast to the preceding species no carotene was seen in the germ tubes which was hyaline and transparent.

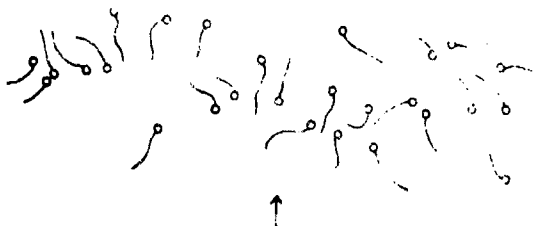


Figure 9

Pucc. antirrhini, 3 hours after insemination, no phototropism (lamplight, about 200 lux). Temp. 21°, Magn. x 45

7. P. suaveolens

The spore material comes from infected thistles from a field near Hohenthurm.

The uredosori are spread over the entire under side of the leaf.

The spores are round to elliptical, spiny, chocolate brown, a diameter of 21-26 μ . After 5-6 hours the germ tube was 80-120 μ long. Here too, negative phototropism under one-sided illumination could not be established (Figure 10). Carotene here too could not be found in the germ tube.

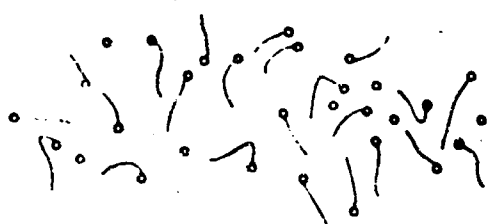


Figure 10

Pucc. guaraniana, 5 hours after insemination, one-sided daylight, no phototropism, Temp. 20°, Magn. x 45

8. P. menthae

The uredospores were obtained from a peppermint field near Sobkeudits. The entire field was infested by this fungus. The cinnamon brown uredosori were especially widespread on the undersides of the leaves. The spores are irregularly round, 18-26 μ long, and 18-20 μ wide; they possess a light yellow to brown exospore with very fine spines. Only after 4-5 hours did the germ tube reach a length of 30-100 μ . A good phototropic reaction consistently took place under one-sided illumination (Figure 11). The germ tube lacked carotene as in Nos. 6 and 7.

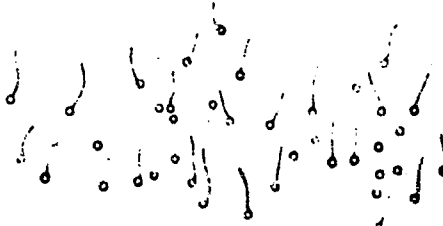


Figure 11

Pucc. menthae, 5 hours after insemination, good phototropic orientation (lamplight about 200 lux). Temp. 20°, Magn x 45

9. Phragmidium subcorticium

This species was found on a wild rose in a Halle garden. The

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leaf undersides were thickly covered by uredospores. The spores are round, oval, finely spined, yellow, 20-24 μ long, and 17-20 μ wide. Only after 4-5 hours did the germ tubes reach a length of about 100 μ . In every case a negative phototropic reaction to one-sided illumination was visible (Figure 12). Carotene was once more present with the same distribution as in P. triticea.

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Figure 12

Phragmidium subcorticium, 5 hours after insemination. Good phototropic orientation (lanplight, about 200 lux). Temp. 20°, Magn. x 45

10. P. poarum

Uredospores were not sought for in this species, but aeciospores which are known to form on Tussilago. The material is from the vicinity of Jena and was collected in August 1951. The spores are polygonal, finely warty, orange yellow, 18-24 μ long, and 16-18 μ wide. Two to three hours after insemination they formed a 50-60 μ length germ mycelium which soon branched. The carotene distribution was as in the uredospores of P. triticea.

The phototropism in this species is not as pronounced as in P. triticea, etc. More than half of ten preparations, however, resulted in the diversion of germ tubes by light (Figure 13). Only after the conclusion of my investigation did I become aware that Robinson (1914) had studied this condition for this species. His results, however, were very brief and only mentioned that in six tests on germ tubes he found them to be "indifferent to light" (1914, page 337, Table 2).

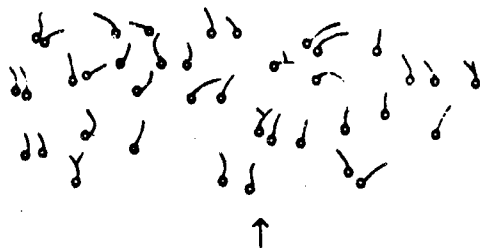


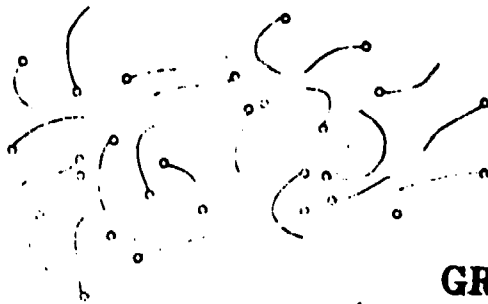
Figure 13

Pucc. pearum, 2 hours after insemination. Negative phototropism (lamplight, about 200 lux). Temp. 20°, Magn. x 45

Since Robinson provided no illustrations for this, one cannot decide whether a great contrast exists between his and my conclusions, as might seem at first glance. Possibly, he considered the delineation of his preparation as insufficient to demonstrate phototropism since this behavior is far less clear for this species than for many others. Nevertheless, I have considered the phototropic characteristic of this species as certain on the basis of statistical analysis.

11. P. magnusiana

Ramunculus ripens, from the vicinity of Halle, was richly covered with aecidosori. The yellowish aecisporae are 16-24 μ wide and 16-26 μ long. After four to five hours the germ tubes reached a noteworthy length of 120-140 μ , without any branching. No phototropism could be established under one-sided illumination in daylight or lamp light in 10 tests. Carotene distribution as in P. triticeina (Figure 14).



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Figure 14

Pucc. magnusiana, 6 hours after insemination. One-sided daylight, no phototropic reaction, Temp. 20°, Magn. x 45

12. Uromyces pisi

The aecidia of these fungi on Euphorbia cyparissias are very commonly found near Halle. The spores are round or elongate, orange-yellow, finely tubercular, with a diameter of 20-24 μ . The spores show a germ-tube length of about 80-120 μ after 4-5 hours. Under one-sided illumination there was no discernible negative phototropism (Figure 15). Carotene distribution as in P. triticea.

