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Gray Leaf Spot in Peppers Caused by *Stemphylium solani* and *S. lycopersici*

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A disease causing severe leaf spots in pepper plants has been observed in northern Gyeongbuk and Gangwon provinces in Korea since 1994. The current study diagnosed the disease as gray leaf spot caused by *Stemphylium solani* Weber and *S. lycopersici* (Enjoji) Yamamoto, both of which are pathogenic in pepper and tomato plants. Although the disease has been found in almost all areas where peppers are grown, it is more severe in mountain terrains where the nights are cool. Both species of pathogenic fungi were found to sporulate profusely on V-8 juice agar in plastic or Pyrex glass Petri dishes, although not in domestically-produced glass Petri dishes, when cultured at 20°C under irradiation from a daylight fluorescent lamp with a 12-hour light and dark alternation. The domestically-produced glass Petri dishes, which are made of window glass, were found to block near ultraviolet wavelengths, around and below 300 nm, which explained why the fungi did not sporulate. However, sporulation decreased at above 25°C and most isolates failed to sporulate above 27°C. The worst level of disease was obtained when the inoculated plants were incubated with a 15°C night and 20°C day temperature regime relative to 4 night/day temperature combinations (15/20, 20/25, 25/30, and 30/35°C).

Keywords **:** *Capsicum annuum* L., pathogenicity, taxonomy

A new and unusual leaf spot disease forming numerous tiny spots on pepper leaves was observed in Youngyang and Cheongsong in Gyeongbuk Province, Korea in 1994. The spots are initially similar to bacterial spots caused by *Xanthomonas campestris* pv. *vesicatoria*. However, the spots become distinct as they remain small and round, no more than 2 mm in diameter, with dry, white, and sunken centers, in contrast to the expanding, brown, and oil-soaked edged bacterial spots. The new disease progresses from the formation of numerous light brown spots to yellowing and severe defoliation, leaving only a few new leaves on the stem tips.

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In this study, spores of the genus *Stemphylium* were found in abundance on infected leaves collected from a greenhouse in Youngyang, yet only occasionally on infected leaves collected from open fields. Two different species of *Stemphylium* were isolated from the diseased leaves depending on the specimen. As such, the causal fungi were isolated and their pathogenicity was tested on pepper and tomato plants.

Gray leaf spot affecting peppers and caused by *S. solani* was first reported in Louisiana by Sinclair et al. (1958) and in Florida by Blazquez (1969). However, both *S. solani* and *S. lycopersici* have been reported to cause gray leaf spot in tomato (Ellis and Gibson, 1975a, 1975b; Hannon and Weber, 1955; Weber, 1930). *S. solani* and *S. lycopersici* have also both been found to cause gray leaf spot in tomatoes in Korea (Kim et al., 1999a; Min et al., 1995).

This study identified the causal fungi of gray leaf spot in pepper plants and their mycological characteristics.

Materials and Methods

Isolation and sporulation of causal fungi. Pepper leaves with spots retaining spores were selected upon examination under a dissecting microscope. The spots were then blotted against water agar plates so that the spores fell onto the agar surface. Thereafter, single spores were picked up from the agar using a needle and transferred to a V-8 juice agar or potato dextrose agar plates for culturing. Spores were produced by incubating mycelial blocks, cut from the edge of an actively growing culture, on a $CaCO_3$ medium at 20°C under dark conditions (Sahin and Shepard, 1979). The spores used for the pathogenicity test were produced by this method at the beginning of the study. Thereafter, the pathogenic fungi were routinely cultured on V-8 juice agar plates in plastic Petri dishes under 12 hours of fluorescent light at 25°C or a lower temperature for sporulation. Spores, conidiophores, and mycelia were taken from 3- to 4-day-old cultures on the V-8 juice agar plates and their dimensions were measured for identification. The size, shape, number of transverse and longitudinal septa, and lateral constrictions were all recorded, and the length/width (l: w) ratio was calculated.

