

Occurrence of Dry Rot on *Cymbidium* Orchids Caused by *Fusarium* spp. in Korea

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Cymbidium orchids with blight and rot symptoms were collected, and a total of 63 isolates of *Fusarium* spp. was obtained from pseudobulbs, roots, and leaves of the diseased plants. The isolates were identified based on their morphological characteristics. Out of the 63 isolates of *Fusarium* spp., 51 isolates were identified as *F. oxysporum*, 10 isolates as *F. solani*, and the rest as *F. proliferatum*. *F. oxysporum* was isolated from all the *Cymbidium* spp., while *F. solani* and *F. proliferatum* were isolated only from *Cymbidium ensifolium* and *C. ginatum*, respectively. Isolates of the three *Fusarium* spp. were tested for pathogenicity to their hosts by artificial inoculation. The strongly pathogenic isolates of *Fusarium* spp. induced severe dry rot of pseudobulbs and roots of the host plants. The symptoms progressed up to the basal part of the leaves, which later caused blight of the entire plant. The dry rot symptoms induced on the plants by artificial inoculation with the isolates of *Fusarium* spp. were similar to those observed in the growers' greenhouses. This is the first report of dry rot of *Cymbidium* spp. caused by *F. oxysporum*, *F. solani*, and *F. proliferatum* in Korea.

Keywords : *Cymbidium* spp., dry rot, *Fusarium oxysporum*, *F. solani*, *F. proliferatum*, orchid.

Cymbidium spp. are native orchids popular worldwide and cultivated extensively in greenhouses for marketing. In Korea, commercial *Cymbidium* orchids are grown in pots under greenhouse conditions. In a disease survey of *Cymbidium* spp. in several locations in the country, severe outbreaks of blight were observed which reached up to 40% infected plants in some greenhouses. The diseased plants showed rot symptoms on basal parts of the leaves, pseudobulbs, and roots. *Fusarium* spp. were consistently isolated from the diseased plant parts, which suggested that the fungi are associated with the disease.

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It has been reported that *Fusarium* spp. cause spot or blight of leaves and rot of seedlings, roots, and pseudobulbs in *Cymbidium* spp. (Benyon et al., 1996; Broadhurst and Hartill, 1996; D'Agliano and Carrai, 1994; Gleason et al., 1966; Honda et al., 1995; Ichikawa and Aoki, 2000; Ichikawa and Saito, 1998). In Korea, Chang et al. (1998) reported that *Fusarium proliferatum* caused leaf spot of *Cymbidium hybrida*. However, there have been no reports on rot of *Cymbidium* plants caused by *Fusarium* spp. This study was conducted to determine the etiological characteristics of *Fusarium* spp. associated with rot symptoms of *Cymbidium* plants.

Materials and Methods

Survey and collection of diseased plants. *Cymbidium* plants grown in greenhouses in six locations in Korea were surveyed from 1996 to 2000. Plants with blight and rot symptoms were collected, and the lesions were anatomically examined.

Isolation of pathogens. Three to five (3-5) mm-pieces of lesions were cut from diseased plants and plated on 2% water agar medium (WA) after surface sterilizing with 1% sodium hypochlorite solution for 1 minute. Fungal isolates obtained from the lesions were transferred to potato dextrose agar (PDA) slants. Isolates of *Fusarium* spp. identified by morphological observation under a light microscope were cultured for sporulation on PDA at 25°C for 10-20 days. Using an inoculating needle, conidia produced on PDA were suspended in 200 µl of sterile distilled water in a 1.5 ml-microtube to make a conidial suspension. A loopful of the conidial suspension was streaked on WA surface with a platinum wire loop to distribute the conidia. After 12-20 h incubation at 25°C, agar fragments with single germinated conidium were transferred to fresh WA and incubated at 25°C for 5 days. Single-conidium isolates obtained from the WA plates were cultured on PDA and used for the identification and pathogenicity tests.

Investigation of morphological characteristics. Each isolate was cultured on carnation leaf agar (Fisher et al., 1982) at 27°C for 20-30 days in alternating cycles of 12 h NUV light and 12 h darkness. The morphological features of each culture on the medium were examined by light microscope. Fifty conidia,



Fig. 1. Symptoms of dry rot on *Cymbidium* spp. grown in the greenhouse. **A-B)** rot of a basal part and a pseudobulb in *C. ensifolium*; **C-D)** rot of a basal part and a longitudinal section showing discoloration in *C. ginatum*; **E-F)** blight of leaves and rot of pseudobulbs and roots in *C. goeringii*; **G-H)** yellowing of leaves and a longitudinal section showing discoloration in *C. kanran*; **I-J)** blight of leaves and longitudinal sections showing discoloration in *C. niveo-marginatum*.

conidiophores, and chlamydo-spores chosen randomly from each culture were observed and measured under the light microscope.