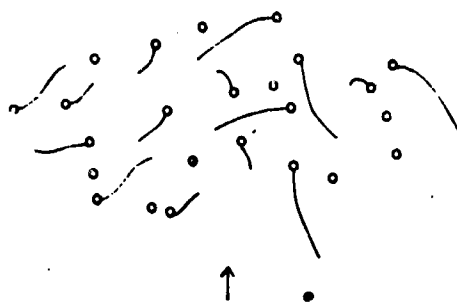


Figure 15

Uromyces pisi, aeciospores, 6 hours after insemination, no reaction. Daylight, Temp. 20°, Magn. x 45

13. Botrytis cinerea

Robinson (1914) observed a negative phototropism for a not closely related Botrytis species. I isolated my source material from rotten cherries and kept it in a culture of 4% malt agar. The conidia are 9-11 μ long and 6.5-9.0 μ wide, tip colorless and completely hyaline in contrast to the rust spores. After 6-8 hours they formed a distinct germ tube on the gelatin, which reacted well phototropically (Figure 16). The germ tube showed no sign of carotene under the microscope, the entire tube remaining crystalline transparent. The mycelium was septate.

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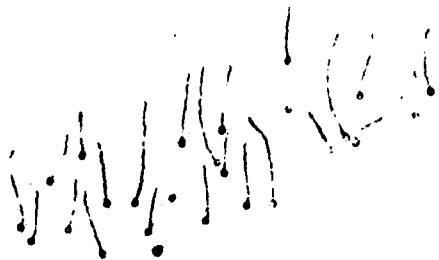


Figure 16

Botrytis cinerea, 12 hours after insemination, good phototropic orientation (lamplight, about 200 lux). Temp. 21°. Magn. x 45

To conclude this chapter, a tabular summary of the phototropic reaction characteristics of the germ mycelia investigated is presented. [See Table 1 on next page]

Table 1

Classification of Light Sensitivity of Germ
Mycelia under Investigation

Species	Author	Spore Form	Negative Phototropic	Caro- tene*
1. <i>P. dispersa</i>	Ward 1902 Stock 1931 Gettkandt 1951	Uredo	+	+
2. <i>P. triticina</i>	Stock 1931 Forbes 1939 Gettkandt 1951	Uredo	+	+
3. <i>P. coronata</i>	Fromme 1915 Mains 1917 Stock 1931 Forbes 1939 Gettkandt 1951	Uredo	+	+
4. <i>P. graminis</i>	Stock 1931 Forbes 1939	Uredo	+	+
5. <i>P. simplex</i>	Gettkandt 1951	redo	+	+
6. <i>P. glumarum</i>	Gettkandt 1951	Uredo	0	+
7. <i>P. antirrhini</i>	Gettkandt 1951	Uredo	0	0
8. <i>P. suaveolens</i>	Gettkandt 1951	Uredo	0	0
9. <i>P. menthae</i>	Gettkandt 1951	Uredo	+	0
10. <i>Phragmidium subcorticium</i>	Gettkandt 1951	Uredo	+	+
11. <i>P. poarum</i>	Robinson 1914 Gettkandt 1951	Aecio Aecio	(0) +	 +
12. <i>P. magnusiana</i>	Gettkandt 1951	Aecio	0	+

[Continued on next page]

Table 1 continued

Species	Author	Spore Form	Negative Phototropic	Carotene*
13. <i>Uromyces pisi</i>	Gettkandt 1951	Aecio	0	+
14. <i>P. malvacearum</i>	Robinson 1914	Basidio	+	?
15. <i>Peronospora parasitica</i>	Robinson 1914	Conidia	0	0
16. <i>Penicillium glaucum</i>	Robinson 1914	Conidia	0	0
17. <i>Botrytis spec.</i>	Robinson 1914	Conidia	+	0
- <i>cinerea</i>	Gettkandt 1951	Conidia	+	0
18. <i>Alternaria spec.</i>	Robinson 1914	Conidia	0	0

* More detailed discussion on the carotene content of germ mycelia is given in Chapter 11.

Chapter 5. Probable Meaning of Negative Phototropism for the Occurrence of Infection

The factors which permit or aid the penetration of fungi into the host plant has previously been discussed. De Bary (1884 p. 394) asked "Do we find specific physical or, somehow through unknown secretions of the host surface, chemical stimuli and a definite specific reaction of the parasite on the host?" Ward (1888) thought along similar lines, and Busgen (1893) actually accepted the effect of chemical stimulus although placing the stress more upon contact stimulation. Ever since the well known investigations of Myoshis (1894) carried out in the Pfeffer-Laboratory, chemical tropism has been considered of decisive importance, and rightly so, for the occurrence of infection (see Pfeffer 1897-Plant Physiology Vol. I, P. 360; Gaumann 1946 Study of Plant Infection, p. 18).

After the negative phototropism of the germ mycelia was observed by Ward (1902) it was suggested that this property should be included in the factors in which aid in the penetration of the mycelia. On this Gaumann (1929) writes as follows: "We must first clearly demonstrate what it is that causes the germ tubes to emerge from the drops of infection, to grow on the leaf, and eventually to penetrate its cracks. In most cases the effect of light must be considered of the utmost importance, in that, among certain rust fungi the germ tubes actually react phototropically negative ... Thus we must do everything in our power to solve the problem presented by the fact that, where heliotropism is not involved, infection prospers at night and in a darkened room, just as well or even better than in full daylight."

It seems to me that previous observations and investigations have not yet led to a conclusive solution of the problem. It is not completely superfluous, however, to examine what conclusions may be drawn from the facts now available and what weight should be given to them.

These facts may be divided into two categories. One includes only the presence of the negative phototropism of germ mycelia. The contributions of the preceding chapter belong here. The second category involves the study of comparative infection in light and in darkness.

We begin with the first category. Phototropism in fungi has been known for a long time from a series of cases. I recall the sporophores of many Conium species, the stem of Peziza fukeliana, the perithecia of Sordaria fimiseda, the asci in the apothecia of many Ascomycetes, and the well known sporophores of the Mucorales such as Phycomyces, Pilobolus, Sporodinia, etc. which show strong positive phototropism. In all of these cases the phototropic reaction ability is limited to the organ of fructification, or its component parts, whereas the vegetative

mycelium by itself or in a medium is always phototropically neutral. This appears to be true for the mycelia of other commonly cultivated saprophytes. Many of these have been cultivated so often in petri dishes that even by chance a clearly defined phototropism must have been observed. Such observation, however, is not known to me either by personal experience or from the literature. I had a communication from Prof. Buder who, with his pupils, has been investigating a group of mucorales whose sporangia display great sensitivity to light stating that research on vegetative mycelia indicated no phototropic reaction ability.