Pathogenicity test. Spores produced by incubating mycelial blocks on a $CaCO_3$ agar at 20°C under dark conditions (Sahin and Shepard, 1979) were used. Spore suspension of each species of

Stemphylium was prepared by rubbing off spores from the surface of the colonies in sterile water using a camel hairbrush and then filtering the spore suspension through four layers of cheesecloth. Thereafter, the suspension of each species was sprayed onto the leaves of 1-month-old pepper and tomato seedlings so that both sides of the leaves got wet. The inoculated plants were placed in a plastic bucket with a small amount of water at the bottom, then the buckets were sealed with plastic film and the plants allowed to be incubated at 20-25°C for 48 hours. Finally, the incubated plants were moved to a greenhouse bench and examined for any symptom development.

Disease survey. A disease survey of the major pepper production regions in Korea was conducted between late summer and fall in 1997, which included the collection of leaf spot specimens and identification of the causal fungi.

Effect of temperature on mycelial growth. Potato dextrose agar plates were seeded with mycelial plugs, 5 mm in diameter and obtained from the edge of an actively growing culture on V-8 juice agar plates of the respective pathogen species. The plates were cultured at 5, 10, 15, 20, 25, 30, and 35°C in incubators. The colony diameters were measured every day, yet only data taken 7 days after inoculation are presented in the results.

Effect of temperature on conidial germination. One (1) ml of conidial suspension (10^3 conidia/ml) of each species of *Stemphylium* was spread over the surface of a plastic Petri dish containing 20 ml of autoclaved 2% water agar. The plates were then cultured at 15, 20, 25, 30, and 35°C in incubators. The conidial germination was examined 1 and 3 hours after treatment.

Effect of incubation temperature on disease development. One-month old pepper seedlings (cv. Gumtap), grown in 12 cm diameter plastic pots, were sprayed with conidial suspensions of the respective species of *Stemphylium*. The conidial suspensions were prepared by collecting spores produced on V-8 juice agar plates in sterile water and by adjusting the spore concentration to approximately 10^5 conidia per ml based on dilution with the aid of a hemacytometer. The inoculated plants were placed in plastic buckets, three plants per bucket, with a small amount of water at the bottom. The buckets were then covered and sealed with plastic film to create an incubation chamber. The buckets with the inoculated plants were subjected to 4 night-day temperature regimes, 15-20°C, 20-25°C, 25-30°C, and 30-35°C. A four-chamber incubator was used to obtain the 4 night-day temperature regimes with a 12-hour alternation. The plants were incubated for 48 hours under each incubation temperature regime, then taken out from the plastic buckets and moved to a greenhouse bench to examine the disease development. A counter was used to determine the number of spots per leaf on the three most diseased leaves on each plant 3 days after completing the incubation. Average number of spots per leaf on the three most diseased leaves from the three plants incubated under each night-day temperature regime was then taken and presented as the result.

Effect of media, light, and temperature on sporulation. To determine the influence of media and light on the sporulation of the two species of *Stemphylium*, they were cultured on V-8 juice agar, potato carrot agar (PCA), and potato dextrose agar (PDA) under complete darkness or alternating light (12 hours) and darkness. For the light source, near ultraviolet (NUV) radiation was provided by Philips TL 40 W/08 black light tubes. Two 40 W fluorescent black light tubes were placed in a horizontal position 20 cm apart at a height of approximately 40 cm above the cultures. Meanwhile, to obtain complete darkness, the Petri dishes were wrapped in thick black paper. Observations were made 6 days after incubation. The number of conidia/unit of measurement (dia. 13 mm agar disk punched out by No. 7 cork borer) was determined using a hamacytometer. The effect of temperature on the sporulation and conidial dimensions of each species of *Stemphylium* was also examined by culturing at 20, 25, and 30°C on a V-8 juice agar under alternating light (12 hours) and darkness for 6 days.

Light transmission of Petri dishes and its effect on sporulation. The light transmission of lids of imported glass ('Pyrex', America), plastic ('Green Cross', Korea), and domestically produced glass Petri dishes was measured at a wavelength ranging from 200 to 400 nm using a UV-VIS-NIR Spectrophotometer (CARY 5G, Varian) at the Institute of Basic Sciences, Kyungpook National University.

