

# On the Life-history of *Macrosporium parasiticum*, Thüm.

BY

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With Plates I. and II.  
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*MACROSPORIUM* is found among a scanty list of the form-genera, whose affinity to certain Ascomycetes has been proved with different degrees of certainty. It is to the labours of Tulasne, Gibelli and Griffini, Bauke, and Kohl, that we owe to a great extent our present knowledge of its relation to the genus *Pleospora*, and also of the rest of the phases of its development, conflicting and unsettled in many important points as these may still be.

Early in the beginning of this year, a specimen of *Macrosporium parasiticum* on onion-plants from Bermuda was kindly placed in my hands for the study of its life-history by Prof. W. G. Farlow, under whose directions the present work has been done.

The Bermuda specimens which I have examined were so far advanced in decomposition that the course of the *mycelium* of the *Macrosporium* in its relation to the internal tissues of the leaves was not clearly definable. The mycelium, which was found in nearly every part of the leaves, sent out through stomata, and sometimes through the ruptured epidermis, small tufts of fertile hyphae. The number of the hyphae in each tuft varied with the size of the aperture, through which they protruded. In the case of the stomata, three to five seemed to be the common number (Fig. 1). These fertile hyphae were simple or occasionally branched, septate and smooth. Their length ranged from fifty to more than a hundred micro-

[Annals of Botany, Vol. III. No. IX. February 1889.]

millimeters. The average length, however, at which the first crop of the conidial spores was formed, was about seventy-five. The diameter of the hyphae at their central portion was five to six micromillimeters. They were generally somewhat swollen at their bases. The swelling was more constant and striking at their free ends, where the spores were borne (Fig. 1 *c*). These spore-bearing cells were always deeper brown in colour than the rest of the hyphae, and their walls were greatly thickened all around their lateral sides in the form of a band. But the wall of their terminal portions remained always thinner in texture and lighter in colour. It was often observed, therefore, that in some of the older hyphae which had ceased to grow after having shed their spores, their terminal walls collapsed and gave to the cells a characteristic cup-shaped form (Fig. 1 *e*). In those which were probably more favourably situated, and abundantly supplied with nourishment, a new growth of hyphae was seen to have taken place from the very spot where the spore had once been borne (Fig. 1 *d*). But a far more common form to be met with in the Bermuda specimens was one where a new growth took its origin, not from the swollen cell, but from the cell next below (Fig. 3). This new hypha grew right through the middle of the former, piercing the wall at its tip, and stopped in growth in most of the cases when two or three septa had been formed. At its free end a new spore was produced. This process could be seen to be repeated several times on a single hypha.

It was not uncommon to see a branch formed also on the upper part of the swollen cell. But in general the branches arose from any of the cells of the primary fertile hypha. The place of their formation on a cell appeared not to be constantly fixed. In some it was produced just below a septum, while in others at about a middle portion of the cell. The branch was usually short, and was given off at an obtuse angle; and on its end a spore was formed.

The spores varied greatly in form and size. In form, they ranged from oblong-obovate to depressed-rotundate, always rounded at both ends. They were furnished with three prin-

cial transverse septa and a longitudinal one, usually with further subdivisions by oblique, transverse or longitudinal partitions, making them into five-, six-, or at times seven-septate muriform spores. Their wall was slightly constricted at the principal septa. It was covered to a greater or less extent all over the surface with very minute projections. This roughness of the spore was quite characteristic; though very rarely one might meet with a spore which looked perfectly smooth. The spore measured 33–43 by 18–23 m.m., and the average size was about 37 by 21 m.m. (Figs. 1 f and 2).

On the Bermuda specimens, besides the *Macrosporium*-spores, a large number of young perithecia were observed. Among numerous sections made on different portions of the leaves, I obtained only once the unripened resting-spores of *Peronospora Schleideniana*. The *Macrosporium*-spores were abundantly seen around the perithecia, with which it was proved that they have organic connection. The perithecia were still too young for the satisfactory determination of species. With regard to our *Macrosporium*, it has been clearly proved to be identical with *Macrosporium parasiticum*, Thümen, by careful comparison with authentic specimens. So far as I am aware, nothing has been published on the life-history of Thümen's species<sup>1</sup>. With a view to determine it as completely as possible, the following cultures were conducted.

The different culture-methods were followed according to the nature of the results to be attained. For observing the germination of the spores under the microscope, the formation of the perithecia, and the rest of the earlier stages of the development of the plant, the Van Tieghem cell was employed. For the study of its further development, the Erlenmeyer flasks proved to be most useful. For nutrient fluids, thin decoctions of onion, of date, of grape, and of horse-dung were used. The onion and date gave the most satisfactory results. Both fluids and apparatus had been carefully sterilised before any sowing was done.

Up to the end of March, fifteen cultures in the Van Tieghem

<sup>1</sup> [See Appendix to this paper. Ed.]

cells and twelve in the flasks were made. Except four failures with the former, the rest of the cultures were successful. Since that time, two more cell- and eight more flask-cultures have been prepared. But they were all poorer in growth, and produced few or no perithecia on the mycelium. By way of comparison, several open cultures on slides and watch-glasses were also prepared.

A large number of young onion-plants were started both from seeds and bulbs. The spores were sown on different parts of the leaves; and the pots were kept moist under bell-jars, with the exception of a few which were left uncovered. The greater part of the young seedlings were badly injured by nematoid worms; but those which survived did not show any sign of the attack. Out of the twelve bulbs, the culture on only two was successful. The spores, however, grew in both cases only on the sheath of the leaves, and not on the active green portions. One of them produced the *Macrosporium*-spores in small quantity, while the other formed in addition a large number of perithecia. These perithecia were arrested in growth when some of them were large enough to form paraphyses in their interior.

The spores sown in pure water germinated within eight hours. The germ-tubes were as a general rule produced from the cells situated on the convex portions of the spores. The number of the tubes from each spore varied a great deal according to its size. In a fully-grown spore, three to six tubes were most commonly seen. These tubes or hyphae grew rapidly in length, but produced lateral branches rather sparingly. The hyphae were colourless and septate, and were filled with refractive contents. The branches were slender and anastomosed readily with each other or with the main hyphae, when they happened to meet. As the hyphae grew in length, many of the cells lost the larger part of their contents, which were carried from one portion to another, until they settled in a certain part of the hyphae. Towards the end of the main hypha a very slender transparent branch was usually observed. The extremity of this branch regularly formed a closely coiled

spiral (Fig. 4). The portion of the hypha adjoining the spore increased in diameter, forming a row of roundish cells for a short distance.

Besides these colourless vegetative hyphae, which grew in the fluid, some of the spores produced directly on their surface short stout filaments of a brownish colour which grew into the air. In cell-cultures, four days after the sowing, secondary spores were observed on their ends. They were then still small, ovate or oblong in form, and one to three septate; and their wall was smooth and light brown in colour. In some way or other their further growth was arrested. The spore-bearing hyphae corresponded very closely in their characters and proportion to those of the Bermuda specimens already described.

In open cultures with pure water the secondary spores were also produced abundantly. On the fifth day they were found to have grown to about one-half the size of mother-spores, and they continued to grow until they were ripe. They showed all the characteristics of the *Macrosporium*-spores. Only in a few cases have I been able to see the spore-bearing filaments produced on the hyphae proceeding from the spores sown. There were no signs of the formation of perithecia in any of the water-cultures.

On the other hand, the spores which were sown in a nutrient fluid presented a widely different result. The main hyphae coming directly out of the spores were vigorous and grew radially at the rate of about 0.6 mm. daily for the first day or two, the rate of growth increasing at quite a rapid ratio until about the fifth day of the culture, when it gradually decreased. On the third day, the average growth during twenty-four hours was 1.8 mm.; on the fifth day, 2.5 mm.; and on the sixth day, about 1.5 mm. They gave out lateral branches in large number, which, interlacing and anastomosing with each other, formed at the end of two days a mycelium of about 3 mm. in diameter. The main hyphae were easily distinguished by their larger size and straighter course, and also by their being copiously filled with fatty globules and

glycogen, as ascertained by the iodine test. Their wall soon assumed a light yellowish brown colour.