It seems that negative phototropism is an unusual characteristic limited to the germ tubes of parasitic fungi. The conclusion must then be drawn that this must have some ecological significance; and it appears probable that this characteristic is present to assist infection. To be sure the number of fungi tested for this characteristic is comparatively small. In Table 1, a summary of all applicable observations is presented. This includes 14 Uredinales of which 9 or 64% show negative phototropism. It appears not only among the germ tubes of uredospores (7 of 10 or 70%) but also in the basidiospores of which only one is known, to be sure; and in the aeciospores where one was discovered among the species under investigation. According to Robinson, I should consider this species neutral, however I was able to establish negative phototropism with certainty even though the phototropism is not as distinct and clear as in the germ tubes of uredospores.

Parasites other than the Uredinales have been little studied, such as Peronospora parasitica which is neutral and Botrytis which reacts phototropically negative. If the Alternaria species referred to by Robinson (1914) is a parasite it is unfortunately not so stated. It is, any event, neutral. Nevertheless, the Botrytis example indicated that phototropic reaction of germ tubes from non-Uredinales may be expected.

Of the 16 certain parasites listed in the table, 10 definitely show negative phototropism. This is about 63% in a batch of about a hundred, so that it is certain that we are not dealing with an accidental circumstance. The probability is, on the contrary, that there is ecological significance in this characteristic and it is related to the parasitic mode.

In order to establish this conclusion firmly, or to limit it to definite fungi groups, a great number of species, parasitic as well as saprophytic, must be tested for the phototropic reaction of their germ mycelia.

Along with negative phototropism there is in every case polarization of the spores before germination. Light, thus, not only exercises a directive effect on the growth of the already-formed germ tubes which

them is in a position to adapt to the epidermis of the host plant, but it also determines the place within the spore where the germ tubes will form. They originate, in every case, on the side of the spore which is not illuminated; this practically is always the side turned toward the epidermis of the host plant. The polarizing effect of light is discussed again in Chapter 11. Here we only note that polarization and negative phototropism work simultaneously. They both must support the effect of infection.

If this is true a clear distinction must be made of the effect of light and darkness on infection using suitable comparative research conditions. Infection should be weaker in darkness than in light. With this we come to the second category of reports which deals with the ecological significance of the phototropism of the germ mycelia. I personally have not instituted any research for this purpose and must be limited to a short comment on the applicable literature. The physiologist would, as a matter of course, be naturally inclined to attribute much greater significance to conclusions based on this type of research than on conclusions that depend merely on ecological considerations. Earlier research has not been hampered by such thoughts as is seen from the passage of Gaumann previously cited.

I am aware of the work of Gassner and Appel (1927) on Pucc. triticea, dispersa, and coronifera and of Forbes on Pucc. coronata. I believe however, that the number and type of investigations are insufficient to permit a definitive conclusion to be drawn. Many more different species must be investigated first of all, and also, conditions of infection must be greatly varied. Finally, in evaluating previous research it should not be forgotten that chemitropism is of importance along with phototropism, and that both operate in the same manner in nature, somewhat in the fashion of phototropism and geotropism in the orientation of the Spermatophytae. In the latter case the effect of each of these factors can be relatively easily distinguished by experimental analysis. For the corresponding separation of the effects of phototropism and chemitropism of the germ mycelia, experimental conditions are definitely far more difficult, but they are probably not as hopeless as it would at first appear.

Because of the view that previous comparable infection investigations did not consider the right things, new research with considerably improved methodology must be carried out. (This research is now being planned at our institute). Until the results of such new investigations are available, we must wait for a final answer. In any event, one can only conclude from previous infection research that experimental data is as yet not available on the development of infection as a direct result of phototropism. It would be quite premature to draw a general negative conclusion, based on previous failures, on the meaning of phototropic characteristics of germ mycelia infections. For the time being, I cannot consider it probable that such a pronounced special

quality could be completely without significant properties, but it is seen rather, as an adaptation within the parasitic way of life.

IV. ANALYSIS OF STIMULUS PHYSIOLOGY OF THE REACTION

Chapter 6. The Minimum Effective Illumination Intensity

After the presence of the phototropic reaction was established or confirmed for a group of species, it seemed of additional value to make a careful analysis of at least some of the representatives of the phototropic reaction. First, the lower threshold of the light sensitivity was determined, that is the least light intensity which still produced phototropic flexures.

All of the following research was carried out in a dark-room at a temperature between 18° and 20°. As a light source I used an osram incandescent lamp (clear glass 40 Watt 32.5 HK at 213 volts), which was placed in a blackened housing and was evaluated photometrically in this mounting with a Lummer-Brodhun contrast photometer against a Hefner candle. The light source was connected to the municipal lighting system. The control of the daily variation in current is accomplished with a rheostat insuring that the lamp operates constantly at under 213 volts.

In front of the light source, situated at the end of a long table, painted black petri dishes are placed within which Uredospores of *Ured. triticea* are inoculated in a gelatin medium. The dishes are at a distance of 50-450 centimeters from the incandescent lamps and are so arranged that they are not in each others shadow and that light falls on the germ tubes at a sharp angle. For optical reasons, the covers of the petri dishes were replaced by a glass plate cover. The loss in illumination resulting from the covering was the same in all tests and was about 20%. Precautions were taken to prevent the shadow of the front edge of the dish from falling on the line of inoculation and that the angle of illumination would be the same for all dishes. A black strip of paper was inserted along the back wall in order to prevent any reflection from it.

The distance of the dishes were so chosen that the illumination intensity would be at a value of 130 lux decreasing to 1.6 lux. From time to time, I observed the reaction after 2 or 3 hours after inoculation and drew the following sketch using the Abbe drawing apparatus.

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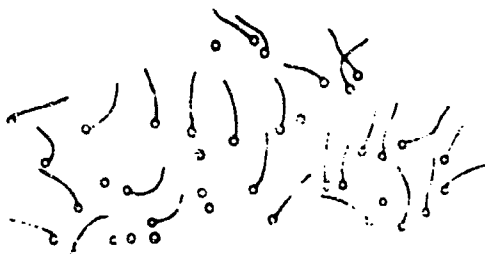


Figure 17

Pucc. tritici 2 hours after insemination, 3.2 lux, Temp. 20°. Magn. x 45

A distinct negative phototropic reaction was visible in every dish located at a distance of 320 centimeters resulting in an illumination intensity of 130 to 3.2 lux (Figure 17). At a distance of 360 centimeters (2.5 MK) the phototropic reaction could only be established by statistical analysis. I consider the hyphae reacting not only when placed directly in the light direction but also when they are deflected as much as 45° to the left or right. The lower limit of the phototropic reaction was taken as that intensity of illumination where 50% of the mycelia react. At all times a dish was placed in darkness as a control. At an illumination intensity of 1.6 MK at 450 centimeters distance, phototropism was no longer apparent, and the condition was the same as if it were in complete darkness. The critical value for white light is 2.5 lux at which point about half of the germ mycelia show negative phototropism. The loss of light resulting from reflection from the cover glass, previously given as about 20%, must be subtracted from this value. The critical value thus is about 2 lux.

