

Newly Discovered Leaf Blight of Citronella Grass Caused by *Curvularia andropogonis* in the Philippines

By TOYOZO SATO and HIROTO OHKUBO

Division of Microbiology, National Institute of Agro-Environmental Sciences
(Tsukuba, Ibaraki, 305 Japan)

Introduction

Citronella grass (*Cymbopogon nardus* Rendle) is one of the raw materials of aromatic essential oil, and commercially cultivated mainly in Sri Lanka, Malaysia and Indonesia at present¹⁾. It was reported that a fungus, *Cochliobolus cymbopogonis* Hall et Sivanesan (anamorph: *Curvularia cymbopogonis* (Dodge) Groves et Skolko) caused seed and seedling blight and leaf spots of citronella grass and lemon grass (*Cymbopogon citratus* Stapf.)²⁾. The senior author encountered severe leaf blight of citronella grass cultivated experimentally in Mindanao Isl., the Philippines, in October, 1987. Results of preliminary study on the spot suggested that the pathogenic fungus was not the species mentioned above, although they seemed to be closely related one another. Therefore, we carried out isolation and identification of the pathogen and inoculation experiments in Japan in order to obtain basic data for controlling the disease. The authors obtained permission of Ministry of Agriculture, Forestry and Fisheries, Japan (Import Permit No. 63-Y-250) to import the materials of the present study.

Observation of the disease in Mindanao Isl.

The leaf blight of citronella grass was observed in a test field of the Philipinas Kao Inc. in Misamis Oriental State of Mindanao

Island. The field is located in a hill area at an altitude of ca. 500 m and about 10 km far from the seacoast. Five strains of citronella grass, each of which had been collected in Bohol, Bukidnon, Cotabato, Leyte in the Philippines, and Taiwan, respectively, were growing in ca. 2 ha of the field. Other aromatic plants and corn were cultivated around the area. Almost all plants of the five strains showed characteristic symptoms of leaf blight. Two types of lesions were observed. One was elongated, large, grayish brown necrosis surrounded by a reddish brown discolored area, and the other was typical small eyespots (Plate 1).

Morphology of the pathogen

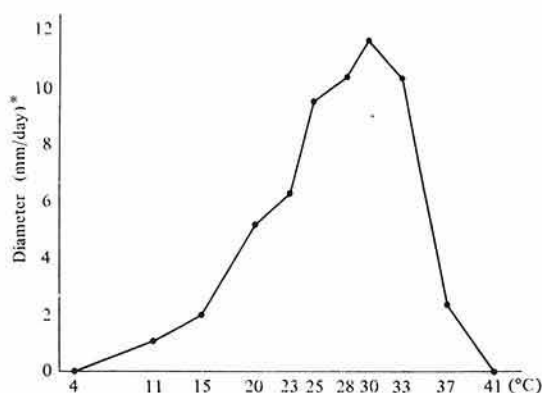
Numerous conidiophores and conidia were produced on the former type of lesions after the diseased leaves were incubated in moist chambers at room temperature for a day. The conidiophores were emerging singly or in groups. They were erect, simple straight or flexuous, sometimes geniculate, 2-17 septate, brown, paler near apex, smooth, up to 450 μm long, often swollen at the base to 9-15 μm , 6-10 μm thick just above the basal swelling, and 4.5-7.5 μm at the apex (Plate 2). The conidia were sometimes straight but more commonly curved, clavate, obconic at the base which has a protuberant hilum, and 3-distoseptate. The third cell from the base was longer and usually darker than others, middle two cells were brown or dark brown, cells at each end were paler, smooth, and 37.5-56.0

(mean 44.8) μm long, 17.5–26.0 (20.5) μm thick in the broadest part (Plate 3). The conidia seemed to be produced sympodially through pores on the conidiophore (Plate 4). These morphological characters just agree with description of the fungul genus, *Curvularia*^{1,2,3,5}.

Isolation and physiological characters of the pathogen

Several isolates of the pathogen were obtained from diseased leaves of five strains by two ways. Conidia on the Taiwan strain incubated for three days were isolated by a dilution plate method and cultured on PDA slants. The pathogen on other four strains of citronella was isolated by another method as follows: after soaking diseased leaf pieces in 70% ethanol and next in 2% sodium hypochlorite solution for several seconds, they were placed on water agar (1.5%) plates containing 100 ppm of streptomycin and incubated at 20–25°C, then hyphal tips elongated from the leaf tissue were transferred to PDA slants. After that, five pure cultures each named Boh., Buk., Cot., Ley., or Tai. were made from conidia of each isolates produced on media by single-spore isolation technique in order to study some cultural characters of the pathogen mentioned below.

