Dieses Mittel ist das allerwichtigste, da die Vorjahrspolster die Hauptquelle der Infection der hervorsprossenden jungen Blüten ist. Es ist sehr wichtig, daß die Reinigung der Bäume vor dem Bersten der Winterknospen geschieht. Betreffs der Kirschbäume ist das Mittel gut durchführbar infolge der Localisation der toten Zweigteile, und speciell die Erfahrung vom Experimentalfältet beweist die Effektivität des Mittels. Die Localisation der toten Blütenbüschel an den Apfelbäumen und das Eindringen des Pilzkörpers auch in zwei- bis mehrjährige Äste macht das vollständige Entfernen aller pilzführenden Astpartien aus den befallenen Apfelbäumen viel schwieriger und unsicherer.

2. Gleich danach bespritze man die Baumkronen sorgfältig und durchgängig mit 2 %iger Bordeauxlösung.

   Diese Bespritzung ist besonders wichtig, wenn es der Dürrekrankheit an Apfel- und Birnbäumen gilt.

3. Wenn trotzdem 2—3 Wochen nach dem Blühen herabhängende, tote Blütenbüschel und Triebspitzen in den Baumkronen sichtbar werden, so muß man alle diese möglichst vollständig sogleich abschneiden und verbrennen. Man wiederhole auch die Durchmusterung und die Reinigung der Baumkronen mehrmals im Laufe des Sommers und des Herbstes, bis in den Winter hinein.

4. Unmittelbar nach der Zerstörung der eventuell angetroffenen toten Blüten- und Triebspitzen im Juni bespritze man die Bäume zum zweitenmal mit 2 %iger Bordeauxlösung.

5. Im Herbste muß man endlich auch alle verfaulten oder mumificierten Früchte, sowohl die zu Boden gefallenen wie die am Baume sitzenden, sorgfältig einsammeln und verbrennen, damit nicht durch die darauf im nächsten Frühjahre hervorsprossenden neuen Conidienpolster oder durch das daraus in darauffolgendem Frühjahre herauswachsende Becherstadium (Sclerotinia) dieser Pilze die neu hervorsprossenden Blüten- und Blattknospen angesteckt werden können.

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

By

W. J. Dowson, B. A. Cambridge.

(Fortsetzung.)

4. Dimensions of spores and mycelial cells of *H. Betae.*

Most of the mycelial cells fig. 12 were barrel shaped and constricted at the ends, some however were constricted at both centre and ends. From a series of measurements the followig was the re sult obtained: 6'5 μ—28,5 μ $\times$ 3 μ—8,5 μ. The mycelial cells varied greatly in length and breadth.

The conidia were of a cylindrical shape with rounded ends; the majority were two-celled; but unicellular, and three-celled spores were also numerous. The spores were not constricted at the junction of the septa with the spore wall (fig. 10). They mensured: 13 μ—24 μ × 6 μ—12 μ. The dimensions of the most numerous were 8 μ × 20 μ.

5. Conidiophores of *H. Betae* and of *H. variabile.*

Reed and Cooley[1]) state that the conidiophores of *H. variabile* produce prolongations from the first head as is the case with *H. from beta* and *H. echinulatum* and *Cladosporium;* but that these prolongations arise from just below the heads; and further that the spores are not produced in chaines; but singly upon each head. The also assert that in old cultures in which the fungus had been growing under saprophytic conditions for some time, the multicellular spiny spores were no louger produced; but in place of these, small unicellular smoth spores were produced by a budding process in chains. Similar spores were never observed in cultures of H. from Beta however old they might be.

In material of *H. variabile* obtained from the herbarium of the „Station für Pflanzenschutz" in Hamburg and mounted in lactophenol, the conidiophore heads appeared to be similar to those of *H. Betae* and *H. echinulatum* with from 1 to 4 articulation points (fig. 25 *a*), thus indicating that in these cases 4 spores had been produced upon one head; a head with a prolongation bearing articulations was also seen (fig. 25 *b*).

