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On two species of *Heterosporium* particularly *Heterosporium echinulatum*.

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(With 3 Photomicrographs and 52 textfigures.)

I.

In October 1911 Prof. Klebahn brought to me some diseased leaves of *Beta vulgaris* taken from the experimental fields belonging to the Landherrenschaften at Hamburg. The outward appearance of the disease took the form of dark brown spots, varying from 5 cm to 1 cm in diameter, which were either isolated or confluent, in which latter case the disease areas were large and irregularly shaped and dark brown to black in colour. The infected leaves were always the lower-most ones. From these diseased areas several members of the Fungi imperfecti were isolated such as *Macrosporium*-sp., *Alternaria*-sp., *Hormodendron*-sp., *Epicoccum*, and a species of *Heterosporium*. In the disease areas were also found small rounded bodies which appeared to be young perithecia; they were unripe and were composed of a pseudoparenchymatous tissue. The disease seems to be that described hy Frank[1]) as „die Schwärze oder Bräune der Runkelrübenblätter“ which according to him is caused by *Pleospora putrefaciens*. Frank gives *Sporidesmium* and *Cladosporium* as additional spore-forms of this *Pleospora;* but Brefeld[2]) has shown that *Alternaria* is the real conidial form. The presence of *Alternaria* and the young perithecia-like bodies taken together with the general appearance of the disease seemed to indicate that the disease here under consideration was the same as that described by Frank, although he confused the conidial forms. The other fungi were probably growing saprophytically upon the disease areas. Prof. Klebahn suggested that some of these forms might be cultured and compared with other nearly related forms, both as to their growth upon nutrient media and upon the *Beta* plant itself. For this purpose he placed at my disposal his spirit material of *Heterosporium Syringae* which he had found some years ago as parasitic upon the lilac. Another easily obtained species of *Heterosporium* was *H. echinulatum* which causes a leaf disease of carnations very prevalent at this time of the year; and was obtained under natural conditions at a nursery at Stellingen near Hamburg. The main part of

1) Krankheiten der Pflanzen (p. 298, second edition).
2) Untersuchungen aus dem Gesamtgebiete der Mycologie, 1891, **10**, 225.

the work centered round the two *Heterosporiums*, which were obtained in the living state. At first a number of cultures were made upon various nutrient media with a view to observing the nature of the growth of these fungi upon different media. On looking through the literature of the genus *Heterosporium* it was found that hardly any of the existing figures were at all accurate, particularly those in connection with such a well recognised form as *H. echinulatum;* and that moreover nothing was known about the inoculation, infection, and nature of the mycelium of this particular parasite. It was therefore decided to go as thoroughly as was possible into these points.

The work was carried out in the Botanic Institute at Hamburg, and I should like to express here my very sincere thanks to Prof. Klebahn for the permission to work under his direction in the Institute and for his continued advice and help during the progress of my work. My thanks are also due to Prof. Brick of the „Station für Pflanzenschutz“ for very kindly affording me every opportunity of examining herbarium material, and also to the director of the Hygienisches Institut for kindly allowing me the use of microphotographic apparatus.

Methods.

The growth of the fungi was observed in petri-dish and hanging-drop-cultures. The germination of the spores and the formation of the conidiophores were observed in damp chambers as described by Prof. Klebahn [1]).

The culture media used were,

Plum-juice agar
Meat-extract agar (Fleischwasseragar)
Liebigs „ „ (Fleischextractagar)
Glucose „
Maltose „
Salep „

The agar for the salep-agar medium was previously treated according to E. Macé [2]). 30 gm. of this prepared agar was gently simmered for $^1/_2$ hour in 1 lit. water (tap). 9 gm. Salep (N. Bernard) [3]) were gradually stirred into 1 lit. tap-water and gently simmered for $^1/_2$ hour; the two liquids were added together and made up to 2 lit. by the addition of tap-water. To this mixture were added the following (Klebahn):

Salep agar. 1 gm. grape sugar,
0,2 gm Monopotassium phosphate,
trace Ammonium-nitrate,
„ „ -sulphate,
„ „ -ferrous sulphate,
„ Magnesium sulphate,
0,2 gm. tartaric acid.

The whole was kept gently simmering for $^1/_2$ hour after which it was filtered in steam till it was quite clear and then poured into soxlet-bottles which were sterilised [4]).

The methods employed in killing and staining are described under the infection experiments and under the examination of the diseased tissues of *Dianthus*.

1. *Heterosporium echinulatum* (Berkeley) Cooke.

The leaf-disease due to this parasite was first described by Berkeley under the name of *Helminthosporium echinalatum* in 1870; with

1) Pringsheims Jahrbücher, **41**, 489.
2) Traité pratique de Bacteriologie, Paris 1889.
3) Rev. gén. de Bot., 1904, **16**, 408.
4) see Himmelbauer, Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten, **28**, 43.

the description is a drawing showing a bundle of conidiophores, but the articulations between the conidia and their conidia-bearing-hyphae are not indicated. The conidiophores were moreover asserted to be colourless and the dimensions were given in inches. In 1873 BERKELEY and BROOME renamed it *Helminthosporium exasperatum* and added a fairly good although short description of the conidiophore. They state that the „Flocci (are) knotted above, each knot bearing an oblong spore." The figures also show the articulations on the heads of the spore-bearing-hyphae and upon the spores.

In 1876 COOKE placed the Fungus in a new genus, namely *Heterosporium*, and called it *H. echinulatum;* he gave no figures, but the dimensions of the spores are given in millimetres and are as follows 0,3—0,5 mm × 0,1—0,125 mm.

In 1881 it was referred to by SACCARDO and ROUMIGIER as *H. Dianthi*. MAGNUS gave an account of the carnation disease in 1888.

In 1888 ROSTRUP published an account of the disease of carnations due to *H. echinulatum* with two figures, one of a *Dianthus* branch with disease spots upon the leaves, the other of conidiophores of the parasite.

