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Zonate Leaf Spot of Sorghum Caused by *Gloeocercospora sorghi* in Korea

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***Gloeocercospora sorghi* caused the zonate leaf spot on sorghum (*Sorghum bicolor* Moench) in fields in Korea. The zonate spots were conspicuous on sorghum leaves as circular, reddish purple bands alternating with tan or straw-colored areas, which formed a coarsely zonate pattern. The lesions often occurred in semicircular patterns along the margin of leaves. The fungus produced sporodochia on the surface of infected leaves in a moist chamber. The conidia, born in a pinkish to salmon-colored slimy matrix, were hyaline, elongate to filiform, straight or slightly curved, 3- to 17-septate, and of variable length (28-197×1.6-3.4 μm). *G. sorghi* isolated from diseased leaves of sorghum caused characteristic reddish-brown water-soaked leaf spots when inoculated to seedlings.**

Keywords : *Gloeocercospora sorghi,* sorghum, zonate leaf spot

Sorghum (*Sorghum bicolor* Moench) is cultivating at present throughout Korea as a food and feed crop, which was traditionally grown on a small scale. The plant may be attacked by various foliar pathogens. During the disease survey in the late summer of 1998 and 1999, we found lesions forming concentric or zonate patterns with irregular borders on the leaves of sorghum in fields.

Some diseased leaves were placed in a moist chamber, while others were plated onto water agar. A fungus consistently isolated from young and mature leaf lesions resembled in many ways *Gloeocercospora sorghi* that is known to cause leaf spots on sorghum. It was first described as *Ramulispora andropogonis* Miura by Miura (1921). Later, following the ideas of Bubak (1916), the name was changed to *Titaeospora andropogonis* (Miura) by Tai (1932). By Bain and Edgerton (1943), the fungus was definitely determined as *G. sorghi.* On sorghum it was reported to occur on both leaf blade and leaf sheath (Odvody and Madden, 1984). In Korea, *G. sorghi* from sorghum has not been reported previously. Instead, *Ramulispora sorghi* (Ellis & Everh.) Olive & Lefferre (Syn., *R. andropogonis* Miura) was recorded as the causal agent of sooty stripe of sorghum in the list of plant diseases in Korea (1998). There has been no report about this disease.

Therefore, the purpose of this study was to identify the causal organism and examine its pathogenicity to sorghum.

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Materials and Methods

Disease survey and collection. Sorghum diseases in farm lands were surveyed from July to mid September of 1998 and 1999 to evaluate disease incidence, symptom expression, and environmental conditions associated with the diseases. The zonate leaf spots were observed in sorghum-growing areas through the coastal and the mountainous regions of Korea. Diseased leaves were collected at maturity stages. Some leaves were placed in a moist chamber and others were dried under paper presses, cut to appropriate sizes, and placed in herbarium packets.

Isolation and identification. Leaf lesions were surface-sterilized with 1% NaOCl for 1 min, rinsed with sterile distilled water, air-dried, and plated onto water agar. They were incubated under longwave UV (F40BLB, black light, 40 W) for 5 days at 24°C. The causal organism was identified by the presence of spore masses with typical conidia produced either on lesion tissue or from sclerotia. Production of typical sclerotia in infested leaf tissues before or during incubation was also used to identify the pathogen.

Pathogenicity. Inoculum was produced by streaking plates of modified V-8 juice agar (200 ml of V-8 juice + 3 g $CaCO_3$/L) with conidia of each isolate (Miller, 1955) and by incubating them under longwave UV at 24°C for 7 days. Conidial masses were the predominant fungal structures formed. Conidia from the plates were suspended in distilled water (6×10^4 conidia/ml) and spray-inoculated onto 30-day-old sorghum seedlings. Inoculated plants were incubated at 100% RH at 27°C for 24 hrs and transferred to a greenhouse bench at 25°C. All inoculated plants were observed for symptom development every day for 1 week, and lesions were periodically selected for reisolation.