The above described mode of development of the conidia-bearing-hyphae with the formation of prolongations from each head, and the origin of the conidia as a budding process (acropetalous) has also been observed for *Cladosporium* by Janczewski and confirmed by Shatokowitsch. It will also he seen to hold true for *H. echinulatum.*

B. *Heterosporium echinulatum.*

The germination upon all solid media and in water was similiar to but not so rapid as that of *H. Betae.* The end cells of the multicellular spores always produced germ hyphae, the first of which corresponded in position with those of *H. Betae* (fig. 26 *a*). After 30 hours nearly every cell of the spore had produced germ hyphae, in some cases more than one hypha was given off from one cell, and in one case as many as four hyphae were counted which arose from an end cell (fig. 26 *b*). After 24 hours the first germ hyphae had branched and produced not only hyphae which grew into the substratum or upon it, but also a very characteristic aerial mycelium, which was very regularly wavy and often spirally coiled (see photomicro B). The bends of the hyphae of which the waves were formed were of equal dimensions, so that a very characteristic appearence was given to all the cultures of *H. echinulatum.* Spores which had germinated at the bottom of a watch glass with a little water in it had also produced these curious aerial hyphae. They were in fact, produced upon all the culture media tried and rendered the growths of the *H. echinulatum* distinguishable from all others even in the early stages of mycelium

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1) Reed, H. S. and Cooley, J. S., Centralbl. f. Bact., 2. Abt., **32**, 40.

formation. The germ hyphae were always recognisable and became by increase in length and breadth, the main hyphae of the mycelium, which

