SOY-BEAN ANTHRACNOSE[1]

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INTRODUCTION

An anthracnose disease of soy bean, *Soja max* (L.) Piper, was found to be present at the North Carolina Agricultural Experiment Station in 1920, in the plots devoted to investigation of this crop. A brief statement was prepared at that time (*7, p. 57*)[2] which is believed by the writers to have recorded for the first time the occurrence of this disease within the United States, and tentatively assigned to the causal organism the name *Glomerella cingulata* (Stonem.) V. Sch. and S. Since then the disease has appeared at the station in succeeding years and has been found in a number of other localities in the State, which has afforded opportunity for a more intensive study of it. It is the present purpose to describe the disease and to record the results of investigations into the morphology and life history of the causal organism.

HISTORICAL

A survey of available literature on soy-bean diseases shows that anthracnose has been noted only in the Orient. Hemmi (*2, 3*) records the isolation by S. Takimoto of an unnamed species of Gloeosporium from diseased soy-bean pods collected in Suigen, Chosen (Korea), in October, 1915. No inoculation experiments were made with this organism, so proof of its parasitism is wanting.

The same investigator isolated in September, 1917, another anthracnose fungus, which was sent to Hemmi (*2, 3*), and which he identified as *Colletotrichum glycines* Hori. Hori has not published a technical description of this species,[3] although its important morphological features, accompanied by appropriate drawings, are given in a report of investigations by Hemmi (*2*). Neither has this anthracnose fungus been employed in inoculation experiments, but its prevalence on stems and pods at harvest time, together with a knowledge of other species of this genus, was taken by Hemmi to indicate that it was actively parasitic.

DESCRIPTION OF SOY-BEAN ANTHRACNOSE

Soy-bean anthracnose has been observed on the stems and pods, but apparently it does not affect the foliage. Plants in all stages of growth are subject to infection, as shown by field observation and

[1] Received for publication Jan. 9, 1926; issued August, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 389.
[3] This statement is taken from a letter from Dr. Takewo Hemmi, Kyoto Imperial University, Kyoto, Japan, dated Aug. 24, 1924.

inoculation experiments. The disease reaches its most destructive stage of development in late summer, when the pods are maturing, especially during rainy periods. At this time no definite lesions such as characterize other anthracnose diseases are present, but the affected portions are thickly covered with setose, black acervuli (fig. 1). The macroscopic appearance somewhat resembles the pod and stem blight, *Diaporthe sojae* Lehm., but the fruit bodies of the two may be distinguished with certainty by the use of a hand lens. Moreover, the acervuli are irregularly disposed at the surface of affected stems, with no tendency to arrangement in lines as occurs with the Phomopsis stage of the pod and stem blight fungus. Affected plants which are scattered irregularly throughout the field may be readily recognized because of their premature death.

The first evidence of disease is the presence of minute pinpoint-like dark discolorations at the loci of infections, which are visible under favorable conditions four days after inoculation. The surrounding tissues become brown and dead, and by the fusion of individual lesions large areas are formed which may involve the entire surface of the stems and pods. If the pod and pedicel are attacked early enough, seed formation may be partly or entirely inhibited. This may be accomplished by the invasion of the tissues of the pedicel, and in consequence the pods fail to fill, or the mycelium may penetrate the pod wall directly and attack the developing seed. Shrunken seed from such pods invariably yield pure cultures of the anthracnose organism when such seeds are subjected to surface disinfection and plantings are made from seed-coat or cotyledonary tissue.

There is little or no shrinking and collapse of stems because of the large proportion of woody tissue and small proportion of parenchyma.

CAUSAL ORGANISM

ISOLATION

Isolations have been made from diseased stems and pods bearing the conidial stage, from infected seed, and from old, decaying stems bearing the ascogenous stage. Suspensions of conidia in sterile water have been employed in plantings in hardened-agar plates, and this has been found to be a satisfactory method of isolation. Stems bearing the Glomerella stage have been placed on bibitory paper in the tops of inverted Petri dishes containing hardened agar. The ascospores are forcibly ejected, lodge on the surface of the agar, and yield pure cultures of the organism by this method. No efforts have been made to make single-spore cultures from either conidia or ascospores, but blocks of agar containing several spores have been transferred to culture tubes. The cultures from all sources, however, have yielded the same type of growth. The mycelium is whitish and rather loose at first, but soon turns dark and the substratum becomes blackened. Within two or three weeks black acervuli bearing pink spore masses and black perithecia have formed. Some strains from conidia have borne conidia alone, even after repeated transfer and cultivation on a variety of media; other strains from ascospores, however, have consistently yielded both the conidial and ascosporic stages when cultivated upon the same kind of media.

