Plant Pathol. J. 22(1) : 16-20 (2006)

The Plant Pathology Journal

Occurrence of Sclerotinia Rot in Four Leguminous Crops Caused by *Sclerotinia sclerotiorum*

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(Received on February 2, 2006; Accepted on February 14, 2006)

Four leguminous crops grown in greenhouses and fields in Korea were surveyed from 2000 through 2002. Sclerotinia rot most severely occurred up to 60% in *Phaseolus vulgaris* grown in greenhouses but occurred as low as 0-0.5% in that grown in fields. Incidence of the disease in *Pisum sativum* grown in greenhouses ranged 1-5%, and that in *Vicia fava* and *Vigna sinensis* grown in fields was 0.8% and 2%, respectively. Symptoms of Sclerotinia rot commonly developed on stems and pods of the crops. A total of 59 isolates of *Sclerotinia* species were obtained from diseased stems and pods of the crops. All of the isolates were identified as *Sclerotinia sclerotiorum* based on their morphological characteristics. Eight isolates of the fungus were tested for their pathogenicity to four host crops by artificial inoculation. All of the isolates induced rot symptoms on stems of the host crops tested, which were similar to those observed in the fields. The pathogenicity tests revealed that there was no significant difference in the susceptibility to the isolates among the leguminous crops tested. This is the first formal report that *S. sclerotiorum* causes the Sclerotinia rot of the four leguminous crops in Korea.

Keywords : leguminous crops, occurrence, pathogenicity, Sclerotinia rot, *Sclerotinia sclerotiorum*

Leguminous crops are grown in the world as an important vegetable or a primary source of oil and food. In Korea, most leguminous crops are cultivated in fields but some of them are in greenhouses. Among leguminous crops, *Glycine max* (L.) Merr. is most widely cultivated in fields in the country, and other leguminous crops are on a small scale. In case of the greenhouse cultivation, the leguminous crops are mostly cultivated for crop rotation with various vegetables depending on the locations.

Severe outbreaks of stem or pod rot symptoms with sclerotial formation were sometimes observed during a disease survey of four leguminous crops in some locations in the country during the cool seasons. *Sclerotinia* sp. was consistently isolated from the diseased plants. The disease of the crops caused by *Sclerotinia* spp. was recorded as Sclerotinia rot, Sclerotinia stem rot, Sclerotinia wilt, stem rot, pod and stem rot, white mold, blight, wilt, etc. (Farr et al., 1989). However, Sclerotinia rot has been most commonly called for the disease name of the crops caused by *Sclerotinia* spp. as the previous workers reported (Kim and Cho, 2002, 2003a, 2003b; Kim et al., 1999).

It has been reported that *Sclerotinia sclerotiorum* (Lib.) de Bary and *S. minor* Jagger cause Sclerotinia rot in *Phaseolus vulgaris* L., and only the former species causes the disease in *G. max*, *Pisum sativum* L., *Vicia fava* L., and *Vigna sinensis* King (Farr et al., 1989). In Korea, few studies have been conducted on the disease occurrence and the pathogenicity of the causal *Sclerotinia* species in leguminous crops. This study was conducted to examine the disease occurrence in four leguminous crops in Korea and the pathological aspects of the pathogen.

Materials and Methods

Disease survey and collection of diseased samples. Four leguminous crops *Phaseolus vulgaris, Pisum sativum, Vicia fava*, and *Vigna sinensis* grown in greenhouses and fields in Korea were surveyed from 2000 through 2002. Incidence of Sclerotinia rot on the crops was investigated, and diseased plants were collected. The severity of the disease was rated in terms of percentage of infected plants among 30 to 50 plants observed with three replicates in each field.