Results and Discussion

Symptoms. The symptoms were characterized by numerous, tiny, mostly less than 2 mm in diameter, round spots that were white to gray with sunken center and brown edge formed on the leaves, resulting in premature defoliation (Fig. 1A). Tiny, round, orange brown spots appeared on the leaves at first, then the center turned gray to white as the disease progressed, while the edge of the spots remained brown to dark brown. The diameter of the spots ranged from 0.5 to 2.5 mm with an average of 1.3 mm. The spots were also often accompanied by a surrounding halo. Unless properly controlled, the spots and halos caused premature defoliation, leaving only a few newly developed leaves at the tip of the stems. Spots also developed on the peduncle and calyx of the fruits (Fig. 1B).

Causal fungi. Two distinct species of *Stemphylium* (Table 1) were isolated from the spots depending on the specimen, and sometimes coincidently from the same location and

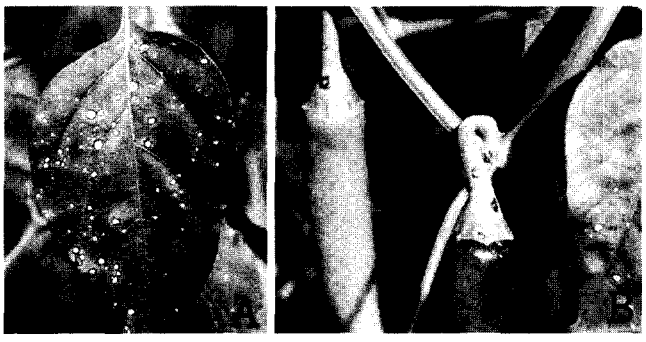

Fig. 1. Symptoms of gray leaf spot in pepper plants. (**A**) Spots on infected leaf; (**B**) Spots on peduncle and calyx.

Table 1. Morphological characteristics of *Stemphylium* spp. causing gray leaf spot in peppers

Character	*S. solani*	*S. lycopersici*
Growth on V8A	Gray	Gray
on PDA	Gray	Gray to light brown with diffusion of brown pigment
Mycelia	hyaline, 3.0-4.5 µm thick septate at 17-107.3 µm interval	hyaline, 3.5-7.8 µm thick septate at 31.2-50.7 µm interval
Conidiophores	tan to light brown range: 107.5-260.2 × 4.3-8.6 µm average: 175.0 ± 31.8 × 7 ± 1.0 µm with 4 to 13 septae vesicular tip: 7.7-8.6 µm in diameter	hyaline to light brown range: 107.5-274.2 × 4.3 µm average: 186.3 ± 38.8 × 4.3 µm with 5-11 septae vesicular tip: 6.5-8.6 µm in diameter
Conidia	oblong, similar to insect pupa, with conspicuous median constriction, 3-4 transverse and 1-2 longitudinal septae, tan to light brown, round basal end, and asymmetrically tapered terminal end forming blunt pointed tip. range: 29.2-51.6 × 15.1-21.5 µm average: 40.6 ± 5.1 × 18.0 ± 1.5 average l/w ratio: 2.25 : 1	elongated elliptic, similar to bullets, light brown to orange brown, with 7-9 transverse septae and 2-3 longitudinal septae, 2-3 conspicuous transverse constrictions in outline, symmetrically pointed apical end, round basal end, and warty surface. range: 30.0-73.1 × 12.9-19.4 µm average: 54.8 ± 8.3 × 16.4 ± 1.3 average l/w ratio: 3.34
Pathogenicity	pathogenic in peppers and tomatoes	Pathogenic in peppers and tomatoes

