LEAFSPOT OF MAIZE CAUSED BY OPHIOBOLUS HETEROSTROPHUS, N. SP., THE ASCIGEROUS STAGE OF A HELMINTHOSPORIUM EXHIBITING BIPOLAR GERMINATION[1]

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INTRODUCTION

For several years the writer has received for identification occasional lots of graminaceous material attacked or suspected of being attacked by members of the genus Helminthosporium. Among the more noteworthy of these are certain collections of maize (*Zea mays* L.) leaves affected with a foliar disease, which, with the parasite responsible for it, this writer has briefly described in an abstract (*3*)[2] and which is more adequately treated in this paper. While the fungus has evidently not escaped observation by previous writers, it has generally been confused with a congeneric form responsible for leaf blight of maize. Additional interest attaches to the fungus under consideration in that it readily yields an ascigerous stage of a type not hitherto recorded as associated with any species of Helminthosporium. The discovery of this association is presumably not without significance in relation to the taxonomic position of a numerous series of apparently allied forms assigned to the genus, including, for example, the parasites causing spotblotch of barley, eyespot of sugar cane, and leafspot of rice, of which the mode of conidial germination by the production of a polar germ tube from one or both end segments represents a common characteristic.

SYMPTOMS

Liberal collections of diseased maize leaves made by A. C. Foster near Sanford, Fla., on September 22, 1923, and by C. Welles near Los Baños, Philippine Islands, in November, 1921, very largely provided the material upon which the present studies were based. The leaves bore numerous lesions, varying considerably in extent, the smaller and evidently incipient ones scarcely discernible and measuring approximately 0.5 mm. in each direction, the larger up to 15.0 mm. in length and from 1.0 to 3.0 mm. in width (fig. 1, A). In the dried condition the interior of the larger spots approximated, on the whole, the cinnamon-buff of Ridgeway's color chart, although this hue was interrupted by rather delicate transverse reddish-brown bands at intervals of 1.0 to 1.5 mm., resulting in a perceptibly zonate appearance. Reddish-brown coloration was equally present also at the margin of the lesion, setting off the latter from the healthy tissue

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[2] Reference is made by number (italic) to "Literature cited," p. 726.

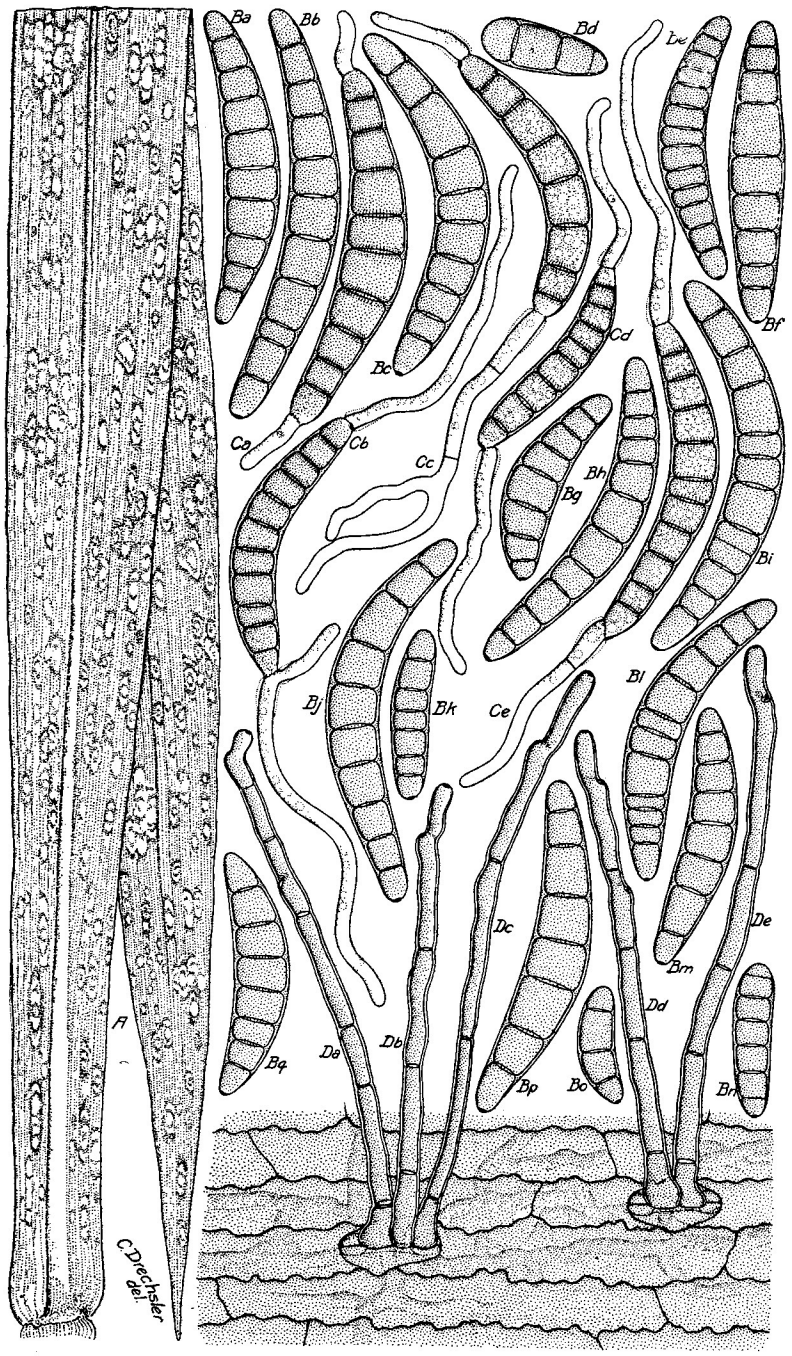


FIG. 1.—A, leaf of maize infected with *Ophiobolus heterostrophus*, showing abundance and size of lesions in specimens collected near Sanford, Fla., Sept. 22, 1923. ×7

Ba–Bq, conidia of *O. heterostrophus* produced on diseased maize leaves in damp chamber at summer temperature (25 to 30° C.) × 474

Ca–Ce, conidia germinating by production of 2 polar germ tubes. × 474

Da–De, conidiophores emerging from stomata as found on material collected in the field. × 474

surrounding it. On some of the less mature foliage a purple tint was frequently evident in lesions that approached the Prussian Red of Ridgway's series. All except the very smallest lesions were elongated in a longitudinal direction, usually with the lateral edges characteristically straight and parallel and coinciding accurately with the course of the veins. Since the latter in the Florida material were spaced at intervals of 1.0 to 1.2 mm., and in the Philippine specimens at intervals of 2.0 to 3.0 mm., the lesions appeared generally as ribbonlike areas of corresponding width. In occasional instances two adjacent intervascular spaces were included in the dead regions and these consequently were double the usual width. The larger regions of discolored foliar tissue, however, had their origin less frequently in such lateral extension of single lesions than in the coalescence of numbers of lesions, side by side and end to end. Owing to the abundance of the spots, which numbered from 200 to 300 on leaves not more than 50 cm. long and 2.5 cm. wide, the morbid parts naturally constituted a considerable portion of the foliar tissue.

Under macroscopic inspection, the appearance of the leaves thus affected does not closely resemble that of specimens affected with blight, a well-known disease widely distributed in maize-producing countries and caused by *Helminthosporium turcicum* Passerini. The blight lesions are relatively few in number, usually not exceeding a dozen to an individual leaf, but their paucity is more than compensated for by their size; they are, when well developed, more than a hundred times larger than even the largest seen in the Florida material. A comparison of Figure 1, A, with Plate 24, A, in a previous publication of the writer (*2*) suggests the difference in number and size of the two types of lesions. Leaf-blight lesions show no indication of zonation other than a reddish-brown marginal border which often is not well developed or is even entirely suppressed. And while the portions of leaf killed by *H. turcicum* are also elongated longitudinally and often bounded on the sides by the veins of the host, more than one intervascular region are usually involved, the veins apparently providing a much less effective barrier to lateral extension than is evidenced in the specimens under consideration.

CONIDIAL STAGE OF THE CAUSAL PARASITE

Macroscopic examination revealed conidiophores and conidia typical of the genus Helminthosporium scattered rather sparsely over the central portions of the larger lesions, the constant positional association strongly suggesting the causal relation subsequently established by inoculation experiments. On the material as obtained from the field, the conidiophores exhibited no unusual peculiarity. Singly, or in groups of two or three, they were found to emerge from the stomata (fig. 1, *Da* to *De*), resembling in the latter detail the homologous structures of *H. turcicum;* although such limitation to stomata is regarded as due to the mechanical firmness of the maize epidermis rather than to an inherent characteristic of the parasites concerned. However, a conspicuous difference distinguishing them from the conidiophores of the leaf-blight fungus was their smaller diameter, in which dimension they were found to measure usually from 4.5 to 7.0 μ instead of 7.5 to 9.0 μ recorded for the other form. In length the conidiophore of the field material measured between 120 and 170 μ, while scars marking the former points of attachment

of spores occurred at fairly wide intervals (10 to 40 μ) at geniculations usually not very pronounced.

Owing to the meager development of the fungus on the material obtained in the field, some of the diseased leaves were incubated in a damp chamber. A luxuriant growth of fructifications at once ensued, with the result that at the end of three days the leaves were covered with a profusion of conidiophores bearing an abundance of fresh conidia (fig. 1, *Ba* to *Bq*). Pure, single-spore cultures of the fungus were obtained by the usual method of transferring individual conidia germinating in poured plates of plain agar to cornmeal agar plates, and then transferring portions of the resultant growth, free of contaminating forms, to cornmeal agar in tubes.

The conidiophores developed in a moist atmosphere on the natural substratum, as in related forms, are not sharply differentiated from the sterile hyphae. Usually they consist of branches or prolongations of subhyaline or fuliginous mycelial filaments, differing from the latter in having a thickened wall dark olivaceous in color. As on the conidiophores found in nature, they develop spores in rather open arrangement, the individual conidia being attached at relatively wide intervals. Branching is not rare although it is more frequent in the sterile portions of the sporiferous elements. The sporophoric filaments, which often attain a length of 1 mm. or more, present, in mass, a brownish-black macroscopic appearance which may become brownish gray or even light gray, depending upon the quantity of subhyaline vegetative mycelium present as an admixture.

