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Gray Mold on Carrot Caused by *Botrytis cinerea* in Korea

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Gray mold caused by *Botrytis cinerea* was found on a carrot seedling in a greenhouse and a field at Daegwallryeong, Gangwon Province in 2007–2009. Symptoms included irregular, brown, blight, or chlorotic halo on leaves and petioles of the carrots. Fungal conidia were globose to subglobose or ellipsoid, hyaline or pale brown, nonseptate, one celled, $7.2\text{--}18.2 \times 4.5\text{--}11 \mu\text{m}$ ($12.1 \times 8.3 \mu\text{m}$) in size, and were formed on botryose heads. *B. cinerea* colonies were hyaline on PDA, and then turned gray and later changed dark gray or brown when spores appeared. The fungal growth stopped at 35°C, temperature range for proper growth was 15–25°C on MEA and PDA. Carrots inoculated with 1×10^5 ml conidial suspension were incubated in a moist chamber at $25 \pm 1^\circ\text{C}$ for pathogenicity testing. Symptoms included irregular, brown, water-soaked rot on carrot roots and irregular, pale brown or dark brown, water-soaked rot on leaves. Symptoms were similar to the original symptoms under natural conditions. The pathogen was reisolated from diseased leaves, sliced roots, and whole roots after inoculation. As a result, this is the first report of carrot gray mold caused by *B. cinerea* in Korea.

Keywords : *Botrytis cinerea*, *Daucus carota* L. subsp. *sativas* (Hoffm), Leaf blight

Gray mold, caused by the important pathogen *Botrytis cinerea*, affects most vegetables and fruit crops as well as a large number of trees, flowers, and weeds (Jarvis, 1977). Carrot (*Daucus carota* L. subsp. *sativas*, Hoffm.), belongs to the family *Umbelliferae*. Carrot roots are consumed as an edible vegetable and contain many nutritive elements such as vitamins, minerals, and fiber (Rubatzky et al., 1999).

Gray mold symptoms were first found on leaves and petioles of carrots in the field. A fungus was isolated from infected tissues and re-isolated from inoculated samples using a conidial suspension. However, gray mold caused by *B. cinerea* on carrot has not been reported in many countries. In many

crops including vegetables the mold causes brown spot on the leaves, petioles, and petals (Ellis, 1971; Hennebert, 1973). The pathogenic fungus, *B. cinerea*, causes a serious gray mold disease wherever umbelliferous crops are grown. This fungus is associated with significant postharvest carrot disease in the USA (Davis and Raid, 2002) but has not been reported on the leaves and petioles of carrots infected in the field in Korea. This fungus has low activity in storage at 0°C but can spread to adjacent tissue at 4°C (Goodliffe and Heale, 1977). Additionally, *B. cinerea* is a problem on fruits and vegetables as well as carrots in cold storage and subsequent shipping, because the fungus is able to remain active at temperatures just above freezing (Hennebert, 1973). Gray mold originates on some parts of the carrot taproot, and the first symptoms are water-

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soaked, light brown to tan lesions. When the fungus expands, the lesions become covered with gray conidia that penetrate into inner tissues (Sharman and Heale, 1977).

This study was conducted to identify the casual agent related with blight lesions on carrot leaves collected from the field in Pyeongchang and to investigate the morphology, cultural characteristics, and β -tubulin analysis of the pathogen and pathogenicity on carrot.

Fungi were isolated from gray mold lesions on carrots grown in Pyeongchang. The surface of the diseased leaf tissue pieces was sterilized with 70% ethanol for 1 minute, rinsed twice with sterilized distilled water, and then air dried on sterilized filter paper. Dried samples were placed on water agar (WA) and then incubated at $25 \pm 1^\circ\text{C}$. After 3 days of inoculation, growing mycelial tips were cultured on potato dextrose agar (PDA) plates for 2 weeks. Single spores were isolated by the dilution method on WA and cultured on PDA for 7 days.

