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Botrysphaeria dothidea, the Causal Organism of Ripe Rot of Kiwifruit (*Actinidia deliciosa*) in Korea

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Ripe rot was commonly found in overripe kiwifruits (*Actinidia deliciosa*) during a disease survey conducted in Jeju and Jeonnam provinces in Korea in 1999. While the disease did not manifest any clear external symptoms on kiwifruits, it caused portion of the fruit surface to collapse. Watersoaked flesh tissue could be seen on the sunken part when the skin of the collapsed portion was peeled off. The milky internal symptom with dark green margin developed concentrically as the fruit ripened. A species of *Botryosphaeria* was consistently isolated from lesions showing typical symptoms of ripe rot on kiwifruit, and its pathogenicity was confirmed by artificial inoculation test on healthy kiwifruits. The fungus was also pathogenic to apple and pear. The mycological characteristics of the fungus were consistent with those of *Botryosphaeria dothidea*. This is the first report on the detailed mycological characteristics of the causal organism of post-harvest ripe rot of kiwifruit in Korea.

Keywords **:** *Botryosphaeria dothidea*, kiwifruit, ripe rot

Post-harvest fruit rot occurs during storage, transportation, marketing, and consumption of kiwifruit [Chinese gooseberry, *Actinidia deliciosa* (A. Chev.) C. F. Liang et A. R. Ferguson]. Many fungi were reported to be associated with post-harvest fruit rot of kiwifruit (Beraha, 1970; Hawthorne et al., 1982; Pennycook, 1981; 1985; Sommer and Beraha, 1975). In Korea, *Phomopsis* sp. was reported to be one of the major pathogens of post-harvest fruit rot in kiwifruit (Chung, 1997; Park et al., 1994; Yi and Lee, 1998). Lee et al. (2001) first reported the teleomorph state of *Phomopsis* sp. as *Diaporthe actinidiae* in Korea. They also confirmed that *D. actinidiae* caused stem-end rot on kiwifruit (Beraha, 1970; Sommer and Beraha, 1975). *Botryosphaeria* sp. was also reported to cause ripe rot, one of the post-harvest fruit rots in kiwifruit (Lee et al., 1998; Ryu et al., 1993), but the detailed mycological characteristics of the fungus including its teleomorph state have not been reported until now in Korea.

Diseased fruits were collected from kiwifruit orchards in Jeju and Jeonnam provinces in 1999. Ripe rot was commonly found in overripe fruits. While the disease did not manifest any clear external symptoms on kiwifruits, it caused portion of the fruit surface to collapse (Fig. 1A). Watersoaked flesh tissue could be seen on the sunken part when the skin of the collapsed portion was peeled off. The milky internal symptom with dark green margin developed concentrically as the fruit ripened (Fig. 1B).

A species of *Botryosphaeria* was consistently isolated from the lesions showing typical symptoms of ripe rot on kiwifruit. The optimum temperature range for mycelial growth of the fungus was 25-35°C, but it did not grow under 10°C and over 40°C. White mycelial colony was formed on potato dextrose agar (PDA) plate, the color of which changed into black after 7-day incubation at 25°C (Fig. 1F). After 4 weeks of incubation, black globose pycnidia were formed on the mycelial colony (Fig. 1G-H) which measured 130 × 260 μm in diameter (Fig. 1I). Conidia were hyaline, unicellular, and fusoid, and 5-7.6 μm wide × 20-30 μm long (Fig. 1J-K). Ascostroma were produced on the border line formed by dual culture of species of *Botryosphaeria* and *Phomopsis* on PDA plates, but not on PDA plates when a singular species of *Botryosphaeria* was incubated. Pseudothecia with ostiolate necks in the ascostroma were black and globose, and measured 200-250 μm in diameter (Fig. 1L). Asci were released from crushed pseudothecia and were filiform, clavate, and bituniate, and 12.5-22.5 μm wide × 127.5-200 μm long (Fig. 1M). These contained hyaline, unicellular, and ovoid ascospores measuring 7.5-12.5 × 27.5-37.5 μm (Fig. 1N-O). The mycological characteristics of *Botryosphaeria* sp. were consistent with those of *Botryosphaeria dothidea* (Table 1) (Punithalingam and Holliday, 1973).