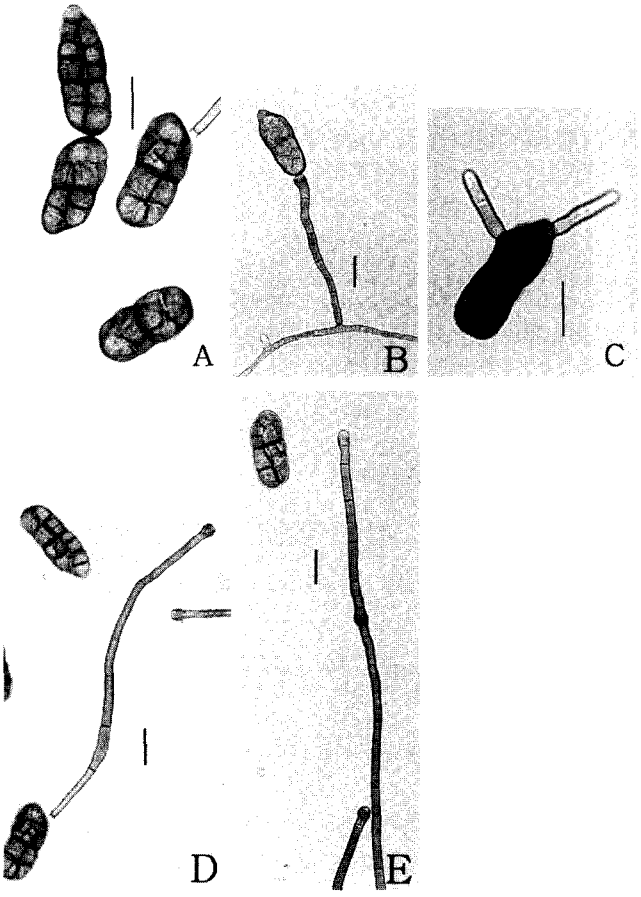

Fig. 2. *Stemphylium solani.* (**A**) Conidia; (**B**) Sporulation on top of vesicular tip of conidiophore; (**C**) Germinating conidia; (**D**) Conidium with secondary conidiophore; (**E**) Conidiophore with secondary growth forming knob between primary and secondary growth. Scale bars indicate 20 µm.

even the same leaf. Both species sporulated abundantly on the V-8 juice agar when cultured at temperatures ranging from 15 to 25°C under 12-hour fluorescent light. The more frequently recovered species produced light brown oblong conidia, similar to an insect cocoon, with one conspicuous median constriction, 3-4 transverse and 1-2 longitudinal septate, a round basal end, asymmetrically tapered terminal end with a bluntly pointed tip, on top of the erect vesicular tip of tan to light brown septate conidiophores (Fig. 2A, B, D). The conidia measured 29.2-51.6 × 15.1-21.5 µm with an average of 40.6 ± 5.1 × 18.0 ± 1.5 µm, yielding an average l/w ratio of 2.25:1. The conidiophores measured 107.5-260.2 × 4.3-8.6 µm, averaging 175.0 ± 31.8 × 5.7 ± 1.0 µm, with 4-13 septate and 7.7-8.6 µm in diameter (Fig. 2D). The species was identified as *S. solani* Weber (Ellis and Gibson, 1975b; Weber, 1930; Yu, 2001). Conidia readily fell off, while the conidiophores often exhibited secondary growth after the drop of the conidia, forming a knob between the initial and secondary growth (Fig. 2E). In addition, the conidia often germinated in situ and produced germ tubes from different cells in the conidia (Fig. 2C).

The other species produced elongated elliptic conidia, similar to bullets, that were light brown to orange brown with 7-9 transverse septate and 2-3 longitudinal septate, 2-3 conspicuous transverse constrictions in outline, an almost symmetrically pointed apical end, round basal end, warty surface, on top of the erect vesicular tip of hyaline to light brown septate conidiophores (Fig. 3A, B). The conidia measured 30.0-73.1 × 12.9-19.4 µm with an average of 54.8 ± 8.3 × 16.4 ± 1.3 µm, yielding an average l/w ratio of 3.34:1. The conidiophores measured 107.5-274.2 × 4.3 µm, averaging 186.3 ± 38.8 × 4.3 µm, with 5-11 septate and a

