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Anthracnose of Perilla Caused by *Colletotrichum* spp. and *Glomerella cingulata*

Wan Gyu Kim*, Byung Dae Lee[1], Weon-Dae Cho and Dong Bum Shin[2]

Plant Pathology Division, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Korea

[1]Biorigin Incorporation, Yongin 449-916, Korea

[2]National Yeongnam Agricultural Experiment Station, Rural Development Administration, Milyang 627-130, Korea

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Severe outbreaks of anthracnose were observed on perilla plants grown in greenhouses and open fields in several locations in Korea during the disease survey from 1997 to 2000. A total of 53 isolates of *Colletotrichum* spp. and *Glomerella* sp. was obtained from diseased perilla plants and identified based on their morphological and cultural characteristics. Forty isolates were identified as *Colletotrichum gloeosporioides*, three isolates as *C. coccodes*, five isolates as *C. dematium*, and the other five isolates as *Glomerella cingulata*, the teleomorph of *C. gloeosporioides*. All isolates of *C. gloeosporioides* tested by artificial inoculation were strongly virulent on perilla plants, but isolates of the other species were weakly or not virulent. Anthracnose symptoms induced on the perilla plants by artificial inoculation with the isolates of *C. gloeosporioides* were similar to those observed in the fields. This study revealed that *C. gloeosporioides* is the main causal fungus of perilla anthracnose.

Keywords : anthracnose, *Colletotrichum gloeosporioides*, *Glomerella cingulata*, pathogenicity, *Perilla frutescens* var. *japonica*.

Perilla [*Perilla frutescens* Britton var. *japonica* (Hassk) Hara] has been widely cultivated as a vegetable or an oil crop in Korea. Leaves of the plant are used for fresh vegetables, while seeds are used for cooking oil. The plant is mostly cultivated in greenhouses for production of leaves during cold seasons, and in open fields for production of seeds during summer.

Recently, spotted lesions on perilla plants were encountered during plant disease survey in several locations in Korea. It has been reported that *Ascochyta*, *Cercospora*, and *Septoria* spp. are associated with the occurrence of spotted lesions on perilla plants (Anonymous, 1990; Anonymous, 1998; Tai, 1979). The authors also isolated not only the fungi but also some *Colletotrichum* spp. and *Glomerella* sp. from the spotted lesions. Fukui (1925) first isolated *Colletotrichum* sp. from perilla stems and named the fungus as a new species, *Colletotrichum yoshinoi* Fukui. Based on the report, the fungus was recorded as a pathogen of perilla anthracnose in Japan (Anonymous, 1990). However, the fungal name has been found to be invalidly published. In Fukui's report (Fukui, 1925), there was no description of the fungus in Latin as well as no nomenclatural type specimen indicated in a herbarium or an institution. Accordingly, this study was conducted to identify the fungus, which causes anthracnose of perilla in the fields. A preliminary study on anthracnose of perilla was previously reported by the authors (Kim et al., 2000).

*Corresponding author.
Phone) +82-31-290-0439, FAX) +82-31-290-0453
E-mail) wgkim@rda.go.kr

Materials and Methods

Field survey. Perilla plants grown in greenhouses and open fields in seven locations in Korea were surveyed from 1997 to 2000. Incidence of anthracnose on 100 perilla plants in each field was investigated in three replicates, and symptoms on the plants were observed.

Isolation of pathogens. Perilla plants with anthracnose lesions were collected from the fields in the locations investigated. Three to 5 mm-lesion pieces cut from the diseased plants were plated on 2% water agar medium (WA) after surface-sterilizing with 1% sodium hypochlorite solution for 1 min. Fungal isolates obtained from the lesion pieces were transferred to potato-dextrose agar (PDA) slants. Isolates of *Colletotrichum* spp. and *Glomerella* sp. identified by morphological observation under a light microscope were cultured for sporulation on PDA at 26°C for 20 days in alternating cycles of 12-h NUV light and 12-h darkness. Using an inoculating needle, spore mass produced on PDA was suspended in 100 μl of sterile distilled water in a 1.5 ml-microtube to make a spore suspension. A loopful of the spore suspension was streaked on WA surface with a platinum wire loop to distribute the spores. After 20-24 h incubation at 26°C, agar fragments bearing a single germinated spore were transferred to fresh WA and incubated at 26°C for five days. Single-spore isolates obtained from the WA plates were cultured on PDA and used for the experiment of identification and pathogenicity tests.