For the pathogenicity test, inoculum of *B. dothidea* was prepared by inoculating mycelial plugs on PDA plates and culturing at 25°C for 4 weeks. Twenty near-ripe healthy fruits were surface disinfected with 70% ethanol for 2

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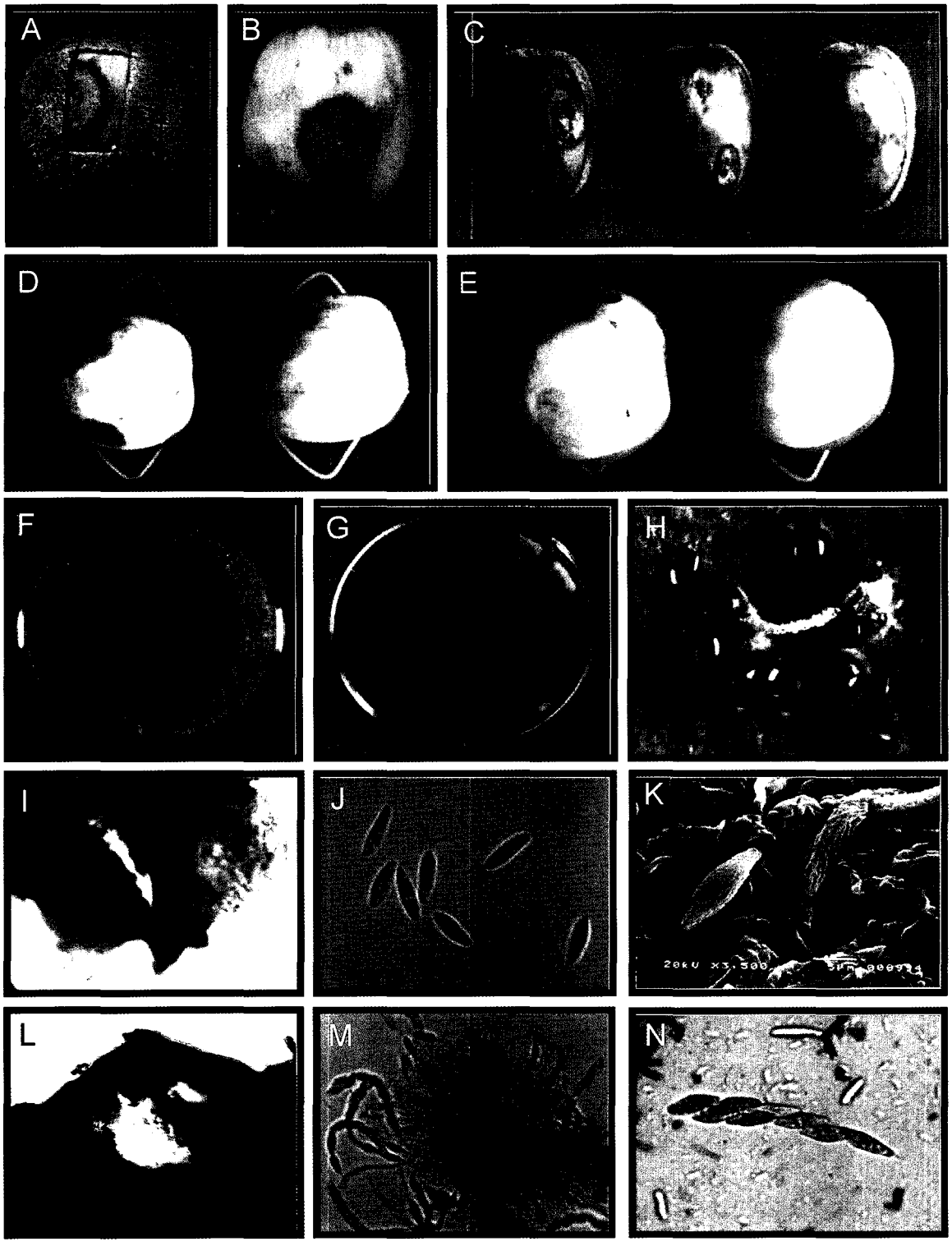