In order to determine more precisely the critical value of the uredospore light sensitivity it was necessary to use Hefner lamps as a source of light. In earlier investigations using white light, which were visually compared with Hefner lamps, the spectrum sensitivity of the human eye was a constant factor in the measurement (the specification of the scale of illumination intensity is based on this fact and is called "visual meter candles"). Buder (1926) noted this important fact and stated: "One cannot establish by this method how much brighter a source of light appears to our eyes than does a Hefner candle, and no quantitative information can be obtained, reporting how much more effective the light source to be tested is than the Hefner candle" (p 7). This is quite true for the problem of the phototropic reaction of the germ tubes. The lamp used

in the investigation and the Hefner lamps used in the measurement differ not only in brightness but also in temperature and in the energy distribution of its spectrum. Because the temperature in individual incandescent bulbs can vary greatly, reproducible values can be obtained only with precisely defined light sources. The Hefner candles serve best of all for this but in addition a source of light which provides the same color can be used as a substitute Hefner lamp. In using this light source during this investigation, the proposal of Buder (1926) suggesting the use of specific meter candles is applied. Detailed information on this is available in the works of Buder (1926), Schrammek (1934), Mosebach (1938), and Dassek (1939). The use of the Hefner candle is inconvenient. Because of this a substitute lamp as used in the course of this investigation. An Incandescent lamp is used here for this purpose whose voltage can be adjusted until the resulting light color is the same as the Hefner candle. This is produced by using a rheostat which is so adjusted that the color of the lamp as tested in the photometer matching field is the same as that produced by a Hefner candle.

I used as a substitute Hefner lamp, an osram opal bulb (60 watt, 220 volt) in a lamp housing whose inside as lined dull ground gypsum plates. The front side of the housing was a milk glass plate 10 x 10 centimeters. The intensity of this light source was 2 specific Hefner candles at 130 volts.

The petri dishes were placed at a distance of from 30 to 50 centimeters from the substitute Hefner lamps, the greater distance was used to make a comparison with a dish in darkness. At a distance of 30 to 50 centimeters (about 22 and 8 specific Hefner candles respectively) negative phototropism was distinct. At a distance of 60 centimeters (5.5 specific meter candles) negative phototropism was noted only with statistical analysis. About 50% showed negative phototropism. At a distance of 70 centimeters the reaction was the same as in darkness. Many repetitions produced the same results.

The critical value of the phototropic reaction as found in these investigation with substitute Hefner lamps can be taken to be about 60 centimeters. This distance corresponds to an illumination intensity of 5.5 specific meter candles. The 20% light loss from the reflection from the cover plate must be subtracted again from this. Thus, the effective critical limit for P. triticea (strain 52) was found to be 4.4 specific meter candles.

The Uredinales are marked by physiological specialization. Based on most recent research 129 physiological strains are known for P. triticea which according to international nomenclature are known as Nos. 1-129 (Chester 1946). In order to establish if a distinguishable phototropic property exists for the separate strains, strain 52 along with strains 20 and 68 were investigated. These strains were obtained

from the local phytopathological institute. The critical value for the negative phototropic reaction was found to be at the distances heretofore noted. The light intensity at this point consists of about 2 lux (visual meter candles) the same value as for white light of the incandescent lamp when used with lowest resistance; or 4.4 specific meter candles when using light equal in color values to the Hefner Lamps.

Chapter 7. The Effective Spectral Region

At this point it is of interest to establish which wave lengths permit the occurrence of the phototropic reaction.

Experimental Procedure:

An osram incandescent lamp (clear glass 200 watt) was used as the source of light, which at a distance of 35 centimeters produced an illumination intensity of about 3,000 visual meter candles. The determination of the wave lengths within which the germ tubes react phototropically was made with Jena light-filter lenses of the Jena Glaswerken Schott and Gen. [Jena Glass Works and Associates]. The filter holder was so attached to the lamp that no undesired additional light fell on the spores. In order to facilitate the observation, I employed glass trays with a bottom surface area of 4.5 x 3.5 centimeters and with a height of 1.5 centimeters each provided with a glass cover. All the tray sides were darkened with black paper. Before each test a 3-5 millimeter thick layer of gelatin was poured into the trays. A straight line of spores were inseminated into the gelatin. To better observe and sketch the reaction the glass trays were placed on the mechanical stage of a microscope (Ortholux of the Leitz Company) at a distance of 35 centimeters from the light source at the height of the incandescent filament. The following filters were used: [See Note]

RG₂ of 2 millimeters thickness

OG₂ of 2 millimeters thickness

GG₁₄ of 2 millimeters thickness

VG₉ of 2 millimeters thickness

BG₁₂ of 2 millimeters thickness

UG₁ of 2 millimeters thickness

[Note]: Details on the light transmissibility of these filters may be obtained from the list of Jena Glaswerken Schott and Gen.

First it was necessary to establish if red light, which in most cases is phototropically ineffective, would also not influence our germ tubes. Tests showed that using the RG₂ filter, only wave lengths greater than 600 millimicrons (m μ) passed through so that, as to be expected, for our subject too, red proved to be phototropically neutral.

The next filter tested was OG₂. It passed wave lengths greater than 550 m μ . Two hours after insemination the spores had well germinated, but showed no phototropic reaction. The germ-tube growth was comparable to the growth of spores simultaneously set out in darkness: no difference could be discerned. On the basis of the fact that orange light was also shown to be phototropically inoperative for the germ tubes, the OG₂ filter was used with the low voltage lamp of the Ortholux microscope since this light was better suited than red light for the sketching of the germ tubes.

Next, filter GG₂ was tested. It permitted rays of 480-500 m μ to pass through in small percentages as well as light of wave lengths greater than 500 m μ . I repeated this test using the same filter several times. The phototropic reaction did not occur in any instance.

Using the next filter VG₉ which passed through rays of 450-650 m μ (maximum about 520) more than half of the germ tubes displayed negative phototropism (Figure 18). [Figure on next page]

In testing the blue filter BG₁₂ which passed wave lengths of 325-520 m μ , good negative phototropism was obtained.

Also the effectiveness of UG₁ was observed. This filter passed wave lengths of 290-400 m μ and 700-1,100 m μ . The wave-length lay in an ineffective area and as a result could be ignored. In this investigation, an Osram violet blue lamp (330 watt, 220 volt) was used in order to ensure that the light source included sufficient ultraviolet. The trays, here, were covered with a quartz glass. Two hours after the insemination a negative phototropic reaction appeared as under the BG₁₂ filter.

