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Stub Dieback of Carnation Caused by *Fusarium graminearum*

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A disease survey on the carnation (*Dianthus caryophyllus* L.) wilt was conducted during the high temperature period (June through August) and the low temperature period (February through May) in 58 greenhouses of its major cultivation areas, including Pusan, Kimhae, and Changwon in Korea from 1998 to 1999. The disease incidence was averaged 5.4% and 11.9% in the low and high temperature periods, respectively. Severe damage was found in summer with high incidences of around 50% in some greenhouses. Close examination of the symptoms and isolation of the causal agent revealed that there was a new disease different from Fusarium wilt caused by *Fusarium oxysporum* f. sp. *dianthi*, which was determined as the stub dieback caused by *F. graminearum* (teleomorph: *Gibberella zeae*). The stub dieback symptoms involved brown rot of stem that started usually from the portion of cutting without discoloration of inner vascular tissues. Seven out of 38 isolates from the wilted plants were identified as *F. graminearum*, while the others as *F. oxysporum* f. sp. *dianthi*. Mycological characteristics of the stub dieback pathogen including colony color, absence of microconidia, and the shape of macroconidia, were consistent with *F. graminearum* previously described. This is the first report of the carnation stub dieback in Korea.

Keywords **:** carnation, *Fusarium graminearum, F. oxysporum* f. sp. *dianthi,* stub dieback, wilt.

The perpetual flowering carnation, *Dianthus caryophyllus* L., is one of five major cut-flower crops in Korea, and cultivated in 149 ha in 2000. There are several diseases in carnation reported in Korea (Korean Society of Plant Pathology, 1998) including rust caused by *Uromyces dianthi*, leaf blight by *Alternaria dianthi*, grey mold by *Botrytis cinerea*, Fusarium wilt by *Fusarium oxysporum* f. sp. *dianthi,* leaf spot by *Cercospora* and *Cladosporium*, and root and stem rots caused by *Rhizoctonia solani* or *F. roseum*. Among them, Fusarium wilt is an important soilborne disease occurring prevalently in carnation fields.

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Diseased plants with the Fusarium wilt initially become less vivid and wither without any visible changes of leaf or stem color. In advanced stages of the disease development, infected plants show chlorosis, accompanying blight of stems whose vascular bundle was discolored to brown. Diseased roots and shoots become dead with dry rot.

Another Fusarium disease found in carnation with similar symptoms to the Fusarium wilt is stub dieback caused by *F. graminearum* (teleomorph: *Gibberella zeae*). Both of them occur on carnation worldwide wherever the plant is cultivating (Nelson et al., 1975; Nilson, 1962). Formerly it had been known to be caused by *F. roseum*, and called branch rot (Peltier, 1919), die-back (Brown, 1938; Dowson, 1929; Wickens, 1935) or stub dieback (Nelson et al., 1971). The species was described and classified as *F. roseum* "Graminearum" or *F. roseum* var. *graminearum* (Snyder and Hansen, 1945). Nelson et al. (1971) reported firstly *F. roseum* "Graminearum" (*Gibberella zeae*) as the causal agent of the carnation stub dieback in the northeastern United States.

Stub dieback occurred mainly as a dieback of stem after cutting or flower harvest (Nelson et al., 1975). The fungus grows down the stub to the main stem or sidebreak, causing death of the plant like Fusarium wilt caused by *F. oxysporum* f. sp. *dianthi*. Under conditions of humidity the fungus mycelium grows profusely on infected stubs. Nelson et al. (1975) reported that stub dieback also occurred when the young carnation plants were pinched to force the production of sidebreaks. Although the stub dieback of carnation is an important disease, there has been no study on its occurrence and the identification of the causal agent in Korea. *F. roseum* is merely listed as one of the causal organisms of "root and stem rot" of carnation (Korean Society of Plant Pathology, 1998).