On these main hyphae, and very rarely on their larger branches, some of the earliest perithecia were formed during the second and third days of the culture. The first sign of their formation was the division of a certain portion of the hypha into a row of short cells. One or usually two or more of these cells began to swell considerably, and each of them sent out one or more hyphal branches, before any division took place in them (Fig. 7). The branches were at first very slender and hyaline; and they grew very rapidly, anastomosing with remarkable readiness with each other, and with any other hypha which happened to lie in their course.

While the branches were thus growing, the initial cell or cells continued to swell and divide, first into two by a transverse or oblique septum, and then into four or more, and so on. There seemed indeed to be no regular directions in the cell-divisions. The resulting tissue was parenchymatous in structure, and the cells composing it were so closely united as to leave scarcely any intercellular space between them. The basal portions of the hyphal branches mentioned above began at the same time to swell and divide (Fig. 8). The groups of cells thus formed, coalescing with the central tissues, gave to perithecia at this stage most irregular forms with several projections (Fig. 9). But as they went on growing and dividing, they sent out from the newly formed outside cells an additional number of similar branches. Their basal parts again by growth and division contributed new groups of cells to the growing central mass; and thus the young perithecia gradually assumed a definite globular shape. At the beginning of the culture, all the changes and growth took place within twenty-four hours in a vigorously growing plant; while in those perithecia formed later on, when the nourishment had become somewhat scarce, it took about three or more days for a similar amount of growth.

On the fourth or fifth day after the sowing, the outside layer of cells of a young perithecium began to assume a dark

colour. The colour was at first deeper on those portions most exposed to light. They were then easily recognisable with the naked eye as small black spots on the mycelium.

While the perithecia were thus being formed, many other important changes were also taking place on the growing mycelium. Besides the growth of the mycelium along the surface of the fluid, a large number of hyphae were sent out both upwards into the air, and downwards into the liquid substratum. Those that were sent down into the fluid were finer than those on which the perithecia were formed. They remained colourless for a long time. In some cases they presented a peculiar undulating appearance, which reminded one of the rhizoids of a *Marchantia*. Generally they formed a loose fringe of a light colour, hanging down into the fluid. But in some of the flask-cultures, where an excessive growth of hyphae took place, they formed a thickly interlaced felt of dark brown colour, with many young perithecia entirely immersed at first in its tissue just under its upper surface.

The aerial hyphae were generally observed two days after the sowing on the central or older part of the mycelium, as short white filaments. They were at first more or less erect, but as they grew on, they began to trail, forming a cobwebby veil over the surface. On these hyphae were produced short branches, which were given out perpendicularly, and which bore on their tips the *Macrosporium*-spores. These fertile hyphae were also formed on the mycelium as well as on some of the perithecia. The aerial hyphae began to assume a light brownish colour at about the time when the spores were forming.

The earlier *Macrosporium*-spores were observed on the third day of a culture. On the fifth day they were ripe, and even produced on their surface the secondary spores having all the characteristics of the spores themselves.

The fertile hyphae were similar in size and characters to those of the Bermuda specimens, with a few minor variations caused by the difference in substrata. They were produced in the present case, not in tufts, but scattered singly on the

hyphae; and there were no swellings to be seen at their bases.

The spores were formed as a general rule from the terminal portions of the fertile hyphae by abstriction. The cell, from which both the spore and swollen spore-bearing cell (basidium) were to be formed, was at first tapering towards its free end (Fig. 5 *a*). The abstriction took place usually at a point a little above its middle part, without being at the same time accompanied by the formation of a partition. The lower half attained its full size while the spore-portion was yet small. The latter was at first ovate in form, colourless, and smooth (Fig. 5 *c*). The roughening and darkening of its cell-wall began to take place when two or three cross-partitions were formed in the spore. In the course of a few days the spore ripened and presented its characteristic muriform shape. The connection between the spore and basidium was very slight. As a consequence, the ripened spore fell off at the least disturbance.

In closed cultures, however, where the plants were kept undisturbed, the ripened spores remained on the hyphae for a long time. It was very rare to see the new spores produced at the ends of the newly extended hyphae proceeding from the tips of the old ones, as commonly observed in the Bermuda specimens. Instead of this process, the new crops of spores were produced here mostly as secondary spores on the surface of the ripened spores. They were produced at the ends of short hyphal branches, which were given out perpendicularly from different parts of the spores. Some of the spores produced three, four, or even more secondary spores on their surface at the same time. On these secondary spores another crop of similar spores was rarely observed while the whole arrangement was still borne on the tip of the hypha.

Another mode of spore-formation, which was far less common than that described above, was one where a new spore was produced on the tip of a fertile hypha by the side of an old spore, which was shifted a little to one side. Here the abstriction of the sprouting hypha took place very close to the surface of the swollen basidium, resembling somewhat the



budding of a yeast-plant. The spore, when it was ripe, could not be distinguished from the older one by its side (Fig. 6).

This 'budding' process may be more common than I am aware in the spore-formation of this plant. I have observed several instances which strongly suggested this process; but, as I did not observe their earliest stages, I cannot state here positively its occurrence beyond the case just described.

Concerning the further development of the perithecia, it will be convenient to take a plant about a week old and follow its development. The mycelium was then blackish in colour, and full of young perithecia and *Macrosporium*-spores in different stages. The oldest perithecia were about 0.2 mm. in diameter. They were generally globular in shape. Their internal structure was still undifferentiated, being composed of cells of similar shape and size, and equally filled with glycogen and fatty matters.

The growth and division of the cells continued to take place in all parts of the perithecium. The cells towards the outside began to grow more rapidly in size than those in the central part, where the cells remained almost at their original size, as they multiplied by constant growth and division. A large number of the cells in the central portion then became markedly filled with very refractive contents. Their number and position in the perithecium were variable, and could not be stated definitely. As to the position, sometimes they were found in the upper portion, sometimes in the lower, but commonly near the centre of the perithecium. These cells soon began to elongate and divide mostly in an upward direction, forming a body of short irregular chains of cells, which might be either simple or branching (Figs. 10 *d* and 11 *b*). From the tip of each of these chains of cells, one, two, or rarely three slender hyphal branches were sent out (Fig. 11, *B*, *C*). The branches or *paraphyses* thus formed in large number, at first crossed each other to a greater or less extent. They, however, soon took a definite upward course in a closely packed bundle towards air and light. The larger part of the parenchymatous cells lying above in the way of these rapidly-

growing paraphyses seemed to be dissolved away, their places being occupied at once by the latter.

This internal change was accompanied by the general growth of the perithecium. The growth was especially active in the upper portion, which was prolonged into a short blunt beak. The bundle of paraphyses grew into the beak, forming a comparatively broad canal, and ceased to grow when it reached the external layer of the obtuse tip, on which usually more than one short papilla was found.

The paraphyses were filiform, septate, and simple, or very rarely branched. They were then copiously filled with glycogen and fatty matters. The perithecia ceased to grow in size when the paraphyses in their interior had reached their full development. All subsequent changes in the interior attending the growth of asci produced little or no effect on the external configuration of the perithecium.

About two weeks after the sowing, a large number of asci began to grow among the rows of paraphyses. The asci were formed as branches on some of those cells from which the paraphyses had sprung (Figs. 10 *c* and 12 *A*). They were at first somewhat club-shaped, and were full of colourless granular protoplasm free from glycogen. The growth of the asci generally began to take place when the bundle of paraphyses had reached its full size. I have observed a few cases, however, in which the asci had grown to about the length of the paraphyses, when the latter were still at about two-thirds of their growth.

In an ascus which had attained the length of about 70  $\mu$ m., that is about one-half its full size, a globular nucleus was observed in its upper portion (Fig. 13 *A*). In one which had progressed a little further, eight spore-primordia made their appearance. When they were surrounded by a cell-wall, they were first divided into two by a transverse septum at the middle, then into four by two septa parallel to the first one (Fig. 12 *B*). At this stage, the spores were colourless and spindle-shaped; and the ascus was still copiously filled with granular colourless protoplasm. Before any further division

in the spores took place, they began to grow noticeably in width, and to assume a yellowish colour (Fig. 12 C). At about this stage, in some of the spores, a longitudinal partition was formed for the first time.

