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Occurrence of Stem and Fruit Rot of Paprika Caused by *Nectria haematococca*

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Since 2000 severe rots on aerial and underground parts of paprika (*Capsicum annum* L.) has occurred in most surveyed glasshouses throughout the country. A total of 56 isolates of a fungus were consistently isolated from various plant parts such as fruit, stem, branch, and root collected from 16 farms in five provinces. Anamorph stage of the fungus was identified as *Fusarium solani* based on its morphological characteristics. However, the fungus readily produced a sexual structure of perithecia on infected plant tissues and on agar media. Since the fungus formed abundant perithecium by a single isolate, it was considered as a homothallic strain of *Nectria haematococca*, the teleomorph of *F. solani*. Irregularly globose perithecia with orange to red color formed sparsely to gregariously on dead tissues of fruits and basal stems at the late infection stage, which is a diagnostic sign for the disease. Perithecia ranged from 125 to 220 μm in diameter varied among isolates. Asci enveloping eight ascospores were cylindrical and measured 60-80×8-12 μm. Ellipsoid to obovate ascospores are two-celled and measured 11-18×4-7 μm. Ascospores were hyaline, slightly constricted at the central septum, and revealed longitudinal striations that is characteristic of the species. This fungus that has never been reported in Korea has previously become a threat to paprika cultivation because of its strong pathogenicity and nationwide distribution.

Keywords : *Capsicum annum*, *Fusarium solani*, *Nectria haematococca*, paprika

Paprika (*Capsicum annum* L. var. *grossum*) produces a highly valuable, colorful, sweet, and nutritious fruit that is an excellent source of vitamin C. However, the plant was introduced into Korea only a decade ago in 1994 and domestic consumption has not been prevalent yet. Though, its cultivation acreage increased rapidly from 4.9 ha in 1997 to 171 ha in 2003 as a profitable exporting crop to Japan (Bae, 2005). The paprika is known sensitive and more vulnerable to various diseases than hot pepper, however, only 15 diseases have been reported to occur on the plant in Korea (Jee, 2005).

From a previous disease survey on paprika, it was found that viral diseases, bacterial wilt, *Phytophthora* blight, and powdery mildew were major problems impeding safe cultivation of the plant. However, a new destructive disease occurred from 2000 and spread throughout the country. The disease causing severe rots on all parts of the plant such as fruit, stem, branch, and root occurred at most investigated farms in five provinces. It was assumed that the causal pathogens infecting root and fruit differ from each other at the early stage of this study. However, the same fungus was consistently isolated from all diseased plant parts regardless of collection areas. Consequently, the causal pathogen encompasses both soil-borne and air-borne properties. In this study, the causal pathogen was identified and Koch's postulates were fulfilled for the first report of the disease in Korea.

Materials and Methods

Survey and isolation. A disease survey on paprika was conducted from April to May in 2003. Seventeen farms located at 5 provinces were engaged in this survey. Plants showing rots on fruit, branch, stem, or root were considered as infected by the disease. Severity of the disease was graded with five scales as 0, < 1, 1-10, 11-20, and > 20% infection incidence. Freshly diseased samples of fruit, branch, and root were collected from the farms and cut into small pieces (5 × 5 mm) for isolation of a causal pathogen. The pieces were semi-sterilized with 75% ethanol for 30 sec and evenly distributed on water agar (WA) and semi-selective medium for *Phytophthora* and *Pythium*. The semi-selective medium consisted of pimaricin 10, rifampicin 10, ampicillin 100, PCNB 50 mg/ml in 5% clarified V-8 juice agar (Jee et al., 2000).

Draining nutrient solution was collected from 8 hydroponic farms to examine contamination by fungal pathogens. Population density of *Phytophthora* and *Pythium* in the solution was examined as follows. Ten to 20 ml of the

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solution directly added into the semi-selective medium described above and incubated for 24-48 hr at 24°C. Colonies formed on the medium were transferred to 10% V-8 juice agar medium for further study. The nutrient solution 0.2 ml was spread onto a Komada's *Fusarium* selective medium to examine the population density of *Fusarium* spp. The medium consisted of L-asparagine 2g, D-galactose 20g, $MgSO_4 \cdot 7H_2O$ 0.5g, K_2HPO_4 1g, KCl 0.5g, Fe (EDTA) 5 mg, and agar 18g per distilled water 1 L.