The conidia of the fungus as produced on diseased maize leaves in a damp chamber (fig. 1 *Ba* to *Bq*) or on cornmeal agar in pure culture (fig. 2), are readily distinguished from those of *Helminthosporium turcicum* in shape, dimensions, septation, and basal modification. While the spores of the leaf-blight fungus are typically straight or slightly curved, widest at the middle and tapering markedly toward the ends, those of the leafspot parasite are typically strongly curved, the curvature being indeed more pronounced than in any large-spored congeneric form studied by the writer, and tapering moderately toward the ends. In respect to size, the conidia of the new foliar parasite appear markedly inferior to those of the other, measuring only 30 to 115 μ in length and 10 to 17 μ in diameter as compared with 45 to 142 μ and 15 to 25 μ for the ranges of the corresponding dimensions in *H. turcicum*. In spite of its inferior length, however, the conidia of the leafspot fungus are the more abundantly septate, the number of cross-walls not infrequently reaching the observed maximum of 12, while in all material of *H. turcicum* examined by the writer, these have never been seen to exceed 8. An additional difference exists, inasmuch as in the fungus under consideration the hilum is represented by a broad flat scar not generally protruding from the rounded contour of the basal end of the conidium, while the basal segment of the spore of *H. turcicum* exhibits a narrow protruding apiculum. With respect to coloration the distinction is less marked, although the light or moderate olivaceous color characteristic of the mature conidia of the leafspot fungus is noticeably darker than the fuliginous tinge generally present in the conidia of the leaf blight parasite. The same mode of germination by the production of two germ tubes, one from the apical end, the other from the region surrounding the hilum, prevails in both species (fig. 1, *Ca* to *Ce*).

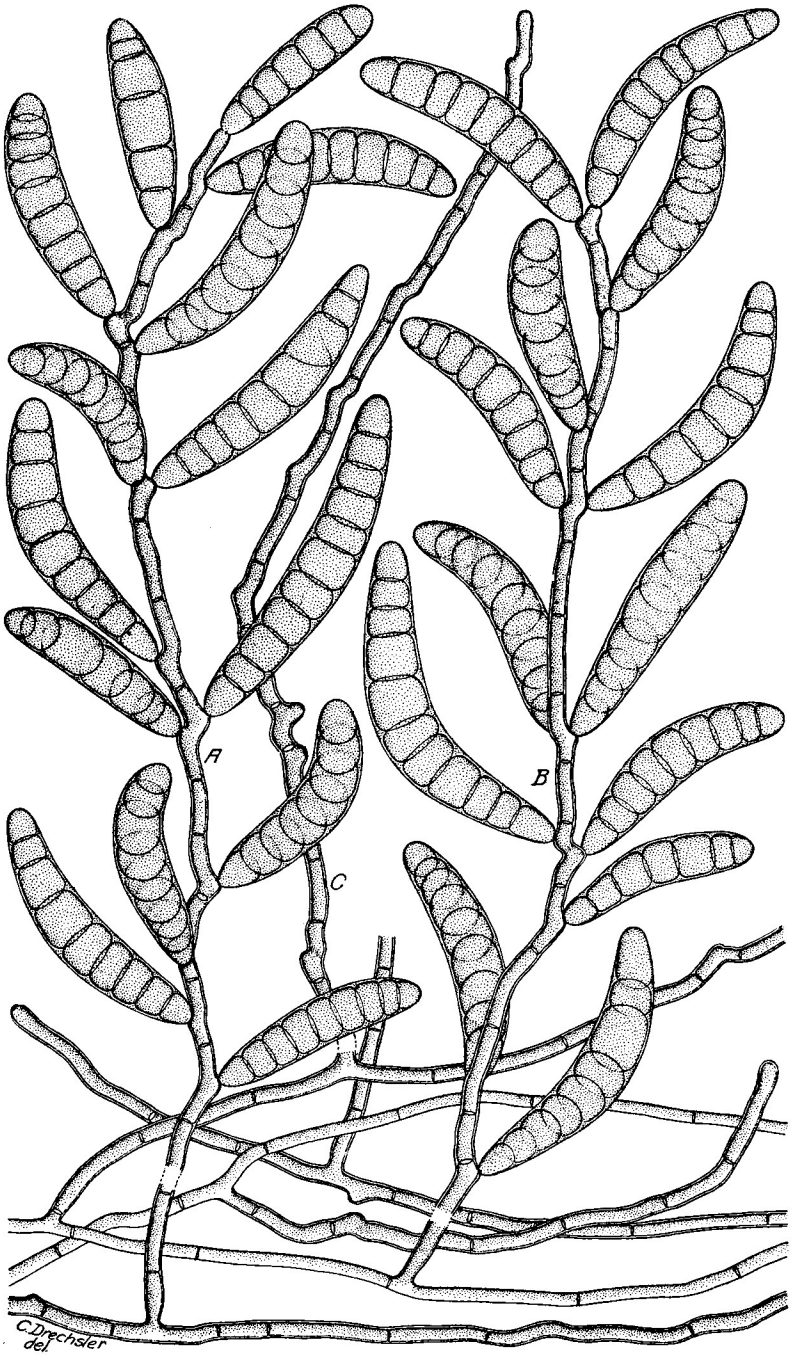


FIG. 2.—Conidiophores and conidia of *Ophiobolus heterostrophus* developed on 30-day-old cornmeal agar plates, arising from somewhat prostrate sporophoric filaments. The dotted lines represent sections of the sterile basal portions of the sporophores omitted for lack of space. ×475

DISTRIBUTION ON MAIZE

In this connection, it may not be amiss to consider some passages in the writings of several investigators working on leaf blight in tropical or subtropical regions, which can scarcely be regarded as aptly descriptive of *Helminthosporium turcicum*. Reinking (*10*), working in the Philippines, described the conidia of *H. inconspicuum* C. and E. (=*H. turcicum* Pass.) as "large, septate, curved brown," a characterization that in regard to coloration and curvature is not generally appropriate. Two figures—one (*10, fig. 40 B*) representing a conidium from a maize tassel, the other (*10, fig. 40, lower c*) representing a conidium from a maize leaf—especially deserve comment. The former is shown as a slightly curved structure with nine cross-walls, diminishing only slightly toward the abruptly rounded end, and, from calculations of the size of the figure and the scale of magnification, measuring approximately 112 μ in length and 17 μ in diameter; the latter is more strongly curved, provided with 10 septa, tapering somewhat more strongly toward the rounded ends, and measuring approximately 128 μ in length by 17 μ in diameter. The spores would appear to be too slender relative to their length, too abundantly septate, and not tapering toward the ends sufficiently to be typical of the leaf-blight fungus. Of the other figures given by the same author, *41b* would evidently seem to represent conidia of *H. turcicum*, while *40 upper c* as well as *43* appear to pertain to this species, though with less certainty. And the habit figure undoubtedly represents the extensive foliar lesions characteristic of leaf blight.

The descriptive passages in Marquez' recent paper (*7*) on leaf blight of corn, incorporating the results of studies carried out at Los Baños, are not free of the same sort of ambiguity.

Since the lesions of both leaf blight and leafspot are elongated in a longitudinal direction and are apt to coalesce, the description of the earlier stages may have been drawn from specimens representing either type or probably both types of infection. The leafspot lesion with its parallel lateral boundaries coinciding with two consecutive veins of the host are more accurately described as "stripes" than the effuse areas killed by leaf blight. They also coalesce with much greater frequency, indeed not attaining any considerable size unless abundant coalescence takes place, since the greatest individual lesions rarely exceed 3.0 mm. in width and 15.0 mm. in length. The leaf blight lesions on the other hand, become relatively very extensive without any coalescence whatever. It is not improbable that the description of the old spots refers to the latter type of lesion, since fructifications of *Helminthosporium turcicum* are always visible as a grayish efflorescence in the central portion of large affected regions. In the material from both Florida and the Philippines as obtained from the field, the fructifications on the leafspot lesions have been too sparse to be macroscopically visible. Since, however, on incubation under moist conditions a copious production of sporulating fructifications ensues, it might be wrong to assume that the "black mold" described by Marquez could not possibly have been produced by the leafspot parasite. The total of microscopic detail given by Marquez is equally difficult to reconcile with the morphology of either species alone. From 20 measurements of conidiophores he found these structures to vary from 96 to 148 μ in length and from

8 to 10 μ in diameter; while 200 spores varied from 36 to 112 μ in length, and from 10 to 18 μ in diameter. As the sporophores and spores of *H. turcicum* do not differ greatly in length from those of the leafspot fungus, the figures given for this dimension might be nearly equally acceptable for either species. On the other hand, the diameter of these structures differs considerably between the two parasites. The range given for the diameter of conidiophores would seem much more acceptable for *H. turcicum* than for the fungus causing leafspot; while the range in spore diameter is certainly characteristic of the leafspot parasite and not at all characteristic of *H. turcicum*. Marquez' statement that "the conidia are wider at the middle and gradually taper toward the two ends; they are septate, and are crescent-shaped" would seem to lend additional support to the assumption that he dealt with the leafspot fungus.

The inferences drawn from the publications of Reinking and of Marquez are altogether in harmony with direct evidence provided by examination of various lots of diseased material originating in the Philippines, mostly in the region in which those writers made their observations. The specimens of affected maize leaves collected near Los Baños in November, 1921, to which reference has already been made, exhibited a number of lesions typical of leaf blight, some of them 15 and 20 cm. long, with an abundance of fructifications in the center of the dead areas. The spores obtained from such areas differed in no wise from the conidia associated with leaf blight encountered in the Middle Atlantic States—broad, mostly straight, tapering markedly toward the basal end, provided with a protruding hilum, and with septa not exceeding eight in number. In addition, the leaves bore a much larger number of spots altogether similar to those present on the Florida material—similar in color and zonation as well as in being delimited laterally, when well developed, by the larger veins, which as the specimens represented more mature foliage, were spaced at intervals of approximately 3.0 mm. Preparations of the discolored areas revealed a relatively sparse development of conidiophores and conidia, which after treatment with chloral hydrate showed, except for the degenerate contents and swollen membranes characteristic of dead structures, complete similarity in morphological detail to the Florida fungus as collected in the field. The specific identity of the two forms was established beyond any question when some of the Philippine material was incubated in damp chambers. Some of the spots gave rise to an outgrowth of sporophoric filaments bearing conidia differing in no particular—size, septation, coloration, curvature, or basal modification—from those resulting from the incubation of Florida material under like conditions.