Fig. 1. Symptoms of ripe rot of kiwifruits and mycological characteristics of*Botryosphaeria dothidea*. (**A**) External symptom on naturally infected kiwifruit. Note collapsed portion of the fruit surface; (**B**) Internal symptom on naturally infected kiwifruit; (**C**) Internal symptoms on artificially infected kiwifruits; (**D**) Internal symptoms on artificially infected apples; (**E**) Internal symptoms on artificially infected pears; (**F**) Mycelial colony of 7-day-old culture on PDA; (**G**) Mycelial colony of 28-day-old culture on PDA; (**H**) Micrograph of pycnidia produced on PDA; (**I**) Micrograph of pycnidia. Bar = 100 μm, (**J**) Micrograph of conidia. Bar = 10 μm; (**K**) Scanning electron micrograph of conidia placed on the surface of pycnidia produced on PDA; (**L**) Micrograph of pseudothecia. Bar = 100μm; (**M**) Asci on stroma; (**N**) Micrograph of ascospores within ascus. Bar = 10μm.

Table 1. Mycological characteristics of the present isolates compared with *Botryosphaeria dothidea* previously described

Characteristic		Present isolate	*Botryosphaeria dothidea*[a]
Pycnidia	Shape	stromatic, globose	stromatic, globose
	Size (μm)	130 × 260	150 × 250
Conidia	Shape	hyaline, fusoid, unicelluar	hyaline, fusoid, unicelluar
	Size (μm)	20-30 × 5-7.5	17-25 × 5-7
Pseudothecia	Shape	ostiolate and darker around the neck	ostiolate and darker around the neck
	Size (μm)	200 × 250	170 × 250
Asci	Shape	filiform, clavate, bituniate	filiform, clavate, bituniate
	Size (μm)	137.5-200 × 12.5-22.5	100-110 × 16-20
Ascospores	Shape	hyaline, unicelluar, ovoid	irregularly biseriate, hyaline, unicelluar, ovoid
	Size (μm)	27.5-37.5 × 7.5-12.5	17-23 × 7-10

[a] Data from Punithalingam & Holliday (1973).

minutes, rinsed in sterile water, and air dried. The conidial suspension (10^6 conidia/ml) harvested from the culture was sprayed onto healthy kiwifruits (cv. Hayward) with wounding (2-3 mm deep by 10 sterile pins) or without wounding. All the fruits inoculated were maintained in a polyethylene bag in dark incubator (25°C) for 3 or 7 days. Five fruits which served as control were treated with sterilized water and maintained under the same condition. Soft rot with dark green margin was observed on the affected flesh tissue when the skin was peeled off 5 days after wound inoculation, but no visible internal symptoms were observed on the fruits inoculated without wounding (Fig. 1C) (Table 2). Pieces of the decayed tissue from the margin of the lesion on the inoculated fruits were transferred to fresh PDA plates, all of which yielded the causal *B. dothidea* organism.

The same methods were applied to healthy pear (cv. Singo) and apple (cv. Hongok) fruits in order to confirm the host range of *B. dothidea*. Similarly, the typical symptoms were observed on the wounded fruits of apple (Fig. 1D) and pear (Fig. 1E) when the skin was peeled off. The disease severity on apple was more or less milder than those on kiwifruit but was similar to that observed on pear (Table 2). *B. dothidea* was reported to cause various diseases in a wide range of hosts (Kim et al., 2003; Punithalingam and Holliday, 1973; The Korean Society of Plant Pathology, 1998). The fungus was also known as the causal organism of stem canker and fruit rot on kiwifruit in Korea (Lee et al., 1998; Ryu et al., 1993). This is the first report on the detailed mycological characteristics of the causal organism of post-harvest ripe rot of kiwifruit in Korea.

Table 2. Pathogenicity of *Botryosphaeria dothidea* isolated from kiwifruit to fruits of kiwifruit, apple, and pear by artificial inoculation

Host	Pathogenicity[a]	
	Wounded	Not Wounded
Kiwifruit (cv. Hayward)	++	−
Apple (cv. Hongok)	+	−
Pear (cv. Singo)	++	−

[a] Pathogenicity was evaluated by the incidence of ripe rot 5 days after inoculation.
++ = Severe symptom; + = Mild symptom; − = No symptom.

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