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Occurrence of Blossom Blight of *Chrysanthemum boreale* Caused by *Didymella chrysanthemi*

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Black blights attacked the blossom and flower buds of wild chrysanthemum (*Chrysanthemum boreale*) in the experimental field in Hamyang in 1998. The infection rate of the disease on the plant ranged from 4.0 to 91.8%. The pathogen isolated from the infected flower buds produced numerous conidia in pycnidia. The pycnidia, which were immersed into the petals, emerged through the epidermis by short ostiolate neck. Conidia had 0-3 septate (mostly uniseptate) and were 10-27.5 × 5-7.5 μm in size. The fungus produced pseudothecia on potato dextrose agar (PDA), and uniseptate ascospores produced in asci were 10 × 2.7 μm in size. The pathogen also produced pycnidia and pycnidiospores on PDA after 4 weeks in the dark condition. The conidia produced on PDA were smaller than those from infected plants. Based on the examined mycological characteristics, the fungus was identified as *Didymella chrysanthemi*.

Keywords **:** blossom blight, *Chrysanthemum boreale*, *Didymella chrysanthemi.*

The wild chrysanthemum, *Chrysanthemum boreale,* is one of the oriental medicinal herbs from ancient times used for headache, sedation, and other purposes. In recent years, new medicinal uses of the wild chrysanthemum such as lowering blood pressure, anti-diabetic, antibacterial activity, and angiogenesis inhibition have been discovered by research workers (Nam and Yang, 1995; Jang, 1998; Jang et al., 1998). For industrial utilization, wild chrysanthemums were collected from various regions in Korea for cultivation and breeding for mass production. However, various pests and diseases were observed during the first year of cultivation. Among them, black blight of the blossom and flower buds was one of the most serious problems. The ray blight of domesticated chrysanthemum was a serious problem of the flower industry in the U.S.A. in the early 1900s (Baker et al., 1949, 1961). Fortunately, in Korea, the disease has not yet been reported to occur in commercial chrysanthemum. The causal agent of the disease was reported as *Ascochyta chrysanthemi* in the conidial state, and *Didymella chrysanthemi* in the perfect state. In this study, the causal agent of wild chrysanthemum flower bud blight has been identified.

During the flowering season of wild chrysanthemum *Chrysanthemum boreale*, black blight attacked the flower buds, thus, infected flowers failed to bloom (Fig. 1). Symptoms on infected leaves also showed brown blight. When infected flower buds were kept in high humidity condition for 12-24 h, the immersed brown pycnidia appeared on the surface of the flower petals and other tissues, from which numerous conidia were released.

The wild chrysanthemum observation field was surveyed for flower bud blight, from which 10 prominent lines were selected. For each line, five plants were inspected randomly for blighted blossom from five corymbs per plant, and the infected rate per line was calculated. The disease incidence ranged from 4.0 to 91.8% in wild chrysanthemum *C. boreale* in the field (Table 1).

Single spore was isolated from released spores from the pycnidia formed in the tissue of infected petals, and placed on moistured filter paper in a petri dish for 24 h at room temperature, on water agar (WA) under a microscope. After germination of the single spore on WA, the hyphal tip was transferred on potato dextrose agar (PDA, Difco). After 5 days, the mycelial colony margin was cut off with a 5-mm cork borer, then transferred onto PDA for temperature profiles, conidiation, ascospore production, and pathogenicity test.

The pathogen produced pycnidia in the tissue of petals of wild chrysanthemum. The brownish pycnidia (sized 140-235 μm in diameter) immersed into the petals emerging through the epidermis by short ostiolate neck. Conidia were hyaline ovoid, cylindrical, straight or slightly curved, with round apices, guttulate, mostly uniseptate, rarely unicellular or biseptate, sized 10-27.5 × 5-7.5 μm (Table 2). The pycnidium formation was induced after a month at room temperature, and ascospore formation was induced after 2 months at the same condition.