Results and Discussion

Symptoms and disease incidences. Disease surveys were carried out in 1998 and 1999 to determine the prevalence of diseases in sorghum-growing areas. In the areas where sorghum is traditionally grown, plants were attacked by as many as five or six foliar pathogens. Leaf blight caused by

Exserohilum turcicum and leaf anthracnose by *Colletotrichum graminicola* were found in many areas. Leaf spot was caused by *Septoria pertusa,* and brown leaf spot was caused by *Mycosphaerella holci.* They were the most common diseases on sorghum in Korea. Also, two or more types of symptoms occurred on one leaf. As shown in Fig. 1A, the zonate leaf spot could usually be distinguished by symptoms from other sorghum diseases. Initial symptoms

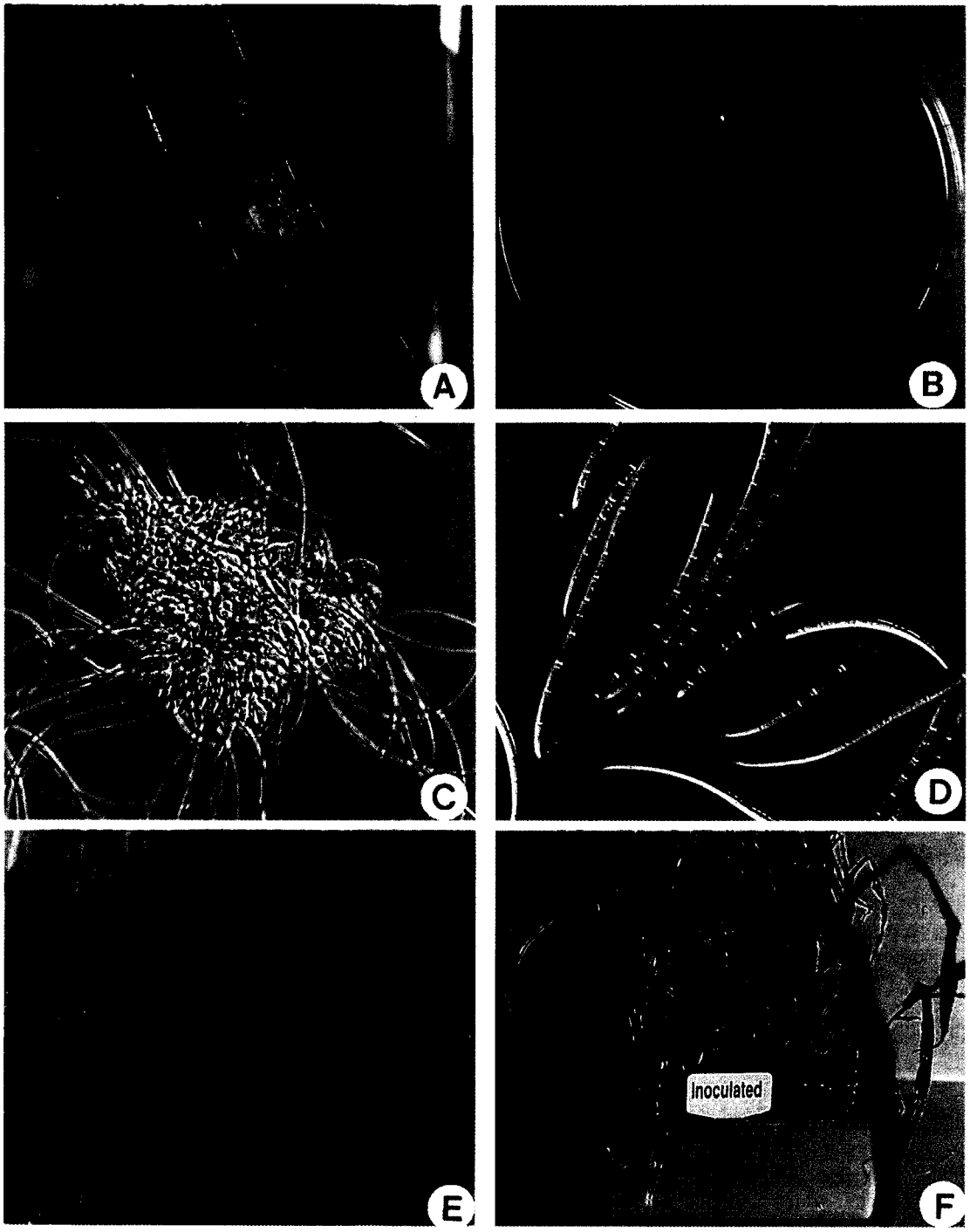

Fig. 1. Zonate leaf spot of sorghum and its causal organism: A, Leaf spots on grain sorghum produced by natural infection; B, Colony of *gloeocercospora sorghi* with young sporodochia and black sclerotia on V-8 juice agar; C, Conidiophores of *G. sorghi,* ×400; D, Conidia of *G. sorghi,* ×500; E, Black sclerotia and sporodochia in necrotic leaf tissues, ×30; F, Leaf spots of sorghum seedlings produced by inoculation with *G. sorghi.*