The five pure isolates were cultured on four kind of media, PDA, PCA, LCA and CMA, at 20–25°C. Their colonies on PCA and PDA were dense, cottony, grayish brown, while those on LCA and CMA were sparse and paler than the former (Plate 5). Although conidial formation was observed on every medium, PCA appeared to be most suitable for sporulation of the pathogen. On PCA conidia were produced from five days after incubation. Stroma also formed on the four media, especially frequently on PDA adjacent to the lateral wall of petri dishes (Plate 6). They were cylindrical, simple or branched, black, 0.45–5.0 mm long and 0.25–0.5 mm thick. Conidiophore and conidia often developed on the stroma. All the pure isolates except for Tai. were cultured on PCA at various tem-

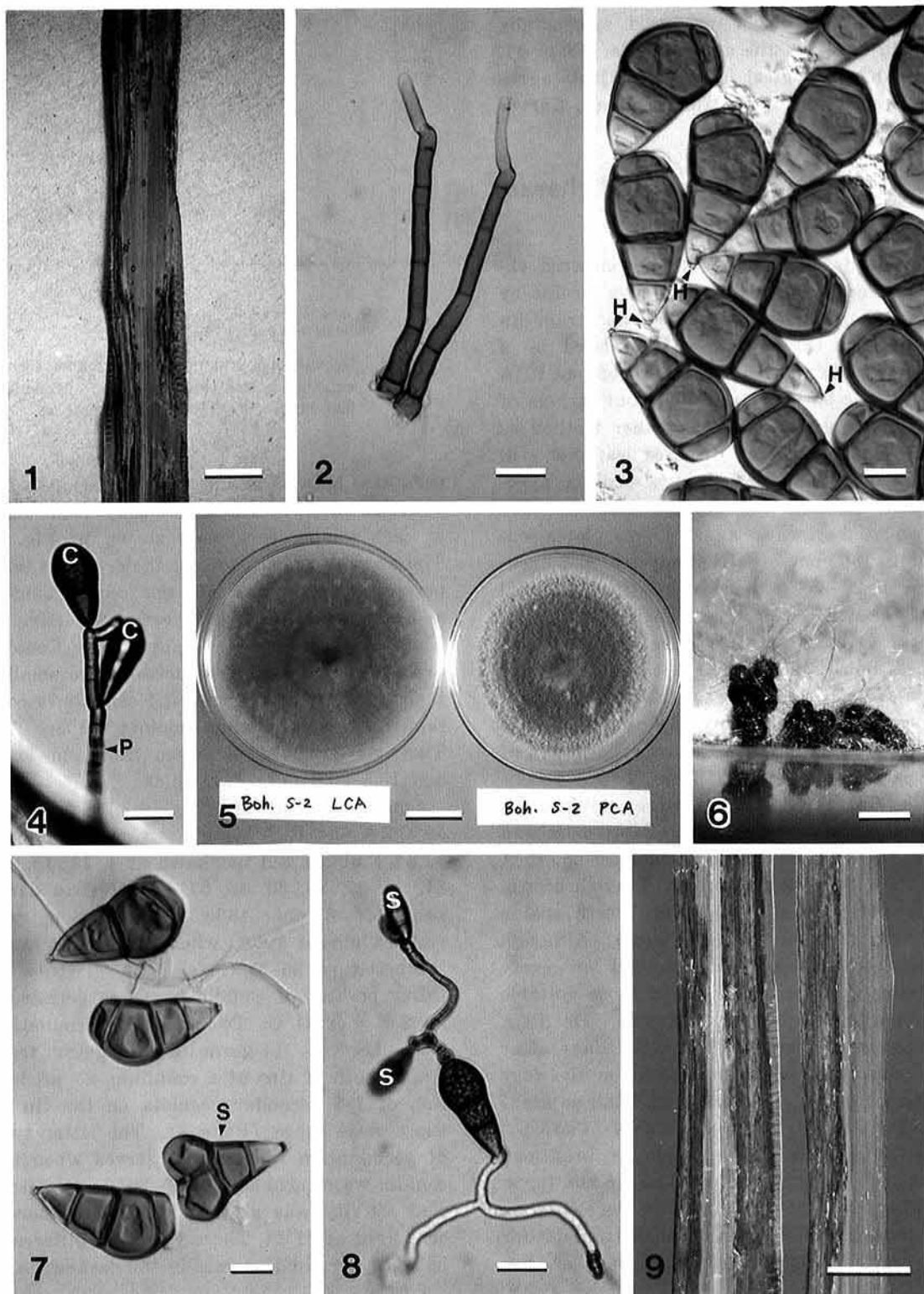


* Mean of colonies of four isolates after culturing for a week on PCA plates

Fig. 1. Growth speed of mycelial colonies *Curvularia* sp. isolated from the citronella leaf blight as related to temperature

peratures between 4 and 41°C in darkness for 2 weeks. Growth rates of colony (mm/day) at each temperature were shown in Fig. 1. The average growth rate at the optimum temperature, 30°C, was 11.6 mm/day. Conidial and stromatal formation were most active at 15–28°C and 25–28°C, respectively. Conidia produced on the artificial media were smaller than those on host leaves and sometimes contained triradiate stauroconidia (Plate 7). They were 23.0–47.5 (mean 34.7) μm long, and 10.5–26.0 (17.6) μm thick.

Conidia of Tai.- and Ley.-isolates produced on CMA and PCA were sown on water agar (1.5%) plates and incubated at 4, 11, 15, 19, 21, 23, 25, 28, 30, 33, 37 and 41°C in darkness for 8 and 16 hr. Germination rate reached almost 100% when the conidia were incubated at 25–33°C for 8 hr. Twenty to thirty percent of conidia began to germinate even at 4 or 41°C. Two types of germination were observed: 1) germ tube elongation from one or both of tips of a conidium, 2) production of 1–5 secondary conidia on the tip of short germ tubes (Plate 8). The latter type of germination was often observed when the conidia were incubated at 15–25°C. Germination activity was also studied under fluorescent light at 21°C. There was little difference between germination rate in the darkness and