All these accounts deal only with the outward appearance of the disease upon the carnation leaves, and the figures of the conidiophores are evidently from material scraped off from the disease spots.

SCHROETER gave a good but short description of the parasite under the name of *H. echinulatum* in 1893, and gave the correct dimensions and colour of the conidia. It was again described by SORAUER in 1898.

Neither BAILEY, DUGGAR, nor FARLOW and SEYMOUR mention *H. echinulatum* as parasitic upon carnations in North America.

2. The species of *Heterosporium* upon *Beta*.

Some difficulty was experienced in assigning a systematic position to the form described here as *Heterosporium Betae*. At first it was considered to he a species of *Cladosporium*; on closer observation, however, the conidia and conidia-bearing-hyphae were found not to agree exactly with the figures given for *Cladosporium* (see JANCZEWSKI on *Cladosporium herbarum*, and SCHOSTAKOWITSCH on the same); but corresponded almost exactly with those of the conidia and conidiophores of *H. Syringae*, KLEBAHN[1]). The variability in the length of the spores (20 μ — 13 μ), their shape which was cylindrical with rounded ends, and not oval as are those of *Cladosporium*, together with the fact that the heads of the conidia-bearing-hyphae and of their prolongations are decidedly swollen spherical structures (fig. 11), which is not the case in *Cladosporium*, decided the genus of this fungus as *Heterosporium* and not *Cladosporium*. The dimensions of the conidia bring it near to such forms as *H. Hordei* BUBÁK, *H. Phragmitis* SACC., *H. proteus* STARB., *H. Laburni* OUDEM., *H. Beckii* BÄUML. None of these, however, occur on *Beta*, nor indeed has any *Heterosporium* been before recorded as growing upon this host. It was not found possible to identify it with any of these forms, nor in fact to say with certainty that it is not identical with any one of them, because of the shortness of the descriptions and of the very small diffe-

1) KLEBAHN, Krankheiten des Flieders, p. 11.

rences which exist between these species. The name of *Heterosporium Betae* was given to the species here described, merely to have a name for it, and not to increase the already large list of insufficiently described or scarcely destinguishable species.

The possibility that the fungus in question might he a *Cladosporium* together with the fact that a species of *Hormodendron* was also found upon the *Beta* leaves raised the question of a connection between the two forms. Janczewski asserts that *Hormodendron* is another Conidial form of *Cladosporium* and this is given as being very probable by many of the text books [1]). The question was not gone into very deeply; but both the *Hormodendron* species and the *Heterosporium* from *Beta* were cultivated in petri-dishes and in damp-chambers for some considerable time and on various media. Neither of them changed into the other on any of the different media used, but each always produced the same conidial form. Perithecia were also never formed. It does not seem very probable therefore, that there may be a conection between the two fungi in the sense of Janczewski.

II. Pure Cultures.

After the two forms had been isolated and obtained pure from other fungi and bacteria, they were grown as pure cultures on various agar media in petri-dishes and in hanging drops in order to determine what differences existed as to form and colour of growth between the two mycelia when growing under similar conditions.

1. Cultures in petri-dishes.

On all the media for the first three or four days of growth the young mycelia of both forms were colourless and to the naked eye presented a very similar appearance. Later, however, differences began to be noticeable, the most marked of which was the very much smaller size of the growth of *H. echinulatum* compared with those of *H. Betae*. From colourless, the mycelia soon changed to a light grey and finally to a green; but the rapidity with which the colour changes took place and the intensity of the final tint varied according to the species and to the nature of the nutrient medium.

The general colour of the growths was due to a blending of the colours of two forms of the mycelium. An aerial mycelium which was either light grey or pure white, and a mycelium sunken in the medium whose hyphae were pigmented by an olive green substance in the membrane, produced together the general appearance of grey-green, In all media the hyphae of the sunken mycelium contained oil-drops. On salep agar the sunken mycelium of *H. Betae* was not produced for some considerable time, long after that of *H. echinulatum* had given a grey-green appearance to its mycelium; the final appearance of *H. Betae* was a light grey-green much lighter than that of *H. echinulatum*.

The two forms on all other media except salep agar, gave rise to dome shaped colonies, which never extended over the whole available area of the nutrient medium. An abundant aerial mycelium was formed by both, and the growths became more and more raised up until they finally cracked the adhearing agar layers which were drawn up underneath the dome-like growths. Between the aerial mycelium and the agar layer a very dark coloured closely interwoven network of hyphae formed a tough skin upon the surface of the agar. In cross sections of month-old colonies of *H. Betae*, this layer was found to be very much folded upon itself, thus accounting for the humped up appearance of the whole growth.

On salep agar the mycelia were much less abundant and only in the case of *H. Betae* did the growth extend over the whole area of the medium; in all other cases long before this happened the agar had dried up so that growth was no longer possible.

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1) Engler u. Prantl, Lindau, in Handbuch der Techn. Mycologie, p. 271.

Conidia-bearing-hyphae were produced by both forms on all media and were scattered regularly over the whole mycelium, they were more numerous upon glucose maltose and plum-juice agar than upon salep and meat-extract agar; but in the case of *H. echinulatum* the conidiophores were far more abundant than in that of *H. Betae*. The conidia produced in petri-dishes were relatively so few in number that for inoculation purposes spores were always taken from damp-chamber cultures in which they were produced in relatively large numbers.

It was always noticed that the hanging-drop-cultures produced a far greater number of spores in proportion to the size of the mycelia than did the cultures in petri-dishes, which observation is in accordance with KLEBS' conclusions. In the hanging-drop-cultures the nutrient-media become exhausted in a comparatively short space of time and the fungus is stimulatied to form reproductive organs by a diminution of the food-supply, whereas in petri-dishes the nutrient media are practically never exhausted [1]).