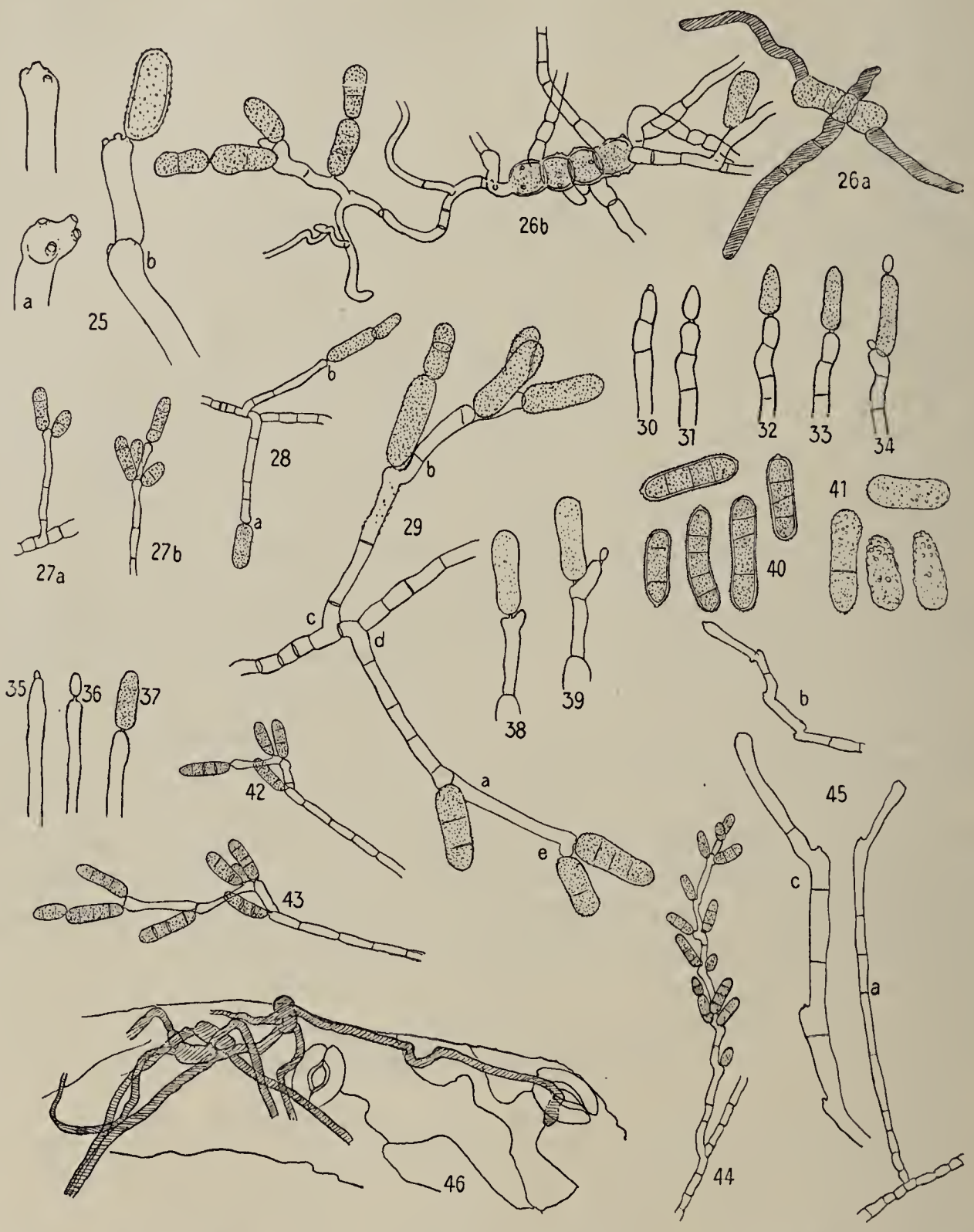


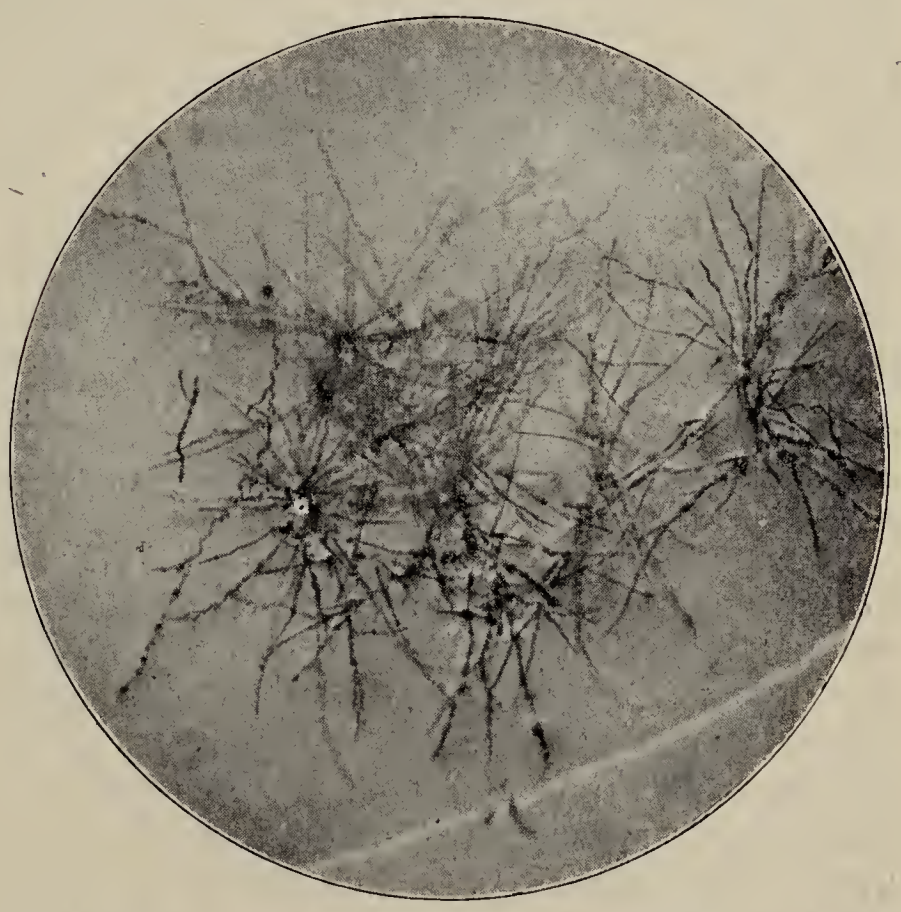
Fig. 25: *Heterosporium variabile.* — Figs. 26—45: *Heterosporium echinulatum.* — Fig. 46: *Heterosporium Betae.*

Fig. 25: Conidiophore heads of *H. variabile* showing articulations taken from herbarium material of *Spinacia oleracea* (× 600). — Fig. 26*a* and 26*b*: Germinating conidia of *H. echinulatum*, *a* on salep agar after a few hours, *b* in water after 2 days (× 215). — Figs. 27*a*—29: Showing the development of the conidiophores. (Figs. 27*a*, 27*b* and 28 × 120. — Fig. 29 × 215. — Fig. 29 was drawn 6 days after fig. 28.) — Figs. 30—34; 35—37; 38, 39: Showing the formation and development of the conidia (× 215). In figs. 34, 37 the spores are not yet mature; but hanc yet to increase in length and breadth. In figs. 38, 39, the spores have nearly reached their mature size. — Fig. 40: Conidia from a clean culture and mounted in lactophenol (× 215). — Fig. 41: Conidia from a diseased leaf and mounted in glycerine, showing unequal distribution of warts in young spores (× 215). — Figs. 42, and 43: One and the same conidiophore (Fig. 42 × 98, Fig. 43 × 120 and drawn 2 days later). — Fig. 44: Mature conidiophore from hanging-drop-culture (× 98). — Fig. 45: Denuded conidia-bearing-hyphae showing the remains of the articulations (*a* × 98, *b* × 120, *c* × 215). — Fig. 46: Spores of *H. Betae* germinating upon dead portion of a wounded *Beta* leaf forming young mycelia (× 215).

was more irregular in outline than that of *H.* from *Beta*. The colonies were most irregular in outline on plum-juice, salep and meat extract agar and least so on glucose agar (see hanging drop cultures). Upon glucose the growth was altogether more luxurious.