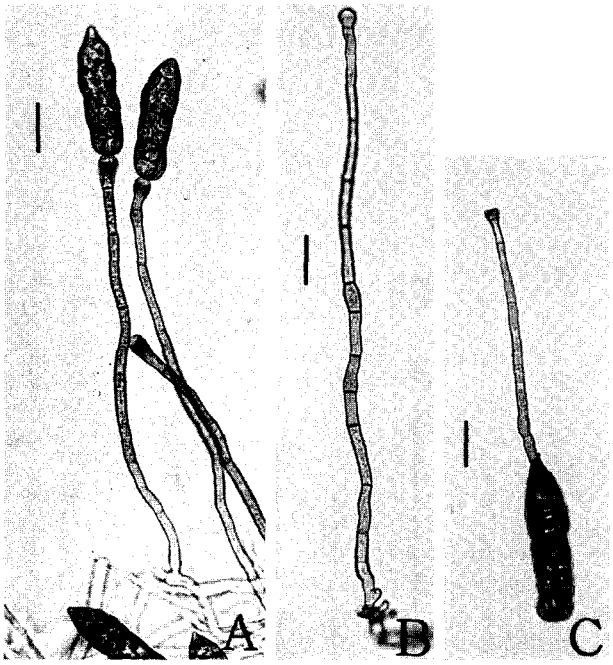

Fig. 3. *Stemphylium lycopersici.* (**A**) Conidia formed on top of vesicular tip of conidiophores; (**B**) Conidiophore with secondary growth; (**C**) Secondary conidiophore produced directly by germination of conidium. Scale bars indicate 20 μm.

6.5-8.6 μm diameter vesicular tip. The species was identified as *S. lycopersici* (Enjoji) Yamamoto (Ellis and Gibson, 1975a; Yu, 2001), which had been named *S. floridanum* once by Hannon and Weber (1955). Conidia dropped easily and often germinated in situ (Fig. 3C). Secondary growth of the conidiophores was also observed as in *S. solani.*

Pathogenicity. Spots began to appear 1-2 days after completing the incubation and became conspicuous after 3 days. Numerous spots developed similar to natural infection, and the leaves with many spots usually defoliated about 7-10 days after inoculation. *S. solani* and *S. lycopersici* developed undistinguishable symptoms on both the pepper and tomato plants. In Korea, the occurrence of gray leaf spot in tomato plants caused by *S. solani* and *S. lycopersici* was previously reported by Kim et al. (1999a) and Min et al. (1995), respectively. As such, the current study is the first time gray leaf spot has been reported in pepper plants in Korea. Braverman (1968) already reported the occurrence of leaf spots caused by *S. botryosum* f. sp. *Capsicum*, yet the present disease differs from what he reported in both the symptoms and morphology of the causal organisms.

Disease occurrence. The disease often occurred during the seedling stage in the nursery, plus seedlings being transplanted to the fields were also occasionally found to carry spots. The disease was checked after transplanting in late April to early May, probably due to the relatively low temperature, yet progressed with the growth of the plants and an increasing temperature. Many infected pepper fields were found in July and August. However, the worst infection occurred mostly in September. The disease was observed in pepper production fields in Gyeongbuk, Gyeonggi, Chungbuk, and Gangwon provinces (Table 2). Although

Table 2. Occurrence of *S. solani* and *S. lycopersici*, and their spore dimensions

Isolate	Location collected	Date collected	Conidial size (μm)	L/W Ratio
S. solani				
SS-01	Youngyang, Kyongpook	1995. 9	25.8-60.2×17.2-25.8(46.8×21.2)	2.21:1
SS-02	Bal'ri, Youngyang, Kyongpook	1995. 9	25.8-51.6×12.9-25.8(38.6×19.3)	2.01:1
SS-03	Euisung, Kyongpook	1996. 6.	30.1-55.9×15.5-30.1(43.7×21.2)	2.07:1
SS-04	Ipseok, Andong, Kyongpook	1996. 8.	30.1-53.8×15.1-21.5(40.6×17.9)	2.28:1
SS-05	Waryong, Andong, Kyongpook	1997. 9.	30.1-55.9×14.6-21.5(45.7×19.0)	2.42:1
SS-06	Daehyun-1ri, Bonghwa, Kyongpook	1997. 9.	32.3-55.9×15.1-21.5(42.2×17.4)	2.42:1
SS-07	Sododong, Taebak, Gangwon	1997. 9.	34.4-55.9×17.2-21.5(44.4×18.6)	2.40:1
SS-08	Yeoha, Taebak, Gangwon	1997. 9.	30.1-55.9×15.1-21.5(42.8×18.6)	2.32:1
SS-09	Dongjeon, Taebak, Gangwon	1997. 9.	30.1-51.6×15.1-21.5(40.0×17.9)	2.24:1
SS-10	Sangdong, Youngwol, Gangwon	1997. 9.	30.1-60.2×17.2-21.5(46.7×19.1)	2.47:1
SS-11	Angsung, Chungju, Chungbuk	1997. 9.	34.4-55.9×15.1-21.5(43.5×18.6)	2.35:1
SS-12	Subi, Youngyang, Kyongpook	1997. 10.	30.1-51.6×12.9-21.5(37.8×16.1)	2.29:1
SS-13	Misung, Wobo, Kunwi, Kyongpook	1997. 10.	30.1-51.6×15.1-21.5(41.4×17.4)	2.38:1
S. lycopersici				
SL-01	Yeoju, Gyeonggi	1996. 2	38.7-68.8×12.9-21.5(51.1×17.4)	2.94:1
SL-02	Cheongsong, Kyongpook	1996. 2	38.7-64.5×12.9-19.4	3.17:1
SL-03	Ipseok, Andong, Kyongpook	1997. 8.	38.7-64.5×12.9-19.4(48.2×15.2)	3.21:1