It took just about a month from the beginning of the formation of a perithecium to the full ripening of its ascospores. A large number of the asci, which were often very closely packed in the cavity of the perithecium, exerted a considerable pressure on the parenchymatous cells along its side, causing them to flatten, and also on the paraphyses between them. The paraphyses lost a considerable part of their contents. Their outline became indistinct, and in some places their cell-wall became completely mucilaginous. The same changes took place in the basal cells, from which both paraphyses and asci were formed. The refractive contents, which had once filled these cells, must have been used up in the formation and growth of the asci and ascospores.

The matured asci were cylindrical-oblong in shape, tapering at one end into short curved pedicels, which were slightly dilated at the point of attachment. Their size ranged from 120 to 160 m.m. in length, and from 25 to 30 m.m. in width. The spores were arranged mostly in two ranks, but towards the base of the ascus they were frequently one-ranked. The spores, even the well-matured, were enveloped in a thin layer of protoplasm, which united the whole into a group. The spores were elliptical or oblong, obtuse at both ends, and 7-septate, with two or three longitudinal partitions at the middle portion, and one or two towards both ends. They were constricted at about the middle. The upper portions were always larger than the lower. They were yellowish-brown in colour, and in size from 30 to 33 m.m. in length, and 12 to 15 m.m. in width (Fig. 13).

In typical cases, the fully matured perithecia were slightly depressed globular in form, with short obtusely-conical beaks, and with flattened bases. Those perithecia which were formed crowded together on a mycelium had longer and narrower forms, with prominent beaks. It was not rare to

see two or more perithecia completely coalesced into one irregular body. The size of the perithecia varied a great deal; but commonly it was between 300 and 450 micromillimeters in diameter.

When a ripened ascus was placed free in water, it began in a few minutes to elongate a little with a corresponding slight diminution in diameter. The internal tension continually increasing by the rapid absorption of water caused finally the rupture of the outer layer of the wall, possessed of a limited power of extensibility, at the apex of the ascus. Relieved of the external resistance, the inner layer elongated in a short time (5–10 seconds) to about two and a-half times the original length of the ascus, carrying with it the spores and protoplasmic envelopes. In none of a large number of the free asci observed under water was there any ejection of the spores from the tips of the elongated tubes. Every one of them germinated in the asci just as they were grouped, sending the hyphae through the delicate wall of the tubes.

Two matured perithecia were placed in a moist chamber, and kept overnight. The next morning it was found that a large number of the spores had been ejected, some to the distance of seven millimeters. But the greater part of the spores dropped near the ostioles. They were scattered, and not in groups of eight. During the night, every one of them had germinated. It is quite probable that the spores in the present case might have been ejected successively from the tips of the elongated asci, which forced their way through the very narrow orifices of the papillae, after the manner of some of the allied plants, as *Sphaeria Lemnaceae*.

The ascospores when sown in nutrient fluids germinated of and grew vigorously, just in the same manner as in the case the *Macrosporium*-spores. In every sowing of the ascospores, only the perithecia and *Macrosporium*-spores were formed on their hyphae. Pycnidia and other forms of conidia, generally attributed to *Pleospora*, have not been observed in any of my closed cultures.

Before determining the specific position of our plant, it may

not be out of place here to state concisely the results of my examination of Thümen's original specimens of *Macrosporium parasiticum*<sup>1</sup>. They are not so much advanced in growth as the Bermuda specimens; still the spores are well matured. Their size and shape correspond very closely with those of the latter. They have also the characteristic minute projections over their surface, though this characteristic is not mentioned in his description. The number of transverse septa is usually five to seven. I have not been able to find a spore which has so many as ten septa. The fertile hyphae correspond exactly in all essential characters in both specimens (Fig. 16). It is beyond doubt that the Bermuda *Macrosporium* is identical with the European form described by Thümen.

From the preceding account of the development of the *Macrosporium parasiticum* it will be clearly seen that its ascosporic stage is a species of *Pleospora*; and furthermore, that it corresponds so closely in every essential character to the descriptions and figures of *Pleospora herbarum* (Pers.), Rabenh., given by Berlese<sup>2</sup> in his recent monograph, and also to those by Tulasne<sup>3</sup>, von Niessl<sup>4</sup>, and Winter<sup>5</sup>, as to leave little doubt in regard to their identity. The comparison with the authentic specimens of *Pleospora herbarum* in the European exsiccati further confirmed the point in question<sup>6</sup>.

<sup>1</sup> F. de Thümen, Mycotheca Universalis, Cent. vii. n. 667, Klosterneuburg, 1887. Accompanied by the following description:—'Maculas atras formans; hyphis abbreviatis breviarticulatis, ramosis, ramis brevibus, griseo-fuscis; conidiis oblongo-ovoideis vel ovoideo-rotundatis vel clavatis, 6-10 septatis, utrinque obtusis, 42-48 × 10-16, fuscis. Hab. in foliis vivis vel languidis *Allii Cepae*, praecique in *Peronospora Schleideniana* parasitans. Bayreuth Bavariae.'

<sup>2</sup> A. N. Berlese, Monografia dei generi *Pleospora*, *Clathrospora* e *Pyrenophora*, in Nuovo Giornale Bot. Ital. vol. xx. 1888, No. 1, p. 91, tav. v. f. 2-6.

<sup>3</sup> L. R. et C. Tulasne, Selecta Fungorum Carpologia, tom. ii. 1863, p. 261, tab. xxxii-xxxiii, Fig. 10-14.

<sup>4</sup> G. von Niessl, Notizen ueber neue u. krit. Pyrenomyceten, p. 29, tab. iv, Fig. 14, 1876.

<sup>5</sup> G. Winter, Rabenh. Kryptog. Flora, Bnd. I. 1885. Pilze, ii. p. 504, Fig. on p. 408.

<sup>6</sup> Some of the specimens on the onion-plants were carefully examined and compared. One published by Cesati and de Notaris under the name of *Pleospora Allii* in Herb. Critt. Ital. ser. ii. fasc. xiii. n. 644, was mostly too young. I saw one or two asci, whose spores were somewhat advanced towards maturity. In some of

The forms of *Pleospora herbarum* growing on onions were once considered by Rabenhorst, Saccardo, and many other authors as a distinct species from, or a variety of, that common fungus. They are, however, at present included under that species without any restriction by the authors who have studied the genus critically, as Berlese, Winter, von Niessl, and some others. The examination of some of the authentic specimens, and also observations on the development of the plant, induce me strongly to accept the latter view.

It has been well established that *Macrosporium Sarcinula* of Berkeley has a genetic connection with *Pleospora herbarum*. Is then *Macrosporium parasiticum*, Thüm., identical with *M. Sarcinula*, or is it another form of the conidial stages of this remarkable plant? Unfortunately, I have not been able to examine the original specimens of Berkeley's species. From his descriptions and figures<sup>1</sup> of the plant, I found it rather hard to make a very satisfactory comparison with our plant. But according to the descriptions and figures of the same plant by Tulasne<sup>2</sup>, there exists such a striking resemblance between these two species of *Macrosporium*, that any one unprejudiced would at once accept them as of one and the same species. Their identity is further confirmed by the fact, that both can produce on their mycelium perithecia which could not be distinguished one from the other.

The only apparent difference that still remains between the *Sarcinula* and *Macrosporium*-forms is their habitat. The former has generally been considered to be entirely saprophytic; while it has been proved that the latter not only thrives on dead vegetable matters, but can also grow on the living plant

these spores the partitions were not fully formed. Still, they were sufficiently grown to exhibit the characteristics of the species (Fig. 15).

*Sphaeria herbarum*, Pers., on *Allium Cepa* in Wartmann and Schenk, Schweiz. Krypt. n. 322, was in a far better condition. It coincides in every respect with my plant (Fig. 14).

<sup>1</sup> M. J. Berkeley, Notices of British Fungi, Ann. Nat. Hist. vol. i. No. 4, 1838, p. 261; No. 125, Pl. viii. f. 10.

