Plant Pathol. J. 22(1) : 7-10 (2006)

The Plant Pathology Journal

First Report of Anthracnose Caused by *Colletotrichum acutatum* on Begonia (*Begonia semperflorens* Link.) Nurseries

Jong Kyu Park[1], Gyoung Hee Kim[1], Gyung Mi Min[1], Hee Jin Park[1], Jae-Seoun Hur[1], Beum Kwan Kang[2], Heung Tae Kim[2], Woobong Choi[3] and Young Jin Koh[1]*

[1]*Department of Plant Medicine and Department of Environmental Education, Sunchon National University, Suncheon 540-742, Korea*

[2]*Department of Plant Medicine, Chungbuk National University, Cheongju 361-763, Korea*

[3]*Departments of Biotechnology and Bioengineering, Biomaterial Control, Dongeui University, Busan 614-714, Korea*

(Received on September 21, 2005; Accepted on November 22, 2005)

Anthracnose severely occurred on begonia (*Begonia semperflorens* Link.) nurseries in Gyeongju, Gyeongbuk in July, 2004. More than 80% of begonia seedlings were diseased in the greenhouse surveyed and diseased leaves per plant were 12.1% in average. Yellowish spots occurred on the leaves of begonia as initial symptoms, and they coalesced irregularly to form large brown pleomorphic lesions. Severely infected leaves were defoliated, resulting in abnormal growth of the entire plant. *Colletotrichum* sp. was repeatedly isolated from the diseased plants and was identified as *Colletotrichum acutatum* on the basis of the mycological characteristics on potato dextrose agar and RAPD analysis. Pathogenicity of the fungus was also confirmed by artificial inoculation on healthy plants. The optimum temperature for mycelial growth of *C. acutatum* was around 25°C. The fungus was sensitive to azoxystrobin, bitertanol, diethofencarb-carbendazim, difenoconazole and tebuconazole. This is the first report on the anthracnose of begonia caused by *C. acutatum* in Korea.

Keywords : Anthracnose, Begonia, *Begonia semperflorens*, *Colletotrichum acutatum*

Begonia (*Begonia semperflorens* Link.) is widely cultivated for ornamental purpose in a garden (Fig. 1A). Outbreak of anthracnose was found on begonia cultivated at nurseries in Gyeongju, Gyeongbuk in July, 2004 (Fig. 1B). More than 80% of the seedlings were infected by the disease and diseased leaves per plant were 12.1% in average. *Colletotrichum* sp. was repeatedly isolated from the diseased plants, which suggested that the fungus is associated with the disease. However, there has been no record of anthracnose on this plant in Korea (The Korean Society of Plant Pathology, 2004). This study was conducted to characterize *Colletotrichum* sp. causing anthracnose on begonia as a basic research for the control of the disease.

*Corresponding author.
Phone) +82-61-750-3865, FAX) +82-61-750-3208
E-mail) youngjin@sunchon.ac.kr

Materials and Methods

Isolation and identification of fungi. After a short disinfection with 70% ethanol, diseased leaf tissues of begonia cut into 5 mm length were placed on potato dextrose agar (PDA) plates at 25°C. Mycelial tips of the fungal isolates grown on the medium were cut and transferred to fresh medium. Single spores isolated by dilution method on water agar were cultured on PDA plates at 25°C for the identification and pathogenicity tests. Conidia produced on PDA plates were examined by light microscope. Appressoria were examined using a modified slide culture method of Smith and Black (1990).

Species-specific PCR amplification. Species-specific primers were tested for the three isolates tested. Mycelia from PDA cultures were collected and grinded in liquid nitrogen for genomic DNA isolation using Genomic DNA purification kit (Promega, USA). RAPD amplification was conducted using Cal-1 (5'-CCAGGGGAAGCCTCTCG-CGGGCCT-3') and CgInt (5'-GGCCTCCCGCCTCCG-GGCGG-3'), *C. acutatum* specific and *C. gloeosporioides* specific, respectively, and ITS4 (5'-TCCTCCGCTTATT-GATATGC-3') as a universal primer for pairing (reference). Each PCR reaction (30 microliter) contained 50 ng of template DNA, 1 M of each primer, and 15 μl of PCR Master mix (Promega, USA). PCR positive and negative controls with or without template DNAs of known species, *C. acutatum* and *C. gloeosporioides*, were included in PCR run. Amplifications were conducted in a program as described : 1 cycle of 4 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 65°C, and 1 min at 72°C, ending with 1 cycle of 7 min at 72°C. PCR products were separated in 2% agarose gels. PCR amplification was repeated at least 3 times with identical results.