The fresh conidia were utilized in making a number of single-spore cultures, which again were completely like those of the Florida parasite. Complete similarity was found to obtain also in regard to perithecia, asci, and ascospores, as well as to the conidial fructifications developed in cultures derived from ascospores or pieces of immature perithecium. The fact that the material collected in November, 1921, gave rise to fresh fructifications when placed in a damp chamber as late as October, 1924, is indicative of a considerable measure of longevity in the mycelium of the fungus found within the tissues of the diseased leaves, since the old conidiophores and conidia could in no instance be made to germinate.

A specimen deposited in the herbarium of the Office of Pathological Collections bears the following label:

Philippine Fungi, Herbarium, College of Agriculture, Los Baños, Philippines No. 5. *Helminthosporium inconspicuum.* On leaf of Zea mays Linn. Los Baños. Laguna, P. I. Date, Dec. 17, 1919. Coll. L. Goco. Det. by O. A. Reinking.

This specimen was found to consist of a portion of maize leaf bearing numerous well-characterized leafspot lesions. A larger discolored region was also present which may have been a leaf-blight lesion, but if so, it had not developed far enough to exhibit the fructifications of *H. turcicum.*

In four collections of material made by W. H. Weston, jr., in the vicinity of Los Baños on July 17, 1918, September 10, 1918, September 18, 1918, and January, 1920, the injury observed could be assigned without difficulty and presumably with certainty, to the parasite under discussion, either alone or together with *Helminthosporium turcicum.* The first of the collections made in a field where three-fourths of the stand had been killed reveals both leafspot and leaf-blight lesions, the former scattered profusely everywhere over the foliar organs, the latter relatively few in number, but frequently very extensive, and often including numbers of leafspot lesions within their boundaries. The second collection made in a test plot where infection was described as rather serious, appeared entirely free of leaf blight, but exhibited an abundance of leafspot lesions. In the third collection, which was made in a field showing "noticeable loss," most of the leaves bore only leafspot lesions, while others were affected with leaf blight as well. On the young foliage that constitutes the collection of January, 1920, only leafspot lesions are evident, these being, however, both numerous and well developed. Although no general conclusions can be based on the few collections examined, it would appear that in 1918, 1919, 1920, and 1921 the leafspot disease occurred abundantly in the Philippines, manifestly being responsible for moderate losses, even in the absence of leaf blight, and, where combined with the latter malady, responsible apparently in large part for instances of more destructive injury.

In a brief discussion of Mitra's interesting paper on the disease of maize and sorghum attributable to *Helminthosporium turcicum* in India (*8*), attention was called to certain discrepancies in morphological detail between the account of the parasite given by that author and the account submitted by the writer from studies made in the Middle Atlantic States. It is believed that a partial explanation of these discrepancies may be found in the assumption that Mitra was dealing not only with the leaf-blight parasite, but with the leafspot fungus as well, notably in the Pusa and Malda specimens, which yielded spores described as "curved and narrow." Certainly some of the figures of spores from the Pusa maize leaves (*8, pl. II, 9, 10, 11, 13*) are more suggestive of the leafspot fungus than of *H. turcicum,* while the others (*8, pl. II, 7, 8, 12*) would seem to be more aptly illustrative of *H. turcicum.* It must be admitted, to be sure, that Mitra's statement that the lesions on the Pusa and Malda material were similar to those on specimens from other localities, and that cultures derived from this material bore spindle-shaped, straight, or rarely curved conidia does not lend support to such an explanation.

In the foregoing, attention has been confined to leafspot and leaf blight of maize, and the congeneric causal organisms responsible for them, without reference to the occurrence of Helminthosporium infection of the male inflorescence of maize, mentioned in several publications based on studies in the Tropics. Reinking in his discussion of *H. inconspicuum* (*10*), states that: "A black mold is produced on the tassel * * *. *Helminthosporium curvulum* Sacc. has been reported from the tassels of corn." In another paper (*11*) the same matter is restated as follows: "A black mold is also produced on the tassel. Saccardo has determined this fungus as *Helminthosporium curvulum* Sacc.; however, it produces a disease identical with the earlier described disease caused by *Helminthosporium inconspicuum*." In a host index of Philippine economic fungi, *H. curvulum* is enumerated among the fungi on corn as "tassel mold."

Mitra, in his description of maize blight, states that—

> In the male inflorescence the disease assumes a blackish mold-like appearance on the surface of the glumes of male spikelets. The attack is not extensive and scattered spikelets here and there are infected. The mycelium ramifies within the tissues of the paleae and the stamens.
>
> * * * Conidia of the fungus from the glumes of male spikelets are a little longer and more curved than those of the leaf fungus and has been named *H. curvulum* Sacc. In the Philippines it is described by this name but here in cultures it resembles the strain from the leaf and, when inoculated on the leaves, produces the same kind of spots as the leaf strain.

To these references may be added a passage from the writer's account (*2, p. 716*) of *Helminthosporium turcicum*, regarding the conidia obtained from affected maize tassels collected in the Philippines by W. H. Weston, jr.:

> Preparations made from the fructifications on the tassel, however, showed conidia which, while of the same color and approximate maximum length, were perceptibly inferior in diameter, measuring approximately 11 to 14 μ in this dimension; more abundantly septate, 12 transverse walls being not uncommon; usually quite distinctly curved; and evidently similar to those figured by Reinking (*10, pl. 20, B, C*). * * * If the forms on the leaves and on the tassels should indeed prove to be identical, the morphology of *Helminthosporium turcicum* as generally understood would stand in considerable need of revision.

Subsequent to the discovery of the leaf-spot disease, the writer took occasion to examine two packets of affected maize tassels deposited in the herbarium of the Office of Pathological Collections by C. F. Baker under the label:

> "*Fungi malayana* No. 239 *Helminthosporium curvulum* Sacc. n. sp. on *Zea mays*. Mount Maquiling, near Los Baños, Province Laguna, Philippines. Det. by Saccardo. Date Aug., 1914."

In general appearance the specimens closely resembled those collected by Weston, the fructifications where best developed being crowded on the scales as a velvety layer (fig. 3, F) very similar to the growth of *Helminthosporium oryzae* B. de H. on the inflorescence of rice. Microscopic examination revealed two species of Helminthosporium, one with relatively small sporophores (4 to 6 by 100 to 225 μ) and small 3-to-4 septate, curved conidia, measuring 9 to 14 μ in diameter by 20 to 30 μ in length, the middle cell of which was considerably smaller and darker than the other segments (fig. 3 *Ba to Bf, Ca to Cm*). The other fungus was apparently identical with the one found on Weston's material, the conidiophores measuring usually from 4.5

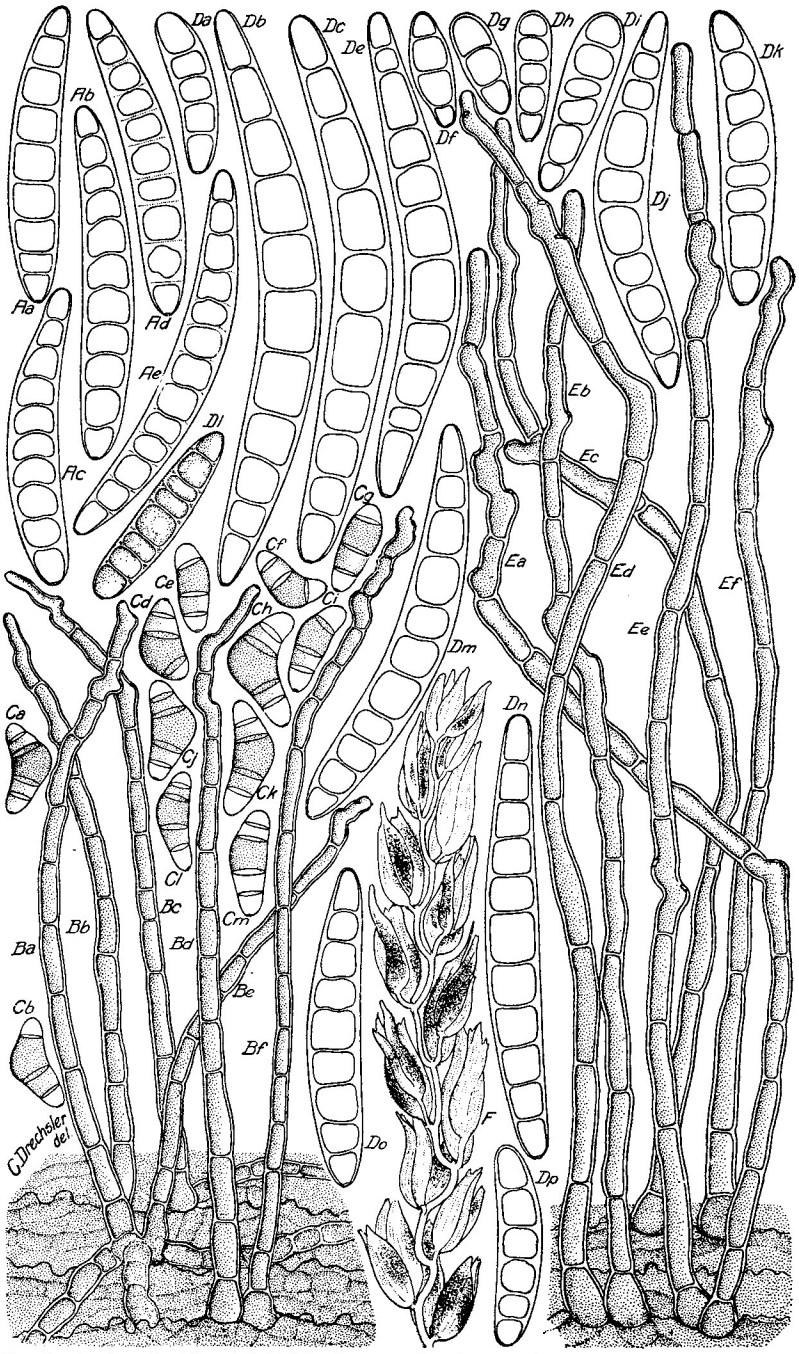


FIG. 3.—Aa–Ae. Conidia of *Ophiobolus heterostrophus* from specimen of maize leaf collected at Brooksville, Fla., June, 1917, deposited in herbarium of the Office of Pathological Collections. × 500

