BROWN-SPOT DISEASE OF SOY BEAN [1]

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INTRODUCTION

The investigations reported in this paper were begun late in the summer of 1922, when the writers' attention was first drawn to a leaf-spot disease of soy bean, *Soja max* (L.) Piper, in North Carolina with which a species of Septoria was associated. The crop was at that time practically mature, and considerable defoliation, beginning with the lowermost leaves, was in progress. During the following season the disease was epiphytotic in character, presumably as the result of excessive rains; two protracted rainy periods occurred during that year, one in June and the other in late August and early September, with much damp, warm weather in the interim.

It was evident early in the course of these studies that the disease was very probably identical with one which had first been described in 1915 in Japan by Hemmi [2] under the appropriate common name of "brown-spot disease." Specimens of this disease were first collected in the Province of Kitamai in September of the previous year by K. Miyabe. Hemmi's report [3] shows that it was very common and very widely distributed in 1914 throughout Hokkaido, in the Provinces of Ishikari, Tokachi, and Iburi. It was present that year in every field and was the cause of very considerable damage. In 1915 S. Ito collected it in Honshu in the Province of Echigo. In all probability brown spot has existed in Japan for years, as pointed out by Hemmi, and it occurs in other parts of Asia where soy beans are grown. Miura [4] observed it in Manchuria, and states that it very probably occurs also in Chosen (Korea). Through the kindness of K. Nakata, Kyushu Imperial University, Fukuoka, Japan, the writers received an unnamed collection made June 18, 1914, by S. Takimoto at Suwon, Korea, which proved to be the brown-spot disease. Brief statements by the writers in the Annual Reports of the North Carolina Agricultural Experiment Station for 1923 and 1924 [5] appear to constitute the only records of the occurrence of this disease within the United States. It no doubt occurs in other States where soy beans are grown, but the only definite information on this point is contained in correspondence with J. F. Adams, who noted it in Delaware in 1923.

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[1] Received for publication Jan. 9, 1926; issued August, 1926.

[2] HEMMI, T. A NEW BROWN-SPOT DISEASE OF THE LEAF OF GLYCINE HISPIDA MAXIM. CAUSED BY SEPTORIA GLYCINES SP. N. Sapporo Nat. Hist. Soc. Trans. 6:12-17. 1915.

[3] HEMMI, T. Op. cit.

[4] MIURA, M. [DISEASES OF THE MAIN AGRICULTURAL CROPS OF MANCHURIA.] Agr. Expt. Sta. So Manchuria Ry. Co. Bul. 11, 56 p., illus. [In Japanese. English abstract in Japan. Jour. Bot. 1: (9), 1923.] 1921.

[5] WOLF, F. A. REPORT OF THE DIVISION OF PLANT PATHOLOGY. N. C. Agr. Expt. Sta. Ann. Rpt. 46: 92. [1923.]

——— and LEHMAN, S. G. REPORT OF DIVISION OF PLANT PATHOLOGY. N. C. Agr. Expt. Sta. Ann. Rpt. 47: 83. [1924.]

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SYMPTOMS OF BROWN SPOT

The disease is most noticeable upon the foliage, where it is characterized by brown or reddish-brown angular spots 2 mm. in diameter. It is primarily a leaf-spot disease and causes severe defoliation. It appears also on the stems and pods as the plants approach maturity.