- Plate 1. The leaf blight of citronella grass showing elongated, large necrosis and typical eyespots (Scale: 1 cm)
- Plates 2-4. The pathogen of the citronella leaf blight, *Curvularia andropogonis*
- 2: Two conidiophores produced on the host leaves (Scale: 20 μ m)
 - 3: Conidia produced on the host leaves showing hila (H) (Scale: 10 μ m)
 - 4: Conidial (C) formation showing sympodial development of a conidiophore (P) (Scale: 20 μ m)
- Plates 5-8. *Curvularia andropogonis* on artificial media
- 5: Colonies on LCA (left) and on PCA (right) incubated at 20-25°C for 27 and 8 days, respectively (Scale: 2 cm)
 - 6: Stromata formed on a lateral wall of a petri dish (Scale: 0.5 mm)
 - 7: A triradiate stauroconidium (S) and normal conidia produced on PCA (Scale: 10 μ m)
 - 8: A conidium developing three small conidia (S) on two branches of a germ tube (Scale: 20 μ m)
- Plate 9. Citronella leaves showing typical lesions 24 days after inoculation with conidia of Tai-isolate produced on PCA (Scale: 1 cm)

that under the light.

Inoculation experiment

Conidia of all the pure isolates were inoculated to citronella grass (*Cym. nardus*) and lemon grass (*Cym. citratus*) growing in clay pots by the method mentioned below. After colonies of each isolate developed all over the PCA plate at 25°C in darkness, pieces of autoclaved filter paper containing PC broth were placed on the colonies and incubated at 20-25°C for 10 days. Young leaves of the plants

were sprayed with autoclaved pure water and smeared with conidia produced on the filter paper. The inoculated plants were kept in a dark moist chamber at 25°C for 40 hr and then transferred to a glasshouse controlled at 25-35°C. Results were shown in Table 1. The citronella grass developed discolored lesions (Plate 9), necrosis and conidia from the necrotic lesion earlier and more frequently than lemon grass (Table 1). It was distinct from the result that the latter was much more resistant to the disease than the former. Reisolation of the inoculated fungus from conidia produced on both plants was successfully done.