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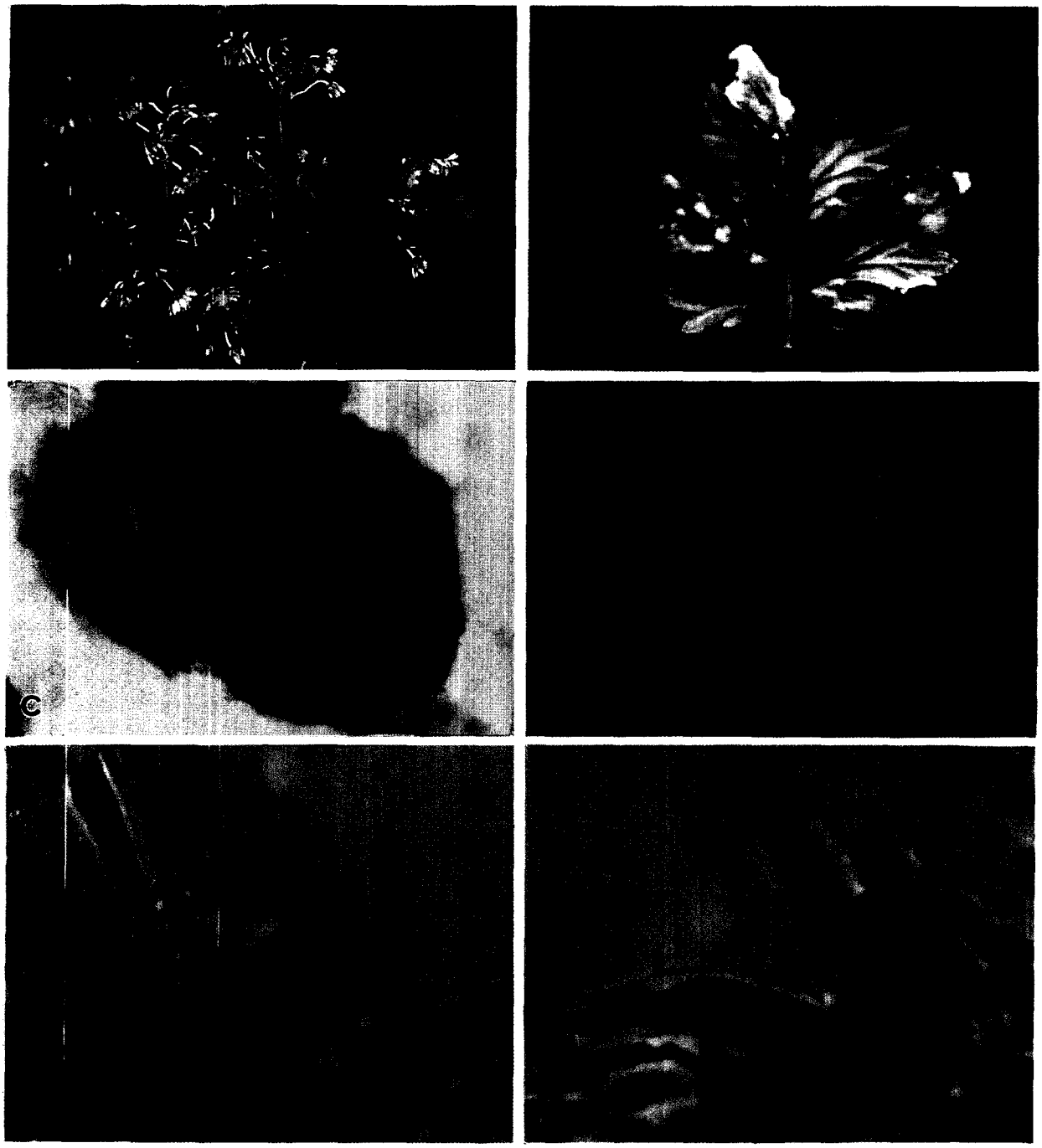

Fig. 1. Symptoms of blossom rot of *Chrysanthemum boreale* and its causal agent, *Didymella chrysanthemi*. (**a**) Symptoms of blossom blight; (**b**) symptoms of leaf reproduced by artificial inoculation; (**c**) pycnidia produced in petal of infected flower (178×); (**d**) conidia released from pycnidia (714×); (**e** and **f**) asci and ascospores produced in PDA (892×).