In all tests where no germ-tube phototropism appeared, that is with the RG₂, OG₂, and GG₁₂ filters, the material involved was checked as a control under white light and each time with phototropic result.

This investigation demonstrates that only wave lengths of the blue, violet, and ultraviolet range are effective for the phototropic reaction of the germ tubes. The red, orange, yellow, and green proved to be ineffective. Only under the VG₉ filter did a weak reaction appear. The limits of the spectral sensitivity as obtained from the results of these tests at a distance of 35 centimeters from a light source of 200 watts is approximately between 450-480 m μ . The effect extends into ultra-violet light.

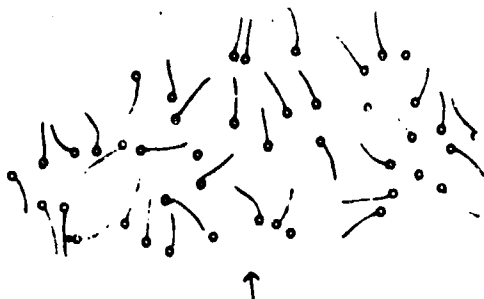


Figure 18

Fus. tritici, 2 hours after insemination under green filter V6, (2 millimeter), weak phototropic adjustment (statistical). Temp. 20°. Magn. x 45

Chapter 8. Inversion of Phototropic Reaction in Liquid Paraffin

The inversion tests made by Bader (1918) in the analysis of phototropic flexures of one-celled organisms has consistently been confirmed. Bader and his assistants found that in all such organisms (apophylls of Phycomycetes, Hepaticae rhizoids), whenever the material is hyaline, positive flexures reverse to negative and vice versa.

It now remains to be seen if the same effect also is present for germ mycelia in liquid paraffin. I used germ tubes of F. tritici which were cultivated for two to three hours in seven percent gelatin. The hyphae, when the test is properly conducted, do not penetrate the gelatin but, as previously stated, grow along the surface of the gelatin only affecting a narrow thin strip. Thus, most of the hyphae is in contact with the air. As a result a necessary condition for the completion of the immersion test is present. The preparation is then covered with liquid paraffin and exposed to one-sided lighting. Next the preparation is so arranged that light falls on the subject material in a direction perpendicular to its previous growth direction. Accidental variations in the growth pattern are of no concern here. After 10-15 minutes a new phototropic reaction commences which soon increases in intensity, so that after 30-45 minutes most have a new orientation to the light and as expected a positive one in relation to the source of light. I permitted the germ tubes to grow for an additional time period and in the usual manner made a sketch for the record (Figures 19 and 20).

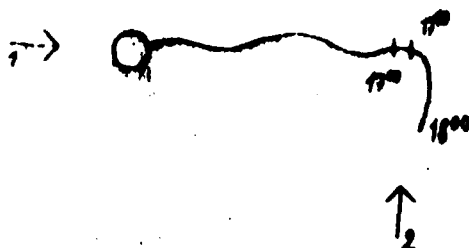


Figure 19

Fig. 19. Tritonia, Inversion test under lamp light (about 200 lux)
 1615 Insemination, Illumination in air (Direction 1)
 1700 Rotated about 90°, Liquid paraffin added. Lighting in Direction 2
 1710 Beginning the of reaction
 1800 Distinct positive phototropism Temp. 20°, Magn. x 150

GRAPHIC NOT REPRODUCIBLE



Figure 20

Fig. 20. Tritonia, Inversion test in daylight
 1000 Insemination, Illumination in air (Direction 1)
 1300 Rotated about 90° and liquid paraffin added, Lighting in Direction 2
 1400 Distinct positive phototropism Temp. 20°, Magn. x 45

From this inversion test it is apparent that the phototropic reaction is the result of a lens effect. The convex lens of the sporephyll is altered to concave by the immersion in liquid paraffin thus changing substantially the character of the light rays through the front and rear walls. The front is more strongly illuminated than the rear (Bader 1920) as a result of the diverging ray directions. Phragmites is demonstrated to have a positive light growth

reaction (Elaauw 1914) when the front side is grown in paraffin leading to a negative phototropic flexure. An additional example is found in the inversion tests of Dassek (1939). The negative phototropic reacting Hepaicae rhizoid shows a positive flexure in liquid paraffin, in this case also the result of a lens effect. In this case, however, there is a negative light direction reaction, so that negative flexures occur in the air which in liquid paraffin are positive.

Our germ mycelia behave, insofar as they are capable at all of reacting phototropically, in the manner of the liverwort rhizoids. The nonphototropic germ tubes including P. glumarum, antirrhini, and suaveolens in contrast grow uninfluenced when covered by paraffin in the once rotated direction. I have convinced myself of this by repeated tests.

It may be concluded from these observations that for the germ mycelia which possess paraboloid and entirely transparent tip the lens effect is of decisive significance. As in the liverwort rhizoids more strongly illuminated sides must be inhibited at times. If this is so a negative light growth reaction should appear.

Chapter 9. The Negative-Light-Growth Reaction

A light growth reaction may be determined by various means. Once can observe, as did Elaauw (1914) in his time, growth in red light which is the same as in darkness, with erroneous conclusions that the growth direction took place as a result of the illumination; a negative growth reaction in the absence of light, positive growth reaction in the presence of light. The method of Bader (1920) utilizing partial illumination can also be used. This method has the advantage of being easily accomplished and extremely fine growth measurements are not called for. First the sporophylls of phycomyces are examined after being placed in a one percent sugar solution. Because the index of refraction of the medium and the sporophyll are almost the same no lens effect appears. It is to be expected, as a result, that under these conditions no flexures would occur if the sporophylls were illuminated in the usual manner from one side. In fact they actually grew without any flexure. It could be seen, however, that despite the lack of flexure the reaction capacity still remained. "If one projected the sharp edge of the longitudinal half of a beam of light using a suitable lens system, on the tips of the sporophylls in the water, they would deviate in a direction perpendicular to the plane of the light rays" (Bader 1920, p. 14).

In a positive light growth reaction the material grows away from the illuminated field; in a negative reaction the flexure is just the opposite (Figure 21).

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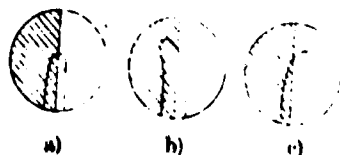


Figure 21

Record of a light growth reaction (LGR) under half side illumination LW (schematic) see text

- a) at outset of observation
- b) reaction with positive LGR
- c) reaction with negative LGR

The method of partial illumination was also employed by M. Dassek (1939), R. Schneider (1942), and H. L. Paul (1950) for various experimental plants and is very well confirmed, as a result. By this means incontestable determination of the site and extent of the light sensitive zone is possible (see Dassek 1939).

