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Anschauung, daß das Vorhandensein oder das Fehlen eines Stromas kein Merkmal darstellt, auf Grund dessen die Gattung *Calonectria* in zwei Gattungen zerlegt werden könnte, wie es z. B. SEAVER wirklich getan hat. Die *C. sulcata* STARB. tritt gewöhnlich mit deutlichem, polsterförmigem Stroma auf und wäre somit nach SEAVER in seine Gattung *Scoleconectria*[1]) zu stellen. Ein von SCHIFFNER auf einer faulenden *Anona*-Frucht in São Paulo gesammeltes, von v. HÖHNEL[2]) studiertes Exemplar zeigt aber Perithecien, die einzeln und ohne Stroma auf dem Substrate auftreten und die unstreitig denselben Pilz darstellen, welcher Pilz aber jetzt nach SEAVER als *Calonectria* bezeichnet werden müßte. Es zeigt sich also hier ganz dasselbe wie in der Gattung *Nectria*, daß die Ausbildung des Stromas je nach den Lebensbedingungen wechselt und somit nicht als Merkmal zur Gruppenbildung herangezogen werden kann.

Aus diesem Grunde lasse ich auch die Gattungen *Creonectria* SEAV.[3]) und *Puttemansia* P. HENN.[4]) (= *Scoleconectria* SEAVER nach v. HÖHNEL) nicht gelten. Der Typus der Gattung *Puttemansia* P. HENN., *P. lanosa* P. HENN. hat daher *Calonectria lanosa* (P. HENN.) WEESE zu heißen.

Puttemansia könnte höchstens für die behaarten *Calonectria*-Arten aufrecht erhalten werden. Doch nach einer solchen Gattung ist auch kein zwingendes Bedürfnis vorhanden. *Trichonectria* KIRSCHSTEIN[5]) (1906) fällt auch mit genannter Gattung zusammen, wenn auch bei diesem Pilz kein Stroma ausgebildet ist.

Zum Schluß danke ich meinem hochverehrten Chef Herrn Hofrat Prof. Dr. v. HÖHNEL herzlichst für die Überlassung seines reichhaltigen Herbariums für meine Studienzwecke.

Life history of a new species of *Sphaerella*.

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(With 2 textfigures.)

While studying *Cylindrosporium* on species of *Prunus* during the summer of 1912, leaves of *Prunus pennsylvanica* were found parasitized by a fungus which was at first thought to be a *Cylindrosporium*. The spores were elongated and slender, resembling those of this genus very much. A short study, however, brought to light some characteristic differences.

The effect on the host was very different. The leaf tissue was killed outright, and by the time the spore bearing fruit bodies appeared the spots had a scalded appearance. The spots were irregularly round

1) SEAVER in Mycologia 1909, p. 197.
2) v. HÖHNEL in Denkschr. d. Kais. Acad. d. Wissensch., Wien 1907, **58** (Erg. d. Bot. Exped. Südbrasilien).
3) SEAVER l. c. p. 183.
4) P. HENNINGS in Hedwigia 1902, p. 112; SACCARDO, Syll. Fung. **18**, p. 98.
5) W. KIRSCHSTEIN in Verhandl. d. Bot. Ver. d. Prov. Brandenb. 1906, **48**, p. 60.

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to elliptical, two to five millimeters in diameter, and often coalescing until the entire leaf was killed and dropped from the tree. Each spot bore many fruit bodies in contrast to the solitary fruit body in *Cylindrosporium*. "Shot hole" was often produced in early summer. When this occurred a separation layer formed outside the dead area and the spot dropped out.

When sectioned the fruit bodies were found to have a distinct pycnidial wall with a rather large pore at the apex. They were small, globose to elliptical, and entirely embedded in the tissue of the leaf barely breaking the epidermis on either surface, but most commonly on the upper surface.

These observations left little doubt of the relation of the fungus to the form genus *Septoria*. Since, however, some confusion has arisen as to the limits of *Septoria* and *Cylindrosporium*[1]) a comparison of its development and life history with that of *Cylindrosporium* was thought desirable.