H. Betae formed zones of buried pigmented hyphae upon all media, most prominent however, were those on salep agar which was perhaps due to the fact of the small amount of surface and aerial mycelia produced on this medium. These zones which are often observed in clean cultures of various fungi [2]) were only produced in the laboratory, that is, under conditions of changing light and temperature; they were not formed in incubators of nearly constant temperature. *H. echinulatum* never produced zones.

In slants of glucose agar in test-tubes *H. echinulatum* formed small dome shaped colonies of a dark grey-green colour, the central portions being covered with an aerial mycelium. *H. Betae* formed colonies much larger in area, but similar in appearance except for a narrow light yellow border round each.

On pieces of sterilised potato *H. echinulatum* formed small humped up colonies of a dark green tint, covered at first with a white aerial mycelium, which later on changed to grey and finally to brown.

After some weeks growth during which time the mycelium had increased somewhat in area and had penetrated the potato tissues a dark brown coloured skin was formed upon the surface. *H. Betae* formed colonies twice as large, dome shaped, and entirely white due to a rich aerial mycelium. After a few days the separate colonies coalesced to form one large irregular growth, the aerial mycelium changed from white to a brown yellow and the whole growth became humped up in parts. After some weeks of growth a skin also formed as in the case of *H. echinulatum* and the aerial mycelium became grey brown, but not so brown as is the case with *H. echinulatum* whose mycelium was also coarser in texture.

H. echinulatum was further grown upon sterilised leaves of *Dianthus* and *Beta*, and *H. Betae* upon sterilised *Beta*-leaves. The leaves were placed with a little water in test-tubes plugged with cotton wool and sterilised in steam. The tubes contained enough water to keep the cultures moist for several weeks. The growths were very similar in all three cases and were marked by a quantity of grey-green aerial mycelium. Upon this culture medium the colonies of *H. echinulatum* were constantly larger and more heaped up than those of *H. Betae*. Upon some of the *Dianthus*-leaves the conidia-bearing-hyphae were produced in concentric circles as they always are upon living leaves, but these patches were considerably larger than those met with in nature, and were coated with a very rich aerial mycelium which was also not the case of the disease spots as seen in the field.

(See Table on p. 6.)

2. Cultures in hanging-drops.

Heterosporium Betae.

1. On glucose agar.

On the third day after inoculation of the hanging-drops the young colonies appeared as pin-points to the unaided eye, and as small regularly shaped stars under a small magnification such as a hand lens. The germ hyphae (fig. 4, 5) were rather

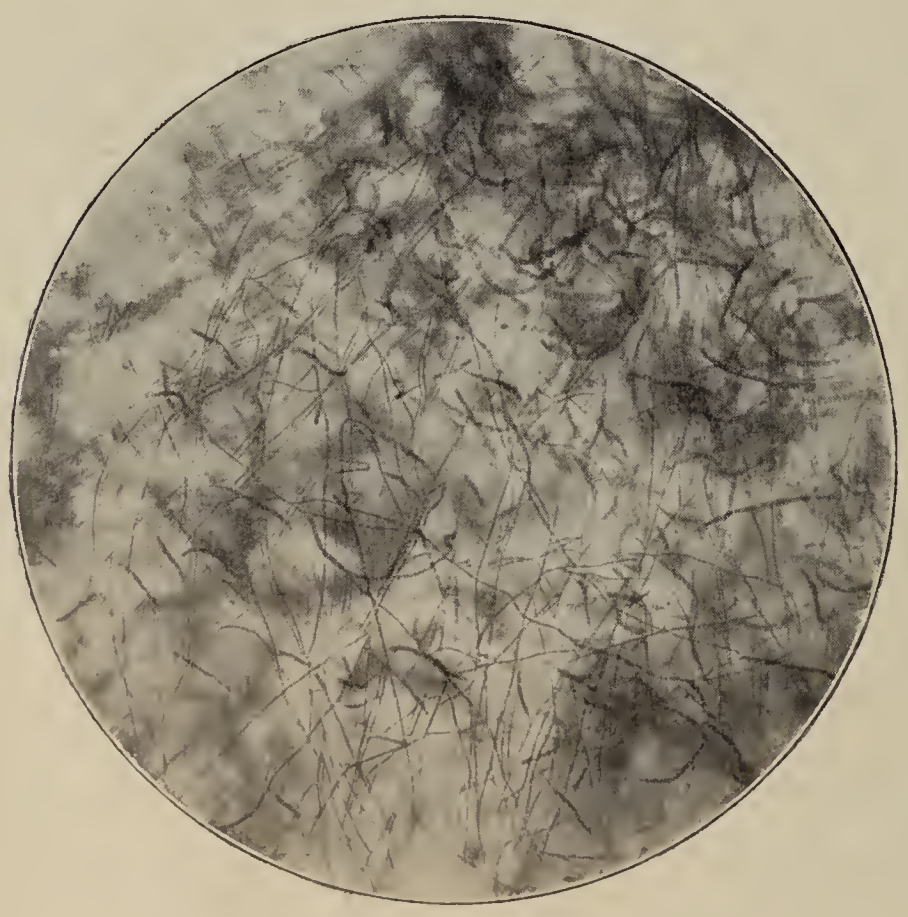
1) KLEBS, Zur Physiologie der Fortpflanzung einiger Pilze, III. Allgemeine Betrachtungen. PRINGSHEIMS Jahrbücher, 1900, **35**.

2) See HIMMELBAUER in the above cited paper and the literature there given; also M. MUNK, Centralbl. f. Bact., II, 1911, **32**, p. 353.

Table comparing the growths of *Heterosporium echinulatum* with those of *H. Betae* upon various nutrient media.

