First report of flower bud rot of hydrangeas caused by *Botrytis cinerea* in Japan

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**Abstract**

Severe flower bud rot was observed in hydrangeas (*Hydrangea macrophylla*) growing in Shimane Prefecture, Japan. This symptom was observed in potted hydrangeas grown under forcing culture in greenhouses during winter. A gray mold pathogen (*Botrytis cinerea*) was isolated from rotten buds, and the original rot symptom was reproduced via inoculation with the isolates. The identification of the pathogen was confirmed by morphology and molecular phylogeny. This is the first report of hydrangea flower bud rot caused by *B. cinerea* in Japan.

**Key words:** *Botrytis cinerea*, flower bud rot, forcing culture, potted hydrangea, winter

**Materials and Methods**

**Investigation of flower bud rot**

From January to February in 2017 and February in 2018, we investigated the emergence of hydrangea flower bud rot in the greenhouses of eight growers conducting forcing culture in winter. We counted the number of plants with flower bud rot and calculated the percentage of diseased plants of the total number of counted plants.

**Isolation**

Fungal isolation was performed from the plants with flower bud rot. The damaged tissues were initially washed with tap water, and the excess water was removed using filter paper. To test for possible bacterial pathogens, 5-mm-long segments of non-sterilized flower bud tissue were mounted in water on glass slides and examined for bacterial streaming with a microscope. For fungal isolation, the segments were soaked in a 0.6% sodium hypochlorite solution for 3 min to sterilize their surfaces and were then rinsed three times with sterile distilled water. The segments were dried on sterilized filter paper, then incubated on potato dextrose agar (PDA) for 5–10 days at 20°C. The isolates were obtained from single-conidia and stored in PDA at 5°C until use. Among the 64 *Botrytis* isolates obtained, three isolates (BK-2, BH-1, and BE-2) were randomly selected and used for the identification.

**Identifications**

The isolates BK-2, BH-1, and BE-2 were grown on PDA at 18°C under constant black light for sporulation and on PDA at 20°C in the dark for sclerotia formation. The conidia (including conidiophores) and sclerotia were measured by a digital microscope after 10 and 42 days of incubation, respectively.

The DNA extraction was performed by the method of

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Nagashima et al. (2021). The polymerase chain reaction (PCR) was conducted using the following primer pairs (Staats et al., 2005): G3PDHfor and G3PDHrev for the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene, HSP60for and HSP60rev for the heat shock protein 60 (HSP60) gene, and RPB2for and RPB2rev for the second-largest subunit of DNA-dependent RNA polymerase II (RPB2) gene. The PCR cycle condition was the same as that described by Staats et al. (2005). Sequencing analyses were performed by the method of Nagashima et al. (2021). The phylogenetic tree of the combined partial sequences (G3PDH 890 bp, HSP60 974 bp, and RPB2 1096 bp) were compared with those of Botrytis species subclade 2 (Zhong et al., 2019). The partial sequences of G3PDH, HSP60, and RPB2 were deposited in the DDBJ/EMBL/GenBank database under the accession numbers LC599392–599394, LC599395–599397, and LC599398–599400, respectively.

Botrytis cinerea identification was also confirmed by a pathogenicity test on the fruits or pods of eggplants, green peppers, and kidney beans. Each isolate was grown on PDA for five days, then was cut out as 4-mm-diameter plugs. One colonized plug was placed in each fruit or pod with a wound 1–2 mm deep and 3 mm in diameter from the surface created using a bundle of 10 pins. The inoculated fruits or pods were placed in a plastic box with saturated humidity at 20°C in complete darkness for three days. Pathogenicity was defined by the presence or absence of rotting lesions that were more than 3 mm in diameter. A sterile PDA plug was used as the uninoculated control. Three fruits or pods per isolate were used for the experiment.

Pathogenicity test on hydrangea

The fungal isolates BK-2, BH-1, and BE-2, and eleven-month-old potted hydrangeas (cv. Mangekyo) were used in the pathogenicity test. The inoculum of the isolates was prepared as described above. One colonized plug was placed at the base of a flower bud that was either unwounded or wounded as described above. The inoculated part was covered by parafilm. The inoculated plants were placed in a growth chamber at 20°C with a 12-h photoperiod for 10 days. The pathogenicity was defined by the method described above. Symptomatic flower buds were analyzed for the presence of fungi as described above. Nine wounded or unwounded flower buds per isolate (i.e., 18 flower buds in total per isolate) were used for the experiment.