Investigation of morphological and cultural characteristics. Fungal structures formed on host plants and PDA cultures were

examined by light microscope. Fifty spores and 25 setae chosen randomly from each lesion or culture were observed and measured under the light microscope. Appressoria of the isolates were examined by using a modified culture method of Smith and Black (1990). A drop of spore suspension (3-5 × 10^6/ml) from each isolate was placed onto 2% WA in a 9 cm-diameter petri dish. A sterile coverslip was placed over the drop and incubated at 26°C for 24-48 h in the dark. Fifty randomly chosen appressoria per isolate were observed and measured by light microscope.

Six millimeter-diameter mycelial disks from PDA cultures of the isolates were transferred to PDA in 9 cm-diameter petri dishes. Colonies of the isolates were observed after 20 days of incubation at 26°C in alternating cycles of 12-h NUV light and 12-h darkness.

Pathogenicity test. Seeds of two local varieties of perilla were sown in circular plastic pots (21 cm in diameter and 29 cm in height) containing sterile soil, and the pots were placed in a greenhouse at 18-32°C. Forty-day-old perilla plants were used for the inoculation tests.

Five isolates of *C. gloeosporoides* and two isolates each of the other species were used for the inoculation tests. Spore suspensions were prepared from 20-day-old PDA cultures, then diluted with sterile distilled water to make a concentration of 3-5 × 10^6 spores/ml. Twenty milliliter spore suspension of each isolate was sprayed onto the perilla plants. Control plants were treated with sterile distilled water. The plants were placed in dew chambers with 100% relative humidity at 26°C for 2 days, then moved into the greenhouse. This experiment was performed in three replicates. Disease severity was rated based on the number of lesions induced on the plants 15 days after inoculation. Re-isolation of the pathogen from the lesions on the plants was conducted as described previously.

Results

Disease incidence and symptoms. Anthracnose was observed on perilla plants grown in greenhouses and open fields in seven locations in Korea during the disease survey from 1997 to 2000 (Table 1). The disease incidence was very severe, reaching up to 90% in Geumsan, 60% in Chuncheon, 40% in Yeongi, and 30% in Busan. In other locations, the disease incidence was as low as 0-2%.

Table 1. Incidence of anthracnose on perilla plants grown in greenhouses or open fields in seven locations in Korea from 1997 to 2000

Location	Pattern of cultivation	No. of fields surveyed	No. of fields infected	% infected plants
Busan	Greenhouse	15	3	5-30[a]
Chuncheon	Open field	26	5	2-60
Chungju	Open field	1	1	Less than 2
Geumsan	Both patterns	48	11	1-90
Gongju	Open field	7	1	Less than 1
Gwangyang	Greenhouse	13	1	Less than 2
Yeongi	Open field	5	2	1-40

[a]One hundred plants in each field were investigated in three replicates.

Symptoms developed on leaves, stems, and petioles of perilla. Symptoms on leaves appeared as circular to irregular, small spots with dark brown to black discoloration, and margins of the lesions turned pale yellow (Fig. 1A, B). Severely infected leaves blighted later. Symptoms on stems and petioles appeared as elliptical to irregular, small spots with dark brown to black discoloration, and the center of the lesions turned gray at the early stage (Fig. 1C-E). The lesions irregularly enlarged with black discoloration at the late stage (Fig. 1F), and severely infected stems rotten and blighted.

Isolation and identification. A total of 53 isolates of *Colletotrichum* spp. and *Glomerella* sp. was obtained from perilla plants: *C. gloeosporioides*; 17 from leaves, 15 from stems, and 8 from petioles, *C. coccodes*; 3 from leaves, *C. dematium*; 5 from leaves, and *G. cingulata*; 5 from leaves. They were identified based on their morphological and cultural characteristics (Table 2 and Fig. 2). The morphological and cultural characteristics of three *Colletotrichum* spp. and *G. cingulata* examined by the authors were consistent with those described by previous researchers (Arx, 1970; Arx, 1981; Mordue, 1971; Sutton, 1980).

Out of the 53 isolates from perilla plants, 40 isolates were identified as *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., 3 isolates as *C. coccodes* (Wallr.) Hughes, 5 isolates as *C. dematium* (Pers.: Fr.) Grove., and the other 5 isolates as *Glomerella cingulata* (Stonem.) Spauld. & von Schrenk, the teleomorph of *C. gloeosporioides*. *C. gloeosporioides* was isolated from leaves, stems and petioles, and the other species only from leaves.