Nutrient medium.	*H. Betae* general appearance.	*H. echinulatum* general appearance.
Plum-juice-agar.	More green than grey, aerial mycelium greyish.	More grey than green, aerial mycelium of a fine velvety nature.
Maltose-agar.	Snow white aerial mycelium.	Circular in outline, light grey, aerial mycelium velvety as before.
Glucose-agar.	Irregular shape, grey-white.	Outline circular, light grey, aerial mycelium as above.
Salep-agar.	Flat and irregular, grey-white.	Flat and irregular, grey-green.
Meat-extract-agar.	Small and regular grey-white.	Very-small, humped up grey-green.
Potato.	Colonies coalesce, white at first, then grey-yellow, and finally grey-brown.	White first, then grey, and finally brown.

longer and more branched than those produced upon plum-juice agar. On the fourth day the colonies appeared pinkish in colour, and measured about 1 mm in diameter. The hyphae which later on would produce both the aerial mycelium and the conidiophores were just emerging from the agar drop into the air space of the culture (fig. 6, 7). The mycelium was composed of hyphae olive green in colour, closely packed together and running almost straight from the germinating spore to the edge of the colony the outline of which was very regular and almost circular. The greater part of the young mycelium was slightly sunk below the agar surface. Short, yellowish hyphae given off from the sunken mycelium invariably took a downward course into the depths of the nutrient medium; these short, nearly vertical hyphae were very noticeable as they were the first to be seen on examining the drop under the microscope, owing to the fact that they were nearest the objective (fig. 8 and photomicro A). An aerial mycelium was produced consisting of long grey hyphae, which, however were a little shorter than those produced upon plum-juice agar. Conidia bearing-hyphae were at first few in number, long, and grew in the radial direction; at this stage the conidiophore heads consisted of not more than two chains of spores, each chain containing two spores (fig. 9). Later on the brown-green sunken mycelium changed in colour to brown, the conidiophores were in some cases long and radially directed,



Photomicro A. *Heterosporium Betae.* Portion of a hanging-dropculture on meat-extract agar, showing the long coloured hyphae penetrating the substratum (× 20).

and in others branches of radiating hyphae. After about a week's growth the origin of the conidiophores and coloured vertical hyphae could no longer be made out, owing to the very compact growth of the mycelium. Finally the sunken mycelium changed from brown to a red brown in colour, thus distinguishing the mycelia on glucose agar from those formed upon the other media. The colonies were small and became dome-shaped, and appeared grey in colour when viewed from above, i. e. from the bottom of the slide containing the hanging-drop. From the opposite side they appeared grey with a decided central orange patch, due to the very numerous coloured vertical hyphae which were not formed at the edges of the mycelia, but only from the middle portions. The conidiophores which were invisible to the unaided eye consisted of one to two, and sometimes three prolongations of the first head.

2. On Maltose agar.

The growth was more abundant than upon glucose both in quantity of spores and the richness of the mycelium which was bright green in colour. Short vertical pigmented hyphae were formed, but were few in number and could not be well seen. The conidiophores were visible from below as bright specks.

3. On Meat extract agar.

The vertical hyphae were very numerous; but were not so deeply coloured as those in glucose or salep agar of the same age; the cytoplasm of the mycelial cells was granular in appearance. Conidiophores began to be produced upon the third day and were short and very numerous. At the end of a fortnight the mycelium was scanty as compared with that on glucose or maltose. Very numerous, long, brown coloured, vertical hyphae were present which were easily seen owing to the thinness of the mycelium; whereas in glucose the reverse was the case. The conidiophores were visible to the unaided eye, many with three prolongations from the first head; the spore chains contained generally three spores each.

4. On Plum-juice agar.

After germination the growth was not so rapid or extensive as in glucose or in salep agar. The mycelial cells contained numerous oil-drops, as indeed was the case on the preceding media; but on plum-juice agar they were very much more noticeable. Later, the colonies increased in size and became larger than those upon other media of the same date of inoculation. The radiating hyphae of the mycelium were not wavy but bent at sharply marked angles and branched. The conidiophores, few in number and placed at the edge of the colony were long and finally produced not more than two prolongations. The sunken mycelium at first green in colour finally became olive green.

5. On Salep agar.

The whole growth consisting of both sunken and aerial mycelia was scanty; the surface mycelium was greyish in colour and contained oil-drops in its individual cells. Very numerous conidiophores and conspicuons short, brown coloured, vertical hyphae were formed. The conidiophores at first colourless became brown-grey and resembled the vertical hyphae in appearance, but were much thinner than these; they formed from two to four prolongations from the first formed head. The growth upon this medium was fairly rapid.

Heterosporium echinulatum.

1. On Salep agar.

The colonies produced spores on the third day after inoculation. The conidiophores took the form of branches given off from the main germ hyphae which had greatly increased in size and formed the main hyphae of the mycelium (fig. 26 *b*). After five days the growth was still scanty and straggling. Very little white aerial mycelium was present; but the young conidiophores were numerous. It was observed that the conidiophores always produced prolongations from the first formed heads (figs. 28, 29). Later on the conidiophores become light-red brown in colour and the scanty sunken mycelium grey-green, the latter consisted of short barrel-shaped cells. The main hyphae of the mycelium, which were considerably branched, were formed from the original germ tubes and remained the largest hyphae of the whole growth. They were always recognizable, as no other thick hyphae were produced, and remained

for the most part short and crowded together, so that the colony always kept its star shaped outline determmind once and for all by the germ hyphae. On the sixth day the conidiophores were still without prolongations; but chains of two spores were oecasionally seen; some times a head bore two spores side by side, in which case it was noticed that one of the two spores was placed upon a little side branch (see figs. 29*e*, 39). The general colour was greyish. In a 15 days old culture the conidiophores were very numerous, and consisted of as many as seven prolongations of the original primary head, and as many as 14 spores (fig. 44). Very little sunken mycelium was present, and the growth consisted chiefly of aerial and surface mycelia besides the numerous conidiophores.

