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Fusarium Wilt of Winter Daphne (*Daphne odora* Thunb.) Caused by *Fusarium oxysporum*

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Severe wilt disease epidemic was found on winter daphnes (*Daphne odora* Thunb.) cultivated in farmers' nurseries in Suncheon, Jeonnam in 2003. Typical symptoms appeared on the leaves of winter daphne as yellowish wilts and turned brown from the lower leaves on the same plant. Severely infected leaves were defoliated, resulting in blight of stems and eventual death of the entire plant. Black decayed vascular tissues were distinctly observed in a wilted plant. *Fusarium* sp. was isolated from the diseased plants repeatedly and its pathogenicity was confirmed by artificial inoculation on healthy plants. The fungus was identified as *Fusarium oxysporum* on the basis of the morphological and cultural characteristics on potato dextrose agar and carnation leaf agar. The optimum temperature for fungal growth was around 25°C and the fungal growth was inhibited by metconazole, triflumizole and trifloxystrobin on potato dextrose agar. This is the first report on the wilt disease of winter daphnes caused by *F. oxysporum* in Korea.

Keywords : *Daphne odora*, *Fusarium oxysporum*, Fusarium wilt, winter daphne

Fusarium species are ubiquitous fungi found in the temperate and tropical areas of the world and cause cortical rots, head blights, leaf spots, root rots, fruit rots, cankers, dieback, and vascular wilt diseases (Nelson, 1981). Of all the diseases caused by *Fusarium*, probably the most important are the vascular wilt diseases on a diverse group of plants caused by *Fusarium oxysporum*. The fungus attacks vegetables, crops, ornamental plants, and trees.

Winter daphne (*Daphne odora* Thunb.) is an ornamental plant with beautiful flowers and fragrant smells. The plant is widely cultivated for ornamental purpose in a garden or for obtaining plant materials as sources for oriental medicines in the nurseries or fields. Wilt disease was found on seedlings of winter daphnes cultivated in farmer's nurseries in Suncheon, Jeonnam in spring, 2003. The disease developed into severe epidemic on winter daphne nurseries during rainy season (Fig. 1A), but there has been no record of fungal diseases on this plant in Korea (The Korean Society of Plant Pathology, 2004). A species of *Fusarium* was consistently isolated from the diseased tissues, which suggested that the fungus was associated with the diseases. This study was conducted to determine the etiological characteristics of *Fusarium* sp. associated with wilt symptoms of winter daphne.

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Materials and Methods

Isolation and identification of fungi. Diseased stem tissues of winter daphne cut into 5 mm length were surface-sterilized with 70% ethanol for 1 minute and placed on potato dextrose agar (PDA) plates at 25°C incubator. Mycelial tips of the fungal isolates grown on the medium were cut and transferred to fresh medium. Morphological characteristics of microconidia of the isolates were examined under the microscope after incubation at 25°C for 10 days on PDA. Cultural characteristics of the isolates were also examined during incubation on PDA. Macroconidia were induced after incubation at 25°C on carnation leaf agar (CLA). Morphological characteristics of macroconidia and chlamydospores produced on CLA after incubation for 7 days were examined under the microscope.

Pathogenicity test. To prove the pathogenicity of 5 mm isolates, the stems of winter daphne with or without pin-pricked wound were inoculated with mycelial plugs 5 in diameter prepared from 7-day-old culture on PDA of each isolate and the inoculated parts were wrapped with plastic film. The inoculated plants were maintained in a moist chamber at 100% relative humidity and 25°C for 24 hours in the dark and then transferred to a greenhouse. Symptom development was checked everyday for 15 days.

Investigation of optimum temperature range. Optimum temperature range for mycelial growth of the fungus was

investigated. Mycelial plugs (5 mm in diameter) were punched out from actively growing mycelial colonies on PDA by a cork borer and placed on the center of PDA plates. The PDA plates were incubated in 9 different temperature regimes (0, 5, 10, 15, 20, 25, 30, 35, 40°C). Mycelial colony diameter measured 7 days after incubation on PDA plates.