For the purpose of studying the fruit bodies a number of the spots which were beginning to assume the scalded appearance were killed, and embedded in paraffin. When sectioned and stained this material showed pycnidia in many primary stages of development.

The development of the pycnidium is very similar to that described for *Diplodia* by Bauke[2]) and called by Zopf[3]) "Knäuelfrucht." It usually begins just beneath the epidermis, more rarely within the epidermal cell or even deep in the palisade layer. A mycelial thread becomes profusely branched, the branches forming a small, round, and rather compact knot (figs. 3, 4). The knot enlarges rapidly and pushes down into the deeper leaf tissues. At the same time the branches which make up the knot coalesce and form an oval or globose hollow structure which is open at the top. At maturity the wall of the pycnidium is pseudoparenchymatous but very delicate. The conidiophores arise as short branches from the inner surface of the wall. The development is therefore "angiocarpous" while that of *Cylindrosporium* is "gymnocarpous".

Another important character in which this fungus differs from *Cylindrosporium* is found in the relation of the mycelium to the host tissue. Instead of being intercellular as in that genus, it passes directly through the host cells. When the end of a thread comes in contact with a host cell a small hole is apparently dissolved through the wall and the thread becoming much constricted passes through and enlarges again on the other side (fig. 1).

The diseased trees were growing in a small thicket made up almost entirely of this species with a few small trees of *Prunus serotina*, which, however, were not affected by the fungus. The infection became very severe toward the end of summer, so that there were very few leaves

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1) A very similar if not idential fungus on leaves of *Prunus pennsylvanica* was distributed in Fungi Columbiani (Nr. 3316) as *Cylindrosporium Padi-cerasina* Peck n. v. So far as I have been able to find, however, no description has ever been published. The pycnidia and spores as well as the effects on the leaf tissue are very similar to those of the fungus under consideration.

2) Bauke, H., Beiträge zur Kenntnis der Pycniden, 1 (Nova Acta, K. Leop. Carol. Deutschen Akad. Naturf. **38**, with 6 plates; Dresden 1876). See Just's Jahresb. **4**, p. 175, 176, 1878.

3) Zopf, W., Die Pilze, p. 60, 1890.

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without several dead spots and they began to turn yellow and drop off during August.

On Sept. 4th some of these fallen leaves contained small black bodies in the spots which had been killed by the *Septoria*. Ten days later these spots were densely dotted with these bodies which were conspicuous because of their black color on the yellow or yellowish brown leaf tissue. Spots on many of the yellowed leaves which were still on the trees also showed these bodies.

Freehand sections showed two kinds of structures. One had the appearance of a small stroma and the other that of a pycnidium containing very small rod-like spores. These last were thought at the time to belong to a saprophytic *Phyllosticta*, and because of the rush of other work were not studied carefully at that time. Some of the leaf tissue containing these structures was killed on Sept. 4th, Sept. 14th, and again on Dec. 18th and embedded in paraffin for further study.

When this material was sectioned and stained, the small spores were found very often forming in the same pycnidium where the typical *Septoria* spores had been produced, which indicated that they were genetically connected with the *Septoria*. They are formed on slender sterigma-like projections arising from the large deeply staining cells which line the pycnidium at this time. These cells often grow out in chain like branches which reach entirely across the pycnidial cavity.

The stroma-like bodies mentioned above were found to be young perithecia. The wall of the perithecium develops in manner very similar to that described for the pycnidium; but they form deeper in the tissue of the leaf, usually in the palisade layer or sometimes in the mesophyll on the underside of the leaf. A dense hyphal knot arises by the branching of one or more hyphae. These branches soon coalesce and form the pseudoparenchymatous wall of the perithecium. The neck of the perithecium is finally formed by coalescence of the more or less spirally wound ends of these branches.

Very early in the formation of the young perithecium from the hyphal knot, a deeply staining central branch is differentiated. The base of this hypha is enlarged and once or twice coiled, and the free end of the coil extends as a slender trichogyne-like structure. It reaches to the ostiolum but has not been seen projecting beyond. These structures are very similar to the carpogonial structures found by Frank [1]) in *Gnomonia erythrostoma* and by Fisch [2]) in *Polystigma rubrum*.