2. On Glucose agar.

On the third-day the colonies were just visible to the unaided eye as very small stars of irregular outline. On the fourth day the aerial hyphae and young conidiophores had begun to emerge from the agar. On the fifth day the central portion of the colony had taken on a grey-green colour; the hyphae of which the mycelium was composed was not wavy, but sharply bent here and there at well marked angles. In petri-dish cultures of the same age it was noticed that the central portion was yellowish as distinct from the grey-green in the hanging-drop cultures. It was noticed that the individual cells of which the sunken mycelium was composed were shorter than those produced in plum-juice agar. A colony 13 days old appeared very nearly black in colour to the unaided eye.

3. On Meat-extract agar.

The growth was very similar to that on salep agar; but neither conidiophores nor their prolongations were so numerous, not more than four spores were observed on any one spore-bearing hypha. The characteristic grey, spirally — coiled hyphae were present as before (see photomicro B). It was observed that whenever these aerial hyphae touched the surface of the agar, which they did in a few cases, that at this spot the hyphae branched, and sent out hyphae into the substratum. The mycelium increased in mass, but very little in area, and the hyphae of which it was composed were packed closely together.

4. On Plum-juice agar.

The colonies after three days growth were just visible to the unaided eye, and could be destinguished from those of *H. Betae* of similar age by their more regular outline. As on Meat-extract and salep agar the outline of the mycelium was determined by the germ-tubes becoming the main hyphae. On the fourth day the sunken mycelium began to turn brown, and on the sixth had become olive-green in colour. Many more conidiophores were produced than by *H. Betae* on the same medium. The colony finally became dome shaped. It was observed in cultures of more than a week old, that many of the spores germinated in situ upon the conida-bearing-hyphae which continued to grow vegetatively and ended in a sharp pointed structure, calling to mind the conidiophores of *Helminthosporium* and of *Heterosporium Syringae* as observed by Klebahn[1]). These vegetative growths were never observed to bear spores.

It may be well here to summarise some of the results obtained from the hanging-drop-cultures, as it will be seen that one and the same form growing upon different media, but otherwise under the same conditions presents well marked differences in colour of the mycelia, its amount, and nature, so much so that at first sight the cultures appear to belong to separate and distinct forms. A culture for instance, of *H. Betae* upon glucose is very different even to the unaided eye, from a culture of the same form upon maltose-agar. Again the absence of the coloured, vertical hyphae in cultures on plum-juice-agar produces a very conspicuous difference when taken in conjunction with the fact that such hyphae were formed upon all the other media tried.

Summary.

Heterosporium Betae.

1. Upon glucose agar.

The germ hyphae are longer and more branched than upon plum-juice-agar; but the aerial hyphae are not so long. The final colour of the sunken mycelium is

1) Krankheiten des Flieders, S. 12.

red-brown, and the colonies become dome-shaped. The conidia-bearing hyphae bear from 1—3 prolongations of the first head; the mature conidiophores are invisible to the unaided eye.

2. On maltose agar.

The growth is more abundant than upon glucose both as regards mycelium and the number of spores. The sunken mycelium is bright green; fewer vertical, sunk, hyphae are formed upon this medium than upon any other. The conidiophores are visible to the naked eye.

3. Upon meat-extract agar.

The vertical, sunken hyphae are very numerous and long, but are not so deeply coloured as they are upon glucose. The surface and aerial mycelia are scanty as compared with those upon other media thus making the vertical sunken hyphae easy to be seen. The conidiophores are visible to the unaided eye.

4. Upon plum-juice agar.

The growth is less rapid than upon glucose or salep-agar; the oil drops contained in the mycelial cells are more noticeable. In size the colonies become more extensive than upon any other medium. The mycelial hyphae are bent sharply and are not wavy. The sunken mycelium is olive-green in colour, and the conidia-bearing hyphae do not produce more than 2 prolongations. No vertical, sunken hyphae are produced upon this medium.

5. On salep agar.

The surface and aerial mycelia are very scanty, but very numerous vertical, sunken hyphae and conidiophores are produced, the latter often bearing as many as 4 prolongations.

Heterosporium echinulatum.

1. On salep agar.

The general colour of the colony is greyish, very numerous conidiophores with as many as 7 prolongations and 14 conidia are produced.

2. On glucose agar.

The central portion of the mycelium is grey-green in hanging-drop-cultures, and yellowish in petri-dish-cultures of the same age. The mycelium is bent at angles and is not wavy, its individual cells are shorter than those produced upon plum-juice agar.

3. Upon meat-extract agar.

The conidiophores are not so numerous neither are their prolongations as upon other media, at most conidia are produced on one conidiophore.

4. On plum-juice agar.

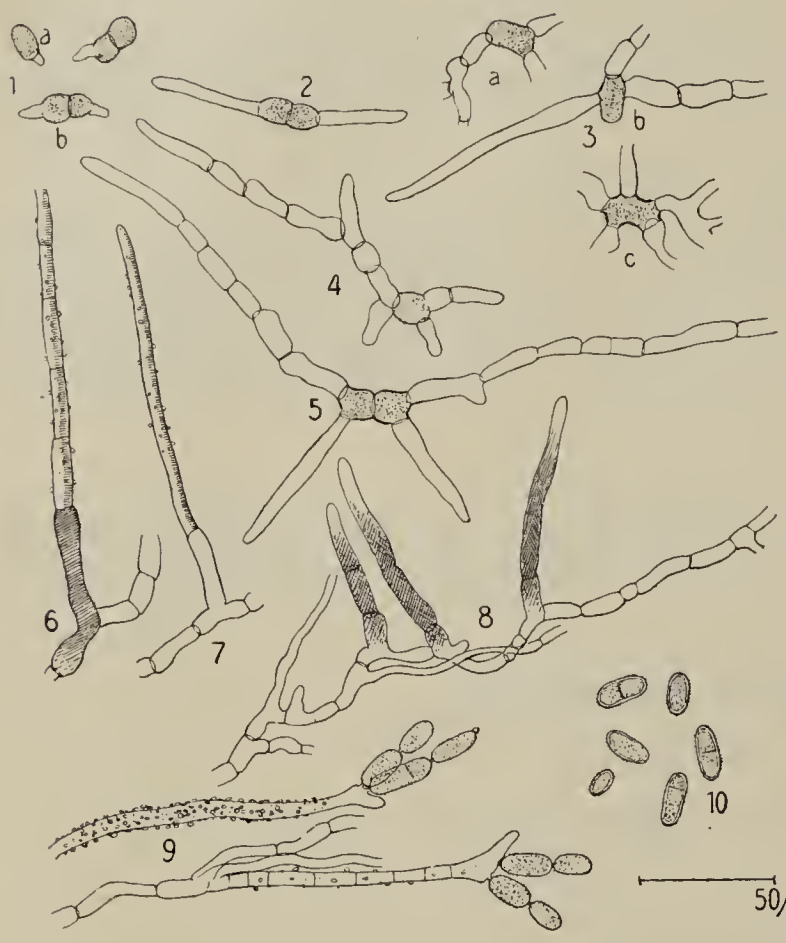
The sunken mycelium is olive-green, and many of the conidia germinate in situ.