Pathogenicity test. Six-month-old to one-year-old healthy plants of *Cymbidium* spp. were used for the pathogenicity tests. Two to four isolates of each *Fusarium* species were used for the inoculation tests to the host plants. Each isolate of *Fusarium* species was cultured on cornmeal-sand medium (23 g cornmeal: 210 g sand: 40 ml distilled water) in 500 ml flasks for 30-40 days to prepare the inoculum.

For the inoculation tests, surface medium around the plant was dug at a depth of 3-5 cm, and 240-250 g of each inoculum was placed on the roots and pseudobulbs. The inoculated plant parts were covered with the original medium. The same quantity of cornmeal-sand medium was used for the control plant. The inoculated and control plants were cultivated in the greenhouse at 18-30°C. The inoculation experiment was performed in three replicates. Symptoms were observed during cultivation of the inoculated plants, and disease rating was made based on the severity of rotting induced on the plants 30 days after inoculation. Re-isolation of the pathogen from the lesions on the plants was conducted as described previously.

Results

Disease incidence and symptoms. Dry rot was observed on *Cymbidium ensifolium* Sw., *C. ginatum* Makino, *C. goeringii* Reichb., *C. kanran* Makino, and *C. niveo-marginatum* Makino during disease surveys in six locations in Korea from 1996 to 2000. Symptoms developed on the basal parts of the leaves, pseudobulbs, and roots of *Cymbidium* spp. (Fig. 1). Symptoms on the basal parts of the leaves appeared as brown to dark-brown, irregularly advancing lesions from the pseudobulbs. Infected leaves generally showed yellowing as the disease developed, then later showed blighting. Infected pseudobulbs and roots turned dark-brown to black and became rotten in dry condition. Severely diseased plants entirely became rotten

Table 1. Isolation of *Fusarium* spp. from diseased plants of *Cymbidium* spp. from 1996 to 2000

<i>Cymbidium</i> spp. (Korean name)	Isolated plant part	Location	No. of isolates
<i>C. ensifolium</i> (Geonran)	Pseudobulb	Gimpo	4
		Jeju	7
	Root	Gimpo	6
<i>C. ginatum</i> (Sagyeran)	Pseudobulb	Cheonan	8
<i>C. goeringii</i> (Chunran)	Root	Hwaseong	6
<i>C. kanran</i> (Hanran)	Pseudobulb	Hwaseong	4
<i>C. niveo-marginatum</i> (Okhwaran)	Pseudobulb	Gwacheon	8
		Gwacheon	9
	Root	Namyangju	5
	Leaf	Gwacheon	6

and blighted.

Isolation and identification. A total of 63 isolates of *Fusarium* spp. was obtained from pseudobulbs, roots, and leaves of *Cymbidium* spp. (Table 1). They were identified based on their morphological characteristics (Table 2 and Fig. 2). The morphological characteristics of three *Fusarium* spp. examined by the authors were consistent with those described by previous workers (Booth, 1971; Nelson et al., 1983).

Out of the 63 isolates of *Fusarium* spp., 51 isolates were identified as *F. oxysporum* Schlecht.: Fr., 10 isolates as *F. solani* (Mart.) Sacc., and the rest as *F. proliferatum* (Matsushima) Nirenberg (Table 3). *F. oxysporum* was isolated from all the *Cymbidium* spp., while *F. solani* and *F. proliferatum* were isolated only from *Cymbidium ensifolium* and *C. ginatum*, respectively.

Pathogenicity. Isolates of the three *Fusarium* spp. were tested for pathogenicity to their hosts by artificial inoculation. Three isolates each of *F. oxysporum* and *F. solani* were weakly to strongly pathogenic to *C. ensifolium*

Table 2. Morphological characteristics of *Fusarium* spp. isolated from *Cymbidium* spp.^a

Structure	Division	<i>F. oxysporum</i>	<i>F. solani</i>	<i>F. proliferatum</i>
Conidiophore	Shape	Monophialide	Monophialide	Monophialide, polyphialide
	Size (µm)	5-27×2-3	12-123×2.5-5.0	12-48×2.5-3.8
Microconidium	Shape	Oval to cylindrical	Oval to ellipsoid	Fusiform to clavate
	Septum	0-2	0-2	0-1
	Size (µm)	3-25×2-5	5-24×2-6	4-12×1.5-3.0
Macroconidium	Shape	Fusoid, falcate	Inequilaterally fusoid	Fusoid, delicate
	Septum	3-5	3-5	2-4
	Size (µm)	16-60×2-6	22-52×4-6	22-53×2-4
Chlamydo-spore	Shape	Globose to ellipsoid	Globose to oval	—
	Septum	0	0	—
	Size (µm)	4-18×4-12	5-10×5-10	—

^aMeasurement was made after 20-30 days of cultivation on CLA. — = no formation.