Pathogen isolation. *Sclerotinia* sp. was isolated from the lesions according to the method described previously (Kim et al., 1999). Nine to 25 mm^2 lesion pieces cut from the diseased plant parts were placed on 2% water agar (WA) after surface-sterilizing with 1% sodium hypochlorite solution for 1 minute. The plates were incubated for 1-2 days at 22°C. The fungi grown from the lesion pieces were

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Table 1. Incidence of Sclerotinia rot in four leguminous crops in Korea from 2000 to 2002

Host	Location	Cultivation pattern	No. of fields infected/surveyed	% infected plants
Phaseolus vulgaris	Gongju	Greenhouse	2/2	20-60[a]
	Hongcheon	Field	0/2	0
	Namhae	Field	1/6	0.5
Pisum sativum	Namhae	Field	0/15	0
	Yesan	Greenhouse	5/7	1-5
Vicia fava	Namhae	Field	1/18	0.8
Vigna sinensis	Paju	Field	1/2	2
	Yecheon	Field	0/1	0

[a]Thirty to fifty plants in each field were investigated with three replicates.

transferred to potato dextrose agar (PDA) slants and cultured for identification.

Examination of morphological characteristics. Each isolate was cultured on PDA in 9-cm-diameter Petri dishes at 22°C in the dark for 12 days for observation of colony morphology. Sclerotia produced on the medium were examined for morphological characteristics and preserved in a low temperature incubator at 0°C for 3 days. Then, the sclerotia were placed in 250-ml flasks with sterile wet sand and incubated at 15°C for 1-3 months in alternating cycles of 12-hr fluorescent light and 12-hr darkness to induce formation of apothecia. Apothecia produced from the sclerotia during the incubation were collected and examined for the morphological features. Nuclei in the ascospores were stained with 1 ppm solution of 4',6'-diamidino-2-phenylindole (DAPI) indicated by Martin (1987) and observed under a compound microscope (Leica Q500MCP & DMRBE) with fluorescent light (Leica Mikroskopie & Systeme GMPH).

Pathogenicity test. A local variety of each leguminous crop was used for pathogenicity tests. Five seeds of each variety was sown in circular plastic pots (29 cm in height and 21 cm in diameter) filled with sterile upland soil in a greenhouse at 18-28°C. After 10 to 20 days of cultivation in the greenhouse, the best healthy plant was selected from among the plants in each pot for continuous cultivation, and the others were removed.

Two isolates of *Sclerotinia* species from each host were used for the pathogenicity tests to the leguminous crops. Fresh mycelial mats (6 mm in diameter) of each isolate grown on PDA were placed on the stems of 5 to 10 cm above ground of 40-day-old plants of the crops grown in the plastic pots. PDA disks of the same size were placed on the stems of the plants as a control. The pots with inoculated plants were placed in a dew chamber with 100% relative humidity at 22°C for 48 hr and then moved into the greenhouse. Virulence of the isolates was rated based on the degree of rot symptoms induced 5 days after inoculation. The inoculation test was performed with three replicates.

Results

Disease incidence and symptoms. Sclerotinia rot commonly occurred in four leguminous crops grown in some locations in Korea (Table 1). The disease most severely occurred up to 60% in *P. vulgaris* grown in greenhouses but occurred as low as 0-0.5% in that grown in fields. Incidence of the disease in *P. sativum* grown in greenhouses ranged 1-5%, and that in *V. fava* and *V. sinensis* grown in fields was 0.8% and 2%, respectively.

Symptoms of Sclerotinia rot usually developed on stems of the leguminous crops and also occasionally on pods (Fig. 1). Infected plant parts rotted with white to gray yellow discoloration. Cottony mycelia frequently developed on the infected plant parts. Globose to irregular black sclerotia were produced on the infected plant parts at the late stages of the disease development.

Pathogen isolation and identification. A total of 59 isolates of *Sclerotinia* species were obtained from lesions of Sclerotinia rot in four leguminous crops (Table 2). The *Sclerotinia* sp. was isolated from stems of all the leguminous crops and pods of *P. sativum, V. fava* and *V. sinensis*. All the isolates were identified as *S. sclerotiorum* based on their morphological characteristics (Table 3 and Fig. 2) as described by previous workers (Kohn, 1979; Willetts and Wong, 1980). Colonies of the fungus on PDA consisted of white to gray mycelia and globose to irregular and black sclerotia (Fig. 2A). Yellowish brown and cup-shaped apothecia were produced from sclerotia (Fig. 2B). Asci were cylindrical and 8-spored, and ascospores hyaline and ellipsoid to ovoid (Fig. 2C). The fungus has two nuclei in the ascospores (Fig. 2D).