The shape, size, color, and conidia of the isolates were investigated under a microscope (Eclipse E600; Nikon, Tokyo, Japan) after a $25 \pm 1^\circ\text{C}$ incubation for 2 weeks on CMA, MEA, PDA, V8A, and WA. Cultural characteristics of the isolates were observed on various culture media incubated for 2 weeks at $25 \pm 1^\circ\text{C}$. Mycelial growth on the media was observed after a 7 day incubation at $25 \pm 1^\circ\text{C}$. Colonies were incubated on PDA from $5\text{--}35^\circ\text{C}$ at intervals of about 5°C with three replications to investigate the temperature range of growth. Agar disks of appropriate size (1 mm thick) were cut from a *B. cinerea* isolate on PDA for scanning electron microscopy (SEM). Samples were immersion-fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2–7.4) for 4 h. The fixed samples were rinsed with 0.1 M phosphate buffer three times for 20 min each. Samples were post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 1 h. Samples were washed in 0.1 M phosphate buffered saline (three time for 20 min each), dehydrated in a graded ethanol series (30, 50, 60, 70, 80, 90, 95, and 100%) for 15 min per step, and then rinsed in absolute ethanol three times

for 30 min each. After critical point drying, the specimens were sputter-coated with gold using a sputter coater and then observed under SEM (Hitachi S-2460N; Hitachi Instruments, Hazelbrook, Australia) at 5–20 kv.

Isolates were used to test carrot pathogenicity. Mycelial agar blocks, made from an 8 mm cork borer, were subcultured on PDA for 14 days. Conidia suspensions were made by adding 3 ml of sterile distilled water in PDA, scraping the colonies with a rubber spatula, and then harvesting them by filtering through four layers of cheesecloth. Leaves, sliced roots, and whole roots of carrot with or without wounds were inoculated with 30 μl drops of conidial suspension (1×10^5 conidia per ml). Inoculated samples were laid in a plastic box ($292 \times 225 \times 120$ mm) under 100% humidity, and incubated at $25 \pm 1^\circ\text{C}$ for 2 weeks.

To identify the isolates, the β -tubulin gene was amplified with the Bcin-366r and BT-2M-up primers (Spotts et al., 2008) and directly sequenced. The sequence was compared with data in GenBank (Accession No. FQ790278 and Z69263). A molecular phylogenetic analysis was carried out with MEGA4 using the neighbor-joining method and the Tajima-Nei distance model.

Gray mold was found on carrot leaves in September 2007. Gray mold originally appeared on the edge of the infected leaves with a small spot or chlorotic halo. Irregularly shaped and water-soaked lesions developed on infected leaves. Subsequently, infected petioles and stems became slender and were totally blighted on the above ground part of the carrot. The fungal colonies were hyaline and then turned gray, and the spores changed dark gray or brown (Fig. 1A, B). Colonies were hyaline or light gray to gray, scarce, and weak on CMA and WA. Sclerotia were black in color and irregular in shape but were absent on CMA and WA. The size of the sclerotia on MEA was larger than that on other media (Fig. 1C). Hyphae were hyaline to gray, with granules, and articulate-septated. Conidia that formed