For the application of this method on our germ tubes without modification, the preparations must be completely covered with water and then sealed with a cover glass. Growth interference would immediately supervene. I abandoned the water covering and arranged for the projection of the edge of the light rays directly on the gelatin preparation. As a result of this simplification small differences in the type and extent of the reaction could be expected but the sense of the flexures would not be changed. And we are only concerned with this sense direction.

Adjustment and observation must be carried out with strong objective lenses because the germ tubes are very thin and delicate (only 4-6 μ in diameter). A Leitz objective 6L with wide working clearance in combination with a strong ocular proved effective.

Germ tubes cultivated for 2-3 hours were observed from time to time under one-sided light. Such slides were placed on the mechanical stage of a microscope whose condenser was replaced by a Winkler-Zeiss objective with a magnification value of 5.5. By means of this objective a thin line at a distance of 16 centimeters is sharply visible on the surface of the field of vision. A total reflecting prism replaced the microscope mirror. To keep the gelatin medium from drying during the investigations, the slides were covered by cardboard the inner side of which was lined with damp blotting paper (Figure 22).



Figure 22

Experimental set-up for investigations on half-sided illumination, see text,

Method of Investigation:

To begin the investigation a straightly grown germ tube was moved to the junction line of the split image by means of the mechanical stage in such a manner that the germ tube is halved lengthwise. This adjustment is made under red light after which the red filter is removed and the half-side illumination begins. The first indication of any flexure usually occurs after 4-10 minutes. The point begins to be unsymmetrical; it is always the dark side which moves and the side in light, which is inhibited. This is then a negative light growth reaction (Figure 23).

GRAPHIC NOT REPRODUCIBLE



Figure 23

Pucc. triticina, negative light growth reaction under half-side illumination

After some increase of the flexure the line of junction is moved so that the point is again half illuminated, half dark. By constant turning it was possible to cause the germ tube to grow in the form of an arc just as Buder (1920) had described for Phragmites and Dasek (1939) for the liverwort rhizoid. In all, I made some 50 tests on Puccinia triticina with the same result produced for each. Puccinia dispersa and Phragmidium subortioium consistently displayed a flexure within the bright field. Pucc. dispersa was tested 25 times; Phragmidium subortioium 15 times. * reaction was found to be always the same.

When a blue light (BG₁₂ laminated filter) is used instead of white in half-side illumination the same reaction occurs. The test with green light (VG₂ laminated filter) did not result in the same distinct effect, where as with yellow light (GG₁ laminated filter) no flexure appeared. The results of this test are completely consistent with those reported in Chapter 7 on the effective wave lengths.

Along with the tests with half-side illumination which showed a distinct negative light growth reaction, direct growth measurements were also made. First the growth was measured under the phototropic-neutral orange light, after which the OG₂ filter was removed and the change in growth determined. A decrease in growth speed regularly occurred after 4-10 minutes.

Puccinia antirrhini, suaveolens, and glumarum were submitted to the same test conditions. As expected the germ tubes grew without any further influence. Direct measurement showed no light growth reaction, no flexure under half-side illumination, and no decrease in growth speed.

Chapter 10. The Light-Sensitive Zone

In the investigation on the site of light sensitivity of one-celled organisms, it has been consistently determined that only the growing zone is light sensitive and that a so-called stimulus conductivity is lacking. This is true for the sporophylls of the Phycmycetes as well as for the liverwort rhizoids. The demonstration of Dassek (1939) is furnished as evidence that only the extreme growing tip is capable of light perception. The method of partial illumination was employed with some modifications. I proceeded in like manner.

The junction line was projected on the germ tube in such a way that only one half of the tip is illuminated. In order to observe and to properly adjust the dark part of the organism a thin glass plate sloping at less than 45° was placed, during these investigations, between the junction line and the image-forming objective. It reflects into the objective the rays of a small, auxiliary lamp which is covered by an OG₂ filter. As a result the entire field of vision is lit up by a weak phototropic-neutral orange light.

A straightly grown germ tube, 2-3 hours old or 4 μ thick was chosen under orange light and its tip zone illuminated for about a length of 4 μ . Each time after 10-15 minutes a flexure directed towards the illuminated side of the germ tube occurred. The same result occurred when only a 2 μ portion of the tip was exposed to half light. If, however, the illuminated zone was only 4 μ beneath the tip (see Figure 24) no flexure occurred.

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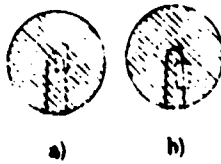


Figure 24

Lighting arrangement for determining the light-sensitive zone.

These test were repeated 25 time with Puccinia triticina and also with P. dispersa each time with the same result.

Since the thorough studies of Reinhardt (1892) it has been well known that the growing zone of the fungus hyphae in all cases is limited to the extreme tip. It was to be expected that our germ tubes were no exception to this generally valid rule. In order to be completely sure, I made some measurements. To mount extremely fine calibrations I used, as did Haberlandt (1889) and Seeman (1936), dried rice starch which was blown carefully with a pulverizer. After several attempts the desired distribution on the hyphae was obtained. The position of the index points were fixed by means of the Abbe camera lucida. Only in the range of less than about 4 μ below the tip did the index point change position. The distance of the starch grainlets lying below remained unchanged. The same results were obtained after many repetitions of the test.

Chapter 11. Discussion of the Results of Part IV

The phototropism of the germ mycelia of parasitic fungi has been very little considered in respect to the physiology of the stimulus. Neither the comprehensive monograph of Du Buy and Huernbergk (1932-1934) nor the recent text of Bunning (1948) provide any information on this matter. Mention is made of this only in the phytopathological literature. Gaumann (1929) in the "Biology of the Parasitic Fungi on Plants" is led to the belief that phototropism is a factor in the occurrence of infection, based on observations of Fromme, Mains, and Robinson. In the new edition of Gaumann's (1951) "Infection Theory," insofar as I can see, phototropism of the germ mycelia is only incidental and is referred to only as a generally known fact which could be of significance to infection. "This may well be (the chemotropic reaction capability), along with a phototropism the reason why the germ tubes do not move about in complete disorder, but grow purposefully on plants

regardless of the position of the leaf and of the drops of water" (Gauermann 1946, page 18).