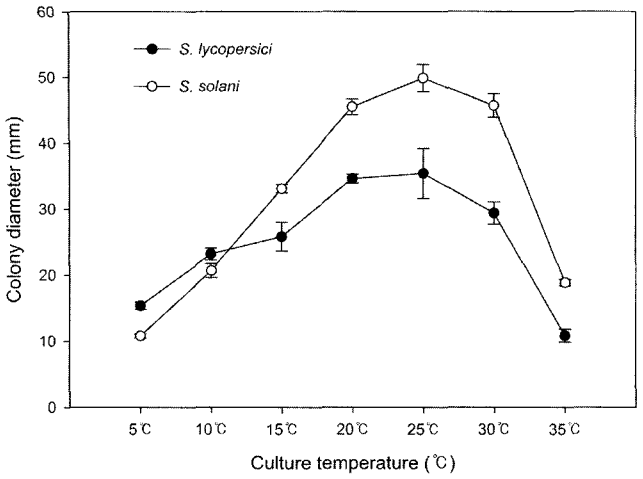

Fig. 4. Mycelial growth on PDA plates of two species of *Stemphylium* causing gray leaf spot in pepper plants as influenced by temperature 7 days after seeding with mycelial plug 5 mm in diameter. Vertical bars indicate 1 standard deviation.

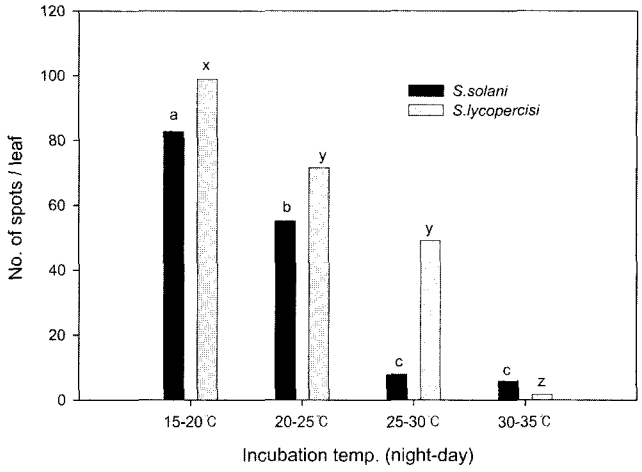

Fig. 5. Effect of incubation temperature on number of spots developed on three most diseased leaves 5 days after inoculation. Mean separation within species by Duncan's multiple range test at $P = 0.05$.

both *S. solani* and *S. lycopersici* were recovered, *S. solani* was predominant. Some variation in spore size and morphology was also observed among the isolates collected. Although the disease was found to occur in almost all areas where peppers are grown, it was more severe in mountainous areas, such as the northern part of Gyeongbuk and Gangwon provinces, where the nights are relatively cooler.

Effect of temperature on mycelial growth. Mycelial growth was observed throughout the test temperature range, 5-35°C (Fig. 4). The optimum temperature for mycelial growth was about 25°C, with severe inhibition at 5 and 35°C.

Effect of temperature on conidial germination. The conidia of both species germinated throughout the test temperature ranging from 15 to 35°C, with the maximum percentage of germination and germ tube length at 25°C. Thus, the optimum temperature for conidial germination was the same as that for mycelial growth.