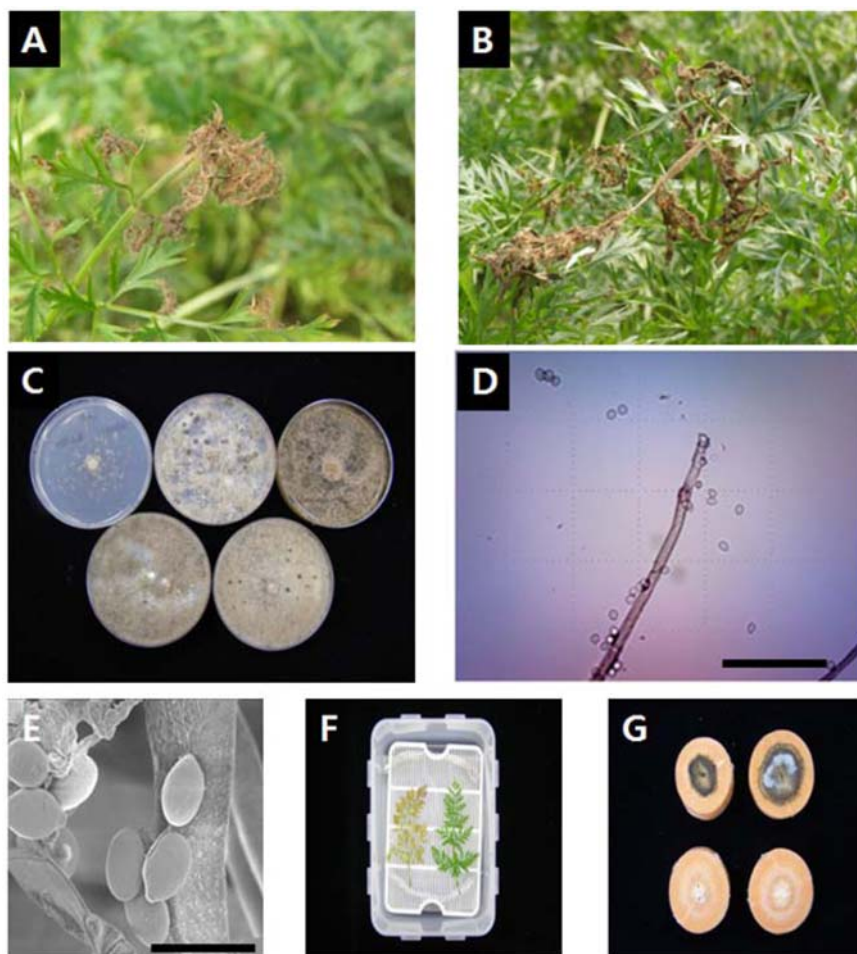


Fig. 1. Symptoms and conidial structure of gray mold on carrot caused by *Botrytis cinerea*. Infected leaves **A**, petiole **B**, colony formed on media after 2 weeks of incubation; **C**, conidia and conidiophore; **D**, scanning electron micrograph of conidia; **E**, pathogenicity test of gray mold on the artificially inoculated leaves; **F**, and sliced roots; **G**, Bar = 100 μ m for D and 10 μ m for E.

Table 1. Morphological characteristics of *Botrytis cinerea* cultured in various media

Characteristics	Description of <i>B. cinerea</i>					
	Ellis(1971)	CMA	MEA	PDA	V8A	WA
Colony color	Pale brown	Light gray	Dark gray	Dark brown	Gray	Gray
Sclerotia	Present	Absent	Present	Present	Present	Absent
Conidia	Abundant	Scarce	Abundant	Abundant	Abundant	Scarce
Color	Pale brown	Gray	Pale brown	Dark brown	Pale brown	Light gray
Shape	Ellipsoidal or obovoid	Ellipsoidal or globose	Ellipsoidal or globose	Ellipsoidal or globose	Ellipsoidal or globose	Ellipsoidal or globose
Size(μ m)	6–18 \times 4–11	9.7–18.9 \times 5.1–12.1	8.1–20.8 \times 5.1–13.2	7.2–18.2 \times 4.5–11	8–20.2 \times 5.4–11.9	6.4–19.1 \times 5.1–12.8
(mean)	–	14.2 \times 8.8	12.6 \times 8.8	12.1 \times 8.3	12.5 \times 8.5	12.2 \times 8.8

on conidiophores in PDA were not in chains, hyaline to pale brown, one-celled, globose to subglobose or ellipsoidal, and measured 7.2–18.2 \times 4.5–11 μ m (12.1 \times 8.3) in size (Fig. 1D). SEM revealed that the conidia of the isolate had

a smooth surface (Fig. 1E). All inoculated samples on the wounded carrot developed gray mold symptoms. No symptoms were observed on the control carrot or whole root without a wound. Typical symptoms on the wounded

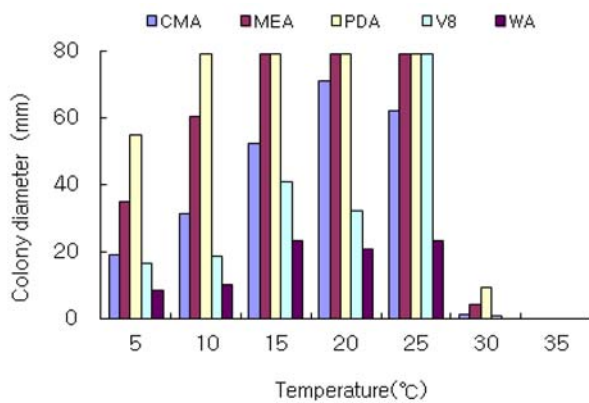


Fig. 2. Colony growth of *Botrytis cinerea* at different temperatures on five kinds of media.