Table 1. Disease incidences of the zonate leaf spot in sorghum-growing regions of Korea in 1998 and 1999

Surveyed area[a]	Collection date	No. of infested sites/ total no. of surveyed sites	% Diseased plants
Yongwol	Sep. 2, 1998	2/7	27.5[b]
Sosan	Sep. 12, 1998	1/3	30.0
Chechon	Aug. 25, 1999	4/13	6.5
Tanyang	Aug. 25, 1999	3/4	1.3

[a] A total of 10 and 17 areas were surveyed in 1998 and 1999, respectively.
[b] One hundred plants in each site were investigated in three replicates.

of zonate leaf spot were less distinguishable from others, but characterized by a typical type of spotting. The symptoms were small reddish-brown water-soaked leaf spots sometimes with a narrow palegreen halo. They were enlarged, became dark red, and were tended to elongate in a direction parallel to the veins. They finally formed large, semi-circular, or irregular lesions several centimeters in diameter. A smaller lesion usually had a light brown center, surrounded by a light to darkred border, but in larger lesions there were frequently alternations of dark and light zones. The leaf lesions occurred along the margins or midrib, or they covered the entire leaf when infection was heavy.

As shown in tabulated data (Table 1), the zonate leaf spot was observed in Yongwol and Sosan among the 10 areas surveyed in 1998, and Chechon and Tanyang among the 17 surveyed areas in 1999. The incidence of zonate leaf spot in fields of Yongwol, Sosan, Chechon, and Tanyang was 27.5%, 30%, 6.5%, and 1.3% respectively, but severity was low and no direct losses were evident.

Fungus Characters. When infected leaves were placed in a moist chamber for 24 to 48 hrs, abundant fruiting bodies appeared in and surrounding necrotic areas. The fruiting bodies were sporodochia, which were formed on the surface of leaves. As shown in Table 2, the sporodochia were pink to salmon-colored gelatinous spore masses and were readily visible to the naked eye. The conidiophores (Fig. 1C) were so densely clustered that it was difficult to determine their length and width. In general, they appeared to be short (5-11 μm), hyaline, and either simple or branched. These clusters of conidiophores became so dense as to form bouquet-like aggregates. The conidia (Fig. 1D) were born in a pinkish to salmon-colored slimy matrix, were hyaline, needle-shaped, straight or slightly curved, tapering somewhat from the base to the apex, 3- to 17-septate, and of variable length (28-197×1.6-3.4 μm). *G. sorghi* grew rapidly and usually sporulated abundantly on V-8 juice agar media, with optimum temperature for growth about 28°C (Table 3). Black sclerotia (Fig. 1E) were about 0.1-0.3 mm in diameter, lenticular-spherical in shape, and tended to develop as small raised bodies in lines parallel to the veins and beneath the stomata. The fungus overseasons as black sclerotia (Dean, 1968) formed within the necrotic tissue of old leaf

Table 3. Mycelial growth and sporulation of *Gloeocercospora sorghi* on ordinary culture media at various temperatures

Temperature (°C)	PDA		V-8 juice agar	
	Colony diameter (mm)[a]	Sporulation[b]	Colony diameter (mm)[a]	Sporulation[b]
4	5	-	5	-
8	5	-	5	-
12	8	-	7	+
16	17	+	22	+
20	45	+	43	+
24	80	+	66	+++
28	87	+	72	+++
32	75	-	70	++
36	5	-	5	-

[a] Average of three colonies formed on agar plates in the dark 7 days after inoculation. Diameter includes the 5-mm-diameter agar plug.
[b] Sporulation was rated at four levels: +++, abundant sporulation; ++, moderate sporulation; +, poor sporulation; –, no sporulation.

Table 2. Morphological comparison of *Gloeocercospora* isolates from sorghum leaf spot with published description of *Gloeocercospora sorghi* Bain & Edgerton

Key character	Morphological characteristics[a]	
	Present *Gloeocercospora* isolates	*Gloeocercospora sorghi* Bain & Edgerton (1943)
Sporodochia	pink to salmon colored gelatinous spore masses	pink to salmon colored gelatinous spore masses
Conidiophores	hyaline, septate, short, 5-1 μm 1in length, simple or branched	hyaline, septate, short, 5-10 μm in length, simple or branched
Conidia	hyaline, elongate to filiform, variable length 28-197 ×1.6-3.4 μm; generally born in a slimy matrix	hyaline, elongate to filiform, variable length 20-195×1.4-3.2 μm; generally born in a slimy matrix, salmon in mass
Sclerotia	0.1-0.3 mm in diameter, black lenticular to spherical, in necrotic host tissue	0.1-0.2 mm in diameter, black lenticular to spherical, in necrotic host tissue
Mycelia	hyaline, septate, branching	hyaline, septate, branching

[a] Sizes of conidia, conidiophores, sclerotia were counted 50 times in each experiment, and values are the means of replicates.