Heterosporium Betae.

1. Germination of Conidia and development of conidia-bearing hyphae.

The germination of the spores was observed in hanging-drop cultures on various media. The spores were obtained from cultures of small pieces of mycelium taken from petri-dish cultures, and transferred to hanging drops. The mycelia which arose from these cultures produced numerous conidiophores after 7—8 days of growth. The material for the study of the germination of the conidia was obtained from these cultures. The spores were taken from the cultures on the end of a blunt platinum needle, previously sterilised by heating to redness in a flame, and dis-

persed in a small drop of sterile water upon a sterile glass slide. This process of gently touching the cultures with the point of the needle, and transferring to the water drop was repeated 3 or 4 times. The drop was then examined under the low power of the microscope to make sure that the spores were present in sufficient quantity to ensure that one dip of a very small platinium loop into the drop would bring away 3 or 4 spores. Sometimes no spores were picked up at all, sometimes only one, and occasionally as many as 9. On an average, however, 3 or 4 spores were delivered by each touch of the charged platinium loop on to the agar drop. Each drop was inoculated at the edge in four different places, corresponding to the cardinal points of the compass. In this way it was easy to distinguish the spores again when observing under the microscope. Care was taken that the agar surface was as little scratched and indented as possible in the process of inoculation as the refraction and shadows caused by these seriously interfered with the accurate observation of the germinating spores. Those spores were observed which lay upon an even surface which was practically parallel to the glass slide. By having thoroughly clean glass cover-slips which acted as the top of the hanging-drop slide, it was found possible to obtain drops of fairly large area in proportion to their thickness, the central portion of the drop being a flat plane only slightly if at all convex. This was only possible when solid media were used. With liquid media, such as normal Pasteur solution, the drops gave convex surfaces, so that the spores very seldom lay in a plane parallel to the bottom of the glass slide. Three slides of each medium were made.



Figs. 1—10: *Heterosporium Betae* (× 215).
Figs. 1 and 3: Spores germinating on salep agar. — 2: Spore germinating in water. — 4 and 5: Young mycelia. — 6 and 7: Hyphae growing into the air studied with water drops. — 8: Short coloured hyphae growing downwards into substratum. — 9: Young conidiophores, showing two chains of two spores each and the beginning of the prolongation of the conidia-bearing hyphae. The acrial portions are covered with water drops. — 10: Conidia.

The germination upon all the solid media, and upon normal Pasteur-solution was the same. After 3—4 hours a germ hypha was pushed out at one end of the spore, a little to one side of the long axis of the spore (fig. 1). Sometimes two germ hyphae were formed, one at one end, and the other at the opposite end of the spore (see fig. 1 *b*). The spores increased a little in size, more in breadth than in length. The germinating spores measured 20 μ—30 μ × 10 μ—13 μ. The first germ hyphae in-

creased in length, and became septate (fig. 4, 5), but did not produce side branches until they had attained a considerable length (100—200 μ). Their course was nearly straight. Soon after the appearance of the first germ hyphae, others made their appearance, to the number of four (fig. 5). Their position was at the end of the spores a little to one side of the long axis. A germinating spore from which 4 germ hyphae had grown, presented the appearance of an x, the spore being in the centre of the x (see fig. 5). This appeared to be the usual method of germination; in some cases, however, only 3 germ hyphae were produced, (fig. 3*a*), taking up the end positions, or sometimes 2 of the 3 germ hyphae were observed to come from the central portion of the spore (see fig. 3*b*). One case was noticed in which 5 germ tubes had been produced, the extra hypha came from the middle of the spore (see fig. 3*c*). After having attained a length of 100 μ the hyphae became septate.

In normal PASTEUR-solution the same x shaped growths were produced, but not to such an extent as in the solid media. In tap-water the spores generally produced two hyphae, one at each end of the spore, almost exactly opposite the ends of the long axis (fig. 2). The hyphae grew greatly in length, and finally became septate, the individual cells of the hyphae being generally longer than those produced on solid media. One case was observed in which two other germ hyphae began to be formed some days after the appearance of the first. In no case were the hyphae of these water cultures seen to branch. In some cases a small conidiophore was produced after a week, directly from the old germinating spore. After 24 hours growth upon solid media the x shape produced by the germinating spores was no longer recognisable. The germ hyphae had repeatedly branched to form mycelia fairly uniformly distributed over the agar surface. It will be seen that this was not the case with *Heterosporium echinulatum*.

2. Origin of Conidiophores.

After 3 days growth the small colony consisted chiefly of radiating hyphae, which originated from the branching of the main (generally 4) germinating hyphae of the spore. The original spore could only with difficulty be made out. The centre of the colony appeared as a tangled mass of hyphae. The edges were composed of radiating hyphae, which had not as yet overlapped. These bore short branches, some of which were conspicuous by their colour and straightness, as compared with others which were not coloured. The distal end of the cell of origin of these coloured hyphae and the middle cells were coloured a greyish-brown. The actual growing tip was colourless (fig. 8).