Pathogenicity. All isolates of *C. gloeosporioides* tested by artificial inoculation were strongly virulent on perilla plants, but isolates of the other species were weakly or not virulent (Table 3). Anthracnose symptoms induced on the plants by artificial inoculation with the isolates of *C. gloeosporioides* were similar to those observed in the fields. The isolates which induced symptoms on the perilla plants were re-isolated from lesions on the plants inoculated.

Discussion

Among the anthracnose pathogens of perilla identified in the present study, *C. gloeosporioides* was most frequently isolated from leaves, stems, and petioles of perilla. The fungal species was also strongly pathogenic to perilla plants. *C. coccodes*, *C. dematium*, and *G. cingulata* were only isolated from leaves of perilla at low frequency and were weakly pathogenic to perilla plants. Accordingly, this study revealed that *C. gloeosporioides* is the main causal fungus

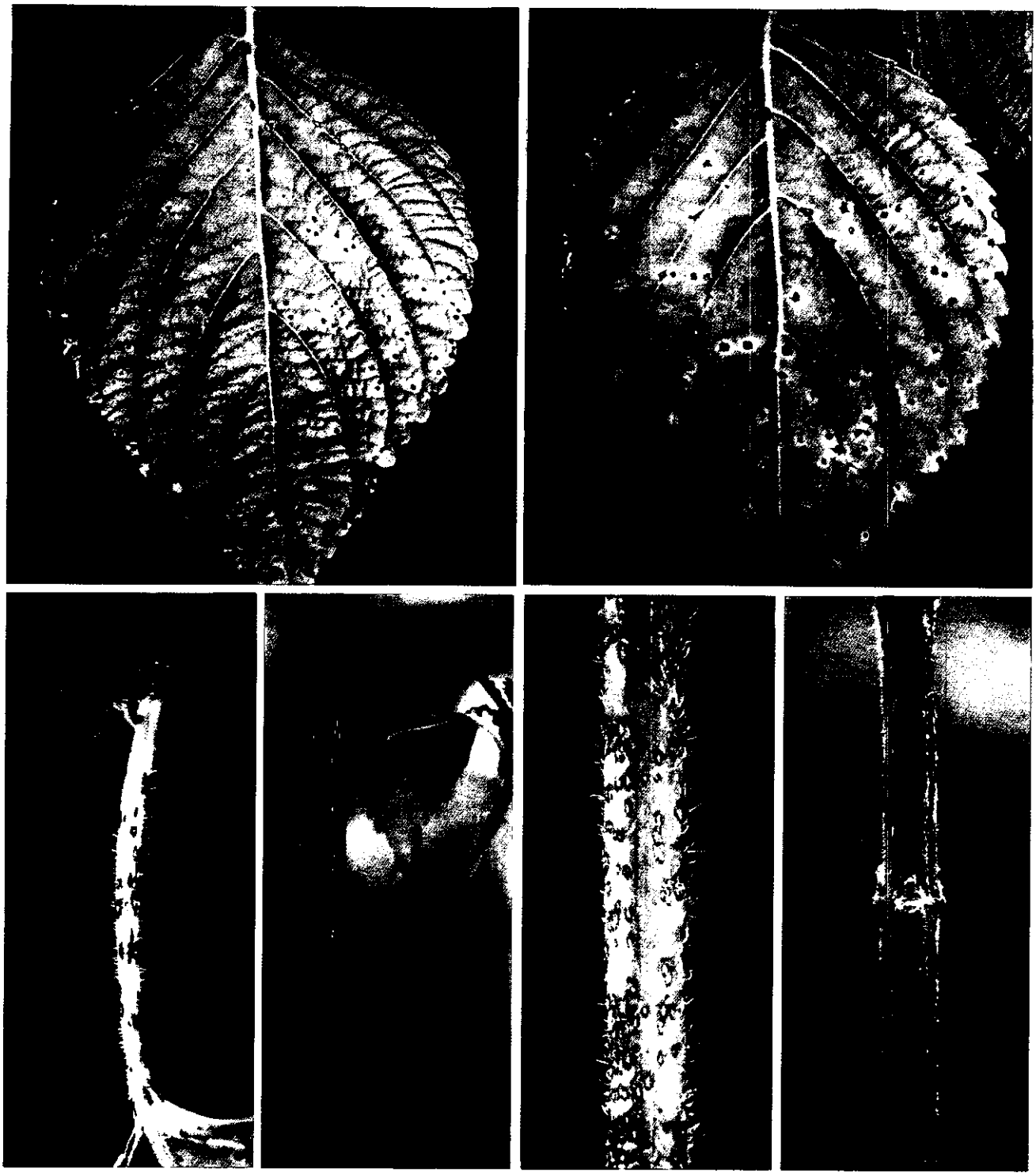

Fig. 1. Symptoms of anthracnose on perilla plants in the field. (**A** and **B**), circular to irregular, small spots on leaves at the early stage and the late stage, respectively; (**C-E**), elliptical to irregular, small spots on petioles and stems at the early stage; (**F**), enlarged lesions on the stem at the late stage.