Identification and pathogenicity. Fungal isolates were cultured on WA and PDA (Difco) for 7-14 days at 24°C. Morphological characteristics of asexual and sexual reproduction structures formed on the medium were examined under a light microscope at 200-1000x. To examine cardinal growth temperature of the fungus, two representative isolates were incubated with 5°C intervals from 5 to 35°C. Mycelial growth was recorded every 24 hr for 7 days.

Three cultivars of paprika such as Special, Spirit, and Fiesta were used for the pathogenicity test. Inoculum of the pathogen was prepared from 14 d old cultures growing on PDA. Sexual and asexual spores produced on the medium were collected with distilled water and filtered through 4 layers of sterilized cheesecloth and adjusted to 10^5 cfu/ml. The inoculum suspension was sprayed to each 60-d old seedlings growing on rock-wool cube and incubated in a humid growth chamber for 24 h and returned to a greenhouse. Five plants were used as replicate for each treatment. Degree of rot was recorded for 14 days. Pathogenicity to wounded or not-wounded fruit was also tested. Cotton plugs soaked in the inoculum was attached to a fruit and kept in a plastic moist box for 14 days.

Results

Disease severity and the causal pathogen. The disease occurred in all surveyed areas except one farm at Jincheon, Chungbuk. The plants growing at the farm without symptoms were early growing stage. Severity of the disease varied among farms; however, it occurred in all parts of paprika such as root, stem, branch, and fruit. Incidence of infected plants showed over 20% in a few field and averaged infection incidence was approximately 10% in the fields (Table 1). The rot occurred the most commonly in root system, followed by fruit and branch at the late growth stage.

A *Fusarium* sp. was constantly isolated from the samples of root, branch, and fruit. Among 58 samples collected from nine areas, the fungus was isolated from 56 (96.6%) samples and *Phytophthora capsici* was isolated only from 2 samples (Table 2). The population density of *Fusarium* in hydroponic nutrient solution of infested fields ranged from 15.5 to 406.3 cfu/ml. Meanwhile, other soil-borne pathogens such as *Phytophthora* and *Pythium* were not detected or at the negligible level (Table 3).

Table 1. Survey on a new disease of stem and fruits rot of paprika

Surveyed		Degree of rot[a]		
Area	No. of farm	Root	Branch	Fruit
Hwasung	1	++	+	+
Goyang	1	++	+	+
Jincheon	1	−	−	−
Buyeo	1	++	+	+
Yesan	1	+++	++	++
Gimjae	2	+++	++	++
Iksan	2	+	+	+
Hwasoon	3	++	+	+
Gangjin	2	++++	+	++
Hadong	3	+	+	+
10 areas	17 farms			

[a]Degree of rot: −, no disease; +, <1%; ++, 1.0-10%; +++, 11-20%; ++++, >20%.

Table 2. Isolation of the causal pathogen of stem and fruit rot of paprika

Sample			No. of isolate		
Area	Part	Number	*Fusarium*	*Phytophthora*	*Pythium*
Hwasung	Root	3	3	0	0
Goyang	Root	3	3	0	0
	Branch	1	1	0	0
Buyeo	Root	3	3	0	0
Yesan	Root	1	1	0	0
	Fruit	8	8	0	0
Kimjae	Root	6	6	0	0
	Branch	4	4	0	0
Iksan	Root	3	2	1	1
Hwasoon	Root	1	1	0	0
	Branch	1	1	0	0
Gangjin	Root	8	7	1	0
	Branch	6	6	0	0
	Fruit	8	8	0	0
Hadong	Root	1	1	0	0
	Fruit	1	1	0	0
9 areas	−	58	56	2	1