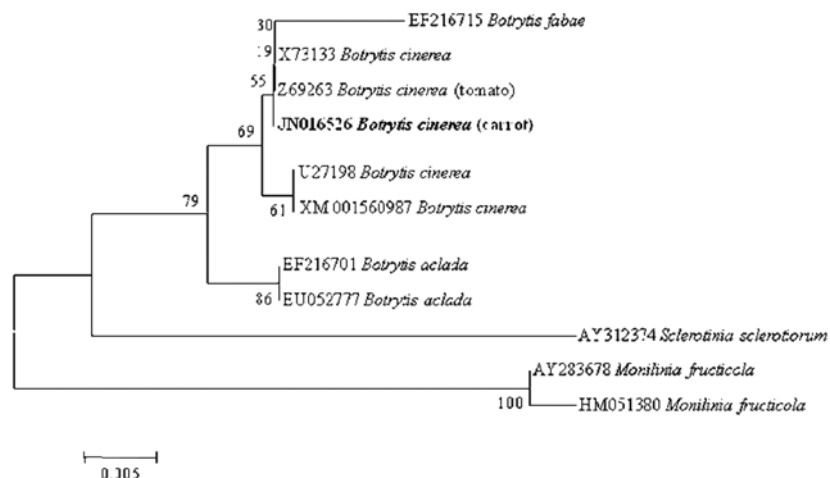
sample appeared 5 days after inoculation. Lesions seemed to expand readily on sliced carrot roots. Symptoms appeared in rot on carrot roots and blight on green leaves as well as petioles (Fig. 1F, G). Conidiophores on PDA were tall, fine, hyaline or pigmented, branched irregularly on the top, apical cells expanded or round-shaped, bearing clusters of conidia on short

denticles, and usually over 1 mm high (Table 1). Fungal colonies reached 8 cm diameter on MEA, PDA, and V8A for 7 days at 25°C. The fungal growth stopped at temperatures > 35°C, and the temperature range for proper growth was 15–25°C on MEA and PDA. However, mycelial growth did not stop at 5°C (Fig. 2). Growth was similar to that of *B. cinerea* at low temperatures reported by Ellis (1971). After 7 days of inoculation, the diseased lesions enlarged, water-soaked, and coalesced in the mass. Common symptoms included a gray to brown discoloration, water soaking, and a pale gray to tan mold growing on the surface of diseased areas. However, the pathogenicity of *B. cinerea* on non-wounded leaves and roots was very weak (Table 2). Hennebert (1977) showed that symptoms of *Botrytis* diseases vary greatly depending on the host and plant part attacked. Gray mold symptoms, which were induced on plants after inoculation with the isolates, were very similar to those observed in the

Table 2. Pathogenicity of *Botrytis cinerea* isolates inoculated on carrot

Lesions	Disease severity ^a	
	Non-wounded	Wounded
Leaf	+	+++
Sliced root	++	+++
Whole root	–	+

^a Length of lesions was measured 7 days after inoculation with 10⁵ conidia/ml. +++, > 21 mm in length; ++, 11–20 mm in length; +, 1–10 mm in length; –, no lesion.



field. The fungus was reisolated from lesions of inoculated plants. No significant difference in pathogenicity was observed among the isolates. Based on the results of α -tubulin analysis, the determined sequence of 530 bp was deposited in GenBank (Accession No. JN016526) and was equal to that of *B. cinerea* with 100% similarity. The fungus clustered with *B. cinerea* in a phylogenetic tree (Fig. 3).

Based on the morphological characteristics, pathogenicity, and the α -tubulin sequence, the fungus was identified as *B. cinerea*. This result is similar to earlier descriptions by Ellis (1971). Gray mold on carrots was not as severe in the field, but the causal fungus could be a latent inoculum to another plant. The fungus *B. cinerea* can overwinter as tiny, black, and irregular sclerotia in dead plant tissue or soil and survives in the soil as mycelium on plant debris. Therefore, eradication of plant debris every autumn may be important for managing gray mold on carrot. Gray mold is not considered a major carrot disease, and it may become more prevalent as a postharvest disease of carrot. Occurrence of the disease has not been reported so far in Korea (The Korean Society of Plant Pathology, 2009). This is the first report of carrot gray mold caused by *B. cinerea* in Korea.

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