Table 1. Results of inoculation experiments using *Curvularia* conidia of isolates obtained from the citronella leaf blight to citronella grass (*Cymbopogon nardus*) and lemon grass (*Cymbopogon citratus*)

Plant inoculated	Days to the first appearance of			
	Eyespot	Large lesion	Necrosis	Sporulation*
Citronella grass (<i>Cym. nardus</i>)	3	8-9	11-21	1
Lemon grass (<i>Cym. citratus</i>)	3	9-17	20-32	4-5

* Conidial formation on the lesion in a moist chamber at 23-27°C.

Identification of the pathogen

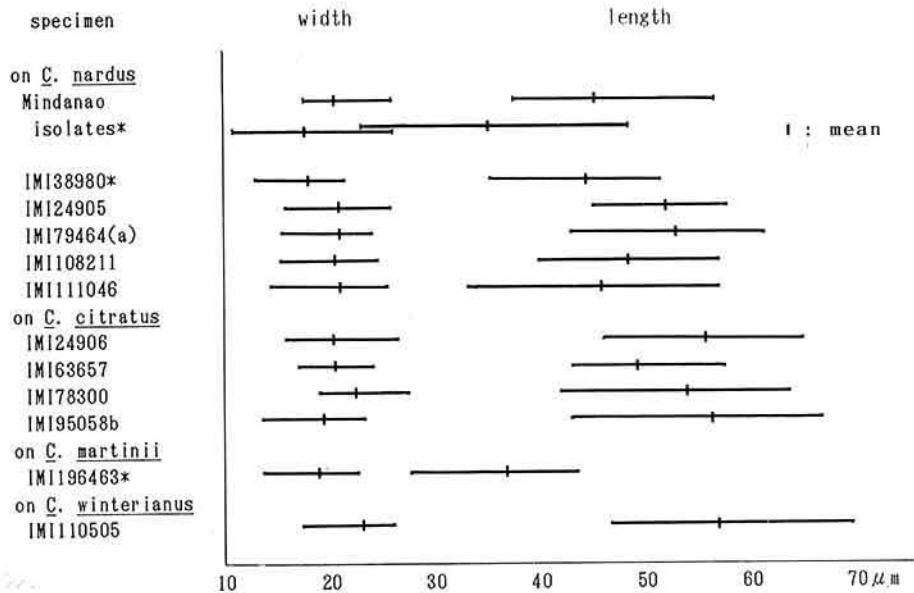
The pathogenic fungi could be regarded as a certain species belonging to the genus *Curvularia* which was parasitic on *Cymbopogon* plants from the results mentioned above. According to Ellis^{2,3}) and Sivanesan⁵), nine species listed in Table 2 have been known for such ones. *Curvularia cymbopogonis*, *C. comoriensis* Bouriquet et Jauffret, *C. senegalensis* (Speg.) Subram. and *C. geniculata* (Tracy et Earle) Boedijn are different from the present pathogen, because of four (3-5) septa and smaller thickness of their conidia

Table 2. Comparison of conidial morphology of *Curvularia* species parasitic on *Cymbopogon* spp.

Species	Conidial			
	Septa	Hilum	Length (μm)	Thickness (μm)
<i>Curvularia</i> sp. (Mindanao collections)	3	+	38-56 (45)*	18-26 (21)*
<i>C. andropogonis</i>	3	+	45-66 (56)	18-28 (22)
<i>C. trifolii</i>	3	+	28-38 (33)	12-16 (14)
<i>C. lunata</i>	3	-	20-32 (25)	9-15 (12)
<i>C. eragrostidis</i>	3	-	22-33 (27)	10-18 (15)
<i>C. pallescens</i>	3	-	21-32 (27)	8-11 (9)
<i>C. cymbopogonis</i>	4	+	35-60 (50)	14-20 (18)
<i>C. comoriensis</i>	4 (3-5)	+	30-55 (44)	13-20 (17)
<i>C. senegalensis</i>	4 (3-5)	-	19-30 (24)	10-14 (11)
<i>C. geniculata</i>	4 (3-5)	-	26-48 (32)	8-13 (10)

* Numerals in parentheses indicate mean (μm).

Data except for those of *Curvularia* sp. (Mindanao collections) were reproduced from Ellis^{2,3} and Sivanesan⁵.