Materials and Methods

We found the stub dieback disease during the survey on carnation wilt caused by *Fusarium* spp. during the years of 1998 and 1999. The disease survey was conducted at major cultivation areas including Pusan, Kimhae, Changwon, Konju and other areas in Chonbuk province. In each carnation field, 100 plants were exam-

ined for the disease symptoms, and diseased stem tissues were collected for the isolation of the causal pathogens. For this, diseased stem tissues were washed in distilled water, cut into pieces, and surface-sterilized with 1% sodium hypochlorite for 1 min. After drying on a filter paper the stem pieces were layed on water agar and Komada's, Fusarium-selective medium, and incubated for 24-48 h at 27°C. Mycelial tips or spores were singly isolated from the fungal colonies formed on the media, and transferred to potato-dextrose agar (PDA) and carnation leaf agar (CLA) for examining cultural and morphological characteristics of the fungi according to the method described by Nelson et al. (1983). On culture test, the optimum temperature for mycelial growth of the isolates was examined in three replicates on PDA at 5°C intervals from 5°C to 35°C.

For pathogenicity tests each isolate was cultured at sand oatmeal medium (150 g sand : 30 g oatmeal : 50 ml distilled water amended with 5% sugar) in 500 ml-flask for 7-10 days at 27°C. Young rooted cutting plants, cultivar "Desio", transferred into pots (5 cm in diameter) and the field. For inoculation to roots, surface soil around the plant was dug by a depth of 2-3 cm, and 20 g of each inoculum was placed around the roots. The inoculated plants were covered with the original soil. The pathogenicity test was performed in five replicates in the pot test and thirty plants in the field test. Symptoms were observed from 3 days after inoculation, and pathogens were re-isolated from the plant showing wilt and root rot. Another pathogenicity test on freshly cut stem stubs was done on flowering stage plants which were grown for 10-12 weeks in the field. Conidial suspension which prepared by a concentration of 1×10^5 conidia/ml from 7-day-old CLA cultures was sprayed onto plants. Control plants were treated with sterile distilled water alone.

Results and Discussion

A total of 58 carnation fields were examined to survey the occurrence of the carnation wilt disease at one time during the high temperature period (June through August) and at another time during the low temperature period (February through May) from 1998 to 1999 (Table 1). Wilt diseases occurred in all of the fields surveyed, of which the incidence ranged from lowest 0.5 to highest 57.0%, and 8.7% in average. Generally the disease incidences were higher in the high temperature period than in the low one, and the disease outbreaks were found only during the high temperature period in Kimhae and Pusan. Nelson et al. (1975) reported the disease occurred especially during the summer when it is not possible to manage greenhouse environmental conditions which favor the disease development.

During the field survey, the stub dieback symptoms could not be clearly differentiated from the Fusarium wilt at often times. Both diseases produced wilt symptoms at the late stages (Fig. 2A-1). However, as based on the key symptoms by Wickens (1935), the Fusarium wilt is a vascular wilt disease caused by a tissue-specific pathogen, *F. oxysporum* f. sp. *dianthi*. The Fusarium wilt disease was also characterized by the vascular discoloration in our study (Fig. 2A-3). On the other hand, specific stub dieback symptoms did not include vascular discoloration of stem tissues and generally showed dieback symptoms starting from the cutting portions (Fig. 2A-1). This is corresponding to the studies of Horst and Nelson (1968) and Nelson et al. (1975), in which the important period of infection for stub dieback or stem rot was propagation, and plants were mainly infected through cuttings. This suggests that careful handling of carnation plants should be needed during the propagation and flower cutting to prevent the fungal infection.

Table 1. Occurrence of carnation wilt in major cultivating areas in 1998-1999

Period of survey	Location	No. of fields surveyed	Incidence of wilt[a] (%)	
			Average	Range
Low temperature period (Feb.-May)	Changwon	6	4.3	0.5-12.8
	Kimhae	9	5.7	0.6-12.5
	Pusan	6	9.9	1.2-6.4
	Koksung	2	4.9	2.3-7.5
	Imsil	1	6.4	–
	Kimjae	1	1.2	–
	Total	25	5.4	–
High temperature period (June-Aug.)	Changwon	5	7.7	1.2-14.8
	Kimhae	12	14.7	2.1-45.5
	Pusan	12	16.7	3.0-57.0
	Kongju	4	8.6	2.2-21.3
	Total	33	11.9	–

[a]One hundred plants in each field were investigated with five replicates.