From the results of our investigation it is to be expected that germ mycelia phototropism of parasitic fungi is a widespread phenomenon of interest not only for phytopathology but also for physiologists concerned with stimuli. We are dealing with organisms of moderate light sensitivity which in this respect are somewhat superior to the liverwort rhizoids.

The critical value of the least yet still effective illumination, (under constant lighting) was determined to be about 4.4 specific meter candles, for the liverwort rhizoids of Dassek (1939) about 6 specific meter candles. The corresponding critical value for the highly sensitive *Phycomyces* sporophylls is many times less. Young sporophylls of *Phycomyces* were found to have a value of 0.000,003 and ripe ones 0.000,025 specific meter candles in tests at the Breslau Botanical Institute (Bernhard 1940) and young sporophylls of *Pilobolus crystallinus* 0.000,003 to 0.000,200, each exposed to prelighting during collection (Schneider 1942).

To date only the work of Forbes (1938), who worked with Corning light filters, is available on effective wave lengths. He found strongest reactions under blue and violet light for *Pucc. triticea*, *ecronata*, and *graminis*. He also still believed that a reaction was possible under a red filter. Because he gave no further information on the transmissibility characteristics of his filter, no inference is possible as to the wave length range of the phototropic effect. From my tests with laminated filters it can be concluded that the value must lie between 450 and 480 m μ . My tests showed that red light was completely neutral. This was true for *Pucc. triticea* and *dispersa* which were very carefully tested. I would not like to draw any conclusion for all the Uredinales based solely on this work. Because in other cases, for example in the *Pilobolus* sporophylls it has been shown, that even among species of the same genus, quite considerable differences may be present. In the same manner Paul (1950) came to the conclusion that the sensitivity of young sporophylls of *Pilobolus crystallinus* was about 625-630 m μ in contrast to the approximate 565-570 m μ obtained by *P. kleinii* and *sphaerosporus*.

The two species of *Puccinia* tested by me were in the 450-480 m μ sensitive area, somewhat in the same sector as the completely hyaline and carotene-free liverwort rhizoids which Dassek (1939) found to be from 470 to 497 m μ . To which wavelengths the completely hyaline germ mycelia of *Botrytis scutellariae* react, could not be tested.

The analysis of phototropic reaction using the method of partial illumination (Buder 1920, Dassek 1939) indicated a negative light growth

reaction in the germ mycelia. A flexure perpendicular to the direction of the light rays and constantly within the bright field occurred when the germ tubes were illuminated on the half side (see Figure 23). From this it is seen that the stronger illuminated side is inhibited in growth. Using the same method the light-sensitive zone was determined to be in the short still-forming tip zone of the germ hyphae.

It can be shown by inversion testing that the lens effect is of decisive importance for the type of phototropic flexures that results here, as it is for all previously investigated hyaline one-celled organisms. By submerging the transparent tip of germ tubes in liquid paraffin the convex lens is changed to concave. As a result the middle area of the posterior side received less light than the corresponding anterior side. Corresponding to the negative light growth reaction the more dimly illuminated side grows at a faster rate producing the effect, when in liquid paraffin, of a positive flexure. In air the illumination characteristics are completely reversed, showing a negative flexure. In this respect the germ mycelia of the Uredinales are completely parallel to the liverwort rhizoids.

Of especial interest is the problem of the carotene content, which for many germ mycelia is quite considerable, in regard to the part it plays in the phototropic reaction capability.

It is known from many authors, particularly Bunning, that carotene is abundantly found in the plasma of Phycomyces and Pilolobus dissolved in many very fine lipid droplets in the vicinity of the light sensitive zone. This is of great importance, it can be shown, for the basic effect of light on the phototropic processes. Because of the obvious soundness of these facts, they will not be further discussed. It may be only pointed out that even in the last article by Bunning (1948) on this subject he presented his repeated apodictic contention (p 332) as follows: "In fact wherever a cell or tissue is especially sensitive to blue-violet light we find carotene," a statement which is not always true as in the case of the completely colorless liverwort rhizoid (Dassek (1939)). To this example we can add the germ mycelia of Botrytis confida which, like the previously mentioned rhizoids, are completely colorless and under the microscope do not show the slightest trace of carotene.

"However even with the carotene-rich sporangiophores of Pilolobus, precise investigation on ripe sporangiophores by Ros. Schneider (1942) and, especially convincing on the young ones, by H. L. Paul (1950), show that the concentration of lipid-dissolved carotene, considered so important by Bunning, was not vital for the reaction as asserted. The growing tip of the young sporangiophores of Pilolobus crystallinus is quite hyaline and transparent, its remarkable carotene content lying at some distance from the apex in a zone where the length growth had already ceased. The young sporangiophores of this species possess a positive

light growth reaction. As a result of the lens effect the hyaline tip of the sporangiophores are led to react negatively phototropically in paraffin oil, positively in air. Such a lens effect can occur only where the conditions are present where the lens does not too greatly absorb the effective blue and violet. In the young sporangiophores of this species there is an absence of a conspicuous carotene content, a prerequisite for the functioning of the organism! Quite different are the conditions for Pilolobus sphaerosporus and kleinii. In these species the carotene content is actually on the extreme tip, however the carotene has quite a different function from that vigorously asserted by Running. That is, it operates solely as a filter which absorbs the short wave rays and by this means prevents their effect on the back side. It is thus entirely consistent that the light growth reaction of the young sporangiophores of this species is negative. The growth of the bright side is thus retarded. Since the 'lens' in this case is not transparent, therefore there can be no 'lens effect.' The sporangiophore bends towards the light, while the diverted flank is constantly the darker side so that it always grows faster than the other side. Regardless, then whether the sporangiophore of these forms are in air, water, or paraffin oil there is always a positive flexure. An inversion in liquid paraffin does not take place.

The internal plasma within the sporophyll plays no part in the determining photochemical processes as Running still maintains (1948 p 339 and also 1953 p 404), but rather obviously in the marginal layer on which the growing wall lies, a concept which from the beginning, I advocated" (Bader).

What then is the distribution of the germ mycelia of our Uredinales? In every place where carotene in visible amounts can be recognized, these accumulations do not occur in the tip zone the only place where growing and reacting occurs, but only somewhat farther down, in an area where length growth has already stopped (see Figure 1). Very similar conditions occur in the young sporangiophores of Pilolobus crystallinus (possessing a hyaline-tip lens effect) with the difference that in P. crystallinus the light growth reaction is positive and, correspondingly in air produced a positive phototropic flexure, whereas in the germ mycelia the negative light growth reaction results in a negative flexure in air.