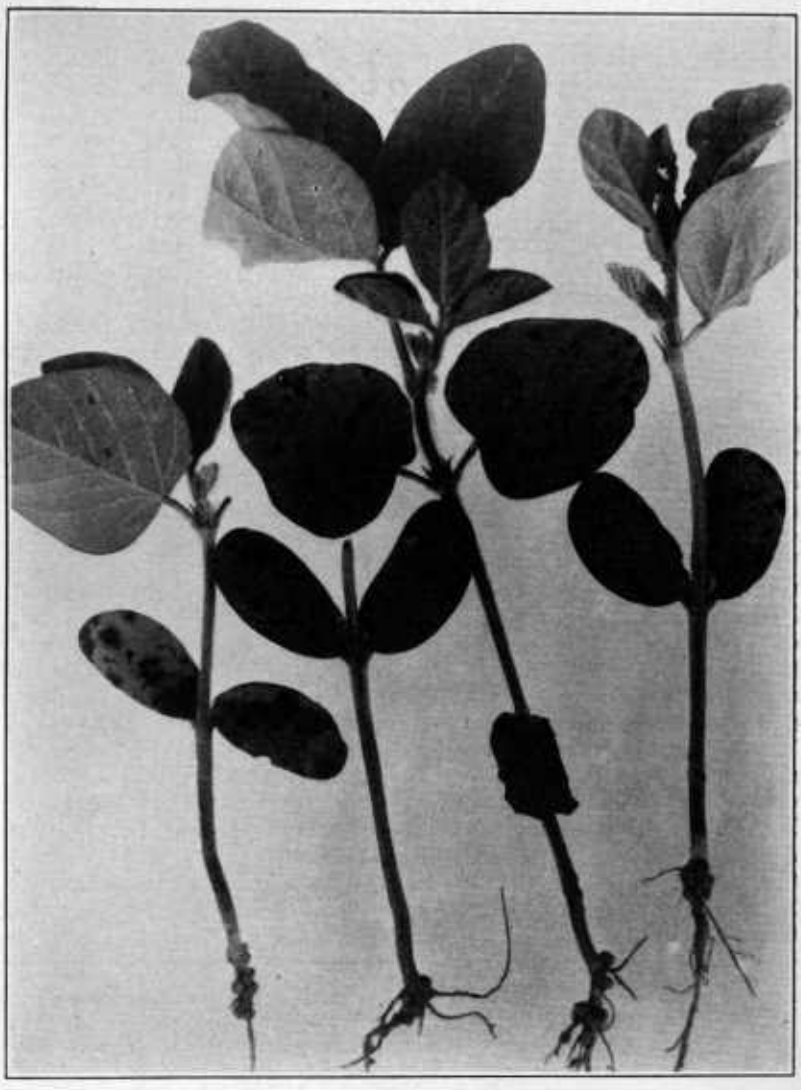

FIG. 1.—Seedling soy beans with brown-spot lesions on cotyledons and first true leaves

The first evidence of disease is apparent early in the season, when the first pair of true leaves have formed. At this time there are irregular dark-brown patches, varying in size from minute specks to areas 4 mm. in diameter, on the cotyledons (fig. 1). Before the cotyledons have shriveled and fallen, infections have become apparent

on the unifoliate or first true leaves. The lesions on these leaves are conspicuously reddish brown on both leaf surfaces. They are angular in outline, being limited by the small veins, and vary in size from 1 to 5 mm. in diameter (fig. 2). The larger ones arise from

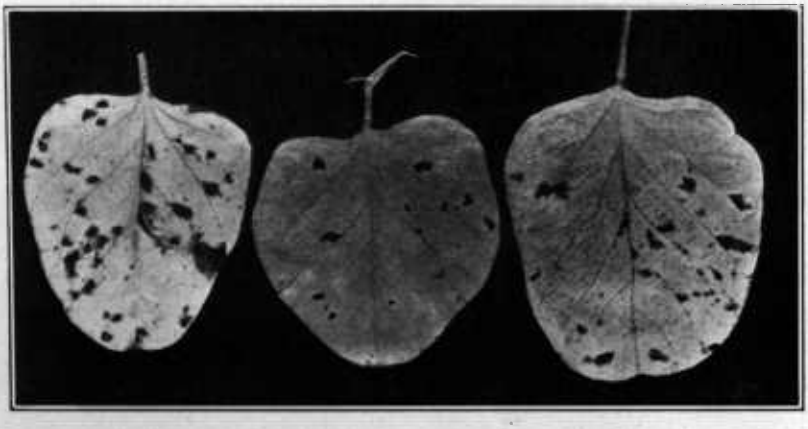

FIG. 2.—Characteristic angular, reddish-brown lesions on unifoliate leaves

coalescence of adjacent spots. The tissues surrounding the diseased areas are pale green at first and then they become decidedly chlorotic, after which the leaves fall off.