MORPHOLOGY

As the soy-bean anthracnose fungus was at first thought to be identical with *Glomerella cingulata*, it was grown in parallel cultures with the apple bitter-rot fungus isolated from lesions on apple fruits. The decaying fruits from which these isolations were made

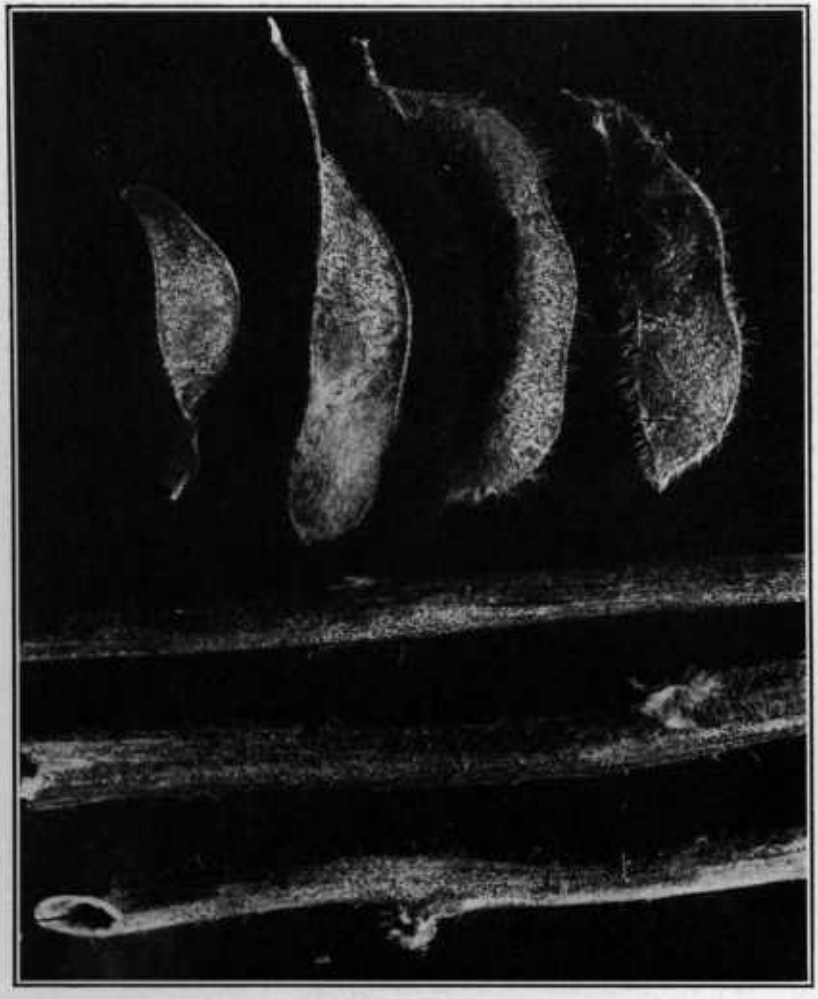

FIG. 1.—Soy-bean anthracnose on pods and stems, showing the distribution of black acervuli

bore at the same time both the conidial and ascogenous stages of the bitter-rot fungus. These in turn fruited in culture with the formation of both stages, so that it was possible to make comparative measurements of the organism from apples and the one from soy beans both from natural sources and from artificial cultures. Table 1 contains a summary of the relative length of ascospores of both organisms from cultures.