of perilla anthracnose. It has been reported that *C. gloeosporioides* causes anthracnose on a large variety of plants (Arx, 1970; Farr et al., 1989; Sutton, 1980; Sutton, 1992). The present study accounts for a new host of the fungus.

Colletotrichum yoshinoi was recorded as a causal fungus

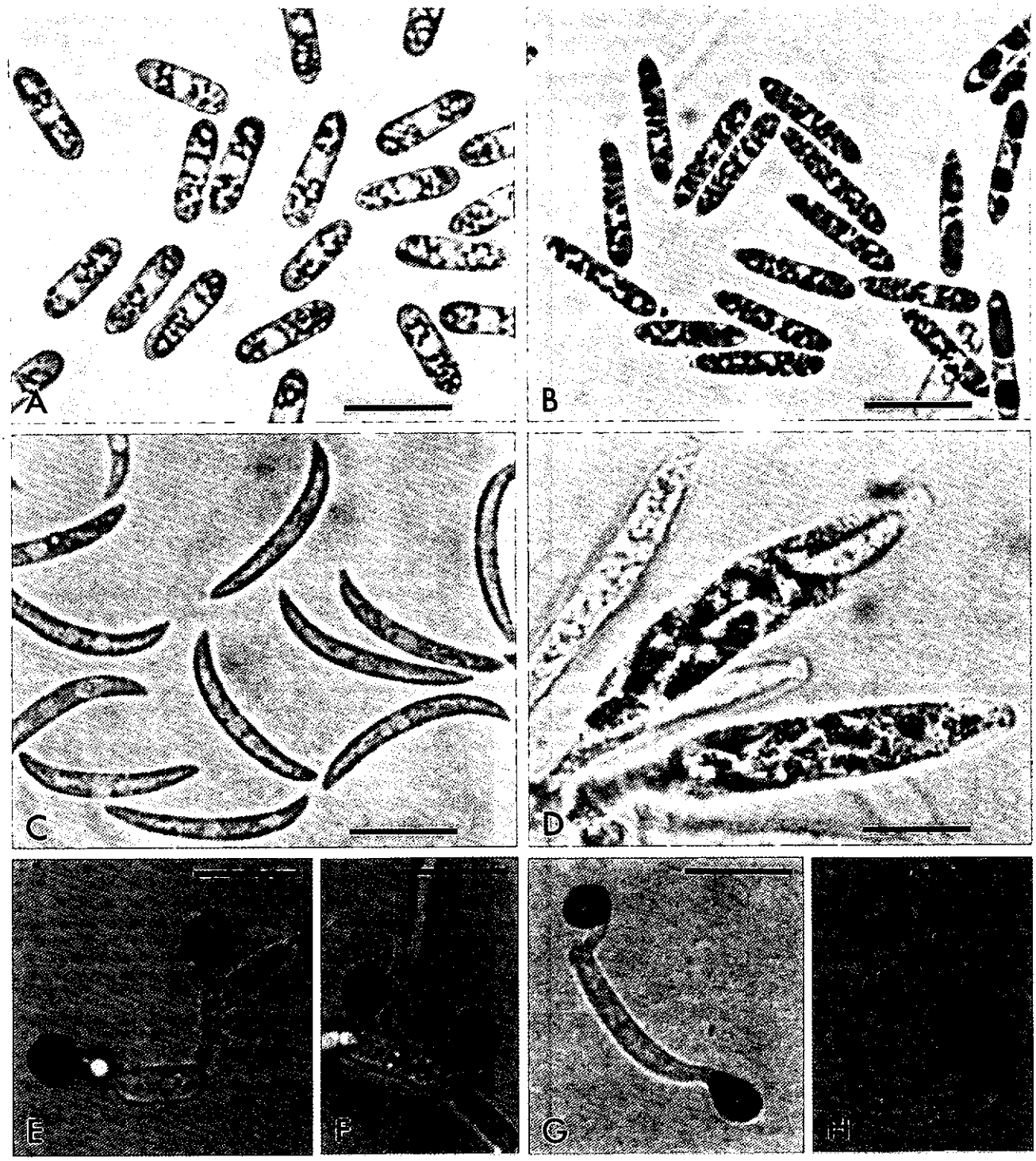

Fig. 2. Morphological features of *Colletotrichum* spp. and *Glomerella cingulata.* (**A-C**), conidia of *C. gloeosporioides, C. coccodes,* and *C. dematium,* respectively; (**D**), asci including ascospores of *G. cingulata*; (**E-H**), appressoria of *C. gloeosporioides, C. coccodes, C. dematium,* and *G. cingulata,* respectively. Each bar represents 15 μm.