From these infected tissues, a *Fusarium* sp. different from *F. oxysporum* f. sp. *dianthi* was isolated. In our study, out of 38 Fusarium isolates, 7 isolates belonged to *F. graminearum*, while the others to *F. oxysporum* (probably *F. oxysporum* f. sp. *dianthi*). This result indicates that the Fusarium wilt may be more prevalent than the stub dieback in the fields examined.

The two fungi were definitely different in cultural and morphological characteristics as shown in Table 2. Fungal colonies were pigmented light pink to dark violet with whitish mycelium for *F. oxysporum*, while *F. graminearum* developed dense mycelium which as colored carmin red with white orange to greyish red, when the fungi were grown on PDA at 28°C for 1 week (Fig. 2B). When they were cultured on CLA at 28°C for 1 week, sporodochia and macroconidia were abundantly formed; macroconidia of *F. oxysporum* were long falcate in shape with 3-5 septa, and $20\text{-}64 \times 2.5\text{-}5.4$ μm in size. While *F. graminearum* spores were slender and falcate with 3-6 septa (Fig. 2F), and $32\text{-}70 \times 3.6\text{-}4.6$ μm in size, and their basal cell was distinctly

Table 2. Morphological and cultural characteristics of *Fusarium* spp. isolated from carnation showing wilt or stub dieback symptoms

Characteristics		Fusarium wilt isolates	Stub dieback isolates
Symptoms		wilt	wilt and stub rot
Color of colony[a]		light pinkdark violet	carmin red
Macroconidium[b]	Shape	long falcate	slender, falcate, foot-cell
	Size (μm)	20-64 × 2.5-5.4	32-70 × 3.6-4.6
	No. of septa	3 (3-5)	4 (3-6)
Microconidium[b]	Shape	monophialides	not produced
	Size (μm)	5-18 × 2.0-3.8	–
Sclerotium		abundant	not produced
Chlamydospore		abundant	formed sparsely
Perithecium[c]	Shape	not produced	ovoid, black-brown
	Size (μm)		150-320 (av. 232.3)
Ascus[c]	Shape	not produced	clavate
	Size (μm)		60.0-77.5 × 8-12 (av. 67.5 × 10.8)
Ascospore[c]	Shape	not produced	hyaline to light brown curved fusoid with rounded ends
	No. of septa		1-3 septa
	Size (μm)		20-28 × 3-4 (av. 23.4 × 3.6)

[a] Grown on potato-dextrose agar at 28°C.
[b] On carnation leaf agar at 28°C for a week.
[c] On carnation leaf agar at 28°C for one month.

foot-shaped. *F. graminearum* did not produce microconidia and sclerotium, and sparsely produced chlamydospores on CLA, but *F. oxysporum* produced abundantly such spores and structures. No teleomorph stage was observed in *F. oxysporum*, but in *F. graminearum*, sexual fruiting structures, perithecia, were formed on the fungal culture one month after incubation on CLA at 27°C (Table 2). Perithecia formed on CLA were characterized by blackbrown in color, and variable sizes of about 150-320 μm (mean value, 232.3 μm) depending on their maturity (Fig. 2C). Shapes and sizes of teleomorph structures were identical to *Gibberella zeae* (Booth, 1973, Burgess, 1981, Vesonder and Hesseltine, 1981). Considering all of the above aspects, the causal agent of stub dieback of carnation was determined as *F. graminearum* (teleomorph: *G. zeae*).