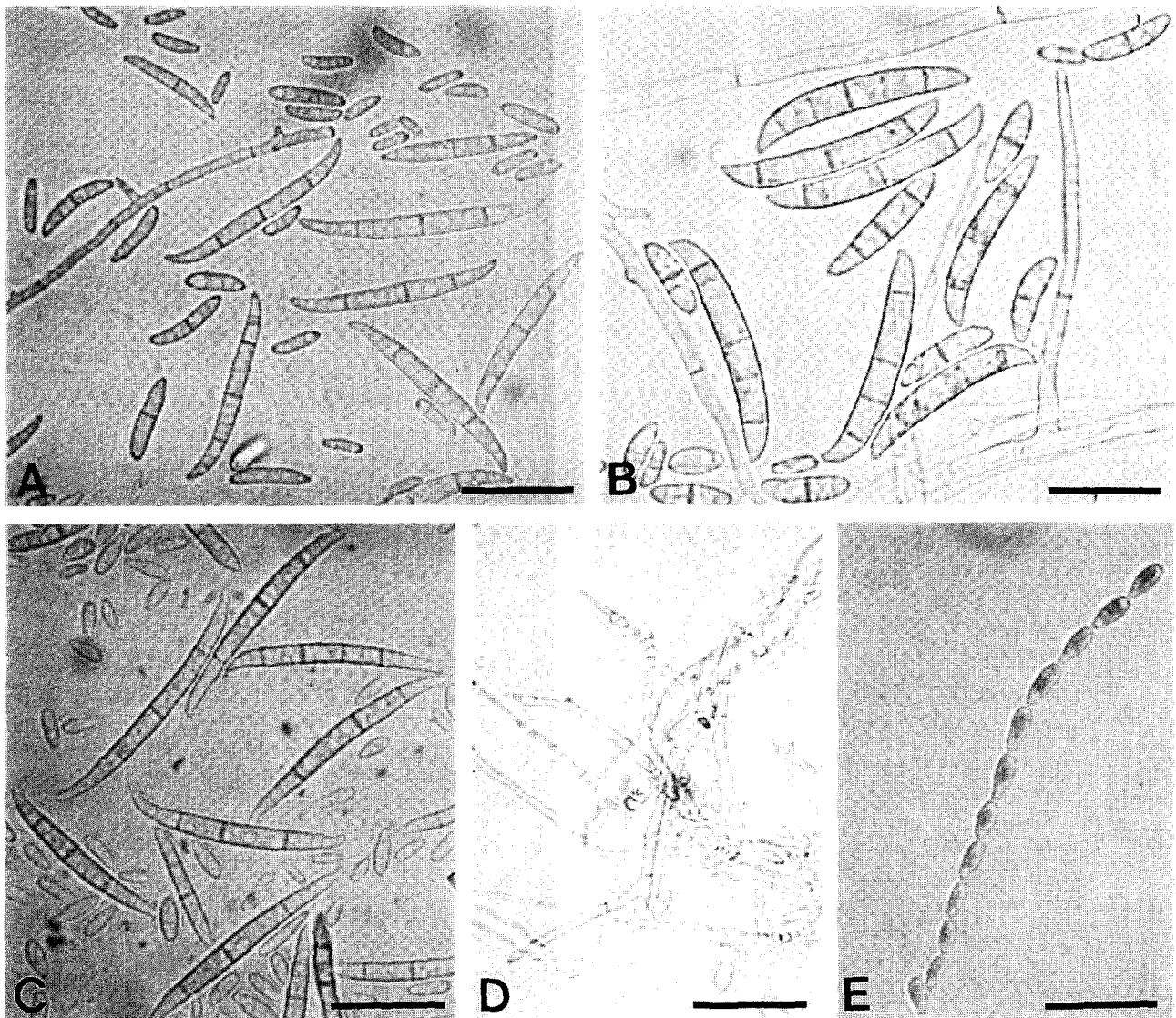


Fig. 2. Morphological features of *Fusarium* spp. isolated from *Cymbidium* spp. A) microconidia, macroconidia, and short monopialides of *F. oxysporum*; B) microconidia, macroconidia, and long monopialides of *F. solani*; C-E) microconidia, macroconidia, polyphialides, and a conidial chain of *F. proliferatum*. Each scale bar = 20 µm.

Table 3. Identification of *Fusarium* spp. isolates from diseased plants of *Cymbidium* spp.