of perilla anthracnose (Anonymous, 1990; Fukui, 1925). However, the fungal name was invalidated because there was no description in Latin and no type specimen indicated. The morphological characteristics of the fungus isolated from perilla stems by Fukui (1925) were similar to those of *C. gloeosporioides* examined by the authors. Therefore, it was assumed that *C. yoshinoi* is a synonym of *C. gloeosporioides.*

Table 2. Morphological and cultural characteristics of *Colletotrichum* spp. and *Glomerella cingulata* isolated from anthracnose lesions on perilla plants

Species	Colony on PDA[a]	Shape and size of conidia/ascospores	Shape and size of appressoria	Shape and size of setae
Colletotrichum gloeosporioides	Dark gray to black	Straight, cylindrical, obtuse or round at ends, 12-18 × 4-6 μm	Circular to ovate, clavate, lobed, 6-14 × 6-10 μm	1-4 septate, 36-102 × 3-6 μm
C. coccodes	Sparse aerial mycelium and black sclerotia	Straight, fusiform, tapered to one end, 16-22 × 3-5 μm	Ovate to elliptical, sometimes lobed, 8-12 × 6-10 μm	1-3 septate, 32-84 × 5-7 μm
C. dematium	Gray to black	Falcate, fusiform, tapered to each end, 20-28 × 3-4 μm	Ovate to clavate, slightly lobed, 6-10 × 5-8 μm	1-5 septate, 40-244 × 3-8 μm
Glomerella cingulata	Gray to dark gray	Slightly curved, elliptical to fusiform, 14-24 × 4-6 μm	Ovate, clavate, slightly irregular, 7-16 × 6-12 μm	1-3 septate, 48-96 × 3-5 μm

[a]Potato-dextrose agar.

Table 3. Pathogenicity of *Colletotrichum* spp. and *Glomerella cingulata* on perilla plants by artificial inoculation

Species	Isolate No.	Disease severity on local varieties	
		Ipdeulkkae 1	Nonggadeulkkae
Colletotrichum gloeosporioides	C99-117	++[a]	++
	C99-120	++	++
	C99-134	++	++
	C00-001	++	++
	C00-005	++	++
C. coccodes	C97-018	−	−
	C97-019	−	+
C. dematium	C97-132	−	−
	C00-027	−	+
Glomerella cingulata	C99-133	+	−
	C00-024	−	−
Control		−	−

[a]Disease severity was rated 15 days after inoculation. ++ = abundant lesions developed on leaves, stems and petioles; + = a few lesions developed on leaves; − = no lesion.

Colletrichum coccodes primarily causes anthracnose and black dot on vegetables in the family Solanaceae (Farr et al., 1989; Mordue, 1967). The fungal species is also associated with the occurrence of anthracnose and root rot of other plants (Dillard, 1992). *C. dematium* has been known as a saprophyte isolated from a wide variety of plants (Arx, 1970; Sutton, 1980). However, it has been recorded that this fungal species causes anthracnose of various plants (Farr et al., 1989). The two *Colletotrichum* spp. are weakly or not pathogenic to perilla plants, suggesting that perilla is a saprotrophic habitat of the two species. They could attack their host plants after saprotrophic habitation on perilla plants.

Glomerella cingulata is the teleomorph of *C. gloeosporioides* but is weakly or not pathogenic to perilla plants. The teleomorphic state also rarely occurs on perilla leaves. It is generally known that the teleomorphic state occurs on diverse plants like the anamorphic state (Bryson et al., 1992). On the other hand, it was reported that pathogenicity of the teleomorphic isolates is weaker than that of anamorphic isolates of the fungus in red pepper fruits (Kim et al., 1986) and Indian fig cactus stems (Kim et al., 2000). There has been no study on the cause of pathological difference between the teleomorphic and anamorphic states of the fungus. Further study is needed to clarify pathological characteristics of the two fungal states in perilla.

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