1. Development of the Conidiophores.

Spores sown on the afternoon of the 29th Jan. produced mycelia with formed spores on 31th Jan. i. e. after about 60 hours. The majority of spores were produced at night and in the early hours of the morning (temp. = 12°—15° C). Those formed in the early morning matured during the day, but no new spores were observed to be formed. During the following night and early morning more spores were formed and so on for 10—12 days. At such a stage the hanging drop cultures showed the original spore, the main hyphae from the germ pores, and branches from these hyphae which were not so thick. The original spores had been set upon the surface or in a dent in the surface of the nutrient medium (agar). The hyphae also grew upon the surface, and were covered with a thin film of water, which on account of refraction, made them difficult to observe under high magnifications. Some, however, grew just below the surface (cf. *H. Betae*), which was easily made out, for those within the agar appeared as flat structures of two dimensions, the coefficient of refraction for agar and for the hyphae and their contents being about the same; whereas that of air is different, and hence the hyphae on the surface appeared as solid, i. e. as cylindrical structures with deeply shaded sides. Upon these main hyphae two sorts of branches were seen to exist, those which grew straight up into the air — the aerial hyphae — which were thin and coiled forming stiff spirals (Microphoto B) and those which, a little thinner than the main hyphae from which they sprang, grew out in a horizontal direction. Some grew in and on the surface of the agar, and were, in fact, new nutrient hyphae, others, however, grew out towards the air. These latter were the young conidiophores, which grew out perfectly straight for some way and were quite colourless. They arose from the main hyphae in two ways, either one cell of the hyphae branching at one end (fig. 27 *a*) or a short cell itself growing out to form the branch (fig. 29 *d*).



Photomicro B. Young colony of *Heterosporium echinulatum* in hanging-dropculture on salep-agar, showing coiled aerial hyphae (× 20).

There seemed nothing in the appearance of these branches when quite young which distinguished those which become conidiophores from those which grew out as nutrient hyphae. On the night of the second day after sowing, spores began to be formed. The ends of the young conidiophores tapered slightly to a blunt point (fig. 30, 35) upon which appeared a transparent papilla which soon swelled up at the distal end to form a spherical body, the young spore. Both papilla and young spore were more refracting than the conidiophore itself. After an hour the young spore swelled up till it became spherical and changed its form when its diametre was about that of the conidiophore into a conical shape with a rounded end (fig. 31, 36). At the same time the end of the conidiophore filled out and swelled slightly to form the head which was only a very little greater diameter than that of the rest of the conidiophore. After this the dimensions of the conidiophore remained unchanged, but the spore grew steadily in size, always cone-shaped and with a rounded base (fig. 32). At this stage a new papilla may appear on the head of the conidiophore, or the whole head itself may begin to swell out to form a short branch (fig. 34, 38). Two spores were never formed at the same time on the same head. The colour of the spores changed from transparent white to a pale brown. At $^3/_4$ mature-size under a high magnification, dots were to be made out upon the surface of the spore, which finally developed into the warts of the mature conidium (fig. 32).