Ba–Bf. Conidiophores of the smaller species of Helminthosporium present on the specimen of maize tassels distributed as "Fungi Malayana No. 239," and evidently the one which Saccardo identified as H. curvulum Sacc. × 500

Ca–Cm. Conidia of the same species. × 500

Da–Dp. Conidia of the larger tassel mold present in the specimens distributed as "Fungi malayana No. 239," after treatment with chloral hydrate; not *H. curvulum*, nor *H. turcicum*, though perhaps *Ophiobulus heterostrophus*. × 500

Ea–Ef. Conidiophores of the foregoing species. × 500

F. Portion of infected tassel from "Fungi malayana No. 239" bearing a mixture of two species of Helminthosporium. × ~~0.1~~ 1.0

to 8.0 μ in diameter, and sometimes more than 340 μ in length (fig. 3 *Ea to Ef*), while the light olivaceous, generally curved 2 to 12 septate conidia, gave as the more extreme measurements, 10 to 17 μ for diameter, and 28 to 155 μ for length (fig. 3, *Da to Dp*). When treated with chloral hydrate, the hilum appeared as a broad, rather inconspicuous scar included within the contour of the basal cell, not as a protruding modification. It was in all probability the latter fungus which Reinking and Mitra regarded as the leaf-blight fungus, and which apparently they believed Saccardo had identified as *H. curvulum*.

As to the correctness of a view holding the larger tassel mold identical with the leaf-blight fungus, no final decision is possible without a comparison of living material of both forms developed under similar conditions. In the absence of cultures of the larger species obtained from diseased tassels, the writer has been unable to make such comparison. He is, nevertheless, quite convinced that the two forms represent altogether distinct species—that, in short, the larger tassel mold is not *Helminthosporium turcicum*. The possibility remains, of course, that *H. turcicum* may occur on male inflorescences of maize to some extent, although the writer has not observed anything he could have identified with this species on any of the specimens examined by him.

In comparing the larger tassel mold with the leafspot fungus, a striking degree of similarity at once appears. In respect to diameter and spacing of septa in conidiophore and conidium, the two forms agree closely. Both are similar in that the conidia are strongly curved, are septate up to twelve times, are from 10 to 17 μ in diameter, and have a broad scar contained within the contour of the basal cell. The only evident difference is the length of conidiophore and conidia, these dimensions attaining considerably greater maxima in the material from affected tassels, although the difference between the general run of spores is not especially marked. It is possible that a condition prevails here similar to that found in the case of *Helminthosporium oryzae*, which produces, on the mats of well-developed sporophoric filaments that constitute the black mold on the inflorescence, conidia considerably longer than those produced on the poorly developed sporophores arising sparsely from the foliar lesions. Although the writer is inclined to regard the larger tassel mold on maize as identical with the parasite causing maize leafspot, he is not prepared to offer this view as a conclusive opinion.

In any case it appears certain that Reinking and Mitra were in error in assuming that the tassel mold which they regarded as identical with *Helminthosporium turcicum* was the one which Saccardo identified as *H. curvulum* Sacc., in spite of the aptness of the latter specific name. As the diagnosis of *H. curvulum* (*14, p. 89*) did not appear until 1919, Reinking probably drew a natural but mistaken inference from an examination of Baker's Fungi Malayana No. 239. The species is based apparently on a fungus on leaves of *Bambusa blumeana*, and in part is characterized as follows:

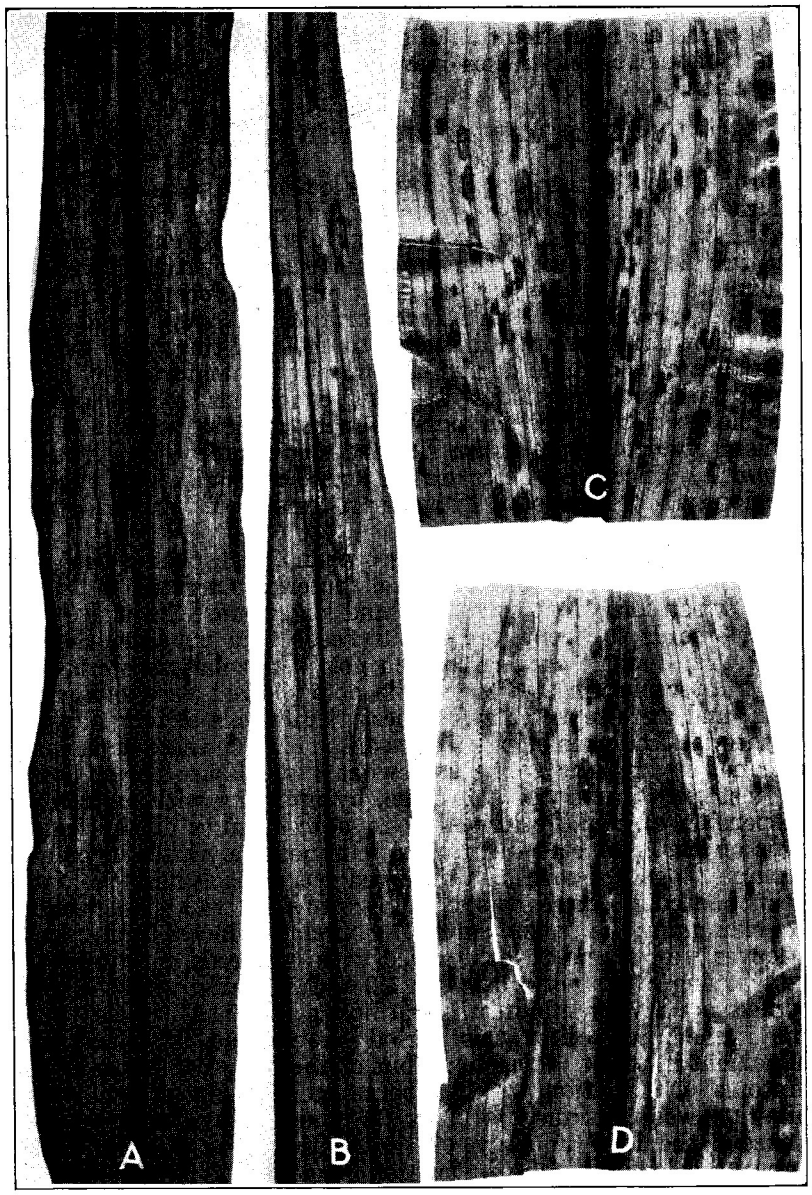
* * * Conidiophoris dense fasciculatis, filiformibus, 90–100=6–7μ, septatis, fuligineis, sursam pallidioribus obsoleteque denticulatis; conidiis obclavatis, distincte curvato-gibbis, 35–40=9–12, utrinque, praecipae basi rotundatis 3-septatis, non constrictis, grosse 4-guttatis, fuligineis, loculis binis mediis obscurioribus.

Unquestionably Saccardo's identification was concerned with the smaller species of Helminthosporium present on Baker's specimens as tassel mold, one of the several types frequently encountered when dead grass leaves are incubated in a damp chamber. In spite of its general distribution in nature, it appears to be of relatively little significance in relation to disease, and is discussed here only to clear up nomenclatorial confusion.

OCCURRENCE ON TEOSINTE

With the several lots of diseased maize leaves kindly sent by Weston were two liberal lots of teosinte (*Euchlaena mexicana* Schrad.) leaves collected in September and November, 1918, in the same locality as the maize collections. These leaves exhibit lesions strikingly similar to leafspot lesions on maize in shape and coloration, though minor differences are apparent in some instances in the less regularly straight lateral margins and the somewhat greater length of the lesions on teosinte, which may measure up to 3 cm. in a longitudinal direction (pl. 1, A, B). The lateral edges, in general, did not coincide as closely with the veins of the host as on maize. Microscopic examination revealed in the central parts of the dead areas, a sparse production of conidiophores and conidia, which when treated with chloral hydrate showed complete similarity to those on maize. Owing evidently to application of preservatives, efforts to recover the parasite in a living condition by incubating infected material in a damp chamber did not succeed. However, because of the close similarity in morphology and pathological habit, there can be little doubt that the same parasite is involved in the foliar disease of the two closely related hosts, which, it may be mentioned, have several other fungous parasites in common.

The occurrence of the leafspot fungus on teosinte raises the question as to the probable identity of the parasite found in the United States and in the Philippines with *Helminthosporium euchlaenae* described by Zimmerman (*17*) as pathogenic to this host in German East Africa. Zimmerman's description of the foliar lesions produced by the African form as light-brown spots with dark-brown margin, mostly elongated in a longitudinal direction, approximately 2 mm. wide and up to 25 mm. long, applies unusually well to the Philippine material. A not unsatisfactory agreement prevails also with reference to the sporophores which, according to the German account, are brown, unbranched, several times septate, 5 to 7 μ in diameter and up to 150 μ in length, and emerge from the stomata singly or in groups of two or three. The characterization of the conidia as brownish, cylindrical, straight or somewhat curved, up to seven times septate, 50 to 60 μ long and 13 to 15 μ in diameter, would seem to represent these structures as considerably inferior in degree of curvature, frequency of septation, and maximum length to the conidia on the Philippine specimens. If Zimmerman's text and figures give a sufficient description of the conidia of the African fungus, it would be necessary to conclude that the latter constitutes a species distinct from the Philippine form. However, with a fungus sporulating as sparingly as the leafspot parasite, the possibility of a description being based on a small number of conidia not altogether characteristic of the species is far from being a remote one. The question concerning the identity of the leafspot parasite with *H.*



A, B.—Portions of teosinte leaves showing lesions of *Ophiobolus heterostrophus*. Photographed from dried specimens originating from the vicinity of Los Baños, P. I., September, 1918.