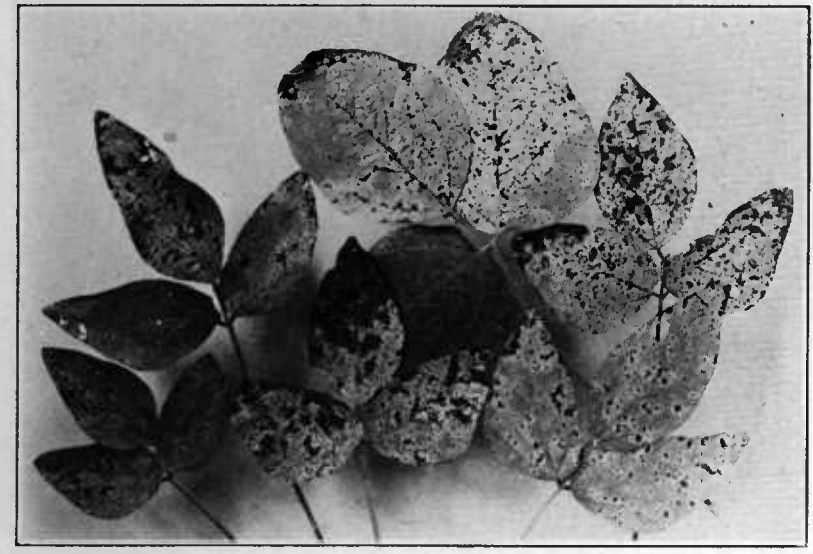

FIG. 3.—Mature leaves showing abundance of lesions, chlorosis, and dead leaf tips

The disease next involves the trifoliate leaves, upon which the spots become more numerous as the season advances and, by confluence of the spots, large, light-brown, irregular areas are formed (figs. 3 and 4). The color gradually deepens until the diseased areas are

dark brown to blackish brown, which appears to be intensified with the loss of the normal green of the intervening tissues. The disease progresses upward on the plants from the lower leaves. By late summer, if conditions are favorable for the development and spread of the causal organism, the older leaves become so spotted that it is difficult to distinguish individual lesions. At this time each leaflet

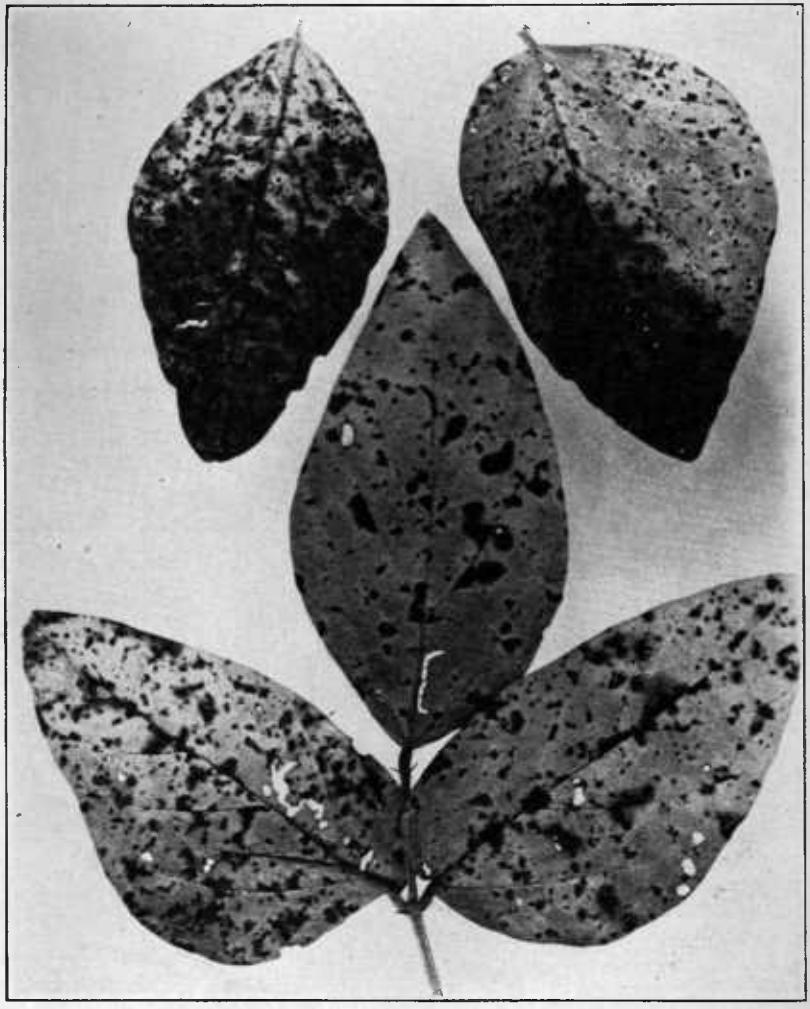

FIG. 4.—A single trifoliate leaf affected with brown spot, and two leaflets with dead tips which would soon have fallen

may bear thousands of specks, with no distinctive microscopic feature except their rusty brown color (fig. 5). Such leaves are prematurely shed from below upward.