The small pycnospores, from their size, mode, and time of formation are probably homologous with what has been termed spermatia. No evidence that they function as male sexual organs has so far been found. They have never been found attached to the trichogyne-like structure. They are, however, often found around the mouth of the perithecium, sometimes in clusters.

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1) Frank, B., Über *Gnomonia erythrostoma*, die Ursache einer jetzt herrschenden Blattkrankheit der Süßkirschen im Altenlande, nebst Bemerkungen über Infection bei blattbewohnenden *Ascomyceten* der Bäume überhaupt (Ber. D. Bot. Ges. **4**, p. 200—205, 1886).

2) Fisch, C., Beiträge zur Entwicklungsgeschichte einiger *Ascomyceten* (Bot. Ztg. **40**, p. 851—870, 875—897, 899—906, pls. 10, 11, 1882).

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The trichogyne and coiled portion of the carpogonial structure soon disintegrate but a part of the coiled basal portion was found in material killed December 18th.

On January 24th more of the material was examined when acsogenous hyphae had aeready formed in the base of the perithecium, but no sections showing the origin of these hyphae have been obtained. The asci are soon formed, but the spores do not mature until about the first of May. In some leaves brought into the laboratory and placed in a moist chamber on December 12th mature asci and ascospores were found the last of January; and in leaves brought in January 24th they had matured after one week in the moist chamber.

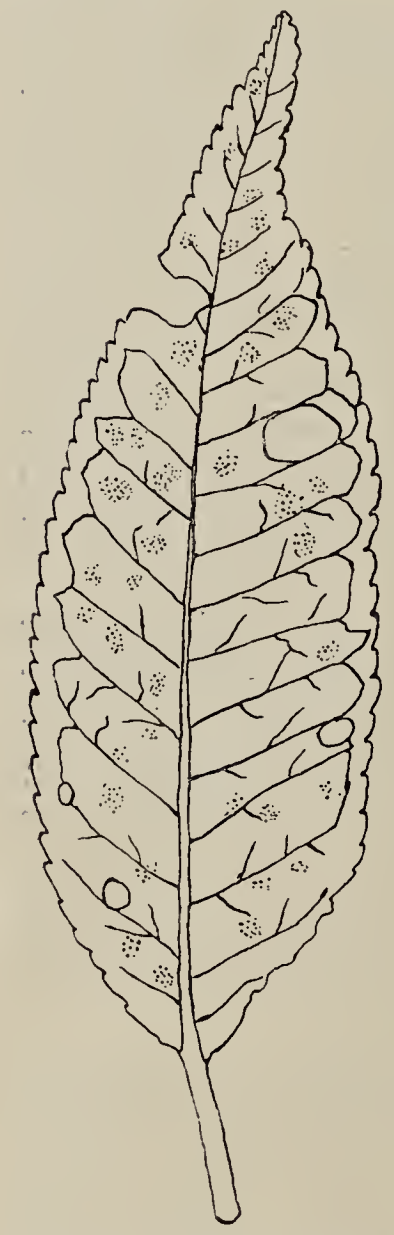

Fig. 1. Fallen leaf of *Prunus pennsylvanica* showing approximately the grouping of perithecia on spots infected during previous summer. (Natural size.)

The asci are eight spored, cylindrical, crowded, without paraphyses, and tend to cling together in a cluster when crushed out of the perithecium. The spores are two celled, colorless and fusiform.

Pure Cultures.

Both pycnospores and ascospores grow readily in nutrient agar; and pure cultures from both sources have been obtained and studied.

The germinating pycnospores were transferred from agar plates to steamed bean pods. The mycelial growth is rather slow and is at first pure white. Very soon, however, some of the hyphae next the bean pods turn dark and also dark colored pycnidia are formed. The mycelium is partly within the tissue of the bean pod, and the pycnidia usually begin their development within the tissue also. Pycnospores similar to those formed on the cherry leaves were found in great abundance. Often the whole colony is covered by waxy appearing masses of spores which have exuded from the pycnidia.