TABLE 1.—*Relative length of ascospores of the soy-bean anthracnose fungus and the apple bitter-rot organism*

[One space=3.75 μ]

Organisms	Number of spores distributed according to length in spaces															Variation in length (μ)	Common length (μ)
	Spaces																
	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	
Soy-bean anthracnose fungus	0	0	6	27	8	74	23	70	30	17	14	3	3	1	1	2	13.12–43.35	18.75–28.12
Apple bitter-rot fungus	16	62	45	61	24	16	0	0	0	0	0	0	0	0	0	0	9.37–18.75	11.25–15.00

Comparing the ascospores of the two organisms grown in parallel cultures, it will be noted that those of the soy-bean organism are considerably larger than those of the apple organism, the former varying in length from 13.12 to 43.35 μ, and the latter from 9.37 to 18.75 μ. These differences are more apparent when it is observed that 75 per cent of the ascospores of the bitter-rot fungus range from 3 to 4 spaces, or 11.25 to 15.00 μ in length, whereas 76.6 per cent of those of the soy-bean anthracnose range from 5 to 7 spaces, or 18.75 to 26.25 μ. They range in width from 3.9 to 5.0 μ, and 4 to 6 μ, respectively.

When ascospores are taken directly from decaying host tissue they appear to be more uniform in size, i. e., there are fewer either longer or shorter than the average, than on artificial media. The average measurements accord, however, without regard to the substratum upon which they are grown, and agree well with measurements of the bitter-rot fungus given by Clinton (*1*) and by Von Schrenk and Spaulding (*4*). Their extremes range from 12 to 22 by 3.5 to 5 μ.

The asci of the soy-bean fungus vary from 70 to 106 μ in length and from 9.5 to 13.5 μ in width, with an average length of 80 μ and an average width of 12 μ. The asci of the apple bitter-rot fungus vary from 55 to 70 μ in length and from 9 to 10 μ in width, with an average length of 60 μ and an average width of 9 μ. The perithecia of the anthracnose fungus vary from 220 to 340 μ in diameter. The perithecia of the apple bitter-rot fungus vary from 125 to 250 μ in diameter, which measurements accord with those given by Clinton (*1*) and by Von Schrenk and Spaulding (*4*).

The Colletotrichum stage of soy-bean anthracnose bears conidia which vary in length from 16 to 25 μ, but the most common size is 20 to 22 μ long by 3.75 to 4.5 μ wide. There are numerous brown setae which vary from 30 to 200 μ in length and from 4 to 6 μ in width. It agrees well with Hemmi's (*2*) description of *C. glycines*, in which the conidial size is given as 16 to 23 by 3.8 to 4.2 μ, with 15 to 40 setae in each acervulus.

The Gloeosporium stage of the bitter-rot fungus is given by Clinton (*1*) and by Von Schrenk and Spaulding (*4*) as having extreme lengths of 10 to 28 μ and extreme widths of 3.5 to 7 μ, with 12 to 16 by 4 to 5 μ as a common size. These measurements agree well with those made by the writers. Measurements of 440 conidia, half of

which were taken from culture on potato-dextrose agar and the other half from a decaying apple, showed an extreme range in length of 6 to 22 μ with 11 to 16 μ as the most common. The conidia grown in culture exhibited a wider range and averaged slightly smaller than those grown on the fruit..

The cultures of soy-bean anthracnose and apple bitter rot exhibit readily distinguishable differences, as shown in Figure 2. Colonies of the former are whitish at first, but soon become smoky black with darkening of the substratum; colonies of the bitter rot are also whitish at first, but become darker (olive tinted) only around the perithecial stromata and acervuli.