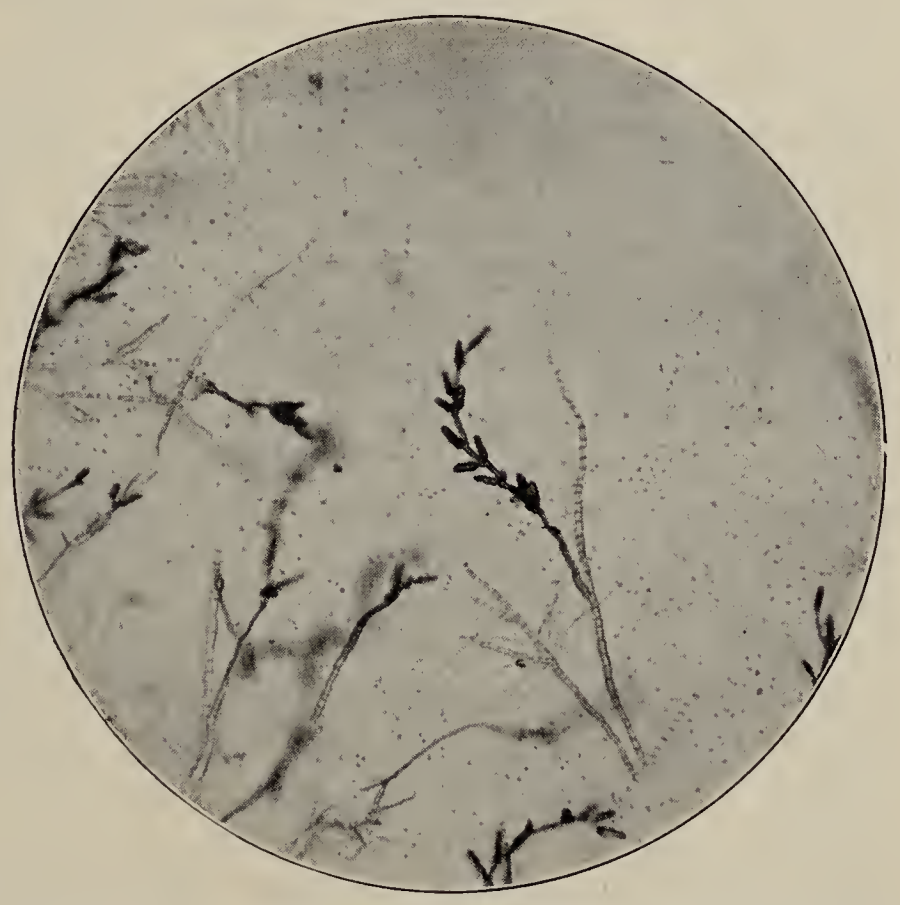
2. Spores of *H. echinulatum.*

The mature spores which vary very greatly in length are long and cylindrical and may be slightly constricted at the centre; the ends which are hemispherical sometimes show the remains of the articulation papillae. The average spore has three septa (fig. 40), some have only one and others two (fig. 40), while as many as 5 have been seen. The colour is reddish-brown and the epispore is studded with small projecting warts. The largest spores were taken from diseased leaves, that is, the greatest number of large spores was found upon *Dianthus*, although spores of the same size were also produced on artificial culture media. Thus the longest spore in a preparation of *Dianthus*-leaf sections measured 12 μ $\times$ 53 μ, and the shortest 3 $\mu \times$ 12 μ. In a preparation taken from a hanging drop culture on salep agar the greatest and smallest spore measured 12 $\mu \times$ 46 μ and 9 $\mu \times$ 30 μ respectively; these were mature spores to judge from their form. From a living culture on salep agar a mature spore measured 12 $\mu \times$ 49 μ, and a young spore, which was yet conical 12 μ to 24,5 μ (at the base). The spores with unequal distribution of spines (see below) measured; from original *Dianthus*-leaf preparations 12—15 μ $\times$ 30—50 μ. On salep agar one spore was found to measure 50 μ in length, but had no septa.

The spores are mature, i. e. have reached their final size in about 12 hours, the distal end being the last to fill up to form the rounded end of the mature spore. The colour also takes on gradually a darker hue until finally it is a reddish-brown. The warts are also developed, but not to the same degree and the septa seem to he laid down after the spore has reached its full size. No young spores which were yet conical in shape were observed to possess septa and many which had reached

their full size were still devoid of any partition walls. In some preparations from a diseased *Dianthus* leaf and also from some clean cultures on agar, it was noticed that one end was more warty than the other (fig. 41). One end was studded with rather fine warts, while the other was quite rough with small blunt spines, placed at somewhat distant intervals. It was further noticed that the spores were immature, to judge from the fact that they were thicker at one end than at the other and that the thicker end was always the rougher, and further that they were still only one-celled. The distribution of warts could only be made out upon preparations which necessitated the disjunction of the spores from the conidiophores, i. e. in glycerine or in lacto-phenol, so that the spores were no longer living and their further development could not be observed. The living cultures in hanging drops did not permit of the use of the high powers of the microscope.

