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First Occurrence of Soybean Southern Stem Canker in Paraguay

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Abstract

Southern stem canker of soybean was detected on more than 10 cultivars in 3 and 13 locations in Itapua and Alto Parana prefectures in Paraguay, respectively. Symptoms and signs on the diseased soybean plants were identical with those reported for the disease previously. Morphology and characteristics of the pathogen both on the host and artificial media agreed with the descriptions of *Diaporthe phaseolorum* f. sp. *meridionalis* (anamorph: *Phomopsis phaseoli* f. sp. *meridionalis*). About 150 isolates were obtained from the diseased soybean plants. Soybean plants inoculated with some of the isolates showed symptoms typical of the disease 2-3 weeks after inoculation. It was shown for the first time that the disease occurred in Paraguay.

Discipline: Plant diseases
Additional key words: *Diaporthe phaseolorum* f. sp. *meridionalis, phomopsis phaseoli* f. sp. *meridionalis, Glycine max*

Introduction

A soybean (*Glycine max*) disease which is similar to stem canker caused by *Diaporthe phaseolorum* var. *caulivora* has occurred and spread over the southeastern part of the United States since 1973[2]. It was designated as "Southern stem canker" because of the physiological and morphological differences between the pathogens of the disease and stem canker, though the pathogen was considered to be the southern (bio)type or isolates of *D. phaseolorum* var. *caulivora* at that time[4]. Later, Morgan-Jones[9] suggested that *Diaporthe phaseolorum* f. sp. *meridionalis* (anamorph : *Phomopsis phaseoli* f. sp. *meridionalis*) and *D. phaseolorum* f. sp. *caulivora* were the pathogens of the former and the latter diseases, respectively, on the basis of the differences between the two fungi reported by several authors[3, 4, 6, 8].

Recently, southern stem canker has been observed in the southern part of Brazil in 1989/1990 and has been one of the most serious constraints on soybean production there[11]. It was assumed that the seed-borne disease[1, 11] had already spread to Paraguay from Brazil, because Paraguay had imported a large number of soybean seeds from Brazil in recent years.

The present study was carried out to analyze the occurrence and distribution of soybean southern stem canker in Paraguay. Results of an inoculation experiment as well as identification of the pathogen are also reported here.

Distribution of soybean southern stem canker in Paraguay

From 4 to 20 February 1992, soybean plants cultivated in various locations in Itapua and Alto Parana prefectures were observed and the diseased plants were collected for examination and isolation of the pathogen in the laboratory. Diseased stems of the collected materials were cut into small pieces and kept in moist chambers at 25 - 32 °C after

Table 1. Locations and soybean cultivars in which southern stem canker was detected in Paraguay, 1992

Location	Diseased cultivar	Date	Collection no.[a)]
(Itapuá pref.)			
Colonia Pirapó	?	4 Feb.	18*
〃	?	13 Feb.	29*
Fuji	IAC-4	18 Feb.	51*
Obligado	Bragg	9 Mar.	57*
(Alto Paraná pref.)			
Naranjal	Bragg	6 Feb.	25*
〃	Bragg	20 Feb.	47
〃	FT-1	6 Feb.	26*
〃	Yguazú	6 Feb.	27*
〃	Paraná	20 Feb.	48*
〃	FT-1	20 Feb.	49*
〃	BR-4	20 Feb.	50*
Hernandaria	Yguazú	18 Feb.	42*
Cruce Gleba 8	Yguazú	18 Feb.	34*
〃	Bragg	18 Feb.	33
〃	BR-4	18 Feb.	32*
Gleba 6	Yguazú	18 Feb.	41
San Roque	Yguazú	18 Feb.	31*
〃	Ocepar 9	18 Feb.	—
Colonia Yguazú	Bragg	19 Feb.	35, 36*
〃	Bragg	1 Mar.	53
〃	Ocepar 9	19 Feb.	37*
Pikyry	Bossier	19 Feb.	38*
Pikyry Gleba 2	Yguazú	19 Feb.	40*
San Esteban	Yguazú	19 Feb.	39*
Campo 8 (km 224)	Paraná	19 Feb.	44
〃	Torcaza	19 Feb.	45*
〃	Bragg	19 Feb.	46
Torocuá	Yguazú	20 Feb.	52*

a) : *Diaporthe phaseolorum* f. sp. *meridionalis* was isolated from the collection marked with*.
Materials of both No. 25 and 47 were collected in the same field.
Materials of No. 52, 53 and 57 were collected by other persons.