C.—Portion of maize leaf showing numerous lesions of *O. heterostrophus* and a much larger lesion caused by *Helminthosporium turcicum* on the upper left margin. Photographed from dried specimens collected near Los Baños, P. I., July, 1918. × ⅔

D.—Portion of maize leaf showing numerous lesions of *O. heterostrophus* and a part of two moderately extensive lesions caused by *H. turcicum*, one immediately adjacent and to the right of the midrib, the other toward the left margin. The border of the second lesion is indicated by a dotted line for greater clarity. The cracks due to the brittle texture of tissues killed by *H. turcicum* indicate approximately the center of the leaf-blight lesion. Photographed from dried specimens collected near Los Baños, P. I., July, 1918. × ⅔

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euchlaenae therefore can not be decided until additional information on the African form becomes available. It may be added that Zimmerman makes no mention of any leafspot on maize, although his account of maize rust makes it evident that this host came under observation.

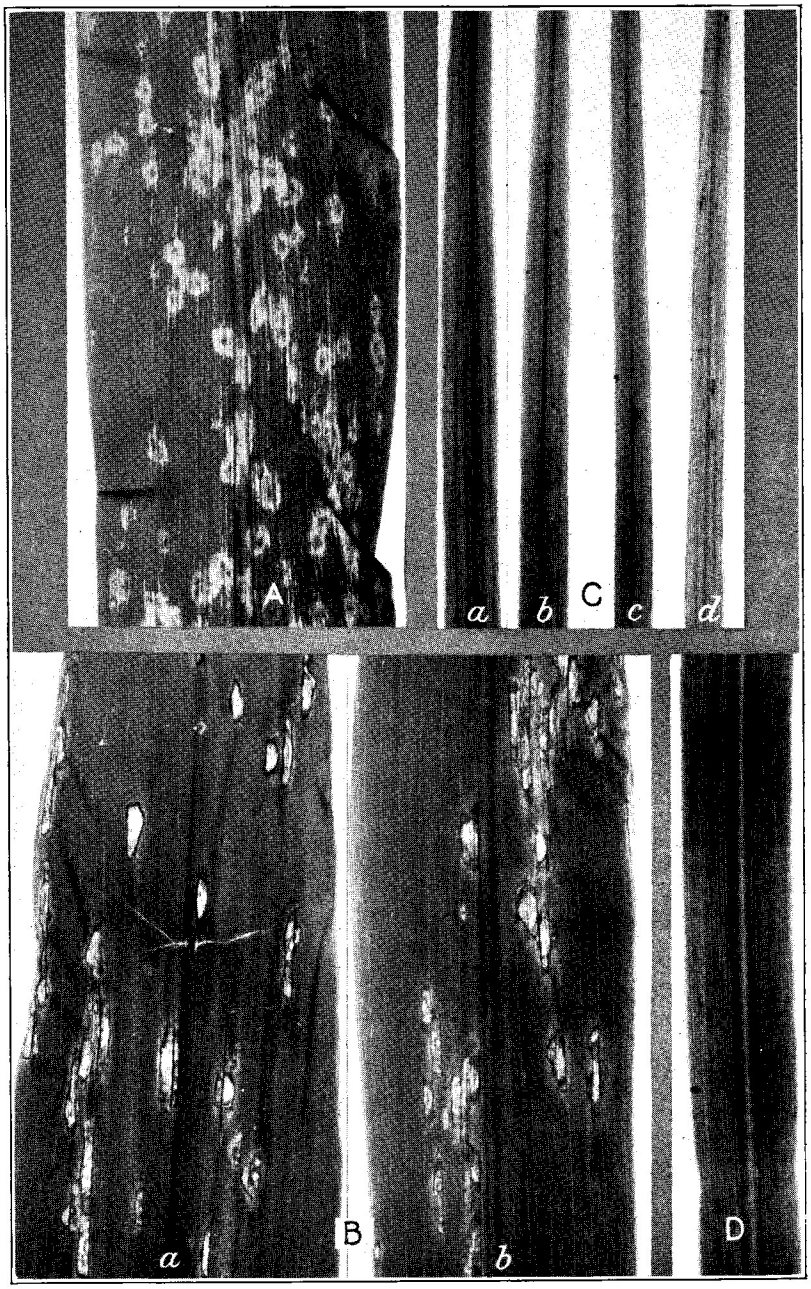
INOCULATIONS ON RICE AND SUGAR CANE

Morphologically, the parasite causing leafspot of maize shows much similarity to *Helminthosporium oryzae*, *H. sacchari* Butler, and *H. leersii* Atk. Perhaps its most obvious distinctive characteristic is found in the curvature of the conidium, this curvature being more pronounced than in any large-spored congeneric form known to the writer. The conidia of both *H. sacchari* and *H. leersii* would appear to be somewhat inferior in diameter to those of the maize parasite. Owing to a considerable degree of variability manifested by species of Helminthosporium under different conditions, it was believed advisable to determine the possible pathogenicity of the Florida organism on rice (*Oryza sativa* L.) and sugar cane (*Saccharum officinarum* L.), since these hosts are cultivated in tropical and subtropical regions where leafspot of maize is presumably of fairly widespread occurrence.

For the inoculation tests, plants grown in 5-inch pots in the greenhouse were used. The experimental material consisted of 24 pots each of sweet corn, dent corn, rice, and sugar cane, the maize being planted 3 seeds to a pot, the rice 10 seeds to a pot, and the cane started with a single cutting to each pot. Inoculations were begun 25 days after planting, when all the pots showed very satisfactory growth, and were repeated five times, at intervals of four days, the last material used thus being 45 days old, and in the case of the maize and cane, so large as to be somewhat unwieldy. Three pots of each host were inoculated at a time, by applying with an atomizer a suspension of conidia derived from pure cultures of the leafspot fungus on plates of corn-meal agar. Immediately after inoculation the plants, together with an unsprayed pot of each host as control, were incubated in a large moist chamber provided with glass windows.

On examining the maize plants 36 hours after inoculation, most of the leaves were found to be covered with hundreds of watersoaked spots varying in diameter between 1.0 and 2.5 mm., with the center occupied by a very minute whitish speck (pl. 2, A). The control plant immediately after this period showed no ill effects from confinement in moist atmosphere, but at the end of 48 hours showed symptoms of etiolation which became increasingly pronounced as incubation was continued to 72 or 96 hours. Even after 96 hours all except the lowest foliage of the controls was still functional, whereas the leaves of the inoculated plants had utterly collapsed and were converted into wet, softened structures drooping flabbily from the stalks.

An apparently more natural course of development of the infection resulted when the experimental plants were removed from the damp chamber to the greenhouse 24 or 36 hours after inoculation. Portions of the leaves in which the infections were very numerous withered very soon, to be sure, the healthy parts dying evidently from an interruption of the water supply, but where a more moderate



A.—Maize leaf 42 hours after inoculation by spraying with conidial suspension of *Ophiobolus heterostrophus*. Photographed by transmitted light, the light areas corresponding to watersoaked spots. × 3/2

Ba–Bb.—Portions of maize leaf inoculated with conidial suspension kept in damp chamber 36 hours, then in greenhouse 15 days. × 3/2

Ca–Cd.—Portions of rice leaf sprayed with conidial suspension and kept in damp chamber 42 hours, the incipient spots originally formed not subsequently increasing in size. × 3/2

D.—Portion of sugar-cane leaf sprayed with conidial suspension and kept in damp chamber 42 hours, the incipient spots originally formed not increasing in size. × 3/2

distribution prevailed the tissues withered only in the regions directly involved in the lesions. The enlargement of the latter took place especially toward the base and the tip of the leaf, lateral extension being in most instances, though not in all, checked by the veins. This elongation resulted in the characteristic lesions found on the original specimens—a striplike spot of uniform width, variegated with delicate, reddish-brown, elliptical or transverse zonation, and surrounded by a narrow reddish-brown marginal zone. The zonation, as expected, was found to have its origin in the intermittent extension of the lesion. During periods of high humidity prevailing often as a result of watering or the lowering of temperature during the night, a zone of healthy tissue immediately below and above the established lesion became watersoaked in appearance. With the recurrence of dry conditions, these portions of tissue withered and appeared as additional zonal increments (pl. 2, B, *a* to B, *b*).

In contrast to the heavy infection brought about on both field corn and sweet corn, most of the rice plants subjected to the same treatment showed no evidence of even incipient lesions. On two of the lots a sparse scattering of minute blackish specks appeared 36 hours after being inoculated. As the rice plants were found to flourish in the damp chambers, better indeed than in the greenhouse, the pots which had yielded the incipient infections were retained in the chamber for 20 days. The spots, however, increased neither in size nor in number, and at the time the material was discarded the foliage was in thriving condition (pl. 2, C, *a* to C, *d*).

The sugar cane responded to the inoculations in a manner somewhat similar to rice. Nearly all the cane plants developed minute, dark, rather indefinitely delimited specks on the foliage 36 hours after being sprayed with the suspension of conidia. As they appeared to suffer no ill effects from being exposed to the humid conditions of the damp chamber they were retained in the chamber for more than 20 days. None of the lesions, however, evidenced any tendency toward further development (pl. 2, D).

The vigorous parasitism of the fungus on maize foliage contrasts strongly with its failure to produce anything but incipient infection, in spite of especially favorable conditions for development, on the leaves of rice or sugar cane. That the organism would infect rice or sugar cane under natural conditions appears highly improbable. Its biological behavior is thus confirmatory of morphological evidence indicating its specific independence of *Helminthosporium oryzae* and *H. sacchari*.