The further development of the conidiophore is very similar to that in *H. Betae*. The first head always sends out a branch, which bears one or two spores together with another branch (fig. 42, 43). In those cases in which 2 spores are produced on one head, one of the spores is nearly always placed upon a very short side branch (fig. 29*e* and 39). The actual prolongation of the first conidiophore was always at the level of the first formed spore. As in *H.* from *Beta* any of the spores may bud off another spore from their distal ends (fig. 29, 34); but this was not often observed, chains of not more than two spores were, however, seen (fig. 28, 29, 43). The process of elongation by the sending forth of a branch from a head of a conidiophore might be continued up to 7 times, so that a mature conidiophore might consist of as many as 7 branches with 13—14 spores (fig. 44). The branches are never produced in the same straight line as the previous one, so that the old conidiophore denuded of its spores appeared as a much bent structure, each bend being at a sharply marked angle. This is well seen in the drawings of old conidiophores (fig. 45) and in those given by other observers.



Photomicro C. Edge of old colony of *Heterosporium echinulatum* in hanging-drop-culture on salep agar, showing mature conidiophore (× 40).

In the origin and development of the conidia the two *Heterosporium* are very similar to each other. The conidia are produced acropetalously, by a budding process from spores already-formed. The conidiophore-heads of *H. Betae* usually produce three chains of three spores each and sometimes four chains can be observed, while those of *H. echinulatum* usually produce not more than two spored-chains. In the early stages of conidia

formation, however, heads hearing a chain of two spores besides one other spore can be observed. Exceptious, however, occur, and in one case a conidiophore head was seen to bear three spores besides a prolongation of the conidia-bearing-hypha (fig. 27 *b*).

The conidia of both are cylindrical with hemispherical and not oval ends; those of *H. echinulatum* are larger and longer in proportion than those of *H. Betae*; and whereas the conidia of the latter are usually two-celled those of the former are generally four-celled.

III. Infection Experiments.

1. On *Beta vulgaris* with species of *Heterosporium, Alternaria* and *Hormodendron*, originally found upon diseased *Beta* leaves.

For this purpose seeds of *Beta vulgaris* were sown in pots, five seeds to each pot. When the young seedlings had produced two foliage leaves besides the cotyledons, they were placed under bell jars, two pots being placed under each bell jar, and six pots being taken in all. The spores were obtained from petri-dish cultures on salep agar, and in the case of *Alternaria* and *Hormodendron* were brushed from off the conidia-bearing hyphae on to the agar surface by means of a little water and a fine paint brush. In the case of *Heterosporium*, however, owing to the great number of aerial hyphae the spores were obtained by means of a needle and placed in water; in this latter case some agar and hyphae came away with the needle. Another lot of spores was obtained in the same way but with a 5 % glucose solution instead of ordinary tap-water. It was known that the spores could germinate in ordinay tap-water; but that they did not form mycelia beyond a few straggling hyphae; in glucose solution, however, well-developed mycelia were found, and it was thought that these well-nourished mycelia might be able to infect the *Betae* leaves; de Bary[1]) showed this to be possible with the ascospores of *Peziza Sclerotiorum* upon carrots and young seedlings of *Petunia violacea*, *Zinnia elegans* etc. The spores were brushed onto both sides of the foliage leaves with a small paint brush, so that under each bell-jar was one pot with plants inoculated with spores suspended in 5 % glucose solution. In the case of *Heterosporium,* a little mycelium, and pieces of agar were present with the spores; the suspension was not filtered owing to the small number of spores of this fungus. On the 16th October 1911 the following inoculations were made:

Experiment I.

Only three plants in each pot were inoculated, the other two acted as controls.

The plants were examined on the 30th October 1911. All those plants inoculted with spores suspended in water did not seem to be infected. In one or two cases a little mycelium was removed by a needle

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1) de Bary, Botanische Zeitung 1886, p. 396—397. See also Brooks, F. T. Annals of Botany, 1908, p. 482.

from a leaf; but no discolouration was observed upon the leaf itself which seemed quite healthy. All those plants, however, inoculated with spores sown in 5 % glucose solution showed moulds upon their surfaces. One or two spots were noticed which extended from one side of the leaf to the other; this was in the case of *Alternaria*. Many of these mould spots were cut out, some being at once fixed in Juels solution, to be later on examined to ascertain if the fungus had penetrated the plant tissues; the rest were examined at once. In the latter case the mycelia could be removed like a sheet from the surface of the leaf. The mycelia of *Heterosporium* had produced numerous spores. No penetration of the *Beta* leaves could be made out in any case.

Experiment II.

On 31st October 1911 a further inoculation was made. Young *Beta* plants consisting of four or five foliage leaves were inoculated in the same manner as in Experiment I with spores of *Heterosporium* sp. and of *Hormodendron* sp. The spores were suspended in (1) tap-water, and (2) 5 % glucose solution. In some cases the leaves were wounded with needles in other cases not. Eight pots were employed.