Mycelial growth on PDA at different temperatures was compared between the two fungi (Fig. 1). The optimum temperature for mycelial growth of both fungi was around 25°C, showing the growth rate of 9.4 mm for 24 h. day at 25°C in case of *F. graminearum*, *F. graminearum* grew more rapidly than *F. oxysporum* on PDA. Considering the optimum temperature of the mycelial growth, high temperatures would be more favorable to the two fungi. Nelson et al. (1975) reported the stub dieback disease occurred more severely during the summer season. In our study, severe incidences of carnation wilt diseases in the high temperature period may be due to the environmental conditions favorable to the fungal growth.

The symptoms on carnation at different growth stages induced by artificial inoculation were similar to those

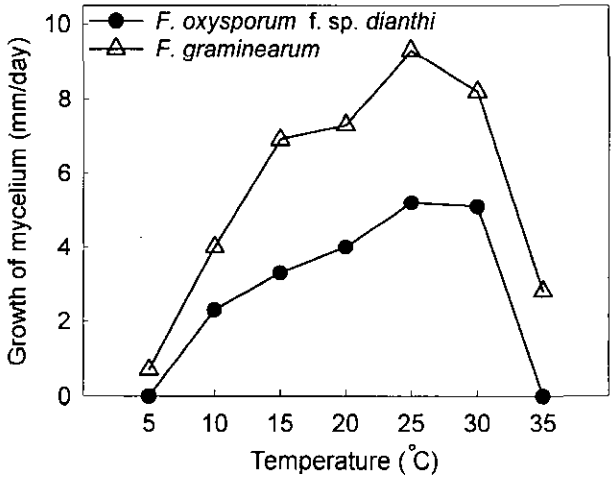

Fig. 1. Mycerial growth of carnation wilt pathogens, *Fusarium oxysporum* f. sp. *dianthi* and *F. graminearum* on potato-dextrose agar.

observed in the fields. Symptoms on the young-rooted plants developed rapidly on the basal stem and root 3 days after inoculation and reddish brown~pink mycelium produced at the soil line and the rotten stem. Symptoms of stub dieback on the flowering stage plants developed firstly 6 days after spray-inoculation. Many of the stubs showed dieback a few centimeters back from the cut surface and the severly infested stubs were girdled. Under high relative humidity conditions, the fungus mycelium was formed on the infected-stem (Fig. 2A-2). Nelson et al. (1975) reported that stub dieback occurred at all stages of plant growth. But