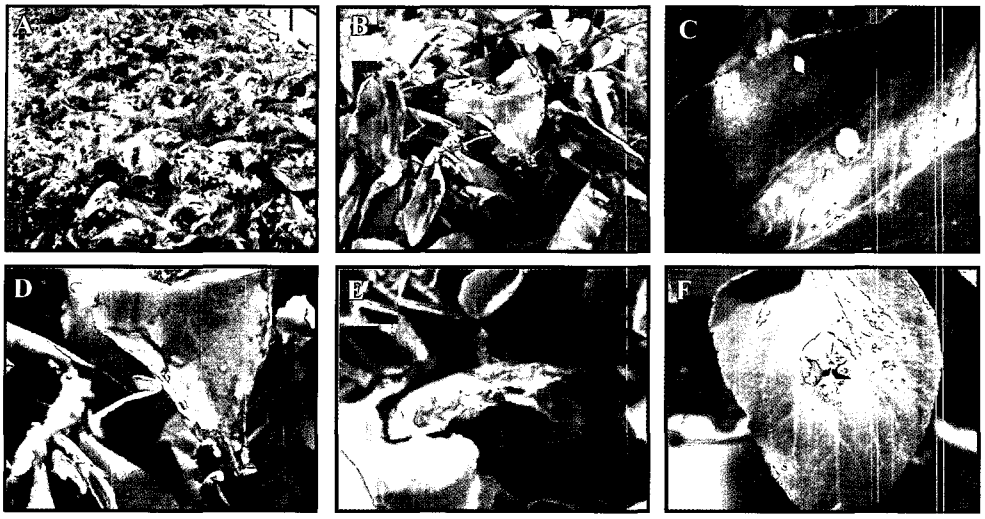

Fig. 1. Symptoms of anthracnose on begonia leaves caused by *Colletotrichum acutatum*. A: Healthy field, B: Infected field, C: Early symptom on a leaf, D: Typical symptom, E: Late symptom, F: Symptom on the leaf by inoculated artificially.