As the culture becomes older "spermogonia" appear bearing rod like "spermatia" indistinguishable from those found on the yellowed cherry leaves in the fall. Also what appears to be abnormal perithecia are formed. These are pseudoparenchymatous structures which apparently begin their formation in a manner similar to the development of perithecia on cherry leaves, but no asci were ever found in them. The walls of the "spermogonia" were also abnormally thick but bore normal appearing „spermatia" in great abundance.

On steamed cherry leaves the mycelium was almost entirely within the leaf tissue. Otherwise the growth was very similar to that on the bean pods.

The ascospores also germinate and grow very readily on nutrient agar, so that asci containing germinating ascopores were readily found and separated from the contaminations. At first long slender conidia (fig. 13) somewhat larger than normal pycnospores were abstricted one after the

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other from the ends of short branches. This conidia formation resembled very closely that found in cultures of certain species of *Fusarium*.

The production of this type of spore soon ceased and the further development is very similar to that described for the cultures of the imperfect stage.

Fig. 2.
1 Mycelium within the cells of the host leaf, showing constrictions where passing through walls of host cells. — *2* A later stage, showing the partial disintegration of cell walls. — *3* and *4* Early stages in the formation of a pycnidium. — *5* Mature pycnidium. — *6* Spermogonium containing spermatia. — *7* Very early stage in the formation of perithecium. — *8* A later stage, showing carpogonical branch surrounded by anastomosing branches which form the perithecial wall. — *9* Section of a perithecium at a still later stage showing ascogenous hyphae arising from the cell in the base of the perithecium. — *10* Mature perithecium with asci and spores in various stages of development. — *11* Mature ascus, showing break in the outer sheath of ascus wall. — *12* Mature ascus, showing broken and collapsed outer sheath and expanded inner membrane of ascus. — *13* Conidiophores and conidia from agar culture of ascospores, seven days old.

Inoculations.

A small plant of *Prunus pennsylvanica*, which had been brought into the green house and planted in a pot, was inoculated by placing drops of water containing pycnospores from an ascosporic culture on the underside of its leaves. This plant with another used as a check were covered with bell jars.

The first inoculation had failed to produce infection at the end of two weeks, so the same plant was used again. This time newly formed pycnospores from an ascosporic culture were used. On the fifth day spots

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began to appear on the inoculated leaves and by the end of eight days nearly every leaf showed one to six spots. Each spot contained numerous pycnidia which bore pycnospores exactly similar in shape, size etc. to those found the previous summer.

To learn the time necessary for infection the check plant was now inoculated with pycnospores from the first plant. Again spots began to appear on the fifth day. This showed clearly that the infections developed from the inoculations rather than from pycnospores which had lodged on the plants during the previous summer; because had such pycnospores been on the plants, they would have produced infections during the first two weeks the plants were under bell jars.

Systematic.

Neither the perfect nor imperfect stage of the fungus so far as I can find has ever been described. Apparently no similar ascomycete has been described on *Prunus pennsylvanica* and the only one described on the genus *Prunus*, whose asci and spores are similar in size, is *Sphaerella cerasella* (Aderh.) Sacc. et Syd.[1]) whose imperfect stage is *Cercospora cerasella* Sacc. according to Aderhold[2]).

The presence of a *Septoria* stage and the carpogonical structures suggested a near relation to *Gnomonia erythrostoma* Pers., but a comparison of the fungus with descriptions of this genus show at once that they are distinct. They differ in shape and size of perithecia, in form of asci, and in their effect on the host. The affected leaves drop prematurely instead of hanging on the tree all winter as in *Gnomonia erythrostoma*. The shape of the perithecium with the very short beak, and the absence of a pore and thickened apex in the ascus would place the fungus in *Sphaerella* rather than *Gnomonia*. Since the fungus agrees with the genus *Sphaerella* Ces. et de Not. it is placed in this genus; and I propose the name *Sphaerella nigerristigma* with the following diagnosis.

Diagnosis.