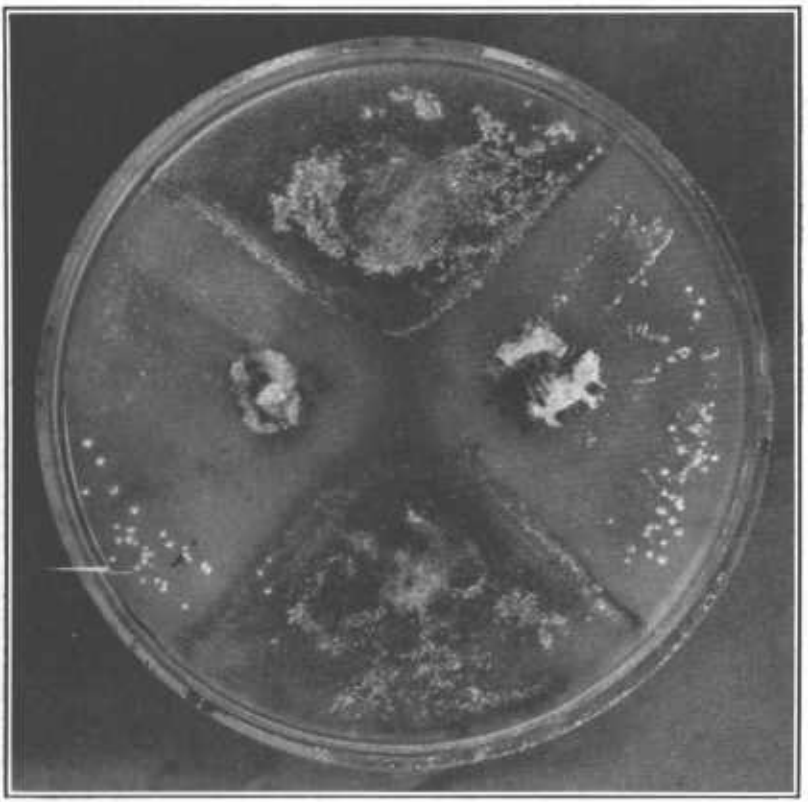

FIG. 2.—The soy-bean anthracnose and apple bitter-rot organism in planted agar plate. The upper and lower quadrants show the bitter-rot fungus, and the right and left quadrants the soy-bean fungus

INOCULATIONS

The inoculation experiments had for their object the determination (1) of pathogenicity of the anthracnose fungus to soy beans, which had not been previously demonstrated, and (2) of the relationship between anthracnose of soy bean and bitter rot of apple. Soy-bean plants, in several series of trials, were inoculated by atomizing them with suspensions of conidia and ascospores from

pure cultures. Certain of the plants were inoculated with the soy-bean fungus, and others with the bitter-rot organism, under identical conditions. Inoculations were made in late afternoon on plants growing in the greenhouse, and the inoculated plants were shaded with newspapers for 24 hours. Each of the series gave uniformly the same results. Within three to four days the first evidences of infection were noted on plants inoculated with the soy-bean organism. Those which were inoculated with apple bitter rot failed to develop any evidence of infection during the entire period in which they were under observation. In another trial three soy-bean plants were inoculated by inserting bits of mycelium and spores into the stems, but here again no infection resulted and the wounds healed promptly.

Reciprocal inoculations were also attempted on several varieties of apples in the following manner: After surface disinfection the fruits were placed in moist chambers and inoculated, on July 2, on opposite sides of the apple. The inoculum, consisting of mycelium and spores from pure culture, was inserted in needle punctures. The tissues surrounding the points of inoculation became involved in decay in the case of all punctures with both organisms. By July 15 the characteristic sunken areas with rings of acervuli had formed in the case of inoculations with bitter rot, whereas the tissues were somewhat darker and less soft in those inoculated with anthracnose of soy bean, and no acervuli had formed on the surface. Furthermore, the characteristic bitter taste was lacking in tissues decayed by the soy-bean fungus.

LIFE HISTORY OF THE FUNGUS

The fungus which causes soy-bean anthracnose has a Colletotrichum or conidial stage and a Glomerella or ascosporic stage. The germination of both types of spores is essentially alike. As an initial step in this process a median septum is generally formed, although many spores remain unicellular. This is followed by the formation of a short germ tube, which is terminated by a brownish appressorium from which the infection tube later arises (fig. 3, F, H). In the inoculation trials in which spores were placed in drops of water on young pods, entrance had been effected within 48 hours by direct penetration of the cell wall (fig. 3, C). The mycelium rapidly extends to adjoining cells and causes their death. It is both inter and intra cellular. Acervuli mature on the lesions in 10 to 14 days.

The fungus passes the winter season either by means of infected seed or of the ascogenous stage. Its occurrence within the tissues of the seed has been established by isolation after surface disinfection, from seed taken from diseased pods. Indirect evidence that anthracnose is seed-borne was obtained by planting seed from affected pods in a situation in which soy beans were not known ever to have been grown, and which was at least half a mile from the nearest soy-bean field. All of the plants in the resultant crop from such seed were seriously affected. In the light of knowledge of other anthracnose diseases it seems reasonable to suppose that the spores which find lodgment on the surface of the seed during harvest could