Symptoms. At the early infection stage, plants showed growth retardation but died eventually after yellowing and wilting (Fig. 1-a). External and internal basal stem tissues turned dark brown to black and pale orange to ochre fruiting bodies of perithecia were occasionally observed on the basal stem (Fig. 1-b). Dark brown to black cankers were developed on branches, usually at nodes or wound sites by

Table 3. Population density of soil-borne pathogens in hydroponic nutrient solution of paprika

Field location	CFU/ml of hydroponic solution		
	Fusarium	*Phytophthora*	*Pythium*
Gimjae 1	33.2±4.6	0	0
Gimjae 2	34.3±8.3	0	0
Gangjin	15.5±5.1	0	0
Hwasoon 1	406.3±94.9	0	0
Hwasoon 2	25.3±5.1	0	0
Hadong 1	37.0±6.1	0	1.0
Hadong 2	73.3±5.5	0	0
Hadong 3	77.3±5.8	0	0

pruning (Fig. 1-c, d). Upper part of the branch died when the canker girdles the branch in later stages of the disease development. Fruits also develop black and water-soaked lesions around the base of fruit stalk (Fig. 1-e). The lesions grow, coalesce and spread down to inside flesh of the fruit. Orange to red fruiting bodies of perithecia also developed on the lesions sometimes (Fig. 1-f).

Identification. The vegetative stage of the causal pathogen of paprika rot was characterized in culture by whitish mycelial growth and asexual reproduction structures. The fungus produced long and branch phialides that measured up to 400mm and attached hyaline to milky-white microconidia (Fig. 1-h). Abundant microconidia were mostly one-celled and sized 9-16 × 2-4 mm. Cylindrical to falcate 3-septate macroconidia with conspicuous foot cell were also readily produced on the medium and sized 40-100 × 5-7.5 mm (Fig. 1-i). Single or grouped chlamydospores were also produced in old culture. Based on the morphological characteristics the fungus was identified as *Fusarium solani* (Booth & Waterson, 1971).

However, the fungus readily produced fruiting bodies like perithecia in old culture and on plant tissues of fruit or basal stem (Fig. 1-g). The orange to light-red perithecia sized as 130-200 mm in diameter contained abundant asci (Fig. 1-j). Asci were cylindrical, clavate, and contained eight ascospores that were ellipsoidal and slightly constricted at the single central septum with longitudinal striations (Fig. 1-k, l). Ascospores were measured 11-18 × 4-7 mm. These mycological characteristics are coincide with *Nectria haematococca* as a teleomorph of *F. solani* (Hanlin, 1990; Booth and Waterson, 1971).

Pathogenicity. Three representative isolates induced severe rots on fruit and young branches of papriaka with or without wounds. No significant difference was found in pathogenicity among the isolate. Meanwhile, susceptibility to the pathogen varied among cultivars. Popular cultivars of Special and Spirit were more susceptible than Fiesta (Table 5).

Discussion

The new disease inducing rots on various parts of paprika spread widely throughout the country and became one of the most serious diseases threatening safe cultivation of the plant. In our previous disease survey on paprika in 1998, *Phytophthora* blight caused by *P. capsici* was the most serious soil-borne disease on the plant (Jee, 2005). However, the *Phytophthora* blight became less common than the disease in recent years (Table 2, 3). Shifting the major disease on paprika could be explained by farmers' cultural practice on metalaxyl application onto rockwool block for control of the *Phytophthora* blight (personal communication). High population density of *Fusarium* and undetectable level of *Phytophthora* in the hydroponic solution also supports this phenomenon (Table 3).