<sup>2</sup> Tulasne, l.c., p. 263, tab. xxxii. Fig. 6.

accompanying a stronger parasite, as *Peronospora Schleideniana*, or by attacking a plant on its less active tissue, as the sheath of the onion-plant. A statement was however made by De Bary<sup>1</sup> on the possibility of species of *Pleospora* being classed among facultative parasites. In fact, observations in regard to the parasitic nature of *Pleospora herbarum* are not wanting. Spegazzini<sup>2</sup> has found this fungus on the living leaves of grape-vine and *Medicago sativa*; Cugini<sup>3</sup> and Passerini<sup>4</sup> on living branches of mulberry-trees; Berlese<sup>5</sup>, on branches of *Sambucus nigra*; and Linde<sup>6</sup>, on roots of clover. These observations however few in number, supported by our own cases on onion-plants, give us sufficient ground to consider *Pleospora herbarum* as a facultative parasite.

It is now convenient to briefly state some of the main points we have arrived at so far. It has been proved that the ascosporous stage of *Macrosporium parasiticum* of Thümen is the common *Pleospora herbarum*, and that the so-called *M. parasiticum* itself is nothing more than *M. Sarcinula* of Berkeley growing on onion-plants.

One seldom meets with a plant whose life-history has been beset with so much confusion, and about which so many controversies have been left unsettled for a long time, as *Pleospora herbarum*, the plant we have just been considering. The first important work on the subject was by Tulasne in 1863<sup>7</sup>. He found in this plant a remarkable illustration of his theory of pleomorphism. He included under the name of *Pleospora herbarum* a perithecial, a pycnidial, and four conidial forms. Among the latter he distinguished, 1st, the *conidia dematiea*,

<sup>1</sup> De Bary, Vergl. Morphol. u. Biol. d. Pilze, p. 409, Leipzig, 1884; Eng. trans. p. 380.

<sup>2</sup> C. Spegazzini, Ampelomiceti italici: Funghi parassiti al grappolo, p. 726, 1878.

<sup>3</sup> G. Cugini, Intorno ad alcune malattie comparse nel 1884 su varie piante coltivate, in L'Agricoltura italiana; an x. Firenze, 1884, Nos. 120, 121.

<sup>4</sup> G. Passerini, Ancora della nebbia o nuova malattia dei gelsi e di alcuni altri alberi, in Bolletino d. Comizio agrar. parmense, Parma, 1884.

<sup>5</sup> Berlese, l. c. p. 99.

<sup>6</sup> S. Linde, Ueber Kleemüdigkeit des Bodens. Leipzig, 1880.

<sup>7</sup> Tulasne, l. c.

which correspond to *Cladosporium herbarum*, Lk.; 2nd, the conidia *didyma*; 3rd, those which correspond to *Macrosporium Sarcinula*, Berk.; and 4th, the *Exosporium conidia* (*Alternaria tenuis*, Nees.)

Fuckel<sup>1</sup>, in 1869, added to the forms of Tulasne *Epicoccum herbarum* as one of the macroconidia of the plant. About the same time Hallier<sup>2</sup> claimed to have proved, by cultivations, that to *Pleospora herbarum* belong not only the six forms of Tulasne, but also a large number of others, namely, *Penicillium grande*, *Rhizopus nigricans*, *Oidium lactis*, an *Aspergillus*, a *Mucor*, *Stachylidium*, *Fumago*, *Micrococcus*, etc.

In 1873 Gibelli and Griffini<sup>3</sup> undertook to prove by means of a large number of careful cultures, in closed chambers, the assertions of the preceding authors. They came to the conclusions that *Pleospora herbarum* of Tulasne is to be divided into two distinct species, one constantly producing the *Sarcinula*-conidia (in which they included the second and third conidial forms of Tulasne) and the larger ascospores named by them *Pleospora Sarcinulae*; and the other, always the *Alternaria*-conidia, accompanied by the perithecia having smaller and less-septate ascospores, called *Pleospora Alternariae*. They obtained pycnidia only twice in the cultures of the ascospores which produced the *Sarcinula*-conidia. The pycnidia thus obtained were shown to be distinct from *Phoma herbarum*; and their pycnospores when sown constantly reproduced the pycnidia and nothing else. *Cladosporium herbarum*, *Epicoccum*, and Hallier's forms, they proved to have no genetic relations at all with either of their species of *Pleospora*.

They made also some observations on the development of

<sup>1</sup> L. Fuckel, *Symbolae mycologicae*, p. 130, 1869.

<sup>2</sup> C. Hallier, *Untersuchungen des pflanz. Organismus, welcher die, unter d. namen Gattine bekannte Krankheit der Leidenraupen erzeugt*. Potsdam, 1868. Die Muscardine des Kieferspinners, in *Zeitschrift für die Parasitenkunde*, Bnd. i. p. 18.

<sup>3</sup> G. Gibelli e L. Griffini, Sul polimorfismo della *Pleospora herbarum*, Tul., in *Archivio Triennale del laboratorio di botanica crittogamica in Pavia*, vol. i. pp. 53-102, tav. v-ix. 1874.



the perithecia of *Pleospora Sarcinulae*<sup>1</sup>. These arose, according to the authors, from two cells or sometimes from a single cell of the moniliform hyphae. The cells swelled up and divided, division taking place in various ways, so that they finally formed spherical cellular bodies. In one case a short lateral hypha incurved over the two initial cells was observed, which also played a part in their further development<sup>2</sup>. They were in doubt whether there was really anything like a fecundation of oogonia by the action of a pollinodium in this case.

In 1876, while Bauke<sup>3</sup> was engaged in the study of pycnidia of Sphaeriaceae, he met a doubtful case in *Pleospora herbarum*, which caused him to make a very large number of cultures. Thus he was led to study the other phases of its life-history at the same time. The preliminary communication<sup>4</sup> of the results he obtained was published in the year following. It is to be regretted that he left the work unfinished.

The conclusions he had arrived at were much nearer to Tulasne's than to Gibelli and Griffini's in regard to the question of pleomorphism. He included under *Pleospora herbarum*, besides an ascosporic stage, a pycnidial, *Sarcinula*-, *Alternaria*-, and microconidial forms. The last form, according to him, has hitherto been overlooked in consequence of the minuteness of its size.

He had some doubts about his pycnidia. He obtained them only twice in a very large number of the sowings of the ascospores. Though he failed to observe the direct organic connection between the latter and the pycnidia, he was convinced of the possibility of their connection by their peculiar forms, which at once distinguished them from all other pycnidia he knew of, and further by the fact that, by sowing the pycnosporos, he obtained, besides the similar

<sup>1</sup> l. c. p. 82.

<sup>2</sup> l. c. tav. vii. Figs. 8-10.

<sup>3</sup> H. Bauke, Beiträge zur Kenntniss der Pycniden, i, in Nova Acta, Bnd. xxxviii. No. 5, p. 443. Taf. 28-33. Dresden, 1876.

<sup>4</sup> H. Bauke, Zur Entwicklungsgeschichte der Ascomyceten. Vorläufige Mittheilung, in Bot. Ztg. 1877, p. 313.

pycnidia, the characteristic *Alternaria*-spores. He never observed the pycnidia accompanied by the perithecia or *Sarcinula*-spores in the same culture.

He further states, that in the cultures of the ascospores obtained from perithecia growing on the same individual host-plant, or even from one and the same perithecium, some produced always the *Sarcinula*-spores and perithecia; while the others, always the *Alternaria*-spores alone, or, in two cases only, with the pycnidia also. The microconidial form finally appeared regularly on both sorts of the cultures. Bauke draws the conclusion from these facts, that mycelia of two different characters belong to this same species. With him the *Alternaria*-spores, when sown constantly, reproduced the *Alternaria* alone; and the *Sarcinula*-spores, regularly the perithecia and the *Sarcinula*.

His account of the formation and development of the perithecia coincides nearly with that given by Gibelli and Griffini. He refuses to consider those hyphae which sometimes happen to fasten on to the primordia of the perithecia as pollinodia. According to him, the formation of the perithecia is entirely apogamic.