<i>Cymbidium</i> spp.	<i>Fusarium</i> spp. identified	Isolated plant part	No. of isolates
<i>C. ensifolium</i>	<i>F. oxysporum</i>	Bulb	7
	<i>F. solani</i>	Bulb, root	10
<i>C. ginatum</i>	<i>F. oxysporum</i>	Bulb	6
	<i>F. proliferatum</i>	Bulb	2
<i>C. goeringii</i>	<i>F. oxysporum</i>	Root	6
<i>C. kanran</i>	<i>F. oxysporum</i>	Bulb	4
<i>C. niveo-marginatum</i>	<i>F. oxysporum</i>	Bulb, root, leaf	28

(Table 4). *F. oxysporum* isolates were weakly to strongly pathogenic to *C. ginatum*, while *F. proliferatum* isolates were strongly pathogenic to *C. ginatum*. All isolates of *F. oxysporum* tested were strongly pathogenic to *C. goeringii* and *C. kanran*. Four isolates of *F. oxysporum* tested were weakly to strongly pathogenic to *C. niveo-marginatum*.

The strongly pathogenic isolates of *Fusarium* spp. induced severe dry rot of pseudobulbs and roots of the host plants. The symptoms progressed up to the basal part of the leaves, which later and caused blight of the entire plant. The dry rot symptoms induced on the plants by artificial inoculation with the isolates of *Fusarium* spp. were similar

Table 4. Pathogenicity of *Fusarium* spp. isolates to the hosts by artificial inoculation

Host (<i>Cymbidium</i> spp.)	<i>Fusarium</i> spp.	Isolate No.	Virulence of isolates to the host
<i>C. ensifolium</i>	<i>F. oxysporum</i>	F6351-1	+ ^a
	<i>F. oxysporum</i>	F6353-1	++
	<i>F. oxysporum</i>	F6355-3	++
	<i>F. solani</i>	F6402-2	++
	<i>F. solani</i>	F6406-1	++
	<i>F. solani</i>	F6408-1	+
<i>C. ginatum</i>	<i>F. oxysporum</i>	F6293-2	++
	<i>F. oxysporum</i>	F6297-1	+
	<i>F. proliferatum</i>	F6286-2	++
<i>C. goeringii</i>	<i>F. proliferatum</i>	F6287-2	++
	<i>F. oxysporum</i>	F6423-1	++
<i>C. kanran</i>	<i>F. oxysporum</i>	F6424-1	++
	<i>F. oxysporum</i>	F6429-2	++
<i>C. niveo-marginatum</i>	<i>F. oxysporum</i>	F6430-1	++
	<i>F. oxysporum</i>	F6288-3	+
	<i>F. oxysporum</i>	F6379-3	++
<i>C. niveo-marginatum</i>	<i>F. oxysporum</i>	F6388-2	+
	<i>F. oxysporum</i>	F6399-3	++

^a ++ = severe rot of bulbs and roots; + = weak rot of bulbs and roots; - = no symptom.

to those observed in the growers' greenhouses. The isolates that induced symptoms on the host plants were re-isolated from the lesions.

Discussion

Fusarium spp. attack a variety of plants as a soil-borne pathogen (Booth, 1971; Nelson et al., 1983; Snyder and Hansen, 1940). It has been reported that *Fusarium subglutinans* (Wollenw. and Reinking) Nelson, Toussoun & Marasas, and *F. proliferatum* cause leaf spot or blight of *Cymbidium* spp. (Broadhurst and Hartill, 1996; Chang et al., 1998; D'Agliano and Carrai, 1994; Honda et al., 1995; Ichikawa and Aoki, 2000), while *F. moniliforme* causes seedling wilt (Gleason et al., 1966). Benyon et al. (1996) reported that *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans* were associated with an increase in root necrosis of *Cymbidium* orchids. Ichikawa and Saito (1998) proposed 'dry rot' as the common disease name for rot of *Cymbidium* plants caused by *F. solani*. The present study revealed that *F. oxysporum*, *F. solani*, and *F. proliferatum* cause dry rot on *Cymbidium* plants.

F. oxysporum was the most frequently isolated from the dry rot lesions of six species of *Cymbidium* plants, while isolation frequency of *F. solani* and *F. proliferatum* from the plants was very low. Results suggest that *F. oxysporum* is the main pathogen of the disease. *F. oxysporum* has a wide host range, and several formae speciales of the fungus have been differentiated based on the pathogenicity of the isolates (Booth, 1971; Snyder and Hansen, 1940). There have been no reports on the formae speciales of the fungus causing dry rot of *Cymbidium* spp. Further study is needed to differentiate the formae speciales of the fungal isolates from *Cymbidium* spp.

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