Screening of fungicides. The effect of fungicides on mycelial growth of the fungus was investigated to screen preventive fungicides of the disease. Mycelial plugs (5 in diameter) were punched out from actively growing mycelial colonies on PDA by a cork borer and placed on the center of PDA plates. Twenty three fungicides were diluted as recommended application rates (Korea Crop Protection Association, 2004). Paper disks (5 mm in diameter) absorbed the fungicides were placed at the edge of the 5-day-old mycelial colony on PDA. Sensitivity of the fungus to fungicides was measured 7 days after incubation at 25°C.

Results and Discussion

Symptom. Typical symptoms appeared on the leaves of winter daphne as yellowish wilts (Fig. 1B). Wilted lower leaves gradually turned brown and severely infected leaves defoliated. Rapid disease progress resulted in blight of stems (Fig. 1C) and eventual death of the entire plant (Fig. 1D). Black decayed vascular tissues were distinctly observed in the infected stem of a wilted plant as seen in the pathological anatomy of several specific host plant-*Fusarium oxysporum* combinations when dissected (Fig. 1E) (Nelson, 1981). Almost all of the plants were

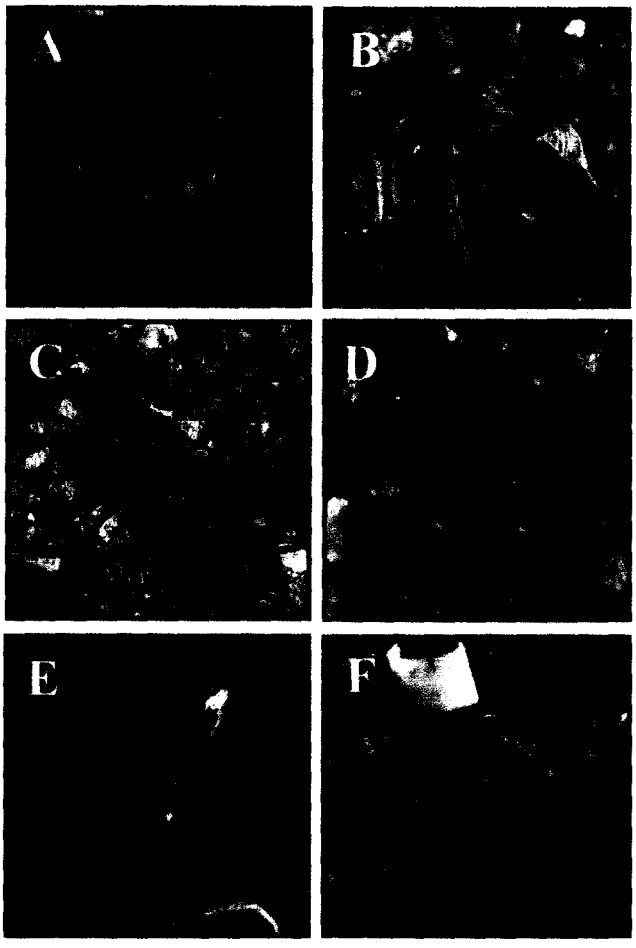

Fig. 1. Symptoms of Fusarium wilt of winter daphne by *Fusarium oxysporum*. (**A**) Seedling blight symptoms on nurseries, (**B**) Typical wilt symptoms on leaves, (**C**) Blighted stem with wilted leaves, (**D**) Dead cutting on nurseries, (**E**) Internal symptom on a wilted plant, (**F**) Dieback symptom in an artificially inoculated stem.