He describes further the inner changes of the growing perithecia, which were scarcely touched upon by the Italian authors. In from three to five weeks the formation of paraphyses began to take place. From a number of parenchymatous cells, situated generally near the base of the perithecia in nearly the same plane, a bundle of thickly crowded hyphae or paraphyses sprang out upwards. The tissue situated in the place, which was eventually to be occupied by the growing paraphyses, was gelatinised and absorbed.

The perithecia formed in early spring produced the asci in the same season; but those formed later on in summer usually refused to grow after the formation of paraphyses had begun. In this state they passed the winter as sclerotia. The asci were formed as the branches of the basal cells of paraphyses. The contents of the latter contributed the nourishment for the growth and ripening of the ascospores.

Besides these, he observed regularly on the *Alternaria*-mycelium resting-hyphae of a very simple nature. Sometimes there appeared later in cultures peculiar hyphal bodies, which were dichotomously branched and parallel to one another. These bodies he considered as diseased formations caused by the nutrient fluids.

In 1882 Kohl<sup>1</sup> started a large number of cultures to settle the disputed problem of pleomorphism of the plant in question. Briefly, he came to the following conclusions: (1) that in pure cultures he obtained, by sowing the ascospores, only the *Sarcinula*-spores and the perithecia, and by sowing those *Sarcinula*-spores he obtained constantly a similar result; (2) that the *Alternaria*-spores when sown always produced the *Alternaria*-spores only; (3) that on the mycelium from the pycnosporos the pycnidia and *Alternaria*-spores were formed; and (4) that *Cladosporium herbarum*, Bauke's *Microconidium*, and *Epicoccum* could not be found in the development-cycle of *Pleospora herbarum*.

He adopts the view of the Italian authors in separating the *Sarcinula*- and *Alternaria*-forms into two distinct species.

Berlese, in his recent monograph of the genus *Pleospora* already referred to, places *Pleospora Sarcinulae* of Gibelli and Griffini under *P. herbarum*, and their *P. Alternariae* under *P. infectoria*, Fuckel. They are the two most common species of the genus, and grow on about the same host-plants.

First, in regard to the question of the pleomorphism, the results of my cultures correspond exactly with those of Kohl. As has already been stated, the *Macrosporium*- or *Sarcinula*-spores, when sown in a nutrient fluid, constantly produced the perithecia and the *Sarcinula*-spores; and the ascospores from the perithecia thus obtained always reproduced similar perithecia and *Sarcinula*-spores. I have also failed to obtain pycnidia in any of my cultures of the ascospores.

The accounts of the pycnidia given by Gibelli and Griffini, and also by Bauke, are far from being convincing. The

<sup>1</sup> F. G. Kohl, Ueber den Polymorphismus von *Pleospora herbarum*, Tul., in Bot. Centr., Bd. xviii. 1883, p. 23.

uncertainty of their appearance, and the lack of definite observations in both cases on the direct organic connection between the ascospores sown and the pycnidia obtained, and also the constant failure in the attempt to reproduce the perithecia and *Sarcinula*-spore by sowing the pycnospores, lead one naturally to suspect that perhaps some foreign pycnospores may have been introduced into their cultures. Moreover, according to Kohl<sup>1</sup>, the pycnospores enveloped in gelatinous substance, after oozing out in a vermiform mass, fasten more or less readily to a substratum, and send out into the air short slender hyphae, which terminate with small secondary spores (Luftsporen). They are very readily blown away by wind, or even by a slight breeze. They germinate on a wet substratum with great readiness.

The evidences of our investigations thus far lead us to the conclusions, that the presence of the pycnidia in *Pleospora herbarum* is very doubtful, and that they may have disappeared altogether from its cycle of development.

During the course of the present experiment an *Alternaria*-form repeatedly appeared on the label-papers pasted on the slides with Van Tieghem cells, which were kept moist under a bell-glass. It was often accompanied by *Penicillium* and *Hormodendron*. The same kind of *Alternaria* made its appearance about a year ago in the same laboratory on the decaying juniper-twigs kept for another purpose under a bell-glass. Its size, form, and habit correspond exactly with those of *Alternaria tenuis*, Nees, a form generally associated with *Pleospora herbarum*.

This *Alternaria*-spore was sown in different nutrient fluids. In flask-cultures it produced a mycelium, which grew with great rapidity, forming a thick and close mycelium. On its upper surface a very large number of aerial hyphae were produced, which were white at first, but gradually turned from green to grey, as the spores were formed and matured on them. The whole surface was literally packed with the rank

<sup>1</sup> Kohl, l. c. p. 30.

growth of long and branching chains of the *Alternaria*-spores, and no other forms of reproductive organs were to be found.

In Van Tieghem cells, the mycelium behaved differently from that of the *Sarcinula*-spore. The hyphae were straighter, and slightly smaller in diameter, and they turned quickly to a brownish colour. *Alternaria*-spores were abundantly formed. No signs of the formation of a perithecium or pycnidium were observed during the whole cultures, except in one instance on the mycelium of a half-starved old culture, when a process which might be taken as an attempt at the formation of either pycnidium or perithecium appeared.

I agree with Gibelli and Griffini and with Kohl in discarding *Alternaria tenuis* as a stage of *Pleospora herbarum*.

In regard to the formation and development of the perithecia, my observations coincide in the main with those of Bauke described in his preliminary communication. Like him I have been unable to observe any sexual process connected with the formation of the perithecia, nor have I been successful in finding any trace of the Woronin's hypha in a young perithecium before the formation of paraphyses and asci. The formation of the perithecium is entirely a vegetative process, which resembles essentially the formation of pycnidia. I do not consider the initial cells of the perithecia as degenerated female organs or ascogonia, but as entirely of vegetative origin.

Both in the *Alternaria*- and *Sarcinula*-cultures I have observed the resting-hyphae, which were briefly described by Bauke. In the *Sarcinula*-cultures started in May these bodies were commonly formed on the mycelium floating free on the surface of the culture-fluid. Besides these common forms of the resting hyphae, I observed in the earlier part of the experiment varieties of abnormal hyphae in the Van Tieghem cell-cultures of the spores of *Pleospora herbarum*. As the growth and appearance of these bodies are very remarkable and interesting, I may give a brief description of them before closing this paper.

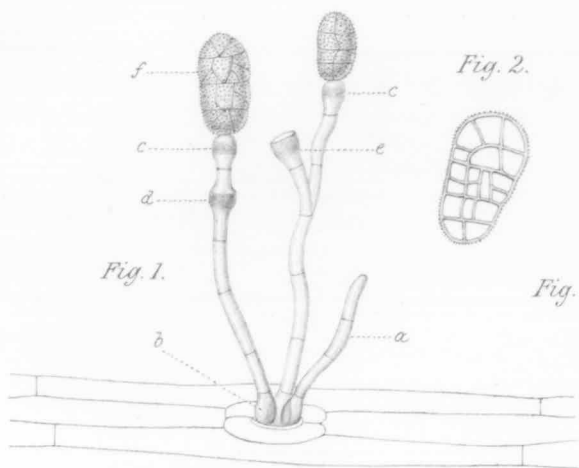
In the *Sarcinula*-cultures, about five days after the sowing, a large number of club-shaped branches, filled with hyaline

highly refractive contents, made their appearance mostly towards the outer edge of the mycelium. These branches were produced close to the under-surface of the cover-glass, from which a drop of the culture-fluid was hanging. Fig. 17 represents one of the typical forms. Two cells at the extremities were still hyaline. Each of them sent out a branch, the lower one touching the upper. Two days after, it presented an unexpected appearance, as shown in Fig. 18. The upper of the two hyaline cells grew and divided, and produced a stout branch almost at a right angle. This branch produced again another branch at about a similar angle. As to the two branches mentioned in the first stage, the upper one did not grow, while the lower grew straight on and anastomosed with another hypha. The cells were soon filled with fatty globules, and ceased to grow. The cell-wall began to assume a brownish colour after some days. The whole arrangement remained unchanged until the end of the culture.