ASCIGEROUS STAGE OF THE PARASITE

In discussing the specific identity of the fungus strains developing on the diseased maize leaves from Florida and from the Philippines, mention was made of their association with entirely similar ascigerous fructifications. The latter develop side by side with the conidiophores when diseased maize leaves are incubated in a damp chamber, although the minute black masses that constitute the young perithecia can not usually be discerned with the naked eye until the third or fourth day. Apparently the initial stages of development take place under the epidermis, which, however, is generally soon broken through and the sclerotiumlike bodies exposed to view. In its later stages

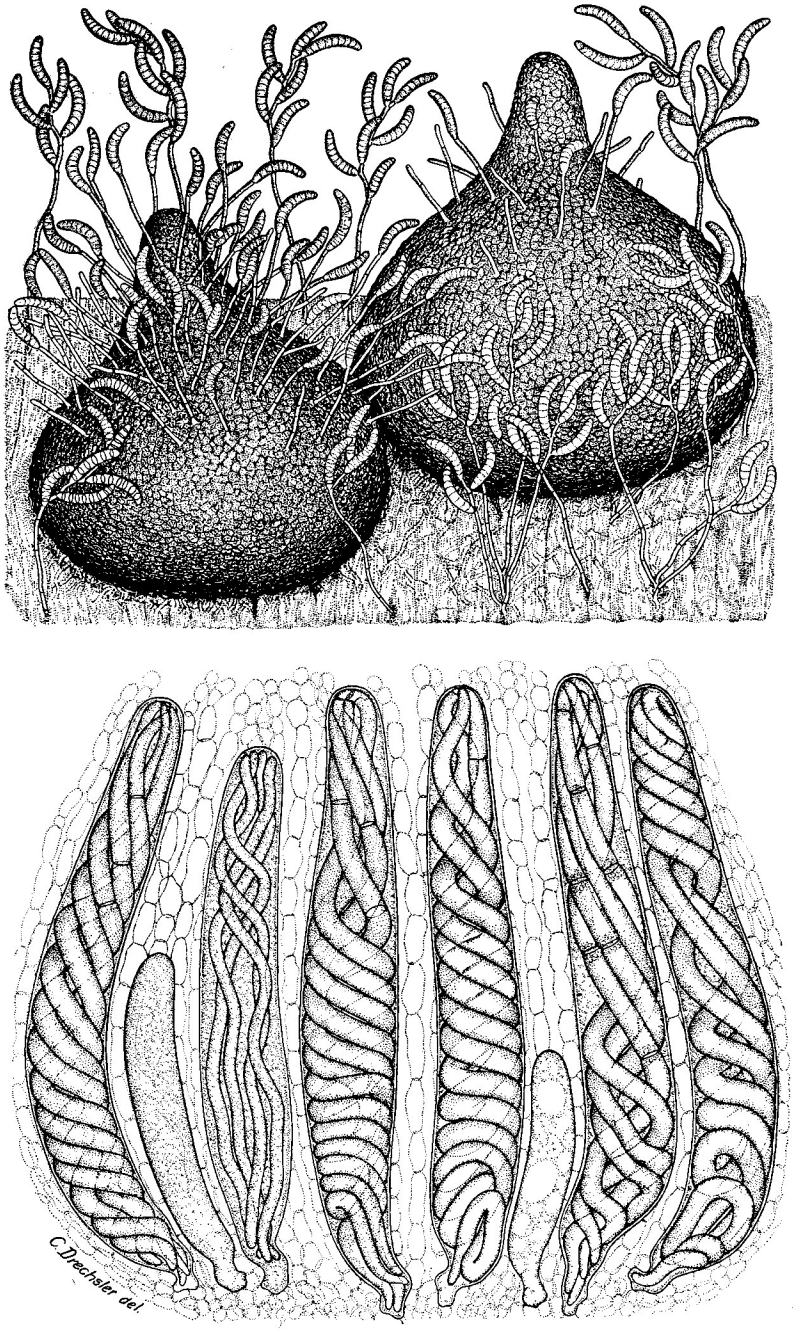


FIG. 4.—A (upper). Two mature perithecia of *Ophiobolus heterostrophus* on diseased maize leaves incubated 20 days in a damp chamber, illustrating some variation in size and shape, and relationship to conidial fructifications. × 120

B (lower). Asci in various stages of maturity together with vertically oriented filaments that compose the colorless pseudoparenchyma in the interior of the perithecium, which diminishes with the development of the asci. × 473

the fruiting body has the appearance of a superficial structure (fig. 4, A, upper). In some instances the rupturing of the disintegrating host tissues is delayed longer, while in the case of perithecia developing on the filter paper over which the fungus spreads from infected material, even the earlier stages are practically superficial, the loose mat of overlying fibers scarcely affording any substantial covering. The globose portion of the perithecium attains its definitive size in about 6 to 10 days, and a few additional days are usually required for the beak to reach its final proportions. Externally the fruiting body is black and relatively smooth, though often beset toward the base with stubby cylindrical projections resembling incipient conidiophores, or, perhaps, the proximal part of fractured conidiophores. The dark pseudoparenchymatous external layer is relatively thin, while the interior consists at first of firm colorless pseudoparenchyma composed of laterally appressed, vertically oriented filamentous elements. Young asci can generally be made out here and there after 12 days, and at the end of 15 days are not at all rare. As the asci increase in size and number the pseudoparenchymatous structures diminish correspondingly. Perithecia from 18 to 20 days old generally yield, on being crushed, a small proportion of approximately mature asci; the condition illustrated in Figure 4, B (lower), where most of the asci are mature, being, however, not attained usually in less than 25 or 30 days.

The mature asci, except for the short, markedly contracted stipe, are roughly cylindrical in shape, though generally widest about one-third of their length from the base, from which point the diminution in diameter toward the apex is more gradual than toward the base. When crushed out individually they frequently show more pronounced distention, evidently as a result of the imbibition of water preliminary to dehiscence, when such imbibition takes place in the absence of pressure normally provided by the surrounding asci and filamentous elements. Those in the center of the fruiting body are usually straight, while others near the periphery are distinctly curved, to conform with the curvature of the subspherical perithecial wall (fig. 5, A). Each ascus contains typically four cylindrical ascospores coiled in a close helix, each spore usually exhibiting approximately four turns, which alternate regularly with the turns of the other spores. As the lower ends of one or more spores thrust well into the short contracted stipe the helicoid arrangement at the base of the ascus is constrained into some irregularity. The upper ends of the spores similarly thrust into the apex of the ascus, yet, owing apparently to the more regular conformation of the apical portion, the departure from the spiral arrangement is usually very slight. In several hundred asci examined the rotation of the helix has been found to be always in the same direction; that is, on an upper focus with the ascus in a horizontal plane following the spore from the base toward the apex the coil appears at right and disappears at the left. The four spores might thus be compared to the threads of a quadruple left-handed screw.

The earlier stages in the development of the ascospores are not easy to make out, at least in unstained preparations, as the young structures are distinguished in the granular protoplasm only with difficulty. The spores first become clearly visible in the still growing ascus as filamentous bodies, approximately 3 μ in diameter and about

125 μ in length, consequently extending nearly the entire length of the ascus within which they follow an irregularly sinuous course. Subsequent growth ensues both in length and diameter, so that the final dimensions are frequently more than double those indicated. The length of the spores thus comes to exceed that of the ascus, a condition which is made possible by the helicoid coiling of the ascospores, the turns of which become increasingly close as maturation proceeds. After a rather tight arrangement has been effected, cross walls make their appearance, beginning at the upper end of the spore and proceeding downward.

When a perithecium containing mature asci is crushed in a water mount, a brisk discharge of ascospores ensues. As might be expected because of their involved arrangement, the spores of each ascus are discharged simultaneously, the discharge always taking place from the apex, and with enough violence to propel the spores a short distance through the water, the helicoid coils thereupon slowly becoming relaxed in loose, sinuous curves. Judging from the instances in which dehiscence is impeded, frequently as the result of immature condition, discharge is preceded by some swelling of the ascus (fig. 5, B, C, D), and circumscissile rupture in the apical portion of the ascus wall (fig. 5, E). Intertwined spores in groups of four have occasionally been observed adhering to the tip of the beak of perithecia examined in place, leaving little doubt that normally the spores in each ascus are discharged through the ostiole simultaneously as a group.

On being liberated, either naturally or by manipulation, the ascospores appear as filamentous structures distinctly fuliginous in coloration, tapering somewhat at the extremities (fig. 5, H. K). The constrictions at the septa which previously were not pronounced usually become more clearly accentuated, evidently as a result of some slight enlargement of the delimited segments. The contents are somewhat granular, especially immediately adjacent to the septa. In some instances a mucous envelope is apparent (fig. 5, L, M). When properly mounted in water the spores germinate very promptly by the production usually of one to eight germ tubes which may originate terminally from the tip or laterally from any part of the spore (fig. 5, F. G).

It should be mentioned that while the number of spores in an ascus is typically four, cases in which only three are present are by no means rare, and even one-spored and two-spored asci may be encountered occasionally. In the latter instances the asci are less completely occupied, the helicoid turns are looser and more irregular, although the direction of rotation remains the same. In cases of frustrated dehiscence, where the spores are only partly liberated from the ascus wall, germination is not materially impeded, the germ tubes arising from the lower segments of the ascospores perforating the surrounding membrane without any apparent difficulty.