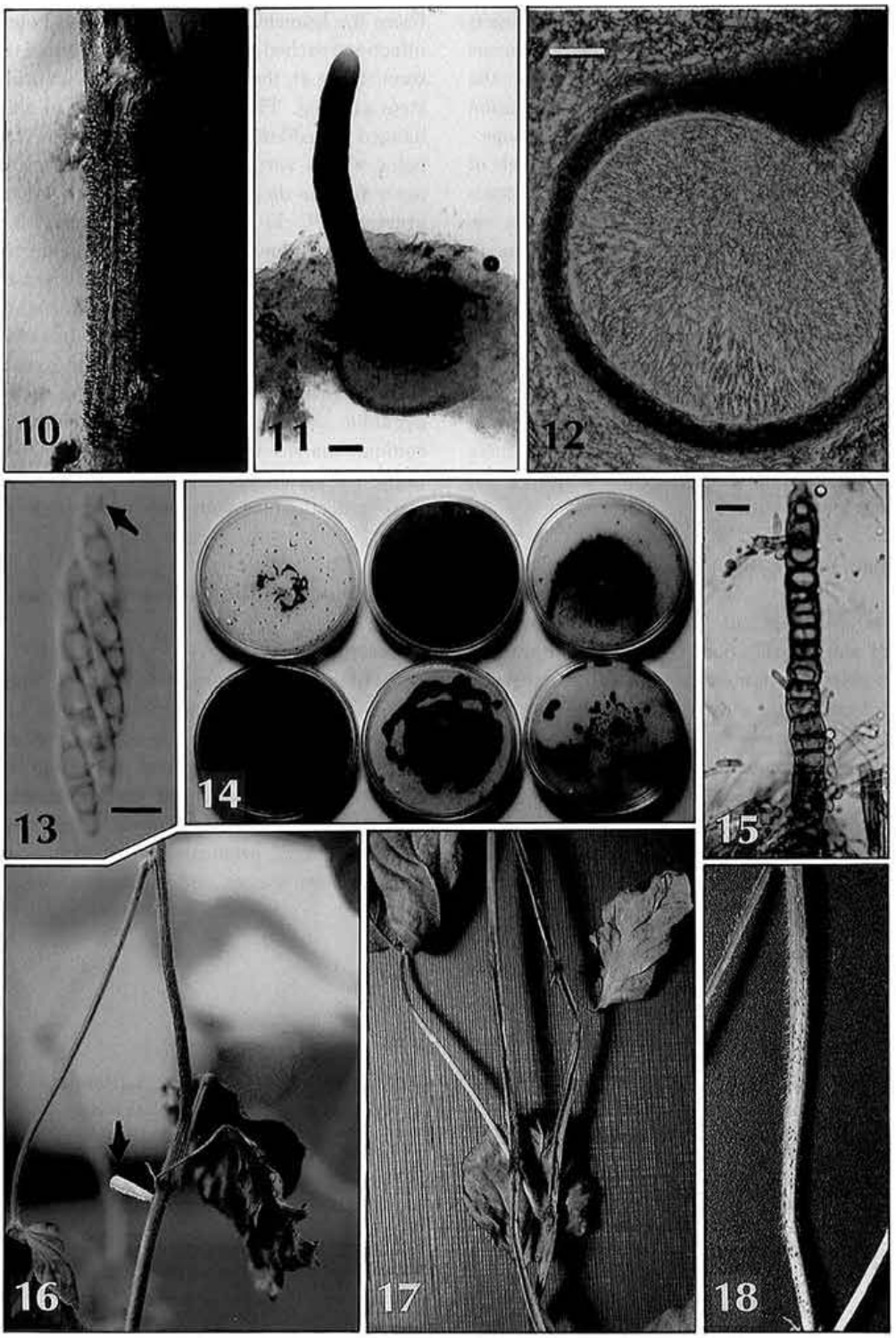
Fig. 1. Distribution of southern stem canker of soybean in Paraguay, 1992
Some locations listed in Table 1 were omitted.

Plate 1. A soybean (cv. Bragg) field in Naranjal, Alto Paraná prefecture damaged by southern stem canker

Plates 2-7. Symptoms of southern stem canker of soybean (cv. Bragg)
2 : Spotty lesions on a stem in early stage of infection
3 : Elongated and slightly sunken lesions on stems with a lighter center and darker margins
4 : A lesion extending over one side of a stem, bearing pycnidial initials
5 : A stem sectioned obliquely, showing browning of pith
6 : Interveinal chlorosis and necrosis of leaves in advanced stage of infection
7 : A dying plant with dead leaves retained

Plates 8-9. Morphology of the pathogen of southern stem canker, *D. phaseolorum* f. sp. *meridionalis*
8 : A cross-section of pycnidium in stem tissues (scale : 50 μm)
9 : Bi-or tri-guttulate conidia produced on host (scale : 10 μm)

Plates 1-9



Plates 10-18

surface-sterilization. The stem fragments that were infected with *D. phaseolorum* f. sp. *meridionalis* showed pycnidia produced within 24 hr. Diagnosis of the disease on the materials was made on the basis of the symptoms as well as by the observation of the pycnidia and conidia under the microscope.