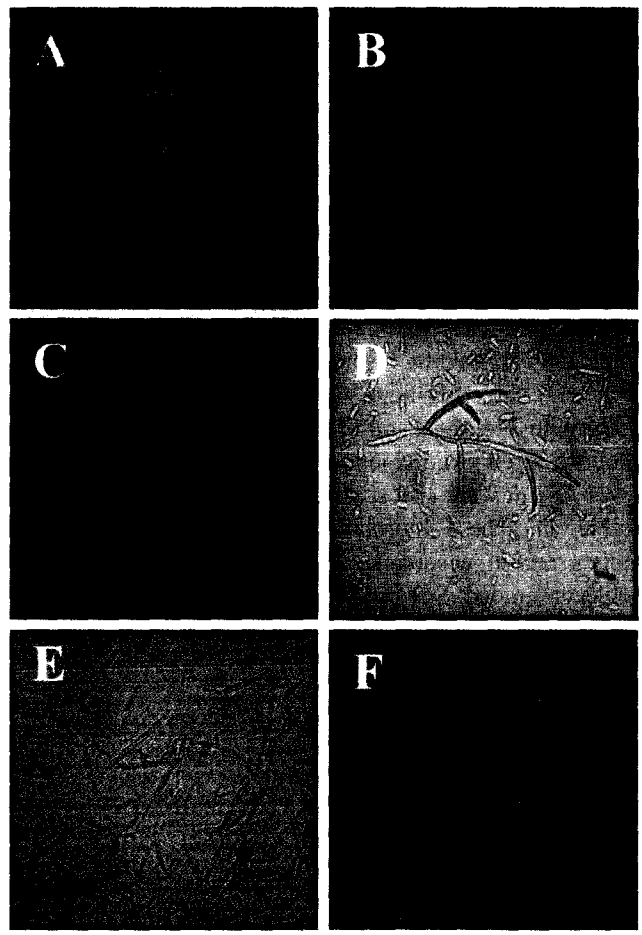

Fig. 2. Morphological characteristics of *Fusarium oxysporum*, the causal organism of wilt on winter daphne. (A) Colony on potato dextrose agar, (B) Microconidiophores on potato dextrose agar, (C) Microconidia on potato dextrose agar, (D) Macroconidiophores on carnation leaf agar, (E) Macroconidia on carnation leaf agar, (F) Chlamydospores on potato dextrose agar. Scale bars represent 10 μm.

Table 1. Comparison of morphological characteristics of the present isolates with *Fusarium oxysporum* previously described

Characters		Present isolates	*Fusarium oxysporum*[a]
Colony on PDA		white with a purple tinge	white or peach but usually with a purple tinge
Macroconidia	Shape	fusoid~falcate	fusoid~subulate and pointed at both ends
	Septum	3(~4)	usually 3~5
	Size (μm)	17~30×2~5	27~46×3~4.5
Microconidia	Shape	oval~ellipsoid cylindrical	oval~ellipsoid cylindrical, straight to curved
	Septum	0	0
	Size (μm)	5~15×2.5~5	5~12×2.2~3.5
Chlamydospores	Shape	globose to ellipsoid	globose to ellipsoid
	Size (μm)	3.8~18×3.8~17.5	4~16×4~12

[a]Both, C. (1970).

destructed by the epidemic in some severely diseased nurseries (Fig. 1A). The grower suspected that such a severe epidemic might be due to introduction of infected cuttings into the nursery. The danger of propagation of symptomless cuttings infected with Fusarium wilt pathogens was also reviewed by Nelson (1981).

Fungal characteristics. On PDA, the isolates grew fast and white aerial mycelia became tinged with light purple (Fig. 2A). The undersurface was dark purple and abundant microconidia were produced on microconidiophores (Fig. 2B). Microconidia were oval to ellipsoid cylindrical measuring 5~15 × 2.5~5 μm (Fig. 2C). When cultured on CLA at 25°C for 1 week, macroconidia of *Fusarium* were abundantly formed on macroconidiophores in sporodochia (Fig. 2D). Macroconidia were long fusoid to falcate in shape with 3~4 septa, and 17~30×2~5 μm in size (Fig. 2E). Globose to ellipsoid chlamydospores measuring 3.8~18 × 3.8~17.5 μm were also sparsely formed on CLA (Fig. 2F) (Table 1). These morphological characteristics of the fungus are in accordance with those of *F. oxysporum* Schlecht. emend. Snyd. & Hans. (Booth, 1970; Nelson et al., 1983).