Cultures made by placing fresh asci on corn-meal agar plates and transferring the resulting growth to sterile agar slants, show the same type of growth and produce the same type of conidiophores and conidia as are found on cultures originating from conidia. Conidia obtained from such cultures when applied to maize foliage have been found to give rise to lesions indistinguishable from the lesions resulting from the use of conidia from other sources. The facts regarding

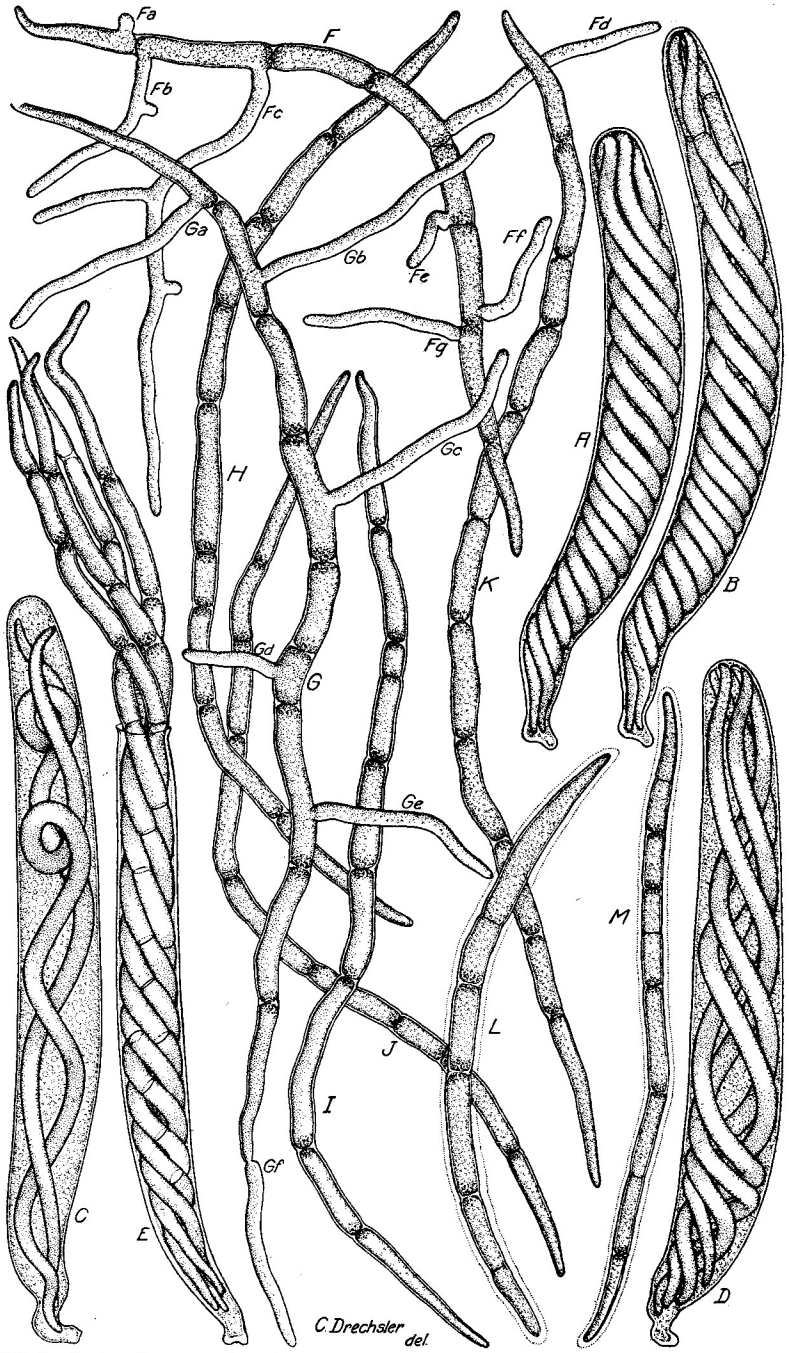


FIG. 5.—A. A mature or nearly mature ascus of *Ophiobolus heterostrophus* as obtained from a freshly crushed perithecium. × 476

B. The same ascus as in A, an hour later, showing elongation presumably preliminary to dehiscence; and the insertion of septa in the apical portion of the spores

C, D. Asci like B, after elongation, C containing only 2 spores. × 476

E. Partial liberation of ascospores by rupture of ascus toward apex; evidently an instance of frustrated discharge. × 476

F, G. Ascospores germinating by production of germ tubes Fa-g, Ga-f, respectively. × 476

H, I, J, K. Ascospores in mature septate condition. × 476

L. M. Mature ascospores inclosed in a mucous sheath. × 476

culture and pathogenicity only serve to confirm, however, the very clear evidence provided by the frequent presence on the perithecium of a larger or smaller number of conidiophores bearing conidia typical of the leafspot fungus and arising unmistakably from the perithecial wall (fig. 4, A). Sometimes the conidial fructifications arising in this manner become very abundant, with the usual result that the internal differentiation of the fruiting body is largely if not entirely suppressed.

CLASSIFICATION OF PARASITE

In reference to Saccardo's system, the fungus is presumably to be reckoned among the scolecosporous Sphaeriaceae, by virtue of the relative dimensions of the ascospores rather than because of any close similarity to the usual filamentous type. Among the definitions of the genera included in this category, none contain any special provision for the most noteworthy characteristic of the fungus—the disposition of the ascospore in a well-defined spiral. A considerable degree of similarity is discernible, however, to a fungus originally described by Saccardo (*12*) as *Rhaphidophora camptospora*, a combination later reduced by the author (*13, v. 2, p. 344*) to a synonym of *Ophiobolus camptosporus* Sacc. Under the latter binomial, Berlese published illustrations (*1, v. 2, pl. 159*) and a somewhat altered description (*1, v. 2, p. 133*) based upon material supplied by Saccardo. Berlese's figures show a short, stipitate, cylindrical ascus containing four spores partly coiled in helices in a manner strongly suggestive of the maize parasite, although the direction of rotation is opposite to that prevailing in the latter. In *Ophiobolus camptosporus* one of the median segments in the ascospores is swollen, which would seem to show relationship to certain other forms assigned to Ophiobolus, as well as to species referred to allied genera exhibiting one or more nodose intermediate segments.

Another fungus that should be mentioned in this connection is *Ophiobolus chaetophorus* (Crouan) Sacc., which, according to the account given by Malbranche and Niel (*6*), exhibits a spiral disposition of its ascospores suggesting the elaters of certain slime molds. In the figures, the direction of rotation seems to be like that found in the maize parasite. As the asci are described as eight-spored, and the perithecia as provided with rigid bristles on the upper part, differences from the latter organism are not lacking. *O. helicosporus* (B. & Br.) Sacc. (*13, v. 2, p. 350*) with "sporidiis linearibus praelongis spiraliter convolutis" and *O. galii* Rich. with "sporidiis spiraliter fasciculatis, hyalinis" represent two imperfectly described species that may possibly be related. Malbranche and Niel state that the perithecia of *O. helicosporus* are glabrous, thus differing in one particular from *O. chaetophorus*, which Saccardo later referred to the genus Ophiochaeta, erected to include definitely setose forms.

According to recent publications by Höhnel (*4, 5*) and Weese (*16*), the genus Ophiobolus, as employed by Saccardo and other writers, is far from being a homogeneous group. Höhnel's recommendations contemplated the maintenance of three genera—(1) Leptospora Rabh., based on *L. porphyrogona* (Tode) Rabh. to include not only "sphaeriaceous" forms having narrow spores with or without a nodose cell, but also species having somewhat broader many-septate, colored spores with nodose segments; (2) Entodesmium Riess, based on *Entodesmium rude* Riess, a "dothidiaceous" type, to include those

species the spores of which become disarticulated within the ascus; and (3) Ophiobolus Riess, established on *O. acuminatus* (Sow.) Duby to include "sphaeriaceous" forms with nodose spores, presumably broader than those referable to Leptospora. An additional and new genus, Leptosporopsis, was proposed for certain other forms like *Ophiobolus rostrupii* Ferd. and Winge, *O. compressus* Rehm and *O. tanaceti* (Fuck.) Sacc. which Höhnel found to possess "dothidiaceous" structure and hence to differ from Leptosphaeria de Not. only in having long narrow spores. Weese adopted Höhnel's revision of the group, suggesting, however, changing the definitions of the closely related "sphaeriaceous" genera Leptospora and Ophiobolus, so that the former is to include only forms with filamentous spores without nodose segments, while the latter is to include the species with nodose spores. For the setose forms, Weese retained two genera, Saccardo's Ophiochaeta, to include forms with erumpent fruiting bodies analogous to Ophiobolus, and Acanthophiobolus Berlese to include species with superficial perithecia and worm-shaped, multiguttulate, subhyaline ascospores. Since the latter combination of characteristics was found represented in *Sphaeria chaetophorus* Crouan, the retention of the combination *Acanthophiobolus chaetophorus* (Crouan) Berlese, abandoned by its author in favor of Saccardo's combination, was indicated.

Although the taxonomy promoted by Höhnel and Weese may redistribute an assortment of fungi into a number of presumably less heterogeneous groups, it is not certain that the rearrangement proposed is, after all, as free of arbitrary features as might be desired. The distinction drawn between the "sphaeriaceous" and "dothidiaceous" types of discrete perithecia, on the basis of differences in internal development and structure, is one that has not hitherto been widely incorporated in mycological literature. In any case, the fungus under consideration would appear not to qualify for inclusion in Entodesmium as interpreted by Höhnel, as its spores show no tendency toward becoming disjointed. Nor could it well be referred to Leptospora, as the type for this genus, with its extremely slender spores, obviously does not represent a closely related form. While it must be admitted that the type of Ophiobolus, *O. acuminatus* (=*Leptosphaeriopsis acuminata* [Sow.] Berlese), with its asci containing eight straight spores, each provided with two swollen segments, does not itself show any close similarity, this species, from the information available, is at least not too obviously different from *O. camptosporus* with its ascus containing four helicoid ascospores each having one nodose intermediate cell to obviate the possibility of their being related more closely than by the arbitrary operations of analytical keys.