The pathogen was detected from the materials of more than 10 cultivars collected in 3 and 13 locations in Itapua and Alto Parana prefectures, respectively (Table 1). Distribution of the disease in the prefectures is shown in Fig. 1. Soybean southern stem canker, therefore, was found to occur in the main-soybean producing areas of Paraguay. The soybean cultivars, Bragg, Bossier, Paraná and FT-1 were severely infected in general and the percentages of diseased plants per field were higher in Naranjal and Pikyry, Alto Parana prefectures (Plate 1) than in the other locations during the period mentioned above.

Symptoms of the disease

Symptoms at various stages of infection were observed during the pod growing stage in the soybean plants cultivated in Naranjal. Progression of the infection was estimated from the results of observation of the diseased plants as follows : discrete fluting and spotting on the stems developed to elliptical or elongated spotty lesions, with a black or reddish-brown color (Plate 2). The lesions generally extended over one side of the stem, reaching a few cm in length and becoming sunken cankers with a light-colored center and darker margins (Plate 3). From the branching point of the lateral stems, the infection reached the top and the bottom of the main stem (Plate 4), then the pith and the plants died by stem girdling. The color of the pith at this stage hanged to reddish-brown (Plate 5). This change of color was a very important and distinctive character for the diagnosis of the disease as reported previously[11]. In this advanced stage of infection, the death of some leaves after interveinal chlorosis and necrosis was also typical of the disease (Plate 6), and the dead leaves remained on the stems even after the whole plant died (Plate 7). In some cases, many black dots appeared in linear rows on the stem lesions (Plate 4). They corresponded to the pycnidia of the pathogen, from which yellow conidial masses were secreted under moist conditions. The symptoms of the disease described above were in accordance with those reported previously[1, 2, 11].

Morphology of the pathogenic fungus

1) Anamorph

Pycnidia were formed singly, their shape was conical to lenticular, they were single- to triple-ostiolate, not beaked at the ostioles, yellowish to grayish brown, darker around the ostiole (Plate 8), 160–450 μm in diameter, 80–150 μm in height, the ostiole was 20–130 μm in diameter. Only the α-type of conidia were produced. They were one-celled, with various shapes and sizes, ellipsoid, oblong,

Plates 10–15. Morphology of the pathogen of southern stem canker, *D. phaseolorum* f. sp. *meridionalis*
10 : Large amount of perithecial necks emerging from an incubated soybean stem
11 : A flask-shaped perithecium with long neck (scale : 100 μm)
12 : A vertical section of perithecia produced on PDA, containing numerous asci (scale : 50 μm)
13 : An ascus with an apical ring (arrow) containing 8 ascospores (scale : 5 μm)
14 : Reverse view of colonies of the pathogen cultured on PDA at 28–33°C for 20 days, forming black stromata
15 : Chlamydospores produced in an agar medium (scale : 10 μm)

Plates 16–18. Symptoms and signs on soybean (cv. Bragg) inoculated with isolates of the pathogen
16 : Wilt of leaves and a brown lesion on stem near the inoculum (arrow), 2 weeks after inoculation
17 : Stem pieces sectioned longitudinally, browning of pith, 2 weeks after inoculation
18 : Pycnidial initials formed on a dead stem, 16 days after inoculation

clavate, or allantoid, hyaline, thin-walled, biguttulate or triguttulate (Plate 9), 4.6-12.0 × 1.7-3.0 μm.

2) Teleomorph

Perithecia were observed only on a few samples under natural conditions in summer. When the materials were kept at 5-10°C for 3 days before incubation in moist chambers at 25-33°C under natural light from windows, several perithecia were produced on the stem pieces of some of the materials 3 weeks after incubation (Plate 10). On the other hand, most of the isolates of the pathogen produced perithecia *in vitro* as mentioned below.

Perithecia were borne singly, embedded in stromata immersed in host tissues or agar media, flask-shaped, each with a long neck, brown to black (Plate 11), containing numerous asci, 280-450 μm in diameter, 250-300 μm in height (Plate 12), the neck was 90-120 μm in width and 120 μm to more than 1 mm in length. Asci were clavate to cylindrical, unitunicate, hyaline, with an apical ring, containing 8 ascospores, 35-41×5.0-7.8 μm (Plate 13). Ascospores were two-celled, fusiform, hyaline, 9.6-12.2×2.9-3.5 μm, biguttulate for each cell (Plate 13).