Mycosphaerella[3]) *nigerristigma* n. sp. Perithecia black, amphigenous but mostly epiphyllous, aggregated on spots killed by pycnidial mycelium or scattered over the entire leaf, ovate, 90—110 × 45—85 μ, immersed with the short beak protruding through the leaf epidermis; asci cylindrical, clustered, aparaphysate, without a pore in the apex, 35—45 × 7 μ, spores fusiform colorless, once septate 16—21 × 2,5—3 μ,

Pycnidial stage: Spots at first glaucous but soon turning brown, often dropping out, 2—5 mm in diameter; pycnidia colorless or light brown, amphigenous, immersed, globose, with a single large apical pore;

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1) Saccardo, Syll. Fung. **16**, p. 469, 1902.

2) Aderhold, R., *Mycosphaerella cerasella* n. spec., die Perithecienform von *Cercospora cerasella* Sacc. und ihre Entwicklung (Ber. D. Bot. Ges. **18**, p. 246—249, 1900).

3) The technical description bears the genus name *Mycosphaerella* Johanson (Svamper fråm Island; Ofversigt af Konigl. Vetensk. Akad. Vöhandl., p. 163, 1884) which is used by Schroeter (Crypt.-Fl. Schlesien **32**, p. 332, 1897), by Lindau (Engler u. Prantl, Natürl. Pflanzenf. **1**, p. 423, 1897), and by many others, though it is desirable that *Sphaerella* Ces. et de Not. may be one of the genera conservanda adopted by the Botanical Congress in London, 1915.

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pycnospores colorless, long, slender, flexuous, 35—56 × 2—3 μ, continuous or 1—4 septate. "Spermatia" produced in "spermogonia" or in the pycnidia in late autumn, 4—5 × 1 μ.

Pycnidial stage parasitic in leaves of *Prunus pennsylvanica*, ascogenous stage saprophytic in leaves of the same host.

Peritheciis amphigenis, gregariis vel sparsis, nigris, immersis, punctiformibus, ovatis, 90—110 × 45—85 μ; ostiolis prominulis; ascis cylindricis, aparaphysatis, 35—45 × 7 μ, octosporis; sporidiis, hyalinis, fusoidiis, 16—21 × 2,5—3 μ, 1 septatis.

Hab. in foliis dejectis *Pruni pennsylvanicae*.

Status conidicus: maculis primum glaucis dein brunneis, 2—5 mm lat., interdum dejectis; pycnidiis amphigenis, immersis, globosis; sporidiis hyalinis, filiformibus, flexuosis, 35—36 × 2—3 μ, continuis aut 1—4 septatis; spermatiis autumno in spermogoniis.

Hab. in foliis vivis *Pruni pennsylvanicae*.

Summary.

A new disease of *Prunus pennsylvanica* caused by the pycnidial stage of a previously undescribed *Sphaerella* is described. The perithecia begin to develop early in the fall often before leaf fall. A single carpogonical (?) branch is found in each young perithecium; and at the same time "spermatia" are produced in pycnidia-like "spermogonia".

The trichogyne-like part of the carpogonial structure disintegrates leaving only a basal cell. Whether or not this basal cell functions as an ascogonium is not known; but in the spring ascogenous hyphae arise from a cell which occupies a position in the base of the perithecium similar to that occupied by this basal cell.

The connection between the pycnidial and ascogenous stages is shown by similarity of cultures from the pycnospores and the ascospores as well as by infection experiments with pure cultures from the ascogenous stage.

It is hoped that sufficient material for a more detailed study of the perithecial development may be obtained in the near future.

The study on which this article is based was carried on in the Botanical laboratory of Cornell University under the direction of Professor George F. Atkinson, whom I take pleasure in thanking for careful consideration and criticism of the work.

Mycologische Notizen über Awamori-Koji-Pilze *(Aspergillus)* und *Rhizopus Delemar*.

Von **K. Usami**, Fukuoka (Japan).

(Mit 8 Figuren.)

Über genannte Pilze liegen schon verschiedene neuere Untersuchungen vor, meine Mitteilung bezweckt lediglich einige Beobachtungen wiederzugeben, die ich schon vor längerer Zeit machte, aus äußeren

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