At another part of the same culture a similar process (Fig. 19) was watched. Here apparently two branches from the adjoining cells formed close spirals; and already several branches were formed upon them. During the next twenty-four hours a great change took place. A stout branch made a remarkable growth, and produced (at Fig. 20 *b*) a fast-growing branch, and (at *c*) a spiral process, which is represented highly magnified in Fig. 21. Two days after, two strong branches were found proceeding from the spiral process (Fig. 22). As in the first case, the cells became filled with fatty globules and stopped their growth. Fig. 23 represents one of the simpler forms of these peculiar bodies.

On the hyphae of the ascospores, these abnormal processes were produced more abundantly. But the spiral process was not observed. Here most of the branches swelled at their basal portions and divided into a row of a few large rounded cells. From the tip of the branches, a hyaline slender hypha, which was commonly slightly club-shaped at the end, was produced. The process at this stage resembled most remarkably a trichogyne and archicarp of some simple Floridean.





*Fig. 1.*

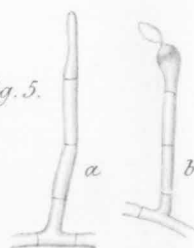
*Fig. 2.*



*Fig. 3.*



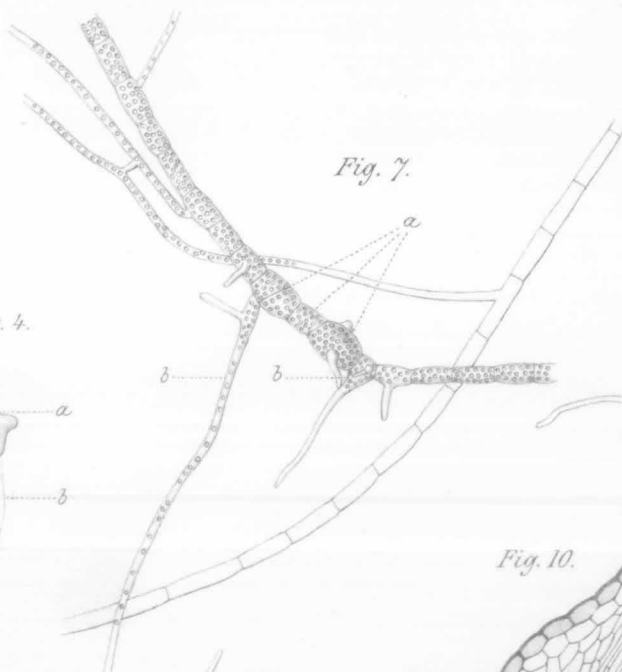
*Fig. 5.*



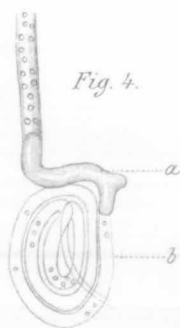
*Fig. 8.*



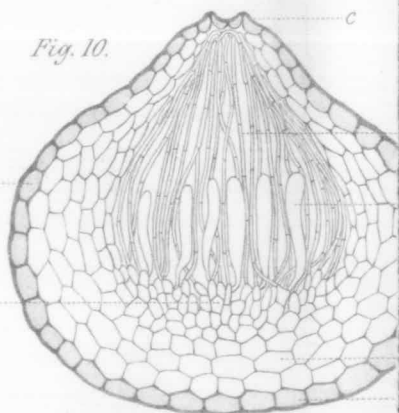
*Fig. 7.*



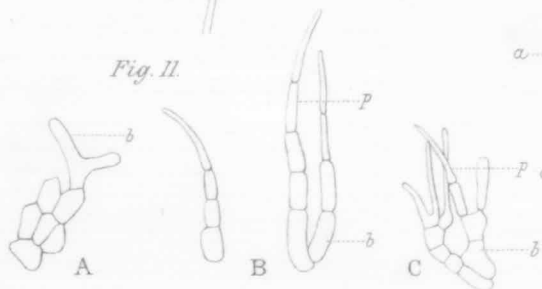
*Fig. 4.*



*Fig. 10.*



*Fig. 11.*



K. Miyabe del.



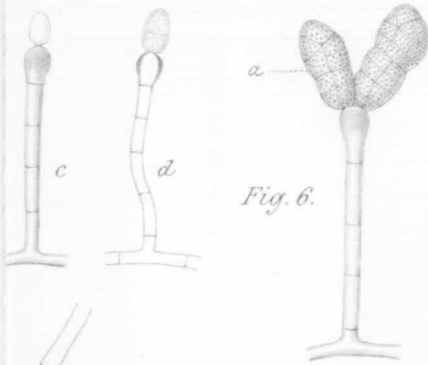


Fig. 6.

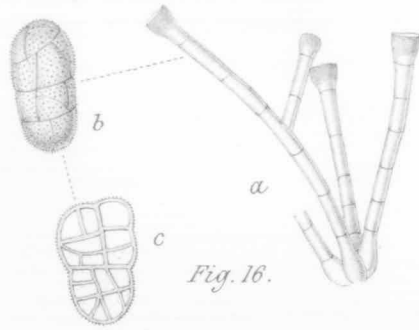


Fig. 16.

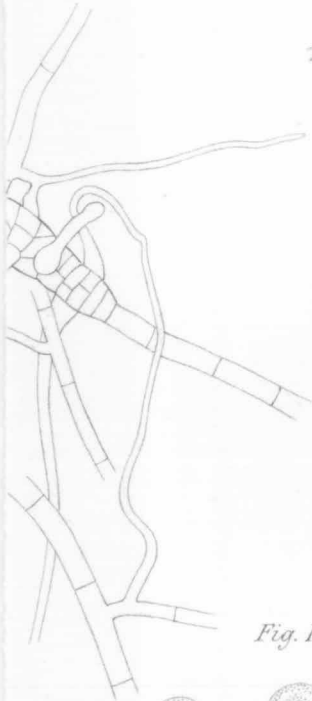


Fig. 12.

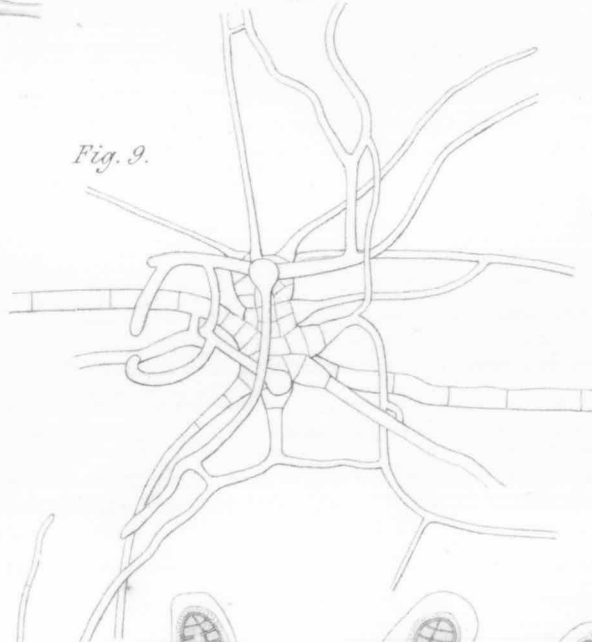


Fig. 9.

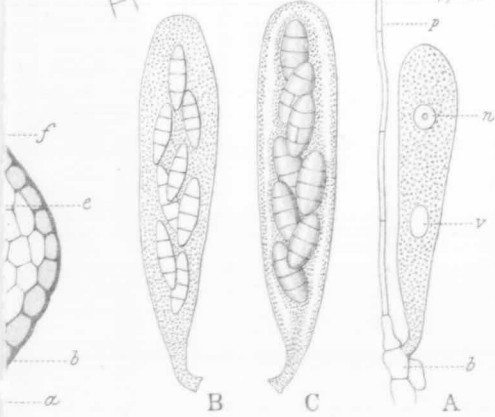


Fig. 13.