Judging from Berlese's figures of *Ophiobolus compressus* (*1, v. 2, pl. 156*) and *Ceuthocarpon brunellae* (Ell. and Ev.) Berl. (=*Leucospora brunellae* Ell. and Ev.) (*1, v. 2, pl. 170*), both of which Höhnel would place in his proposed genus Leptosporopsis, these fungi exhibit a degree of similarity to the maize parasite about equal to that shown by the general run of forms referred to Ophiobolus, though not as great a similarity as is evidenced by *O. camptosporus*. Indeed, neither Leptosporopsis Höhnel, nor Ophiomassaria Jaczewski, based on *O. selenospora* (Otth.) Jacz., a fungus with 2 to 3 septate ascospores in a mucus sheath (*13, v. 11, p. 353*); nor Acerbia as exempli-

fied in *A. therryana*, illustrated by Berlese (*1, v. 2, pl. 167*); nor Leptosporella Peng. and Sacc. (*13, v. 14, p. 619*), would seem to offer any species as similar to the maize parasite as *O. camptosporus*. In the absence of a more appropriate genus to which it could be referred, and in order to conserve for the time being its proximity to the latter form, the leafspot fungus is tentatively assigned to Ophiobolus. Such assignment is not intended to imply close relationship to certain species referred to this genus that are frequently mentioned in the literature pertaining to the diseases of cereal crops, as, notably, *O. cariceti* (Berk. and Br.) Sacc., and *O. herpotrichus* (Fr.) Sacc. It may be superfluous to add that the parasite is undoubtedly different from *Acerbia maydis* Rehm, described from dead remains of maize in the Philippines (*9*), as well as from *Leptosphaeria orthogramma* (B. and C.) Sacc., reported on dead maize culms in Pennsylvania, South Carolina (*13, v. 2, p. 60*), and the Philippines (*9*), for in respect to dimensions of spore, for example, the latter forms are decisively inferior to the fungus under consideration.

As far as the writer is aware, the ascigerous stage of the parasite has not been described before. There is scarcely any doubt that the conidial stage has been encountered by pathologists, but with the possible exception of the somewhat problematical *Helminthosporium euchlaenae*, it has apparently not served as type for any species designated by a binomial. As has been pointed out, Saccardo evidently did not intend to apply to it his binomial *H. curvulum*. The combination *H. turcicum* has long been applied, and seemingly altogether correctly, to the fungus causing leaf blight of maize and several other graminaceous hosts. The excessively brief diagnosis of *H. inconspicuum* applies better to the leaf-blight fungus than to the parasite causing leafspot, especially in the details concerning the 3 to 5 septate condition of the conidia and their diameter of 20 μ, although the passage "effused, but so thinly as not to be visible to the naked eye" is not readily reconciled with the actual appearance of *H. turcicum* developing luxuriantly on its host in nature. More direct evidence that *H. inconspicuum* is indeed synonymous with *H. turcicum* is provided by the specimen in the herbarium of the Office of Pathological Collections under the label:

Ellis. North American Fungi. 45. *Helminthosporium inconspicuum* C. & E. Grev. vol. 6, p. 88. On leaves of Indian corn.

Which, judging from the date of issuance, 1878, presumably represents authentic material from approximately the same collection as the type specimens. The conidia present in abundance on the very extensive affected area were found to be altogether of the broad, straight, markedly tapering, relatively sparingly septate type characteristic of *H. turcicum*.

The specific name *heterostrophus*, descriptive of the ascospores, is suggested for the fungus.

DIAGNOSIS

Ophiobolus heterostrophus n. sp.

Occurring on maize (*Zea mays* L.), as the cause of a destructive disease manifested by the appearance on the leaves of numerous dead cinnamon-buff or purplish areas surrounded by a darker reddish brown margin, and often delicately variegated with brownish zonate bands; the lesions longitudinally elongated, first elliptical, later long-rectangular, typically limited to a single intervascular region, usually 1 to 3 by 5 to 15 mm., often coalescing to form more extensive dead portions.

Perithecia developing on disintegrating host tissues, usually early erumpent, black, often bearing a variable number of conidiophores, but no differentiated sterile setae; ascigerous portion subglobose or more frequently somewhat ellipsoidal, measuring usually 0.4 to 0.6 mm. in transverse diameter and approximately 0.4 mm. in vertical diameter; ostiolate beak well defined, subconical or paraboloid, approximately 0.15 mm. at base and 0.15 mm. in length; interior composed of colorless pseudoparenchymatous tissues consisting of vertically oriented appressed filaments, diminishing usually with the development of the asci.

Asci numerous, short-stipitate, with rounded apex, subcylindrical but sometimes becoming more inflated before discharge; 160 to 180 μ in length by 24 to 28 μ in diameter; containing 1 to 4, typically 4, spores. Ascospores filamentous, fuliginous, thin-walled; in somewhat immature condition, of uniform diameter of 6 to 7 μ, except at extremities which are somewhat attenuated; later becoming five to nine times septate, the septa usually associated with perceptible constrictions, the delimited segments becoming somewhat swollen so as to attain in places a diameter up to 9 μ; thrusting firmly against apex and into base of stipe in multiple heterostrophic helicoid arrangement with approximately four turns to each spore; measuring 130 to 340 μ in length; discharged simultaneously, often with mucous envelope; germinating promptly by producing indiscriminately from any or all segments, either laterally or terminally, germ tubes up to eight in number, from 3.5 to 5.0 μ in diameter.

Conidiophores arising singly or in groups of two or three from stomata in center of killed foliar parts; olivaceous, septate at intervals of 15 to 60 μ; bearing the first conidium after attaining a length of 50 μ or more; points of attachment of successive spores marked by scars occurring at intervals from 10 to 40 μ at geniculations not always pronounced; in nature measuring usually 120 to 170 μ in length, but under moist condition occurring as irregularly branching sporophoric filaments, often exceeding 1 mm. in length. Conidia developed at 25° C. on diseased maize leaves in damp chambers or in pure culture on artificial media, fuliginous to light olivaceous, measuring 30 to 115 μ in length by 10 to 17 μ in diameter; often strongly curved, usually widest near the middle and tapering perceptibly toward the rounded ends; peripheral wall thin, especially in the apical and basal regions; basal scar broad, not conspicuous, contained within rounded contour; germinating promptly by the production of two polar germ tubes.

Found on diseased leaves of *Zea mays* L. collected at Sanford, Fla., September 22, 1923 (type), Brooksville, Fla., June, 1917 (fig. 3, *Aa* to *Ae*), and at Los Baños, P. I., in 1918, 1919, 1920, and 1921. Perhaps identical with the more luxuriant Helminthosporium form widely occurring on the inflorescence of maize in tropical and subtropical regions. Found also on leaves of *Euchlaena mexicana* Schrad. near Los Baños, P. I., September and November, 1918; and possibly to be identified with *H. euchlaenae* Zimm.

TAXONOMIC CONSIDERATIONS

The pleomorphism of the maize parasite casts light on the affinities of a large proportion of the fungi referred to Helminthosporium. In their excellent account of *Pleospora polytricha* (*15, v. 2, p. 269–271, pl. 29*), the Tulasne brothers set forth an association with one representative of the genus which has since been paralleled by the discovery of analogous relationships in other graminicolous forms—*Pyrenophora teres* (Diedicke), *P. tritici-repentis* (Diedicke), and *P. bromi* (Diedicke) found in Europe and America, as well as *Pleospora graminea* Diedicke, the ascigerous stage of the parasite causing the widely distributed stripe disease of barley, which stage, however, has hitherto been reported only from Europe. A significant fact concerning all the species of Helminthosporium achieving their perfect form as members of the genera Prenophora or Pleospora is that their conidia are of the straight cylindrical type, germinating by the production of germ tubes laterally or obliquely from intermediate as well as end segments. In pure culture, on many artificial media, under ordinary laboratory conditions, these species are characterized usually by abundant anastomoses in the submerged portion of the mycelium, resulting in the production of complexes of inflated elements plausibly inter-

preted as incipient perithecia, and by conidial apparatus either being absent, or, if present, not infrequently represented by proliferous atypical structures in which sporophoric elements and spores are poorly differentiated.

The maize parasite, like *Helminthosporium sativum* P. B. and K., *H. sacchari* and *H. oryzae*—to mention only a few of the more important species—represents a type of Helminthosporium with mostly elliptical, curved conidia, germinating by the production of two germ tubes, both from small, thin-walled regions, one at the apex and the other immediately adjacent to and surrounding the basal scar. In artificial culture, sporulation is usually abundant and not markedly abnormal, although the conidia may be shorter and less regularly curved than those produced under natural conditions. There can be little doubt that the species adhering to this type, including many parasites of higher plants and very probably a considerable number of small-spored forms often referred to the conidial genus Brachysporium, are closely related with one another, and that the latter, when connected with ascigerous stages, be found not without similarity to *Ophiobolus heterostrophus*. It is apparent that a proper taxonomic disposition of the general run of species of Helminthosporium with bipolar germination is contingent on the discovery of the perfect condition of additional forms, from the morphology of which the common characters may be deduced for incorporation in the definition of a suitable genus, or, if possible, for appropriate revision of a genus already established.

SUMMARY

A foliar disease has been found to occur on maize in Florida and on maize and teosinte in the Philippines, which is characterized by cinnamon-buff lesions which are considerably smaller and much more numerous than those of leaf blight, and are also distinguished by being usually confined to a single intervascular region.

The disease is associated with a fungus which, in its conidial condition, differs from *Helminthosporium turcicum* in the smaller diameter of its conidiophores, as well as in the smaller diameter, more abundant septation, and greater curvature of its elliptical conidia, which, moreover, have an internal basal scar rather than a protruding modification.

The fungus produces discrete, subglobose perithecia with a well defined beak, and bearing asci containing typically four multiseptate, fuliginous, filamentous spores, each coiled in a heterostrophic helix of approximately four turns. It is described tentatively as *Ophiobolus heterostrophus*, n. sp., though not obviously closely related to several well-known species of Ophiobolus parasitic on grasses.

The morphological difference between this ascigerous stage and Pyrenophora or Pleospora lends support to the view that the species of Helminthosporium with straight subcylindrical conidia germinating laterally from end and intermediate segments constitute a natural group distinct from the group of species producing curved elliptical conidia germinating by two polar germ tubes.

The leafspot disease is probably widely distributed in tropical and subtropical maize-growing regions, having evidently been confused with leaf blight, which occurs in the same territory. It appears not improbable that one type of tassel mold will prove to be identical with the foliar parasite, in spite of the greater length of its conidia.

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