Condidia were seldom induced on PDA culture, however, few culture produced pycnidia in one month. The pycnidia and conidia formed on PDA were morphologically same as those of infected plant tissue. However, the pycnidia (sized 150-340 μm in diameter) produced on PDA were slightly bigger than those in host plant; Pycnidiospore (10-27.5 × 5-7.5 μm in diameter) produced in host plant were slighty larger than those on PDA. After 2 months, several hard black spots appeared on the PDA media, which were confirmed as pseudothecium containing asci and ascospores by microscopic examination. Ascospore was hyaline, fusiform, and uniseptate, sized 10 × 2.7 μm.

Table 1. Disease incidence of blossom blight on *Chrysanthemum boreale* in the experimental field

Entry No.	Flowering date	Plant height (cm)	Incidence of blossom blight (%)
97001	Oct. 21	145	35.2
97002	Oct. 28	131	24.0
97004	Oct. 23	154	60.3
97022	Nov. 1	83	4.0
97025	Oct. 23	148	46.9
97028	Oct. 23	120	91.8
97030	Oct. 28	93	20.2
97095	Oct. 28	133	7.9
97100	Oct. 28	145	4.5
97101	Oct. 23	133	39.5

The optimum temperature for growth of the pathogen was 25°C (maximum of 30°C and minimum of 5°C). Morphological and physiological characteristics of sexual and asexual organs of the pathogen agreed well with *Didymella chrysanthemi* reviewed by Punithalingam (1980). The pathogen in this study was identified as *Didymella chrysanthemi.*

The pathogenicity of the isolates was tested by spraying the conidial suspension (ca. 10^4 spores/ml) on the young leaves of wild chrysanthemum, and kept in moisture chamber for 24 h. Pathogenicity of the isolate was confirmed on *C. boreale* either by conidial inoculum in 6-7 days or by inoculating mycelial agar disc (Fig. 1b); the inoculated leaf revealed brown blight in 2-3 days.

This is the first report of *Didymella chrysanthemi* in Korea, and the first original report of the *Didymella* disease of *Chrysanthemum boreale* as a new host.

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Table 2. Description of asexual and sexual organs of the pathogen *Didymella chrysanthemi*

Organs		Size and morphological traits	
Asexual organ produced in plant	Pycnidia	181 μm (140-210) × 188 μm (150-235)	Spherical, brownish and short ostiolate neck, immersed in tissue
	Pycnidiospore	Unicellular : 11 μm (10-12.5) × 5(5-6)	Hyaline, 1-4 cells, straight or slight curve cylindrical,
		Uniseptate : 17 μm (15-20) × 5	Round apices, guttulate
		2-septate : 21 μm (20-22.5) × 6(5-7)	
		3-septate : 26 μm (25-27.5) × 7.5	
Asexual organ produced on PDA	Pycnidia	212 μm (150-260) × 228 (150-340)	Spherical, brown, short ostiolate neck
	Pycnidiospore	Unicellular : 7 μm (6-10) × 3(2-3)	Hyaline, 1-3 cells, straight or slightly curved cylindrical,
		Uniseptate : 13 μm (10-15) × 3(2-5)	Round apices, guttulate
		2-septate : 17 μm (16-17) × 4(3-5)	
Sexual organ produced on PDA	Pseudothecia	119 μm (70-180) × 130 μm (100-150)	
	Ascospore	10 μm × 2.7	Hyaline, fusiform, uniseptate