Fig. 1. Symptoms of Sclerotinia rot in four leguminous crops grown in greenhouses and fields. A, *Phaseolus vulgaris*; B, *Pisum sativum*; C, *Vicia fava*; D, *Vigna sinensis*.

Table 2. Isolation of *Sclerotinia* sp. from diseased plant parts of four leguminous crops

Host	No. of isolates obtained		
	Stem	Pod	Total
Phaseolus vulgaris	19	0	19
Pisum sativum	12	10	22
Vicia fava	10	4	14
Vigna sinensis	1	3	4
Total	42	17	59

Pathogenicity. All the isolates of *S. sclerotiorum* induced rot symptoms on stems of the four leguminous crops inoculated (Table 4). The symptoms were similar to those observed in the fields. The fungus was re-isolated from the lesions of the plants inoculated. The pathogenicity tests revealed that there was no significant difference in the susceptibility to the isolates among the leguminous crops tested.

Discussion

The present study showed that *S. sclerotiorum* is the causal fungus of Sclerotinia rot in four leguminous crops in Korea. The fungus has been reported to cause Sclerotinia rot of *G. max* which is most widely cultivated in Korea among leguminous crops (Park, 1961; Sung, 1982). Sclerotinia rot of *P. vulgaris* caused by *S. sclerotiorum* was also recorded in Korea (Cho and Shin, 2004). However, it is only a record of the pathogen for the disease occurrence in *P. vulgaris* without descriptions on the symptoms and the pathogen. There has been also no report on the disease occurrence in the other leguminous crops in Korea. Accordingly, this is the first formal report that *S. sclerotiorum* causes Sclerotinia rot of the four leguminous crops in Korea.

It was reported that *S. sclerotiorum* and *S. minor* caused Sclerotinia rot of *P. vulgaris* (Farr et al., 1989). However, the former species is known to be the main pathogen of the disease in leguminous crops. *S. minor* is known to have

Table 3. Morphological characteristics of *Sclerotinia sclerotiorum* isolated from four leguminous crops

Structure Examined	Division	Description of characteristics	
		Present isolates	Kohn (1979)
Sclerotium	Color	Black	Black
	Shape	Globose to irregular	Globose to cylindrical, quite variable
	Size	1-10 × 1-7 mm	(2-)5-15(-30) × 2-8 (-15) mm
Apothecium	Shape	Cup-shaped	Cup-shaped
	Diameter of disks	1.7-5.0 mm	2-8(-10) mm
	Size of stipes	1.2-5.0 × 0.5-1.0 mm	3-20(-30) × 1-2 mm
Ascus	Shape	Cylindrical	Cylindrical
	Size	127.5-175.0 × 7.5-10.0 μm	(110-)130-150(-160) × 6-10 μm
Ascospore	Shape	Ellipsoid to ovoid	Ellipsoid
	Size	11.5-17.5 × 5-8 μm	(9-)10-14 × 4-5(-6) μm
	Nuclear number	2	2