Each pot as before contained five plants; one plant in each pot was inoculated with a suspension of spores in water, and one plant with a suspension of spores in 5 % glucose solution. The remaining three plant in each pot were not treated in any way.

In one pot the inoculated plants were first of all wounded by means of a needle. There were thus four pots in all; in two pots two plants were inoculated with *Heterosporium*; in one of the two pots the leaves were wounded, in the other not. One of the plants in each of the two pots was inoculated with spores suspended in water, spores suspended in 5 % glucose solution were used in the other. In the other two pots the plants were treated in the same way but were inoculated with *Hormodendron.*

Upon those plants inoculated with spores in water no fungus growth appeared at all; but upon those which were treated with spores suspended in glucose solution, one or two mould spots did make their appearance. In these cases the fungi were growing upon small portions of killed tissue at the edge of the wound. No penetration of healthy tissue was found; and it was concluded that in these few cases the fungi were growing as saprophytes upon tissue killed as the result of the wounding operation, and nourished by the remaining glucose.

Experiment III.

On 16th January 1912 *Beta*-plants were again inoculated with spores of the *Heterosporium*-species originally found upon *Beta.* The plants used were those of the former experiment; they now consisted of from six to seven leaves about three to four inches in length, not counting the petiole.

One plant was chosen in one of the pots and three of its leaves were wounded, the mid-rib being torn across by means of a needle, the rest of the leaf was not touched. This plant was perfectly healthy.

From another pot three plants were selected which were yellow and wilted. The spores were taken from a petri-dish culture on salep agar and placed upon the leaves by means of a small paint brush and a little water. In the case of the plant with the severed mid-ribs, both the leaf blade and the cut portions were inoculated:

1 plant, 3 leaves wounded,
3 plants in low state of vitality.

The plants were kept under bell-jars during the whole time.

On 20th January the 3 plants were found to have little growths of mould upon their surfaces. These growths upon examination proved to be *Heterosporium*. The growths were found upon parts of the plants which were evidently dead, the discoloration being dark brown; upon the yellow and wilted portions no fungus growth made its appearance. Subsequent microscopical investigation upon the wounded and inoculated leaves showed that the spores had indeed germinated (see fig. 46), and had produced small mycelia which ramified over dead tissue at the edge of the torn mid-rib. In one or two instances the hyphae had entered the stomata of this dead tissue.

Good preparations were obtained by treating portions cut out of the leaves and including the torn place, with lactophenol and staining in Bleu coton G4B [1]). The cells of the healthy tissue took up the blue stain, but those of the dead tissue did not, and remained almost transparent, while the hyphae of the fungus were stained a deep blue. By this means it was easy to trace the course of the hyphae which appeared as blue threads upon a transparent area of dead tissue surrounded by healthy tissue stained light blue. In no case were hyphae observed among the cells of the healthy tissue. It was concluded from this that the *Heterosporium* under the conditions of the experiment behaved purely as a saprophyte.

Experiment IV.

In March 1912 it was decided to try inoculation experiments with the *Heterosporium* upon starved *Beta* plants. For this purpose plants were selected from a number planted during the previous autumn which were now a fair size, the leaves having attained a length of six or seven inches. Ten plants were taken and replanted into two pots in sand and were kept under a bell-jar for three weeks without water. At the end of this time there was no visible difference between these plants and those growing in earth and not kept under bell-jars. On examination it was found, however, that the leaves of the starved plants were thinner and more delicate than those kept under normal conditions. The two pots were then left over night in a warm room; the following morning the plants were found to be completely wilted, all the leaves had become flaccid, and were hanging over the edges of the pots. After standing in the cold house for a few days they partially recovered, the youngest leaves became once more turgescent, while the older and outer ones remained more or less wilted, but were by no means dried up. On 19th

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1) See Klebahn (3), p. 23.

April 1912 these lower leaves were inoculated with spores taken from two damp-chamber cultures of salep agar and dispersed in tap-water. Owing to the fact that the *Heterosporium* sp. formed very poor growths at this time on culture media, only a comparatively small number of spores were attainable for inoculation purposes. The plants were kept under bell-jars. One pot was taken in which the plants were growing in sand, the other pot contained healthy plants growing in earth.

On May 1st 1912 these plants were examined, but no Heterosporium was found. These experiments seemed to show that *Heterosporium* sp. and *Hormodendron* sp. behaved as saprophytes, and *Alternaria* sp., perhaps, as a weak parasite.