Morphology of the pathogen described above agreed well with that reported previously for *D. phaseolorum*[7, 10] and the "southern (bio)type or isolates" of *D. phaseolorum* var. *caulivora* (=*D. phaseolorum* f. sp. *meridionalis*)[1, 2, 8].

Characteristics of the pathogen on artificial media

After the transfer of conidial masses from the incubated stem pieces of the diseased soybean plants infected with the fungus to an agar medium, ca. 150 isolates of *D. phaseolorum* f. sp. *meridionalis* were obtained from the 21 samples listed on Table 1.

Colonies of the isolates cultured on PDA plates at 30-33°C for 20 days showed a well-developed aerial mycelium with a buff to tan color (Plate 14). A large number of chlamydospores were observed among the submerged hyphae of the dark-pigmented isolates (Plate 15). In most of the isolates, pycnidia and relatively large stomata with an irregular shape were produced on the medium 1-2 weeks after incubation at 28-33°C under black light (12 hr/day) (Plate 14). Perithecia also began to develop more than 3 weeks after culture on PDA at 15-20°C. These characteristics were in accordance with those reported previously[1, 8], though the morphological appearance of the pathogen, depending on the isolates, appeared to vary on artificial media.

Thirteen isolates representative of the sources sampled which were cultured on PDA plates at various temperatures are shown in Fig. 2. Comparison of the diameter of the colonies grown after 70 hr, indicated that the optimum temperature for mycelial growth of the fungus ranged between 28

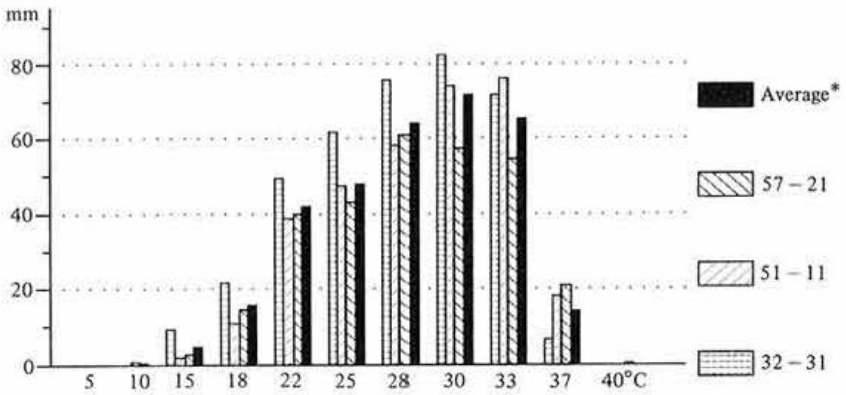


Fig. 2. Mycelial growth of isolates at *D. phaseolorum* f. sp. *meridionalis* at various temperatures
* Average diameter of 13 isolates cultured on PDA plates in darkness for 70 hr.
Isolate 57−21 : a slow grower, 51−11 : the most thermophilic isolate, 32−31 : the fastest grower.

and 33°C (Fig. 2). The pathogen grew better at a relatively high temperature as reported by Keeling[6].

Pathogenicity of the isolates

A soybean cultivar, Bragg, which was known to be susceptible to southern stem canker[2,11], was seeded in plastic pots. Twenty five days old plants were inoculated with 5 isolates each from the materials of the collections No. 25, 26 and 27 (Table 1) by the toothpick method[5]. The inoculated plants were covered with plastic bags for 3 days and kept in a green house controlled at 28-33°C. In most cases, the symptoms and signs such as brick-colored lesions on stems (Plate 16), browning of pith (Plate 17), death of whole plant and pycnidial formation on stems (Plate 18) were observed 2-3 weeks after inoculation. The same fungus was isolated again from some of the inoculated plants after the appearance of the symptoms. The No. 27 isolates caused rapid death of the plants, whereas the death of the plants was delayed in the case of No. 26 isolates. The findings suggested the presence of a differentiation of pathogenicity among the isolates of the pathogen, though they were all pathogenic to soybean.

Conclusion

The present studies revealed for the first time that southern stem canker of soybean, "cancro del tallo de la soja" in Spanish, caused by *D. phaseolorum* f. sp. *meridionalis* (*P. phaseoli* f. sp. *meridionalis*) occurred in the main soybean-producing areas of Paraguay.

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