However, not all Uredinales tested carry visible amounts of carotene in their germ tubes. Also not all react phototropically. Of 14 species tested 5 were found to be neutral. Is there at least a relationship between carotene content and the reaction capability? Table 1 gives us some information on this. The last column indicates the presence of carotene in recognizable amounts under the microscope by a (+), the absence by (0). Such a relationship is a fact in the majority of the species investigated. I found carotene in Puccinia dispersa, triticea, coronata, Graminis, poarum, and Phragmidium siccorticium. These species are phototropic and carry carotene which migrates from the spore into the germ

tube wherein, to be sure as previously stated, the accumulation of the carotene-containing lipid droplet is in no case within but below the only light sensitive growth zone. These species are in contrast to Puccinia antirrhini and guaveolens, which are phototropically neutral and lack any carotene content. The relationship extends to this extent. It must not be overlooked, but by no means is it without exception. Puccinia menthae does not react less phototropically than the other species and it possesses no carotene at all in the germ tube. It is precisely as hyaline and colorless as Botrytis. On the other hand, carotene is always as abundantly present in the phototropically neutral germ mycelia of Puccinia glumarum, magnusiana, and Uromyces pisi, as it is in the species with good phototropic reaction. A clear parallelism between carotene content and phototropism thus does not exist for the Uredinales.

Our investigations have yielded further information in reference to the carotene problem, which provide evidence counter to the view that the carotene dissolved in lipid within the inner plasma is of vital concern to the photic primary reaction. However it should not be stated that phototropic and phototaxic reactions have nothing at all to do with carotenoids. Such a relationship is entirely possible, even certain, in many cases. This is also the opinions of Buder whose ideas in conjunction with the work of Paul I repeat briefly here. It was Buder himself who first precisely reported on the significance of the pigment group, specifically the carotenoids of the purple bacteria (1919 p 575 and 1932 p 434). These reports deserve to be brought into prominence rather than to be completely ignored in the discussion of this subject. In contrast to Bunning, Buder however considers that the lipid-dissolved carotene which are so concentrated in the sporangiophores of Phycomyces, Pilolobus, and in our germ mycelia are phototropically inactive. At most, in particular cases, it can play a purely passive role as a light shield. The pigments, carotenoids or other types, which control the phototropic and phototaxic reactions, he believes, are closely tied to the albumin chain of the plasma. They may very well occur in such small concentrations that they may avoid direct microscopic observation. In the case of one-celled organisms everything suggests a site of the photochemical system which is the basis of the phototropic reaction, in the plasmatic marginal layers which determine the growth of the young cell walls. This view is also confirmed among other light effects, specifically in the polarization of spores and oozygotes by light. Mosebach arrived at the same conclusion for this process and sees the site of the photosensitive structure in the "dermal layer of the cytoplasm" (Mosebach 1938, 1942; Buder 1944).

I was also able to observe such polarization in spores of fungi worked with for this report. In the description of the germination of uredospores of Puccinia triticina it was briefly noted that light not only exerted a directive effect on the already-formed germ tubes, but also that the spores were polarized by light falling on one side previous to their emergence from the germ tubes. This polarization manifested itself by

the fact that the germ tubes constantly formed on the side away from the source of light.

I have been able to establish a polarization of spores in every case where subsequently a negative phototropism of the germ tubes was observed. This is true for such spores which were equipped with germ pores as well as for uredospores and aecospores, also some which displayed no membrane position of this type such as the conidia of Botrytis. The germ tube, in each case, appeared on the side of the spore in shadow. After these facts were determined for all tests, a survey of the older literature yielded the information that Fromme (1915) reported the same for Puccinia coronata even though the concept of polarization was not specifically mentioned. He says (1915) p 84: "The incidence of light, therefore, not only had a pronounced effect in determining the direction of growth of germ tubes but also determined to a considerable degree the approximate part of the spore wall at which the germ tubes issued, i.e., the part farthest from the light." With the report on the polarization of fungi spores by light, the number of the previously known cases of polarization of individual cells are enriched by a group of further examples. This should be of interest because of the exclusion of the wall-formation, conditions are much simpler than in the case of the Equisetales spores and of the oocytes of the Fucaceae. For this reason it seems to me that these fungi spores are very much suited for a further comprehensive study of problems dealing with the polarization of individual cells by light.

V. SUMMARY OF THE MOST IMPORTANT RESULTS

1. The germ mycelia permit easy and good cultivation using 7% gelatin (Chapter 2)

2. The germ mycelia of 12 Uredinales and Botrytis cinerea were tested for their phototropic reaction capabilities. Of these the following were for the first time: Puccinia simplex, glumarum, antirrhini, suaveolens, menthae, magnusiana; Phragmidium subcorticium, and Uromyces pisi. Only the following were found to be phototropically neutral: the germ tubes of the uredospores of P. glumarum, antirrhini, suaveolens, the aeciospores of P. magnusiana and Uromyces pisi. All others reacted well phototropically except P. poarum where the reaction was somewhat weaker; all without exception flexed negatively (Chapter 4 and Table 1).

3. It seems quite certain that the special germ mycelia characteristic of reacting with a negative flexure in the presence of one-sided light is an adjustment to the parasitic mode of life. No compelling conclusion on the promotion of infection by this negative phototropism can be drawn from experimental reports available in the literature to this date. On the contrary very little is to be found. So that now new infection investigations can be carried out under suitable conditions to

provide conclusive decisions on these question (Chapter 5).

4. The threshold of the lighting intensity whereby a phototropic reaction is still visible is 4.4 specific meter candles for Puccinia triticina (Chapter 6).

5. The limit of spectral sensitivity of wave length lies between 450 and 480 m μ for Puccinia triticina and dispersa (Chapter 7).

6. The germ mycelia flex phototropically positive (phototropic inversion) (Chapter 8).

7. Under the application of half-side illumination (after Buder) flexures occur perpendicular to the direction of the light, by means of which the tips curve away. From this it can be concluded that there is present a negative growth reaction which can be determined by direct measurement (Chapter 9).

8. The mechanics of the reaction of the phototropic flexures corresponds to that of the liverwort rhizoids, i.e., hyaline operating tip region, negative light growth reaction (Chapter 10).

9. The tip area, which is the only phototropically sensitive area, is free from carotene accumulation. The carotene content is present only below the growing point.

This work was carried out in the Botanical Gardens in the years 1950 -- 1951 and was accepted by as a dissertation by the Mathematical and Scientific Faculty of the Martin-Luther University of Halle in 1952. I am indebted to my honored teacher Prof. Buder for encouragement and advice. In addition I owe thanks to Prof. Gassner and Dr. Noll for literature and advice how to obtain literature not easily accessible, to Dr. Nover and Miss Simon of the local phytopathological institute for making available research and insemination material, and to Senior Assistant Dr. Handke for much friendly advice.

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