The disease on the stems manifests itself by the presence of indefinitely margined, brown discolorations. These lesions vary in size from tiny specks to areas several centimeters in length, and they

may nearly or completely encircle the stems. Tissues adjacent to these discolorations are less green than normal tissues, and as chlorosis advances the diseased areas become more conspicuous (fig. 6). Main stalks, branches, and leaf stalks are all involved in the same manner.

The spots on pods are similar in all respects to those on stems The smaller areas are pinpointlike, whereas the larger ones may come to involve more than half the surface of the pods (fig. 7).

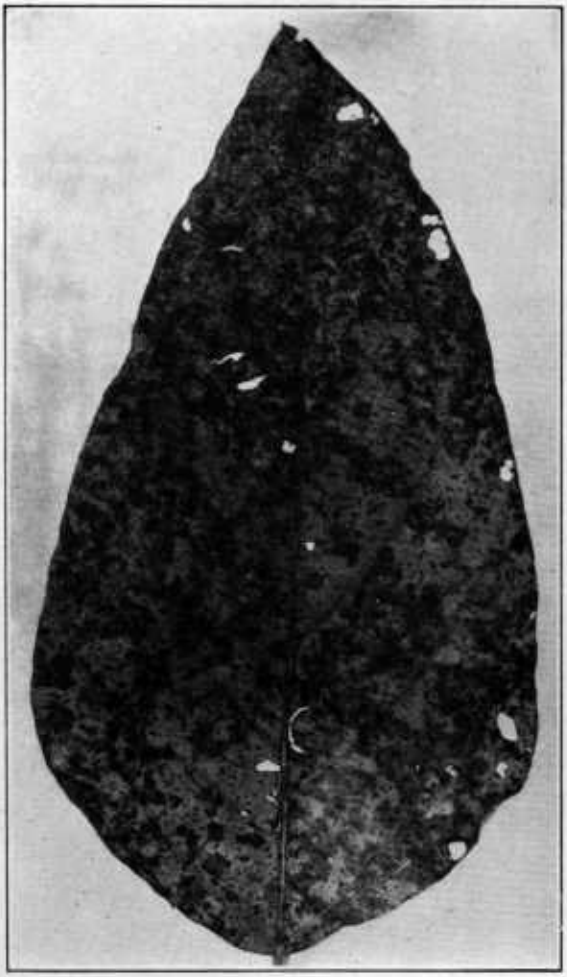

FIG. 5.—A single leaflet in late summer, when the lesions are so abundant that irregular brown patches are formed by confluence of adjacent spots

THE BROWN-SPOT FUNGUS

ISOLATION

Mature lesions on leaves, stems, and pods bear innate, brown pycnidia, and each source has been found to serve equally well as the others in isolation trials. The fungus may readily be isolated when tissues bearing pycnidia are macerated in sterile water and drops of the suspension are spread over the surface of hardened-agar plates. Conidial germination occurs promptly, but it is two or three days before the colonies become visible to the unaided eye. The colonies are olive brown from the first, they develop slowly and remain small. Within three weeks they reach a diameter of 5 to 10 mm. (fig. 8). The mycelium is dense and stromalike. Pycnidia form in abundance on such media as potato cylinders, potato agar, steamed corn meal, steamed tapioca, and sterile bean pods.

MORPHOLOGY

Lesions on stems and leaves sectioned in paraffin and stained show that the pycnidia are immersed and open to the surface by a large pore (fig. 9, D, E). Those in the leaves are globose to conico-globose,

and open mainly to the upper leaf surface. Those on the stems are flattened, because of their position between mechanical tissues. They commonly measure 90 to 100 μ in diameter, varying from 60 to 125 μ. They are either scattered or quite densely gregarious, and when two are sufficiently close they are flattened on the sides in contact. The pycnidial wall is thin and membranaceous (fig. 9, E).