Effect of incubation temperature on disease development. The maximum number of spots was developed with the 15°C night and 20°C day temperature regime (Fig. 5). The number of spots formed on the three most diseased leaves decreased with an increase in the incubation temperature, thus, very few spots were observed with the 30°C night and 35°C day temperature regime. This was consistent with the observation that the disease was more prevalent in mountainous areas, such as the northern part of Gyeongbuk and Gangwon provinces, where the night temperature is lower than in other production areas.

Effect of media, light, and temperature on sporulation. Both species sporulated best on the V8 juice agar plates under 12-hour near ultraviolet (NUV) light at 20°C (Table 3). Near ultraviolet light was not a requisite for sporulation, as both species also sporulated well under fluorescent light. Yet, neither species sporulated much under continuous darkness. Thus, the sporulation behavior of the two *Stemphylium* species was the same as that of *S. botryosum* and *S. trifolii* in response to light (Leach, 1962a). Sporulation decreased as the air temperature rose above 25°C, and no sporulation occurred above 28°C. It is generally known that irradiation at 20°C induces the sporulation of many fungi, yet this effect is lost at high temperatures for some fungi (Johnston and Booth, 1983), which appeared to be the case with the two species of *Stemphylium*. Temperature was also found to influence the shapes of the spores produced (Table

Table 3. Sporulation of *Stemphylium* spp. produced on three culture media at 20°C 7 days after transfer

Culture conditions	Sporulation ($\times 10^3$)/dia. 13 mm agar disk)					
	S. solani			*S. lycopersici*		
	V8A[z]	PCA[z]	PDA[z]	V8A	PCA	PDA
Light and darkness (12 hours/12 hours)	14.2 a[y]	2.5 b	0.8 b	31.8 a	3.0 b	3.1 b
Continuous darkness	0	0	0	5.5 a	0.4 b	0.2 b

[z] V8A: vegetable 8 juice agar; PCA: potato carrot agar; PDA: potato dextrose agar.
[y] Mean separation within culture condition by species by Duncans multiple range test, $P \leq 0.05$.

Table 4. Effect of temperature on morphology and conidial dimensions of *S. solani* and *S. lycopersici*

Stemphylium spp.	Conidial characters	Temperature		
		20°C	25°C	30°C
S. solani	Dimension (μm)	35-58×17-23 (Av. 6.0×19.2)	37-53×17-25 (Av. 44.8×21.5)	No sporulation
	L/W ratio	2.4 : 1	2.0 : 1	
S. lycopersici	Dimension (μm)	37-75×15-30 (Av. 5.3×17.4)	35-68×12-25 (Av. 49.5×18.0)	20-67×15-25 (Av. 48.5×18.5)
	L/W ratio	3.3 : 1	3.0 : 1	2.6 : 1

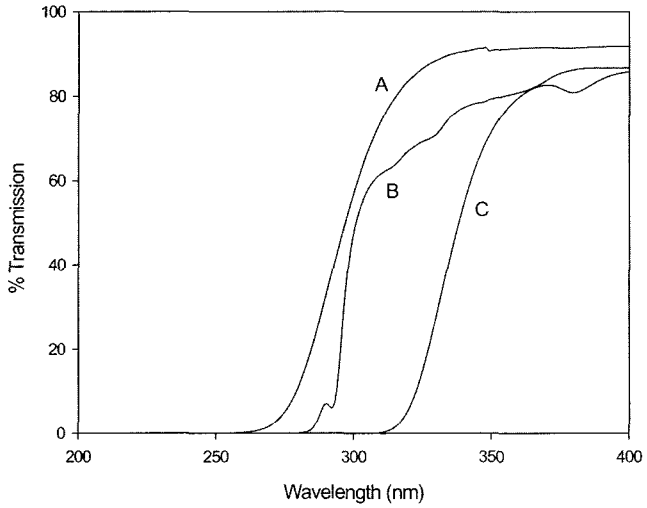

Fig. 6. Light transmission by Pyrex (**A**), plastic (**B**), and domestically produced glass (**C**) Petri dishes according to light wavelength.

4). The conidia became slim as the culture temperature decreased to within 20-30°C.