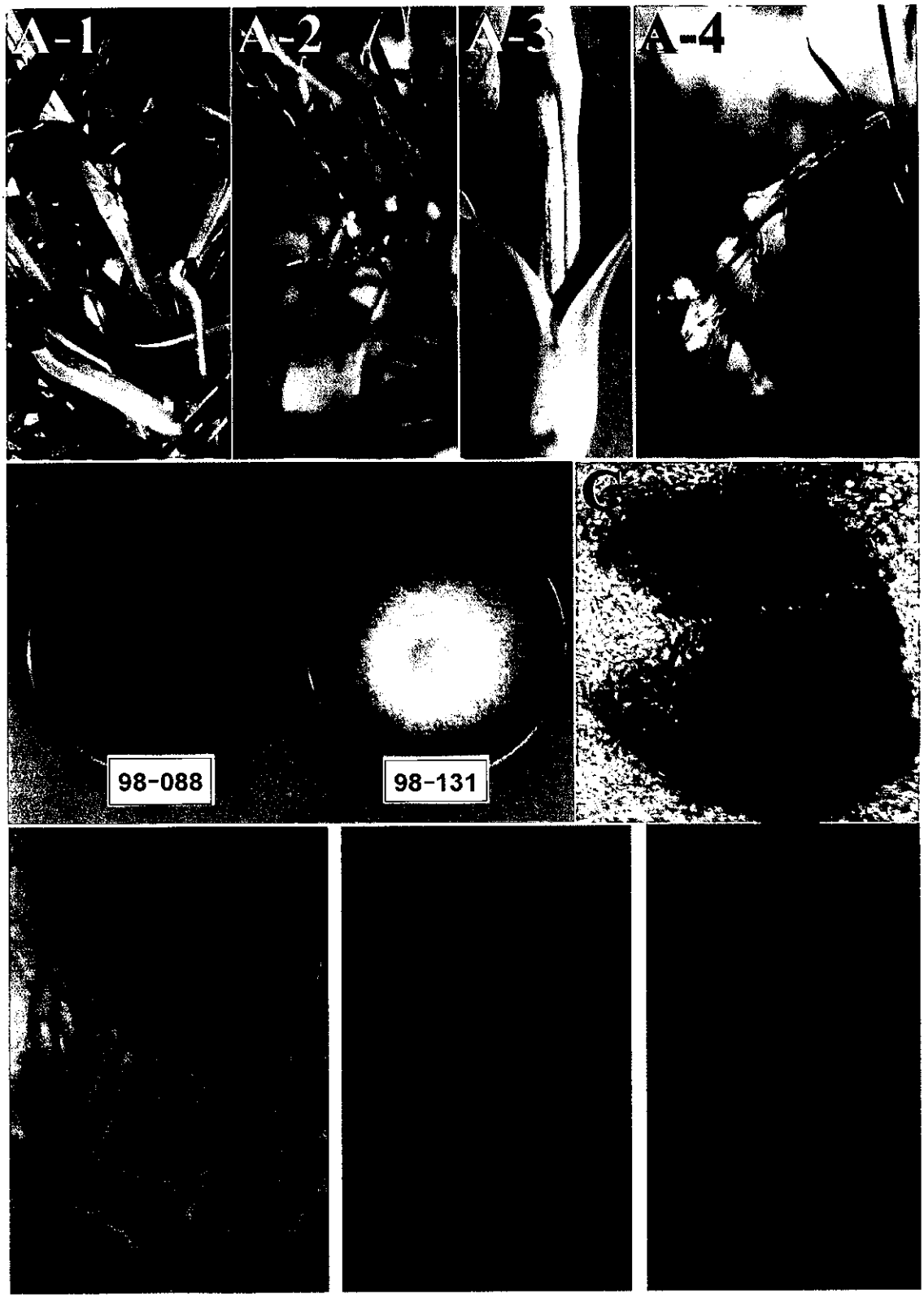

Fig. 2. Stub dieback caused by *Fusarium graminearum* and morphological characteristics of the fungus in sexual and asexual stages. **A-1**; Carnation plant showing a stub dieback symptom. **A-2**; The fungus growing on the surface of stem stub. **A-3**; Symptoms of carnation wilt. **A-4**; Carnation blossom and flower stem showing girdling. **B**; Cultural characteristics of *F. graminearum* (left) and *F. oxysporum* f. sp. *dianthi* (right). **C**; Perithecia of *F. graminearum* formed on carnation leaf agar. **D**; Asci. **E**; Ascospores and conidia. **F**; Macroconidia.

the most severe losses were sustained during the flower harvest, reducing the number of flower, shoot, and productivity of the plant.

In this study, pathogenicity to carnation's blossom as well as to stub was observed (Fig. 2 A-4). Generally stub dieback was extended from the upper to lower parts, and sometimes flower stems were girdled by the fungus and dead, showing flower blight symptoms. This fungus was reported worldwide as an airborne pathogen, and the occurrence of head blight in wheat crops by ascospores originated from perithecia (Burgess et al., 1975). Actually *G. zeae* caused seedling blight, brown rot, head or kernel blight (scab on ear scab) on a wide range of gramineous hosts. Also this fungi induced leaf and flower rot of many ornamentals as well as carnation (Booth, 1973). In Korea, diseases caused by this pathogen were reported as patch on wheat, brown leaf blight on rice, Gibberella stalk rot on maize, ear blight on barely and other cereals, and Fusarium rot on sweet potato (Korean Society of Plant Pathology, 1998). *Gibberella zeae* was reported as an airborne pathogen and its infection occurred widely by water droplets under humid conditions or by ascospore discharge (Booth, 1973). However, we could not observed any of the sexual fruiting structures in nature.

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