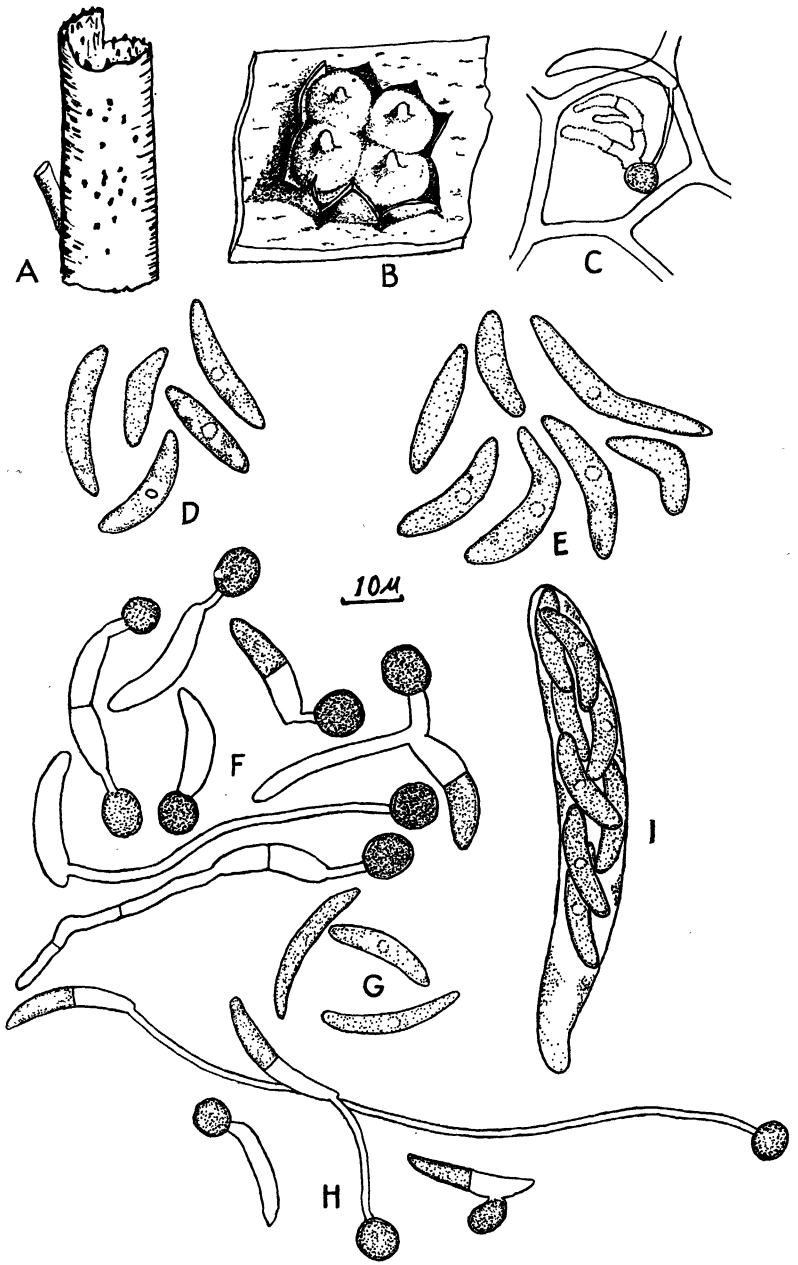

FIG. 3.—A, Diagram of soy-bean stem showing disposition of perithecial stomata; B, diagram of a single stroma bearing four innate perithecia of *Glomerella glycines;* C, germination of conidium and penetration of pod wall by infection tube 48 hours after inoculation, surface view; D, ascospores of *Glomerella glycines* from decaying soy-bean stems; E, ascospores from artificial cultures; F, types of germination of ascospores after 16 hours in water; G, conidia from the Colletotrichum stage; H, germination of conidia with resultant appressoria; I, ascus of *Glomerella glycines*

also serve as a means of carrying the organism over the winter period and of disseminating it in new fields or localities.

In March, 1924, stems of diseased soy beans from the crop of the previous year which had remained in the field during the winter were found to bear the Glomerella stage. The stromata in which the perithecia are embedded arise within the cortex and are at first covered. Each stroma gives rise to one or several perithecia, whose short beaks protrude (fig. 3, A, B). The ascospores are ejected as the asci mature, as previously shown by the isolation trials with inverted agar plates. It is very probable that some of these would come to lodge on plants of the next crop if soy beans were planted in the same field the succeeding year.