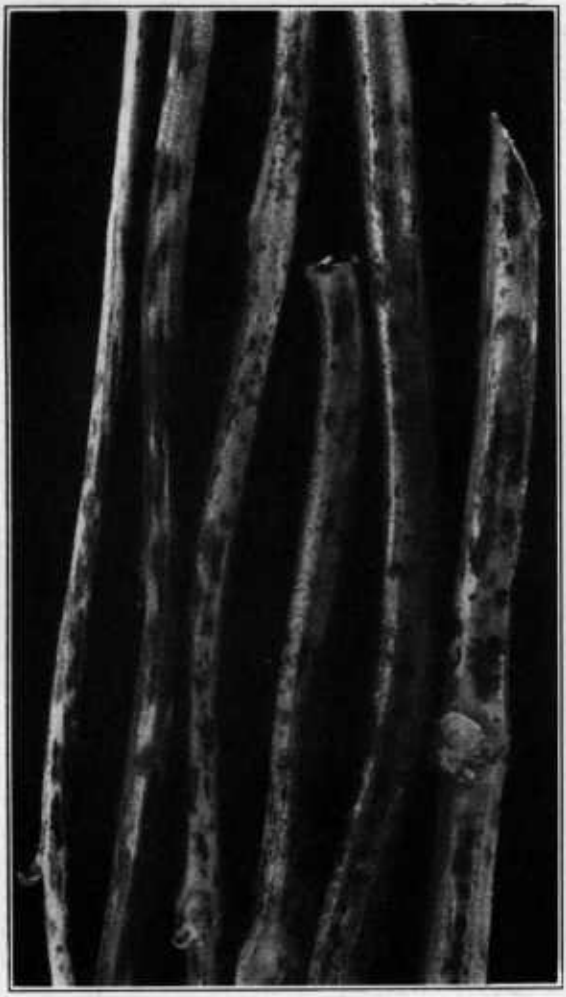

FIG. 6.—Brown spot on stems and branches

The conidia are hyaline, filiform, curved, and indistinctly 1 to 3 septate. The length varies between extremes of 21 and 50 μ; but most of them are 35 to 40 μ in length, with diameter varying from 1.4 to 2.1 μ. On germination there is first an increase in size and the septa become more clearly visible because of the constrictions (fig. 9, B). The first germ tubes usually arise at the ends of the conidia, after which one or two hyphae arise from each of the cells. The mycelium is delicate at first, but in mature colonies it is composed of densely branched, interwoven hyphae the cells of which are brown, thick-walled, and beadlike (fig. 9, C).

INOCULATION EXPERIMENTS

The brown-spot fungus appears never to have been proved, by inoculation trials, to be parasitic. The writers attempted to prove its pathogenicity by use of suspensions of conidia from pure cultures. These suspensions were atomized, in late afternoon, upon healthy plants which had just begun to set seed. Plants growing in beds in the greenhouse were employed in some of the trials, and plants growing out of doors were used in others. When it was found that infections developed so promptly no attempt was made to conserve high degrees of humidity around inoculated plants, and only a few trials were made. In all cases by

the fifth day small, brown discolorations were present in abundance. Characteristic brown-spot lesions had developed a week later, while nearby plants which served as controls remained free from disease. The organism was reisolated from certain of these artificially inoculated plants by use of tissue plantings.

The method of entrance of the fungus was determined by sowing conidia in drops of water on leaves. Then after 16 to 20 hours, by examination in surface view of the epidermis stripped from these leaves, it was observed that the germ tubes enter the stomates (fig. 9, A). As growth continues the hyphae become much branched and ramify between the cells of the leaf tissues.

IDENTITY

The fungus which causes brown spot was compared by Hemmi [6] with *Septoria sojina* Thüm., the only other species known to be parasitic on soy bean, and was found to differ in several essential respects.