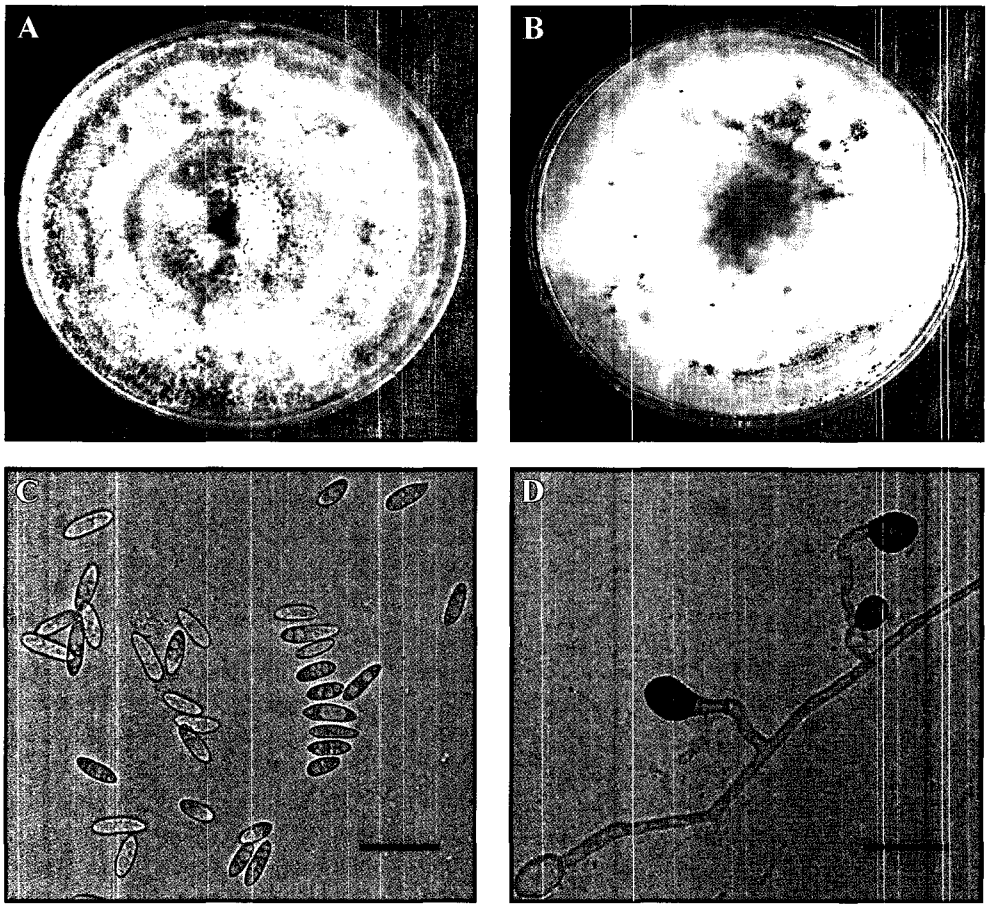

Fig. 2. Morphological characteristics of *Colletotrichum acutatum* isolated from begonia. A: Diseased leaf, B: Upper surface of colony incubated on potato dextrose agar (PDA), C: Lower surface of colony incubated on PDA, D: Conidia (scale bar: 20 μm), E: Pycnidia (scale bar: 30 μm), F: Appressoria (scale bar: 10 μm).

Pathogenicity test. To confirm the pathogenicity of the fungus, the leaves of begonia with or without wound were inoculated with conidial suspension (10^6 conidia/ml) prepared from 7-day-old culture on PDA at 25°C. The inoculated plants were maintained in a moist chamber and 25°C for 24 hours in the dark and then transferred to a greenhouse.

Investigation of optimum temperature range. The effect of temperatures on mycelial growth of the fungus was investigated. Mycelial plugs 5 mm in diameter were punched out from actively growing mycelial colonies on PDA by a cork borer and placed on the center of PDA plates. The PDA plates were incubated in 9 different temperature regimes (0, 5, 10, 15, 20, 25, 30, 35, 40°C). Mycelial colony diameter measured 7 days after incubation on PDA.

Screening of fungicides. The effect of fungicides on mycelial growth of the fungus was investigated to select preventive fungicides of the disease. Mycelial plugs 5 mm in diameter were punched out from actively growing mycelial colonies on PDA by a cork borer and placed on the center of PDA plates. Sixteen fungicides were diluted as recommended application rates as shown in Table 2. Paper disks (5 mm in diameter) absorbed the fungicides were placed at the edge of the 5-day-old mycelial colony on PDA. Sensitivity of the fungus to fungicides was measured 48 hours after incubation at 25°C.

Results and Discussion

Symptom. Yellowish spots occurred on the diseased leaves of begonia plants as initial symptoms (Fig. 1C), and they coalesced irregularly to form large brown pleomorphic lesions (Fig. 1D). Severely infected leaves were defoliated (Fig. 1E), resulting in abnormal growth of the entire plant (Fig. 1B).

Morphological characteristics. The isolates formed white to grayish aerial mycelia (Fig. 2A) and the under-surface of the plate was dark gray (Fig. 2B). Conidia were aseptate, hyaline, mostly fusiform, and measured 10.0~20.5 × 3.5~5.0 μm (Fig. 2C). Dark brown and obovoid or clavate shape of appressoria developed from germ tubes, ranging from 7.5~12.5 × 5.0~7.5 μm in size (Fig. 2D). These morphological characteristics (Table 1) of the fungus are in accordance with those of *Colletotrichum acutatum* J. H. Simmonds (Dyko and Mordue, 1979).