Mycological characteristics of the fungal anamorph stage coincided with *Fusarium solani*, while orange to ochre perithecia and ascospores described in Table 4 and Fig. 1 are typified by *N. haematococca* described by others (Booth and Waterson, 1971; Hanlin, 1990). *F. solani* widely is distributed in soils with high saprophytic ability. Its parasitic capability is suspicious to some extend, however, it has been reported as a causal pathogen on various host plants (Barritt et al., 2002; Cerkauskas, 2001; Lee and Park, 2001). Especially, the stem and fruit rot on paprika caused by the fungus has been reported in many countries such as Canada, USA, Europe, New Zealand, and Japan (Guiderdone and Buonaurio, 2000; Jarvis et al., 1994; Lamb, 2001; Takahashi et al., 1999; Tyson, 2001). The disease was first reported in greenhouse cultivation of pepper in Ontario and British Columbia, Canada in 1991 and followed by England in 1994 and in Florida USA in 1999. Up to 40% of the plants in the affected greenhouse range were infected in that outbreak in Florida, USA (Lamb, 2001).

Since the causal pathogen has two stages in the growth cycle, the disease usually started from roots or basal stems as a soil-borne pathogen and spread to aerial parts as an air-borne pathogen. The anamorph stage *Fusarium solani* is a typical soil-borne pathogen infecting roots or basal stems of plants. It has no active dissemination and dispersal mechanism into air (Booth, 1977). While, the teleomorph *N. haematococca* infects aerial parts of plant since ascospores of the fungus are forcibly ejected into air from the perithecium (Hanlin, 1990). However, high relative humidity or even dew occurring at night is the most important environmental factor for perithecium formation and ascospore release. Also, a prolonged period of high humidity, over 95% RH is required for ascospore germination and