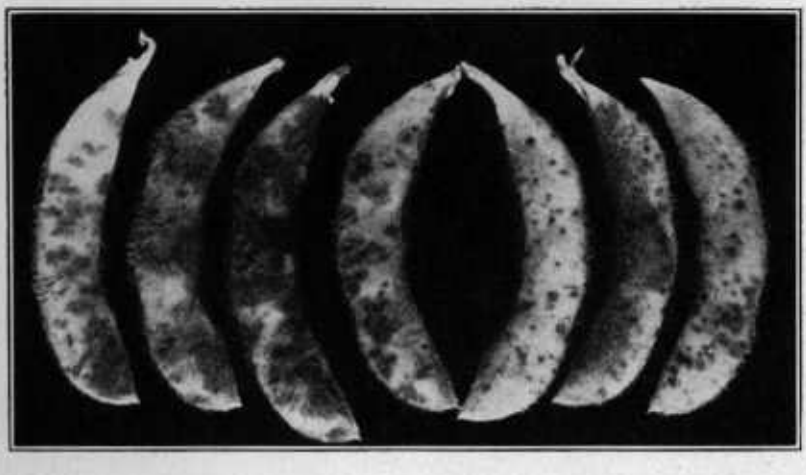

FIG. 7.—Soy-bean pods attacked by the brown-spot fungus

He therefore described it as a new species, *Septoria glycines*. The similarity between the pycnidial and conidial measurements of this fungus and those of the fungus with which the writers have been dealing led to the opinion that they were identical. Fortunately it was possible to establish this identity by comparison with Hemmi's type material, collected July 19, 1914, at Tsukisappu, Ishikari, which was sent to the writers. Specimens collected at Raleigh, N. C., were in turn sent to Hemmi for identification. He stated: "I am of the opinion that the fungus on your specimen is macroscopically as well as microscopically the same as my species, *Septoris glycines* Hemmi." [7]

[6] HEMMI, T. A NEW BROWN-SPOT DISEASE OF THE LEAF OF GLYCINE HISPIDA MAXIM. CAUSED BY SEPTORIA GLYCINES SP. N. Sapporo Nat. Hist. Soc. Trans. 6 : 12-17. 1915.

[7] From a letter dated Mar. 15, 1924, from Takewo Hemmi, Kyo o Imperial University, Kyoto, Japan.

OVERWINTERING AND DISSEMINATION

Diseased leaves and stems from the crop of 1922 and that of 1923 were collected and stored out of doors in unsuccessful attempts to obtain an ascogenous stage. The conidia in such material have been found to survive the winter and be still viable, as shown by germination tests made during March. Refuse from a diseased crop of the preceding year could therefore be expected to serve as a source of inoculum when the same field is returned to soy beans.

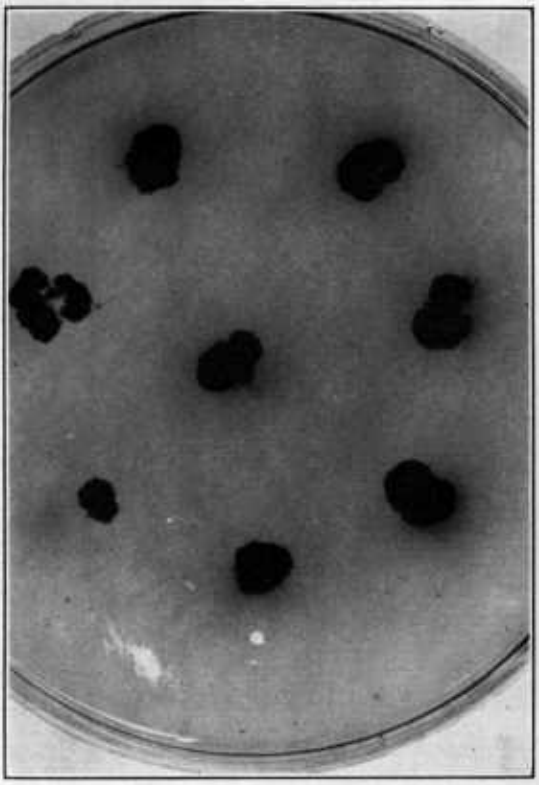

FIG. 8.—Three-weeks-old colonies of *Septoria glycines* on potato agar

The fact that the pods are subject to attack suggests the probability that brown spot is seed-borne and that contaminated seed are the agency by which it is disseminated. To date, the writers have not been able to demonstrate by cultures and by the aid of a microscope the presence of the fungus within the tissues of the seed or of the conidia on the surface of the seed coats. It has been pointed out, however, that the cotyledons bear lesions. This evidence of seed-borne infection is supplemented by the occurrence of brown spot in a small planting in 1924 from seed from a severely diseased crop in 1923. This planting was made on freshly cleared land, and was well isolated from other soy-bean fields. The presence of brown spot in this field appears to be most reasonably accounted for by its introduction with the seed.