IDENTITY OF THE FUNGUS

Plant pathologists would place one or the other of two interpretations upon the foreging studies on the morphologic and pathogenic characters of the soy-bean anthracnose fungus. Some would regard this fungus as specifically identical with *Glomerella cingulata* from apples. Such an opinion is indicated from Shear's (*5*) studies on anthracnose of a large number of different plants, in which he concludes that "all are perhaps only slightly specialized physiological forms of one omnivorous species." The same idea is expressed by Taubenhaus (*6*) from his studies on Gloeosporium on sweet pea, in which he states (1) "that the anthracnose disease of the sweet pea is due to the same organism, *Glomerella rufomaculans* (Berk.) Spauld. and Von Sch., that causes the bitter rot of the apple," and (2) "that *Gloeosporium officinale* E. and E., *Gloeosporium gallarum* Ch. Rich., and *Gloeosporium sp.* from May-apple fruit are also the same as *Glomerella rufomaculans*, since they are all able to produce the typical anthracnose disease of the sweet pea and the bitter rot of the pear."

There are others, among whom are the present writers, who would conclude that soy-bean anthracnose is specifically distinct from the apple bitter-rot fungus. The reasons for this may be summarized as follows: (1) The perithecia, asci, ascospores, and conidia of the soy-bean fungus are larger than those of the apple bitter-rot organism; (2) the conidial stage of the former belongs to the form genus Colletotrichum, the latter to Gloeosporium; (3) when the two are grown on the same substratum the colonies present readily distinguishable differences; (4) the soy-bean organism is pathogenic to soy beans whereas the fungus from decaying apples failed to produce infections on soy beans; (5) the type of decay produced by the fungus from soy beans on apple fruits possesses none of the characteristics associated with apple bitter rot. The fact that the soy-bean fungus causes a decay of apple fruits need not be taken to prove its parasitism, since a mature apple is essentially a culture medium. As is well known, apples can be rotted by a considerable number of fungi not regarded as parasites. More significance certainly should be attached to the fact that the bitter-rot fungus will not attack soy beans than that the anthracnose fungus will produce a rot of apples.

Since it has thus far been impossible to make comparison with specimens from Hori's original collections or with any other collections of *Colletotrichum glycines* from the Orient, the writer does not know with certainty whether the form with which they have been working is identical with *C. glycines*. The descriptive characters mentioned by Hemmi (*2*) accord sufficiently well, however, to lead to the belief that they are one and the same species. Since the perfect stage is herein appropriately described for the first time, and its relationship to *Colletotrichum glycines* established, a brief technical summary is given, as follows:

Glomerella glycines (Hori.) n. n.

Syn. *Colletotrichum glycines* Hori.

PERITHECIAL STAGE.—Perithecia membranaceous, rostrate, caespitose, 220 to 340 μ in diameter, immersed in a stroma. Asci oblong to bluntly clavate, a paraphysate, 70 to 106 by 9.5 to 13.5 μ; ascospores hyaline, slightly curved, blunt-pointed, unicellular, 13.12 to 43.35 μ in length, chiefly 18.75 to 28.12 μ, by 4 to 6 μ in width.

Hab.: On decaying stems of *Soja max* (L.) Piper.

CONIDIAL STAGE.—Lesions on stems and pods indefinite in outline. Acervuli black, setae numerous, brown, 100 to 200 μ in length, Conidia hyaline, curved, bluntly tapered, unicellular, 16 to 25 $\times$ 3.75 to 4.5 μ, with 20 to 22 μ as the most common length.

Hab.: Parasitic on stems and pods of *Soja max* (L.) Piper.

SUMMARY

Soy-bean anthracnose affects the stems and pods of this crop and its presence in North Carolina was first observed in 1920. It is believed to be identical with a disease collected in Chosen in 1917 and ascribed to *Colletotrichum glycines* Hori.

The disease is characterized by the presence of numerous black acervuli, uniformly scattered over the surface of the affected parts. It causes premature death of the plants and failure of the pods to fill properly.

The organism is seed-borne, and exists as a mycelium within the seed and as spores adhering to the exterior.

When the soy-bean anthracnose fungus is compared with the fungus which causes apple bitter rot, with which it was at first believed to be identical. it is found to be morphologically distinct, to be of different appearance in culture, and to react differently in reciprocal inoculations.

The ascogenous stage has been found on diseased stems which overwintered in the field, and has been developed in culture. The name *Glomerella glycines* (Hori) n. n. is therefore proposed as a synonym for the conidial stage name *Colletotrichum glycines*.

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