Up to this point nearly the whole colony was beneath the agar surface, as was proved by the fact that the cell walls were sharply defined, whereas walls of the original spore, and of some of the germ tubes which lay upon the surface of the agar were thick in appearance and shaded, showing them at once to be cylindrical structures. These latter appeared to be covered with a film of moisture.

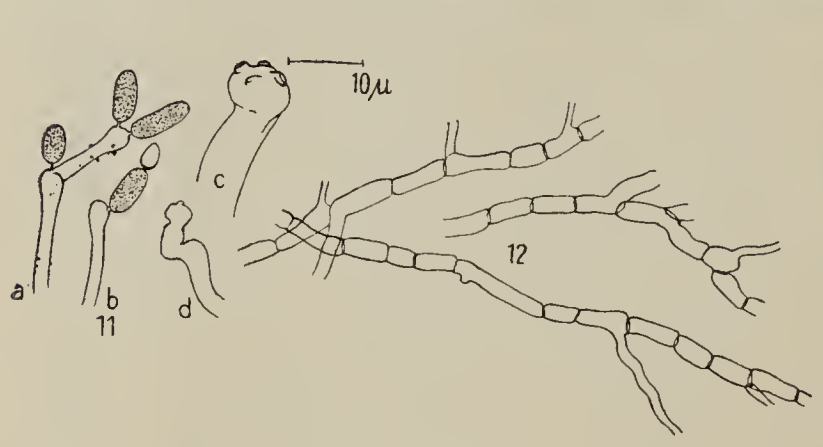
It was noticed that the tips of some of the uncoloured branches above-mentioned, were shiny and shaded, showing them to have reached the surface. The actual point of departure from the agar, was marked by

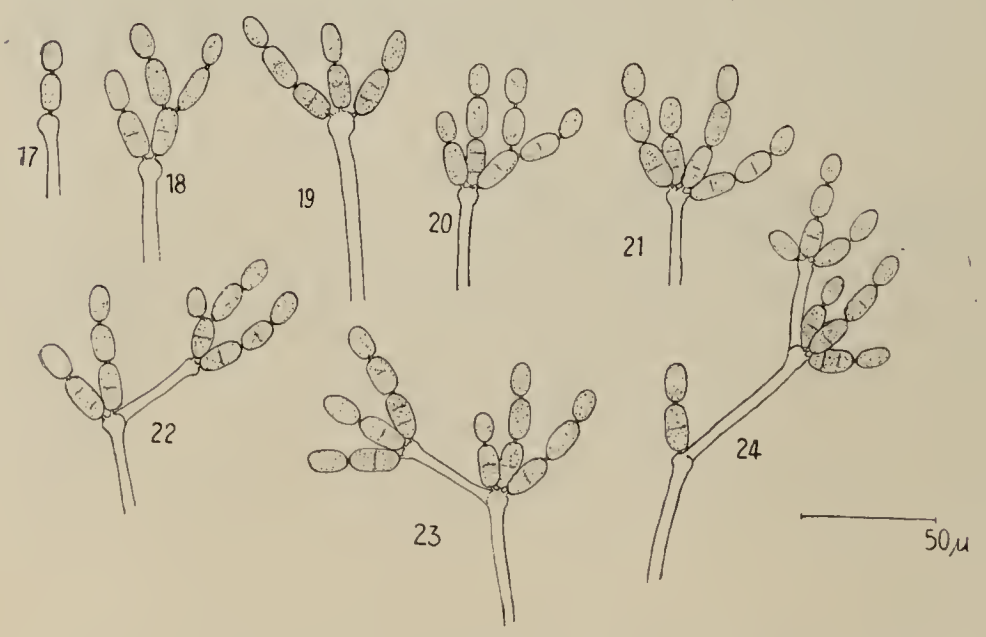
a lessening in the diameter of the projecting hyphae (fig. 6). In some cases these aerial hyphae had reached a considerable length (fig. 7), and were studded with minute water drops, so much so that it was not at all easy to make out the septa (fig. 9). These, however, as could be seen in favourable cases, were numerous, and closer together than in the buried hyphae (fig. 9). Very often in an old culture in which the agar had dried somewhat, and had contracted or had been used up by the fungus, the junction of the conidiophores with the nourishing mycelium was very noticeable. The thinner conidiophores arose almost direct from the thicker basal portion which was formerly buried (fig. 6).

The spores and spore-bearing-branches were formed at night.

3. Development of the conidiophores.

On the 10th Jan. 1912 observations were made upon a young colony sown on the 6th inst. Young conidiophores were seen all around the circumference of the colony; but judging from their length, and comparing them with those of colonies 2 days older; it was thought that these would not form spores during the night; this proved to be correct. However, where the colony came in contact with water and air at one edge of the agar drop, one hypha of the mycelium was seen bearing much longer conidial branches, two or three of which bore one spore at their tips (see fig. 13). The spore was joined to the swollen head of the conidiophore by a definite stalk, which was quite colourless and transparent, while the conidiophore was greyish, and the spore a chestnut brown. A drawing was made at this stage at





Figs. 11—24: *Heterosporium Betae* (× 215).

Fig. 11: Distal portion of conidia-bearing-hyphae, showing swollen head of the conidiophore. *a* and *b*: from hanging-drop-cultures, *c* and *d*: from diseased Beta leaf (*c* × 600). — 12: Portion of mycelium from a hanging-drop culture. — 13—16: Origin and development of the conidia. — 17—24: Various forms of the conidiophore; for explanation see text. (Drawn without the help of the drawing apparatus.)