RAPD analysis. The primer pair of Ca1-1 and ITS4 successfully amplified ITS region of three isolates in a species-specific manner with PCR products around 500 bp as expected (Fig. 3). The results from positive and negative reactions showed the reliability of PCR amplification conducted. As shown in the morphological analysis, the three isolates were considered to be *C. acutatum*.

Pathogenicity. Typical dark brown necrotic lesions appeared on the wounded leaves of begonia 5 days after artificial inoculation (Fig. 1F) but no symptom developed on control plants. The fungus was re-isolated from lesions on the plants inoculated. Thus the causal fungus was identified as *C. acutatum* based on the pathogenicity and morphological traits (Dyko and Mordue, 1979).

Optimum temperature. Mycelial colony diameter measured 7 days after incubation on PDA is shown in Fig. 4.

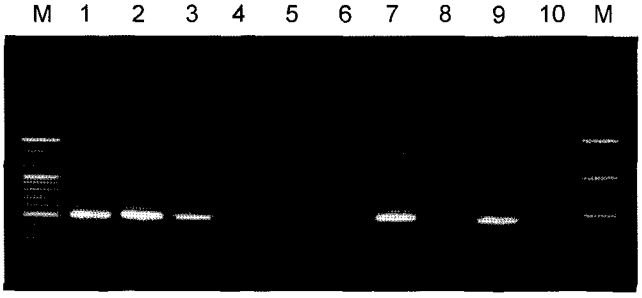

Fig. 3. RAPD patterns of ITS regions of rDNA of *Colletotrichum* isolated from begonia in Korea using species specific primers Ca1-1 and Cglnt with ITS4 as a universal primer. Size markers are 100 bp ladder at both sides (M). Lanes1-3, tested isolates with Ca1-1 and ITS4 pair; lanes 4-6, tested isolates with Cglnt and ITS4 pair; lane 7 and 8, *C. acutatum* isolate positive and negative, respectively; lane 9 and 10, *C. gloeosporioides* isolate positive and negative, respectively.

Table 1. Comparison of morphological characteristics of the present isolates with *Colletotrichum acutatum* previously described

Characteristics		Present isolates	*Collectotrichum acutatum*[a]
Colony	Shape	dense, aerial mycelium	dense, aerial mycelium
	Color	white, pinkish grey, green grey	white, pinkish grey
Conidia shape		fusiform, straight, obtuse at the apex	fusiform, occasionally medianly constricted
Conidia size (μm)		10.0~20.5 × 3.5~5.0	8.0~16.0 × 2.5~4.0
Appressoria size (μm)		7.5~12.5 × 5.0~7.5	6.5~11.0 × 4.5~7.4

[a]Data from Dyko and Mordue (1979).

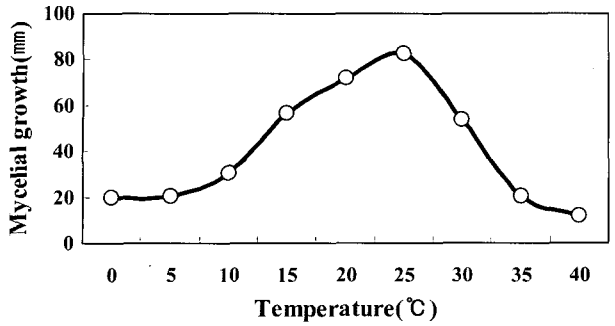

Fig. 4. Effect of temperature on mycelial growth of *Colletotrichum acutatum*, causing begonia anthracnose. Linear mycelial growth was measured 7 days after incubation on potato dextrose agar. Data are means of three replicates.