Light transmission of Petri dishes and its effect on sporulation. Both species of *Stemphylium* sporulated abundantly when cultured in plastic or imported glass Petri dishes (Pyrex) under fluorescent light, yet did not sporulate when cultured in domestically produced glass Petri dishes, regardless of the culture media used. When measuring the wave length of the light transmitted by the lids of the three brands of Petri dish, the domestically produced glass Petri dishes exhibited very poor or no transmission at a wavelength range of around or below 300 nm (Fig. 6) when compared to the Pyrex glass and plastic, yet it had similar transmission to the plastic and Pyrex glass Petri dishes at 350 nm or above. Leach (1962a, 1962b) reported that the sporulation of many fungi, including *Stemphylium*, was significantly reduced or inhibited when near-ultraviolet and blue wavelengths were filtered from daylight fluorescent lamps, and that window glass superimposed over a Pyrex Petri dish lid greatly reduced the sporulation of *Ascochyta posii*, which was attributed to the absorption of the shorter wavelengths of near ultraviolet radiation by the window glass. For *A. pisii*, two wavelengths, 237.8 and 313.1 nm, were notably effective in inducing pycnidial formation (Leach, 1962b). However, in the current measurements, the transmission of those wavelengths through the lids of domestically produced glass Petri dishes was non-existent or negligible (Fig. 6). After investigation, it was discovered that domestically produced glass Petri dishes are made by cutting sheets of window glass into the necessary pieces

Table 5. Conidial germination of *Stemphylium* spp. as influenced by temperature

Stemphylium spp.	Incubation temp. (°C)	% conidial germination[z]		Length of germ tube (μm)	
		1 hour	3 hours	1 hour	3 hours
S. solani	15	0.2	11.0	2.5	6.9
	20	0.3	21.0	3.8	10.1
	25	0.9	40.0	17.5	27.3
	30	0.3	0.8	5.8	23.1
	35	0.1	0.7	2.5	22.5
S. lycopersici	15	20.0	46.5	12.3	12.5
	20	31.0	57.4	19.3	23.8
	25	39.0	62.0	34.0	40.8
	30	20.0	36.6	24.2	38.0
	35	15.0	33.0	24.0	29.5

[z] Based on 50 conidia observed.

and welding them together. Accordingly, this explains why neither species of *Stemphylium* sporulated in the domestically produced Petri dishes, as the near ultraviolet wavelengths, around 300 nm and below, from the fluorescent lamp were blocked by the lids made of window glass. It is already known that 'Pyrex' Petri dishes and many colorless plastics transmit near ultraviolet radiation quite effectively, in contrast to standard window glass, which is not recommended for interposing between lamps and Petri dishes (Johnston and Booth, 1983; Leach, 1961, 1962a, 1962b). However, since transmission data on the near ultraviolet wavelengths of window glass is unavailable, the current measurement data is valuable, especially as it was not previously known that domestically produced glass Petri dishes are made of window glass.

For the routine culture of *Stemphylium* spp. for sporulation, the following CMI recommendations may be used with the substitution of domestically available lamps with an equivalent light quality: (1) A light-bench is constructed with a holder for three fluorescent tubes, then a near ultraviolet (black light) tube (Philips TL40W/08) is placed in the center with a daylight tube on either side (Philips cool white MCFE 40W/33). (2) All three tubes are controlled by a time switch with a 12-hour on/off cycle. (3) Polyethylene Petri dishes containing the fungi on nutrient media are placed on the bench about 35 cm below the fluorescent tubes. The edges of the dishes are sealed with adhesive tape to prevent the agar from drying out.

For the rapid and mass production of spores in the lab, V8 juice agar plates were flooded with spore suspensions and cultured in a 15°C dark and 20°C light incubator with 12-hour alternation. To prepare the spore suspensions, pure isolates were cultured on V8 juice agar plates for 3-4 days, and then culture blocks with many spores were extracted from sporulating colonies, placed in test tubes with sterile water, and the tubes agitated by hand to dehisce the spores in the water. Finally, the V8 juice agar plates were flooded with the spore suspensions.

To control the disease, fungicides can be applied either at the beginning of the disease or in advance for protection. Many fungicides sold for the control of anthracnose are effective in controlling the disease (Kim et al., 1999b). Resistance to the disease has also been found in a few pepper accessions (Cho et al., 2001). Therefore, resistant varieties may be available in the future if breeding efforts are undertaken.

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