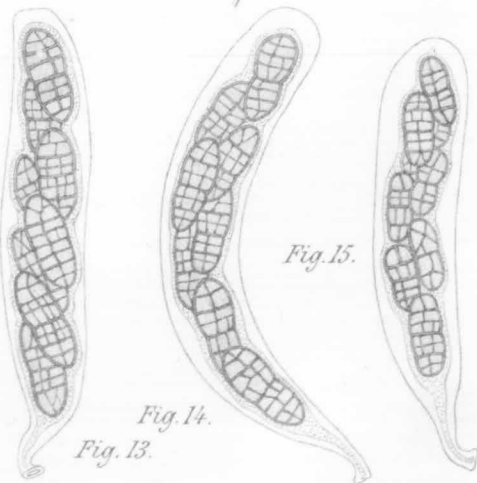


Fig. 14.

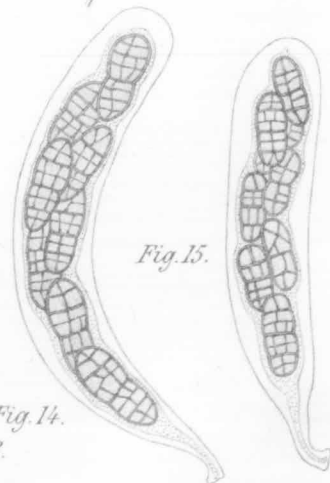


Fig. 15.





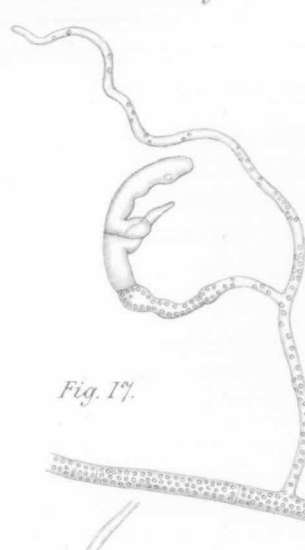


Fig. 17.

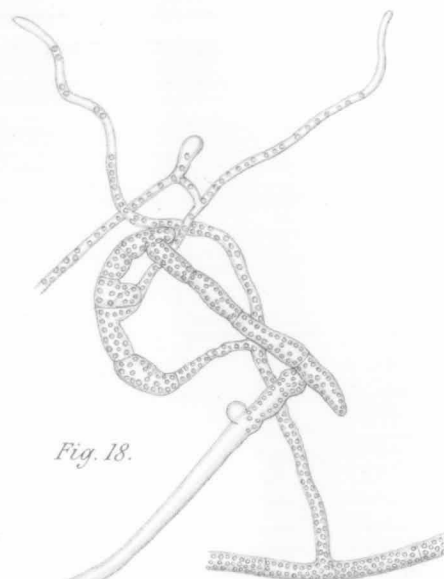


Fig. 18.

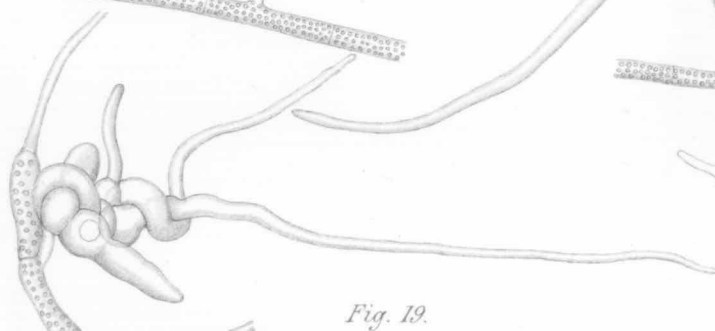


Fig. 19.

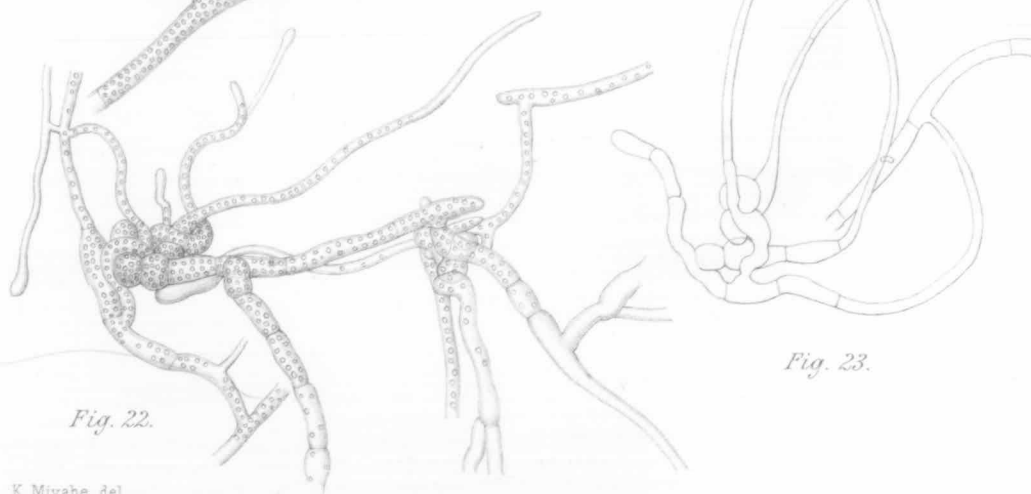


Fig. 22.

K. Miyabe del.



Fig. 23.

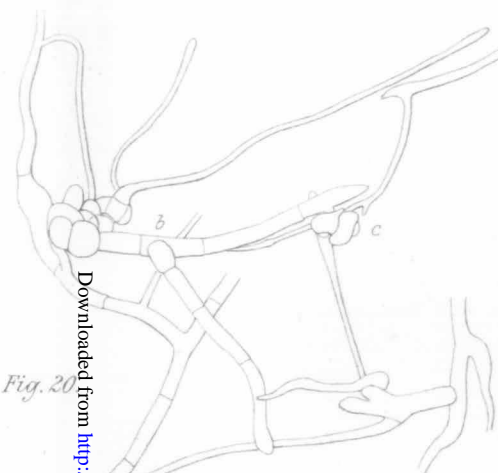


Fig. 20.

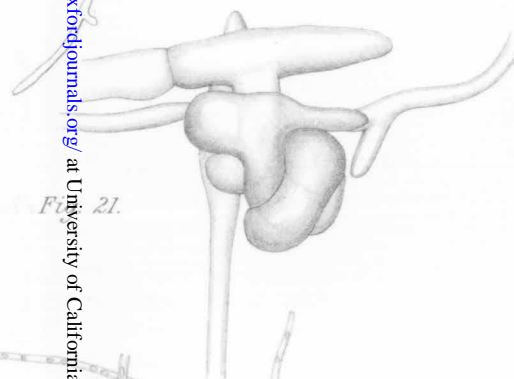


Fig. 21.

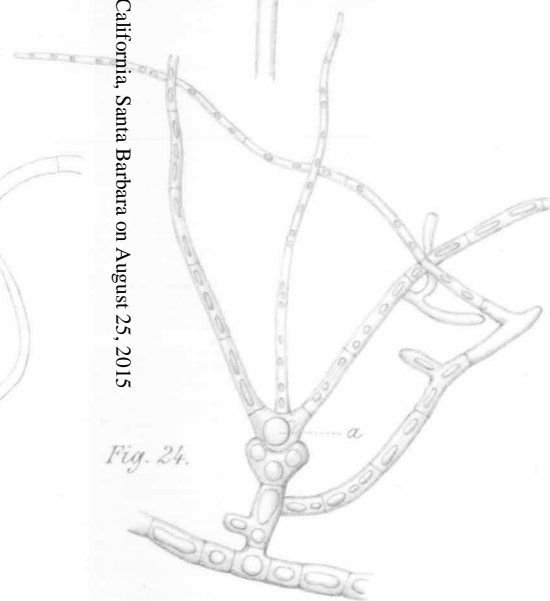


Fig. 24.

University Press, Oxford.



Usually, in a day or two, some branches were sent out from the rounded cells at the base. These branches grew and anastomosed freely with other hyphae. The cells, as in the other cases, were filled with fatty globules, when they ceased to grow, and gradually assumed a brownish colour. Fig. 24 represents one of these trichogyne-like processes in a culture which has been kept for about four months.

It must be noted here, that in all the cultures, in which these abnormal hyphae appeared, an abundance of perithecia were also formed on the main hyphae in the manner already described.