11 p. m. (fig. 13). The conidiophore-bearing hyphae exposed to the saturated air of the chamber were studded with numerous minute water-drops. At 12.30 p. m. the conidium had moved a little to one side out of the axis of the conidiophore and had also budded off a small oval colourless cell (fig. 14 *a*); at its distal end the head of the conidiophore had also pushed out a papilla on the opposite side of the conidium (see fig. 14 *b*). At 2.30 a. m. the first formed conidium had moved still further out of the axis of the conidiophore, and the budded off cell at its tip was plainly seen to be a young conidium, and was now of the same colour as the first. The papilla *(b)* on the opposite side of the conidiophore head had also increased in length, and was now seen to be a small colourless oval body (see fig. 15 *b*). At 4.30 a. m. the first budded off spore was almost mature, while that on the conidiophore head had become a spore whose dimensions exceded those of the other two which were older (fig. 16 *a* and *b*).

At 9.30 a. m. Another spore had been produced on the head of the conidiophore, while the previous ones had again budded. So that finally the head of the conidia-bearing-hypha bore two chains of spores one containing 3, the other 2 spores; and a third spore which had not yet budded any conidia. This was not drawn as the chains lay in three dimensions and could not he made out all at once.

The other coloured hyphae which were noticed at first (see fig. 8) were observed day by day. They never reached the surface, but on the the contrary grew vertically down into the substratum and took on a brown colour. After having attained a length of 100—200 μ growth in length ceased. These coloured vertical hyphae were produced upon salep, meat extract, maltose, and glucose agars; but not on plum-juice agar. The ripe conidiophore heads bore from 1—4 spores, which could bud off spores at their distal ends so as to form chains of usually 3, sometimes of 4 spores, so that 1—4 chains might be present of 3—4 spores each. The lowest spores of these chains might bud off a spore at their tips and this spore might also bud off spores so that secondary chains were formed. No one spore, however, bore more than 2 spores at its tip, wheras the conidiophore head bore as many as 4 at times (see fig. 21).

But instead of the conidiophore head bearing chains of spores, it may also send out a branch in place of a spore. Only one such branch was thus formed, no matter what the number of spores (fig. 11 *a*, 22—24). A conidiophore head may bear one spore chain and one branch (fig. 11 *a*, 24), or 2 spore chains and one branch (fig. 22), or 3 spore chains and one branch (fig. 23). These branches produce swollen heads in the same manner as the primary conidiophore, and may in their turn bear from 1—4 spore chains, each chain consisting of 3 spores at maturity, or 3 chains and another branch, which repeats the process. These branches are usually set off at angles to one another, so that the mature conidiophore bears a jointed appearance, each joint resembling in its swollen ends a bone. It is rare for any one of these branches to lie in the same axis as the one preceding it, but it does sometimes occur (see fig. 24).

The spores of a chain are joined together by small transparent stalk-like articulations in the same way as the first-formed conidium is joined to the conidiophore head. On the heads of old conidiophores after the spores have fallen off little papillae are to be seen in the position of the articulations, and are probaly their remains; the same structures

can also be made out upon the ends of the individual conidia. They are indicated by Berkeley and Broome in their figure of *H. echinulatum*[1]).

(Fortsetzung folgt.)

Referate.

Bataille, F., Miscellanées mycologiques. (Bull. Soc. Mycol. France, 1912, **28**, 127—130, pl. 8.)

L'auteur signale quelques réactions colorées produites par l'action de NH_3 sur divers Champignons. Il signale ensuite la réviviscence [déjà étudiée par Buller] des Champignons marcescents, et quelques exemples du polymorphisme des pores chez les Polypores. Il décrit ensuite le *Chamonixia caespitosa* Roll., qui n'était connu que par un seul individu et qui vient d'être retrouvée dans une nouvelle localité.

R. Maire (Alger).

Grandjean, M., Causerie mycologique. (Bull. Soc. Mycol. France, 1912, **28**, 195—198.)

Considérations sur l'utilité des marchés de champignons contrôlés. La douceur de l'hiver 1911—1912 a amené une poussée précoce de champignons printaniers en janvier, en même temps qu' une prolongation de la poussée automnale. R. Maire (Alger).

Le Fort, R., Un curieux cas de production de la Morille. (Bull. Soc. Nationale d'Acclimatation, 1912, **59**, 502—503.)

Des Morilles se sont développées à l'ombre d'un *Picea* au pied du quel on déverse tous les ans les résidus de cidre restés dans l'alambic après la fabrication de l'eau-de-vie de cidre. Elles étaient jusque là inconnues dans la région. R. Maire (Alger).

Konwiczka, H., Bekannte eßbare und giftige Pilze, 70 pp., 44 farb. Abb., 2 Textfig. (Ernst'sche Verlagsh., Leipzig 1912.)

Eines der volkstümlichen Pilzbücher: Die Pilze als Nahrungsmittel. Wie sammelt man Pilze? Die Zubereitung der Pilze als Speisen. Richtige Verwendung der Pilze usw. Einteilung der Pilze. Beschreibung der wichtigsten eßbaren und giftigen Pilze. — Beschrieben werden 75 Arten, darunter 37 eßbare; diejenigen Pilze, welche durch die Marktordnungen der Städte Berlin, München, Breslau, Leipzig, Dresden und Chemnitz für den freien Handel zugelassen sind, werden dabei besonders kenntlich gemacht. 33 Zeilen (!) behandeln (ohne Abbildung!) die Entstehung, Entwicklung und das Leben der Pilze, sowie die für die grobe Einteilung wichtigen Merkmale. Tabellen zum Bestimmen der Gattungen bzw. Arten fehlen. Die Abbildungen sind wenig befriedigend.

Leeke (Neubabelsberg).

Molz, E., Bemerkungen zur Arbeit Max Munks: Bedingungen der Hexenringbildung bei Schimmelpilzen. (Centralbl. f. Bact. II, 1912, **34**, 40—42.)

Verf. legt Verwahrung ein gegen eine falsche Interpretation eigener Angaben durch Munk (in obiger Arbeit, Centralbl. f. Bact. II, 1912, **32**,

1) The Annals and Magazine of Natural History, Vol. XI, 4th Series, p. 345, 1873).