2. Infection experiments on *Dianthus Caryophyllus* with *H. echinulatum*.

Experiment V.

On 19th December 1911 two small plants of *Dianthus* („Queen Louise") in separate pots, and under separate bell-jars were inoculated with spores of *Heterosporium echinulatum* taken from a pure culture on salep agar. The spores were dispersed in tap water, and were applied to the upper surface of certain leaves by means of a small paint brush. On the lower leaves of these plants the upper surface was easily wetted when touched by the brush charged with the water containing the spores; but it was found impossible to leave drops of water upon any of the young upper leaves unless the upper surface had previously been rubbed over. In this experiment, however only the lower wettable leaves were inoculated. The leaves so treated were marked with little loops of copper wire so placed that they indicated the place where the brush had been. Six leaves on one plant, and seven on the other were inoculated in this manner.

On December 20th 1911 the inoculated areas of two leaves were cut out and killed in acetic alcohol; this process was repeated upon the 21st, 22nd and 23rd December. The four lots of two pieces each were washed in alcohol, and finally imbedded in paraffin in the usual way with a view to subsequent microscopial examination of the germination and development of the germ tubes, and the penetration of the host tissues.

On January 3rd the plants were examined; they had been kept under bell jars the whole time in order to imitate as far as possible the conditions under which the carnations are grown during the winter months in the nurseries. Both plants were found to be covered in places with moulds of various kinds, notably a species of Botrytis. One plant was so rotted that upon being touched it fell into two pieces close to the earth. No trace of *H. echinulatum* was observed. One leaf which had been inoculated was cut off from the more healthy of the two plants, and placed in a test tube with a little water in the bottom in order that the enclosed atmosphere might be kept moist; the tube was finally corked up. On January 15th this leaf showed a typical disease spot with two concentric rings of conidiophores upon the inoculated area. It was observed that the greatest amount of aerial hyphae was upon the lower side of the leaf. Microscopical examination showed that this spot was indeed due to *H. echinulatum*, spores and aerial hyphae being present in great quantity

Thus the inoculation of the 19th December had been effective in this case, and the time between the inoculation and the appearance of spore-producing organs was something less than twenty-eight days.

The imbedded material fixed upon the 22nd December was sectioned, and stained with safranin and gentian violet and with gentian violet and orange G.; but neither germinating spores nor mycelium were visible anywhere. From this it was suspected that the fungus conidia took longer to germinate, and further, to penetrate the host tissues than they did when growing upon nutrient media in pure cultures.

Experiment VI.

On January 17th 1912 two larger and stronger *Dianthus* plants in separate pots were placed under one large bell-jar and inoculated with spores taken from a petri-dish-culture on salep agar. This culture had been made upon the 4th December 1911, so that the spores were about six weeks old. The leaves chosen were chiefly the lower ones upon those parts of the plants facing the observer; but one or two upper ones were also inoculated. These latter were young and small, and it was with considerable difficulty that a drop of water containing spores was lodged upon their surfaces. The leaves were marked with two parallel ink lines drawn across the leaf at right angles to the mid-rib.

These lines marked the places and boundaries of the inoculations. The spores were applied in the same manner as before with a small paint brush. Several leaves were treated in this way, care being taken that only sound healthy leaves were used. The plants were kept under bell jars.

On February 9th 1912 the plants were examined. Upon many of the inoculated leaves small grey spots from 1—1,5 mm in diametre were seen. Some of these spots were cut out, and examined under the microscope; from others sections were cut which were killed and stained; the stains used being Bleu coton G 4 B, and the combination Fuchsin and Lichtgrün. These preparations showed mycelium to be present, and spores of *H. echinulatum*. Other pieces were also cut out, and fixed in dilute Flemming solution under the air pump for the purpose of imbedding in paraffin, and obtaining microtome sections.

On the 12th February the plants were again examined. Definite spots could clearly be seen upon both old and new leaves. The spots possessed a diametre of from 1—3 mm, and were of a greyish green colour with a central portion of olive-green composed of bundles of conidia-bearing hyphae of *H. echinulatum*. The small spots, those namely of from 1—2 mm in dianietre showed very small central portions which appeared as mere dots to the naked eye; the larger spots of 3 mm diametre showed centre spots of 1—2 mm diametre. As the grey spots were first seen upon the 9th February, the fungus had taken twenty-three days from inoculation to reappearance; that is 23 days had elapsed from the sowing of spores to the time when spores were produced from the mycelium developed from the infecting spores. (Schluß folgt.)