Table 4. Pathogenicity test of *Gloeocercospora sorghi* to seedlings by inoculating conidia suspensions in the greenhouse

Host (cultivar)	Pathogenicity
Sorghum (Grain sorghum)	+++[a]
Sorghum (Broom sorghum)	+++
Corn (Suwon 19)	+
Uninoculated	–

[a] Signs of +++, +, –, indicate pathogenic, weakly pathogenic, and non-pathogenic, respectively.

lesion. Also sclerotia may function in the survival of the pathogen (Dean, 1968). It germinates sporogenically and forms conidia, which infect the next crop. During wet weather, conidia are produced on the new lesions and cause further spread of the disease.

The fruiting body of *Gloeocercospora* is a sporodochium-like structure and the fungus should be placed in the Tuberculariaceae. The fruiting structure originates between the guard cells or slightly below them, and emerges through the stomata. As the spore characters of *Gloeocercospora* resemble those of some species classified in the genus *Cylindrosporium* (Barnett and Barry, 1998), it sould be carefully examined whether a fungi has the fruiting structure characteristic to *Gloeocercospora*. In many respects, *G. sorghi* resembles *Ramulispora sorghi* (*Titaeospora andropogonis*), the cause of sooty stripe disease of sorghum (Olive et al., 1946). Both fungi attack sorghum, causing leaf lesions and forming sporodochial-like fructifications and small black sclerotia. *Ramulispora* is distinguished by its branched spores and by the fact that its conidial fructifications arise from a subepidermal stroma and later become erumpent through the stomata, whereas those of *Gloeocercospora* are superficial. *Gloeocercospora* also differs from *Cercospora* (Chupp, 1954; Deighton, 1973) and *Cercosporella* (Deighton, 1973) in that its fruiting body is definitely a sporodochium and the conidia are born on short conidiophores in a slimy matrix.

Therefore, based on the morphological characteristics, the pathogen causing the zonate leaf spot on sorghum was identified as *G. sorghi,* and accorded to that stated by Bain and Edgerton (1943). So, this fungus was identified as *G. sorghi* Bain and Edgerton ex Deighton.

Pathogenicity of *G. sorghi.* As shown in Table 4, an isolate of *G. sorghi* from sorghum was highly pathogenic to sorghum cultivars, Grain sorghum and Broom sorghum, but only slightly so to corn (*Zea mays* L.). Lesions produced by *G. sorghi* first were observed at 12 to 18 hrs after inoculation. The initial lesions appeared as chlorotic, water-soaked lesions with 1-3 mm in diameter. On infected leaves, lesions were enlarged, coalesced, and became reddish brown spots with some necrosis by 72 hrs after inoculation. Many lower leaves were killed within 7 days (Fig. 1F) and sclerotia of *G. sorghi* were evident in some necrotic lesions (Fig. 1E). Conidial masses developed after 24-48 hrs on either surface-sterilized or nonsterilized lesions incubated at 24°C under longwave UV. Many plants sprayed with conidia of *G. sorghi* developed both blade and sheath lesions similar to those observed in the fields. *G. sorghi* was reisolated from lesions inoculated seedlings.

The multicellular, hyaline conidia germinated readily in water generally in less than 5 hrs. Frequently, the conidia produced one to seven germ tubes of highly variable lengths. Bain and Edgerton (1943) and Dean (1965) reported that *G. sorghi* entered leaves with germ tubes only through stomata and produced no appressoria; they also observed no subcuticular hyphae. In contrast, Myers and Fry (1978) reported that the fungus produced appressoria, appressorium aggregations, and subcuticular hyphae and gained ingress via stomata or by direct penetration into non-specialized epidermal cells and leaf trichomes, which differs from other reports.

Zonate lead spot is common on sorghum during wet season and may also infect maize, millet, sugarcane, and numerous other grasses (Tarr, 1962). This disease has been record-ed in the United States, South America (Argentina, Venezuela), Central America (Salvador, Panama, Nicaragua), West Indies, India, Pakistan, Japan, and several African countries (Tarr, 1962). Although it was widely distributed all over the world, there has been no report in Korea. This is the first report of zonate leaf spot on sorghum caused by *G. sorghi* in Korea.

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