Though there are great differences in appearance and mode of growth between these abnormal hyphae and the hyphal clusters observed by Brefeld<sup>1</sup> on the mycelium of *Peziza tuberosa*, it is certain that they were alike produced under similar stimuli,—the presence of plentiful nourishment, and of a solid impenetrable substratum.

Whatever the function of these abnormal bodies may be under such a circumstance, one cannot help recalling, on seeing these hyphae in a young growing stage, some of the sexual organs represented in other groups of Ascomycetes. So striking is the resemblance between them, that I venture to suggest that under undue stimuli the hyphal branches of this fungus might have produced by reversion traces of their long-lost character, which became useless and disappeared on the acquisition of the power of the purely non-sexual formation of its perithecia.

Recapitulation of the principal results obtained.

1. The ascosporous stage of *Macrosporium parasiticum*, Thüm., is the common *Pleospora herbarum* (Pers.), Rabenh.
2. *Macrosporium parasiticum*, Thüm., is identical with *Macrosporium Sarcinula*, Berk.
3. *Pleospora herbarum* is decidedly a facultative parasite.

<sup>1</sup> O. Brefeld, Untersuch. üb. Schimmelpilze, Heft 4. p. 112. t. ix. Fig. 15 a, b. 1881.

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4. There are only two stages in the development-cycle of *Pleospora herbarum*, the ascosporous stage and the *Sarcinula*-stage.

5. The presence of pycnidia in *P. herbarum* is very doubtful, and they may have entirely disappeared from its cycle of development.

6. An *Alternaria*-form does not belong to *P. herbarum*.

7. The formation of the perithecium is purely non-sexual.

8. No Woronin's hyphae or similar spiral processes are found in the perithecia before the formation of asci and paraphyses. The asci and paraphyses are produced from the same short chains of parenchymatous cells, which are formed by elongation and division of the pre-existing cellular group of parenchymatous nature filled with highly refractive contents, and situated generally in a central portion of the perithecium.

In conclusion, I wish to express my thanks to Prof. W. G. Farlow, who has helped me throughout my work with valuable suggestions, and allowed me also to make a free use of his library and collection.

THE CRYPTOGAMIC LABORATORY OF HARVARD UNIVERSITY,  
CAMBRIDGE, MASS., U.S.A., July 20, 1888.

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EXPLANATION OF FIGURES IN PLATES I AND II.

Illustrating Mr. Kingo Miyabe's paper on the Life-history of *Macrosporium parasiticum*, Thüm.

PLATE I.

Fig. 1. A tuft of fertile hyphae of *Macrosporium parasiticum*, Thüm., on the Bermuda onion-plant, showing the manner of their protrusion through its stomata. *a*, young hypha; *b*, swellings at base; *c*, swollen basidium; *d*, old basidium; *e*, old cup-shaped one; *f*, matured spore. × 400.

Fig. 2. Optical section of a matured spore of the same. × 500.

Fig. 3. An old fertile hypha of the same, showing a series of renewed growths of spore-bearing cells. × 400.

Fig. 4. A spiral formation of a very slender and transparent hyphal branch, formed on the mycelium of *Macrosporium*-spores sown in water. *a*, portion filled with fatty matters; *b*, spiral portion.  $\times 800$ .

Fig. 5. Process of formation of the spores by abstriction. *a*, young fertile hypha before abstriction; *b*, after abstriction, basidium-portion growing; *d*, the spore dividing, and the wall of the basidium considerably thickened  $\times 400$ .

Fig. 6. Twin-spores, the spore *a* formed later.  $\times 400$ .

Fig. 7. Youngest stage of a perithecium formed on the mycelium of the *Macrosporium*-spore. *a*, initial cells; *b*, hyphal branches.  $\times 500$ .

Fig. 8. A similar perithecium more advanced.  $\times 500$ .

Fig. 9. A similar one still more advanced.  $\times 500$ .

Fig. 10. Diagrammatic longitudinal section of the perithecium. *a*, darkened outer layer of cells; *b*, closely united parenchymatous wall; *c*, papilla; *d*, basal cells of asci and paraphyses; *e*, young asci; *f*, paraphyses.

Fig. 11. Young paraphyses, *p*, and their basal cells, *b*. *A*, young basal cell, forked.  $\times 500$ .

Fig. 12. *A*, a young ascus; *n*, nucleus; *v*, vacuole; *p*, paraphyses; *b*, basal cells, *B*, one with very young spores, still colourless. *C*, somewhat advanced, spores with a light yellowish colour.  $\times 500$ .

Fig. 13. Ripened ascus obtained by sowing the *Macrosporium*-spore.  $\times 440$ .

Fig. 14. Ripened ascus from the specimen of *Sphaeria herbarum*, Pers., in Wartmann and Schenk, Schweiz. Krypt. n. 322.  $\times 440$ .

Fig. 15. Unripened ascus from the specimen of *Pleospora Allii*, Ces. et de Not. in Herb. Critt. Ital., Ser. II. n. 644.  $\times 440$ .

Fig. 16. Fertile hyphae, *a*, and conidial spores, *b* and *c*, of *Macrosporium parasiticum*, Thüm., from the authentic specimens in Myc. Univ. n. 667.  $\times 400$ .

## PLATE II.

Abnormal hyphae formed on the mycelium of *Pleospora herbarum*, in Van Tieghem cell-cultures. Figs. 17-23 obtained by sowing *Sarcinula*-spores.

Fig. 17. One drawn on Feb. 14, 4.25 P.M.  $\times 500$ .

Fig. 18. The same on Feb. 16, 3.30 P.M.  $\times 500$ .

Fig. 19. A similar process in the same culture, Feb. 15, 10 A.M.  $\times 800$ .

Fig. 20. The same on Feb. 16, 10 A.M. At *c*, another spiral-process was formed.  $\times 500$ .

Fig. 21. The spiral-process *c*, of Fig. 20, highly magnified.

Fig. 22. The same arrangement on Feb. 18, 12 M. From the spiral *c*, two stout branches were formed.  $\times 500$ .

Fig. 23. One of simpler nature.  $\times 500$ .

Fig. 24. A trichogyne-like process formed on the mycelium of the ascospores of *Pleospora herbarum*, drawn after having been kept for about four months in the culture. *a*, fatty globules.  $\times 500$ .



## APPENDIX.

The material studied by Mr. Miyabe was sent to me by the Rev. George Tucker of Smith's Parish, Bermuda. I visited Bermuda in January 1882, and at that time the onions were free from disease; but in 1886 I received a letter from Mr. Tucker, saying that a serious disease had attacked the onions, and he forwarded some diseased plants soon afterwards. I had expected that I should find either *Peronospora Schleideniana* or the *Urocystis* common on onions in the United States; but to my surprise no trace of either was seen. I noticed there was an abundance of *Macrosporium parasiticum*, Thümen. Early the following year more material was sent by Mr. Tucker, and, although a very large number of specimens was examined, with the rare exception mentioned by Mr. Miyabe, there was no trace of the *Peronospora*, a species readily recognised by its conidia, oospores, and characteristic mycelium. As in the material of the preceding year, the diseased plants were covered with *Macrosporium*. As *Peronospora Schleideniana* certainly does produce a serious disease of onions in Bermuda, as has been shown by Mr. Arthur E. Shipley, who visited Bermuda in 1887 for the purpose of studying the subject<sup>1</sup>; and as, with a rare exception, all of the numerous specimens of diseased onions from Bermuda which I had examined with great care showed no trace of *Peronospora*,—the question naturally arose whether the *Macrosporium* was merely a fungus which had attacked plants previously suffering from *Peronospora*, as most botanists would suppose, or whether it might not of itself cause a disease of onions. It was for the purpose of settling this point, if possible, that Mr. Miyabe, at my suggestion, undertook his investigation from which the possibility that *Macrosporium* can grow on the tissues of living plants free from *Peronospora* seems to have been demonstrated. I should here like to express my thanks to Mr. Tucker for the material which he kindly furnished.

W. G. FARLOW.

<sup>1</sup> Kew Bulletin of Miscellaneous Information, No. 10, Oct. 1887; also Proc. Camb. Phil. Society, vol. vi. Part 3 (1887).