Results and Discussion

The percentage of diseased hydrangeas (Fig. 1a) ranged from 0.5 to 11.5% of the total number of plants in the green-
houses. The flower bud rot symptom was usually evident around two weeks after beginning the forcing culture. Dark-brown lesions initially appeared on the surface of the basal part of the flower buds. Then, the lesions gradually enlarged by spreading both upward to the surface and toward the inside of the buds (Fig. 1b). Under high-humidity conditions, the damaged plants exhibited gray-mold sporulation (Fig. 1c), followed by bud dropping (Fig. 1d). A mild symptom that only caused bud surface discoloration followed by delayed sprouting was occasionally observed in the hydrangeas (Fig. 1e).

No bacterial streaming was observed when the small segments were examined microscopically. *Botrytis* isolates were consistently recovered from the damaged hydrangeas with flower bud rot in all eight commercial greenhouses during both years of the experiment.

The fungal isolates BK-2, BH-1, and BE-2 were morphologically identified as *B. cinerea* by comparison with the species description of Ellis (1971) (Table 1). This identification was confirmed by the phylogenetic analysis (Fig. 2) and pathogenicity tests with the fruits or pods of eggplants, green peppers, and kidney beans (data not shown). The isolates used in this study were deposited to the NIAS Genebank, Tsukuba, Ibaraki Prefecture, Japan (MAFF) under the accession numbers shown in Table 1.

All three *B. cinerea* isolates (BK-2, BH-1, and BE-2) caused flower bud rot symptoms when they were inoculated on the hydrangea plants. All tested isolates caused similar damage to the flower buds of potted hydrangeas (Fig. 1i–j). The symptoms developed on both the wounded and unwounded buds. All the isolates tested were re-isolated from the inoculated flower buds, thus fulfilling Koch’s postulates.

Arai (1996) reported that *B. cinerea* was the causal agent of gray mold on the leaves and blooms of hydrangeas in summer under field conditions. Occurrences of *B. cinerea* on the flower buds of hydrangeas can cause serious problems in greenhouses (Peterson and Davis, 1970) and during cold storage (Bailey, 1989). As far as we know, this is the first report of flower bud rot caused by *B. cinerea* in hydrangea in Japan.

*Botrytis cinerea* epidemics are highly influenced by the relative humidity and the leaf wetness in greenhouse crops (Dik and Wubben, 2004). These two environmental factors also likely affect the development of the present symptom of bud rot in hydrangeas. Based on our observations in the greenhouses, this symptom is probably enhanced by 1) a low

### Table 1. Morphological characteristics of the hydrangea isolates BK-2, BH-1, and BE-2

|          | BK-2 (MAFF247421) | BH-1 (MAFF247422) | BE-2 (MAFF247423) | *Botrytis cinerea*
|----------|-------------------|-------------------|-------------------|----------------------|
| Conidiophore Length (mm) | 0.9–2.6           | 0.7–2.3           | 1.0–2.3           | >2 (Frequently)
| Conidia Length (μm)      | 6.6–11.0          | 7.3–10.8          | 7.1–12.6          | 6–18                 |
| Breadth (μm)              | 4.6–6.8           | 4.1–6.8           | 5.0–8.0           | 4–11                 |
| l/b ratio                 | 1.56              | 1.62              | 1.57              | 1.35–1.5             |
| Sclerotia Color           | Black             | Black             | Black             | Black                |
| Length (mm)               | 1.4–7.0           | 1.3–5.4           | 1.9–4.4           | ND                   |
| Breadth (mm)              | 1.3–5.0           | 1.3–5.0           | 1.5–3.6           | ND                   |

*Ellis (1971)*

*Not described*
night temperature accompanied by high relative humidity, and 2) wetting of the basal surface of the flower buds by sap exudations from the phloem and xylem. Further inoculation tests should confirm the effects of these two environmental factors on development of the present bud rot symptom.

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