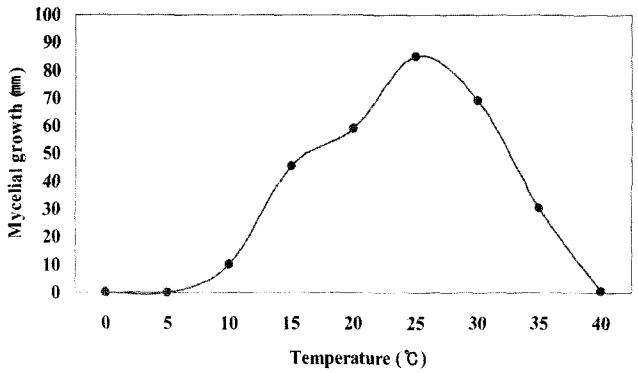

Fig. 3. Effect of temperatures on mycelial growth of *Fusarium oxysporum*, the causal organism of winter daphne wilt. Linear mycelial growth was measured 7 days after incubation on potato dextrose agar. Data are means of three replicates.

Pathogenicity. All of the isolates caused typical symptoms on wounded plants. Dark brown dieback lesions appeared on the pin-pricked stems of winter daphne 5 days after artificial inoculation (Fig. 1F) but no symptom developed on control plants. Leaves on the infected plants were defoliated 15 days after artificial inoculation, resulting in

Table 2. Sensitivities of *Fusarium oxysporum* isolates to various fungicides on potato dextrose agar

Fungicides	Application rate (mg/l)	Sensitivity to fungicides
Azoxystrobin WP	1,000	–
Chlorothalonil WP	1,650	–
Copper hydroxide WP	1,000	–
Difenoconazole WP	500	+
Dimethomorph WP	1,000	–
Dithianon-copper WP	2,000	–
Iprodione WP	1,000	–
Oxadixyl-mancozeb WP	2,000	–
Polyoxin B WP	1,000	–
Triflumizole WP	500	++
Thiophanate-methyl WP	1,000	–
Etridiazole EC	500	–
Fenarimol EC	335	–
Tetraconazole EW	500	–
Cyproconazole SL	250	–
Propamocarb hydrochloride SL	1,450	–
Cyazofamid SC	500	–
Ethaboxam·dimethomeph SC	1,000	–
Ethaboxam SC	1,000	–
Metconazole SC	335	++
Tribasic coppersulfate SC	2,000	–
Metalaxyl WP	500	–
Trifloxystrobin SC	500	++

[a]++, inhibition zones were 5 mm or more; +, inhibition zones were 1~5 mm; –, no inhibition zone was formed.

complete blight of stems and eventual death of the entire plant as observed in naturally infected nurseries. The fungus was re-isolated from the lesions on the plants incoculated. Thus the causal fungus was identified as *F. oxysporum* based on the pathogenicity and morphological traits (Booth, 1970; Nelson et al., 1983).

Optimum temperature. Mycelial colony diameter measured 7 days after incubation on PDA is shown in Fig. 3. The optimum temperature for mycelial growth of *F. oxysporum* on PDA was around 25°C, indicating that severe epidemic of wilt disease on winter daphne resulted from the environmental conditions favorable to the fungus during the rainy season with high temperatures and humidities in Korea. Similar results were reported from various host plant-*F. oxysporum* combinations (Nelson, 1981).

Effective fungicides. The fungus was highly sensitive to metconazole SC (suspension concentrate), triflumizole WP (wettable powder) and trifloxystrobin SC and moderately sensitive to difenoconazole WP. However, mycelial growth of the fungus was not affected by the other fungicides (Table 2). Even though severe disease epidemics caused by *Fusarium* spp. were reported in many plants, efficient fungicides for controlling the Fusarium diseases have not been developed until now (Korea Crop Protection Association, 2004). This makes it difficult to control the Fusarium diseases. However, it is expected that some of the selected fungicides in this study will be developed as promising preventive fungicides to alleviate the disease progress of Fusarium wilt on winter daphne in the future.

Mosaic disease is recorded on winter daphne in Korea (The Korean Society of Plant Pathology, 2004), but there is no previous report of wilt disease on winter daphne caused by *F. oxysporum*. Therefore, we propose that the disease be named as Fusarium wilt of winter daphne.

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