Table 2. Sensitivities of *Colletotrichum acutatum*, causing begonia anthracnose, to various fungicides on potato dextrose agar

Fungicides tested	Concentration (ppm)	Sensitivity
Azoxystrobin SC	2,000	+[a]
Bitertanol WP	1,000	–
Diethofencarb-carbendazim WP	1,000	–
Difenoconazole WP	2,000	–
Dithianon SC	1,000	–
Iminoctadine tris(albesilate) WP	1,000	–
Iminoctadine tris(albesilate)-thiram WP	1,000	–
Iminoctadinetriacetate SL	1,000	–
Iprodione WP	1,000	–
Metiram WG	500	–
Polyoxin B-iminoctadine tris(albesilate) WP	1,000	–
Procymidone WP	1,000	–
Propineb WP	500	–
Tebuconazole WP	1,000	+
Thiophanate-methyl WP	1,000	–
Triflumizole WP	1,000	–
Control	–	–

[a]+, Sensitive; –, Insensitive.

The optimum temperature for mycelial growth of *C. acutatum* on PDA was around 25°C, indicating that high temperatures would be more favorable to the fungus. In our survey, outbreak of anthracnose on begonia was observed in July. High temperature and humiditiy favorable to the fungus during rainy season of July in Gyeongju seemed to predispose the epidemic.

Effective fungicides. The fungus was sensitive to azoxystrobin and tebuconazole (Table 2). This suggests that the disease will be alleviated by specific fungicides.

Soft rot caused by *Erwinia carotovora* subsp. *carotovora* and gray mold by *Botrytis cinerea* are recorded on begonia in Korea (The Korean Society of Plant Pathology, 2004), but there is no previous report of anthracnose on begonia caused by *C. acutatum*. *C. acutatum* was reported in mungbean sprout (*Phaseolus radiatus*) (Kim et al., 2003), safflower (*Carthamus tinctorius*) (Kim et al., 1999; Kwon et al., 1998), red pepper (*Capsicum annuum*) (Park and Kim, 1992), cosmos (*Cosmos bipinnatus*) (Kwon et al., 1999), and apple (*Malus pumila* var. *dulcissima*) (Lee, 1994) in Korea. Therefore, this is the first report on the anthracnose of begonia caused by *C. acutatum* in Korea. We propose that the disease be named as anthracnose of begonia.

Acknowledgements

This research was supported by the Korea Science and Engineering Foundation Grant No. R01-2003-000-10898-0.

References

Dyko, B. J. and Mordue, J. E. M. 1979. *Colletotrichum acutatum*. CMI Descriptions of Pathogenic Fungi and Bacteria. Kew, Surey, England. No. 630.

Kim, D. K., Lee, S. C., Kang, J. H. and Kim, H. K. 2003. Colletotrichum disease of mungbean sprout by *Colletotrichum acutatum*. *Plant Pathol. J.* 19:203-203.

Kim, W. G, Moon, Y. G., Cho, W. D. and Park, S. D. 1999. Anthracnose of safflower caused by *Colletotrichum acutatum*. *Plant Pathol. J.* 15:62-67.

Kwon, J. H., Kang, S. W., Kim, H. K. and Park, C. S. 1998. Safflower (*Carthamus tinctorius* L.) anthracnose caused by *Colletotrichum acutatum* in Korea. *Korean J. Plant Pathol.* 14:651-653.

Kwon, J. H., Kang, S. W., Son, K. A. and Park, C. S. 1999. Anthracnose of cosmos caused by *Colletotrichum acutatum* in Korea. *Plant Pathol. J.* 15:172-174.

Lee, D. H. 1994. Etiological characteristics of *Colletotrichum acutatum, C. gloeosporioides* and *Glomerella cinglulata* isolated from apples. *Korean J. Plant Pathol.* 10:105-111.

Park, K. S. and Kim, C. H. 1992. Identification, distribution and etiological characterization of anthracnose funigi of red pepper in Korea. *Korean J. Plant Pathol.* 8:61-69.

Smith, B. J. and Black, L. L. 1990. Morphological, cultural, and pathogenic variation among *Colletotrichum* species isolated from strawberry. *Plant Dis.* 74:69-76.

The Korean Society of Plant Pathology. 2004. List of Plant Diseases in Korea. 4th edition. The Korean Society of Plant Pathology. 779 pp.