(* conidia produced on artificial media)

Fig. 2. Comparison of conidial sizes of *Curvularia* collected in Mindanao with those of *Curvularia andropogonis* specimens of IMI

(Table 2). *Curvularia trifolii* (Kauffm.) Boedijn, *C. lunata* (Wakker) Boedijn, *C. eragrostidis* (P. Henn.) J. A. Meyer and *C. pallescens* Boedijn are also distinguished from it by smaller conidial size and/or absence of hila (Table 2). The present species is similar

to *Curvularia andropogonis* (Zimmermann) Boedijn most. However, its average conidial length is shorter than that of *C. andropogonis* reported by Boedijn¹, Ellis^{2,3} and Sivanesan⁵, whereas it is longer than that appeared in the original description by Zimmermann⁷

(Table 2). Since it was hard to decide identity of them on the basis of comparisons with these descriptions, we tried to compare the present species directly with collections of *C. andropogonis* deposited in CAB International Mycological Institute (IMI). Eleven herbarium specimens listed in Fig. 2 as well as all collected in Mindanao Isl. were examined in detail and 50 conidia of each specimen were measured to obtain range and mean of their sizes. Results were shown in Fig. 2. It was evident that conidial size of *C. andropogonis* varied depending on collections. Generally speaking, conidia produced on media and on citronella grass were inclined to be smaller than those on host plants and on lemon grass, respectively. Conidial sizes of the present pathogen agreed very well with those of a short conidial strain, IMI111046. Thus it could be regarded as one of such strains of *C. andropogonis*. Four of the specimens collected in Mindanao Isl. were deposited in the herbaria of National Institute of Agro-Environmental Sciences (NIAES) and IMI with the following access numbers: NIAES 10431 (=IMI327409), NIAES10432 (=IMI 327410), NIAES10433 (=IMI327411) and NIAES10434 (=IMI327412).

Discussion and conclusion

The present pathogen was identified as a strain of *C. andropogonis* producing short conidia from the results mentioned above. Severe leaf blight of citronella caused by the present species has not been reported before, though *C. cymbopogonis* was known for a pathogen of other diseases of citronella and lemon grass⁶⁾. On the other hand, no one has collected *C. andropogonis* in the Philippines before, although it has been reported from India, Indonesia and Malaysia^{1,2,3,5,7)}. This is, therefore, the first report of citronella leaf blight caused by this fungal species in the Philippines.

The severity of the present disease observed in Mindanao Isl. suggested that the disease be likely to become one of the important obstructive factors to commercial production of citronella grass in the Philippines. It is necessary to clarify the life cycle of the pathogenic fungi and environmental conditions favorable for the occurrence of the disease on the spot, and to develop means to control it. In Mindanao Isl., lemon grass cultivated in another test field was observed to be healthy. This observation and the result of inoculation experiments in this study indicate that lemon grass is relatively resistant to the disease. Thus lemon grass can be utilized as a breeding material to build up cultivars resistant to the disease, providing that interspecific crossing is possible between lemon grass and citronella grass.

References

- 1) Boedijn, K. B.: Ueber einige phragmosporen Dematiaceen. *Bull. Jard. Bot. Buitenzorg, Serie III*, 13, 120-134 (1933).
- 2) Ellis, M. B.: Dematiaceous hyphomycetes. VII. *Curvularia*, *Brachysporium*, etc. *Mycol. Pap.*, 106, 2-43 (1966).
- 3) Ellis, M. B.: Dematiaceous hyphomycetes. C·A·B International, Wallingford, UK, 452-459 (1971).
- 4) Iwasa, S.: Useful tropical crops. Tropical Agriculture Research Center, Tsukuba, 92-94 (1974) [In Japanese].
- 5) Sivanesan, A.: Graminicolous species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and their teleomorphs. *Mycol. Pap.*, 158, 1-261 (1987).
- 6) Sivanesan, A. & Holliday, P.: *Cochliobolus cymbopogonis*. *CMI Descr. Path. Fungi and Bact.* No. 726 (1982).
- 7) Zimmermann, A.: Ueber einige an tropischen Kulturpflanzen beobachtete Pilze. II. *Centralb. Bakt. Parasitkde. Infektionskrank. Abt. 2*, 8, 216-221 (1902).

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