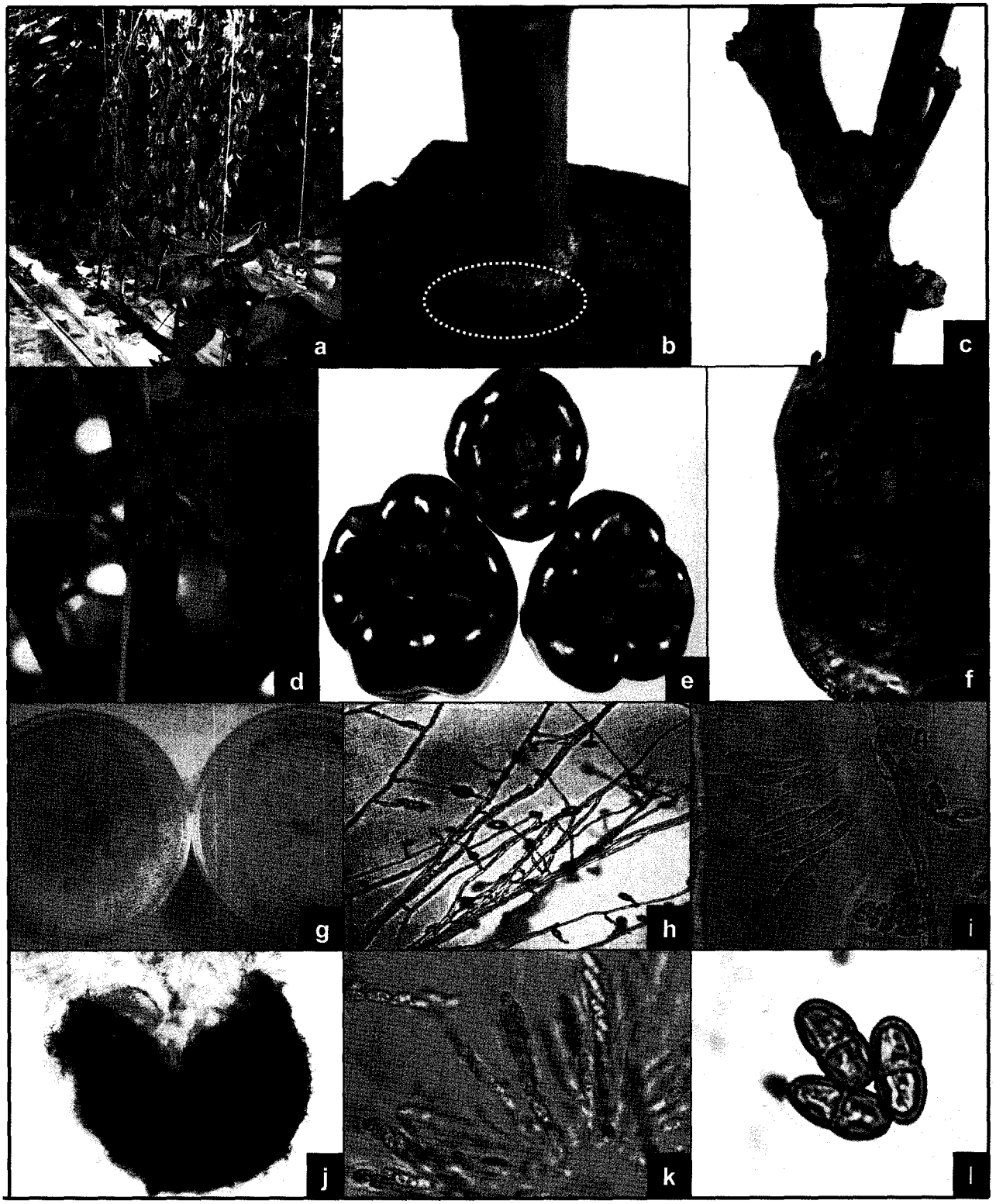

Fig. 1. Symptoms of stem and fruit rot of paprika and morphology of the causal fungus, *Nectria haematococca*. Severely diseased plant (a) and abundant perithecia formed on its basal stem (b). Dark red to black cankers developed on a node (c) and wound site by pruning (d). Infected immature fruits (e) and abundant perithecia were developed on a mature fruit (f). Colony pattern of the causal fungus on PDA with abundant perithecia (g). Asexual reproduction structures of micro- and macroconidia (h and i). Sexual reproduction structures including a perithecium (j), asci (k), and ascospores (l).

Table 4. Mycological characteristics of the causal pathogen of stem and fruit rot of paprika

Item	Characteristics
Growth	Grow well on common media, Bluish to brown color Moderate aerial grayish-white mycelia Cardinal temperatures : min 8, opt. 25-28, max. 33°C
Asexual stage	
Microconidia	Abundant, ellipsoidal to cylindrical, born on long phialides in false head, 1(-2)-celled, 9-16×2-4 μm
Macroconidia	Abundant, cylindrical to falcate, conspicuous foot cell, born on monophialides, 40-100×5-7.5 μm
Chlamydospore	Single or in groups, globose, 9-12×8-10 μm
Phialide	Elongated, narrow slightly in apex, up to 400 μm
Sexual stage	
Perithecia	Readily form on agar and plant tissues, pale orange to ochre, 13-200 μm in diameter
Ascus	Cylindrical, clavate, 8 ascospores
Ascospore	Ellipsoid, slightly constricted at single central septum, longitudinal striations, 11-18×4-7 μm

Table 5. Pathogenicity of the causal pathogen, *Nectria haematococca*, to paprika

Tested isolate	Degree of branch rot						Degree of fruit rot					
	Special		Spirit		Fiesta		Special		Spirit		Fiesta	
	W	NW	W	NW	W	NW	W	NW	W	NW	W	NW
H1	+++	++	+++	+++	+	+	+++	++	+++	+++	+	+
K7	+++	++	+++	+++	++	++	+++	++	+++	+++	++	++
K1	+++	++	+++	+++	+	+	+++	++	+++	+++	+	+
Control	–	–	–	–	–	–	–	–	–	–	–	–

infection into plant (Jarvis et al., 1994).

Fusarium solani is a widespread soil-borne fungus comprising at least 50 genetically distinct species that exhibit a strong biogeographic structure world-wide (Kerry, 2000). The original source of primary inoculum is not understood in this study. However, paprika seeds imported totally from foreign countries are not ruled out as the origin of *Nectria haematococca* since the fungus commonly occur in the seed exporting country. Control measures of the disease including seed disinfection and fungicide application to be investigated in a further study.

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