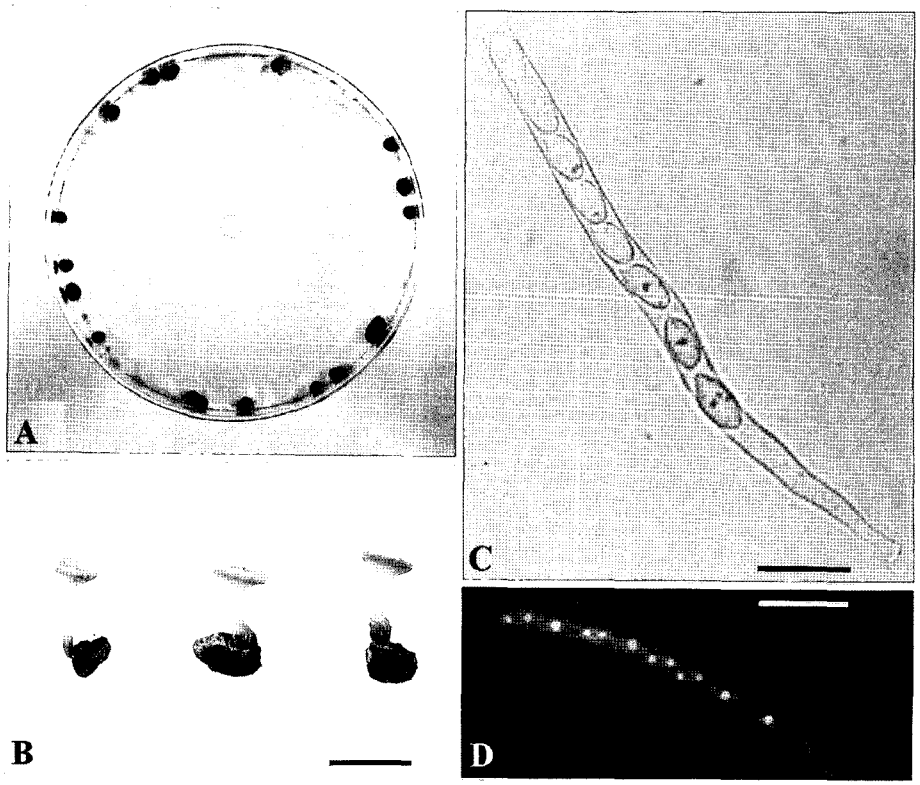

Fig. 2. Morphological features of *Sclerotinia sclerotiorum* isolated from four leguminous crops. A, 12-day-old colonies on PDA at 22°C in the dark; B, apothecia produced from sclerotia (scale bar = 5 mm); C, ascospores in an ascus (scale bar = 20 μm); D, nuclei in the ascospores stained with DAPI (scale bar = 40 μm).

somewhat narrower host range than *S. sclerotiorum* (Kohn, 1979; Willetts and Wong, 1980). Therefore, *S. minor* may cause Sclerotinia rot in leguminous crops but be a minor pathogen of the disease in the field.

In the present study, Sclerotinia rot of the leguminous crops more severely occurred in greenhouses than in fields, suggesting that the greenhouse conditions are favorable for occurrence of the disease in the crops due to the high humidity. It has been also reported that Sclerotinia rot is apt to readily occur under cool and moist conditions (Purdy, 1979; Willetts and Wong, 1980).

There have been reports on differences in virulence of *S. sclerotiorum* isolates to individual plants and in susceptibility of some plants to the isolates (Kim et al., 1999; Price

Table 4. Pathogenicity of *Sclerotinia sclerotiorum* isolates in four leguminous crops by artificial inoculation

Isolate	Isolate source	Virulence of isolates on stems of hosts[a]			
		PV[b]	PS	VF	VS
S02-15	*Phaseolus vulgaris*	+	+	+	+
S02-21	*Phaseolus vulgaris*	+	+	+	+
S00-28	*Pisum sativum*	+	+	+	+
S02-77	*Pisum sativum*	+	+	+	+
S02-79	*Vicia fava*	+	+	+	+
S02-89	*Vicia fava*	+	+	+	+
S00-43	*Vigna sinensis*	+	+	+	+
S00-46	*Vigna sinensis*	+	+	+	+
Control		−	−	−	−

[a]Disease severity was rated 5 days after inoculation. +, above 2 cm of lesion length or wholly rotted; −, no symptom.
[b]Abbreviation for hosts. PV, *Phaseolus vulgaris*; PS, *Pisum sativum*; VF, *Vicia fava*; VS, *Vigna sinensis*.

and Calhoun, 1975). It has been also reported that there are differences in susceptibility of cultivars or lines of some crops to the pathogen (Cassells and Walsh, 1995; Grau and Bissonnette, 1974; Orellana, 1975; Porter et al., 1975). However, the present study showed that there was no significant difference in virulence of *S. sclerotiorum* isolates to leguminous crops, as reported by previous workers in other crops (Kim and Cho, 2002; Kim and Cho, 2003a; Kim and Cho, 2003b).

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