VARIETAL RESISTANCE

The observations on this phase of the problem are very meager and cover only the year 1923, when brown spot was epiphytotic. Just as is the case with other diseases of this crop there are very manifest differences in varietal resistance. Among the varieties grown in the experimental breeding plots which were noted to be severely affected are Black Eyebrow, Virginia No. 12, and a considerable number of hybrids of Virginia. Austin, Wilson Black, Midwest,

and Ito San exhibited only a moderate degree of infection. Mammoth Yellow and Haberlandt 38, the two most commonly grown varieties in North Carolina, were only slightly affected. In this last group belong also Laredo, Biloxi, Lexington, Tokyo, Tar Heel Black, and Chiquita.

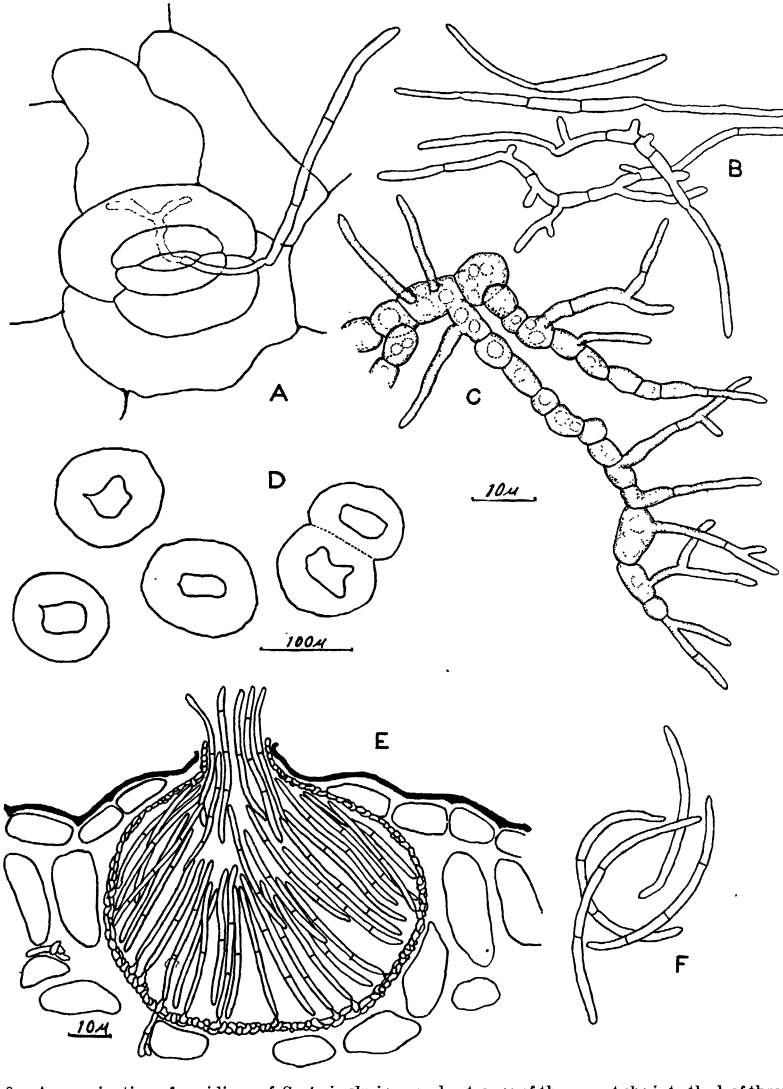

FIG. 9.—A, germination of conidium of *Septoria glycines*, and entrance of the germ tube into the leaf through a stoma; B, germination of conidia of *S. glycines*; C, hyphae from old cultures, with beadlike thick-walled cells; D, surface view of pycnidia of *S. glycines*, showing large ostiola; E, pycnidium in section which opens to the upper leaf surface; F, conidia of *S. glycines*. (A, B, C, and F are drawn to the same scale)

SUMMARY

This investigation deals with a disease of soy beans commonly called "brown spot," first described in Japan in 1915, where it was

collected in several Provinces. It occurs also in Manchuria and Korea, and was first noted in North Carolina in 1922 and in Delaware in 1923.

Brown spot manifests itself by the presence of angular brown or reddish-brown lesions on the foliage. It attacks also the stems and pods.

The disease seems to be seed-borne. It appears first on the cotyledons, spreads thence to the unifoliate leaves, from these to the trifoliate leaves, and causes defoliation from below upward.

Pathogenicity has been established by artificial inoculation. Infection occurs through the stomates, and the mycelium is intercellular.

Brown spot is caused by the imperfect fungus, *Septoria glycines* Hemmi. No ascogenous stage is known. The conidia remain viable during winter on decaying stems and leaves.